



Coping with temperature extremes: thermal tolerance and behavioral plasticity in desert leaf-cutting ants (Hymenoptera: Formicidae) across an altitudinal gradient

Natalia I. YELA*, Luis A. CALCATERRA* & Adriana ARANDA-RICKERT*

Abstract

Current approaches to assess and predict the impact of climate warming on ectotherms are largely based on their physiological sensitivity to temperature. However, these physiological studies provide little insight into the mechanisms by which particular species respond to increasing temperatures through behavior, phenotypic plasticity, or genetic adaptation. In this work, we focus on the potential of thermoregulatory behaviors of terrestrial ectotherms to buffer the impact of climate change. Using as models two sympatric species of leaf-cutting ants (*Acromyrmex lobicornis* and *A. striatus*), we attempt to investigate whether their altitudinal distribution across an arid-base mountain is predicted by their physiological critical thermal limits (CT_{max} and CT_{min}), temperature of foraging activity, and fungus garden depth. We found that both species differed in their critical thermal limits, but this difference did not explain their pattern of abundance along the altitudinal gradient. Both species showed plasticity in their behavioral responses to temperature changes across the elevational gradient. The onset and daily pattern of foraging activity changed with altitude, such that the range of temperatures at which both species foraged as well as the temperature of maximum foraging activity were maintained across all elevations. The depth of the fungus chamber changed as a function of the environmental temperature, being deeper during the summer compared with the winter, and at the base of the mountain compared with the highest elevation. Our results show that we need to go beyond thermal physiology to predict how some ectotherms species respond to climate change and that the plasticity in behavioral responses to extreme temperature could allow species to persist in warmer habitats despite increasing temperatures.

Key words: *Acromyrmex*, critical thermal limit, northwestern Argentina, elevational gradient, fungus garden depth, activity pattern.

Received 10 October 2019; revision received 3 March 2020; accepted 18 March 2020

Subject Editor: Jens Dauber

Natalia I. Yela (contact author) & Adriana Aranda-Rickert, Centro Regional de Investigaciones Científicas y Transferencia Tecnológica de La Rioja (CRILAR-CONICET), Anillaco, La Rioja, Argentina.

E-mail: natalia.yela@gmail.com

Luis A. Calcatera, Fundación para el Estudio de Especies Invasivas (FuEDEI) and CONICET, Hurlingham, Buenos Aires, Argentina.

*These authors contributed equally to this work

Introduction

Global warming has stimulated worldwide studies aiming to assess or predict the impact of raising environmental temperatures on faunas (SALA & al. 2000). Most of such studies have focused on thermal tolerances of terrestrial ectotherms (e.g., DEUTSCH & al. 2008, DIAMOND & al. 2012, HOFFMAN & al. 2013) because they represent the vast majority of terrestrial biodiversity (WILSON 1992) and are especially likely to be vulnerable to climate warming due to the strong influence of environmental temperature on their physiological and behavioral functions (HUEY & STEVENSON 1979).

How ectotherms respond to ambient temperature change depends largely on their physiology. Simple metrics such as critical thermal maximum and minimum limits (CT_{max} and CT_{min}), which are experimentally measured as the upper and lower temperatures at which individuals lose essential motor functions (LUTTERSCHMIDT & HUTCHISON 1997), are often used to estimate thermal performances of individual species as well as to assess their current and predicted geographical distribution (CALOSI & al. 2010, REZENDE & al. 2011, SUNDAY & al. 2011, BIRKETT & al. 2018). A number of studies have shown that CT_{max}

commonly declines with increasing latitude and elevation, leading to the assumption that thermal regimes in which populations are found shape thermal tolerances and thus dictate species' latitudinal and elevational range limits in evolutionary and / or ecological times (GASTON & CHOWN 1999, ADDO-BEDIAKO & al. 2000, TERBLANCHE & al. 2006, HOFFMANN & al. 2013).

However, the generality of the relationship between thermal physiology and geographic range limits is not supported by many ectotherms' groups, in which CT_{max} shows little variation across temperature gradients (SUNDAY & al. 2011, ARAÚJO & al. 2013, BISHOP & al. 2017, NOWROUZI & al. 2018). Therefore, for a complete understanding of ectotherms vulnerability to climate change we need to consider other factors than their physiological sensitivity. A little-explored key point for predicting species range responses to climate change is the extent to which behavioral responses to temperature could buffer physiological impacts of warmer temperatures (KEARNEY & al. 2009, SUNDAY & al. 2014).

Social insects, such as ants, provide a unique opportunity to study how behaviors can safeguard the impact of warming, as temperature affects not only the performance of individuals but also the entire colony, which is the unit of selection. Among behavioral responses of ants to temperature, shifts in the place (open habitat / shade) and times of activity (nocturnal / diurnal) appear to be critical to avoid thermal stress (BRIESE & MACAULEY 1980, JAYATILAKA & al. 2011, SUNDAY & al. 2014). As central place foragers, ants can also behaviorally regulate microclimatic conditions by selecting particular microenvironments for their nests (BLÜTHGEN & FELDHAAR 2009), by altering nest architecture (BOLLAZZI & al. 2008) and by relocating the broods within the nest to regions with adequate temperature (ANDERSON & MUNGER 2003, PENICK & TSCHINKEL 2008).

Leaf-cutting ants (hereafter LCA) in particular, also need certain temperature and humidity conditions to cultivate their mutualistic fungus, the food source for their larvae, and these can be achieved by an active choice of proper locations of the fungus chamber / chambers inside the nest (NAVARRO & JAFFÉ 1985, BOLLAZZI & ROCES 2002, MUELLER & al. 2011, BRANSTETTER & al. 2017). For instance, as soil temperature is negatively correlated with soil depth (HILLEL 1998), LCA in the *Acromyrmex* genus relocate their fungus and brood to the temperature range in which maximal growth of ant fungus is achieved (BOLLAZZI & ROCES 2002) and build subterranean nests in warm soils to avoid the higher temperatures near the soil surface and superficial nests in cold ones (taking advantage from the milder temperatures at the superficial soil layers) (BOLLAZZI & al. 2008).

In this work, we attempt to investigate the extent to which altitudinal distribution pattern of ants is influenced by their thermal physiology and behavior, using as model two sympatric species of LCA (*Acromyrmex lobicornis* and *A. striatus*). Previous studies have shown that *A. lobicornis* reaches higher latitudes (FARJI-BRENER & RUGIERO 1994, SÁNCHEZ RESTREPO & al. 2019) and forages at

lower temperatures than *A. striatus* (NOBUE-BEHRMANN & al. 2017), therefore suggesting a preference for colder temperatures. However, to our knowledge no study has been carried out to relate thermal tolerances and behavior with the variation of temperature along an altitudinal gradient. Specifically, we set the following questions: 1) Does physiological thermal tolerance predict elevational distribution?; 2) Does the temperature of foraging activity predict elevational distribution?; and 3) Does nest architecture and fungus garden depth predict elevational distribution?

We expect that: 1) both species will differ in their thermal limits, with *Acromyrmex lobicornis* being more cold tolerant (lower CT_{max} and lower CT_{min}) than *A. striatus*, therefore *A. lobicornis* will increase its abundance towards the higher (colder) elevations of the mountain gradient, while *A. striatus* will do it towards the lower (warmer) elevations; 2) *A. lobicornis* will forage at lower temperatures and therefore will be more abundant at higher elevations, while *A. striatus* will forage at warmer temperatures and therefore will increase its abundance towards the base of the mountain gradient; and 3) *A. lobicornis* fungus garden will be more superficial than that of *A. striatus*.

Material and methods

Study site: The study was conducted between 2015 and 2018 along an elevational gradient from 800 to 1900 m of altitude, in the eastern slope of the Sierra de Velasco mountain range, near Anillaco (28° 48' S, 66° 56' W), La Rioja Province, northwestern Argentina. The mountain is located within the northern portion of the Monte Desert, the most arid rangeland of Argentina (ABRAHAM & al. 2009). The climate is arid with a marked seasonality. Average annual temperature is 16.9 °C, and average annual precipitation is 233 mm, falling mainly during the December-March summer wet season (Anillaco Meteorological Station, data from 2000 to 2010).

The elevational transect covers three vegetation types from the base to the top of the mountain (ARANDA-RICKERT & al. 2014, CABIDO & al. 2018, Fig. 1): 1) the base (800 - 1300 m above sea level (a.s.l.)) occupies the dry intermountain valleys with the characteristic vegetation of the Monte Desert, an open shrubland dominated by *Larrea cuneifolia* ("jarilla", Zygophyllaceae) and shrubby Fabaceae and Cactaceae species; 2) middle elevations (1300 - 1800 m a.s.l.) are located on a mid-slope (3 - 4% steepness). It is a closed shrubland dominated by *Flourensia fiebrigii* (Asteraceae) and characterized by the presence of columnar cacti (*Trichocereus terscheckii*); and 3) high elevations (above 1800 m a.s.l.) correspond to a Chaco Serrano vegetation type, and are characterized by a high steepness (ca. 50%) and riparian open forests with *Parasenegalia visco* (Fabaceae) and *Lithraea molleoides* (Anacardiaceae) as dominant tree species and closed shrublands dominated by Asteraceae and Verbenaceae species. The sites selected for sampling have relatively low anthropogenic disturbance. Field work was carried out along the altitudinal transect of 1100 m, with six sampling stations spaced at

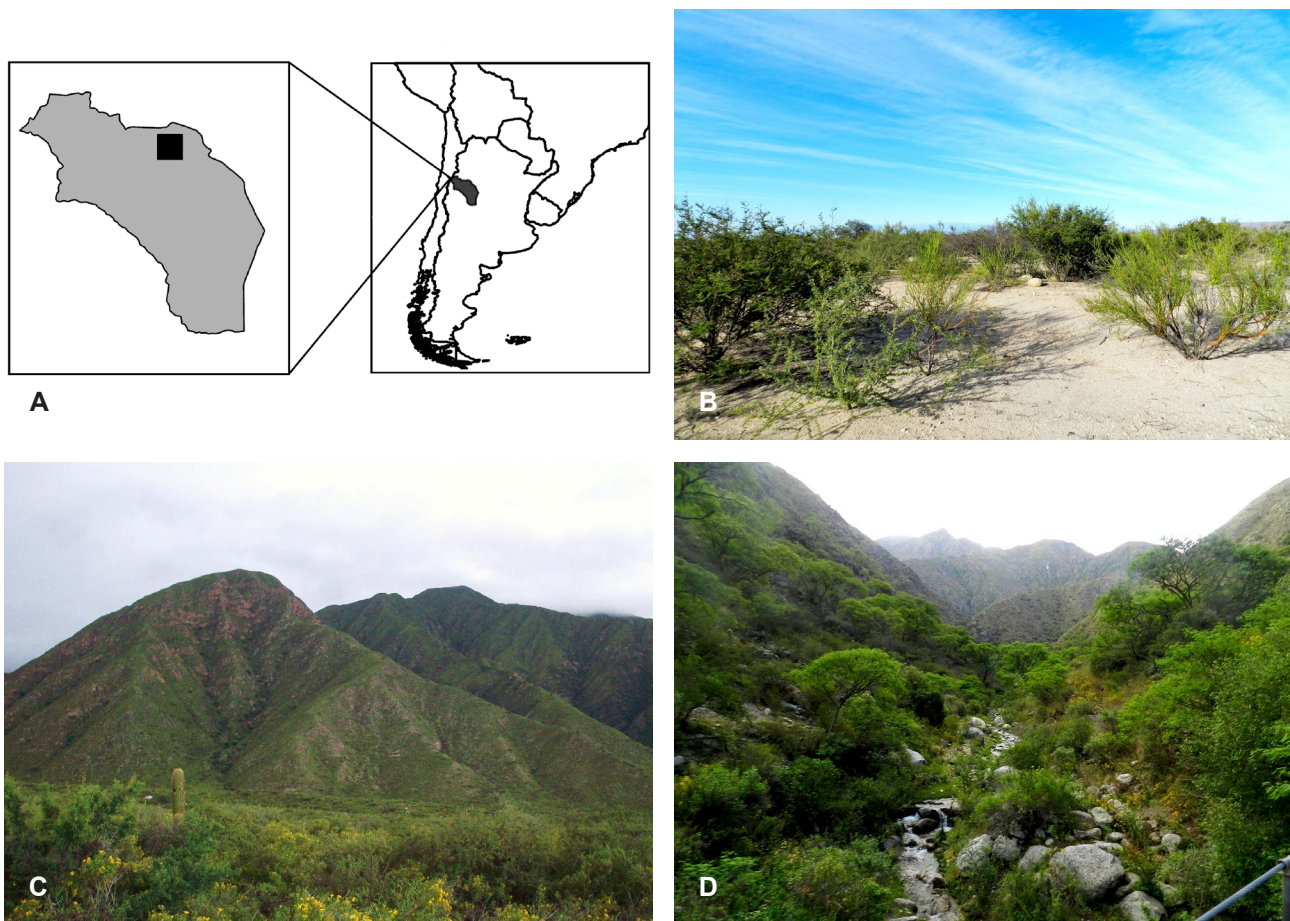


Fig. 1: (A) Location of the study area in La Rioja, northwestern Argentina. (B - D) Low, mid, and high elevation vegetation types for the altitudinal transect in the Sierra de Velasco. (B) Open shrubland at low elevations (800 - 1300 m a.s.l.); (C) Closed shrubland at mid elevations (1300 - 1800 m a.s.l.); (D) Chaco Serrano vegetation at high elevations (above 1800 m a.s.l.).

elevational intervals of 100 - 300 m (altitudes 800, 1100, 1300, 1600, 1800 and 1900 m a.s.l.) and separated linearly between 3 and 10 km (at the top and the base respectively). Although the elevation of the highest peak of the mountain is 4189 m, the last sampling point was at 1900 m a.s.l. due to the difficulty for accessing and monitoring additional stations at higher elevations, and the presumable absence of LCA above 2400 m a.s.l. (L.A. Calcaterra, unpubl.). Ant samplings and observations were performed during the summer, and nest excavations during summer and winter.

At each elevation, climatic variables (temperature and relative humidity) were recorded approximately 1 m above ground every 1 h from September of 2015 to December of 2017 using data loggers (HOBO H8 logger) placed in an open plastic shelter, but coated with an aluminum membrane to reflect the sun's rays. Although soil surface temperatures would be more relevant to ants than air temperatures (KASPARI & al. 2015), temperature measured at 1 m was highly correlated with soil temperature and logistically easier to measure with the available instruments. The mean annual temperature, the mean maximum daytime temperature of the warmest months (December - January), the mean minimum daytime temperature of the coldest months (June - July), the absolute minimum and

maximum temperatures, and the mean annual relative humidity were calculated for each station.

Ant species: *Acromyrmex lobicornis* and *A. striatus* are the only LCA species inhabiting the study region (ARANDA-RICKERT & al. 2014). Both are similar in size and use gramineae and dicotyledons (leaves, flowers and fruits) as substrate for culturing their fungus (NOBUA-BEHRMANN & al. 2017). Both species coexist over a wide latitudinal range, covering arid and semi-arid regions from the south of Brazil and Bolivia to the center-western of Argentina, with *A. lobicornis* reaching slightly higher latitudes (extending across the Monte ecoregion up to 44° S) than *A. striatus* (until 42° S in the Río Negro province) (FARJI-BRENER & RUGGIERO 1994, ARMANI & QUIRÁN 2007, SÁNCHEZ-RESTREPO & al. 2019). The two species have been found in Argentina at a maximum altitude of ~ 2400 m a.s.l. (L.A. Calcaterra, unpubl.). As it happens with all other ant species of the study area, the highest LCA activity occurs during spring and summer, with an almost hibernating state during winter (ARANDA-RICKERT & al. 2014).

Species abundance: The relationship between LCA species abundance and elevation across the entire gradient was assessed using an abundance index. The abundance

index was calculated as the product of LCA nest density \times average nest size. Nest density was estimated in December 2016 at each altitudinal station using six transects of 100×10 m, at least 100 m apart from one another and perpendicular to the slope. Along each transect, the nests were located through visual inspection and the species identity confirmed by manual collection of workers, which were placed in eppendorf tubes with 96% alcohol for later identification in the laboratory using available keys (KUZNEZOV 1978, CUEZZO & FUENTES 2004). LCA's density at each station was expressed as the number of nests / ha. Nest size was estimated as the nest surface area approximated to an ellipsoid, by measuring the maximum distance between the four farthest nest entrances along two perpendicular axes. Nest size was expressed as m^2 and averaged for each elevation.

Thermal tolerance: The CT_{min} and CT_{max} for each LCA species were measured using a temperature ramping method based on WARREN II & CHICK (2013) but using a growth chamber with controlled temperature instead of a water bath. In February 2017, live LCA workers were collected in five elevations (800, 1100, 1300, 1600, and 1800 m a.s.l.). *Acromyrmex* ants are polymorphic, and since body size affects many physiological traits (CHOWN & GASTON 2010), small (3 - 4 mm) and large workers (6 - 7 mm) (approx. 50 individuals each) were collected from three different nests of each species per elevation. The length of each individual was measured from the mandibular apex to the gastral apex.

Ants were kept in separate plastic boxes (one per each size group and colony) partially filled with plaster and transported to the lab, where testing began within 3 h of field collection. Ants' CT was measured using two separate growth chambers, one for the CT_{max} (San Jor SL 70C) and one for the CT_{min} trial (Bio-Control 1000 Model). Ants from each group were individually placed in Petri dishes and acclimatized for 20 min at $15^\circ C$ or $40^\circ C$ for CT_{min} or CT_{max} respectively. After the acclimation, the temperature was progressively lowered (for CT_{min}) or raised (for CT_{max}) by $\sim 1^\circ C / min$, and the temperature at which each individual lost mobility and righting response was recorded as its CT_{max} or CT_{min} .

For each trial, ants were haphazardly selected from different groups (size, colonies and stations). The test was ran as many times to obtain five replicates per colony ($5 individuals \times 2 sizes \times 3 colonies \times 5 sites = 150 individuals$ per species for each variable (CT_{max} and CT_{min})). For each CT_{max} and CT_{min} experimental run, five individuals were used as control of each group, which were individually placed in Petri dishes and maintained after acclimation at room temperature. New individuals were used for each trial.

Foraging activity: Foraging activity was evaluated in five or six colonies of each species randomly selected at the lowest and highest altitudinal stations where LCA were found (800 and 1800 m a.s.l.) during February of 2016 (summer). Colonies were monitored for 5 min every 3 h for a 24 h period. The aboveground activity at trails was quantified by counting the number of workers returning to

the nest that walked across a marked point located on the main trail 1 m from the main nest entrance. The ranges of temperatures at which *Acromyrmex lobicornis* and *A. striatus* colonies were active were estimated by averaging the range temperature limits at which each colony was found foraging at each station. For each colony, the temperature at which it showed its maximum activity was also determined. Temperature data were obtained from the data loggers.

Nest structure: Three mature nests of each species were selected in the lowest, mid and highest altitudinal stations where ants occurred (800, 1300 and 1800 m a.s.l.) in summer and winter 2018, for a total of 36 nests ($3 nests \times 3 elevations \times 2 seasons \times 2 species$). Nests were carefully excavated inwards and downwards and the number, depth and dimensions (length, height and width) of the fungus chambers were recorded.

Statistical analyses: Generalized Linear Models (GLM) were used to test the relationship between the abundance indexes of each LCA species and elevation, considering elevation as a quadratic, exponential or linear term during model selection.

Differences on CT_{max} and CT_{min} between the two LCA species were tested using a Linear Mixed Model, including the elevation from which the ants were sampled as a random effect. GLM were also used to test for the effect of elevation and ant size on CT_{max} and CT_{min} of each species separately. The relationship between critical thermal limits and elevation was further analysed separately for each species by linear regressions.

Differences on the minimum and maximum temperatures of foraging activity were evaluated between species and altitudes with Kruskal-Wallis tests, since data did not satisfy the assumptions of normality and homocedasticity to perform an ANOVA test. Temperatures of maximum foraging activity in the trails were compared between species and among altitudes with GLM.

Differences in fungus chamber depth were tested between seasons (winter and summer) and among elevations (low, mid and high) using two-way ANOVA tests. First, differences in fungus garden depth between LCA species and seasons were evaluated, and then separately for each season, differences between species and among elevations. Tukey multiple comparison tests were used to assess pairwise differences at $p < 0.05$.

For all the GLM analyses, a multi-model selection based on Akaike's information criterion corrected for small samples (AICc) was used to search for the most parsimonious model ($AICc < 2.0$). Data were checked graphically for influential data and outliers by Cook's Distance. All analyses were performed in R version 3.6.1 (R DEVELOPMENT CORE TEAM 2019). All data are presented as mean values \pm S.E.

Results

Climatic variables: Mean annual and mean maximum temperatures decreased linearly with elevation (linear regressions of temperature on elevation: $r^2 = 0.72$ and

Tab. 1: Summary statistics for climatic variables along the elevational gradient. Temperatures are given in °C as means over three years (2015 - 2017); minimum temperatures were measured during the coldest months (June - July), and maximum temperatures during the warmest months (December - January).

Altitude (m a.s.l.)	800	1100	1300	1600	1800	1900
Mean temperature	17.18	18.94	16.62	13.41	14.53	11.69
Absolute minimum temperature	-4.30	-1.51	-2.19	-1.10	-4.20	-6.82
Absolute maximum temperature	42.94	42.94	37.65	35.00	38.17	39.22
Mean maximum temperature	32.96	33.13	29.36	26.32	27.68	27.62
Mean minimum temperature	2.84	3.79	3.51	4.80	2.54	0.95
Mean relative humidity (%)	46.29	48.93	57.06	53.45	60.72	62.76

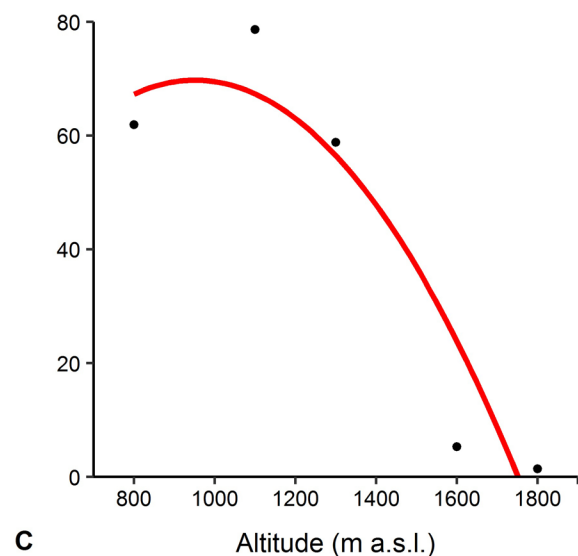
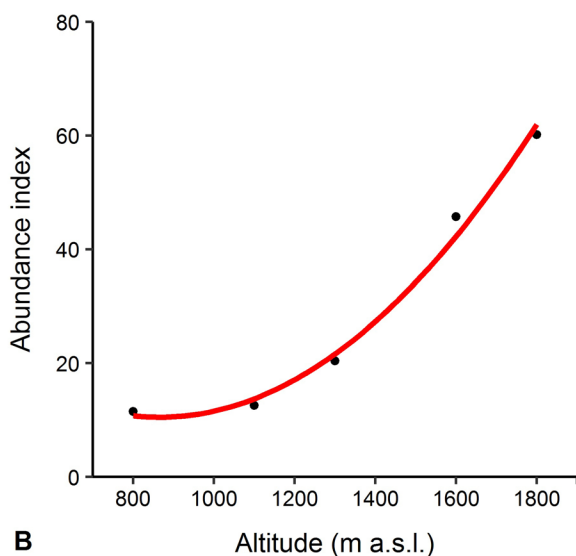
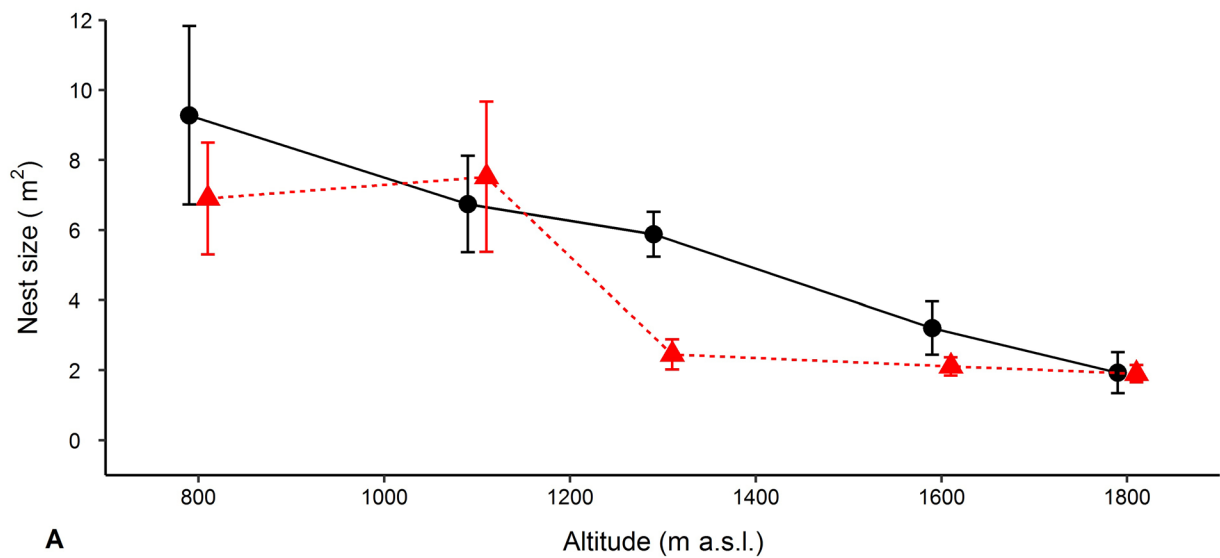


Fig. 2: (A) LCAs nest sizes by elevation along the altitudinal transect for *Acromyrmex striatus* (red) and *A. lobicornis* (black). Nest sizes are means \pm S.E. of 10 nests of each species per altitude. Abundance index (LCA nest density \times nest size) along the elevational gradient for (B) *A. striatus* and (C) *A. lobicornis*. Each point represents the number of nests ha^{-1} \times the average size of the nests at each altitude. Lines represent significant trends. The fitted equations of the GLMs are $y = 54.82 - 0.10x + 5.90E^{-5}x^2$ and $y = -28.65 + 0.21x - 0.0001x^2$ for *A. striatus* and *A. lobicornis* respectively.

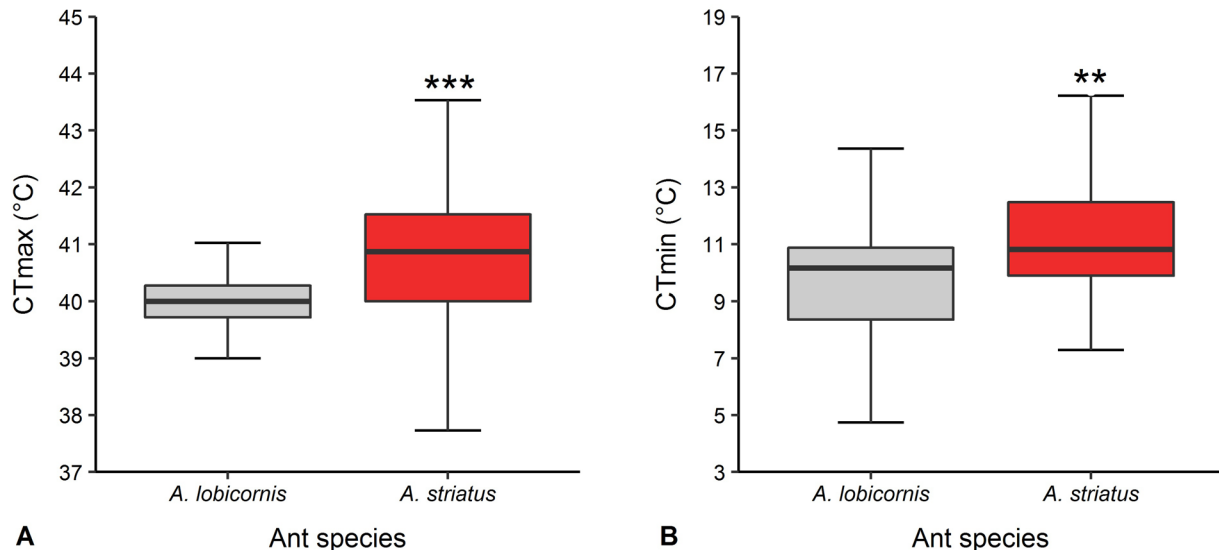


Fig. 3: (A) Maximum (CT_{max}) and (B) minimum (CT_{min}) thermal tolerance limits of the two LCA species. Data correspond to 150 individuals of each species (5 individuals \times 2 sizes \times 3 colonies \times 5 sites along the elevational transect). The color of the boxplot indicates the species: *Acromyrmex lobicornis* (gray) and *A. striatus* (red). Boxplots display the median (central line), 25th and 75th percentiles (bottom and top boxes) and the largest and smallest values (whiskers). *** $p < 0.001$, ** $p < 0.01$.

0.77 respectively, $p < 0.05$), by 4.90 and 4.85 °C for every kilometer increase in elevation, respectively (Tab. 1). The mean minimum temperatures did not vary with elevation ($r^2 = 0.14$, $p = 0.46$) as did the maximum temperature, though tended to decline with the increase in elevation from 2.84 °C at the base up to 0.95 °C in the top (1.72 °C / km). Absolute maximum and minimum temperatures showed greater variation, being 42.94 and -4.30 °C at the base and 35.00 and -6.82 °C at the top, respectively (7.20 °C / km and 2.30 °C / km). Mean relative humidity was low grouping all sites (54.86%) and tended to increase with elevation (from 46.29% at the base to 62.76% at the top (15.00% / km)), though this relation was not significant ($r = -0.75$, $p = 0.08$).

Species abundance: The size of nests for both LCA species decreases from the base to the top of the gradient (Fig. 2A). Abundance significantly increased with elevation for *Acromyrmex striatus* (pseudo- R^2 (explain deviance) = 0.94, $df = 4$, $p < 0.01$; Fig. 2B). *Acromyrmex lobicornis* showed a distinct pattern, with higher abundances at the base and middle elevations (800 - 1300 m a.s.l.) and a decrease towards the top (pseudo- R^2 (explain deviance) = 0.81, $df = 4$, $p < 0.05$; Fig. 2C). *Acromyrmex lobicornis* nests are between $9.28 \pm 2.55 \text{ m}^2$ and $1.93 \pm 0.59 \text{ m}^2$ (means \pm S.E.); while *A. striatus* nests are between $6.90 \pm 1.6 \text{ m}^2$ and $1.90 \pm 0.25 \text{ m}^2$ (means \pm S.E.). The last sampling station in which we found *A. lobicornis* was at 1600 m a.s.l., but further sampling (not included in this study) showed that the highest elevation reached by this species was 1730 m a.s.l. At 1800 m a.s.l., only *A. striatus* was present, and this was the upper elevation range limit for this species.

Thermal tolerance: Thermal tolerance differed significantly between the two LCA species, both for

CT_{max} ($\chi^2 = 3.76$, $p < 0.001$) and CT_{min} ($\chi^2 = 3.468$, $p < 0.01$). *Acromyrmex striatus* had higher CT_{max} and CT_{min} than *A. lobicornis* (CT_{max} : 40.68 ± 0.11 °C vs. 39.87 ± 0.08 °C, $P < 0.001$; CT_{min} : 11.42 ± 0.19 °C vs. 9.82 ± 0.18 °C, $P < 0.001$, respectively; means \pm SE; Fig. 3). Both thermal limits are above the mean minimum temperature and below the mean maximum temperature recorded over the study period at the highest and lowest elevation (0.95 and 32.96 °C respectively). The elevation had no significant effect on CT_{max} of both species ($p > 0.05$) and only an unexpected positive effect on CT_{min} of *A. striatus* ($p < 0.01$, see Fig. S1, Tab. S1, as digital supplementary material to this article, at the journal's web pages). However, when analyzing the relationship between CT_{min} and elevation for this species, the variation in elevation explained only 15% of the variation in CT_{min} ($r^2 = 0.1538$, $p < 0.0001$). Ant body size had no significant effect on CT_{max} and CT_{min} of both species ($p > 0.05$, see Tab. S1).

Foraging activity: The temperature range of foraging activity during summer (minimum and maximum foraging temperature limits) did not differ significantly between both species, ranging between 20.60 and 27.00 °C for *Acromyrmex lobicornis* and between 22.50 and 29.20 °C for *A. striatus* ($\chi^2 = 0.83$, $p = 0.36$ for minimum temperature and $\chi^2 = 0.21$, $p = 0.64$ for maximum temperature). The temperature of highest foraging activity (24.82 ± 0.89 °C and 25.44 ± 0.78 °C, mean \pm SE for *A. lobicornis* and *A. striatus*, respectively) did not differ significantly between species ($Z = 1.254$, $p = 0.229$) and altitudes ($Z = 1.850$, $p = 0.084$). We found differences in the daily activity pattern between the two LCA species only at the highest elevation. At the lowest altitudes (800 m a.s.l.) both species showed an overlapping and unimodal activity pattern. They foraged during the night, starting at

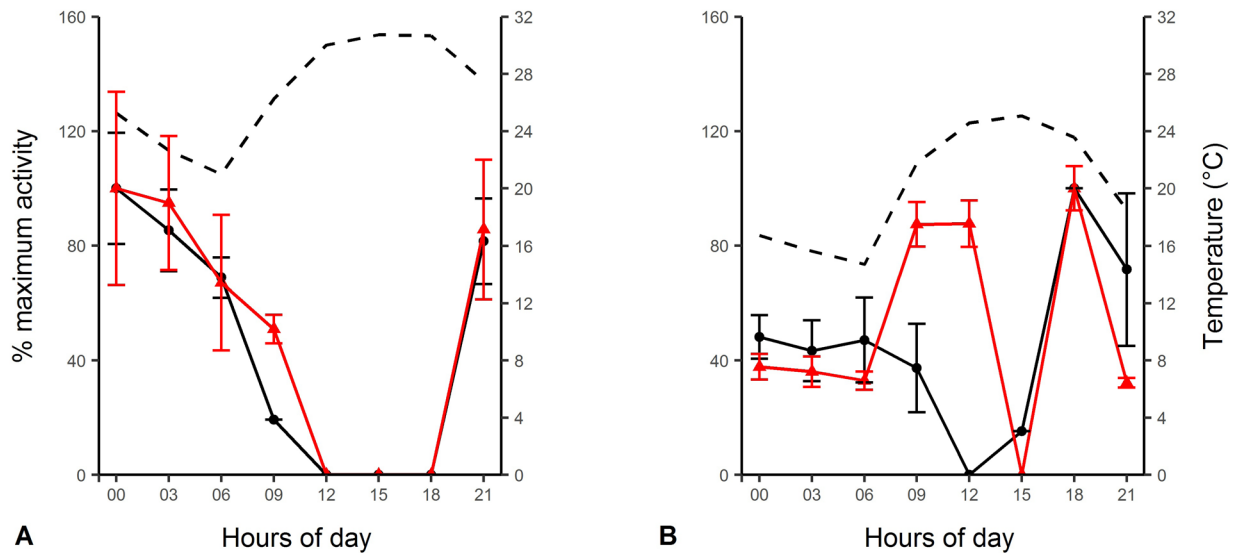


Fig. 4: Daily foraging activity patterns of the two LCA species in summer at the (A) lowest (800 m a.s.l.) and (B) highest (1800 m a.s.l.) elevations along the gradient. Foraging activity is estimated from the mean number (\pm SE) of workers returning to the nest for 5 min every 3h for a 24h period ($n = 6$ colonies per species) and expressed relative to the maximum activity value for each species (100% of activity) observed during each sampling day. The color of the line indicates the species: *Acromyrmex lobicornis* (black line) and *A. striatus* (red line). Average ambient temperature throughout the day is also shown (dashed line).

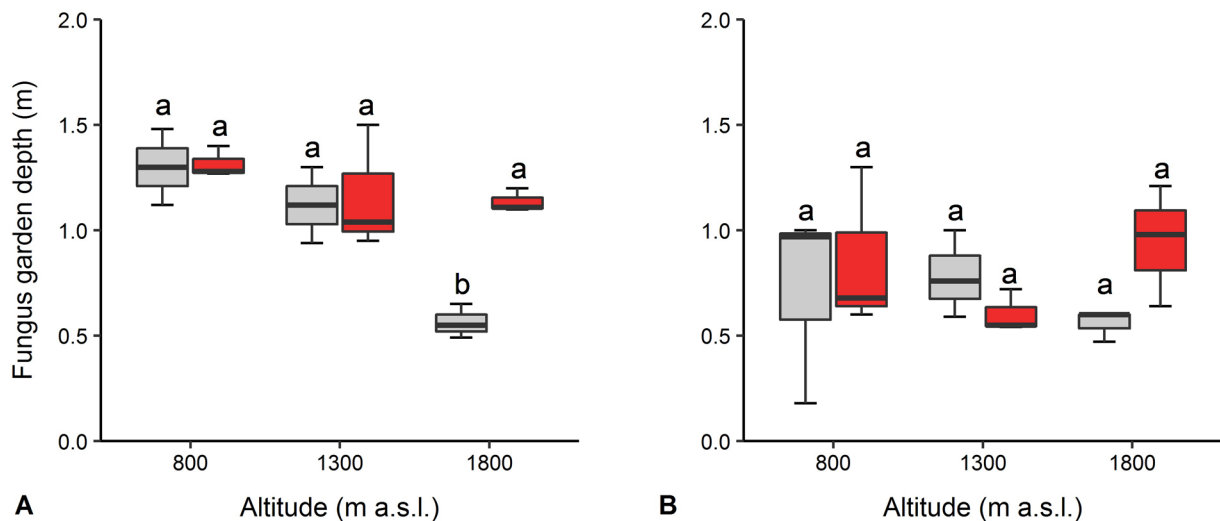


Fig. 5: Comparison of fungus garden depth of *Acromyrmex lobicornis* (gray) and *A. striatus* (red) among low (800 m a.s.l.), mid (1300 m a.s.l.) and high (1800 m a.s.l.) elevations during (A) summer and (B) winter. Boxplots display the median (central line), 25th and 75th percentiles (bottom and top boxes) and the largest and smallest values (whiskers) of three nests of each species per elevation. Boxplots sharing the same letters indicate no significant differences ($P > 0.05$).

dusk (21:00h) and continuing until next morning (the coldest daily period), with a peak of activity at midnight and no activity during the hours of the day (Fig. 4A). At 1800 m a.s.l., a bimodal pattern emerged for *A. striatus*, with peaks of activity during the morning (from 09:00 to 12:00 h) and the afternoon (around 18:00h) and avoiding the hottest summer midday and early afternoon. On the other hand, *A. lobicornis* maintained at this altitude the unimodal pattern, though showing the maximum activity at 18:00h and continuing during the night and the next

morning (Fig. 4B). At the highest elevation, both species began their foraging activity approximately 3 hours earlier in the afternoon compared to the lowest elevation.

Nest structure and fungus garden depth: Nest structure is quite different in both *Acromyrmex* species. Externally, *A. lobicornis* nests have an above-ground mound made of loose soil, twigs and some debris, with the main entrances located beneath shrubs or big rocks. In contrast, *A. striatus* nests are found on bare soil, have no mound, and nest entrances are directly exposed on

the surface. Regarding the fungus chamber, *A. lobicornis* builds a large fungus chamber (mean \pm SE dimensions: $10.28 \pm 6.73 \text{ cm}^3$, $n = 22$), often found interwoven with roots when next to shrubs. In almost half of the nests (10 of 22), we also found 1 to 9 satellite small fungus chambers variable in size (1 - 5.4 cm^3) and always more superficial than the main fungus chamber. *Acromyrmex striatus* has multiple (between 5 and 27) small fungus chambers, all similar in size (mean \pm SE: $1.09 \pm 1.14 \text{ cm}^3$, $n = 19$) and with the fungus gardens hanging from the ceiling of the chambers (Supplementary material Fig. S2).

We found significant differences in the depth of the fungus chambers between seasons ($F = 13.47$, $p < 0.001$). In average, both species locate their fungus chambers approximately 0.4 m deeper in summer than in winter (mean \pm SE: summer: *Acromyrmex lobicornis* 0.98 ± 0.13 , *A. striatus* 1.20 ± 0.06 ; winter *A. lobicornis* 0.68 ± 0.09 , *A. striatus* 0.80 ± 0.10 ; $F = 13.47$, $p < 0.001$). Significant differences among elevations ($F = 10.51$, $p < 0.01$) and between species ($F = 7.19$, $p < 0.05$) were found only during the summer (Fig. 5A). Tukey HSD tests revealed that during this season, at the lowest and mid elevations both species locate the fungus chamber at a similar depth (between 1.3 - 0.93 m, $p > 0.05$); whereas at the highest elevation *A. lobicornis* locates it 60 cm shallower than *A. striatus* ($p < 0.01$). The fungus chambers depth of *A. striatus* did not vary significantly across all elevations ($p > 0.05$), but tended to decrease with increasing elevation for *A. lobicornis*, being in average almost 0.7 m shallower at the top (1800 m a.s.l.) than at the base (800 m a.s.l.) ($p < 0.05$). In winter, we found no significant differences in the fungus chamber depth between species ($F = 0.73$, $p = 0.41$) and among elevations ($F = 0.16$, $p = 0.85$) (Fig. 5B).

Discussion

Our results show that we need to go beyond thermal physiology to predict how some ectotherms species respond to climate change. A dominant assumption in thermal biology is that a higher, lower or both critical temperatures (CT_{\max} and / or CT_{\min}) confer greater thermal tolerances in the field. However, the ecological critical temperatures, that is, the temperature extremes at which organisms are in fact exposed in the field, might be avoided by behaviorally modulating their exposure to extreme temperatures through an active selection or creation of appropriate thermal microenvironments, as seems to be the case of social insects as the two LCA sympatric species of our study.

Does physiological thermal tolerance predict elevational distribution?: Our results do not sustain that thermal tolerances predict LCA elevational distribution. Though statistically significant, the difference in the CT_{\max} between the two LCA species was small (in average $0.81 \text{ }^\circ\text{C}$), whereas the difference in CT_{\min} was more pronounced ($1.60 \text{ }^\circ\text{C}$). However, these differences do not explain their elevational ranges and pattern of abundance along the altitudinal gradient, as the more heat tolerant species increases in abundance toward the top and the

more cold-tolerant species peaked at low and mid altitudes.

These results are consistent with previous studies showing that for ants, as well as for other terrestrial ectotherms, CT_{\max} shows little variation across elevational gradients and that the altitudinal distribution of ants is not primarily driven by physiological thermal limitation (NOWROUZI & al. 2018). The upper elevational limit in the distribution of both species along the mountain gradient was relatively similar for both species, though ~ 600 m lower than the maximum altitude observed in other regions of Argentina (2400 m a.s.l.; L.A. Calcaterra, unpubl.), suggesting that cold temperatures impose a similar constraint to both LCA species. This pattern is not surprising given the widespread understanding that ants are generally thermophilic (HÖLLEDBLER & WILSON 1990), and that the CT_{\min} of ants should be more important than the CT_{\max} as a driver of their geographical distribution (BISHOP & al. 2017, COULIN & al. 2019). Cold temperatures at the upper gradient can reduce local ant species richness by direct effects that limit ant activity, such as changing the time of day when workers are active or reduced foraging time (BRÜHL & al. 1999, AZCÁRATE & al. 2007), which is supported by the reduction in the size of ant nests we found from the base to the top of the gradient.

It is worth to mention that the LCA are one of the worst pests of Argentina in vineyards implanted in the Monte Desert (SÁNCHEZ RESTREPO & al. 2019). Pests and infections that were limited by the cold of winter have expanded their distribution areas in the last 10 years in Europe by the climate warming, affecting vineyards with greater intensity (HANNAH & al. 2013). As consequence of this, some vineyards are being moved into higher latitudes and altitudes (e.g., until 3100 m a.s.l. in the Salta Province, L.A. Calcaterra, unpubl.). Thus, it would be also interesting to determine if LCA could be able to spread to higher altitudes with the increase in the ambient temperature, as has been reported for *Aphaenogaster* ant species of the Appalachian mountains; though in this case the heat tolerant species moved upwards and replaced the cold tolerant species (WARREN II & CHICK 2013).

There was no effect of natural summer acclimation at each elevation on the thermal tolerances at the colony-level for both species (i.e., colonies of higher elevations and consequently lower temperatures being more cold tolerant than colonies of lower elevations where temperatures are higher, and vice versa). In contrast with previous reports in polymorphic ants showing that larger workers have higher CT_{\max} than smaller subcastes (RIBEIRO & al. 2012, BAUDIER & al. 2015), we did not find a clear effect of ant size on the critical thermal limits of both species. Finally, we need to consider that thermal tolerance values in insects can differ between laboratory and field studies, as reported by TRATTER KINZNER & al. (2019), thus questioning the ecological relevance of laboratory approaches. Also, it is important to note that the methodology used to assess thermal tolerances in laboratory studies, for instance

how the thermal challenge is applied, can yield different tolerance estimates (CHICK & al. 2017).

Does the temperature of foraging activity predict elevational distribution?: Our findings do not support that the most frequent temperature of foraging activity can predict elevational distributions, that is, that the species that usually forages at colder temperatures will be more abundant at the highest elevations and the species that frequently forages at warmer temperatures will increase its abundance at the base of the elevational gradient. In our study, neither the temperature range of foraging activity nor the temperature of highest activity during summer differed significantly between both species and among altitudes. Instead, our results show that, by behaviorally adjusting the onset and daily pattern of foraging activity, the range of temperatures at which both species forage as well as the temperature of maximum foraging activity was maintained along the whole altitudinal gradient.

When comparing the time of the day at which both species began their foraging activity along the mountain, the colonies at the base began approximately three hours later in the afternoon than those at the top. By doing this, they adjust their foraging activity to those hours of the day with optimum temperatures (between 20 and 25 °C) and avoid thermal extremes, mainly the high temperatures from midday to afternoon during the summer.

Our results are in contrast with those reported by NOBUA-BEHRMANN & al. (2017) for the central Monte Desert (at 34° S, and 500 - 600 m a.s.l.) when studying these two same species, where they found that *Acromyrmex lobicornis* foraged at lower temperatures than *A. striatus* and that the difference in their thermal tolerance ranges resulted in temporally separated foraging activities. These authors suggested that the avoidance of competitive interactions might explain the temporal niche partitioning of these species. In ant species that coexist and compete for the same food resources, it is expected a dominance-thermal tolerance trade-off, in which the dominant species forage at moderate temperatures while subordinate or subdominant species forage at temperatures closer to their tolerance limits to avoid competition (CERDÁ & al. 1998, 2013). In our study, the activity curve of the two species overlapped at 800 m a.s.l., where temperatures and habitat are very similar to that of the central Monte Desert. Our contrasting results could be explained by the lack of interspecific competition between both species, at least from what we have observed in our study site. Although *A. lobicornis* has some characteristics of an ecologically dominant species, such as big colonies, mass recruitment and the ability to monopolise resources (STUBLE & al. 2017), the dominance-thermal tolerance trade-off may not operate in our site, and the main factor explaining the pattern of foraging activity should be temperature, as *A. striatus* avoids the extreme hot temperatures at the base as well as *A. lobicornis* does. Finally, our findings suggest that the plasticity in the foraging behavior could allow

LCA to persist in warm habitats despite increasing temperatures under a climate warming scenario (especially *A. lobicornis*, which is more abundant in the low and mid part of the gradient).

Does nest architecture and fungus garden depth predict elevational distribution?: Our third question and its prediction did not find support from our results, which show that the depth of the fungus chamber varies for both species with the ambient temperature but did not explain the differences in their abundances along the elevational gradient. Colonies of both species at the base have deeper fungus chambers compared to that at the top of the gradient, thus suggesting that the fungus (and queens and brood) location is a plastic thermoregulatory behavior, and that when facing increasing temperatures, LCA colonies should be able to achieve an optimal temperature for the growth of their symbiotic fungus (between 25 and 30 °C, POWELL & STRADLING 1986) by relocating it deeper in the ground. This is explained because the more distance to the surface (and therefore to the direct influence of the ambient temperature) allows a control of the temperature inside the nest through the buffering effect of the surrounding soil (KLEINEIDAM & ROCES 2000, BLÜTHGEN & FELDHAAR 2009). Also, the shallower fungus garden location of *Acromyrmex lobicornis* at higher elevations agrees with previous studies that found that LCA's species with higher latitudinal distributions have more superficial nests compared to those of lower latitudes (SEELEY & HEINRICH 1981, BOLLAZZI & al. 2008).

Nest architecture is also considered as a behavioral adaptation to temperature (KADOCHOVÁ & FROUZ 2014). Here, LCA species show differences in their nesting habits: while *Acromyrmex lobicornis* builds nests with mound and locates them under shrubs (in the lower elevations) or rocks (in the higher elevations), *A. striatus* nests lack mounds and are located on bare soils. The construction of nest mound by ants is considered as a behavioral adaptation to low environmental temperatures (GÖSSWALD 1989, HEINRICH 1993), and for LCA mound construction may favor fungal growth during cold winter periods by working as a solar collector (WEBER 1972). Indeed, *Acromyrmex* species inhabiting mound nests, as *A. lobicornis*, have a more southerly distribution than those living in subterranean nests, as *A. striatus* (FARJI-BRENER 2000). In our study, both nesting strategies seem to be suitable for maintaining stable temperatures inside the nest. The nest mound of *A. lobicornis* as well as the nest location (under rocks that are heat collectors and shrubs that provide shade) act as isolators from external temperatures. The more exposed nests to external temperatures of *A. striatus* seem to base their thermoregulation alone on the buffering effect of the soil. Building deeper nests implies a higher energetic cost, which might partly explain the decrease in abundance of this species towards the base of the mountain, where temperatures are high.

In conclusion, the poor predictive power of thermal physiological limits in explaining LCA distribution along

our elevational gradient point out that, to predict responses of some ectotherms to rising temperatures, we need to consider species-specific behaviors, as well as the ability to modify these behaviors. Neither the temperature of foraging activity nor the fungus garden depth explained the differences in the pattern of abundance between the two *Acromyrmex* species in our elevational gradient, suggesting that other factors than temperature and beyond the scope of this work might be shaping their distribution. However, both species behaviorally adapt to the variation in temperature along the gradient by modifying their foraging activity rhythm and their fungus garden location, two main colonies' features that ensure their survival as colonies. Behavioral plasticity will help some species to mitigate the adverse impacts of climate change and thus should be an important predictor of organism's climate warming vulnerability as well as extinction risk (KEARNEY & al. 2009). As it states by the climatic variability hypothesis, species occurring at higher latitudes and larger elevational ranges have wider thermal tolerances because they are subjected to a greater spatial and temporal variability in climatic variables (STEVENS 1992, GASTON & CHOWN 1999). At a small spatial scale, the difference in altitude is the dominant factor affecting differences in mean annual near-surface air temperature, followed by differences of latitude (e.g., $-4.80^{\circ}\text{C} / \text{km}$ elevation, as in our study, versus $-0.87^{\circ}\text{C} / \text{latitude}$ across the Qinghai-Tibet Plateau, WANG & al. 2011). Further, this greater altitudinal variability in climatic conditions should select for more plastic phenotypes compared with species in more unseasonal environments (as tropical regions at lower latitudes and altitudes) (GHALAMBOR & al. 2006). In this sense, species inhabiting large altitudinal and latitudinal gradients, like the two LCA species studied in the Monte Desert, should be excellent models for future studies on the plasticity for behavioral thermoregulation under different climate change scenarios.

Acknowledgments

We thank J. Barros, J. Mulet, and C. Herrera for field assistance. We are very grateful to the journal editor F.M. Steiner and X. Cerdá and T.R. Bishop for their helpful suggestions on an earlier draft of the manuscript.

Funding

This research was supported by the Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET) of Argentina (PIP 2014-2016GI CONICET, PUE0125 CONICET) and the Agencia Nacional de Promoción Científica y Tecnológica (ANPCyT) of Argentina (PICT2013-3214). NY has a PhD fellowship from CONICET.

References

ABRAHAM, E., DEL VALLE, H.F., ROIG, F., TORRES, L., ARES, J.O., CORONATO, F. & GODAGNONE, R. 2009: Overview of the geography of the Monte Desert biome (Argentina). – *Journal of Arid Environments* 73: 144-153.

- ADDO-BEDIAKO, A., CHOWN, S.L. & GASTON, K.J. 2000: Thermal tolerance, climatic variability and latitude. – *Proceedings of the Royal Society B-Biological Sciences* 267: 739-745.
- ANDERSON, K.E. & MUNGER, J.C. 2003: Effect of temperature on brood relocation in *Pogonomyrmex salinus* (Hymenoptera: Formicidae). – *Western North American Naturalist* 63: 122-128.
- ARANDA-RICKERT, A., DIEZ, P. & MARAZZI, B. 2014: Extrafloral nectar fuels ant life in deserts. – *AoB Plants* 6: art. plu068.
- ARAÚJO, M., FERRI-YÁÑEZ, F., BOZINOVIC, F., MARQUET, P., VALLADARES, F. & CHOWN, S. 2013: Heat freezes niche evolution. – *Ecology Letters* 16: 1206-1219.
- ARMANI, A. & QUIRÁN, E. 2007: Evaluación cualitativa y cuantitativa de la oferta y cosecha de biomasa herbácea por *Acromyrmex striatus* ROGER (Hymenoptera: Formicidae) en la provincia de La Pampa, Argentina. – *Gayana* 71: 203-206.
- AZCÁRATE, F.M., KOVACS, E. & PECO, B. 2007: Microclimatic Conditions Regulate Surface Activity in Harvester Ants *Messor barbarus*. – *Journal of Insect Behavior* 20: 315-329.
- BAUDIER, K.M., MUDD, A.E., ERICKSON, S.C. & O'DONNELL, S. 2015: Microhabitat and body size effects on heat tolerance: implications for responses to climate change (army ants: Formicidae, Ecitoninae). – *Journal of Animal Ecology* 84: 1322-1330.
- BIRKETT, J., BLACKBURN, G. & MENÉNDEZ, R. 2018: Linking species thermal tolerance to elevational range shifts in upland dung beetles. – *Ecography* 41: 1510-1519.
- BISHOP, T.R., ROBERTSON, M.P., RENSBURG, B.J. & PARR, C.L. 2017: Coping with the cold: Minimum temperatures and thermal tolerances dominate the ecology of mountain ants. – *Ecological Entomology* 42: 105-114.
- BLÜTHGEN, N. & FELDHAAR, H. 2009 [2010]: Food and shelter: how resources influence ant ecology. In: LACH, L., PARR, C.L. & ABBOTT, K.L. (Eds.): *Ant ecology*. – Oxford University Press, Oxford, UK, pp. 115-136.
- BOLLAZZI, M., KRONENBITTER, J. & ROCES, F. 2008: Soil temperature, digging behaviour, and the adaptive value of nest depth in South American species of *Acromyrmex* leaf-cutting ants. – *Oecologia* 158: 165-175.
- BOLLAZZI, M. & ROCES, F. 2002: Thermal preference for fungus culturing and brood location by workers of the thatching grass-cutting ant *Acromyrmex heyeri*. – *Insectes Sociaux* 49: 153-157.
- BRANSTETTER, M.G., JEŠOVNIK, A., SOSA-CALVO, J., LLOYD, M.W., FAIRCLOTH, B.C., BRADY, S.G. & SCHULTZ, T. 2017: Dry habitats were crucibles of domestication in the evolution of agriculture in ants. – *Proceedings of the Royal Society B-Biological Sciences* 284: art. 20170095.
- BRIESE, D.T. & MACAULEY, B.J. 1980: Temporal structure of an ant community in semi-arid Australia. – *Australian Journal of Ecology* 5: 121-134.
- BRÜHL, C.A., MOHAMED, M. & LINSENMAIR, K.E. 1999: Altitudinal distribution of leaf litter ants along a transect in primary forests on Mount Kinabalu, Sabah, Malaysia. – *Journal of Tropical Ecology* 15: 265-277.
- CABIDO, M.R., ZAK, M.R. & BIURRUN, F.N. 2018: La vegetación y el ambiente de la Provincia de La Rioja: una guía ilustrada para viajeros y ecólogos. – *Chilecito, UNdeC, Córdoba: Editorial Ecoval*, 135 pp.
- CALOSI, P., BILTON, D.T., SPICER, J.I., VOLTIER, S.C. & ATFIELD, A. 2010: What determines a species' geographical range? Thermal biology and latitudinal range size relationships in European diving beetles (Coleoptera: Dytiscidae). – *Journal of Animal Ecology* 79: 194-204.

- CERDÁ, X., ARNAN, X. & RETANA, J. 2013: Is competition a significant hallmark of ant (Hymenoptera: Formicidae) ecology? – *Myrmecological News* 18: 131-147.
- CERDÁ, X., RETANA, J. & CROS, S. 1998: Critical thermal limits in Mediterranean ant species: trade-off between mortality risk and foraging performance. – *Functional Ecology* 12: 45-55.
- CHICK, L.D., PEREZ, A. & DIAMOND, S.E. 2017: Social dimensions of physiological responses to global climate change: what we can learn from ants (Hymenoptera: Formicidae). – *Myrmecological News* 25: 29-40.
- CHOWN, S.L. & GASTON, K.J. 2010: Body size variation in insects: a macroecological perspective. – *Biological Reviews* 85: 139-169.
- COULIN, C., DE LA VEGA, G.J., CHIFFLET, L., CALCATERRA, L.A. & SCHILMAN, P.E. 2019: Linking thermo-tolerances of the highly invasive ant, *Wasmannia auropunctata*, to its actual and potential distribution. – *Biological Invasions* 21: 3491-3504.
- CUEZZO, F. & FUENTES, M.B. 2004: Hymenoptera: Formicidae. In: CORDO, H.A., LOGARZO, G., BRAUN, K. & DI IORIO, O.R. (Eds.): – Catálogo de insectos fitófagos de la Argentina y sus plantas asociadas. – Sociedad Entomológica Argentina, Argentina, pp. 395-408.
- DEUTSCH, C.A., TEWKSBURY, J.J., HUEY, R.B., SHELDON, K.S., GHALAMBOR, C.K., HAAK, D.C. & MARTIN, P.R. 2008: Impacts of climate warming on terrestrial ectotherms across latitude. – *Proceedings of the National Academy of Sciences of the United States of America* 105: 6668-6672.
- DIAMOND, S.E., SORGER, D.M., HULCR, J., PELINI, S.L., DEL TORO, I., HIRSCH, C., OBERG, E. & DUNN, R.R. 2012: Who likes it hot? A global analysis of the climatic, ecological, and evolutionary determinants of warming tolerance in ants. – *Global Change Biology* 18: 448-456.
- FARJI-BRENER, A. 2000: Leaf-cutting ant nests in temperate environments: mounds, mound damages and mortality rate in *Acromyrmex lobicornis*. – *Studies on Neotropical Fauna and Environment* 35: 131-138.
- FARJI-BRENER, A.G. & RUGGIERO, A. 1994: Leaf-cutting ants (*Atta* and *Acromyrmex*) inhabiting Argentina: patterns in species richness and geographical range sizes. – *Journal of Biogeography* 21: 391-399.
- GASTON, K.J. & CHOWN, S.L. 1999: Elevation and climatic tolerance: a test using dung beetles. – *Oikos* 86: 584-590.
- GHALAMBOR, C.K., HUEY, R.B., MARTIN, P.R., TEWKSBURY, J.J. & WANG, G. 2006: Are mountain passes higher in the tropics? Janzen's hypothesis revisited. – *Integrative and Comparative Biology* 46: 5-17.
- GÖSSWALD, K. 1989: Die Waldameise, Band 1: Biologische Grundlagen, Ökologie und Verhalten. – Aula Verlag, Wiesbaden, 660 pp.
- HANNAH, L., ROEHRDANZ, P.R., IKEGAMI, M., SHEPARD, A.V., SHAW, M.R., TABOR, G., ZHI, L., MARQUET, P.A. & HIJMANS, R.J. 2013: Climate change, wine, and conservation. – *Proceedings of the National Academy of Sciences of the United States of America* 17: 6907-6912.
- HEINRICH, B. 1993: The hot-blooded insects – strategies and mechanisms of thermoregulation. – Springer, Berlin, 601 pp.
- HILLEL, D. 1998: Environmental soil physics. – Academic Press, London, UK, 771 pp.
- HOFFMANN, A.A., CHOWN, S.L. & CLUSELLA-TRULLAS, S. 2013: Upper thermal limits in terrestrial ectotherms: How constrained are they? – *Functional Ecology* 27: 934-949.
- HÖLLDOBLER, B. & WILSON, E.O. 1990: The ants. – Cambridge University Press, Cambridge, MA, 746 pp.
- HUEY, R.B. & STEVENSON, R. 1979: Integrating thermal physiology and ecology of ectotherms: a discussion of approaches. – *American Zoologist* 19: 357-366.
- JAYATILAKA, P., NARENDRA, A., REID, S.F., COOPER, P. & ZEIL, J. 2011: Different effects of temperature on foraging activity schedules in sympatric *Myrmecia* ants. – *The Journal of Experimental Biology* 214: 2730-2738.
- KADOCHOVÁ, Š. & FROUZ, J. 2014: Thermoregulation strategies in ants in comparison to other social insects, with a focus on red wood ants (*Formica rufa* group). – *F1000 Research* 2: art. 280.
- KASPARI, M., CLAY, N.A., LUCAS, J., PYANOVIAK, S.P. & KAY, A. 2015: Thermal adaptation generates a diversity of thermal limits in a rainforest ant community. – *Global Change Biology* 21: 1092-1102.
- KEARNEY, M., SHINE, R. & PORTER, W.P. 2009: The potential for behavioral thermoregulation to buffer “cold-blooded” animals against climate warming. – *Proceedings of the National Academy of Sciences of the United States of America* 106: 3835-3840.
- KLEINEIDAM, C. & ROCES, F. 2000: Carbon dioxide concentrations and nest ventilation in nests of the leaf-cutting ant *Atta vollenweideri*. – *Insectes Sociaux* 47: 241-248.
- KUSNEZOV, N. 1978: Hormigas argentinas. Claves para su identificación. – *Miscelánea* 61. Fundación Miguel Lillo, Tucumán, 147 pp.
- LUTTERSCHMIDT, W.I. & HUTCHISON, V.H. 1997: The critical thermal maximum: history and critique. – *Canadian Journal of Zoology* 75: 1561-1574.
- MUELLER, U.G., MIKHEYEV, A.S., HONG, E., SEN, R., WARREN, D.L., SOLOMON, S.E., ISHAK, H.D., COOPER, M., MILLER, J.M., SHAFFER, K.A. & JUENGER, T.E. 2011: Evolution of cold-tolerant fungal symbionts permits winter fungiculture by leafcutter ants at the northern frontier of a tropical ant-fungus symbiosis. – *Proceedings of the National Academy of Sciences of the United States of America* 108: 4053-4056.
- NAVARRO, J. & JAFFÉ, K. 1985: On the adaptive value of nest features in the grass-cutting ant *Acromyrmex landolti*. – *Biotropica* 17: 347-348.
- NOBUA-BEHRMANN, B.E., LOPEZ DE CASENAVE, J., MILESI, F.A. & FARJI-BRENER, A. 2017: Coexisting in harsh environments: temperature-based foraging patterns of two desert leafcutter ants (Hymenoptera: Formicidae: Attini). – *Myrmecological News* 25: 41-49.
- NOWROUZI, S., ANDERSEN, A.N., BISHOP, T.R. & ROBSON, S.K.A. 2018: Is thermal limitation the primary driver of elevational distributions? Not for montane rainforest ants in the Australian Wet Tropics. – *Oecologia* 188: 333-342.
- PENICK, C.A. & TSCHINKEL, W.R. 2008: Thermoregulatory brood transport in the fire ant, *Solenopsis invicta*. – *Insectes Sociaux* 55: 176-182.
- POWELL, R.J. & STRADLING, D.J. 1986: Factors influencing the growth of the *Attamyces bromatificus*, a symbiont of Attine ants. – *Transactions of the British Mycological Society* 87: 205-213.
- R DEVELOPMENT CORE TEAM 2019: R: A language and environment for statistical computing. – R Foundation for Statistical Computing, Vienna, Austria; <<https://www.R-project.org/>>, retrieved on 5 July 2019.
- REZENDE, E.L., TEJEDO, M. & SANTOS, M. 2011: Estimating the adaptive potential of critical thermal limits: methodological problems and evolutionary implications. – *Functional Ecology* 25: 111-121.
- RIBEIRO, P.L., CAMACHO, A. & NAVAS, C.A. 2012: Considerations for assessing maximum critical temperatures in small ectothermic animals: insights from leaf-cutting ants. – *Public Library of Science One* 7: art. e32083.

- SALA, O.E., CHAPIN, F.S., ARMESTO, J.J., BERLOW, E., BLOONFIELD, J., DIRZO, R., HUBER-SANWALD, E., HUENNEKE, L.F., JACKSON, R.B., KINZIG, A., LEEMANS, R., LODGE, D.M., MOONEY, H.A., OESTERHELD, M., LEROYPOFF, N., SYKES, M.T., WALKER, B.H., WALKER, M. & WALL, D.H. 2000: Global biodiversity scenarios for the year 2100. – *Science* 287: 1770-1774.
- SÁNCHEZ-RESTREPO, A.F., JIMENEZ, N.L., CONFALONIERI, V.A. & CALCATERRA, L.A. 2019: Distribution and diversity of leaf-cutting ants in Northeastern Argentina: species most associated with forest plantations. – *International Journal of Pest Management* 65: 244-257.
- SEELEY, T.D. & HEINRICH, B. 1981: Regulation of temperature in the nest of social insects. In: HEINRICH, B. (Ed.): *Insect thermoregulation*. – Wiley, New York, NY, pp. 160-234.
- STEVENS, G.C. 1992: The elevational gradient in altitudinal range: an extension of Rapoport's latitudinal rule to altitude. – *The American Naturalist* 140: 893-911.
- STUBLE, K.L., JURIC, I., CERDÁ, X. & SANDERS, N.J. 2017: Dominance hierarchies are a dominant paradigm in ant ecology (Hymenoptera: Formicidae), but should they be? And what is a dominance hierarchy anyways? – *Myrmecological News* 24: 71-81.
- SUNDAY, J.M., BATES, A.E. & DULVY, N.K. 2011: Global analysis of thermal tolerance and latitude in ectotherms. – *Proceedings of the Royal Society B-Biological Sciences* 278: 1823-1830.
- SUNDAY, J.M., BATES, A.E., KEARNEY, M.R., COLWELL, R.K., DULVY, N.K., LONGINO, J.T. & HUEY, R.B. 2014: Thermal-safety margins and the necessity of thermoregulatory behavior across latitude and elevation. – *Proceedings of the National Academy of Sciences of the United States of America* 111: 5610-5615.
- TERBLANCHE, J.S., KLOK, C.J., KRAFSUR, E.S. & CHOWN, S.L. 2006: Phenotypic plasticity and geographic variation in thermal tolerance and water loss of the tsetse *Glossina pallidipes* (Diptera: Glossinidae): implications for distribution modelling. – *American Journal of Tropical Medicine and Hygiene* 74: 786-794.
- TRATTER KINZNER, M., KINZNER, M.C., KAUFMANN, R., HOFFMAN, A.A., ARTHOFER, W., SCHLICK-STEINER, B.C. & STEINER, F.M. 2019: Is temperature preference in the laboratory ecologically relevant for the field? The case of *Drosophila nigrosparsa*. – *Global Ecology and Conservation* 18: art. e00638.
- WANG, K., SUN, J., CHENG, G. & JLANG, H. 2011: Effect of altitude and latitude on surface air temperature across the Qinghai-Tibet Plateau. – *Journal of Mountain Science* 8: 808-816.
- WARREN, R.J. II & CHICK, L. 2013: Upward ant distribution shift corresponds with minimum, not maximum, temperature tolerance. – *Global Change Biology* 19: 2082-2088.
- WEBER, N.A. 1972: Gardening ants, the Attines. – *Memoirs of the American Philosophical Society* 92: I-XVII, 1-146.
- WILSON, E.O. 1992: *The diversity of life*. – Harvard University Press, Harvard, MA, 432 pp.