



## RESEARCH ARTICLE

# Does social thermal regulation constrain individual thermal tolerance in an ant species?

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**Abstract**

1. In ants, social thermal regulation is the collective maintenance of a nest temperature that is optimal for individual colony members. In the thermophilic ant *Aphaenogaster iberica*, two key behaviours regulate nest temperature: seasonal nest relocation and variable nest depth. Outside the nest, foragers must adapt their activity to avoid temperatures that exceed their thermal limits.
2. It has been suggested that social thermal regulation constrains physiological and morphological thermal adaptations at the individual level. We tested this hypothesis by examining the foraging rhythms of six populations of *A. iberica*, which were found at different elevations (from 100 to 2,000 m) in the Sierra Nevada mountain range of southern Spain. We tested the thermal resistance of individuals from these populations under controlled conditions.
3. Janzen's climatic variability hypothesis (CVH) states that greater climatic variability should select for organisms with broader temperature tolerances. We found that the *A. iberica* population at 1,300 m experienced the most extreme temperatures and that ants from this population had the highest heat tolerance (LT50 = 57.55°C). These results support CVH's validity at microclimatic scales, such as the one represented by the elevational gradient in this study.
4. *Aphaenogaster iberica* maintains colony food intake levels across different elevations and mean daily temperatures by shifting its rhythm of activity.
5. This efficient colony-level thermal regulation and the significant differences in individual heat tolerance that we observed among the populations suggest that behaviourally controlled thermal regulation does not constrain individual physiological adaptations for coping with extreme temperatures.

**KEYWORDS**

ants, *Aphaenogaster iberica*, climate variability hypothesis, elevational gradient, foraging activity rhythms, thermal tolerance

## 1 | INTRODUCTION

Almost all biological phenomena, from molecular stereochemistry to species interactions, are conditioned by temperature. Consequently, the rise in the earth's temperature, which is

taking place in association with other elements of anthropogenic climate change, is predicted to have major consequences for the future of biodiversity (Bellard, Bertelsmeier, Leadley, Thuiller, & Courchamp, 2012). It has already been shown that global warming is shifting species ranges and distributions towards cooler habitats

and leading to phenotypic changes, via rapid genetic adaptation and phenotypic plasticity (Anderson, Inouye, McKinney, Colautti, & Mitchell-Olds, 2012; Angilletta et al., 2007; Brans et al., 2017; Chen, Hill, Ohlemüller, Roy, & Thomas, 2011; Henning-Lucass, Cordellier, Streit, & Schwenk, 2016; Oliver & Palumbi, 2011; Tattersall, Arnaout, & Symonds, 2017). That said, there are few examples of population extinctions being driven by climate change: a direct causal link has rarely been established (Cahill et al., 2013). However, this apparent species resilience could, in fact, hide a profound extinction debt. It might also indicate that species have evolved efficient adaptations for coping with spatial and temporal thermal heterogeneity. As Kaspari, Clay, Lucas, Yanoviak, and Kay (2015) have stated, thermal adaptation can occur through a variety of mechanisms ranging from behaviour to natural selection and thus encompass a variety of time-scales. Understanding how these adaptations function and evolve is crucial to predicting the effects of global warming.

The body temperature of ectotherms fluctuates based on environmental conditions and, consequently, such organisms may be particularly sensitive to the impacts of climate change (Paaijmans et al., 2013). However, many species have evolved behavioural thermoregulatory mechanisms that allow them to maintain a near-optimum body temperature (Sunday et al., 2014). This phenomenon has been particularly well studied in reptiles (Bogert, 1949; Caldwell, While, & Wapstra, 2017; Dubois, Blouin-Demers, Shipley, & Thomas, 2009; Seebacher, Grigg, & Beard, 1999). For example, it has been shown that some lizard species that occur across broad altitudinal gradients can use behavioural thermal regulation to compensate for the decrease in environmental temperature that accompanies increasing elevation (Hertz & Huey, 1981; Theisinger, Berg, & Dausmann, 2017). However, efficient behavioural thermal regulation might also constrain the evolution of other thermal physiological mechanisms that could contribute to species survival over the long term (Huey, Hertz, & Sinervo, 2003). Bogert (1949) was the first to propose that efficient behavioural thermal regulation could reduce the degree of natural selection on physiological mechanisms that prevent overheating. Although evidence for this hypothesis has been found in reptiles, the same cannot be said for other ectotherms. Moreover, differences in thermal tolerance among populations along a temperature gradient may not necessarily arise from natural selection; they could also be due to differences in acclimation resulting from phenotypic plasticity (Oms, Cerdá, & Boulay, 2017).

Janzen (1967) stated that tropical organisms, which experience a relatively uniform set of temperatures, would be more acclimated and evolutionarily adapted to a narrower absolute range of temperatures. As a consequence, mountain passes in the tropics would present greater hurdles to dispersal than would mountain passes of similar elevations in temperate zones. The climatic variability hypothesis (CVH) posits that as climatic variability increases with latitude and/or elevation, it favours the selection of terrestrial species with broader thermal tolerance ranges (Stevens, 1989).

Ants are an interesting model system for studying thermal adaptations (Chick, Perez, & Diamond, 2017; Diamond et al., 2012)

and for testing the predictions of the CVH. They occur in almost all terrestrial ecosystems and are ecologically dominant organisms (Wilson, 1990). Some species have evolved particular adaptations that allow them to live in the warmest deserts on earth (Boulay et al., 2017). Heat tolerance is an ecological benefit for these species because it reduces competition and predation risk (Wehner, Marsh, & Wehner, 1992). However, because ants are eusocial, thermal adaptations have evolved at both the individual level and the colony level (Cerdá & Retana, 2000). At the individual level, workers can reduce body temperature by convectively cooling themselves via rapid movements and by exploiting thermal refuges (Sommer & Wehner, 2012; Wehner et al., 1992). In some desert ants, workers have been shown to act pre-emptively: they produce heat shock proteins before leaving the nest for a foraging trip (Gehring & Wehner, 1995; Willot, Gueydan, & Aron, 2017). At the colony level, ants can adjust their activity to forage during the most favourable hours of the day (Cerdá, Retana, & Cros, 1998). Ant colonies are also highly mobile and may colonize microclimates that best fit their environmental and temperature preferences (Warren & Chick, 2013). Consequently, many ants have been shown to shift their foraging activity across seasons, elevations or latitudes (Amor, Ortega, Cerdá, & Boulay, 2011; Azcárate, Kovacs, & Peco, 2007; Cros, Cerdá, & Retana, 1997; Jayatilaka, Narendra, Reid, Cooper, & Zeil, 2011). However, the ecological performance of this strategy has rarely been quantified, and it remains unknown if ants are able to keep their daily resource intake constant despite changes in environmental conditions. Moreover, depending on the species, ant colonies can take advantage of worker polymorphism to send out larger workers at the warmest hours of the day. From the colony's perspective, large workers are costlier to produce than small workers, but they also display greater thermal tolerance because of their longer legs (which hold their bodies farther up off the ground), their lower mass-specific metabolic rates (Shik, 2010) and their lower surface-to-volume ratios, which all reduce the risk of desiccation (Cerdá & Retana, 1997, 2000).

When Sinclair, Williams, and Terblanche (2012) assessed the variability seen in the thermal performance of insects, they discovered many examples of differences among populations. However, they highlighted that the link between the patterns observed and the proposed underlying mechanisms was poorly defined, and they called for more targeted research. The aim of our study was to test whether links existed between mechanisms of thermal tolerance and ant foraging activity and to determine whether support existed for the CVH. The main questions were (a) whether efficient behavioural thermal regulation at the colony level could constrain individual-level mechanisms of thermal tolerance in an ant species and (b) whether populations experiencing greater thermal variability displayed broader thermal tolerance (as per the CVH). More specifically, we analysed differences in foraging activity in the Spanish gypsy ant, *Aphaenogaster iberica*, along an elevational gradient ranging from 100 to 2,000 m a.s.l. We tested whether the ant's rhythm of relative activity was bimodal at lower elevations (where midday temperatures can climb high enough to preclude

foraging) versus unimodal at higher elevations. The main question was as follows: if this change in activity occurs, are populations able to maintain daily activity, the number of food items retrieved, and the temperature of maximum activity regardless of elevation and mean daily temperature? We therefore first assessed whether the size of the workers out foraging changed over the course of the day and whether such differences in worker size could potentially help populations deal with diurnal variation in environmental temperatures. We also compared the thermal tolerance of individual ants taken from populations at different elevations to examine the relationship between an ant's physiological thermal tolerance and the temperature range at the ant's elevation of origin. More broadly, using an elevational and temperature gradient, we examined whether behavioural thermal regulation in a social ectotherm constrained physiological mechanisms that could contribute to species survival over the longer term.

## 2 | MATERIALS AND METHODS

### 2.1 | Species and study sites

*Aphaenogaster iberica* is a thermophilous ant species that is widely distributed in xerophilous habitats across the Iberian Peninsula. Colonies are monogynous and reproduce by fission. New queens do not fly but rather leave the mother nest with a group of workers to create a new independent colony of a few hundred workers nearby. This mode of dispersal generates isolation by distance among nearby populations, as shown in the congeneric species *Aphaenogaster senilis* (Galarza et al., 2012). *A. iberica* is omnivorous and collects both animal matter (arthropod corpses) and plant matter (seeds and petals). Workers forage individually. However, they can recruit nestmates when they find prey too large to carry individually; in these cases, the recruiter leads a group of up to 10 nestmates to the food source (Lenoir et al., 2011).

This study was conducted in the Sierra Nevada mountain range of southern Spain, near the village of Lanjarón (36°54'58.3"N, 3°28'41.5"W). A transect running from south to north was established, and six populations were identified at the following elevations: 100, 600, 1,000, 1,300, 1,700 and 2,000 m a.s.l. The closest populations were 1.5-km apart, while the farthest populations were 30-km apart. They were located in habitats characterized by typical Mediterranean vegetation, which was dominated by small shrubs and scarce pine and olive trees, whose densities decreased with elevation (see Villalta, Rami, et al., 2020 for more details on the study sites).

### 2.2 | Elevational variation in ground temperature and microclimatic variability

Differences in the ground temperature experienced by the different populations along the elevational gradient were measured using CEM DT171 data loggers. The data loggers were buried at a depth of 2–5 cm in order to keep the temperature sensors on the

ground surface. The sensors were covered by small caps to prevent overheating. Temperature was recorded three times per hour for 122 days, from 1 June to 30 September 2015.

We also measured the temperature at ant nest entrances (see Section 2.3) during the foraging activity period (from 8:00 to 20:00, from May to September), using a Reed R2002 infrared thermometer. We employed the standard deviation (SD) of the nest entrance temperatures and the ground temperature ranges (from the data loggers) to characterize the climatic variability experienced by each population.

### 2.3 | Colony thermal regulation: Ant foraging activity

We studied the daily rhythm of foraging activity for the six *A. iberica* populations over the course of five field sessions in 2015: these sessions took place once every month from May to September. For a given session, observations for the six populations were made on consecutive days. During each session, we started by identifying 15 nests within each population. On the following day, for each nest, we counted the number of workers entering (with and without food items) and exiting for a 5-min period every hour from 8:00 to 20:00 LST (=GMT + 1). *A. iberica* does not regurgitate food, so all food items are transported with the mandibles and were thus visible to the observers. We had to exclude data from several colonies that relocated their nests during the observation period or engaged in nest maintenance activity. By the end of the study, we had collected foraging activity data for 323 different nests (9–13 nests per population and field session). As mentioned above, we also measured nest entrance temperatures at the beginning and end of each 5-min observation period using a Reed R2002 infrared thermometer.

On the same days that we measured ant foraging activity, we placed 21–30 pitfall traps at least 6-m away from the target nests (i.e. two traps per nest), including nests whose activity data had been excluded. This distance is greater than the mean foraging distance of *A. iberica*, so the pitfall trapping did not interfere with the activity measurements. The pitfall traps were 25-cl white plastic cups that were buried in the ground and filled two thirds of the way with soapy water. They were opened at 8:00 and their contents were collected at 12:00, 16:00 and 20:00. In total, we ran 712 pitfall traps. After separating out the biological material found in the traps, we counted the number of *A. iberica* workers captured during each time interval.

### 2.4 | Morphological measurements

We measured the mesosoma length and hind leg tibia length of the ants collected in the pitfall traps to compare the size of *A. iberica* workers foraging in the morning, at midday and during the afternoon. We stored the 1,314 workers from the traps in 70% ethanol until the measurements could take place. We also carried out measurements on foragers sampled from 10 to 13 colonies (1,090 ants)

immediately after they arrived in the laboratory (October 2015) to compare forager size among populations.

First, we mounted the thorax and hind leg of a given individual ant on a microscope slide using petroleum jelly. We then photographed the body parts alongside a millimetre ruler using a Leica binocular microscope equipped with a digital camera (Canon 30D with an MP-E 65-mm macro lens). We used ImageJ software (v. 1.8; Schneider, Rasband, & Eliceiri, 2012) to measure mesosoma length and tibia length. Mesosoma length was defined as the diagonal length in profile from the anterior edge of the pronotum to the posterior corner of the metapleuron (also known as Weber's length).

## 2.5 | Individual heat tolerance

At the end of the last field session (September 2015), we excavated a total of 71 nests, although we only found the queen in 50 of them. All the resulting colonies were taken to the laboratory, but only those with a queen were used in further experiments and to estimate demography. We placed the colonies in  $20 \times 15 \times 12$  cm plastic boxes; the inner walls of the plastic boxes had been coated with Fluon® to prevent any ants from escaping. The colonies were kept at 25°C and 50%–60% relative humidity (RH). The ants could take shelter in one or two  $20 \times 2$  cm test tubes that were half filled with water located on the other side of a cotton plug. The ants also had ad libitum access to food (dead mealworms [*Tenebrio molitor*] and pieces of fruit). After the colonies had spent 2 months in the laboratory, we selected six of median size (= ~500 workers) per population to evaluate workers' thermal tolerance. We evaluated forager half-lethal temperatures (LT50) as described in Oms et al. (2017): LT50 is the temperature at which 50% of the ants exposed to a specific temperature die. Briefly, ants collected outside the test tubes were placed in groups of three on a Selecta Plactronic hot plate set to a temperature of 50°C. We recorded whether the ants had died or were still alive after 10 min. We then increased the hot plate temperature by 1°C and repeated the same procedure with another group of three ants from another colony until we reached a temperature of 62°C. We exposed 18 ants from each population (three ants  $\times$  six colonies) to temperatures ranging from 50°C to 62°C and then calculated the temperature at which 50% of the ants from a specific population had died. Overall, we tested 1,402 workers.

## 2.6 | Statistical analyses

We calculated the mean hourly ground temperature using the measurements from the data loggers. We then calculated the mean daily temperature and daily temperature range and compared these variables for the six populations using linear mixed-effect models. We treated day as a random factor and colony as a replicate. We employed the lmer function in lme4 package (Bates, Mächler, Bolker, & Walker, 2015). Analyses were conducted in R software version 3.4.3 (R Development Core Team, 2017).

We defined the relative foraging activity of each colony as the sum of entries (with and without food items) and exits observed during each 5-min time period divided by the total number of entries and exits observed during the day. We then fitted a linear mixed-effects model with time period (13 levels—the 13 1-hr periods between 8:00 and 20:00) and population (six levels) as fixed effects and field session as a random effect. We used the default contrasts in R to examine differences among time periods and populations. In the model, a significant intercept indicated that mean relative foraging activity at 8:00 for the population at 100 m was significantly different from 0. The other coefficients for the population at 100 m indicated whether the mean relative activity for other time periods was significantly different from that at 8:00. Finally, the coefficients for the other populations indicated whether there were significant differences in relative activity between each time period between the population at 100 m compared to the other populations.

We also examined absolute foraging activity for each colony, which was defined as the sum of entries (with and without food items) and exits observed during each 5-min time period. Furthermore, we counted the number of food items retrieved using the same raw data. For each population, we tested the goodness-of-fit between absolute foraging activity and nest entrance temperature by constructing various models in which the dependent variable was absolute ant activity and the independent variable was nest entrance temperature. Using a model selection procedure, we compared four models: the null model (with no independent variables); a model with a linear relationship between ant activity and temperature; a model with both a linear and quadratic relationship between ant activity and temperature; and a model with a linear, quadratic and cubic relationship between ant activity and temperature. The quadratic and cubic terms were tested because curvilinear relationships could potentially result from the negative effects of high temperatures on foraging activity. The best models for each population for each month were selected based on Akaike information criterion values ( $\Delta AIC \leq 2$ ). GLMs were performed with GENMOD in SAS package version 9.4 (SAS Institute Inc., 2016).

We tested whether the mean daily temperature at the nest entrance, population and their interaction affected absolute daily foraging activity, the total number of food items retrieved and the temperature of maximum activity using linear mixed-effect models in which field session was a random factor. Similarly, we performed linear mixed-effect models in which *A. iberica* abundance in the pitfall traps, mesosoma length and tibia length were dependent factors. The fixed independent factors were population, time period and their interaction. The random factors were field session and pitfall trap nested within field session.

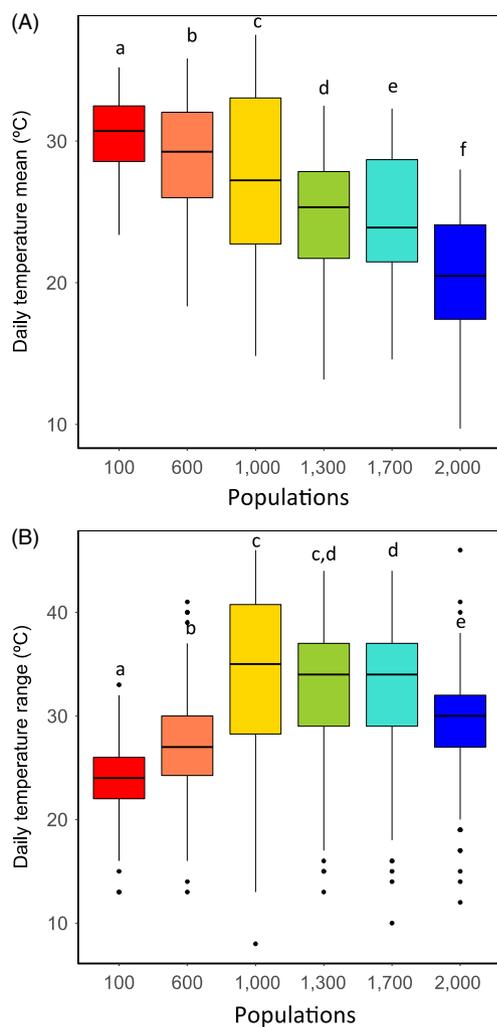
Finally, we tested whether the hind leg tibia and mesosoma lengths of foragers for a given population were positively correlated with elevation.

The LT50 values were analysed using the DRC package in R (Ritz, Baty, Streibig, & Gerhard, 2015). We first tested whether temperature responses differed among populations by comparing two logistic models including and excluding population. We then calculated the LT50 values, and we examined the 95% confidence intervals to determine which differences were significant.

### 3 | RESULTS

#### 3.1 | Elevational variation in ground temperature and microclimatic variability

The analysis of the ground temperature for the 122 days of the field study showed that there were significant differences among the conditions experienced by the six populations (Figure 1A;  $F_{5,605} = 426, p < 0.0001$ ). The mean daily ground temperature ( $\pm SE$ ) was  $30.45^\circ\text{C} \pm 0.39^\circ\text{C}$  at 100 m; it was  $10.09^\circ\text{C} \pm 0.24^\circ\text{C}$  lower at 2,000 m (Table 1). The range of daily temperatures also varied significantly (Figure 1B;  $F_{5,605} = 85.20, p < 0.0001$ ). The greatest daily range (i.e. the highest degree of diurnal climatic variability) was recorded at intermediate elevations (1,000 and 1,300 m), where the



**FIGURE 1** (A) Mean daily ground temperature at the different elevations at which *Aphaenogaster iberica* populations were studied; (B) mean daily temperature range experienced by each study population. Temperature range was used as a proxy for the climatic variability experienced by the populations. The boxplots show the median (line in the centre) and the interquartile range (upper and lower box boundaries). The letters indicate significant ( $p < 0.0001$ ) differences between populations. Using data loggers, temperature was recorded three times per hour for 122 days, from 1 June to 30 September 2015

ground temperature climbed from  $15.52^\circ\text{C} \pm 0.33^\circ\text{C}$  in the early morning to  $48.59^\circ\text{C} \pm 0.78^\circ\text{C}$  in the early afternoon (15:00).

The mean ( $\pm SE$ ) nest entrance temperature, which was associated with ant foraging activity (i.e. only diurnal temperatures, from 08:00 to 20:00), was  $33.11^\circ\text{C} \pm 0.29^\circ\text{C}$  at 100 m and  $8.00^\circ\text{C} \pm 0.16^\circ\text{C}$  at 2,000 m. The greatest daily range of nest entrance temperature was recorded in July at 1,300 m, where the temperature climbed from  $17.2^\circ\text{C}$  at 8:00 to  $71.2^\circ\text{C}$  at 14:00 (Table S1).

The standard deviation of nest entrance temperature was also used as a proxy for the climatic variability experienced by each population; it was greatest at 1,300 m (Table 1).

#### 3.2 | Colony-level thermal regulation

There are two non-exclusive mechanisms that the ant colonies could use to thermoregulate: shifts in daily foraging activity and the recruitment of larger foragers.

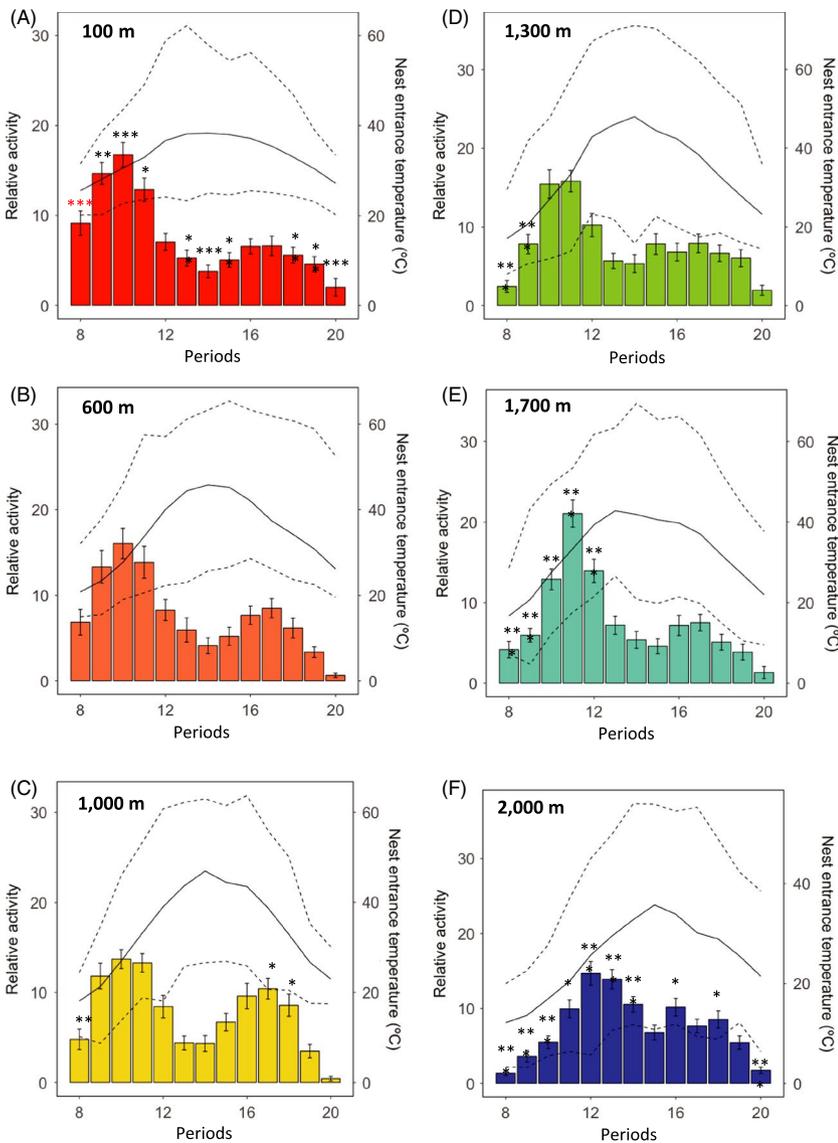
First, the ant colonies could thermoregulate by shifting their daily foraging activity towards the morning and/or the evening when mean daily temperature increases (Figures 2 and 3). If this mechanism was being used, we would expect to see unimodal activity patterns at higher elevations and bimodal activity patterns at intermediate and lower elevations, where the midday heat may constrain foraging. Indeed, we found that relative foraging activity differed among time periods ( $F_{12,4,121} = 59.68, p < 0.0001$ ) and populations ( $F_{65,4,121} = 5.72, p < 0.0001$ ). At 100 m, colonies exhibited a bimodal pattern of foraging activity—the first peak occurred at 10:00 and a second, less pronounced peak occurred at 17:00. The foraging activity pattern at 600 m was not significantly different from that at 100 m. However, as elevation increased, there was also an increase in the number of time periods for which relative foraging activity differed significantly from that at 100 m (Figure 2). At intermediate elevations (1,000 and 1,300 m), foraging activity was largely bimodal, and there was a shift towards foraging in the morning at higher and lower elevations. In particular, at higher elevations (1,700 and 2,000 m), relative foraging activity tended to start later, stop earlier and primarily occur during the warmest periods of the day (Figure S1).

Seasonality also had a differential effect (Figure 3). For colonies at lower elevations, absolute foraging activity displayed a bimodal pattern in both the spring (May) and summer (July). In contrast, colonies at intermediate elevations (1,300 m) shifted from unimodal to bimodal activity between seasons, and colonies at high elevations (2,000 m) always had unimodal activity, regardless of the season (Figure S1).

If thermal regulation is efficient, we predicted that absolute foraging activity, the number of food items retrieved and the temperature of maximum activity should be independent of mean daily temperature; in other words, the thermal reaction norms should be similar along the elevational gradient. We found that absolute foraging activity did not differ significantly among populations (Figure 4A;  $F_{5,307} = 1.09, p = 0.3683$ ) based on the mean daily nest entrance temperature ( $F_{1,310} = 0.52, p = 0.4712$ ) and the lack of interaction between the two factors ( $F_{5,307} = 1.32, p = 0.2534$ ). However, the number

**TABLE 1** Temperature-related characteristics of the study populations. The mean temperature ( $\pm SE$ ), the maximum temperature, the diurnal temperature range and the standard deviation ( $SD$ ) of temperature were calculated based on the nest entrance temperatures, which were characterized during the field sessions focused on daily foraging activity (from 8:00 to 20:00 from May through September). The  $SD$  was used as a proxy for the climatic variability experienced by the populations. The total temperature range was determined using the data-logger measurements (taken three times per hour continuously from June to September) and was defined using the maximum and minimum temperatures observed in each population. The  $LT_{50}$  values were experimentally determined in the laboratory

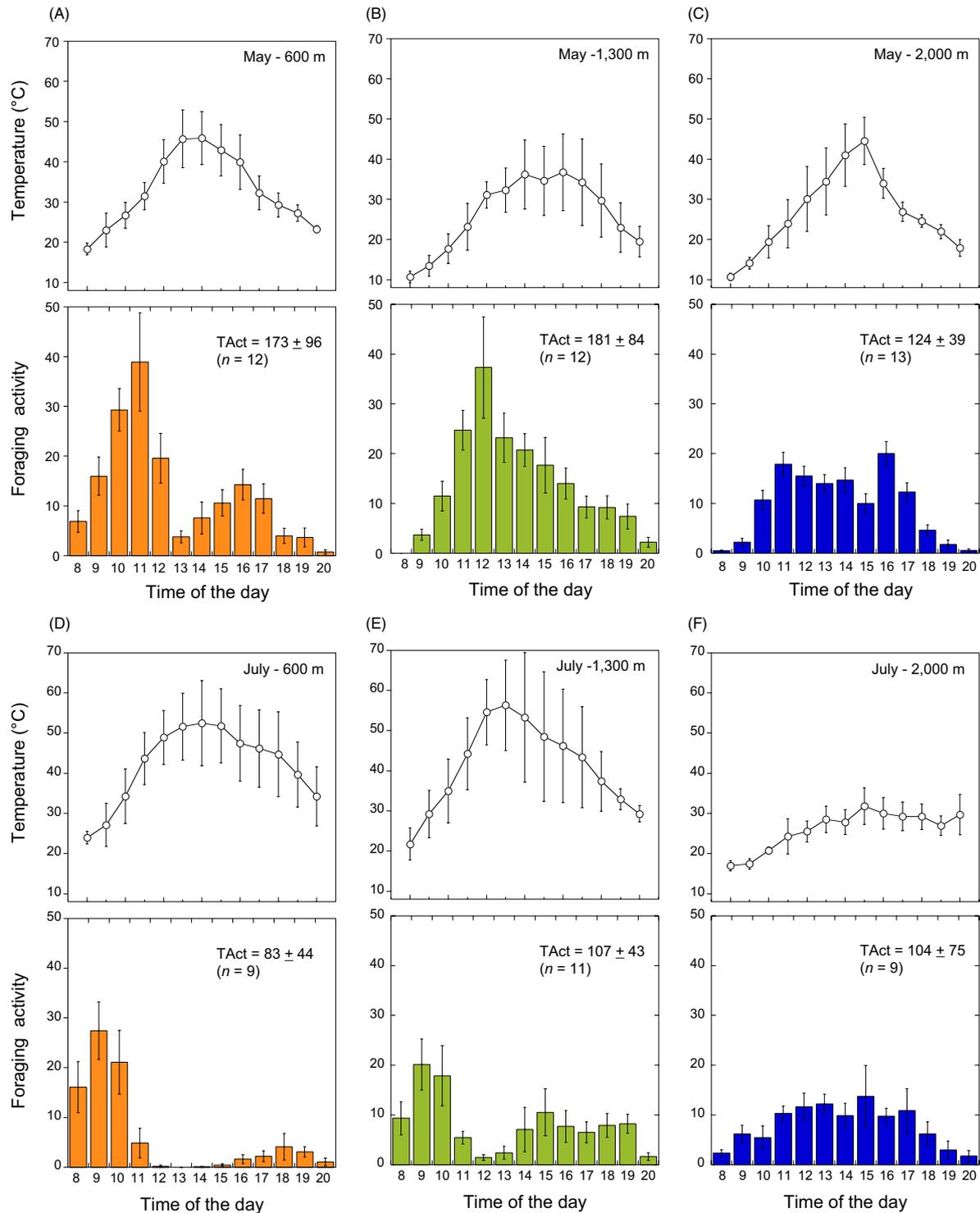
Population	100 m	600 m	1,000 m	1,300 m	1,700 m	2,000 m
Mean temp $\pm SE$	33.1 $\pm$ 0.29	34.8 $\pm$ 0.44	33.8 $\pm$ 0.45	34.2 $\pm$ 0.52	32.4 $\pm$ 0.46	25.1 $\pm$ 0.45
Max temp	62.2	65.4	63.7	71.2	69.5	56.0
Diurnal temp range	33.0 $\pm$ 6.8	42.5 $\pm$ 0.8	45.2 $\pm$ 2.8	45.0 $\pm$ 8.3	42.9 $\pm$ 8.3	35.5 $\pm$ 13.0
Total temp range	42.1	50.4	55.0	63.2	64.8	52.8
$SD$ (CV)	7.56	11.40	12.04	13.92	12.19	12.12
$LT_{50}$	55.20	56.05	57.67	57.55	55.68	55.35



**FIGURE 2** Relative foraging activity (mean  $\pm SE$ ), defined as the sum of entries and exits observed during a given 5-min observation period divided by the total number of entries and exits observed during the day. The 5-min observation periods were carried out once per hour over the course of 13 hr at each study elevation (A–F) for the 5 months of field observations. The solid lines represent the mean nest entrance temperature, while the dashed lines represent the hourly maximum and minimum nest entrance temperatures. For the population at 100 m (A), the asterisks for relative foraging activity at 8:00 (in red) indicate whether there was a significant difference from 0; the asterisks for the relative foraging activity at other time periods indicate whether there was a significant difference from relative foraging activity at 8:00. For the other populations (600–2,000 m, B–F), the asterisks indicate whether there was a significant difference in relative foraging activity at that time period compared to the relative foraging activity at the equivalent time period for the 100-m population. \* $p < 0.05$ , \*\* $p < 0.01$ , \*\*\* $p < 0.001$

of food items retrieved increased significantly with the mean daily nest entrance temperature (Figure 4B;  $F_{1,304} = 6.59$ ,  $p = 0.0108$ ). This effect did not differ among populations (population:  $F_{5,308} = 0.88$ ,  $p = 0.4949$ ; population  $\times$  temperature:  $F_{5,308} = 1.88$ ,  $p = 0.09718$ ).

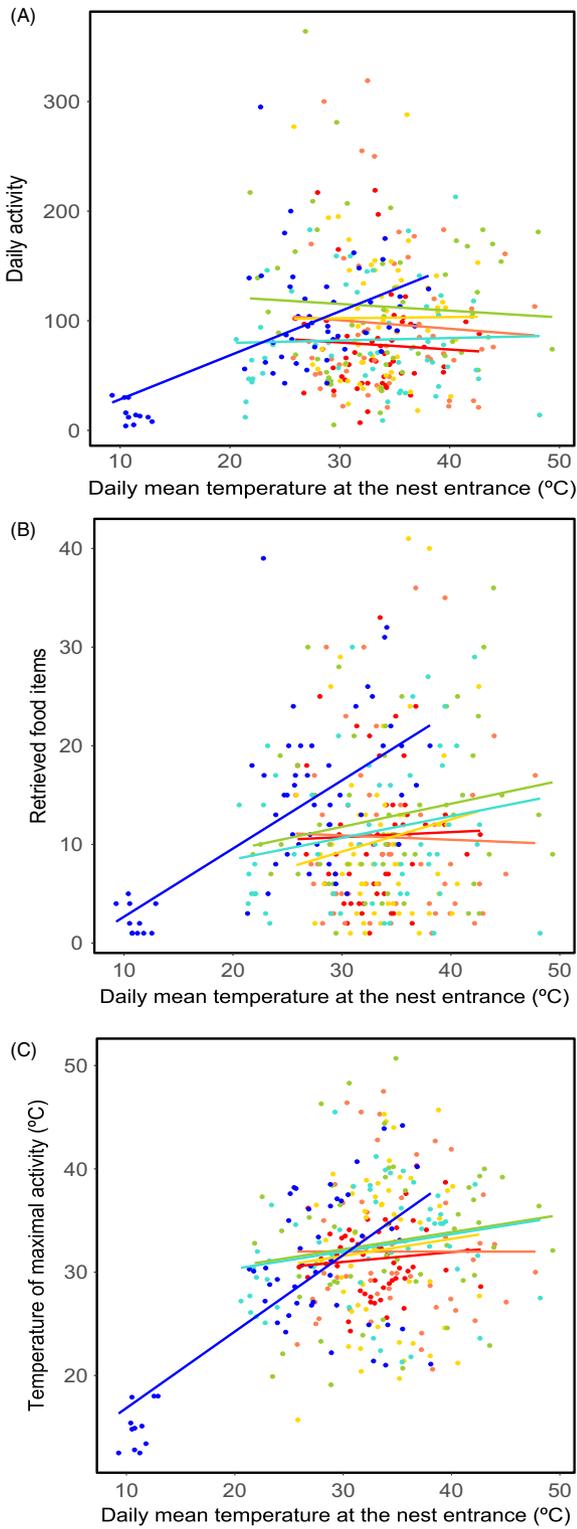
Thus, overall, there was no evidence of a reduction in absolute foraging activity or in the number of food items retrieved as elevation changed. However, the temperature of maximum activity varied among populations and with mean daily nest entrance temperature



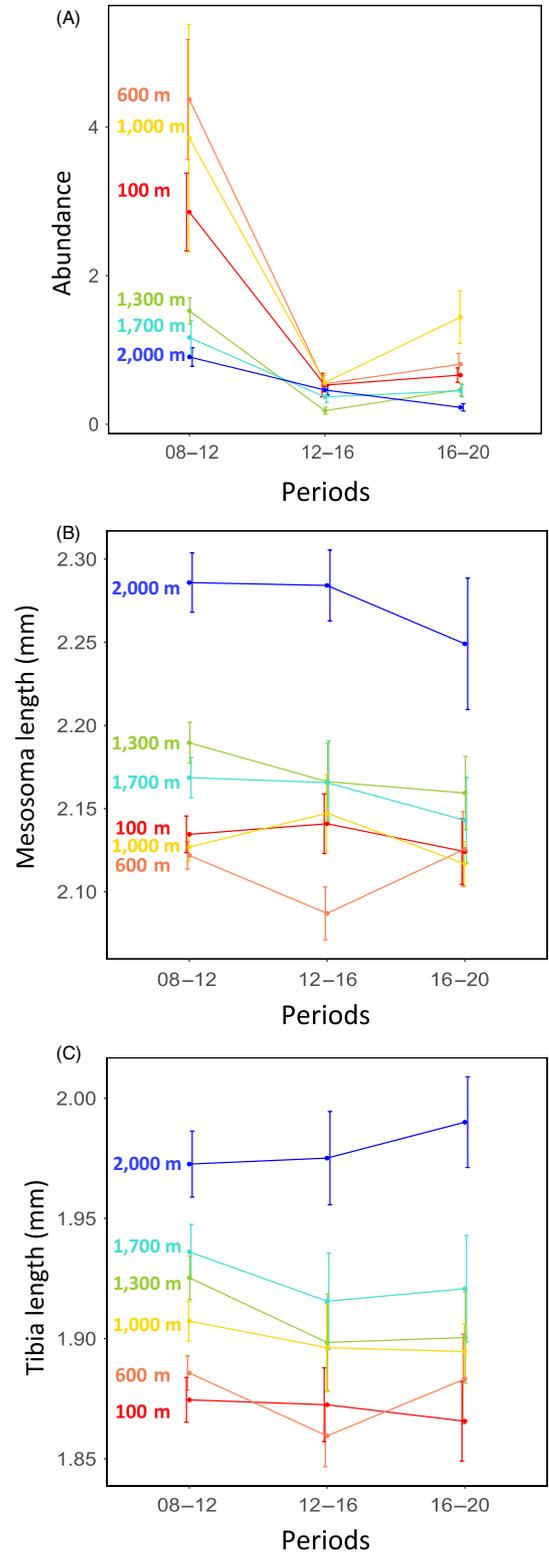
**FIGURE 3** Mean nest entrance temperature (°C) and absolute foraging activity (sum of entries and exits for a 5-min time period; mean  $\pm$  SE) for populations at three elevations (600 m - A,D; 1,300 m - B,E; and 2,000 m - C,F) during 2 months (May, A-C, and July, D-F). TAct is the mean ( $\pm$ SE) absolute foraging activity for the full 13-hr observation period and  $n$  is the number of colonies sampled for each population and month

(Figure 4C; population:  $F_{5,309} = 4.01$ ,  $p = 0.0015$ ; temperature:  $F_{1,242} = 21.12$ ,  $p < 0.0001$ ; population  $\times$  temperature:  $F_{5,309} = 4.22$ ,  $p = 0.0010$ ). The slope of the relationship between mean daily temperature and the temperature of maximum activity was significantly steeper at 2,000 m than at the lower elevations. Nevertheless, it is

important to note that this difference was strongly driven by a group of 11 colonies in the population at 2,000 m, which were observed to be active on particularly cold days at the end of the summer (temperature  $< 15^{\circ}\text{C}$ ). Model selection revealed that the best models selected following the Akaike information criterion contained the



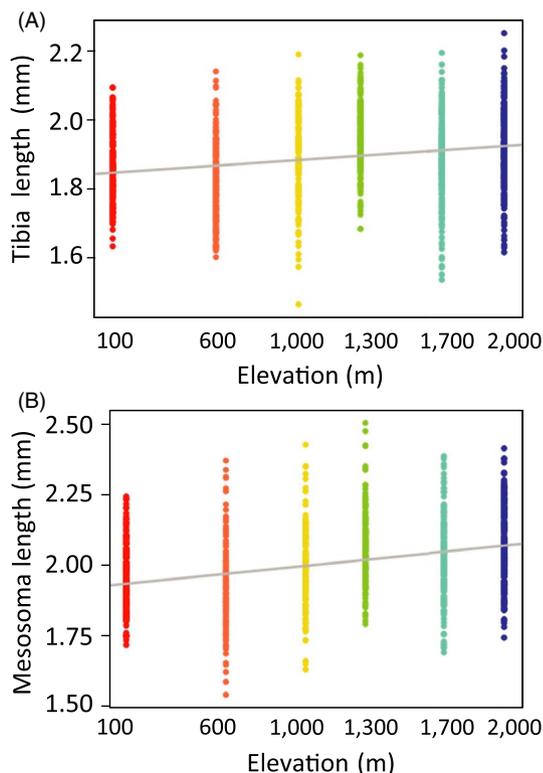
**FIGURE 4** (A) Relationship between absolute daily foraging activity and mean daily nest entrance temperature for all the study colonies; (B) relationship between the number of food items retrieved daily and the mean daily nest entrance temperature for all the study colonies; (C) relationship between the temperature of maximum activity and the mean daily nest entrance temperature for all the study colonies. Different colours were used for the various study populations: red-100 m, coral pink-600 m, yellow-1,000 m, green-1,300 m, turquoise-1,700 m and blue-2,000 m



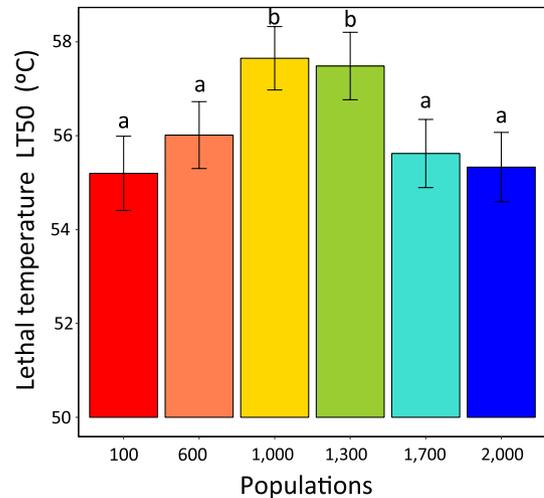
**FIGURE 5** (A) Abundance of *Aphaenogaster iberica* (mean  $\pm$  SE) in pitfall traps during different time periods for the different study populations; (B) mesosoma length (mean  $\pm$  SE) for *A. iberica* captured during different time periods for the different study populations; (C) tibia length (mean  $\pm$  SE) for *A. iberica* captured during different time periods for the different study populations. Different colours were used for the various study populations: red-100 m, coral pink-600 m, yellow-1,000 m, green-1,300 m, turquoise-1,700 m and blue-2,000 m

curvilinear term, showing a curvilinear relationship between absolute foraging activity and hourly nest entrance temperature in each population (Figure S2; Table S2), although relationship strength was low ( $R$  values: 0.2094–0.6205; Figure S2). At this fine-grain scale, there were no marked differences among populations in their responses to temperature.

Second, ant colonies could thermoregulate by recruiting larger foragers at the warmest hours of the day. To test this hypothesis, we analysed the pitfall trap contents (collected at 12:00, 16:00 and 20:00). The abundance of *A. iberica* foragers in the traps varied among time periods and populations (Figure 5A; time period:  $F_{2,2,542} = 41.98$ ,  $p < 0.0001$ ; population:  $F_{5,23} = 4.96$ ,  $p = 0.0025$ ; time period  $\times$  population:  $F_{10,2,542} = 2.90$ ,  $p = 0.0013$ ). At the three lowest elevations (100, 600 and 1,000 m), the abundance of *A. iberica* foragers decreased significantly from the morning to the afternoon and evening. At the three higher elevations (1,300, 1,700 and 2,000 m), forager abundance was lower overall and did not vary significantly among time periods. We found no significant difference in mesosoma length or tibia length among time periods (Figure 5B,C;  $F_{2,1,289} = 1.99$ ,  $p = 0.1360$  and  $F_{2,1,295} = 0.60$ ,  $p = 0.5492$  respectively). However, both measures differed significantly among populations (mesosoma:  $F_{5,24} = 6.26$ ,  $p = 0.0008$  and tibia:  $F_{5,26} = 2.83$ ,  $p = 0.0361$ ) and they both increased with increasing elevation. The interaction between population and time period was not significant for either trait (mesosoma:  $F_{10,1,286} = 0.51$ ,  $p = 0.8846$  and tibia:



**FIGURE 6** Variation in ant hind leg tibia length (A) and mesosoma length (B) along the elevational gradient. Different colours were used for the various study populations: red–100 m, coral pink–600 m, yellow–1,000 m, green–1,300 m, turquoise–1,700 m and blue–2,000 m



**FIGURE 7** Half-lethal temperatures (LT50) for the different *Aphaenogaster iberica* study populations (mean  $\pm$  SE). Different colours were used for the various study populations: red–100 m, coral pink–600 m, yellow–1,000 m, green–1,300 m, turquoise–1,700 m and blue–2,000 m. Bars that share the same letters were not significantly different ( $p > 0.05$ )

$F_{10,1,294} = 0.98$ ,  $p = 0.4597$ ). Moreover, we found that mesosoma and tibia lengths measured using colonies collected within each population ( $n = 10$ –13 each; Figure 6) were significantly correlated with elevation ( $p < 2.2e-16$  and  $p < 6.092e-14$  respectively), although the strength of the correlation was rather weak (0.3156 and 0.2220 respectively).

### 3.3 | Demography and individual thermal tolerance

Mean colony size was 585 workers (ranging from 154 to 2,186 workers per colony) and was not significantly different among populations ( $F_{5,44} = 0.99$ ,  $p = 0.4321$ ). There was a significant difference in individual ant response to heat among populations (Figure 7; log-likelihood ratio = 74.52 for 5  $df$ :  $p < 0.0001$ ). The analysis of the 95% confidence intervals revealed that ants from the populations at 1,000 and 1,300 m were significantly more thermally tolerant than those from the other populations (Table 1; Figure 7).

## 4 | DISCUSSION

Temperature is one of the most important factors acting on organisms because it determines both macro- and microclimatic conditions, thus affecting species success, species interactions and ecosystem services (García, Bestion, Warfield, & Yvon-Durocher, 2018; Gottfried, Pauli, Reiter, & Grabherr, 2002; Waldock, Dornelas, & Bates, 2018). Ectotherms are especially sensitive to temperature variability and, therefore, to climate change (Deutsch et al., 2008; Sunday, Bates, & Dulvy, 2012). As a result, they have developed adaptive behavioural and physiological coping

mechanisms (Abram, Boivin, Moiroux, & Brodeur, 2017; Chown & Terblanche, 2007; Paaijmans et al., 2013; Willmer, 1982). Ants are subject to somewhat different constraints than most other ectotherms because they are eusocial. In terms of physiology, behaviour and genetics, the colony is the unit of selection for ants (Chen & Robinson, 2014) and thermal adaptation can thus occur via a variety of mechanisms. Here, we observed that the ant *A. iberica* displayed efficient behavioural thermal regulation at the colony level. We observed shifts in the rhythm of foraging activity in populations at different elevations, which appears to be a way of compensating for decreasing temperatures at increasing elevations. This mechanism allowed ants to maintain similar levels of daily foraging activity and food intake regardless of elevation and mean daily temperature. Moreover, we found that ants from populations at intermediate elevations, which experienced the most extreme temperatures, displayed higher heat tolerance than did ants from populations at lower and higher elevations. These results therefore suggest that efficient behavioural thermal regulation does not hinder the emergence of physiological differences in thermal resistance among populations.

*Aphaenogaster iberica* is distributed over a particularly large elevational gradient. In the Sierra Nevada mountain range of southern Spain, where we conducted our study, it is the only ant species that is present starting at sea level and that occurs all the way up to 2,000 m. Mean ground temperature was observed to decrease by 10°C along our study gradient, but both the daily temperature range and microclimatic variability in temperature were higher at intermediate elevations than at lower or higher elevations. There is a geographical explanation for why the intermediate-elevation populations experienced greater thermal variability. All the study populations were located on sunny, south-facing slopes. However, the lower elevation populations (100, 600 m) were relatively close to the Mediterranean Sea (between 5 and 25 km away; see fig. 2 in Villalta, Rami, et al., 2020) and thus benefitted from a thermal buffer effect. In contrast, intermediate-elevation populations were subject to both heat fluxes from the lowlands and cold fluxes from highlands; as a consequence, temperatures could climb very high during the day but drop precipitously at night (information about Sierra Nevada mountain range of southern Spain: <https://deims.org/e51cee43-dc12-4545-8e5b-dad35431e3f7> and <https://obsnev.es/>).

To cope with such variation in temperature, colonies found at different elevations can thermoregulate by adjusting their rhythm of foraging activity. Previous studies on other ant species have already shown that ants shift their rhythm of activity across seasons and locations (Amor et al., 2011; Azcárate et al., 2007; Cerdá, Retana, & Manzaneda, 1998; Cros et al., 1997; Jayatilaka et al., 2011; Lázaro-González, Arnan, Boulay, Cerdá, & Rodrigo, 2013). However, our study goes one step further by showing that such shifts can allow colonies to maintain similar levels of foraging activity regardless of elevation and mean daily temperature during the summer. The only exception was the highest elevation population (2,000 m): its foraging window was constrained by mean daily temperatures,

particularly in the late summer. The ants had no other choice than to forage at low temperatures, and daily foraging activity was thus reduced.

Although the observed shift in foraging activity was a collective strategy, it arose from information being processed at both the individual and colony levels. Hence, an ant's decision to leave or return to the nest depends on its propensity to take thermal risks, a trait that varies among colonies within populations (Blight, Díaz-Mariblanca, Cerdá, & Boulay, 2016).

In all populations, foraging efficiency (measured by the number of food items retrieved irrespective of their nutritional value) was affected by mean daily temperature, perhaps because prey availability or the ant's ability to capture prey was greater on the warmest days. The populations at intermediate elevations (1,000 and 1,300 m) foraged at temperatures that were very close to their critical thermal limits, which raises questions about how these ants will cope with climate change. High temperatures are not the only risk that ants face. The presence of predators and competitors may also have a large impact on foraging decisions (Wehner et al., 1992). Such decisions are also affected by the recent experience of foragers (e.g. whether they have been successful during previous foraging trips) and the intensity of pheromonal recruitment by scouts that have discovered a food resource (Cerdá, Angulo, Boulay, & Lenoir, 2009). The propensity to take thermal risks may be greater if pheromonal recruitment by scouts is more intense. However, an increase in ground temperature is also likely to increase pheromone volatility and thus hamper communication between scouts and recruits, which can reduce colony-level activity (van Oudenhove, Boulay, Lenoir, Bernstein, & Cerda, 2012). Trail pheromones are useless to several *Cataglyphis* species because of the high temperatures at which they forage; as a consequence, these ants must instead utilize individual strategies, such as spatial orientation. They have developed several other adaptations for dealing with extreme temperatures, notably modulating their rhythm of foraging (Wehner & Wehner, 2011) and maintaining extreme worker polyphenism in colonies that produce small and large workers. While large workers are more costly to produce, they are more heat tolerant and can forage even during the hottest hours of the day (Cerdá & Retana, 2000), an advantage in the thermally extreme environments inhabited by these species.

In our study, we found no evidence for the hypothesis that *A. iberica* exploits morphological differences among workers to deal with thermal variability: foragers present in the morning, afternoon and evening were the same size. Consequently, colonies do not appear to rely upon worker polymorphism to continue foraging activity during the warmest hours of the day. In other ant species, thermal tolerance has been shown to be correlated with worker size (Cerdá & Retana, 1997; Sommer & Wehner, 2012). Moreover, in the highly polymorphic species *Cataglyphis velox*, large workers predominantly forage during the hot part of the day, while median-sized workers forage during cooler periods (Cerdá & Retana, 1997, 2000).

However, our results highlight that worker size differs dramatically with elevation. A similar result was obtained by Bernadou, Römermann, Gratiashvili, and Heinze (2016), who studied *Leptothorax acervorum* populations along an elevational gradient in the Alps. It is not known whether these size differences are genetically determined, or whether they result from phenotypic plasticity. In contrast, studies in other ant species did not find a significant relationship between thermal tolerance and body size (Foucaud et al., 2013; Johnson & Stahlschmidt, 2020). When Modlmeier, Pamminger, Foitzik, and Scharf (2012) studied plasticity in cold tolerance in the ant *Temnothorax nylanderii*, they found no correlation between worker size and the ability to recover from a chill-induced coma; they did, however, highlight the importance of acclimation and suggested that cold tolerance was determined more by differences in physiology than by differences in behaviour or worker size. In a recent study on the congeneric species *A. senilis*, Oms et al. (2017) showed that cooler environmental temperatures tended to slow down larval development, which led to the production of larger individuals. Furthermore, Dahlhoff et al. (2019) showed that development was slower at higher elevations in the leaf beetle *Chrysomela aeneicollis* because of a reduction in metabolic rate. That said, in *A. iberica*, the opposite was observed along the same study gradient as ours: Shik, Arnan, Oms, Cerdá, and Boulay (2019) discovered that the metabolic rates of ants from higher elevations increased faster at low temperatures but that the temperature at which metabolic rates levelled out was the same across populations. They also observed that body size (mg of dry mass) was significantly larger for higher elevation ants than for lower elevation ants. If worker size is a plastic trait, as we have seen, phenotypic plasticity could explain why workers in higher elevation populations were larger than workers in lower elevation populations.

Another important finding of our study is that workers from intermediate-elevation populations (1,000 and 1,300 m), who experienced the greatest temperature variability, displayed significantly greater thermal tolerance (had higher LT50 values) than workers from lower and higher elevation populations. These results support the CVH, which posits that exposure to greater temperature variability selects for greater thermal tolerance (Baudier, D'Amelio, Malhotra, O'Connor, & O'Donnell, 2018; Chan et al., 2016) or for more plastic phenotypes (Kellermann & Sgrò, 2018). Different recent studies have also found support for the CVH in a variety of organisms: tropical tadpoles (Gutiérrez-Pesquera et al., 2016); Andean frogs (Pintanel, Tejedo, Ron, Llorente, & Merino-Viteri, 2019); snails and vascular plants in the Caucasus (Mumladze, Asanidze, Walther, & Hausdorf, 2017); tropical aquatic insects (Shah et al., 2017); and tropical army ants (Baudier et al., 2018). However, an experimental study using *Drosophila* found no association between thermal plasticity and climatic variability, and it was thus suggested that thermal plasticity could not be solely determined by the climatic variability (Kellermann & Sgrò, 2018).

Although the specific mechanisms that enhance thermal tolerance in *A. iberica* have not yet been identified, they might include

dietary effects. For example, Bujan and Kaspari (2017) recently showed that carbohydrate-rich diets increased thermal tolerance in *Azteca chartifex* and Nelson et al. (2019) observed that, at low elevations, *Formica podzolica* colonies consumed twice as many carbohydrates. In the case of *A. iberica*, workers only collect solid prey, and so the species' diet is relatively poor in carbohydrates. It is more likely that thermal tolerance is enhanced by mechanisms such as the production of heat shock proteins (Gehring & Wehner, 1995; Helms Cahan et al., 2017; Ślipiński, Pomorski, & Kowalewska, 2015; Willot et al., 2017), more efficient convective cooling or constrained cuticular transpiration (Cerdá & Retana, 2000; Gibbs & Pomonis, 1995). These differences could be the result of natural selection acting differentially on intermediate-elevation populations, creating a pattern of local adaptation. Indeed, because of the colony founding mode used by *A. iberica*, populations separated by even small physical distances are likely to be genetically isolated (Villalta, Rami, et al., 2020). Consequently, populations exposed to more extreme temperatures could have evolved more efficient physiological mechanisms for protecting themselves against elevated temperatures. Alternatively, differences among populations could be due to phenotypic plasticity. For example, in *Camponotus rufipes*, *Temnothorax curvispinosus* and *A. senilis*, workers that develop at warmer temperatures have higher temperature response thresholds and temperature preferences than those raised at cooler temperatures (Diamond, Chick, Perez, Strickler, & Martin, 2017; Oms et al., 2017; Weidenmüller, Mayr, Kleineidam, & Roces, 2009). These results suggest that the differences in thermal tolerance observed among *A. iberica* populations in this study could be due to acclimation during development. However, further studies with transplant or common garden experiments are necessary if we wish to tease apart how the local environment, natural selection and phenotypic plasticity affect thermal tolerance.

Here, we studied the foraging ecology of *A. iberica* along an elevational gradient in the field and characterized the thermal tolerance of workers from populations at different elevations in the laboratory. We found that efficient colony-level thermal regulation does not appear to limit individual thermal tolerance. However, our findings raise new questions related to the decision-making processes involved in foraging and the role-played by phenotypic plasticity versus natural selection in generating population-level differences in thermal tolerance. Further studies conducted over large elevational gradients will increase our understanding of the behavioural, physiological and evolutionary mechanisms that could allow ants to withstand the effects of climate change.

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## AUTHORS' CONTRIBUTIONS

R.B. and X.C. came up with the ideas and designed the methodology; C.S.O., C.R.M.-G. and S.D. collected the data; R.B., I.V., E.A. and X.C. analysed the data; R.B., I.V., E.A. and X.C. led the writing of the manuscript. All the authors made important contributions to the manuscript drafts and gave final approval for publication.

## DATA AVAILABILITY STATEMENT

Data available from CSIC repository: <http://dx.doi.org/10.20350/digitalCSIC/12516> (Villalta, Sánchez Oms, et al., 2020).

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## SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section.

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