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Author for correspondence:
Rafael C. da Silva
e-mail: rcswasp@gmail.com

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Thermal stress impairs survival and immune responses in ant founding queens

Rafael C. da Silva and Thibaud Monnin

Sorbonne Université, Université Paris Cité, Université Paris Est Créteil, CNRS, INRAE, IRD, Institut d'Écologie et des Sciences de l'Environnement de Paris (UMR7618), 4 place Jussieu, Sorbonne Universite, Paris, Île-de-France 75005, France

RCdS, 0000-0003-2674-8976; TM, 0000-0003-3762-942X

Rising temperatures associated with global warming affect all organisms, particularly ectotherms such as insects. Ant queens provide an ideal model for studying the effects of thermal stress because of their distinctive life-history traits. After mating, nest-founding queens of many species undergo a critical phase in which they search alone for a suitable site to establish a new colony. Despite growing concern about the effects of thermal stress on organisms, we still lack detailed understanding of how heat intensity and exposure duration influence mortality and immune function in founding queens, both of which are key to successful colony establishment. Here, we tested how these factors affect mortality and encapsulation, a proxy for immune response in insects, in queens of the common garden ant *Lasius niger* during colony founding. We found that heat stress increased queen mortality, regardless of exposure duration to heat, and decreased their encapsulation responses, indicating compromised immune function. These results highlight the detrimental effects of high temperatures on ants, organisms that provide essential ecological services.

1. Introduction

Global climate change has already altered the living conditions of nearly all terrestrial organisms [1]. Increases in mean temperature associated with global warming can negatively affect ectotherms across multiple levels of biological organization [2]. Even more concerning is the impact of episodic extreme heat events on these organisms [3–5]. This dynamic raises the following question: how do organisms cope with the substantial challenge linked to heat stress? [6]. In ants from temperate regions, nuptial flights of males and virgin queens, followed by colony founding by inseminated queens, typically occur during summer, triggered by environmental cues such as temperature and light intensity [7]. Colony founding represents the most vulnerable and lethal phase of an ant colony life cycle, with failure rates reaching up to 99% in some species [8,9]. Whether queens found solitarily or in groups of cooperating queens [10], they face a narrow temporal window of exposure to predation, starvation, disease, competition, and mostly overheating under high and fluctuating summer temperatures [11,12].

Although ants are generally regarded as thermophilic (e.g. adapted to warm conditions) compared with other insects [13–15], temperature strongly affects their physiology and behaviour [15–19]. Their fitness typically increases up to a species-specific optimum but declines sharply beyond it as vital processes become impaired [2,20,21]. Thermal stress can compromise energy balance, reproduction and physiological functions, including immune responses [15]. Even sudden temperature fluctuations can induce physiological stress in insects [22–24], although some studies have reported that

urban thermal gradients do not necessarily drive changes in immune system activation in honeybees [25]. The insect immune system comprises multiple responses, including the encapsulation–melanization reaction (hereafter encapsulation), which targets parasitic protozoa and nematodes [24,26]. This response combines cellular and humoral processes, producing haemocyte aggregation and melanin deposition around the target, which becomes visible as a dark encapsulation [26].

Given the thermal conditions queens experience after mating and during nest site selection, and considering the increasing frequency and intensity of summer heat waves over recent years [27], it is particularly pertinent to ask how heat intensity and exposure duration interact to influence immune function and survival during colony founding. Addressing thermal sensitivity by considering both the magnitude and duration of heat exposure is especially relevant, as the effects of temperature on organisms are inevitably shaped by this interaction. Frameworks such as thermal death time (TDT) and thermal load sensitivity (TLS) explicitly incorporate both aspects, providing a conceptual basis for predicting organismal responses under variable thermal regimes [28].

Ants are an ideal model system, with diverse life-history strategies, global distribution (except Antarctica), and high abundance and biomass in terrestrial ecosystems [29]. As social insects, the effects of rising temperatures can be buffered at the colony level, at least to some extent and within the confines of the nest [16], although foragers remain exposed to ambient temperature. Nevertheless, each ant is an individual organism, allowing us to study environmental stress mechanisms at the individual level. We addressed this question in founding queens of the black garden ant, *Lasius niger*, focusing on the pre-nesting founding phase. In this species, the optimal temperature for oviposition ranges between 23°C and 28°C. Pupae can withstand higher temperatures (29–30°C) [30], and workers have a critical thermal maximum (CT_{max}) of approximately 47°C (personal data). However, specific data for queens are lacking. It remains unclear how heat stress may affect immune responses in ant queens during this highly sensitive life stage, despite their potential to impose simultaneous physiological and energetic stress. While previous studies have examined thermal effects on queen mortality and colony success upon foundation [11], or mortality and immunity during the winter quiescent phase [18], no study has tested the combination of these factors shaping queens' immune responses during early colony founding. We hypothesized that both survival and immune response would be negatively affected by heat and longer exposure. Specifically, we predicted increased mortality and reduced immunocompetence in queens subjected to prolonged thermal stress.

2. Material and methods

(a) Queen collection

Lasius niger is a common European ant found in natural and urban habitats [31]. Mated queens found colonies alone or in small groups without worker support, making them particularly vulnerable [32]. A total of 242 queens were collected in Paris in July 2025 after a nuptial flight and were brought to the lab for thermal and encapsulation assays. Queens were randomly assigned to six experimental groups (three heat intensities × two exposure durations). Queen size (head width) did not differ among groups (all $p > 0.05$) and was therefore excluded from subsequent analyses.

(b) Thermal stress assays

The thermal stress treatments consisted of three heat intensities (25°C as control, 35°C, 40°C) applied for two durations (1 h or 5 h). The temperature and time of exposure chosen are expected to be ecologically relevant for those queens [11,33,34]. The temperature range falls within that experienced by *L. niger* queens during nest founding (30.7–57.5°C) and is even conservative [11]. Exposure duration also reflects natural conditions for solitary foundresses [33,34]. Queens were gently handled with soft forceps and placed on a high-precision thermal plate (PZ28-1, Gestigkeit, Germany; 200 × 280 mm heating surface) set to the target thermal stress temperature. Humidity was not controlled during thermal exposure, and queens had no access to water during treatment or the following 24 h observation. This set-up was designed to mimic expected natural conditions experienced by founding queens, combining thermal and desiccation stress during nest founding. Each queen was positioned inside a bottomless circular plastic tube (5.2 cm diameter × 6 cm height, open at both ends) coated with Fluon® to prevent her from escaping, with a maximum of three queens per tube. Queens of the 1 h treatment were individually removed from the thermal plate after 1 h and transferred to clean glass containers (sanitized with 70% ethanol) and maintained at 25°C for 4 h, until 5 h treatments were complete. This procedure ensured that both treatments were standardized with regard to handling and experimental time prior to the encapsulation assays, thereby controlling for potential confounding effects of time and handling. The number of queens initially assigned to each treatment was n = 36, 41 and 42 for exposures at 25°C, 35°C and 40°C during 1 h, and n = 30, 49 and 44 for exposures at 25°C, 35°C and 40°C during 5 h, respectively. The number of queens used in the subsequent encapsulation assays was slightly lower as 11 queens died during the heat exposure (all but one had been exposed to heat, and all were from the 5 h treatment, electronic supplementary material, table S1).

(c) Encapsulation assays

(i) Nylon filament insertion and post-exposure handling

A total of 231 queens survived heat exposure (95.4%) and were used for encapsulation assays. We adapted the following protocol of queen handling and subsequent analysis from [35,36]. A 1.5 mm segment of nylon monofilament (0.2 mm

diameter), previously roughened with fine-grit sandpaper (600 grit) and knotted at one end to facilitate insertion and later removal, was inserted into the haemocoel through a small puncture in the pleural membrane between the second and third abdominal tergites using cleaned forceps (70% ethanol) and entomological pins. Queens were then maintained individually at 25°C : 20°C (day : night) for 24 h to allow encapsulation. They were kept in glass vials closed with cotton and provided with no water or nesting substrate. Relative humidity in the thermal chambers was approximately 30% but not actively controlled.

At the end of the encapsulation assays, after 24 h, a few queens had lost their implant (n = 10, electronic supplementary material, table S1) and were excluded from the analysis. For the remaining queens, we recorded mortality (n = 79, electronic supplementary material, table S1) and quantified the encapsulation response of the queens that had survived (n = 142, electronic supplementary material, table S1).

(ii) Image acquisition and processing

Filaments were photographed against a standardized Kodak 18% grey card, serving as a neutral reference for lighting and white balance. Each image included at least one filament from each temperature treatment. Images were captured in RAW format using a digital camera mounted on a stereomicroscope (Zeiss Discovery.V12) with consistent fibre-optic lighting and the grey card as the background. White balance was calibrated using a region of interest (ROI) of the grey card. Subsequently, the RAW images were converted to uncompressed TIFF format and analysed in Fiji/ImageJ (https://imagej.net/Fiji). Images were transformed to 8-bit greyscale, and for each filament (the part inserted inside the queens' body) and the grey card, a region of interest (ROI) was manually selected and measured using the Freehand Selection Tool.

(iii) Encapsulation index calculation

The mean grey value (MGV) of each filament was determined by taking three measurements on a 0–255 greyscale (0 = black, 255 = white), and the average was used to quantify each queen's encapsulation response. The grey card included in each image was measured in the same way, and its mean MGV served as a reference for standardization. To account for differences in lighting and exposure, a standardized encapsulation index was calculated as the ratio of the grey card MGV to the filament MGV. Higher values indicate stronger melanization and thus stronger encapsulation.

(d) Statistical analysis

All statistical analyses were performed in RStudio (version 2025.09.2 [37]). Mixed-effects models were fitted using *lme4* [38] and *lmerTest* [39], *post hoc* comparisons were performed with *emmeans* [40]. To assess whether queen mortality during the encapsulation assays varied as a function of heat (25°C, 35°C and 40°C) and exposure duration (1 h versus 5 h), we initially fitted a generalized linear model (GLM) with a binomial error distribution. However, these models exhibited perfect separation, preventing reliable estimation of model parameters. Mortality differences among treatment groups were therefore analysed using Fisher's exact tests, which compare the proportions of surviving versus dead queens and are appropriate for categorical data with small sample sizes or cases of complete or quasi-complete separation. Fisher's tests were complemented with binomial GLMs using temperature as a continuous predictor of mortality (see electronic supplementary material).

For the encapsulation data, normality was also checked using Shapiro–Wilk tests and visual inspection of Q–Q plots, confirming that residuals were normally distributed. Encapsulation responses were analysed with an additive linear mixed-effects model (since including the interaction did not improve model fit), including temperature and exposure duration as fixed effects. *Post hoc* pairwise comparisons among treatment combinations were performed using estimated marginal means, with p-values adjusted for multiple testing using the false discovery rate (FDR) method. As a complementary analysis, we examined temperature–encapsulation relationships using separate linear models for each exposure duration (1 h and 5 h), with temperature treated as a continuous variable (see electronic supplementary material).

3. Results

(a) Mortality during the encapsulation assay

Queen survival during the encapsulation assay differed significantly among treatments (figure 1). No significant mortality was observed following exposure at 25°C during either 1 h or 5 h (Fisher's exact test: 1 h, p = 0.11; 5 h, p = 0.11). By contrast, significant mortality occurred following exposures at 35°C and 40°C, during both 1 h and 5 h (all $p < 0.001$; figure 1a,b). At 35°C, queen survival was reduced by approximately 42% after 1 h and 33% after 5 h. At 40°C, survival declined more sharply, with reductions of approximately 54% after 1 h and 50% after 5 h. Consistent with this pattern, mortality differed among temperature treatments within each exposure duration (figure 1a,b). For the 1 h exposure, queen survival declined with increasing temperature, decreasing from 87.5% at 25°C to 57.9% at 35°C and 46.2% at 40°C. This corresponds to reductions of approximately 30% and 41% in survival at 35°C and 40°C, respectively, relative to 25°C. For the 5 h exposure, queen survival also declined with increasing temperature, decreasing from 86.2% at 25°C to 66.7% at 35°C and 52.6% at 40°C. This corresponds to reductions of approximately 19% and 33% in survival at 35°C and 40°C, respectively, relative to 25°C. The reduction at 40°C was statistically significant, whereas the effect at 35°C was marginal and not significant after multiple comparisons. When temperature was treated as a continuous variable, logistic regression analyses revealed a significant negative effect of

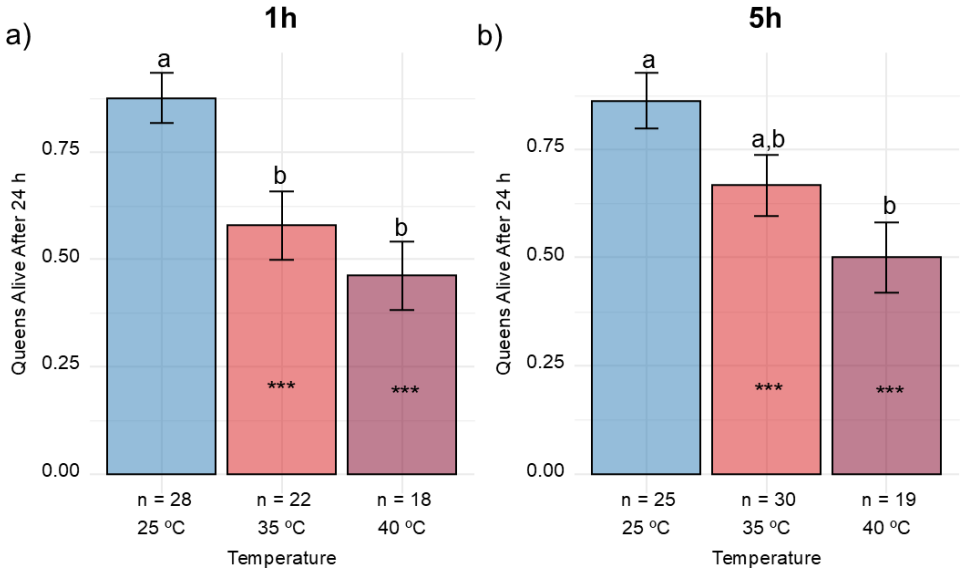

Figure 1. Queen survival during the encapsulation assay. Bars show the proportion of surviving queens at the end of the encapsulation assay (24 h) across temperatures (25°C, 35°C, 40°C) and exposure durations of (a) 1 h and (b) 5 h. (***) Represents *p*-value < 0.001 for comparisons between the number of queens alive at the onset and end of the assay for each treatment; different letters indicate significant differences at the end of the assay among groups. Error bars represent standard errors of proportions.

temperature on queen survival for both exposure durations (1 h: estimate = –0.138, z = –3.43, $p < 0.001$ (electronic supplementary material, figure S1a)); 5 h: estimate = –0.123, z = –2.92, $p = 0.003$ (electronic supplementary material, figure S1b), indicating an increase in mortality with increasing temperature.

(b) Encapsulation responses following thermal stress

Encapsulation responses were significantly affected by both temperature and exposure duration. Queens exposed to higher temperatures showed reduced encapsulation, with a pronounced decrease at 40°C compared with 25°C (estimate = −0.053, t = −3.03, p = 0.003), corresponding to an approximate 6.2% reduction in encapsulation, whereas responses at 35°C were intermediate and only marginally different from those at 25°C (estimate = −0.028, t = −1.71, p = 0.089; approx. 3.2% reduction). Exposure duration also influenced encapsulation, as queens exposed for 5 h exhibited significantly lower encapsulation than those exposed for 1 h (estimate = −0.033, t = −2.39, p = 0.018), representing an approximate 3.9% decrease relative to the control condition (figure 2a,b).

Post hoc comparisons supported these patterns. Encapsulation was consistently lower at 40°C than at 25°C within both exposure durations (1 h: p = 0.012; 5 h: p = 0.012), corresponding to reductions of approximately 6% in encapsulation performance (figure 2a,b). Comparisons between 25°C and 35°C revealed weaker and generally non-significant differences, although queens exposed to 35°C for 5 h showed reduced encapsulation relative to queens exposed to 25°C for 1 h (p = 0.012). Encapsulation was reduced by approximately 10% when comparing the two extreme groups (25°C, 1 h versus 40°C, 5 h).

By assessing the relationship between temperature (treated as a continuous variable) and encapsulation, we found no significant effect of temperature on encapsulation for the 1 h treatment (estimate = −0.002, t = −1.27, p = 0.21) (electronic supplementary material, figure S2a). By contrast, for the 5 h treatment, encapsulation decreased significantly with increasing temperature (estimate = −0.004, t = −3.31, p = 0.001) (electronic supplementary material, figure S2b).

4. Discussion

Our results show that both higher temperatures and longer exposure durations increase mortality and reduce encapsulation in *L. niger* founding queens during the pre-nest founding phase. In particular, queens suffered a higher mortality under 40°C compared with 25°C, regardless of the exposure duration. Encapsulation declined with higher temperature, with correlation analyses confirming a negative relationship particularly for 5 h exposure treatment, indicating that prolonged and intense heat exposure has a stronger negative effect on encapsulation. These findings indicate that increased temperature and exposure duration jointly induce mortality and decrease immune performance of founding queens, highlighting how critical the short window between nuptial flight and nest excavation is for colony establishment in summer.

The elevated mortality observed cannot be explained solely by acute thermal exposure, as most queens survived immediately after treatment. Similarly, it is unlikely to be attributed solely to filament insertion, given the high survival of control queens (electronic supplementary material, table S1) (although we cannot rule out a possible interaction between nylon injury and high heat). Delayed mortality at elevated temperatures likely reflects carry-over effects of thermal stress. Our results are in line with a recent study that demonstrated that acute exposure of queens to either 25°C or 43°C in a long-term context affects survival differently, with those exposed to 43°C prior to colony establishment being less likely to survive [11]. In insects, and

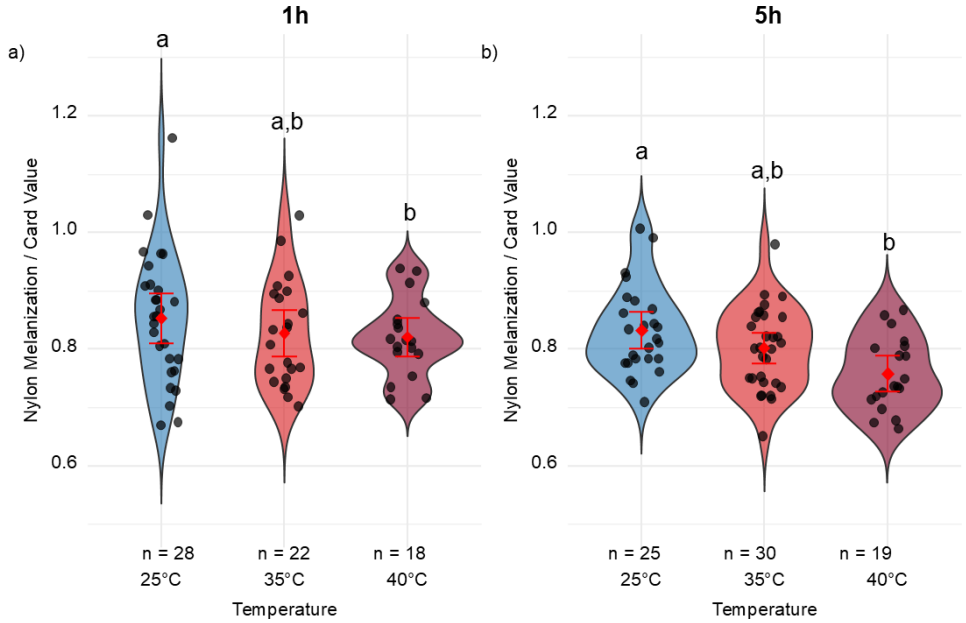

Figure 2. Encapsulation response of queens following thermal stress. Data are shown for (a) 1 h exposure and (b) 5 h exposure across three temperatures (25°C, 35°C, 40°C). Encapsulation was quantified as the ratio between the mean grey reference card and the mean encapsulation of the filament implant (higher encapsulation = higher index). Violin plots show the distribution of individual values, with red diamonds indicating group means and red vertical lines representing 95% confidence intervals. Different letters indicate statistically significant differences among groups within each exposure duration.

particularly queens undergoing prolonged periods of food deprivation, exposure to higher temperatures accelerate metabolism and deplete fat body reserves [41–43], ultimately compromising survival. The effects of raising temperature on *L. niger* queens appear to depend strongly on seasonal context. Under summer conditions, a 10°C increase relative to control temperature significantly increased queen mortality within 24 h. By contrast, during overwintering, smaller but prolonged temperature increases (approx. 5°C for 165–191 days) had little effect on survival (82.8% and 99.2% [18]). These results suggest that rising temperature is more detrimental during summer, when resulting temperatures may approach the critical thermal maximum (CT_{max}), than moderate winter fluctuations.

In insects, survival is influenced by multiple factors, including intrinsic species traits and environmental conditions such as humidity, habitat and parasite infection, as well as interactions among these factors, particularly the interplay between thermal stress and pathogen exposure [44–46]. Studies have highlighted the connection between heat shock proteins, which are activated in response to heat stress, and immune function: pathogen infection induces heat shock protein activation, and exposure to specific thermal regimes can enhance resistance to pathogens [47–50]. However, such beneficial effects are limited and depend on the intensity and duration of the thermal challenge [51,52], underscoring that the effects of heat stress on the insect immune system are highly variable [46]. Immune gene expression in *Bombyx mori* can be reduced following exposure to moderately elevated, non-extreme temperatures (e.g. 30°C) [53]. Exposure to elevated temperatures can reduce aphid resistance to parasitoid infection [54]. In some cases, the effects of heat exposure are temperature-dependent. An exposure to 36°C increases the expression of immune genes such as *cecropin*, *defensin* and *transferrin* in *Aedes aegypti* larvae, whereas exposure to 40°C does not [55]. The effects of heat shock on insect immune responses are thought to result from cross-talk and cross-protection mechanisms, involving heat shock protein pathways that act as danger signals and stimulate immune activation [24,56]. Immune traits such as encapsulation, phenoloxidase activity, antimicrobial peptide expression and haemocyte responses may therefore show both similar and contrasting responses to temperature depending on the thermal regime [46]. Although we focused on acute thermal stress and short-term immune responses, it remains possible that in *L. niger* founding queens, moderate thermal exposure could prime immunity against pathogens encountered during nest excavation. However, this possibility remains speculative and warrants further investigation.

Reduced encapsulation at high temperatures may reflect energetic trade-offs between immunity and other costly processes [57–59]. We hypothesize that in *L. niger* queens this is especially relevant during nest excavation, when resources are limited and social immunity is absent [60], or due to increased activity at 35–40°C. Encapsulation is an energetically costly immune response [58,61], and its activation varies with individual traits, including age, individual quality and rearing environment [35,36,62]. In mosquitoes, elevated temperatures not only weaken encapsulation but also accelerate age-related declines in melanization-based immunity [62,63], suggesting that thermal stress can compromise both the magnitude and persistence of this immune response. Similar phenomena may occur in founding queens under challenging high thermal regimes. Together, increased energetic expenditure and weakened immune defences may increase queen mortality, which would impair colony establishment, and ultimately influence long-term ecological persistence. It is important to note that we did not control humidity, and queens were not provided with water during exposure or post-exposure periods. Therefore, the observed effects may also reflect combined heat and desiccation stress during natural nest-founding conditions.

The identification of heat intensity and exposure duration as key determinants of queen mortality and immune function highlights the need to examine these factors across the colony cycle. Several questions remain. For queens that survive early high-temperature exposure during summer colony founding, do heat intensity–duration interactions affect key behaviours such

as nest excavation? Does milder chronic warming enhance immunity via thermal priming, even though extreme temperatures impair it? Whether such effects extend to offspring, shaping colony-level immunity and resilience, remains to be tested.

Ethics. This work did not require ethical approval from a human subject or animal welfare committee.
Data accessibility. Data can be accessed online [64]. Supplementary table and figures are provided in the electronic supplementary material [65].
Declaration of AI use. We have not used AI-assisted technologies in creating this article.
Authors' contributions. R.C.d.S.: conceptualization, data curation, formal analysis, funding acquisition, investigation, methodology, writing—original draft, writing—review and editing; T.M.: conceptualization, funding acquisition, project administration, resources, supervision, writing—review and editing.

Both authors gave final approval for publication and agreed to be held accountable for the work performed therein.

Conflict of interest declaration. We declare we have no competing interests.
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