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Heatwave-induced demographic variation across populations is decoupled from thermal tolerance in a widespread ant

Tania Tyczynski [1], Romain Libbrecht[1]*, Irene Villalta[1]*

[1]Insect Biology Research Institute (IRBI, UMR CNRS 7261) University of Tours, France

*These authors contributed equally to the work

Correspondence:

Tania Tyczynski: tania.tyczynski@gmail.com

Romain Libbrecht : romain.libbrecht@univ-tours.fr

Irene Villalta : irene.villalta@univ-tours.fr

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Credit Authorship Statement

TT: Data curation; Investigation; Methodology; Validation; Writing - review & editing. RL & IV: Conceptualization; Data curation; Formal analysis; Funding acquisition; Investigation; Methodology; Project administration; Resources; Supervision; Validation; Visualization; Writing - original draft; Writing - review & editing.

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Data accessibility

The datasets and the R script are available in a public repository at: https://doi.org/10.5281/zenodo.22814191

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Abstract

Extreme heat events are increasing under climate change, yet their effects on social insect population dynamics remain poorly understood, particularly at early stages of colony development. We experimentally exposed colonies of the black garden ant (*Lasius niger*) from three European populations to a short-term heatwave (30 °C for 4 days) to assess how acute thermal stress influences colony demography, functional traits, and thermal tolerance. Heat exposure accelerated egg production and brood development, with the strongest response observed in the northernmost population, indicating population-dependent plasticity in developmental timing. However, heat events experienced as larvae reduced thermal resistance in newly emerged workers independently of body size, revealing a trade-off between accelerated growth and physiological performance. These findings demonstrate that, sublethal heat events can simultaneously enhance colony growth and compromise worker thermal performance, with population-dependent variation in developmental sensitivity that is cryptic under benign conditions. Our results highlight the importance of integrating demographic and physiological responses across populations and life stages when assessing insect vulnerability to climate extremes.

Keywords: critical thermal maximum, developmental plasticity, thermal reaction norm, heatwave, latitudinal gradient, *Lasius niger*

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Introduction

Climate change is increasing the frequency, intensity, and duration of extreme heat events worldwide, with important consequences for biological systems (IPCC, 2023). Heatwaves act as acute environmental stressors that can disrupt survival, development, and reproduction, thereby influencing population dynamics and ecosystem functioning (Bellard et al., 2012; Parmesan and Yohe, 2003). Understanding how organisms respond to these events is therefore essential for predicting ecological impacts under ongoing global change.

Insects, particularly widely distributed species, provide powerful systems for investigating biological responses to environmental gradients and stressors (Castañeda et al., 2015; Savolainen et al., 2013; Schmidt et al., 2005). While thermal traits such as developmental rate or thermal tolerance are often treated as species-level constants in ecological models, increasing evidence shows that they can vary among populations through local adaptation and phenotypic plasticity (Dixon et al., 2009; Hoffmann et al., 2013; Sunday et al., 2019). Accounting for such variation is critical for understanding how species respond to climate stressors, particularly extreme events such as heatwaves.

To explain patterns of thermal adaptation, two major hypotheses have shaped ecological research. The Climatic Variability Hypothesis (CVH) suggests that thermal tolerance evolves in response to the amplitude of environmental temperature fluctuations, predicting a positive correlation between thermal limits and local thermal extremes (Addo-Bediako et al., 2000; Stevens, 1989). Complementing this, Janzen's hypothesis (Janzen, 1967) posits that organisms inhabiting thermally stable environments evolve narrower thermal niches,

leading to reduced temperature overlap across elevational gradients—a pattern supported across ectothermic taxa, including insects (Polato et al., 2018; Sunday et al., 2011). However, these macroecological predictions do not always translate directly to the conditions that organisms experience *in situ*. Microclimatic variation, shaped by fine-scale features such as nesting depth, vegetation structure, and soil moisture, can buffer or amplify thermal exposure (Potter et al., 2013; Woods et al., 2015), potentially decoupling selective pressures from broader climate trends. Adding to this complexity, numerous studies have shown that upper thermal limits (CTmax) are often more phylogenetically constrained and less plastic than lower thermal limits (CTmin), resulting in asymmetric evolutionary responses across thermal gradients (Addo-Bediako et al., 2000; Araújo et al., 2013; Gunderson and Stillman, 2015; Sunday et al., 2011). This pattern, formalized in the Niche Asymmetry Hypothesis (NAH; Araújo et al., 2013), raises concerns about the limited adaptive potential of species to cope with increasingly frequent and intense high-temperature events.

Bridging these macroecological insights to organismal performance, however, requires considering how thermal stress translates into demographic outcomes. While physiological limits such as CTmax are widely used to assess vulnerability to climate extremes, they capture only part of the ecological consequences of heat stress (Diamond et al., 2013, 2012; Penick et al., 2017). Population persistence depends on demographic processes — reproduction, brood development, and survival — that can be disrupted well before critical physiological thresholds are reached, and whose sensitivity to heat may vary across developmental stages and among populations with different thermal histories.

Most mechanistic work on developmental thermal stress has focused on *Drosophila* and aquatic ectotherms (Kellermann et al., 2017; Overgaard et al., 2011; Pottier et al., 2022), whereas comparable research in social insects has received comparatively little attention despite their ecological dominance in terrestrial systems (Pelini et al., 2012; White and Dillon, 2023). Social insects provide a particularly informative model because colony performance emerges from integrated processes operating across multiple levels of biological organization, including queen reproduction, brood development, worker activity, and colony-level regulation. Thermal stress can therefore act concurrently on these levels, linking physiological responses of individuals to emergent colony-level outcomes. In ants, colony viability is tightly linked to reproductive output and early-life development (Asano and Cassill, 2011; Hölldobler and Wilson, 1990; Ingram et al., 2013; Tschinkel, 1993). These processes are strongly temperature-dependent and can be disrupted by elevated temperatures (Abril et al., 2010; Kipyatkov et al., 2004; Penick et al., 2017; Porter, 1988). In addition, thermal conditions during development can influence functional traits such as body size or morphology (Angilletta., 2009; Atkinson, 1994), which in turn affect worker performance and thermal tolerance (Diamond et al., 2012; Penick et al., 2017). Because ants contribute to key ecosystem processes, including soil turnover, trophic interactions, and nutrient cycling, such temperature-driven changes across levels of biological organization may have broader ecological consequences.

Here, we investigate how exposure to a short-term heatwave affects early colony development, adult morphology, and thermal tolerance in the black garden ant *Lasius niger*, a widespread species inhabiting diverse thermal environments across Europe. Using colonies from three urban populations distributed along a latitudinal gradient across

Europe (Santander, Tours, and Warsaw; ~8.8° latitude; ~2,080 km), we assess whether responses to heat stress vary across populations originating from contrasting climatic conditions. Specifically, we addressed three questions: (i) Does short-term heatwave exposure accelerate brood development, and do populations from contrasting thermal environments differ in the magnitude of this response? Based on the temperature dependence of metabolic rates and evidence that northern populations exhibit steeper thermal reaction norms, we predicted that heat exposure would accelerate egg production and larval development, with the strongest response in the Warsaw population. (ii) Does early-life thermal stress affect adult thermal tolerance, and if so, is this effect mediated by changes in body size? Based on the temperature–size rule, we predicted that heat-exposed larvae would develop into smaller workers, and that any reduction in CTmax would be potentially associated with this size reduction rather than direct physiological adjustment. (iii) Does the thermal cost of a heatwave during development — if present — vary among populations from contrasting climatic origins? We asked whether the physiological cost imposed on newly emerged workers is population-dependent, or whether CTmax reduction is uniform across the latitudinal gradient, suggesting a shared physiological constraint rather than a locally adapted response. By integrating demographic, morphological, and physiological responses across populations from a broad climatic gradient, this study examines how short-term heatwave exposure shapes multiple components of colony performance during a critical early stage of social organization, and whether the benefits and costs of heat thermal events are distributed symmetrically or differentially across populations with contrasting thermal histories.

Materials and Methods

Queen collection and colony establishment

Founding queens of *L. niger* were collected during nuptial flights in summer 2023 from three geographically distinct populations: Santander, Spain, on 28 June 2023; Tours, France, on 21 June 2023; and Warsaw, Poland, on 30 June and 8 July 2023. Collection locations and geographic coordinates are detailed in Table S1 and Fig. S1. Each queen was housed individually in a standard test tube nest (12.5 cm length × 1.5 cm diameter), half-filled with water and sealed with two cotton plugs—one separating the water reservoir from the nesting space and another closing the tube. All colonies were maintained in constant darkness at 20 °C in a breeding room.

Once workers emerged, colonies were provisioned weekly with a drop of commercial lizard jelly (composition: water 65%, glucose syrup 28%, sugar 3.9%, gelatin 1.7%, fruit juice concentrate 0.4%, citric acid 0.4%, artificial flavors 0.2%, protein 0.2%).

Experimental colony setup

In February 2024, 178 experimental colonies were established: 58 from Santander, 60 from Tours, and 60 from Warsaw. Colony composition was standardized to facilitate comparisons across populations and treatments: each experimental colony included one queen, five workers, and five larvae visually identified as being approximately at the second larval instar (L2). These larvae were still developing and were not close to pupation. The experimental colonies represented incipient colonies at an early post-founding stage maintained in test tubes (7 cm length × 1 cm diameter) under the same 20 °C dark conditions.

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Thermal treatment

Control colonies remained at 20 °C throughout the experiment, while treatment colonies were exposed to 30 °C for 4 consecutive days, three days after colony setup. Colonies were randomly assigned to either control (20 °C) or heatwave treatment (30 °C). The Santander population comprised 29 control and 29 heatwave-exposed colonies, whereas the Tours and Warsaw populations each comprised 30 control and 30 heatwave-exposed colonies.

Thermal treatments were carried out in complete darkness using two incubators. Colonies were processed in six weekly batches comprising 30, 30, 30, 30, 30 and 28 colonies, respectively, with treatment order alternated weekly to control for batch effects. After the 4-day period, all colonies were returned to the breeding room at 20 °C.

The experimental design was chosen to approximate thermal conditions experienced by young colonies during heatwave events, when limited worker numbers and shallow nests constrain thermoregulatory buffering. Reanalysis data indicate that soil temperatures at shallow depths can reach or exceed 30°C across Central Europe during summer heatwave events (García-García et al., 2023; Muñoz-Sabater et al., 2021)

Demographic monitoring

Colony demography was monitored weekly for 11 weeks following treatment. Counts of eggs, larvae, cocoons, and workers were recorded from February 26 to June 10, 2024. Because treatments were applied sequentially across batches, time series were aligned relative to the onset of treatment (defined as Day 0).

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Thermal tolerance assays

Critical thermal maximum (CTmax) was measured to assess worker thermal tolerance in response to the heatwave treatment. Measurements were performed on up to two workers per colony:

For adult workers, CTmax was measured eight days after the beginning of the thermal treatment in 269 individuals from 156 colonies. The control treatment included 142 workers from 80 colonies, whereas the heatwave treatment included 127 workers from 76 colonies. Overall, 113 colonies contributed two workers and 43 colonies contributed one worker.

For newly emerged workers, CTmax was measured in 193 individuals from 118 colonies. The control treatment included 76 workers from 48 colonies, whereas the heatwave treatment included 117 workers from 70 colonies. Overall, 75 colonies contributed two workers and 43 colonies contributed one worker. Sample sizes for each population × treatment combination are provided in Table S2.

For newly emerged workers, pupae were monitored daily, and newly emerged workers were distinguished from older workers by their lighter cuticle and reduced mobility and were removed from the colony on the day they were detected. They were maintained individually for 2–3 days in Eppendorf tubes containing a water reservoir separated from the ant by cotton. CTmax was measured once the workers had become fully active. Assays were conducted using a Peltier element connected to a manually regulated temperature controller, allowing precise ramping of temperature. Individual ants were placed in 0.3 ml aluminum wells covered with transparent plastic. Temperature was increased from 25 °C

at 1 °C min^{-1} (±0.1 °C), a ramping rate commonly used in ant thermal biology to minimize acclimation and measurement artefacts (Roeder et al., 2021). CTmax was recorded with a HOBO Onset UX120-014M logger using a thermocouple placed at the base of a reference well. CTmax was defined as the temperature at which individuals lost muscular coordination and could no longer maintain posture. After measurements, ants were preserved in 70% ethanol for morphometric analysis. In total, CTmax was measured for 454 individuals from 169 colonies.

Morphometric measurements

To assess whether developmental temperature affected worker morphology and to examine the relationship between body size and thermal tolerance, morphometric traits were measured in newly emerged workers collected during the CTmax experiment. Four traits were quantified: head length (HL), inter-ocular distance (HW), hind femur length (FL), and hind tibia length (TL). Measurements were obtained from high-resolution images taken with a Leica DMS1000 stereomicroscope using Leica Application Suite v4.12.0. (Fig. S2). Complete measurements for all four traits were available for 210 workers from 134 colonies. The control treatment included 92 workers from 57 colonies, whereas the heatwave treatment included 118 workers from 77 colonies. Overall, 76 colonies contributed two workers and 58 colonies contributed one worker. Sample sizes for each population × treatment combination are provided in Table S2.

Statistical analyses

All statistical analyses were performed in R version 4.3.2 (R Core Team, 2022). Demographic dynamics (eggs, larvae, cocoons, and workers) were analyzed using linear

mixed-effects models (LMMs) fitted with the lmer function from the lme4 package (Bates et al., 2024) with time since the start of the heat event as a continuous predictor, and treatment, population of origin, and their interactions as fixed effects. To capture non-linear temporal dynamics, time was modelled using natural cubic splines with increasing degrees of freedom (df = 1–10); for each response variable we selected the model with the lowest AIC where increasing df no longer substantially improved the fit. Batch was included as a random effect in all models to account for the staggered implementation of treatments, and colony identity was added as an additional random factor in time-series analyses to account for repeated measures within colonies. To determine whether the models should account for temporal autocorrelation, we tested whether incorporating a temporal correlation structure improved the fit of our models.

For each selected demographic model, we performed Type-II F-tests (Wald tests) using the Anova function of the car package (Fox et al., 2019) to quantify the main effects and interactions, focusing particularly on terms involving time (time : treatment, time : population, time : treatment : population) to describe differences in demographic trajectories. Where a significant time : population interaction was detected, we further examined population differences by fitting models to pairwise population subsets and correcting p-values using Holm's method. To check model predictions, we used the emmip function of the emmeans package (Lenth et al., 2024) to obtain estimated marginal means over time.

To summarize treatment and population effects on overall brood production, we also analyzed the maximum number of eggs, larvae and cocoons reached by each colony during the experiment. For each trait, we fitted LMMs with treatment, population and their

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interaction as fixed effects, experimental batch as a random effect, and used Type-II likelihood-ratio χ^2 tests to assess significance. Post hoc pairwise contrasts among populations were obtained using estimated marginal means with Tukey adjustment.

Thermal tolerance (CTmax) was analyzed separately for workers present at the start of the experiment and newly emerged workers. For each worker class, we fitted LMMs with treatment, population and their interaction as fixed effects, and colony identity and batch as random intercepts. We used Type-II χ^2 tests to assess the effects of treatment and population and obtained treatment contrasts from the emmeans of the final model.

Morphological traits of newly produced workers (femur length, tibia length, head length, head width) were measured on 1 or 2 workers from each colony. To avoid pseudo replication, we first averaged each trait at the colony level. We conducted a principal component analysis (PCA) on centered and scaled colony means to summarize overall morphological variation and visualize patterns across populations and treatments. Because the four traits were strongly correlated, we used a multivariate analysis of variance (MANOVA) with treatment, population and their interaction as predictors and the four traits as multivariate responses, summarized using Pillai's trace. When a significant overall effect of population was detected, we repeated the MANOVA on pairwise population subsets and adjusted p-values using Holm's method.

Model assumptions were checked by visual inspection of residuals. All visualizations were generated using ggplot2 (Wickham, 2024), as well as emmip plots from the emmeans package (Lenth et al., 2024).

Results

Demographic colony dynamics

Egg production

Egg numbers increased more rapidly in heat-exposed colonies (30 °C) than in controls (20 °C), with the strongest acceleration in the Warsaw population. This was reflected in a robust time : treatment interaction (Fig. 1a; $F_{4,1915}$ = 105.73, p < 0.001). Temporal trajectories also differed markedly among populations (Fig. 1b; $F_{8,1915}$ = 15.37, p < 0.001): Warsaw colonies showed the steepest increase in egg numbers, followed by Tours and then Santander. Post hoc comparisons confirmed strong population-level divergence across the full time series (Santander vs Tours: $F_{4,1264}$ = 5.90, p = 1.0×10^{-4}; Tours vs Warsaw: $F_{4,1297}$ = 9.56, p <0.001; Santander vs Warsaw: $F_{4,1271}$ = 29.56, p< 0.001).

A significant three-way interaction (time : treatment : population; Fig. 1c; $F_{8,1915}$ = 3.10, p = 0.002) showed that populations responded differently to the heat event. Separate analyses revealed population differences in the 20 °C treatment (time : population: $F_{8,950}$ = 4.87, p < 0.001): Santander differed from Tours ($F_{4,624}$ = 3.76, p = 0.005) and Warsaw ($F_{4,630}$ = 9.80, p < 0.001), whereas Tours and Warsaw did not ($F_{4,646}$ = 1.95, p = 0.10). In the 30 °C treatment, the effect size of population differences was stronger (time : population: $F_{8,965}$ = 11.43, p < 0.001), and all pairwise contrasts were significant (Santander–Tours: $F_{4,639}$ = 2.67, p = 0.032; Tours–Warsaw: $F_{4,650}$ = 8.82, p < 0.001; Santander–Warsaw: $F_{4,641}$ = 20.65, p < 0.001), again with Warsaw showing the steepest increase in egg number.

These temporal patterns translated into clear differences in overall egg production. Heat-exposed colonies reached significantly higher egg maxima than controls (Fig. S3a; χ^2_1 = 25.49, p < 0.001). Populations also differed (Fig. S3b; χ^2_2 = 21.09, p < 0.001): Warsaw

264 colonies produced the highest maxima (37.2 ± 3.1), followed by Tours (29.0 ± 2.3) and
265 Santander (22.4 ± 1.7). Post hoc contrasts confirmed that Warsaw produced significantly
266 more eggs than Santander (t_{167} = 4.58, p < 0.001) and Tours (t_{167} = 2.58, p = 0.029). The
267 Santander–Tours comparison remained non-significant (t_{167} = 2.02, p = 0.111). No
268 treatment : population interaction was detected (χ^2_2 = 4.21, p = 0.122), indicating that the
269 heatwave increased the maximum number of eggs similarly across populations.

270 Larval production

271 We first restricted our analyses to the initial 35 days to capture the decline of the first larval
272 cohort as it developed into cocoons (Fig. S4). During this early period, the heatwave
273 strongly altered larval trajectories (Fig. 2a; $F_{3,871}$ = 194.38, p < 0.001), and populations also
274 differed (Fig. 2b; $F_{6,871}$ = 4.67, p < 0.001). Post hoc tests showed that Santander differed
275 significantly from both Tours ($F_{3,577}$ = 3.86, p = 0.019) and Warsaw ($F_{3,577}$ = 8.73, p <
276 0.001), while Tours and Warsaw did not ($F_{3,588}$ = 1.40, p = 0.241). We found weak evidence
277 for a three-way interaction (Fig. 2c; $F_{6,871}$ = 1.88, p = 0.082).

278 When examined separately by treatment, no population differences were detected for
279 colonies exposed to a 20 °C treatment ($F_{6,435}$ = 0.86, p = 0.524), whereas trajectories
280 diverged significantly among populations in the 30 °C treatment (Fig. 2c; $F_{6,436}$ = 4.21, p <
281 0.001). At this temperature, Santander differed clearly from Tours ($F_{3,289}$ = 4.40, p = 0.010)
282 and Warsaw ($F_{3,289}$ = 7.34, p < 0.001), whereas Tours and Warsaw remained similar ($F_{3,294}$
283 = 1.40, p = 0.489). Thus, heat exposure amplified population-level differences in early larval
284 development.

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After day 35, larval numbers rose again as newly laid eggs hatched, generating non-linear patterns that differed between treatments (Fig. 3a). Heat-exposed colonies produced many more larvae during this second wave, as shown by the very strong time : treatment interaction ($F_{3,856}$ = 107.21, p < 0.0001). This treatment effect was consistent within each population (Santander: $F_{3,275}$ = 33.24, p < 0.001; Tours: $F_{3,287}$ = 31.56, p < 0.001; Warsaw: $F_{3,294}$ = 44.60, p < 0.001).

Population differences were also detected (Fig. 3b; $F_{6,856}$ = 6.64, p < 0.001). Santander diverged significantly from both Tours ($F_{3,562}$ = 5.72, p = 0.0015) and Warsaw ($F_{3,569}$ = 12.80, p < 0.001), while Tours and Warsaw remained similar ($F_{3,581}$ = 2.01, p = 0.11). A significant three-way interaction (time : treatment : population; Fig. 3c; $F_{6,856}$ = 2.48, p = 0.022) showed that populations responded differently to the heat event.

When examined separately by treatment, population structure was weak in the controls (Fig. 3c; $F_{6,422}$ = 2.34, p = 0.031), but became stronger for heat-treated colonies (Fig. 3c; $F_{6,434}$ = 5.12, p < 0.001): Santander differed sharply from Warsaw ($F_{3,289}$ = 9.74, p < 0.001), whereas contrasts involving Tours were marginal (Santander–Tours: $F_{3,287}$ = 2.95, p = 0.066; Tours–Warsaw: $F_{3,292}$ = 2.51, p = 0.066). Thus, population-level differences were most pronounced after heat exposure.

These temporal patterns translated into clear differences in overall larval output. Colonies exposed to 30 °C produced significantly more larvae than controls (Fig. S3c; χ^2_1 = 57.80, p < 0.001). Populations also differed (Fig. S3d; χ^2_2 = 17.65, p < 0.001): Warsaw reached the highest maxima (20.8 ± 1.3 se), followed by Tours (17.6 ± 1.3) and Santander (14.0 ± 1.3). Warsaw produced significantly more larvae than Santander (t_{167} = 4.20, p < 0.001), whereas

contrasts involving Tours were weaker and non-significant (Santander–Tours: $t_{167} = 2.21$, $p = 0.073$; Tours–Warsaw: $t_{167} = 2.01$, $p = 0.112$). No treatment : population interaction was detected ($\chi^2_2 = 4.52$, $p = 0.104$).

Cocoon production

Cocoon numbers showed non-linear dynamics that depended heavily on heat exposure (Fig. 4). Across all populations, heat-exposed colonies produced cocoons earlier and in greater numbers, as reflected in the very strong time : treatment interaction (Fig. 4a; $F_{4,1916} = 206.42$, $p < 0.001$). This pattern was consistent within each population (Santander: $F_{4,620} = 49.97$; Tours: $F_{4,645} = 77.79$; Warsaw: $F_{4,652} = 83.16$; all $p < 0.001$).

Temporal trajectories also differed among populations (Fig. 4b; $F_{8,1916} = 6.77$, $p < 0.001$). Pairwise comparisons showed divergence between Santander and Tours ($F_{4,1265} = 3.18$, $p = 0.013$), between Tours and Warsaw ($F_{4,1297} = 5.38$, $p < 0.001$), and especially between Santander and Warsaw ($F_{4,1272} = 11.63$, $p < 0.001$). A significant three-way interaction (time : treatment : population; Fig. 4c; $F_{8,1917} = 2.55$, $p = 0.009$) showed that populations responded differently to the heat event.

When analyzed separately by treatment, populations did not differ in the control treatment (Fig. 4c; $F_{8,951} = 1.11$, $p = 0.354$) but showed strong divergence after the exposure to 30 °C ($F_{8,965} = 6.72$, $p < 0.001$). In this treatment, all pairwise contrasts were significant (Santander–Tours: $F_{4,639} = 3.90$, $p = 0.004$; Tours–Warsaw: $F_{4,650} = 4.38$, $p = 0.002$; Santander–Warsaw: $F_{4,641} = 11.73$, $p < 0.001$), with particularly strong divergence between Santander and Warsaw.

Heat exposure strongly increased maximum cocoon production (Fig. S3e; $\chi^2_1 = 62.56$, $p < 0.001$), whereas neither population nor the treatment : population interaction influenced cocoon maxima (population: $\chi^2_2 = 0.95$, $p = 0.622$; interaction: $\chi^2_2 = 1.34$, $p = 0.511$). Estimated maxima were similar across populations (Santander: 2.61 ± 0.21; Tours: 2.87 ± 0.21; Warsaw: 2.67 ± 0.21; all pairwise contrasts $p \geq 0.62$; Fig. S3f), indicating that heat exposure increased cocoon output to a similar extent across populations, despite their distinct temporal dynamics.

Thermal Tolerance

Thermal tolerance (CTmax) of workers that experienced the heatwave as adults did not differ between treatments or populations. (Fig. 5; treatment: $\chi^2_1 = 0.54$, $p = 0.463$; population: $\chi^2_2 = 0.16$, $p = 0.922$; interaction: $\chi^2_2 = 0.52$, $p = 0.773$). CTmax of newly emerged workers showed a significant treatment effect (Fig. 5; $\chi^2_1 = 7.36$, $p = 0.007$), but no population effect ($\chi^2_2 = 2.48$, $p = 0.290$) or treatment : population interaction ($\chi^2_2 = 0.55$, $p = 0.761$). On average, newly emerged workers from heat-exposed colonies (30 °C) had slightly lower CTmax than those from control colonies (20ºC) (20 °C: 46.06 ± 0.04 °C; 30 °C: 45.93 ± 0.04 °C; $t_{99} = 2.65$, $p = 0.009$). Although this difference is small in magnitude, it suggests a modest but consistent reduction in thermal tolerance following heat exposure during larval development.

Morphometric Analysis

We quantified the morphology of newly produced workers (femur length, tibia length, head length, head width) averaged per colony. The four traits were strongly correlated and largely captured by the first principal component (PC1, 80.9% of total variance), consistent

with a major axis of overall body size (Fig. S5). Multivariate analysis revealed no effect of the heatwave treatment on worker morphology (Fig. S5a; Pillai's trace = 0.043, $F_{4,125}$ = 1.39, p = 0.241) and no treatment : population interaction (Pillai's trace = 0.062, $F_{8,252}$ = 1.00, p = 0.436), but a significant population effect (Fig. S5b; Pillai's trace = 0.142, $F_{8,252}$ = 2.41, p = 0.016). Pairwise MANOVAs showed that workers from Tours differed significantly from those from Santander ($F_{4,83}$ = 3.44, Holm-corrected p = 0.024) and Warsaw ($F_{4,85}$ = 3.70, Holm-corrected p = 0.024), whereas Santander and Warsaw did not differ ($F_{4,79}$ = 0.28, Holm-corrected p = 0.888). PCA biplots confirmed these patterns, with populations separating along PC1 while 20 °C and 30 °C ellipses largely overlapped, indicating that population-level differences in worker morphology are not detectably altered by the heatwave treatment (Fig. S5b).

Discussion

As climatic extremes intensify, predicting biological responses to heat stress requires integrating physiology, development, and demography across populations (Jentsch et al., 2007; Vasseur et al., 2014). Thermal traits and developmental responses can vary substantially within species, particularly across environmental gradients (Angilletta., 2009; Durmaz Mitchell et al., 2025; Kingsolver et al., 2013; Overgaard et al., 2011; Walter et al., 2025), highlighting the need for multi-population approaches. Our results show that even a short heatwave event can reshape colony demography and trait expression in a widespread temperate ant, with responses that depend on both developmental stage and geographic origin. Because ants function as ecosystem engineers — influencing soil turnover, nutrient cycling, seed dispersal, and trophic interactions — shifts in brood

development rates and worker thermal tolerance could alter activity and colony growth trajectories, with cascading consequences for the ecosystem processes they sustain.

Short-term heat exposure (30 °C for 4 days) accelerated brood development across all populations. Queens laid more eggs, and larval and pupal stages progressed more rapidly, responses consistent with established patterns in ants and other ectotherms where warming typically shortens development and stimulates oviposition (Abril et al., 2010; Kipyatkov and Lopatina, 2010; Porter and Tschinkel, 1993). These effects reflect the temperature dependence of metabolic processes, which increase in efficiency up to a species-specific optimum (Angilletta, 2004; Howe, 1967; Kingsolver and Huey, 2008; Taylor, 1981). The present experiment was specifically designed to isolate the effect of temperature by maintaining standardized water and food conditions, thereby avoiding confounding thermal effects with simultaneous variation in hydration or resource availability. Determining whether drought, desiccation risk or resource limitation amplify or counteract these temperature-driven changes in brood development will require future factorial experiments.

Importantly, population divergence was most apparent after the 30°C treatment, whereas differences were weak or absent under control conditions. This condition-dependence suggests that heat exposure can reveal otherwise cryptic population differences in brood development, which may remain undetected in experiments conducted under thermally benign conditions alone. These findings reinforce the potential of using stress treatments to evaluate intraspecific variation in climate sensitivity and potentially adaptive differences among populations.

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Geographic origin strongly modulated developmental sensitivity. Colonies from Warsaw, originating from the coolest environment, showed the strongest response to heat exposure, reaching the highest egg, larval, and cocoon maxima. This pattern is consistent with evidence that populations from cooler or more thermally variable environments often exhibit steeper thermal reaction norms, enabling rapid exploitation of favorable conditions (Kipyatkov and Lopatina, 2004; Nilsson-Örtman et al., 2012; Tüzün et al., 2017). Such responses may reflect greater metabolic flexibility maintained at the cost of reduced performance at higher temperatures (Addo-Bediako et al., 2000; Klockmann et al., 2017), a pattern also observed across broader latitudinal gradients in other insects (Durmaz Mitchell et al., 2025; Walter et al., 2025). Conversely, populations from warmer and more thermally stable environments — such as our Santander population — tend to show flatter reaction norms, that could reflect canalization of developmental trajectories around higher thermal optima that may limit their capacity to exploit brief warm periods (Kellermann and Van Heerwaarden, 2019; Svensson et al., 2020).

Despite accelerating brood production, heat exposure experienced as larvae carried a physiological cost: workers that experienced the heatwave event at the larval stage showed modest but consistent reductions in CTmax across all populations, whereas workers exposed to heat only as adults showed no change. This life-stage specificity indicates that responses to thermal stress depend strongly on developmental conditions. Newly emerged workers were temporarily isolated to ensure their identification and standardize their post-emergence age at testing. Because callow workers normally receive social care, this procedure may have influenced their maturation and absolute CTmax, although its standardized application across populations and treatments preserves the

relative comparisons among experimental groups. Developmental thermal exposure has been shown to influence adult CTmax in both directions. Warmer rearing temperatures typically elevate CTmax through beneficial acclimation (Kellermann et al., 2017; Pottier et al., 2022), yet sublethal heat stress during sensitive developmental windows can instead reduce adult thermal resistance — a pattern increasingly documented across insects (Alston et al., 2025; White and Dillon, 2023). Our results align with this latter scenario, suggesting that the pace of larval development under heat stress comes at the cost of impaired physiological performance in the resulting workers. However, the ecological significance of this shift remains to be established. Future research should determine whether this moderate change in CTmax of young workers has biologically meaningful effects on worker survival, foraging performance or long-term colony success.

The absence of population-level divergence in CTmax aligns with macroecological patterns: high-latitude insects may evolve broader tolerance ranges (Deutsch et al., 2008), but this expansion typically occurs through changes in lower—not upper—thermal limits (Addo-Bediako et al., 2000; Sunday et al., 2011). This asymmetry reflects fundamental differences in underlying mechanisms: while cold tolerance can be extended through membrane remodelling and metabolic adjustments, heat tolerance depends on the maintenance of protein stability and membrane integrity under acute thermal stress, processes that are difficult to upregulate and rapidly overwhelmed when temperatures rise (González-Tokman et al., 2020; Klok et al., 2004; Neven, 2000). Beyond these potential evolutionary constraints, an experimental factor may have further contributed to the homogeneity of CTmax values in our study: common-garden rearing conditions at 20°C standardized developmental thermal history across populations and may have reduced reversible plastic

differences in CTmax that might otherwise be expressed under field conditions (Kellermann et al., 2017; Roeder et al., 2021). Together, common-garden standardization and evolutionary constraints may have contributed to the convergence in CTmax values observed across populations with contrasting climatic origins — a pattern consistent with broader evidence for CTmax conservatism across ant communities (Bujan et al., 2020; Diamond and Chick, 2018).

Contrary to our prediction that accelerated development would produce smaller workers with reduced CTmax, heat exposure did not affect worker morphology. This outcome is consistent with mixed support for the temperature–size rule (Gardner et al., 2011; Kingsolver and Huey, 2008) as thermal effects on body size depend strongly on timing and duration (Bowler and Terblanche, 2008; Kingsolver et al., 2013; Kingsolver and Buckley, 2020). The absence of a detectable effect on worker morphology may indicate that the four-day treatment was too brief to affect final body size or that it did not fully overlap with the developmental window during which temperature has its strongest influence on adult morphology (Atkinson, 1994; Kingsolver and Huey, 2008). In addition, nutritional dynamics during larval development may have contributed to the lack of size differences, as nutritional inputs often interact with thermal conditions to shape adult morphology (Davidowitz and Nijhout, 2004; Nijhout, 2003).

Instead, reduced CTmax in workers following larval heat exposure likely reflects sublethal disruption of developmental processes that underpin heat resistance, including membrane remodeling, protein homeostasis, and stress-response pathway investment, without leaving any detectable morphological signature (González-Tokman et al., 2020; Neven, 2000). These outcomes support a developmental trade-off: accelerated maturation under

heat may come at the cost of investment in physiological systems that sustain heat resistance

Carry-over effects of developmental temperature on subsequent physiological performance have been reported in a range of ectotherms, including insects, as well as aquatic invertebrates, where accelerated development under elevated temperatures can reduce stress tolerance or survival (Harvey et al., 2020, Klockmann et al., 2017; Sasaki and Dam, 2019). These parallels suggest that the patterns observed here are not restricted to social insects but may reflect a more general response of ectothermic organisms to acute thermal stress. As such, developmental-stage-dependent trade-offs may represent a widespread mechanism linking climate extremes to population-level outcomes across taxa.

Taken together, our findings demonstrate that even brief heatwave events can simultaneously accelerate colony development and reduce worker thermal resistance, with responses that are both stage-specific and population-dependent. These results highlight a key limitation of approaches that rely solely on adult CTmax to assess vulnerability: demographic and trait-based responses to heat can diverge substantially from physiological limits, particularly during early development. For widespread species already experiencing heterogeneous thermal regimes, such within-species variation may play an important role in shaping population dynamics and influencing the stability of ecosystem processes under increasingly frequent climate extremes.

Conclusion

Our results highlight that, sublethal heatwave exposure can simultaneously accelerate colony development and reduce worker thermal resistance through a developmental

485 trade-off that operates independently of body size and is consistent across populations
486 from contrasting thermal environments. Notably, whereas the demographic benefits of
487 heat exposure — accelerated brood production and egg laying — were strongly
488 population-dependent, with northern colonies showing the greatest response, the
489 physiological cost — reduced CTmax in newly emerged workers — was uniform across the
490 latitudinal gradient. This asymmetry between population-dependent benefits and
491 population-independent costs suggests that the developmental trade-off between growth
492 rate and thermal resilience reflects a shared physiological constraint rather than a locally
493 adapted response, and that demographic and physiological outcomes of heatwave
494 exposure can be decoupled across populations from contrasting thermal environments.
495 Incorporating population-specific developmental dynamics and their asymmetric costs and
496 benefits into thermal performance frameworks will be essential for understanding how
497 developmental thermal trade-offs scale from individual physiology to population
498 vulnerability under an increasingly variable climate. Further research should determine
499 whether these responses are mechanistically linked and whether they have biologically
500 meaningful consequences for worker performance and long-term colony dynamics under
501 natural thermal conditions.

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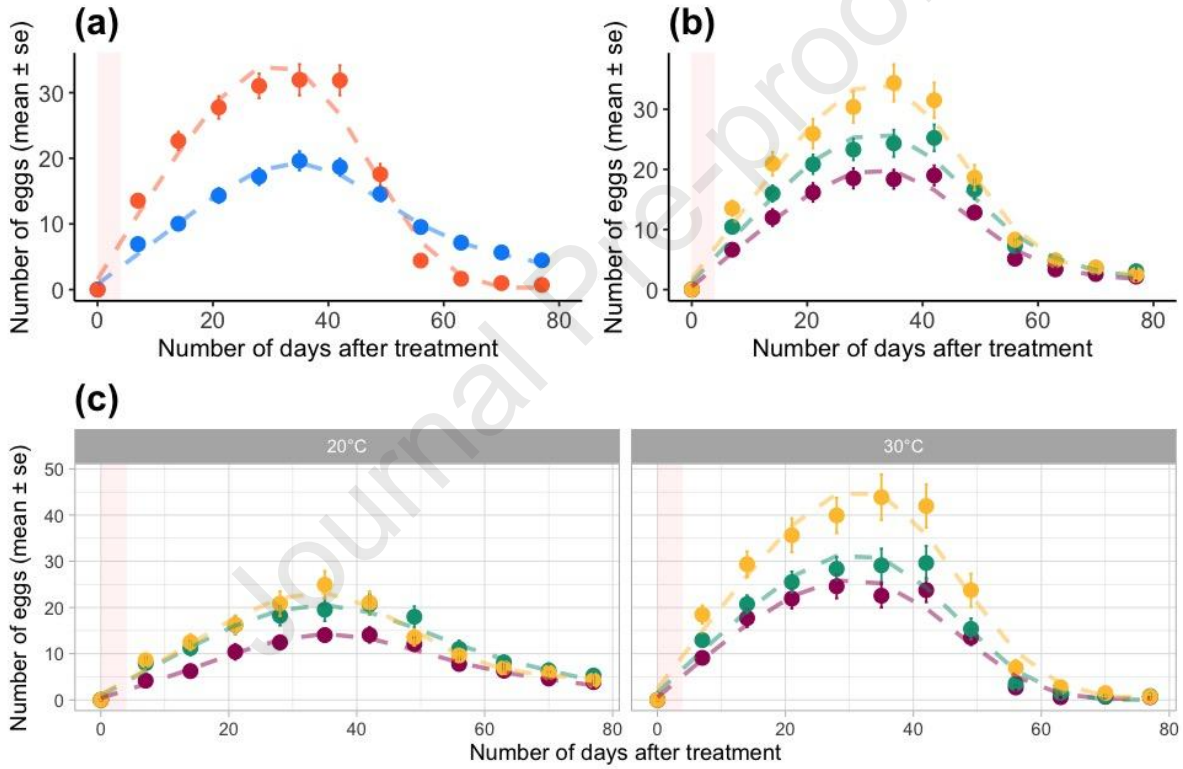
740 Woods, H.A., Dillon, M.E., Pincebourde, S., 2015. The roles of microclimatic diversity and
741 of behavior in mediating the responses of ectotherms to climate change. J. Therm. Biol.
742 54, 86–97. https://doi.org/10.1016/j.jtherbio.2014.10.002.

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Figure 1. Temporal patterns of egg production following heatwave exposure across temperatures and populations.

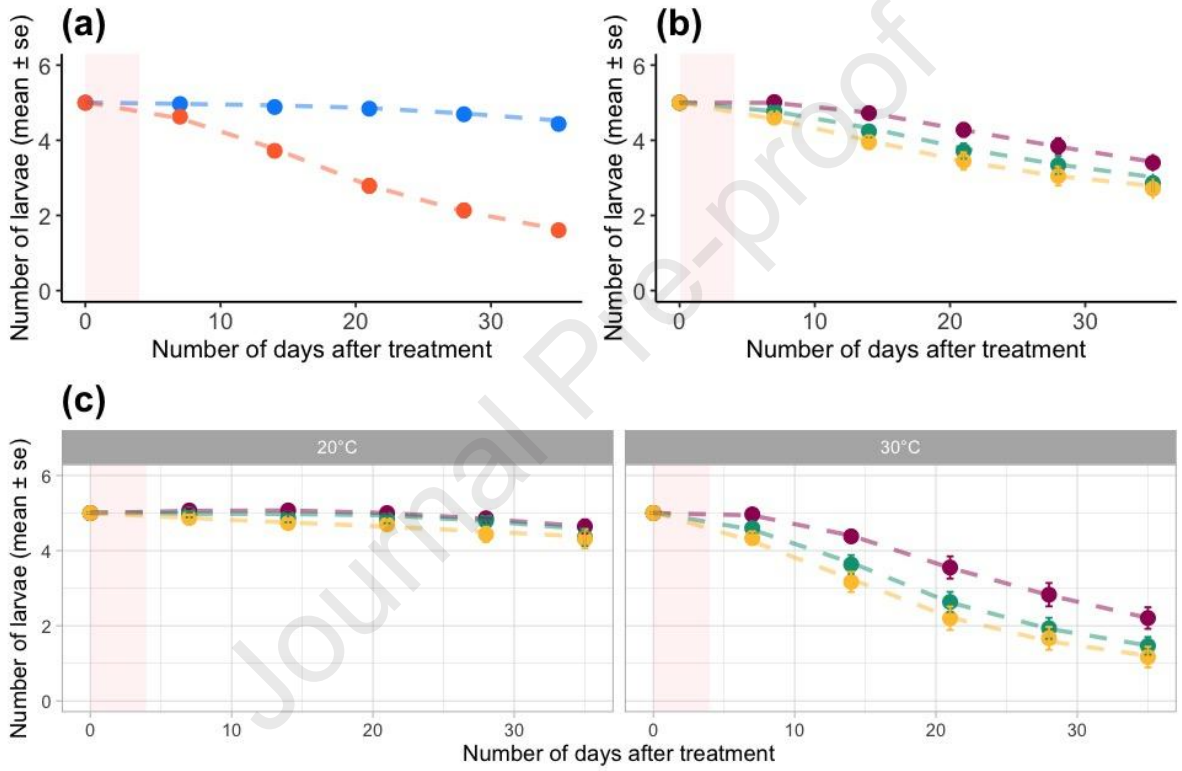
(a) Mean (± SE) number of eggs produced per colony over time under the two temperature treatments (blue: control 20 °C; orange: heatwave 30 °C).
(b) Mean (± SE) egg production over time for the three populations (purple: Santander; green: Tours; yellow: Warsaw), averaged across treatments.
(c) Mean (± SE) egg production over time for each population under each temperature treatment (20 °C left panels; 30 °C right panels).
Shaded region marks the heatwave. Dashed lines indicate model-predicted trajectories. n = 29–30 colonies per population × treatment combination (total N = 178 colonies)



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Figure 2. Early larval dynamics (0–35 days) following four-day heat event exposure.

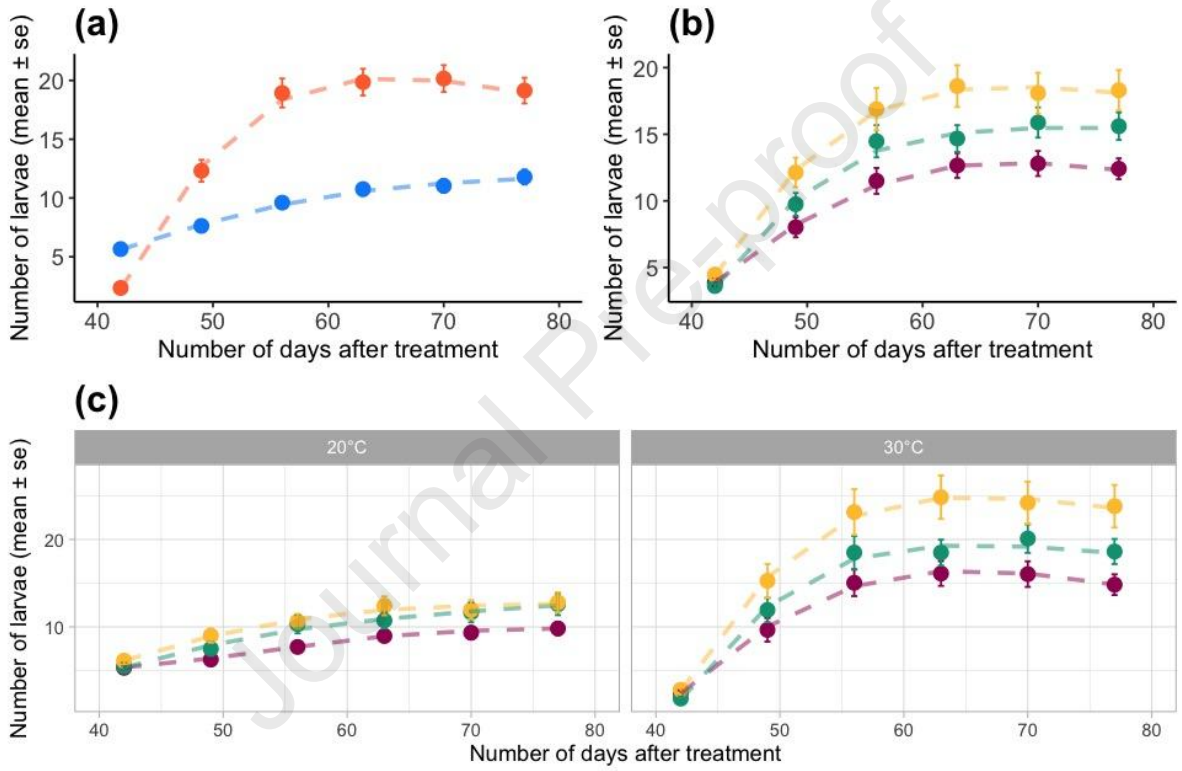
(a) Mean (± SE) larval numbers during the first 35 days under control (20 °C, blue) and heatwave (30 °C, orange) treatments.
(b) Mean (± SE) early larval trajectories for the three populations (purple: Santander; green: Tours; yellow: Warsaw), averaged across treatments.
(c) Population-specific trajectories separated by temperature treatment (20 °C left; 30 °C right). Shaded region marks the heatwave. Dashed lines indicate model-predicted trajectories. n = 29–30 colonies per population × treatment combination (total N = 178 colonies)



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Figure 3. Late larval dynamics (>35 days) as queen laid eggs started hatching.

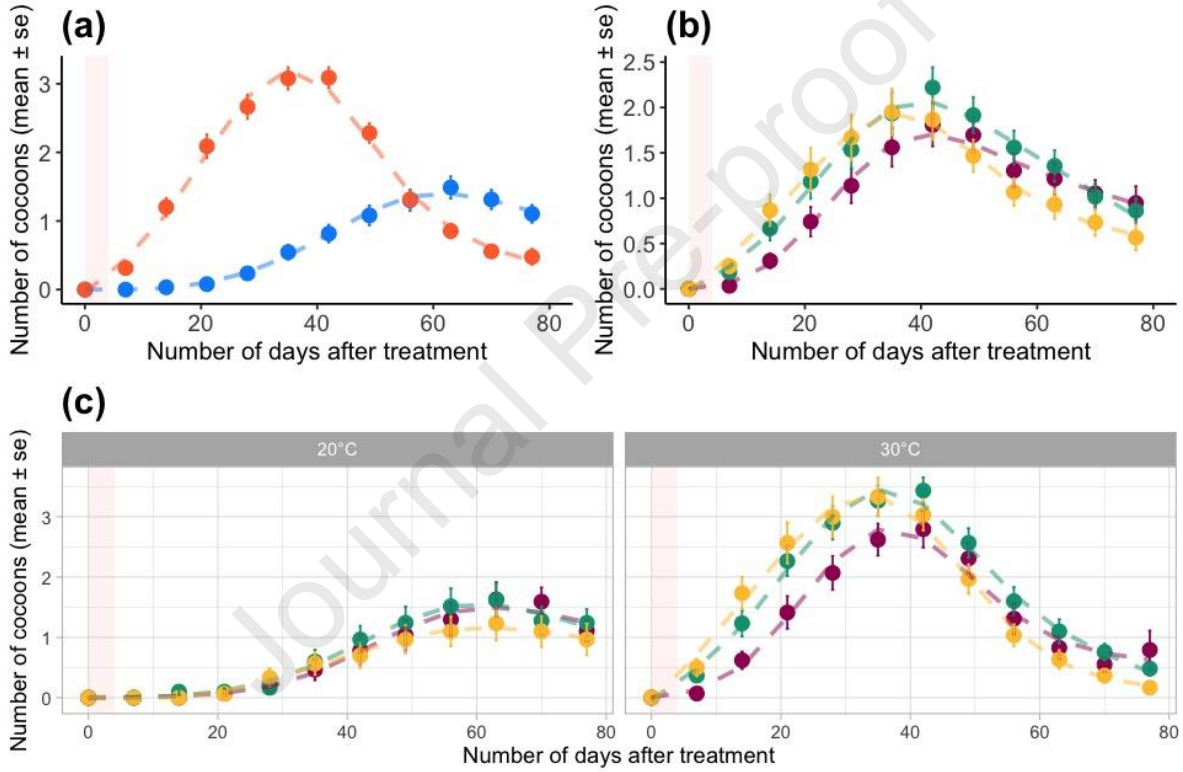
(a) Mean (± SE) larval numbers after day 35 in control (20 °C, blue) and heatwave (30 °C, orange) colonies. Heat-exposed colonies exhibit a pronounced second larval wave.
(b) Mean (± SE) late-phase larval dynamics for the three populations (purple: Santander; green: Tours; yellow: Warsaw), averaged across treatments.
(c) Population-specific late-phase trajectories at 20 °C (left) and 30 °C (right).
Dashed lines indicate model-predicted trajectories. n = 29–30 colonies per population × treatment combination (total N = 178 colonies)



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Figure 4. Cocoon production dynamics across treatments and populations.

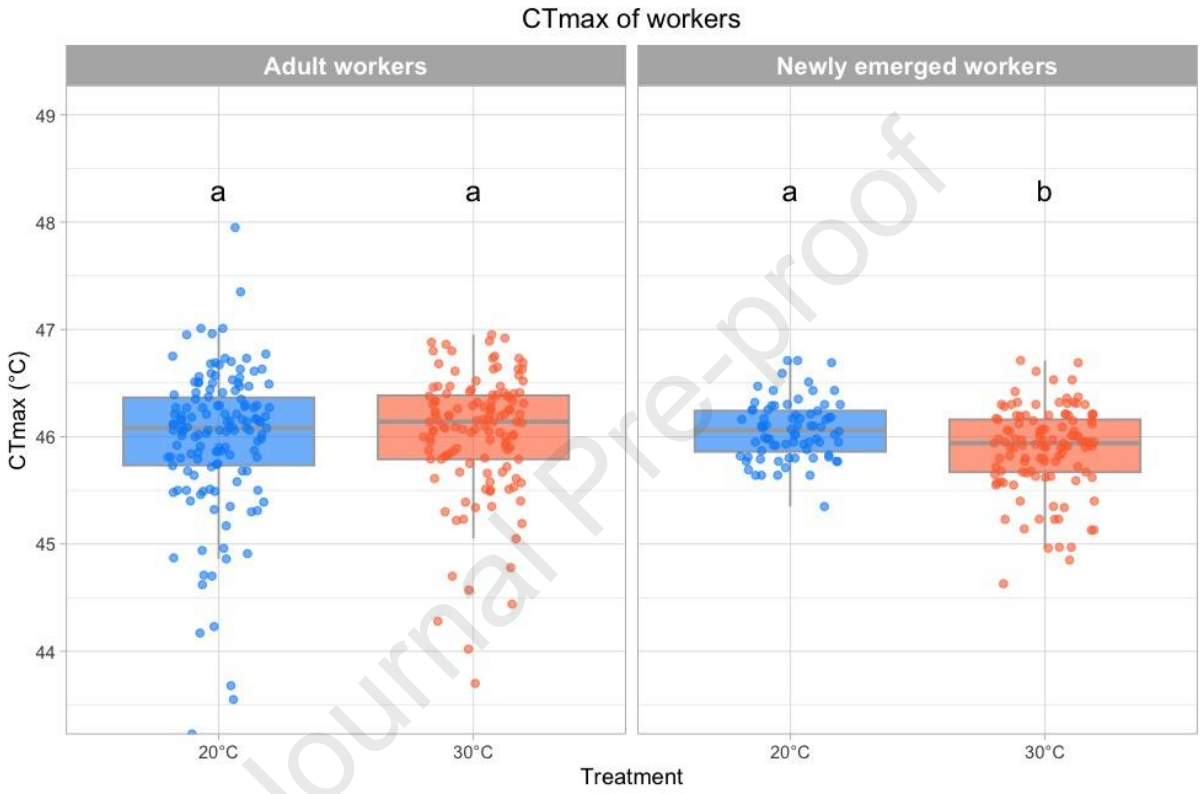
(a) Mean (± SE) cocoon numbers over time under the two temperature treatments (20 °C blue; 30 °C orange).
(b) Mean (± SE) cocoon production for the three populations (purple: Santander; green: Tours; yellow: Warsaw).
(c) Cocoon trajectories at 20 °C (left) and 30 °C (right) for each population, illustrating stronger divergence under heatwave conditions.
Shaded region marks the heatwave. Dashed lines indicate model-predicted trajectories. n = 29–30 colonies per population-treatment combination (total N = 178 colonies).



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Figure 5. Thermal tolerance (CTmax) of adult and newly emerged workers.

CTmax of workers exposed to control (20 °C, blue) or heatwave (30 °C, orange) treatments. CTmax did not differ between treatments or populations for workers that experienced the heat wave as adults (Adult workers). Newly emerged workers that experienced the heatwave as larvae exhibited reduced CTmax. Letters denote significant treatment differences within worker types (n =269 for adult workers, and n =193 for newly emerged workers).



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Highlights

- Experimental heatwaves accelerated brood production and egg laying in ant colonies
- Northern populations showed the strongest demographic response to heat exposure
- Heat exposure at the larval stage reduced worker CTmax independently of body size
- Demographic benefits and physiological costs of heat stress were decoupled across populations

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Declaration of Competing Interests

The authors declare no competing interests.

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