
Social Factors in Heat Survival: Multiqueen Desert Ant Colonies Have Higher and More Uniform Heat Tolerance

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ABSTRACT

Investigations of thermally adaptive behavioral phenotypes are critical for both understanding climate as a selective force and predicting global species distributions under climate change conditions. Cooperative nest founding is a common strategy in harsh environments for many species and can enhance growth and competitive advantage, but whether this social strategy has direct effects on thermal tolerance was previously unknown. We examined the effects of alternative social strategies on thermal tolerance in a facultatively polygynous (multiqueen) desert ant, *Pogonomyrmex californicus*, asking whether and how queen number affects worker thermal tolerances. We established and reared lab colonies with one to four queens, then quantified all colony member heat tolerances (maximum critical temperature [CT_{max}]). Workers from colonies with more queens had higher and less variant CT_{max}. Our findings resemble weak link patterns, in which colony group thermal performance is improved by reducing frequencies of the most temperature-vulnerable individuals. Using ambient temperatures from our collection site, we show that multi-queen colonies have thermal tolerance distributions that enable increased midday foraging in hot desert environments. Our results suggest advantages to polygyny under climate change scenarios and raise the question of whether improved thermal

tolerance is a factor that has enabled the success of polygynous species in other climatically extreme environments.

Keywords: climatic variability, individual variation, matriline, *Pogonomyrmex californicus*, polygyny, social insects, thermal performance, weak link hypothesis.

Introduction

Cooperation can evolve as a consequence of climatic selective pressures. Associations between climate and sociality have been described broadly across animal taxa, including birds (Jetz and Rubenstein 2011; Lin et al. 2019), mammals (Lukas and Clutton-Brock 2017; Firman et al. 2020), and insects (Brady et al. 2006; Kocher et al. 2014; Sheehan et al. 2015; Lukas and Clutton-Brock 2017; Groom and Rehan 2018; Lin et al. 2019). Comparative studies suggest that cooperation is more likely to occur in climates that are especially hot (Arnold and Owens 1999) or particularly cold (Heinze 1993; Heinze and Hölldobler 1994; Heinze and Rueppell 2014; Groom and Rehan 2018). Increased cooperation has also been associated with particularly stochastic climates (Jetz and Rubenstein 2011; Lukas and Clutton-Brock 2017). The underlying factors that drive the adaptiveness of cooperation in these particularly harsh climates, however, remain an area of ongoing study. Because this topic lies at the interface of thermal physiology and social behavior, there is a gap in our understanding of potential mediators that span both fields. In this study we test for the first time whether cooperation affects individual to group thermal tolerance. By exploring the potential direct link between social strategy, climatic conditions, and thermal performance, we elucidate previously unknown interactions between climate and cooperation.

Because of the established link between sociality and climate, changes to the adaptiveness of social strategies are a predicted consequence of climate change (Schürch et al. 2016). As such, investigation of the social dimensions of animal thermal tolerance is vital for understanding and predicting global species distributions. Social organisms comprise some of the most successful and abundant animal lineages on earth. Eusocial insects in particular comprise a tremendous portion of global animal biomass, including some of the most ecologically and agriculturally important pollinators, ecosystem engineers, and invasive species (Wilson 1971; Hölldobler and Wilson 1990; Folgarait

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1998; Holway et al. 2002; Thomson 2004; Slaa et al. 2006; Requier et al. 2019). Elucidating widespread patterns in functional thermal limitations of social insects can therefore inform conservation and land management decisions in the face of a changing climate. Ants are particularly tractable models for this type of work because they are highly social, globally abundant, and, as small-bodied poikilotherms, highly affected by environmental temperatures (Dunn et al. 2009; Diamond et al. 2012, 2016; Diamond and Chick 2017; Penick et al. 2017; Yilmaz et al. 2019). Unlike individual organisms, social insect colonies can either benefit from or be constrained by the distributions of thermal capacities across colony members. This effect of within-group variation is not captured in most studies of thermal tolerance in social species. Within a single colony, thermal tolerances of workers can vary widely, and the distribution of this within-group variation is important for accurate assessment of accessible thermal environments at the colony level (Cerdá and Retana 1997, 2000; Baudier et al. 2015; Baudier and O'Donnell 2017). Here we investigate whether polygyny (colonies with multiple queens) is a factor affecting the distribution of worker thermal tolerances within the colony.

Eusocial insect colonies vary in queen number both among and within species (Thorne 1982, 1984; Hölldobler and Wilson 1990; Cameron and Jost 1998). The presence of multiple, usually unrelated, reproductives is known as polygyny and can impact genetic and behavioral variation among colony members (Herbers 1986; Hölldobler and Wilson 1990). In ants, polygyny appears to be particularly associated with colony survival in climatically harsh environments, such as in deserts (Cahan 2001; Johnson 2004; Haney and Fewell 2018), at high latitudes (Heinze 1993; Heinze and Hölldobler 1994), or at high altitudes (Heinze and Rueppell 2014), where colony or foundress mortality risk is heightened. Many successful exotic ant species are also polygynous in their introduced ranges, in which ants may experience different environmental conditions than they would in their native environments (Mescher et al. 2003; Tsutsui and Suarez 2003).

Previous studies aiming to elucidate the underlying causes of this climate-dependent benefit to polygyny have focused on colony growth and division of labor under harsh or unpredictable conditions. For instance, colonies with cooperative foundresses can grow more rapidly and with decreased founding mortality risk (Mintzer 1987; Vargo and Fletcher 1989; Deslippe and Savolainen 1995; Johnson 2004; Clark and Fewell 2014). However, these harsh environments—deserts, urban landscapes, high-latitude habitats, and high-elevation habitats—are also in many cases more thermally extreme. We tested whether and how these nuanced social strategies directly affect within-colony thermal tolerance distributions, another possible factor selecting for polygyny in harsh environments.

Because of the established association between polygyny and climatic extremes, we hypothesized that colonies with multiple queens have members with greater thermal tolerances. However, what form might worker variation in thermal tolerance take for polygynous colonies living in harsh environments? Mounting theory suggests both disadvantages and advantages of within-colony thermal tolerance variation in thermally extreme envi-

ronments. First, there is empirical evidence of selection against high interworker variation in thermal tolerance for species and populations adapted to more extreme thermal environments (independent of queen number). In these cases, the “best” colony strategy may not be diversity in worker thermal tolerances but instead production of a set of workers that have similarly high thermal tolerances, enabling them to function simultaneously and coordinately in the same set of relatively extreme thermal environments. For instance, worker thermal tolerances in above-ground army ant colonies are more extreme and less variable than those of subterranean army ant colonies, the result of higher selective pressure against more thermally vulnerable phenotypes within the colony, as these most negatively impact group performance in challenging thermal environments (Baudier et al. 2015, 2018). A similar pattern of selection against the most thermally vulnerable colony members can be seen in leaf-cutting ant populations (*Atta cephalotes*) that inhabit thermally extreme low-elevation rain shadows compared with more moderate climates of windward mountainsides and mountaintops (Baudier and O'Donnell 2020). In both cases, selection against the weakest links (the most performance-limiting individuals) caused an asymmetric reduction in tolerance variation in environments with more extreme temperatures (Baudier and O'Donnell 2017), environments similar to those that select for polygyny. Under this weak link hypothesis, we anticipated that part of the adaptiveness of polygyny in climatically extreme environments is that it causes a similarly beneficial set of uniformly robust thermal tolerances across the worker force.

However, that there are benefits to lower worker tolerance diversity may also seem at odds with a major paradigm in social behavior, namely, that variation in functional attributes among workers can be beneficial for achieving stable colony-level functions, such as nest site selection (Hui and Pinter-Wollman 2014), brood thermoregulation (Jones et al. 2004), and efficient allocation of workers to foraging and/or defense (Cole et al. 2010; Modlmeier and Foitzik 2011). Consistent with this but in the context of thermal tolerance, studies of desert ants in the genus *Cataglyphis* suggest that workers foraging in competitive, high-heat environments may benefit from having workers with bimodal thermal tolerances, enabling specialization on foraging at different times of day (Cerdá and Retana 1997, 2000). Under this beneficial tolerance diversity hypothesis, we might expect polygynous colonies in harsh environments to have higher thermal tolerance variation within a colony than monogynous colonies in similar environments.

Another, more proximate reason to expect higher tolerance diversity in polygynous colonies is that colonies with multiple queens are predicted to be more genetically diverse than those with a single queen because of increases in both matriline and patriline with each additional queen that cooperatively founds a nest in this highly polyandrous species (Overson et al. 2016). Polygyny and polyandry in ants at large have been shown to increase colony genetic and phenotypic diversity, allowing colonies to adaptively respond to environmental challenges (Mattila and Seeley 2007; Oldroyd and Fewell 2007, 2008; Wiernasz et al. 2008). However, the degree to which thermal tolerance as a

functional trait is predicted by matrilineal or patrilineal descent is unclear.

Intraspecific comparisons of species that vary in their expression of cooperative behavior provide valuable alternatives to biogeographic comparative studies by enabling direct comparison of sympatric social and solitary strategies, thus controlling for environmental context (Eickwort et al. 1996; Hirata and Higashi 2008; Sheehan et al. 2015). We used the facultatively polygynous desert ant *Pogonomyrmex californicus* to examine the effects of queen number on thermal tolerance distributions within colonies. Across its range in arid regions of southwestern North America, certain populations of this species display primary polygyny, in which nonkin queens found nests cooperatively and cohabit throughout the life of the colony (Johnson 2004; Overson et al. 2016; Shaffer et al. 2016; Haney and Fewell 2018). Despite being relatively thermophilic, harvester ants inhabiting these arid regions are often challenged by midday high temperatures that can limit diurnal foraging (MacKay and MacKay 1989; Cole et al. 2010; Friedman et al. 2019). We reared *P. californicus* ant colonies founded by single queens or groups of queens to assess the effects of polygyny on thermal tolerance. Using standard dynamic thermal ramping assays (Lutterschmidt and Hutchison 1997), we estimated the upper thermal limits of queens and workers. We then used environmental temperatures to estimate the impact that these differences in heat tolerance have on worker foraging potential in the present environment. In doing so, we assessed potential long-term contributions of queen cooperation to adaptive worker thermal phenotypes and predict effects of changing climate on global distributions of this behavioral phenotype.

Material and Methods

Foundress Treatments and Colony Rearing

On June 24 and 25, 2018, we collected newly mated *Pogonomyrmex californicus* foundresses before nest excavation from a population previously characterized as predominantly polygynous (Johnson 2004; Overson et al. 2014; Haney and Fewell 2018) in Pine Valley, San Diego County, California (32.822°N, 116.529°W; elevation: 1,136 m). Colonies in this population naturally range from one to eight queens, with an average near four (Overson et al. 2016; Haney and Fewell 2018). Within 36 h of collection, we established foundresses in artificial nests consisting of two plastic chambers (diameter: 9 cm; height: 3.5 cm) connected by vinyl tubing (as in Ostwald et al. 2021). One closed chamber simulated a nest environment, in which foundresses laid brood. The second chamber was open on top and simulated a foraging arena, in which we provided ad lib. food in the form of Kentucky bluegrass and sesame seeds (1:1 by volume), fruit flies (*Drosophila melanogaster*), and agar-based ant diet (Bhatkar and Whitcomb 1970). Collected foundresses were randomly assigned to one of three treatment groups with a single foundress, paired foundresses, or foundresses in groups of four. We reared 30 colonies of each queen number treatment for 10.5 mo in the lab, kept at a steady temperature between 29°C and 31°C. At the end

of 10.5 mo, workers and queens were weighed live, then subjected to a heat tolerance assay.

At the end of 10.5 mo, 36 colonies (40%) had at least one surviving queen, and the average worker number (\pm SE) for colonies without queen death was 28.3 ± 4.4 ants. High foundress mortality is typical in both the field and the lab, particularly for smaller, semiclaustal harvester ants like *P. californicus* (Wiernasz and Cole 2003; Johnson 2004; Enzmann et al. 2014). While there are no published estimates for expected survival at 10.5 mo, in this study queen survival between 49 and 56 d exceeded that of two previous studies that used similar rearing methods (table S1).

Because it is difficult to assign a queen number to a colony in which a queen died at some time point throughout the rearing period, we analyzed tolerance data only from colonies with no queen mortality ($N = 3$ one-queen colonies, $N = 5$ two-queen colonies, $N = 7$ four-queen colonies). The number of workers at 10.5 mo in colonies without queen death varied from 8 to 76. This wide variation in subject colony size is similar to that reported by Holbrook et al. (2011) in 12-mo-old colonies, suggesting that variability in colony size at this stage was typical as well. For further details on growth, mortality, and division of labor in these subject colonies, see Ostwald et al. (2021).

Heat Tolerance (CT_{max}) Assays

We used a standard dynamic method for estimating maximum critical temperature (CT_{max}) of each ant (Lutterschmidt and Hutchison 1997). Ants were placed individually in 1.5-mL microcentrifuge tubes situated in Thermal-Lok dry heat blocks (USA Scientific). Cotton in the top one-third of each tube prevented thermal refuge in the cap. We preheated blocks to 32°C. Temperature was increased at a rate of 1°C every 10 min. Ants were checked for loss of mobility at the end of each 10-min interval. Tubes containing ants not visibly moving were lightly tapped to elicit movement response. The highest temperature at which mobility was retained was considered each ant's CT_{max} .

In each subject colony, all queens and workers were assayed. Because of limited space within heat blocks, the assay period lasted approximately 6 wk. However, colony order was randomized across queen number treatments to prevent directional discrepancies in final colony age across treatment groups. We noted whether each ant was visibly callow (recently eclosed, determined by pale integument) at the time of the assay. Together, this enabled the evaluation of whether two common covariates of individual critical temperatures, ant mass and age (Oberg et al. 2012; Baudier and O'Donnell 2016), explained patterns in CT_{max} with respect to queen number.

Environmental Temperatures

In very sunny environments, ants forage in boundary layer microclimates created by superheated substrate surfaces (Kaspari et al. 2015). Soil surface temperature is therefore an ecologically relevant metric to compare with upper critical temperatures. In June 2015 we placed three iButton (Maxim Integrated, San Jose,

CA) temperature loggers in the northern, southern, and central portions of the Pine Valley meadow from which the foundresses were collected (fig. S1) to record soil surface temperatures experienced by *P. californicus* in the summer, when temperatures are highest. We wrapped iButtons in thin plastic bags and buried them 5 cm underground in areas with light vegetation, near where *P. californicus* colonies were abundant. Burying iButtons in the upper layers of soil is standard practice when using these devices to estimate ground temperature (Basurto-Lozada et al. 2020). Distance between any two iButtons was greater than 185 m. The iButtons recorded temperatures every 20 min continuously from midnight on July 5 to 2340 hours on July 26. This yielded 4,752 temperature logs across all days and iButtons.

Analyses

All analyses were performed in R version 4.0.0 (R Core Team 2020). To test for differences in magnitude of CT_{max} according to queen number, we fitted a linear mixed effects model using the lmer function (Bates et al. 2015). This model included queen number, worker mass, the interaction between worker mass and queen number, and whether the worker was callow as fixed predictors, with colony included as a random factor and transformed CT_{max} (TCT_{max}) included as the response variable. Factor significance was assessed using a type II Wald χ^2 test. CT_{max} was transformed to improve normality and homoscedasticity using the equation $TCT_{max} = \log_{10}(-CT_{max} + \text{maximum } CT_{max} + 1)$, where maximum CT_{max} was the highest collected CT_{max} in the study.

We compared worker variation in CT_{max} across colonies with different queen numbers by using pairwise Brown-Forsythe tests for heteroscedasticity, with *P* values adjusted to account for multiple comparisons using the Benjamini-Hochberg method (Brown and Forsythe 1974; Benjamini and Hochberg 1995). As an additional group-level test of how CT_{max} variation differed according to queen number, we regressed colony standard deviation of CT_{max} against queen number.

We further tested whether differences in worker size, age, and number were mechanistic explanators of polygyny-associated differences in CT_{max} . To test whether worker mass varied with queen number, we fitted a linear mixed effects model with queen number as a fixed predictor variable, colony ID as a random factor, and worker mass as a response variable, then tested for significance of predictors using a type II Wald χ^2 test. We used a Pearson's χ^2 test to determine whether the portion of callow workers differed across queen treatment groups. To test whether total number of workers per colony differed according to queen treatment, we regressed total colony worker number against queen number.

To test for possible predictors of queen thermal tolerance, we also fitted a linear model with queen CT_{max} as a response variable and queen mass and queen number as predictor variables, then tested significance of predictor variables using a type II test ($N = 41$ queens). We used a Wilcoxon rank sum test with continuity correction to compare worker and queen CT_{max} ($N = 41$ queens, $N = 424$ workers).

We used a subset of daylight temperatures (0540–2000 hours) to estimate the percentage of diurnal temperature logs that did not exceed the CT_{max} of each worker ant (henceforth, “tolerable diurnal temperatures”). Because data were nonparametric, we compared percentage of tolerable diurnal temperatures across worker groups with different queen numbers using two separate analyses: (1) a Kruskal-Wallis test followed by a post hoc Wilcoxon rank sum test with Benjamini-Hochberg correction and (2) a Cox proportional hazard survival analysis that included colony as a random factor.

Results

CT_{max} according to Queen Number

Colonies with more queens had workers with significantly higher CT_{max} (table 1; fig. 1A). Variation in worker CT_{max} decreased with increasing queen number according to Brown-Forsythe tests of homoscedasticity ($F_{2, 169.0} = 6.64$, $P = 0.002$; table 2; fig. 1A) and according to a regression of colony-level standard deviation in CT_{max} versus queen number ($F = 4.94$, $df = 13$, $R^2 = 0.220$, $P = 0.045$; fig. 1B). This reduction in heat tolerance variation with increasing queen number occurred predominantly from decreased incidence of especially low CT_{max} values. As such, mean colony CT_{max} and colony standard deviation in CT_{max} showed a strong negative relationship that was significantly curvilinear (odds ratio test: $F = 31.55$, $P < 0.001$; linear model AIC = 18.17, quadratic model AIC = 0.84; quadratic formula: $y = 48.35 + 0.89x - 0.58x^2$; $F_{2, 12} = 151.5$, $R^2 = 0.956$, $P < 0.001$; fig. 2A) and with four-queen colonies occupying the less variable and higher mean end of this curve.

Effect of Queen Number on CT_{max} Is Not Explained by Mass, Age, or Colony Size

Individual worker mass did not explain the effects of queen number on CT_{max} . Worker CT_{max} increased weakly with body mass, but the slope was unaffected by queen number (table 1; fig. 3). Individual worker mass also did not differ with queen number ($\chi^2 = 1.99$, $df = 1$, $P = 0.159$). Queens were larger bodied than workers and were also more heat tolerant ($W = 14, 258$, $P < 0.001$; fig. 3B). However, variation in queen mass was not predictive of queen CT_{max} (table 3).

Table 1: Mixed effects analysis testing factors in worker heat tolerance

Fixed factor	χ^2	df	<i>P</i>
Queen number	5.3629	1	.021*
Mass	4.3955	1	.036*
Age	2.4557	1	.117
Queen number : mass	.1572	1	.692

Note. Output of type II Wald χ^2 test on a fitted mixed effects model of structure $TCT_{max} \sim \text{queen number} \times \text{mass} + \text{age} + (1|\text{colony})$. TCT_{max} = transformed maximum critical temperature.

*Significant ($\alpha = 0.05$).

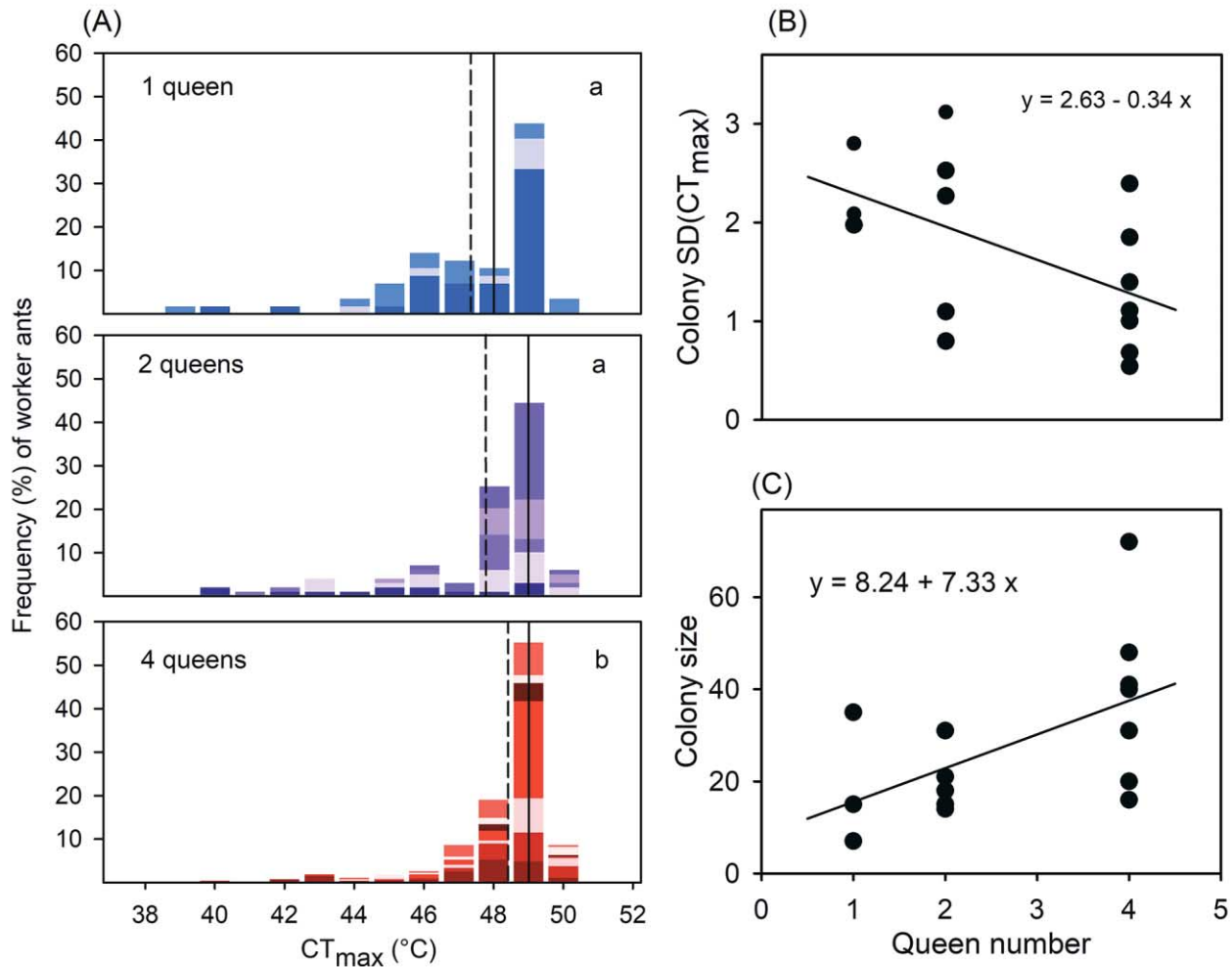


Figure 1. Variation in maximum critical temperature (CT_{max}) decreased as queen number increased. A, Stacked histograms of worker ant frequencies across CT_{max} values for each queen number treatment group ($N = 424$ worker ants). Different tones within each histogram represent different colonies within each treatment ($N = 3$ one-queen colonies, $N = 5$ two-queen colonies, $N = 7$ four-queen colonies). The solid line indicates the treatment median, and the dashed line indicates the treatment average. Lowercase letters in the top right of each histogram signify results of pairwise Brown-Forsyth tests of homoscedasticity, with adjusted P values for multiple comparisons (summarized in table 2). B, Significant decrease in colony standard deviation of CT_{max} as queen number increased ($N = 15$ colonies). C, Significant increase in colony size as queen number increased ($N = 15$ colonies; $\alpha = 0.05$).

Differences in worker CT_{max} among queen treatment groups were also unrelated to the presence of callow workers (young workers with pale integument). Callow workers did not significantly differ from mature workers in CT_{max} (table 1; fig. 3B), but even if they had, only 7.9% of all workers were callow at the time of assay, a proportion that did not differ with increasing queen number (Pearson's χ^2 test: $\chi^2 = 0.79$, $df = 2$, $P = 0.675$). Differences in CT_{max} variation across colonies with different queen number additionally do not appear to be the result of differences in colony size (worker number). Although colonies with more queens had significantly more workers at the end of the rearing period (linear regression: $F = 5.69$, $df = 13$, $R^2 = 0.304$, $P = 0.033$; fig. 1B), colony size was not a significant predictor of colony standard deviation in CT_{max} (linear regression: $F = 2.65$, $df = 13$, $R^2 = 0.105$, $P = 0.128$; fig. 2B) or mean colony CT_{max} (linear regression: $F = 2.18$, $df = 13$, $R^2 = 0.078$, $P = 0.163$; fig. 2C).

Tolerable Diurnal Temperatures

Average ground temperature (\pm SD) for July in our field site was 28.80°C ($\pm 9.13^\circ\text{C}$), with a range across days and probes of 12.60°C – 54.53°C . Maximum temperatures surpassed the tolerance

Table 2: Pairwise Brown-Forsyth tests of worker maximum critical temperature homoscedasticity

Contrast	Statistic	df	Raw P	Adjusted P
1 vs. 2 queens	.740	114.06	.391	.391
1 vs. 4 queens	9.183	66.76	.003	.010*
2 vs. 4 queens	6.938	132.98	.009	.014*

Note. Adjusted P values in pairwise Brown-Forsyth tests of homoscedasticity across queen number treatments account for multiple comparisons using the Benjamini-Hochberg method.

*Significant ($\alpha = 0.05$).

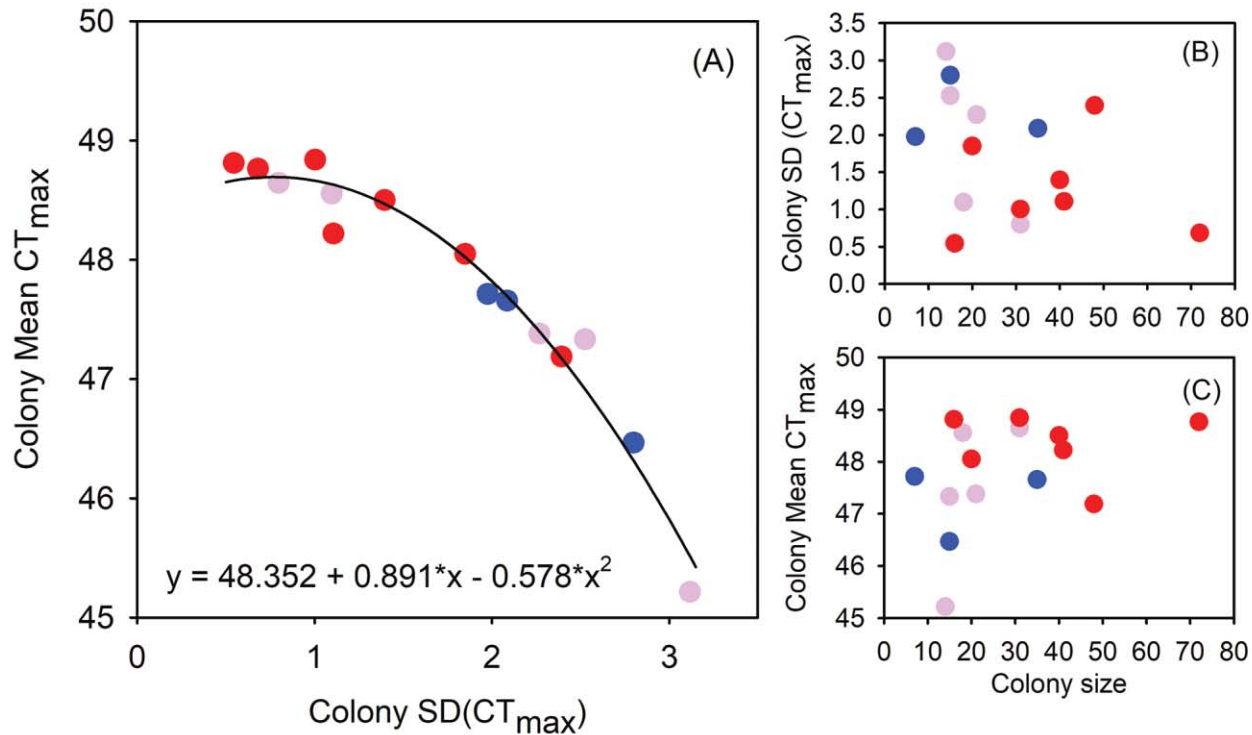


Figure 2. Colony-level thermal tolerance patterns ($N = 15$ colonies). *A*, Curvilinear negative relationship between mean colony maximum critical temperature (CT_{max}) and within-colony CT_{max} variation. The asymmetry of individual tolerance variation appears to underlie this relationship, explaining both inter- and intratreatment variation in colony average performance. *B*, Nonsignificance of colony size as a predictor of standard deviation of CT_{max} . *C*, Nonsignificance of colony size as a predictor of colony mean CT_{max} . In all panels, point color indicates queen number of each colony (red indicates four queens, light purple indicates two queens, and blue indicates one queen), and $\alpha = 0.05$.

of all ants in this study, but average midday temperatures exceeded the heat tolerance of only the ants with the lowest CT_{max} (fig. 4). Percentage of tolerable diurnal temperatures (calculated for each ant, equal to the percentage of diurnal temperatures that did not exceed each ant's CT_{max}) was higher in multiqueen colonies (Kruskal-Wallis: $\chi^2 = 13.98$, $df = 2$, $P = 0.001$; fig. 4B). Workers in four-queen colonies had higher percentages of tolerable diurnal temperatures compared with two-queen workers ($P = 0.018$) or one-queen workers ($P = 0.003$), though one-queen and two-queen workers were not significantly different ($P = 0.304$). In survival analyses (fig. 4C), hazard nonproportionality (assessed by Schoenfeld residuals) motivated separate assessments of survival below and above the 95th percentile of diurnal temperatures. At temperatures less than or equal to the 95th percentile, survival was significantly lower for workers from one-queen colonies than for four-queen workers ($z = -1.98$, $P = 0.047$) but not two-queen workers ($z = -0.98$, $P = 0.330$). Above the 95th percentile of environmental temperature (representative of the hottest midday temperatures recorded in the study), workers from all queen treatments had equally low probability of survival ($P < 0.05$).

Discussion

In this study we explored the thermal performance consequences of nonkin cooperation (primary polygyny) in members of a

cooperative society. We asked whether polygynous ant colonies containing multiple unrelated queens benefit from improved heat tolerance in thermally harsh environments. The advantages of polygyny are often linked to worker diversity (reviewed by Oldroyd and Fewell 2007). However, while we do report that increasing queen number coincided with greater worker heat tolerance, we show no evidence of increased variation in worker heat tolerance within multiqueen colonies. Rather, worker heat tolerance was both greater and less variant in colonies with more queens. Specifically, colonies with more queens had fewer extremely heat-intolerant workers.

When comparing thermal tolerances to environmental temperatures, we found that the most sensitive workers were those whose thermal tolerances would be challenged by common midday summer temperatures. Worker ants in multiqueen colonies therefore had higher portions of the day when they could be active outside of the nest, suggesting that they would have had higher potential colony productivity via greater possible foraging ant-hours than single-queen colonies under recorded conditions. In addition to benefits from foraging period length, colonies more capable of midday foraging may also face less competition with behaviorally dominant but lower-tolerance competitors, as reported in the desert ant *Cataglyphis cursor* (Cerdá et al. 1997). These benefits offer a new and potentially important example of the adaptiveness of social cooperation in harsh environments.

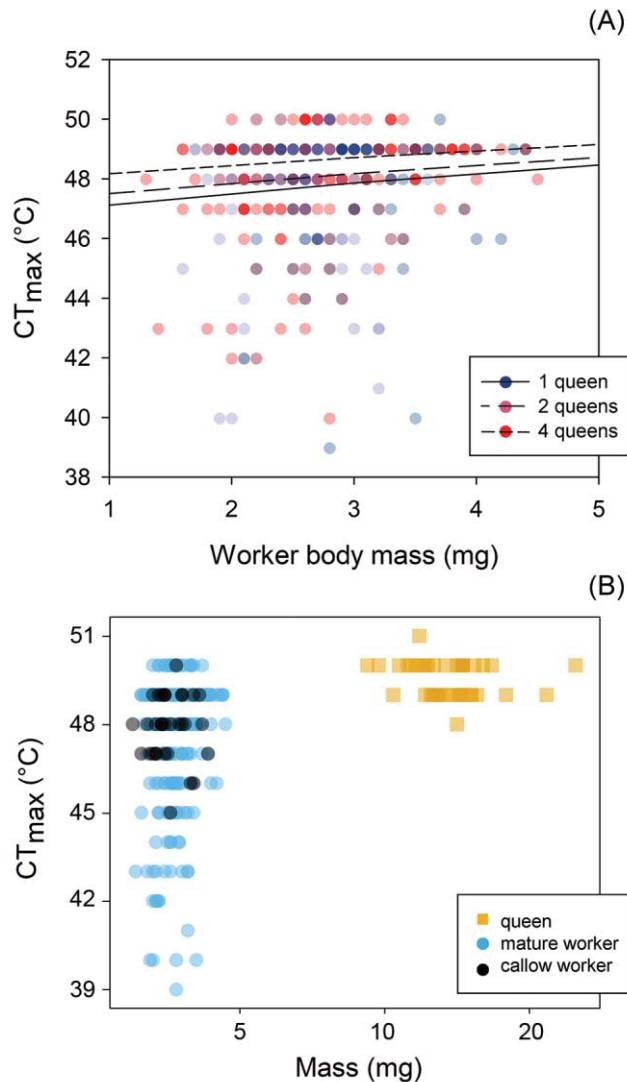


Figure 3. A, Relationship between mass and maximum critical temperature (CT_{max}) across worker ants ($N = 424$) in treatment groups with different numbers of queens. Trend lines for treatment groups with different queen numbers did not differ in slope but significantly differed in intercept (table 1). B, Thermal tolerances according to body size, reproductive caste, and age. Black circles represent callow (young) workers with pale integument ($N = 34$). Blue circles represent mature (non-callow) workers ($N = 390$). Orange squares represent queens ($N = 41$). Workers were significantly less heat tolerant than queens, but whether a worker was callow did not affect their CT_{max} . In both panels, symbol opacity is 50% to improve visibility of overlapping data points.

Our findings offer a new example in which elimination or alteration of predominantly the most thermally sensitive weak link phenotypes within a highly cooperative social group can lead to adaptive outcomes in thermally extreme environments (Baudier and O'Donnell 2017).

Potential Pattern Drivers

Despite the evidence supporting this pattern, the question of how multiqueen colonies achieve less variant and more robust

worker heat tolerance remains. Through the course of this study, we tested and found no support for several intuitive mechanistic hypotheses. Namely, colony differences in worker age (assessed by integument color), worker body size, and total worker number did not appear to drive the effect of queen number on heat tolerance. The absence of a colony size effect on mean or variation in CT_{max} is also evidence against the idea that colony-size-associated benefits lead to more heat-tolerant workers. For example, it did not appear that bigger colonies with more nurses had better brood care leading to higher thermal tolerances.

We also found no evidence at the colony level that patterns between queen number and thermal tolerance were explained by worker genetic variation. Among social hymenopterans, within-colony genetic variation can be generated by the presence of multiple matrilines (due to polygyny) or patriline (due to polyandry; Keller and Reeve 1994). Queens of *Pogonomyrmex californicus* in Southern California are estimated to mate with about eight males each (regardless of whether they cooperatively found), and when founding nests together, they show multiple matriline contributions to worker production (Overson et al. 2016). Because we collected founding queens that had mated and assigned them randomly to queen number treatment groups, patriline number per queen should not have differed directionally across treatments. As such, worker genetic variation was almost certainly higher in the four-queen colonies. Despite this, we observed significantly higher functional trait variation among single-queen colonies, where worker genetic diversity is expected to have been the lowest. This suggests that thermal tolerance variation among colony members is generated largely by factors other than patriline or matriline.

These results have stimulated the formation of two main mechanistic hypotheses that may underlie a reduction in thermally vulnerable workers in multiqueen colonies. First, the strain of founding nests solitarily may have had lasting energetic effects on the queens themselves, leading to lower-quality (low- CT_{max}) offspring production. Our experimental design sought to reduce these costs. Foundresses were presented with nest chambers and proximate food sources, so they did not dig or gather distant seeds. However, solitary foundresses were alone in performing essential functions of reproduction, brood care, and processing seeds before the emergence of the first workers (Ostwald et al. 2021). As such, we cannot rule out this possible explanation.

Second, it is also possible that polygynous colonies, which produce more eggs in total, can more selectively regulate brood care to eliminate lower-quality individuals during development. In another lab study of this species, colonies with two queens were found to produce a lower proportion of adults (workers) relative

Table 3: Factors in queen heat tolerance

Factor	F	df	P
Queen number	.5108	1	.4792
Mass	1.3797	1	.2475

Note. Output of a type II test on a fitted linear model of structure $CT_{max} \sim \text{mass} + \text{queen number}$. CT_{max} = maximum critical temperature.

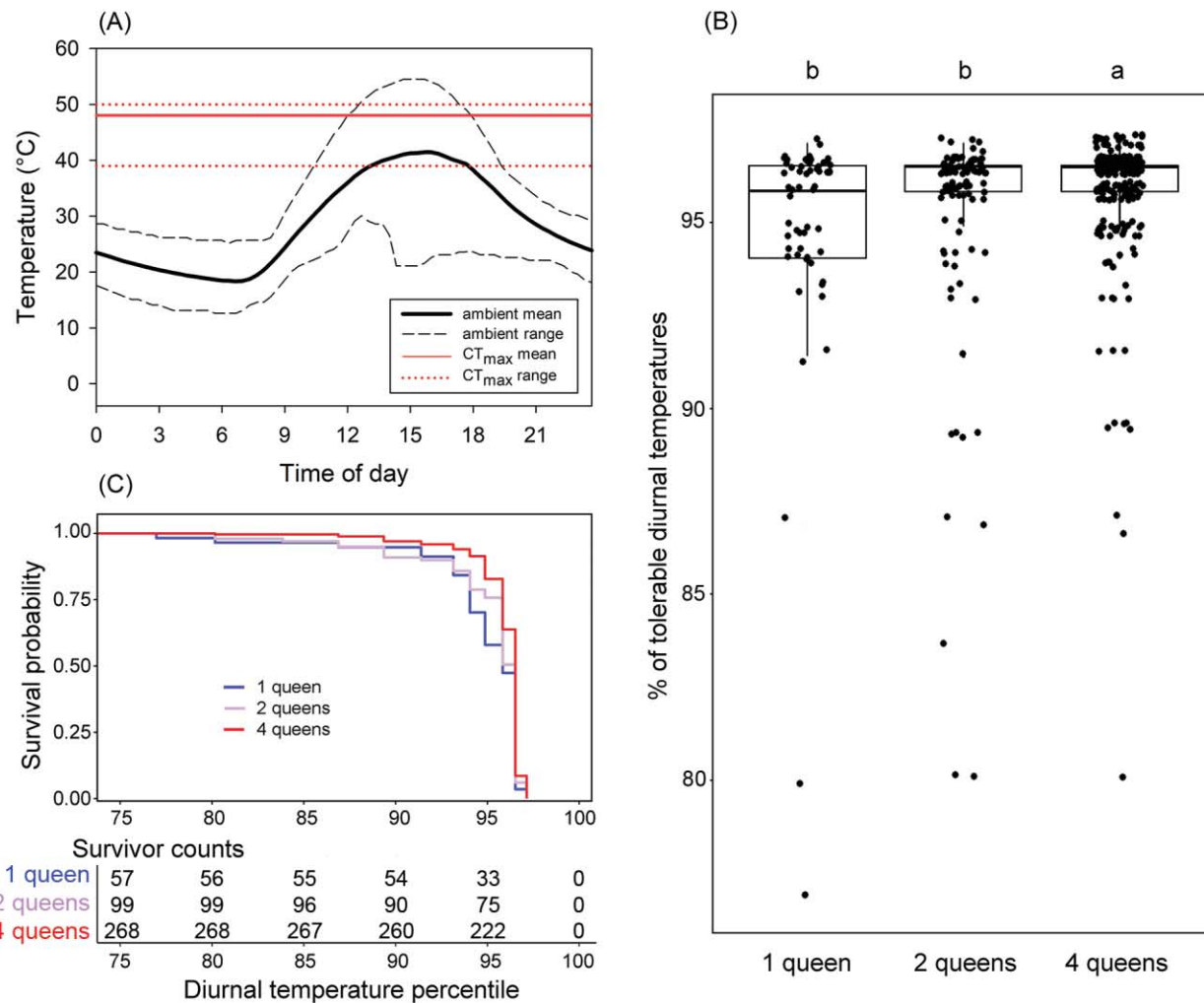


Figure 4. Proximity of ground temperatures to worker maximum critical temperature (CT_{max}). $N = 4,572$ temperature logs. $N = 424$ for worker ant CT_{max} values. *A*, Although maximum recorded midday summer temperatures would have been limiting to all of the ants assayed in this study, average midday ground temperatures exceeded CT_{max} of only very heat-sensitive (low- CT_{max}) workers. *B*, Percentage of diurnal ground temperatures tolerable by each worker (those not exceeding each ant's CT_{max}) across queen treatment groups. Inner and outer quartiles are represented by boxes and whiskers, with median values shown as bold lines. Lowercase letters denote results of post hoc Wilcoxon rank sum tests. *C*, Survival of ants across recorded environmental temperatures (reported as percentiles). Cox proportional hazard analysis revealed higher survival probability of workers from four-queen colonies in these environments.

to per capita egg production during colony founding than did single-queen colonies, suggesting social regulation of brood production (Clark and Fewell 2014). In these lab colonies, eggs and young larvae are regularly cannibalized, often by other larvae (Clark and Fewell 2014; J. H. Fewell and R. M. Clark, personal observation). Brood cannibalism is common in ant colonies in general (Bourke and Franks 1995) and can be used to selectively regulate brood production around nutrition (Nonacs 1991). Brood differentiation in culling can occur on the basis of variation in chemical cues (Schultner and Pulliainen 2020) and even by genotype (Clark et al. 2006; Schultner et al. 2013). However, whether this underexamined context for selective regulation of worker phenotypes is responsible for the contrasts we observe remains to be directly tested.

Implications

Potential mechanisms aside, the existence of a connection between queen number and worker thermal physiology raises questions about the adaptiveness of polygyny across past, current, and future thermal regimes. We report that colonies with more queens produced more thermally tolerant workers that perform better under current extreme summer temperatures. A recent study suggests that these multiqueen founding strategies dominate more southerly populations such as the one sampled here (Overson et al. 2016). We predict that hotter summer temperatures associated with climate change would select for higher incidences of polygyny in our focal population, potentially increasing average queen number for this population. Whether and how climate

change will drive shifts in the distributions of polygynous populations within the range of *P. californicus* remains an area meriting future biogeographic study.

Polygyny is a trait shared by most invasive ant species (Passera 1994; Tsutsui and Suarez 2003), as well as some species that are highly successful in urban heat island environments (Menke et al. 2010). Whether higher worker heat tolerances associated with increased queen numbers are an important part of the success of these species remains an intriguing direction for future study, as is the generalizability of these trends across different forms of polygyny. Still more is to be learned about the potential effects of polygyny on variation in cold tolerance.

There are high fitness costs associated with nonkin cooperation under favorable and stable environmental conditions, but in harsh environments cooperation can afford social groups higher rates of growth and improved odds of survival, offsetting these costs (Arnold and Owens 1999; Jetz and Rubenstein 2011; Lukas and Clutton-Brock 2017; Groom and Rehan 2018). Polygyny in ants is one well-studied example of this (Suarez and Goodisman 2021; Ostwald et al. 2022). We suggest that robust polygynous worker thermal tolerance can be one such offsetting factor in harsh environmental contexts. This then enables the consideration of environmental temperatures experienced by workers as a potential factor affecting the context-dependent adaptiveness of polygyny. As such, future research investigating the link between biogeographic contrasts in environmental temperatures and tolerance-associated incidence of multiple-queen founding has the potential to expand our understanding of when and why nonkin cooperation is adaptive.

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