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ORIGINAL PAPER

Body mass and cuticular hydrocarbon profiles, but not queen number, underlie worker desiccation resistance in a facultatively polygynous harvester ant (*Pogonomyrmex californicus*)

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Abstract

As small-bodied terrestrial organisms, insects face severe desiccation risks in arid environments, and these risks are increasing under climate change. Here, we investigate the physiological, chemical, and behavioral mechanisms by which harvester ants, one of the most abundant arid-adapted insect groups, cope with desiccating environmental conditions. We aimed to understand how body size, cuticular hydrocarbon profiles, and queen number impact worker desiccation resistance in the facultatively polygynous harvester ant, *Pogonomyrmex californicus*. We measured survival at 0% humidity of field-collected worker ants sourced from three closely situated populations within a semi-arid region of southern California. These populations vary in queen number, with one population dominated by multi-queen colonies (primary polygyny), one population dominated by single-queen colonies, and one containing an even mix of single- and multi-queen colonies. We found no effect of population on worker survival in desiccation assays, suggesting that queen number does not influence colony desiccation resistance. Across populations, however, body mass and cuticular hydrocarbon profiles significantly predicted desiccation resistance. Larger-bodied workers survived longer in desiccation assays, emphasizing the importance of reduced surface area-to-volume ratios in maintaining water balance. Additionally, we observed a positive relationship between desiccation resistance and the abundance of *n*-alkanes, supporting previous work that has linked these high-melting point compounds to improved body water conservation. Together, these results contribute to an emerging model explaining the physiological mechanisms of desiccation resistance in insects.

Keywords Pleometrosis · Water balance · *n*-alkanes · Gas chromatography

Introduction

Maintaining water balance is a major homeostatic challenge shared by all organisms. As small-bodied terrestrial organisms, insects face particular water balance challenges due to their larger body surface area to volume ratios (Hadley 1994; Kühsel et al. 2017). Despite the critical ecological value of insects in terrestrial ecosystems, the mechanisms by which they resist desiccation remain underexplored. As a complex functional trait, desiccation resistance is shaped by interactions between phylogeny, environment, and life history traits (Chung and Carroll 2015; Hadley 1994; Sprenger et al. 2018; Wang et al. 2021). Here, we experimentally link major physiological and colony characteristics of harvester ants, a common arid-adapted insect, to their survival outcomes under low humidity conditions, with the aim of disentangling the physiochemical and behavioral mechanisms governing insect desiccation resistance.

The capacity to withstand desiccating conditions can vary widely among and within species (Bujan et al. 2016; Hadley 1994; Hoffmann and Harshman 1999). Animals living in arid environments, in particular, may reduce water loss through behavioral and/or morphological adaptations, for example,

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by restricting activity to the coolest times of day (Schultheiss and Nooten 2013), or by exploiting body morphologies that reduce their surface area-to-volume ratio (Hood and Tschinkel 1990; Prange and Pinshow 1994). Larger-bodied organisms not only have a reduced surface area, they also may have proportionally larger water reserves, additionally buffering against desiccation (Lighton et al. 1994; Prange and Pinshow 1994). Positive relationships between body size and desiccation resistance have been demonstrated across insect taxa (Chown and Klok 2003; Fouet et al. 2012; Kærsgaard et al. 2004; Le Lagadec et al. 1998) suggesting potential selective pressures for increased body size in insects experiencing water conservation challenges.

Other physiological adaptations to resist desiccation are determined by the chemical composition of the insects' epicuticle, which consists predominantly of structurally diverse lipids known as cuticular hydrocarbons (CHCs). This waxy barrier serves as the primary interface through which insects exchange water with the environment (Blomquist and Bagnères 2010). The chemical composition of CHC profiles can vary considerably among species and populations, as do their waterproofing capabilities (Blomquist and Bagnères 2010; Sprenger and Menzel 2020). CHCs generally protect against water loss below a certain critical temperature, which is determined by the chemical structure of individual CHC compounds (Gibbs 1998, 2002). Generally, molecules with longer chain lengths, fewer methyl groups, and fewer double bonds have higher melting points (Gibbs and Pomonis 1995). Several studies have associated particular CHC signatures with desiccation resistance (especially linear alkanes; Chung 2014; Chung and Carroll 2015; Ferveur et al. 2018; Whyte et al. unpub.) or with adaptation to arid environments (Buellesbach et al. 2018; Menzel et al. 2018; Sprenger et al. 2018), though these patterns appear to vary considerably across taxa.

Seed harvester ants represent the numerically dominant animals in arid ecosystems globally (Brown et al. 1979; La Richelière et al. 2022), and thus hold particular promise for illuminating the adaptive and physiological mechanisms underlying desiccation resistance. One such seed harvester, *Pogonomyrmex californicus* (Formicidae: Myrmicinae), is abundant in arid to semi-arid environments including deserts, grasslands, and scrublands of southwestern North America. This species has served as a valuable model for studying the evolution of cooperation due to its intraspecific variation in queen social strategy (i.e., cooperative/primary polygynous vs. non-cooperative/monogynous); (Cahan and Fewell 2004; Clark and Fewell 2014; Haney and Fewell 2018; Overson et al. 2014, 2016; Shaffer et al. 2016). Polygyny has the potential to interact with worker and colony-level functional traits (Greenberg et al. 1985; Haney and Fewell 2018; Hölldobler and Wilson 1990; Pisarski 1981), potentially by increasing worker genetic diversity or by mediating offspring quality, though this relationship has rarely been examined for physiological traits. A recent study showed that multi-queen colonies of *P. californicus* produced workers with higher and more uniform heat tolerance (Baudier et al. 2022), raising questions about implications for desiccation resistance, which interacts with thermal tolerance in complex and variable ways not yet fully elucidated (Bujan et al. 2016; Klok et al. 2006; Renault et al. 2005), Importantly, the relationship between nest founding strategy (polygynous vs. monogynous) and worker desiccation resistance has not been previously studied. Despite the prominence of this model system, the chemical composition of *P. californicus*' CHC profile has also not yet been formally described. Likewise, despite suggestions in other harvester ants that water may be limiting during colony founding (Johnson 2000, 2021), little is known of the chemical mechanisms workers employ to resist desiccation.

In this study, we use *P. californicus* as a model for investigating the physiological, chemical, and behavioral drivers of desiccation resistance. We assay survival under desiccation stress and characterize chemical profiles of worker ants sampled simultaneously across distinct populations that vary in queen number (monogynous vs. polygynous) within a small geographic area with homogenous environmental conditions (Overson et al. 2016). In doing so, we examine effects of queen number on colony desiccation resistance while controlling for major seasonal and habitat effects. Across this variation, we highlight physiological and chemical correlates of desiccation resistance with the aim of clarifying the integrated set of mechanisms these animals use to cope with water balance challenges.

Methods

Study populations and field collections

To assess desiccation resistance in *P. californicus* workers, we sampled worker ants from three sites in San Diego County, California: at Lake Henshaw (33.23176°, − 116.7553°, elevation: 825 m), Pine Valley, CA (32.82342°, − 116.5278°, elevation: 1130 m), and Cuyamaca Rancho State Park, hereafter "Cuyamaca" (32.93798°, − 116.5625°, elevation: 1295 m). The three sites span a ~ 46 km range of *P. californicus* populations that vary in queen number, from predominantly single-queen colonies at Lake Henshaw to predominantly multi-queen colonies at Pine Valley (Overson et al. 2016). The Cuyamaca site is situated geographically between these two regions and contains a relatively even mix of monogynous and polygynous colonies (Haney et al. 2017). We did not quantify queen number in colonies of this site, which can range from one to eight in mature colonies, and which could impact worker genetic diversity (beyond the effects of polyandry, as queens are multiply mated, Overson et al. 2016). However, queen number in polygynous populations is both relatively uniform (mean = 4.1; std. error = 0.66) and temporally stable (Haney and Fewell 2018).

We collected workers for desiccation assays and chemical analyses from a total of 30 colonies across the three sites. For desiccation assays, we collected foraging workers from 10 colonies at each site on the mornings of June 22 and 23, 2021. Workers were immediately placed in a high humidity chamber (a 15 mL tube containing 5 mL water separated by a cotton ball, from which ants could drink ad libitum) with only their nestmates. For chemical analysis, we additionally collected foraging workers from each of the same 30 colonies across the three sites between June 20 and 28, 2021. Workers were collected into glass vials and freeze-killed within 2 h of collection then stored at − 20 °C for later analysis.

Desiccation resistance assays

To standardize the initial hydration level of individual ants, we kept all field-collected workers in tubes with ad libitum water for approximately 5 h following collection (described above). We then marked all workers for individual identification with a single, small mark on the gaster using oil-based paint pens (Sharpie®, Newell Brands, Atlanta, GA, USA). Because the paint mark covered only a small proportion of total body surface area, we do not expect it to meaningfully impact desiccation resistance. We assayed ten workers from each colony, for all ten colonies from each of the three sites, for a total treatment sample size of 300 workers.

Immediately after marking, ants were placed in desiccation treatment chambers consisting of 15 mL tubes containing 5 mL calcium sulfate (Drierite, W.A. Hammond Drierite Co Ltd, Xenia, OH, USA). Ants were placed in a 5 mL space in the uppermost portion of the tube, separated from the Drierite by two cotton balls, between which was an additional layer of air (as in Whyte et al. unpub.). Each desiccation chamber held five nestmate workers, such that we had two treatment chambers per colony to hold 10 workers. We chose to maintain workers in groups to mitigate the confounding negative effects of social isolation on ant survival and energy consumption (Boulay et al. 1999; Koto et al. 2015).To control for other possible causes of death over the experimental period, we additionally established a high-humidity control in the same design as our desiccation chambers, but replacing Drierite with water. We used Hygrochron humidity-logging iButtons® (DS1923, Maxim Integrated, San Jose, CA, USA) to confirm that desiccation chambers consistently provided 0% relative humidity conditions.

We visually inspected all treatment and control workers for survival every three hours from the start of the experiment until the death of the final surviving treatment ant. We defined the time of death as the point at which an individual ant did not move in response to agitation of the chamber. Ants from all treatment were then dried in a drying oven at 55 °C for 3 days and weighed with a microbalance (AandD GR-200; repeatability 0.0001 g) to obtain dry mass as a proxy for body size and therefore surface area to volume ratio (Kühsel et al. 2017).

Chemical analysis

We extracted chemical surface profiles from 150 freeze-killed, field-collected ants from 30 colonies (5 workers from 10 colonies at each of 3 sites). We added 100 µL HPLC-grade hexane to 2 mL HPLC screwcap vials (Agilent Technologies, Waldbronn, Germany) containing single ants, and extracted their surface profiles for 10 min on an orbital shaker at 240 revolutions/min (IKA KS 130 Basic, Staufen, Germany). We then transferred extracts to a conical 250 µL GC insert (Agilent Technologies, Waldbronn, Germany) and evaporated the solvent completely under a flow of CO_2. We then resuspended the dried extracts in 10 µL of a hexane solution containing 7.5 ng/µL dodecane (C12) as an internal standard.

To identify and quantify chemical compounds from the surface profiles, we used a gas chromatograph (GC: 7890B) coupled to a flame ionization detector (FID: G3440B) and a tandem mass spectrometer (MS/MS: 7010B, all from Agilent Technologies, Waldbronn, Germany). The system was equipped with a fused silica column (DB-5MS ultra inert; 30 m × 250 µm × 0.25 µm; Agilent JandW columns, Santa Clara, CA, USA) at a temperature of 300 °C with helium used as a carrier gas under a constant flow of 1.8 mL/min. The FID had a temperature of 300 °C and used nitrogen with a 20 mL/min flow rate as make-up gas and hydrogen with a 30 mL/min flow rate as fuel gas. The column was split at an auxiliary electronic pressure control (Aux EPC) module into an additional deactivated fused silica column piece (0.9 m × 250 µm × 0.25 µm) with a flow rate of 0.8 mL/min leading into the FID detector, and another deactivated fused silica column piece (1.33 × 250 µm × 0.25 µm) at a flow rate of 1.33 mL/min into the mass spectrometer. An initial column temperature of 60 °C was maintained for 5 min and then gradually increased (20 °C/min to 240 °C, 1 °C/min to 294 °C, 4 °C/min to 325 °C) to a final temperature of 325 °C held for 2 min.

We used the resulting chromatograms to identify cuticular hydrocarbon (CHC) compounds from their mass spectra, diagnostic ions, retention indices using the program Agilent MassHunter Workstation Software (Version B.09.00/Build 9.0.647.0, Agilent Technologies, Santa Clara, CA, USA). We included only compounds identified as CHCs in our analysis. Absolute CHC quantities (in ng) were obtained by calibrating each compound according to a dilution series at four concentrations (5 ng/µl, 10 ng/µl, 20 ng/µl, 30 ng/µl) based on the closest eluting *n*-alkane from a C21-40 standard series (Merck, KGaA, Darmstadt, Germany). We excluded five of 150 chromatograms from analysis due to apparent extraction failure, leaving a final sample size of 145 workers (Lake Henshaw $n = 49$; Cuyamaca $n = 49$; Pine Valley $n = 47$).

Environmental conditions at sampling sites

The three sampling sites are situated in chaparral-dominated, lower montane shrub and woodland ecoregions in southern California. We characterized the environmental conditions at these three sampling sites using publicly available bioclimatic data (WorldClim2, (Fick and Hijmans 2017). We analyzed average monthly climatic patterns over a 30-year time period (1970–2000) and found that the three sites do not differ significantly in annual precipitation (Kruskal–Wallis test: $P = 0.388$), water vapor pressure ($P = 0.320$), or temperature ($P = 0.615$); (Supplementary Fig. 1; Supplementary Table 1). This confirmed our assumption that the three sites did not substantially differ in climatic conditions related to desiccation.

Statistical analysis

To understand predictors of survival in the desiccation resistance assays, we performed a nonparametric mixed effect Cox regression ("coxme" and "survival" packages; (Therneau 2020, 2022) with survival time in desiccation assays as our response variable, population and log dry mass as our predictor variables, and colony as a random effect. We log-transformed the dry mass data to improve normality. We calculated lethal time (LT_{50}) as the median time to death. We used a Kruskal–Wallis test to compare colony-level standard deviation in survival time across populations. Colony-level standard deviation was the standard deviation of the survival time values across all workers in each colony. We then used post-hoc pairwise Wilcoxon tests with a Bonferroni correction for multiple comparisons.

To visualize CHC data, we used a principal component analysis (PCA) of absolute CHC abundances with centered log-ratio transformation using the R package "vegan" (Oksnen et al. 2020). To assess the relationship between desiccation resistance and CHC profiles, we used a linear model with mean colony LT_{50} as our response variable and with the log abundance of linear *n*-alkanes and the log abundance of methyl-branched alkanes (the two major structural classes of compounds we found in the CHC profiles) as predictor variables. Because the desiccation assays are intrinsically stressful (and ultimately lethal), and likely to impact workers' CHC composition (Menzel et al. 2018), we did not extract CHCs from the same individual workers that had undergone desiccation assays. Instead, we extracted CHCs from workers that were frozen immediately after field sampling. As a consequence, our CHC and desiccation resistance were not taken from the same individual ants. Instead, we compared individual ant CHC profiles (predictor variable) to the mean colony-level LT50 value for a given worker's colony of origin (response variable). We log-transformed CHC abundance data to improve normality, as assessed by Q-Q plots of model residuals, and confirmed that data met assumptions homoscedasticity by inspecting plots of fitted values vs. residuals.

Finally, to analyze WorldClim data to assess environmental conditions at sampling sites, we used the R packages "raster" (Hijmans 2021), "rgdal" (Bivand et al. 2021), and created maps using the package "maps" (Becker et al. 2018). All statistical analyses were conducted in R 4.1.9 (Team, 2021).

Results

Effects of population and body mass on desiccation resistance

All ants in the 0% humidity treatment had died by 81 h post-introduction to the desiccation chambers ($LT_{50} = 36$). Survival was high (86.7%) for workers in our high-humidity controls over the 81-h experimental duration, suggesting that the majority of death in our treatment groups can be attributed to desiccating conditions, as opposed to other stressors such as starvation. Resistance to desiccation increased significantly with log dry mass (Mixed-effect Cox regression: $X^2 = 44.36$; df = 4; $P = 0.004$; mean dry mass = 1.595 ± 0.019); (Fig. 1). The effect of population on desiccation was non-significant ($P = 0.096$), with workers from Lake Henshaw (majority monogynous pop.) and

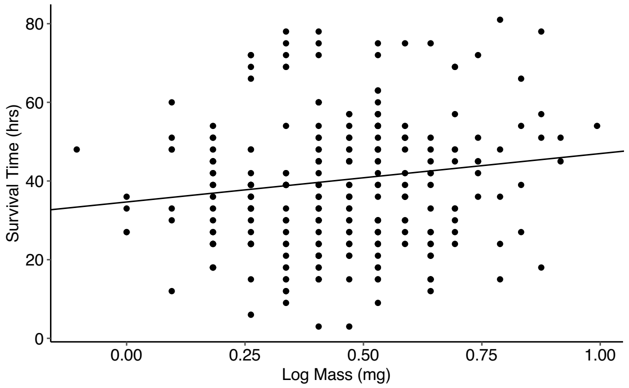

Fig. 1 The log dry mass of workers (mg) was positively related to survival time (hrs) in desiccation assays (slope = 12.305; Mixed-effect Cox regression: $X^2 = 44.36$; df = 4; $P = 0.004$)

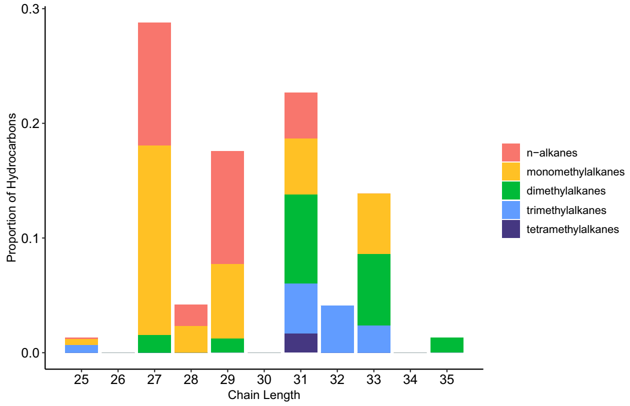

Fig. 2 Cuticular hydrocarbon profiles of *P. californicus* workers. Bars indicate proportions of the total hydrocarbon profile represented by each compound class for a given chain length (i.e., number of carbon atoms in the hydrocarbon chain). Proportions were calculated by pooling data from all 145 individuals

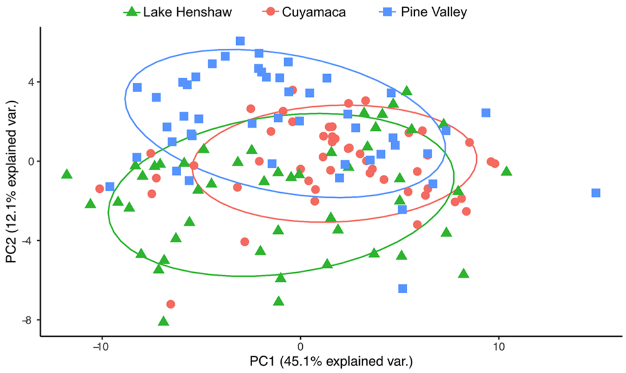

Fig. 3 Principal component analysis of the cuticular hydrocarbon profiles in workers from three sampling populations, plotted for the first two components, which explain 44.4% and 12.3% of the total variance, respectively. CHC abundance data are center log-ratio transformed. Ellipses show the 95% confidence intervals around the centroids for each population

Cuyamaca (mixed mono/polygynous pop.) surviving slightly longer than workers from Pine Valley (majority polygynous pop.); ($LT_{50,LH} = 45$; $LT_{50,CM} = 45$; $LT_{50,PV} = 39$); (Supplementary Fig. 2). Within-colony variance in desiccation resistance did not differ across populations (Wilcoxon tests: Cuyamaca vs. Lake Henshaw, $P = 0.157$; Cuyamaca vs. Pine Valley, $P = 1.000$), though there is a trend toward differentiation in the comparison between Pine Valley and Lake Henshaw colonies (Wilcoxon test: $P = 0.056$).

Cuticular hydrocarbon profiles of P. californicus workers

We identified 48 unique CHC compounds in the cuticular surface profiles of *P. californicus* workers (Supplementary Table 2). Compounds consisted of linear and methyl-branched alkanes, ranging in carbon chain length from 25 to 35 (Fig. 2). CHC profiles were dominated by methyl-branched alkanes (72.0% of CHCs), especially monomethylalkanes, which account on average for 38.1% of the total CHC profile of analyzed workers. Principal component analysis revealed similarities in CHC profiles across sampling populations, shown by overlap in 95% confidence intervals (Fig. 3). In the PCA, the first two principal components explained the majority of variation in CHC abundance (PC1: 44.4%; PC2: 12.3%).

Effects of CHC profiles on colony desiccation resistance

Cuticular hydrocarbon profiles significantly predicted desiccation resistance in our model of absolute CHC abundances by compound class (linear and methyl-branched alkanes; Adj. $R^2 = 0.064$; $P = 0.004$). Colony LT_{50} was significantly and positively correlated to the log absolute abundance of linear *n*-alkanes (ANOVA: Adj. $R^2 = 0.062$; $P = 0.002$); (Fig. 4), but not to the log abundance of methyl-branched alkanes ($P = 0.474$).

Discussion

Water conservation presents a fundamental physiological challenge for terrestrial organisms, particularly small-bodied ectotherms like insects. Desiccation is one of the principal risks of insect foraging, especially in arid environments and for insects that forage on exposed ground surfaces in high-temperature boundary layers (Bernstein 1974; Oke 1978). As such, activity and distributions of insects are often shaped by moisture availability (Kaspari and Weiser 2000; Menke and Holway 2006), though the mechanisms supporting these

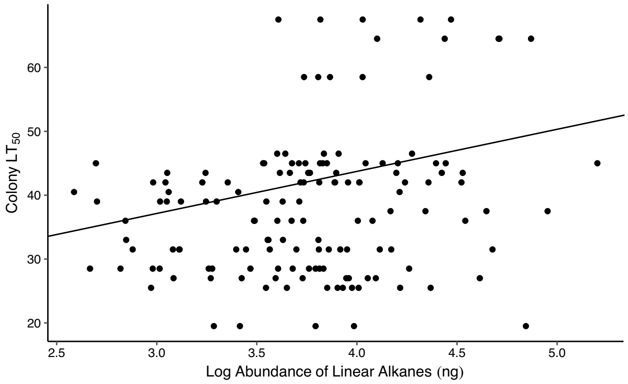

Fig. 4 The log abundance of linear alkanes (ng) in worker cuticular hydrocarbon profiles significantly predicted colony LT_{50} in desiccation trials (ANOVA: Adj. $R^2 = 0.062$; $P = 0.002$)

patterns have received little attention relative to temperature effects (Chown et al. 2011). Here, we present evidence for the roles of body mass and cuticular hydrocarbon (CHC) composition in determining the desiccation resistance of *P. californicus* workers from a semi-arid habitat. These results provide insights into the physiological mechanisms supporting insect survival in low-humidity conditions.

One of the clearest patterns in insect desiccation resistance is a positive relationship between body size and water conservation (Chown and Klok 2003; Fouet et al. 2012; Hadley 1994; Kærsgaard et al. 2004; Le Lagadec et al. 1998). We experimentally assayed desiccation resistance in *P. californicus* workers and found that larger ants survived longer at 0% humidity. Differential survival according to body size was likely driven by reduced surface area-to-volume ratios in larger-bodied ants (Hood and Tschinkel 1990; Kühsel et al. 2017), and perhaps proportionally larger water reserves (Lighton et al. 1994; Prange and Pinshow 1994). Unlike ant species that forage heavily on water-based foods like nectar (Nicolson 2007), *P. californicus* colonies subsist primarily on seeds, and to a lesser extent, other arthropods (Macmahon et al. 2000). This seed-based diet suggests that water intake during foraging is minimal. Future work measuring body water content in *P. californicus* in tandem with desiccation resistance could disentangle these effects.

The chemical composition of epicuticular lipids, especially CHCs, influences water loss in insects by determining the permeability of the cuticle to water (Gibbs 2002; Rourke and Gibbs 1999). Lipid melting increases transpiration of water through the cuticle, rendering insects vulnerable to desiccation (Gibbs 1998). Melting occurs over a range of temperatures determined by the chemical structure of CHC compounds; methyl-branching and double bonds both disrupt hydrocarbon packing, leading to lower melting points (Gibbs and Pomonis 1995). In line with this proposed mechanism for cuticular permeability, we found that desiccation resistance was positively correlated with absolute abundances of *n*-alkanes, but not methyl-branched alkanes. This association between desiccation resistance and the abundance of saturated, straight-chain molecules emphasizes the link between the physical properties of CHCs and their waterproofing performance under desiccating conditions.

These results also highlight the taxon-specific nature of chemical mechanisms for desiccation resistance. In line with our findings, foragers of the congener *Pogonomyrmex barbatus* have been found to possess higher proportions of *n*-alkanes than nest workers, likely as an adaptation to hot/dry conditions outside of the nest (Wagner et al. 1998). Menzel et al. (2018) exposed *Temnothorax* ants to low humidity stress and observed resulting increases in the abundance of *n*-alkanes and decreases in methyl-branched alkanes. Conversely, in other species, methyl-branched alkanes may play an important role in desiccation resistance. For example, long-chain methyl-branched alkanes were positively associated with desiccation resistance in Argentine ants (*Linepithema humile*; Whyte et al. unpub.) and *Drosophila serrata* (Chung 2014). Despite containing methyl groups, which are associated with lower melting points (Gibbs and Pomonis 1995), the length of these compounds may lower the melting point sufficiently to protect against desiccation at typical environmental temperatures (Whyte et al. unpub.). It may be that the quantities of *n*-alkanes observed in our study are sufficiently protective against desiccation regardless of the quantities of methyl-branched alkanes in the rest of the CHC profile.

This interspecific variation in chemical mechanisms for desiccation resistance may be a consequence of constraints arising from the dual role of CHCs in waterproofing and communication (Gibbs 2002; Menzel et al. 2019). In social insects, CHCs additionally play a major role in nestmate recognition (Blomquist and Bagnères 2010; Leonhardt et al. 2016). In Argentine ants, methyl-branched alkanes are important both for colony recognition and desiccation resistance (Whyte et al. unpub., (Brandt et al. 2009; van Wilgenburg et al. 2010). The use of a particular compound class for one function (e.g., nestmate recognition) may constrain its use in another role (e.g., waterproofing). Our study represents the first characterization of the CHC profile of *P. californicus*. As such, it is unclear at this stage how these compounds relate to other behaviors (colony recognition, individual recognition, division of labor, etc.) in this species. Further, we present CHC information only for workers, not queens or males. Compared to workers, queens may exhibit different or additional chemical mechanisms for desiccation resistance, likely due to the additional role of CHCs in fertility signaling (Menzel et al. 2018). Characterizing the CHC profiles of *Pogonomyrmex* queens would be particularly interesting given their vulnerability to desiccation via cuticular abrasion during nest excavation (Johnson 2000; Johnson and Gibbs 2004). This desiccation stress during colony founding may represent an important selective pressure leading to the formation of foundress associations in this species (Cahan and Fewell 2004). As such, future studies more broadly clarifying the chemical/behavioral ecology of *P. californicus* have potential to shed additional light on the physiology of desiccation resistance in this species.

Interestingly, we observed relative homogeneity in desiccation resistance and CHC profiles across populations differing in queen social organization (monogyny vs. polygyny). The evolution of polygyny has often been linked to harsh environmental conditions (Bourke and Franks 1995; Heinze et al. 2017), but the physiological mechanisms underlying the adaptive benefits of polygyny remain unclear. In our study, worker desiccation resistance did not differ significantly across populations despite strong population-level variation in queen number (Overson et al.

2016). Importantly, we were not able to characterize individual colonies in our study as monogynous or polygynous. Instead, we took advantage of known variation in colony founding life history across a small and climatically homogenous geographic region, with populations varying from predominantly monogynous (Lake Henshaw) to predominantly polygynous (Pine Valley); (Haney and Fewell 2018; Overson et al. 2016). A minority of colonies in these populations adopt the opposite founding strategy, and our study does not identify these cases. However, if queen number were an important driver of desiccation resistance in this species, we would still expect to see strong differentiation in survival outcomes across populations, a pattern we did not observe.

Polygyny can shape colony phenotypes not only by directionally shifting worker trait distributions, but also by impacting behavioral and genetic variation (Herbers 1986; Hölldobler and Wilson 1990). Little is known about how polygyny might affect variance in worker physiological tolerance, though it is feasible that a workforce derived from multiple matrilines could show greater variation in physiological traits. Contrary to this expectation, colonies from the Pine Valley (polygynous) population were no more variable in desiccation resistance than colonies from Lake Henshaw (monogynous). Indeed, a marginally non-significant trend suggested the opposite: that colonies from Lake Henshaw may be more variable in their desiccation responses than Pine Valley colonies. A similar pattern was observed in a comparison of experimental associations of Pine Valley queens, where single-queen colonies were more variable in worker heat tolerance relative to multi-queen colonies (Baudier et al. 2022). It may be that within-colony genetic diversity is already high due to high levels of polyandry in this species (Overson et al. 2016), such that multiple matrilines do not meaningfully increase worker phenotypic diversity. Together, these results call into question the intuition that multiple matrilines should increase phenotypic variation in workers and highlight the need for further investigation of the relationship between polygyny and variation in environmental tolerance.

In conclusion, we found that body size and the abundance of *n*-alkanes predicted survival responses in low humidity conditions, across three populations of *P. californicus*. Despite associations between polygyny and survival in harsh conditions (Cahan 2001; Heinze 1993; Heinze and Hölldobler 1994; Rueppell et al. 2015), we found that workers from a majority-polygynous population were no more resistant to desiccation than those in a majority-monogynous population. Together, these results suggest that individual physiology and CHC composition are more important drivers of desiccation resistance than queen social phenotype. These results align with previous studies highlighting the role of body size and unsaturated, straight-chain hydrocarbons in adaptative responses to water conservation challenges.

Supplementary Information The online version contains supplementary material available at https://doi.org/10.1007/s00360-023-01488-3.

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Data availability Data is made available in the supplementary information.

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