

RESEARCH ARTICLE

Cuticular hydrocarbons and collective response to water stress in a desert ant

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Abstract

1. With the accelerating pace of climate change, we urgently need to understand how physiological traits shape behavioural plasticity in response to environmental stress. In social insects, collective behaviour operates without central control but through interactions among individual participants. In the aggregate, this produces a collective response to environmental conditions.
2. Here we consider how variation among desert ant colonies in the cuticular hydrocarbons (CHCs) that prevent water loss is associated with variation among colonies in the collective behaviour that manages water stress. Colonies of the desert ant, *Pogonomyrmex barbatus*, differ in the collective regulation of foraging activity to manage water loss to evaporation while foraging. Foraging is regulated through olfactory interactions between outgoing and returning foragers, which determine a forager's decision whether to leave the nest on the next trip. Some colonies are risk-averse, with foragers less likely to make foraging trips in dry conditions, while others are risk-tolerant, with foragers who do not reduce foraging trips in dry conditions.
3. We found that behavioural differences among colonies are associated with the capacity of their foragers' CHCs to prevent water loss. In risk-averse colonies whose foragers make fewer trips in dry conditions, the abundance of alkenes was significantly higher. High abundance of alkenes, with a low melting point, makes the CHC layer more permeable, increasing susceptibility to water loss. In one of 2 years of this study, we found that workers in risk-averse colonies also had significantly shorter *n*-alkanes, which further enhance water permeability of the CHC layer and thus desiccation risk.
4. To our knowledge, this is the first report of variation among conspecific colonies in CHC profile that is linked to colony differences in collective behaviour.

KEYWORDS

behavioural physiology, climate change, cuticular hydrocarbons, desiccation risk, drought resistance, foraging behaviour, Formicidae

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1 | INTRODUCTION

As the pace of climate change accelerates, it has become urgent to learn how physiology shapes the behavioural flexibility of animals in changing conditions (Beever et al., 2017). Collective behaviour operates without central control, through interactions among individual participants. Physiology may shape how individuals respond to those interactions. Here, we consider how variation among desert ant colonies in the cuticular hydrocarbons (CHCs) that prevent water loss is associated with variation in the collective behaviour that changes in response to water stress.

Desiccation resistance is crucial for desert organisms (Chown et al., 2011). Drought and high temperatures require animals to seek shelter and to conserve water, which may influence their ability to compete for resources. Animals lose water mainly through evaporation, respiration and excretion. Water loss via evaporation is especially high in small-bodied animals, such as insects, with a high surface-to-volume ratio (Gibbs & Rajpurohit, 2010; Hadley, 1994). In insects, CHCs form a physical barrier to water loss, acting as functional traits that affect survival in dry environments (Wong et al., 2019). An individual insect's CHC profile may include more than 100 compounds that differ in melting point and viscosity (Blomquist & Bagnères, 2010; Sprenger & Menzel, 2020). Compounds with low melting points increase the water permeability of the CHC layer, making the insect more susceptible to drought (Gibbs, 2002; Gibbs & Mousseau, 1994; Gibbs & Rajpurohit, 2010; Menzel et al., 2019).

The water permeability of a CHC layer depends on its chemical composition (Huthmacher et al., 2025). Most insect hydrocarbons have 20–40 carbon atoms in their molecular backbone and fall into 3 classes that differ in melting point. First, *n*-alkanes between C20 and C40 are solid at room temperature. Second, methyl-branched alkanes have lower melting points because methyl branches disrupt molecular aggregation. Third, unsaturated hydrocarbons (alkenes and alkadienes) melt at even lower temperatures (down to -20°C), because their double bond hinders tight aggregation of the molecules even more (Gibbs, 2002; Gibbs & Pomonis, 1995), and melting point is higher for more tightly aggregated molecules. Melting point also increases with chain length, but chain length has less influence on melting point than CHC class (Gibbs, 2002; Gibbs & Rajpurohit, 2010). Thus, high abundances of alkenes, and, to a lesser degree, short-chain CHCs, make a CHC layer more permeable to evaporative water loss.

Ants are abundant and diverse in arid environments (Feener & Lighton, 1991), with a high tolerance for heat (Perez & Aron, 2020). Heat drastically increases vapour pressure deficit, and thus the risk of desiccation, so rising temperatures enhance desiccation risk (Botsch et al., 2024). Differences among ant species in CHC composition are associated with the humidity conditions and precipitation levels of their habitats (Menzel et al., 2017; van Wilgenburg et al., 2011). In addition, differences in CHCs among colonies, within a species, are linked to drought resistance (Menzel et al., 2018; Ostwald et al., 2023). There is evidence both for heritability of CHC variation from parent to offspring colony in ants (Walsh et al., 2020),

and of plastic CHC changes, in response to ambient conditions, that enhance drought survival (Gefen et al., 2015; Hadley, 1977; Menzel et al., 2018; Sprenger et al., 2018). Experiments in humid conditions show that warm-acclimated ants are more resistant to drought than cold-acclimated ants (Baumgart et al., 2022). Finally, CHCs vary within colonies among workers of different task groups, in response to differences in exposure to microclimatic conditions. For example, exterior workers of *Pogonomyrmex barbatus* (Wagner et al., 2001), *Myrmica rubra* and *Myrmica ruginodis* (Sprenger et al., 2018) have more *n*-alkanes than interior workers, increasing their resistance to drought conditions.

Ant colonies also adjust water loss via evaporation by regulating foraging activity in hot, dry conditions (Lighton & Feener, 1989). Differences among sympatric species in foraging activity, due to differences in thermal tolerance, influence resource competition (Bestelmeyer, 2000; Cerdá, Retana, & Cros, 1998; Cerdá, Retana, & Manzaneda, 1998; Lessard et al., 2009).

Here we investigate how differences among colonies in foraging response to dry conditions are associated with the composition of CHCs. We asked how variation among colonies in the collective behaviour that affects colony-level water loss is associated with variation in CHC profiles in the red harvester ant, *P. barbatus*. Colonies of this desert species must spend water to obtain water and food: ants lose water while foraging, but like many desert ant species, capture water metabolically when digesting the seeds that they eat (Feener & Lighton, 1991; Lighton & Feener, 1989). Deepening drought across the southwest US (Cayan et al., 2010; Williams et al., 2020) has reduced the food supply and intensified competition among colonies (Sundaram et al., 2022). This may influence selection on the collective behaviour that regulates foraging in dry conditions, which is associated with colony reproductive success (Gordon, 2013).

In *P. barbatus*, foraging is regulated through olfactory interactions between outgoing and returning foragers. A forager does not leave the nest unless it has experienced a high enough rate of antennal contact with foragers returning with seeds (Davidson et al., 2016; Pinter-Wollman et al., 2013). In the course of an antennal contact, an ant uses the CHC profile of the other ant to assess whether it is a nestmate and to identify its task group (Greene & Gordon, 2003). Outgoing foragers respond both to the CHC profile of returning foragers and the odour of the food the returning forager carries (Greene et al., 2013). Because each forager searches until it finds a seed, and then returns to the nest, this process sets up positive feedback in response to food availability: the more food is available, the more quickly foragers find it and return to the nest, and the more they stimulate outgoing foragers to leave on their next foraging trip. Foraging is also regulated in response to current humidity conditions. As the environment inside the nest remains cool and humid, foragers respond to the conditions they experienced on previous trips outside the nest (Pagliara et al., 2018).

Colonies vary in how they manage the trade-off between water loss and food intake (Gordon et al., 2023). In some colonies,

foragers are more averse to the risk of water loss in dry conditions. In these risk-averse colonies, foragers make fewer trips in dry conditions, so the colony reduces foraging activity, thus sacrificing food intake, and rely instead on stored food, so as to conserve water lost to evaporation when foraging. In other colonies, foragers are more risk-tolerant, maintaining foraging activity in dry conditions. These differences among colonies persist over years in successive cohorts of workers (Gordon et al., 2023). Foraging activity is lower in risk-averse than in risk-tolerant colonies only on dry days (Gordon et al., 2023; Figure 1), when foragers in risk-averse colonies take fewer trips; on humid days, foraging activity is similar in both risk-averse and risk-tolerant colonies. Workers of risk-averse colonies are more sensitive to water loss, losing water more rapidly in dry conditions, than workers of risk-tolerant colonies (Friedman et al., 2019).

We examined how risk-tolerant and risk-averse colonies differ in the abundance of alkenes and *n*-alkanes, and CHC chain length, the CHC traits that most strongly influence water permeability. We compared the CHC profiles of foragers from risk-averse colonies that reduce foraging in dry conditions with those of foragers from risk-tolerant colonies that do not. We also compared the profiles of risk-averse and risk-tolerant colonies for two other task groups that spend less time outside the humid nest than foragers and hence are less exposed to evaporative water loss (Wagner et al., 1998, 2001): patrollers, who make short trips outside the nest before foraging activity begins, and whose return triggers the onset of foraging (Greene & Gordon, 2003); and nest maintenance workers, who make short trips outside the nest to carry out refuse and deposit it on the nest mound.

2 | MATERIALS AND METHODS

2.1 | Sampling and behavioural observations

Workers from colonies of *P. barbatus* were collected at a long-term study site near Rodeo, New Mexico, where the ages and locations of all colonies are known (Sundaram et al., 2022). No permit was required to work at the long-term study site, and no ethical approval was required to work with these ants. In August 2022, we collected workers from 7 risk-averse colonies that had been found in previous work to consistently reduce foraging in dry conditions and 12 risk-tolerant colonies that were found not to do so. Whether a colony was considered to be risk-averse, reducing foraging in dry conditions, depended on how much its foraging was reduced on dry days relative to humid days in 3 or more years of observation in the summer (Gordon et al., 2023). In 2022 we collected workers from 3 task groups: 22 foragers, 17 patrollers and 33 nest maintenance workers, defined as in (Gordon, 1986) (Table S1), for an average of 1.67 ± 0.12 SE workers per colony (total $N=72$). In August 2023, we collected foragers only, from 6 risk-averse and 8 risk-tolerant colonies, for a total of 24 samples. The total number of colonies sampled in both years was 11 risk-averse and 16 risk-tolerant colonies.

We measured the foraging activity of the colonies we sampled, to compare the foraging activity of the two types on dry and humid days. Foraging activity was measured on 14 days in August 2022, and 8 days in August 2023. To measure foraging activity, the observer counted the number of ants travelling in both directions, towards and away from the nest entrance, across an imaginary line bisecting each foraging trail in 30 s. Early in the foraging period, most foragers

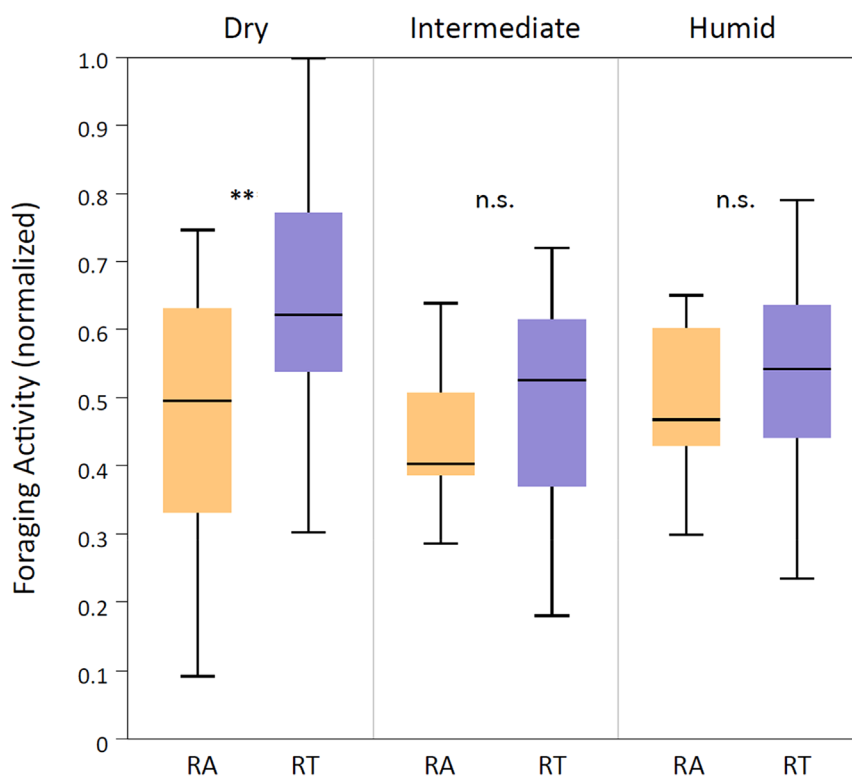


FIGURE 1 Foraging rate by relative humidity. Foraging rate is the mean normalized number of ants entering and leaving the nest; days were grouped by maximum daily relative humidity as dry, intermediate or humid (see text for details). Comparison of foraging activity in risk-averse (RA) and risk-tolerant (RT) colonies with Wilcoxon signed ranks test, ** $p < 0.01$; 'n.s.' $p > 0.05$.

are leaving the nest; at the end, around noon, most are returning, while in between, the number of ants entering and leaving the nest is about the same (Pagliara et al., 2018). Foraging counts were made during this steady-state period, daily once per colony. As a measure of foraging rate, we calculated the sum, across all trails, of the counts of outgoing and returning ants in 30s. To compare the two types in foraging activity we normalized for colony size, by dividing each measure of foraging rate by the largest foraging rate counted for that colony in that year. To estimate how much the two types differed in the number of foraging trips, we found for each colony the average foraging rate on dry days and then the mean for risk-averse and risk-tolerant colonies.

The 2 years of our study, 2022 and 2023, differed in weather conditions. Weather data were obtained from Weather Underground (The Weather Company, L. L. C., 2024), and we calculated the average daily temperature and humidity through the month of August in each year. In 2022, there was considerable rainfall. Average daily temperature in August 2022 was $75.0^{\circ}\text{F} \pm 1.3^{\circ}\text{F}/23.9^{\circ}\text{C} \pm 0.7^{\circ}\text{C}$, and average daily relative humidity was $65.5\% \pm 2.6\%$. In August 2023, conditions were hot and dry with little to no rainfall, with daily average temperatures of $83.1^{\circ}\text{F} \pm 1.0^{\circ}\text{F}/28.4^{\circ}\text{C} \pm 0.5^{\circ}\text{C}$ and average humidity of $42.0\% \pm 1.9\%$.

2.2 | Replication statement

Scale of inference	Scale at which the factor of interest is applied	Number of replicates at the appropriate scale
Colonies	Colonies	11 risk-averse, 16 risk-tolerant
Task groups \times foraging type	Individual worker	6–21 per factor combination, total $N=96$

2.3 | Chemical analysis

Workers were killed by freezing and stored at -20°C until extraction within 1–3 days of collection. CHCs were extracted by immersing the workers in ca. $300\ \mu\text{L}$ *n*-hexane for 10 min and then analysed using gas chromatography–mass spectrometry (GC–MS). First, the samples were concentrated under a gentle nitrogen flow down to approx. $20\ \mu\text{L}$. The 2022 and the 2023 samples were analysed with different machines. For each sample from 2022, $2\ \mu\text{L}$ were then injected into a GC–MS (GC: 7890A, MS: 5975C, Agilent Technologies, Santa Clara, CA, USA). Injection was performed at 320°C in splitless mode. We used Helium as carrier gas ($1.2\ \text{mL}/\text{min}$) and a Zebron Inferno DB5-MS capillary column ($30\ \text{m} \times 0.25\ \text{mm} \times 0.25\ \mu\text{m}$, Phenomenex, Aschaffenburg, Germany). The oven temperature started at 60°C for 2 min and then was increased at $60^{\circ}\text{C}/\text{min}$ to 200°C and afterwards at $4^{\circ}\text{C}/\text{min}$ up to 320°C , where it was held constant for 10 min. The

molecules were fragmented with an ionization voltage of $70\ \text{eV}$. The detector scanned for molecular fragments in a range of $40\text{--}550\ \text{m}/z$.

For the 2023 samples, $1\ \mu\text{L}$ was injected at 300°C in splitless mode, using a GC-2030 NEXIS and a single quadrupole mass spectrometer (QP-2020; Shimadzu, Nakagyoku, Kyoto, Japan). Separation occurred on an Rxi-1ms column ($30\ \text{m} \times 0.25\ \text{mm} \times 0.25\ \mu\text{m}$; Restek, Bellefonte, PA) at a flow rate of $0.94\ \text{mL}/\text{min}$. The temperature program began at 170°C for 2 min, then increased by $25^{\circ}\text{C}/\text{min}$ until 220°C (maintained for 1 min) and then increased by $3^{\circ}\text{C}/\text{min}$ until 310°C , where it was maintained for 8 min. Ion source temperature and interface temperature were 200°C and 280°C , respectively. The mass spectrometer operated in full scan mode, performing a scan every $0.2\ \text{s}$ using a mass range of $35\text{--}500\ \text{m}/z$.

We identified the hydrocarbons based on a retention index (Carlson et al., 1998) and diagnostic ions. Non-hydrocarbon substances, hydrocarbons with a maximum (across all samples per species) below 0.5% and hydrocarbons that occurred in less than 20% of the samples of either species or treatment were excluded.

2.4 | Statistical analysis

We extracted from each CHC profile the relative abundance (proportion) of *n*-alkanes, alkenes, monomethyl alkanes and dimethyl alkanes, as well as the average chain length of *n*-alkanes. Average chain length was calculated for each sample as the scalar product of the relative abundance of each *n*-alkane and their respective chain lengths. The scalar product was then divided by the total abundance of all *n*-alkanes in the respective sample, viz.

$$\text{avCL} = \frac{\sum p_i \cdot \text{CL}_i}{\sum p_i}$$

where avCL is the average chain length of all *n*-alkanes in a certain sample, p_i is the proportion of the *i*th *n*-alkane in the sample and CL_i the chain length of the *i*th *n*-alkane. We created separate statistical models for each of the four CHC traits. Chain length was analysed for *n*-alkanes only because the position of a double bond or a methyl group strongly influences its melting point (Gibbs, 2002), so a comparison across CHCs with different positions would not yield meaningful results. These position effects are stronger than chain length effects (Gibbs & Rajpurohit, 2010). In contrast, *n*-alkanes only differ in chain length and nothing else; hence, an average chain length can provide information about average melting points.

We first compared the profiles of foragers in the two sets of colonies, using linear mixed-effects models with *year* and *foraging type* (risk averse, reducing foraging in dry conditions or risk-tolerant, not reducing foraging in dry conditions) as independent variables (interaction allowed) and *colonyID* as a random factor (command *lmer*, package *lme4*).

Next, we tested for differences among the three task groups, foragers, patrollers and nest maintenance workers, for ants collected in 2022. These models contained the variables *task group* and *foraging type* as independent variables, with an interaction allowed, and *colonyID* as a random factor as above. The impact of each variable was assessed using ANOVA (R package *car*).

3 | RESULTS

Foraging activity was significantly lower in risk-averse than risk-tolerant colonies only on dry days (Wilcoxon rank sum test, dry days $W=1751$, $p=0.0086$; intermediate days $W=3361$, $p=0.11$; humid days: $W=1911$, $p=0.18$; Figure 1) as in previous work (Gordon et al., 2023). This is due to a small difference of about 11% fewer foraging trips on dry days in risk-averse colonies: the mean on dry days of all risk-averse colonies' average foraging rate in 30s was 41.5 ± 29.4 SD trips; the same mean for risk-tolerant colonies was 46.8 ± 25.8 SD trips.

Across all samples, we detected 32 CHCs, including 7 *n*-alkanes, 5 alkenes, 11 monomethyl and 9 dimethyl alkanes (Figure 2; Table S2). By far the most abundant compound was *n*-C25 (median abundance 35.2%).

CHCs of risk-averse colonies, which make fewer foraging trips in dry conditions, differed from those of risk-tolerant colonies. The abundance of alkenes was significantly higher in foragers of risk-averse than foragers of risk-tolerant colonies (LMER: $\chi^2_1=15.2$, $p<0.0001$; Figure 3). This was true in both years that we sampled, 2022 and 2023 (*year* × *foraging type*: $\chi^2_1=1.0$, $p=0.31$). By contrast, the relative abundance of monomethyl alkanes, dimethyl alkanes and *n*-alkanes did not differ between foraging types (Table 1). A higher abundance of alkenes was detected in 2023 than in 2022 ($\chi^2_1=9.3$, $p=0.0023$; Table 1; Figure 3), but a comparison of analyses from the two labs in which samples were analysed suggests this might be an artefact of different GC-MS machines.

Another difference in CHC profile between the two foraging types was in *n*-alkane chain length. This difference occurred in only one of the 2 years. In 2022, foragers from risk-averse and risk-tolerant colonies did not differ in *n*-alkane chain length (*foraging type* $\chi^2_1=2.4$, $p=0.12$; *year* $\chi^2_1=59.9$, $p<0.0001$; *interaction* $\chi^2_1=5.5$, $p=0.019$; *year effect for 2022*: $t=0.70$, $p=0.51$; Table 1). By contrast, in 2023, risk-averse colonies had significantly shorter *n*-alkanes than risk-tolerant ones (*year effect for 2023*: $t=-2.7$, $p=0.035$; Figure 4).

Task groups within colonies differed in CHC profile. Nest maintenance workers had more alkenes, monomethyl alkanes and dimethyl alkanes and less *n*-alkanes compared to foragers and patrollers

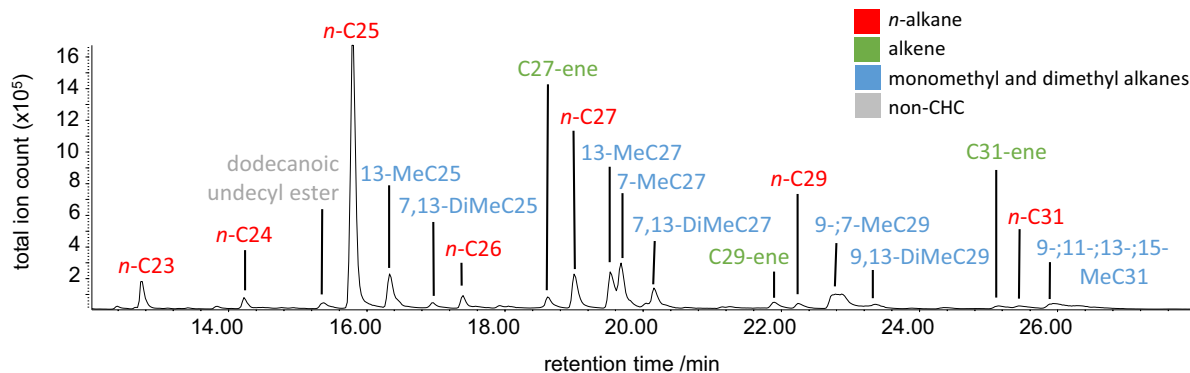


FIGURE 2 Chromatogram of cuticular hydrocarbons of one forager of *Pogonomyrmex barbatus*.

(Figure 1; Figure S2; Table 2). Nest maintenance workers of risk-averse colonies had less monomethyl alkanes and dimethyl alkanes than nest maintenance workers of risk-tolerant colonies (Table 3). Foragers of risk-averse colonies showed a strong increase in alkene abundance; there was no significant difference between patrollers or nest maintenance workers of the two foraging types (Figure 5; Table 3).

4 | DISCUSSION

Foragers from risk-averse colonies, which reduce foraging in dry conditions, have a lower abundance of alkenes than foragers of risk-tolerant colonies. This probably makes them more susceptible to water loss. Of the classes of compounds present in the CHC profile of *P. barbatus*, alkenes have the lowest melting point (Sprenger & Menzel, 2020). Alkenes decrease the overall viscosity of the CHC layer, thereby increasing the diffusion rates of molecules (Einstein, 1905) and enhancing water loss through the CHC layer (Gibbs, 1995, 2002). This explains the result from previous work that foragers from risk-averse colonies are more sensitive to water loss than those from risk-tolerant colonies (Friedman et al., 2019).

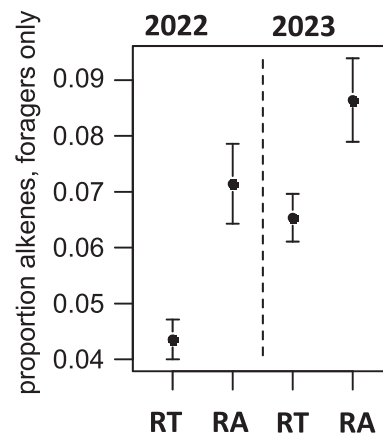


FIGURE 3 Proportion of alkenes in foragers of risk-tolerant (RT) and risk-averse (RA) colonies. Each point shows the mean \pm standard error by foraging type and year.

TABLE 1 Model results for the comparison of cuticular hydrocarbon traits by foraging type (risk-tolerant or risk-averse) and year (for foragers only).

Proportion of alkenes	χ^2	df	<i>p</i>
Year	9.27	1	0.0023
Foraging type	15.25	1	9.43E-05
Foraging type × year	1.02	1	0.31
Chain length of <i>n</i> -alkanes	χ^2	df	<i>p</i>
Year	59.85	1	1.02E-14
Foraging type	2.36	1	0.12
Foraging type × year	5.50	1	0.019
	<i>t</i>		<i>p</i>
Foraging type	2022	0.70	0.51
	2023	-2.71	0.035
Proportion of <i>n</i> -alkanes	χ^2	df	<i>p</i>
Year	32.50	1	1.19E-08
Foraging type	1.52	1	0.22
Foraging type × year	0.90	1	0.34
Proportion of dimethyl alkanes	χ^2	df	<i>p</i>
Year	5.74	1	0.017
Foraging type	0.15	1	0.70
Foraging type × year	0.04	1	0.85
Proportion of monomethyl alkanes	χ^2	df	<i>p</i>
Year	47.86	1	4.57E-12
Foraging type	1.39	1	0.23
Foraging type × year	0.25	1	0.62

Note: Significant effects are shown in bold. Since there was a significant interaction of foraging type and year for the chain length of *n*-alkanes, we report effects of foraging type on *n*-alkane chain length separately for 2022 and 2023 (*t* and *p* values). These values were obtained from model summaries.

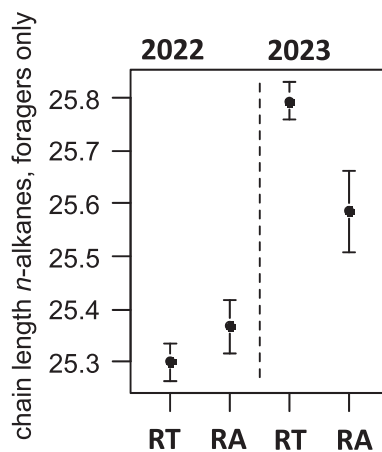


FIGURE 4 Average chain length of *n*-alkanes of foragers from risk-tolerant (RT) and risk-averse (RA) colonies. Each point shows the mean ± standard error by foraging type and year.

TABLE 2 Model results for the comparison of cuticular hydrocarbon traits by foraging type (risk-tolerant or risk-averse) and task group (2022 samples only).

Proportion alkenes	χ^2	df	<i>p</i>
Foraging type	3.89	1	0.049
Task group	8.50	2	0.014
Foraging type × task group	4.84	2	0.089
Chain length of <i>n</i> -alkanes	χ^2	df	<i>p</i>
Foraging type	4.97	1	0.026
Task group	1.60	2	0.45
Foraging type × task group	1.55	2	0.46
Proportion <i>n</i> -alkanes	χ^2	df	<i>p</i>
Foraging type	0.0062	1	0.94
Task group	8.54	2	0.014
Foraging type × task group	6.71	2	0.035
Proportion dimethyl alkanes	χ^2	df	<i>p</i>
Foraging type	1.03	1	0.31
Task group	6.55	2	0.038
Foraging type × task group	6.18	2	0.045
Proportion monomethyl alkanes	χ^2	df	<i>p</i>
Foraging type	0.07	1	0.79
Task group	7.21	2	0.028
Foraging type × task group	7.53	2	0.023

Note: Significant effects are shown in bold. See Table 3 for task group-specific differences between RA and RT colonies.

Risk-averse colonies also had *n*-alkanes with lower average chain length in one of the 2 years; this further promotes water loss.

Our results suggest how differences among colonies in foraging behaviour can be shaped by differences in CHC composition. While foragers in risk-averse colonies are more sensitive to water loss than foragers of risk-tolerant colonies in severe experimental conditions (Friedman et al., 2019), the dehydration experienced during a foraging trip is not lethal. A forager's decision whether to leave the nest on another trip depends on the desiccation it has experienced on recent trips (Pagliara et al., 2018). Within a colony, foragers are similar in CHC profile (Sturgis & Gordon, 2013) and make similar decisions about when it is too dry to leave the nest on another foraging trip (Nova et al., 2022). A forager of a risk-averse colony returning from a trip outside is likely to have lost more water to evaporation than a forager of a risk-tolerant colony, because its CHCs were less effective at preventing water loss. As a result, the forager may be likely to decide not to leave the nest on another foraging trip.

We do not know how the differences in CHC profile, and the associated foraging behaviour, affect colony fitness. The production of more desiccation-resistant CHC profiles by risk-tolerant colonies could have higher physiological costs than the production of less desiccation-resistant profiles. However, this is unlikely because CHC production takes up only a small fraction of an insect's resting

TABLE 3 Effects of foraging type, separately for each task group (2022 data only).

	Foragers		Patrollers		Nest maintenance workers	
	<i>t</i>	<i>p</i>	<i>t</i>	<i>p</i>	<i>t</i>	<i>p</i>
Proportion of alkenes	2.95	0.0052	0.50	0.62	0.90	0.37
Proportion of <i>n</i> -alkanes	-0.85	0.40	-1.51	0.14	1.85	0.071
Proportion of dimethyl alkanes	-0.11	0.91	1.21	0.23	-2.31	0.026
Proportion of monomethyl alkanes	0.59	0.56	1.69	0.096	-2.04	0.047

Note: These effects were not calculated for chain length of *n*-alkanes because the interaction had a *p* value >0.1. All effects were obtained from model summaries. Significant effects are shown in bold.

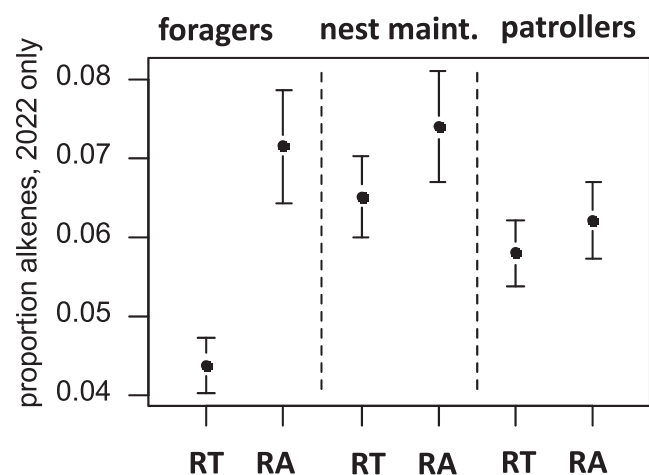


FIGURE 5 Proportion of alkenes in three task groups of risk-tolerant (RT) and risk-averse (RA) colonies. Each point shows the mean \pm standard error for foragers (F), nest maintenance workers (N) and patrollers (P) in 2022.

metabolism (Dirks & Federle, 2011), and alkanes and alkenes are probably equally costly to produce. In 2010, colonies that reduced foraging in dry conditions, thus conserving water, were more likely to have offspring colonies (Gordon, 2013); however, the effects of reduced foraging on colony survival and reproduction may be shifting as the food supply decreases in intensifying drought conditions (Sundaram et al., 2022). In addition, there may be other benefits of less desiccation-resistant profiles, for example through enhanced foot adhesion (Dirks & Federle, 2011).

While exposure to warm or dry conditions can trigger acclimatory CHC changes (Baumgart et al., 2022), this is unlikely to explain the differences in CHC profiles of risk-averse and risk-tolerant colonies. Foragers of risk-averse colonies do not experience much less exposure to conditions outside the nest than foragers of risk-tolerant colonies. Risk-averse colonies do not stop foraging altogether on dry days; instead, they decrease foraging activity (Gordon et al., 2023). In this study, on dry days foragers of risk-averse colonies made about 11% fewer trips than foragers of risk-tolerant colonies (Figure 1). This small decrease is spread over about 2000 foragers in a mature colony (Adler & Gordon, 2003). A forager makes up to 6–8 trips per day, and the average foraging trip lasts 20 min (Beverly et al., 2009; Nova et al., 2022). If a forager reduces its foraging by 11% from 8

to 7 trips, it would reduce its exposure, on dry days only, by 20 min, from 160 to 140 min outside the nest. It is very unlikely that such small differences in exposure, only on the driest days, would suffice to produce detectable differences in the CHC profiles of a small sample of foragers.

Previous work shows that successive cohorts of workers of a given colony show consistent foraging behaviour (Gordon et al., 2023), indicating that workers inherit the process that determines forager CHC profiles from the queen and her mates. However, we do not know whether differences among colonies in CHC profile are heritable from parent to offspring colony, as in other ant species (Walsh et al., 2020). Our results here show that these differences, whatever their origin, are linked to differences in foraging behaviour.

Within a colony, task groups differed in CHCs. As in previous work (Sturgis & Gordon, 2013; Wagner et al., 2001), we found that foragers and, to a lesser extent, patrollers had more *n*-alkanes than nest maintenance workers, who had more monomethyl and dimethyl alkanes. Patrollers and foragers spend more time outside the nest than nest maintenance workers and so experience higher drought stress; *n*-alkanes decrease water loss. Previous experiments show that the proportion of *n*-alkanes increases in only 11 days of exposure to high temperature and low humidity (Wagner et al., 2001). Other species often show similar differences between foragers and interior workers (Sprenger et al., 2018), though some ant species do not (Beros et al., 2017; Menzel et al., 2018). These differences among task groups probably reflect requirements for preventing water loss while executing particular tasks, which vary widely across species.

In other studies of CHC plasticity, abundances of *n*-alkanes and alkenes tended to be correlated, either positively (Sprenger et al., 2018) or negatively (Baumgart et al., 2022). Here, while risk-tolerant and risk-averse colonies differ in alkene abundance, the abundance of *n*-alkanes did not differ. Further work is needed to determine whether in this species, some CHC traits such as *n*-alkane abundance are more plastic than others, such as the abundance of alkenes, which may be more biosynthetically constrained.

Year-to-year changes in conditions apparently influence CHC profiles. The difference between the two sets of colonies in *n*-alkane chain length shifted from 2022 to 2023. During the time that ants were sampled in 2022, temperatures were low and humidity was high, with heavy rainfall; 2023 had high temperatures and low humidity, with no rainfall. Foragers live only a year (Gordon & Hölldobler, 1987), so ants collected in one summer are unlikely to

have experienced the conditions of the previous summer. In 2023, the risk-averse colonies had significantly shorter *n*-alkanes, which reduces drought protection. In contrast, in the more humid conditions of 2022, risk-averse colonies had longer *n*-alkanes. Perhaps risk-averse and risk-tolerant colonies differ in plasticity, which allows them to adjust chain length to conditions so that in hot, dry conditions risk-tolerant colonies can increase chain length and thus protection from water loss more than risk-averse colonies. However, this effect of chain length on water permeability is much weaker than the effect of the proportion of alkenes (Gibbs & Rajpurohit, 2010), which was consistent in both years. We also found that alkene proportions differed between years, but we cannot rule out the possibility that both differences between years are artefacts, as 2022 and 2023 samples were analysed on different machines, and differences in sensitivity between different GC-MS machines are common. However, our finding that risk-averse and risk-tolerant colonies differed in *n*-alkane chain length in 2022, but not in 2023, cannot be a lab artefact because these effects only concern comparisons within the same year.

Variation in physiology, and its effects on behaviour, will shape adaptation to climate change. In desert ant colonies, variation among colonies in collective behaviour is linked to the physiology of water loss. To learn how selection on colony variation in CHC profile may allow adaptation to drought and rising temperatures, further work is needed to determine how colonies differ in plasticity of CHC profiles in response to conditions, and how colony differences in CHCs are inherited from parent to offspring colonies. To our knowledge, this is the first study that links intraspecific CHC differences both to the collective behaviour that regulates exposure to water stress and to drought resistance. Understanding how physiology is linked to behavioural flexibility will allow us to learn how animals can deal with the changing climate.

AUTHOR CONTRIBUTIONS

Florian Menzel and Deborah M. Gordon conceived the ideas and designed methodology; Katherine Fiocca, Deborah M. Gordon and Florian Menzel collected the data; Katherine Fiocca, Florian Menzel and Erik B. Steiner analysed the data; Florian Menzel and Deborah M. Gordon led the writing of the manuscript. All authors contributed critically to the drafts and gave final approval for publication.

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CONFLICT OF INTEREST STATEMENT

All authors declare that they have no conflict of interest.

DATA AVAILABILITY STATEMENT

All data are available from the Stanford digital repository: <https://doi.org/10.25740/kp523dt7562> (Menzel et al., 2025).

STATEMENT OF INCLUSION

Our study brings together authors from two different countries. Two of the three authors, including the lead author, are based in the country where the study was carried out. All authors were engaged early on with the research and study design to ensure that the diverse sets of perspectives they represent were considered from the onset. Whenever relevant, literature published by scientists from the region was cited.

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SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

Table S1. Sample sizes for the analyses.

Table S2. Cuticular hydrocarbons detected in *Pogonomyrmex barbatus*.

Figure S1. CHC traits by task group and foraging type.

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