

Original Article

# Aggression is task dependent in the red harvester ant (*Pogonomyrmex barbatus*)

Shelby J. Sturgis and Deborah M. Gordon

Department of Biology, Stanford University, 371 Serra Mall Gilbert Building rm. 410, Stanford, CA 94305-5020, USA

In many animal species, the propensity for aggression is linked to social context. In social insects, aggressive interactions between colonies are related to the assessment of cuticular hydrocarbon profiles. We investigated how, in the red harvester ant, *Pogonomyrmex barbatus*, an individual ant's task environment contributes to its hydrocarbon profile, and how task-specific differences in profiles are linked to aggression. We compared the hydrocarbon profiles of 200 individuals from 4 task groups and 10 colonies, and measured aggression of ants of specific task groups toward non-nest mates of neighboring and distant colonies. We found that task groups can be distinguished by their hydrocarbon profiles. Hydrocarbon profiles are more closely associated with task group membership than with colony identity. Ants of the patroller task group were more likely to be aggressive to non-nest mates than ants of any other task group. There was no correlation between the extent of aggression between task groups and the magnitude of difference in their hydrocarbon profiles. Nearby nests did not differ more in hydrocarbon profile than distant nests. Our results suggest that aggression in *P. barbatus* is task dependent and is not directly correlated with the magnitude of difference in hydrocarbon profiles. **Key words:** aggression, hydrocarbons, nest-mate recognition, *Pogonomyrmex barbatus*, task. [*Behav Ecol*]

## INTRODUCTION

In many animal species, the extent of aggressive behavior depends on individual social status and social context (Griskevicius et al. 2009; Humfeld et al. 2009; Tanner and Adler 2009). In social insects, aggression toward non-nest mates can be context dependent (reviewed in Sturgis and Gordon 2012). For example, in the honeybee *Apis mellifera*, aggression by nest guards depends on food availability (Downs and Ratnieks 2000). In several ant species, aggression toward conspecifics depends on the environment in which encounters occur (Jaffe and Puche 1984; Wenseleers et al. 2002).

Individuals of certain task groups, such as those that guard the nest, may be especially likely to act aggressively toward conspecifics (Polizzi and Forschler 1999; Newey et al. 2010; Modlmeier and Foitzik 2011), whereas workers of less aggressive task groups may display other behaviors in response to non-nest mates (Bjoerkman-Chiswell et al. 2008). Task group differences in aggression may be due to differences in experience with non-nest mate conspecifics (van Wilgenburg et al. 2010) in the developmental changes (Muscedere and Traniello 2012) as workers move from one task to the next (Calabi and Traniello 1989; Gordon 1989a; Robinson et al. 2009) or in the motivational state of workers (Boulay et al. 2003).

Nestmate recognition in social insects is based on the detection of colony-specific hydrocarbon (HC) profiles (Guerrieri et al. 2009; van Wilgenburg et al. 2010). An ant's task influences its HC profile (Bonavita-Cougourdan et al. 1993; Wagner

et al. 1998; Martin and Drijfhout 2009a) because of chemical changes induced by the environmental conditions associated with particular tasks (Kaib et al. 2000; Wagner et al. 2001). In termites, worker caste-specific HC profiles appear to be more distinct than colony-specific HC profiles (Sevala et al. 2000). Little is known about the extent to which task group and colony identity contribute to HC profile differences in ants.

Recognition cue similarity models propose that an ant remembers a neural template of its colony's HC profile and that it is not aggressive toward another ant if that ant's profile does not deviate more than a threshold quantity from this mean (Breed and Bennett 1987; van Zweden and d' Ettore 2010). Newey's (2011) model of this process suggests that an ant has 2 profiles, a colony or gestalt profile and an individual profile. In Newey's model, the more a worker's individual profile matches that of its colony, the more likely it is to detect a non-nest mate as different, and thus the more likely it is to be aggressive to the non-nest mate. This would explain why within a colony, ants differ in the extent of aggression toward non-nest mates (Newey et al. 2010; Johnson 2011).

In this study, we consider whether similarity of HC profiles predicts aggression in the seed-eating red harvester ant, *Pogonomyrmex barbatus*. Colonies of *P. barbatus* build nests with large mounds, about 1 m in diameter in a mature colony, covered with small pebbles that contain colony-specific HCs (Sturgis et al. 2011). The youngest ants in a *P. barbatus* colony are interior workers (Gordon et al. 2005). Brood workers stay inside the nest, tending brood. Nest maintenance workers work inside the nest constructing and repairing chambers and tunnels, making short trips outside the nest to deposit debris on the nest mound, but do not leave the nest mound. Midden workers manipulate pebbles and other materials on the nest mound that contain the colony-specific HC profile (Sturgis et al. 2011); like nest maintenance workers, midden workers do not leave the nest mound.

Address correspondence to S.J. Sturgis. E-mail: [ssturgis@stanford.edu](mailto:ssturgis@stanford.edu)  
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Ants of 1 *P. barbatus* colony do not venture onto the mound of another, so ants of neighboring colonies meet only if they leave the nest mound. Only patrollers and foragers travel off the nest mound and encounter non-nest mates. Patrollers explore the foraging area early in the morning, and it is their return that stimulates the onset of foraging (Greene and Gordon 2003a). The patrollers respond to incursions on the nest mound by ants of other colonies (Gordon 1986, 1987, 1989a) and set the foraging directions each day using a secretion of the Dufour's gland (Greene and Gordon 2007a), apparently avoiding those directions that would lead to overlap with neighbors in foraging area (Gordon and Kulig 1996; Greene and Gordon 2007a). Foragers search for seeds, and occasionally for dead or dying insects, making trips of about 20 min and up to 20 m outside the nest (Gordon 1986; Adler and Gordon 2003).

Workers of *P. barbatus* have task-specific HC profiles, which are used in the interactions that determine colony activity (Wagner et al. 1998; Greene and Gordon 2003b, 2007b). Workers switch tasks according to colony needs (Gordon 1986, 1989a), and task-specific profiles change as a worker moves from one task to the next (Wagner et al. 1998). Fighting between colonies is rare in most mature colonies, which avoid confrontations when neighboring colonies overlap in foraging area (Gordon 1992; Gordon and Kulig 1996). Foragers are more likely to avoid ants from neighboring colonies than ants from more distant colonies (Gordon 1989b), which suggests a nasty neighbor effect (Dimarco et al. 2010; Newey et al. 2010). Patrollers respond when defense is required (Gordon 1987). In combination, these observations suggest that aggression may be highest in patrollers and that neighboring colonies may show larger differences in HC profile than more distant colonies.

To investigate how differences in HC profiles are related to aggression, we first ask how individuals vary in the extent to which each ant's profile matches that of the colony. If the relation between an ant's profile and that of its colony accounts for task-specific differences in aggression, then ants of more aggressive task groups should show smaller differences between individual and colony HC profiles. We then examine differences between colonies, to determine whether the difference between colonies in HC profiles is associated with the amount of aggression when ants of the 2 colonies interact, and whether colony differences in HC profiles are related to distance between nests. Finally, we ask how task groups differ in aggression toward ants of other colonies, and how the amount of aggression according to task group depends on how particular task groups differ among colonies.

## METHODS

### *P. barbatus* collection

All *P. barbatus* workers were collected from colonies at a long-term field site near Rodeo, NM, USA, in which colonies have been censused since 1985, so the ages of all colonies are known (census methods in Gordon and Kulig 1996).

### Comparison of HC profiles among task groups

To compare differences in HC profiles among task groups, workers of *P. barbatus* were collected from 10 mature colonies (ages 6–18 years) for HC extraction. From 12 to 24 August 2008, we collected 5 workers from each of 4 task groups (foragers, nest maintenance workers, midden workers, and patrollers, defined as in Gordon 1986, 1989a), for a total of 20 workers per colony ( $N = 200$  workers). All ants were collected while performing the task for which they were identified, placed in glass vials, and stored at  $-20^{\circ}\text{C}$ . HCs were extracted from each individual worker, for a total of 200

samples. HC extraction and identification were performed as in Sturgis et al. (2011).

To determine whether there were differences in HC profiles among the 4 task groups, we performed first a principal component analysis (PCA) and then a linear discriminant analysis (LDA) on the full HC profile, consisting of the 21 most prevalent HCs previously identified on the cuticle of *P. barbatus* workers (Sturgis et al. 2011).

### HC profiles as predictors for colony and task group membership

To determine whether task groups or colonies are more similar in HC profiles, we performed PCA prior to quadratic discriminant analysis (QDA) with leave-one-out cross validation (LOOCV) with either colony or task as the grouping variable, and all combinations of HC classes (the full HC profile, alkanes, methyls, alkenes, alkanes + methyls, alkanes + alkenes, and methyls + alkenes) as predictor variables. We compared the classification tables from the LOOCV of both colony and task. The grouping variables "colony" and "task" differ in number; there were 10 colonies and 4 task groups. There is thus a higher probability of assigning a given worker's profile correctly to task than colony. We corrected for this using Cohen's Inter Rater Agreement (Fleiss 1975) to calculate "z," a distribution-free value. The percentages of ants correctly classified to colony and task differed significantly if z values were greater than 1.96 or 1 standard deviation (SD) apart.

The number of principal components used in the QDA was chosen based on the Kaiser (1958) criterion. The chi-square values for all discriminant functions for colony and task were significant ( $P < 0.001$ ). Data were transformed according to Aitchison (1986) and Reyment (1989), and all statistical analyses were performed using the statistical package R version 2.13.0.

### Relationship between task and aggression

To compare aggression levels among task groups, we performed nestmate recognition assays using 2073 foragers and patrollers, and 304 interior workers, collected from a total of 36 colonies. Patrollers and foragers were collected on the nest mound and in the foraging area. Interior workers were collected from brood chambers near the surface of the nest mound, on hot, sunny days (Jones and Oldroyd 2006). An average ( $\pm$ SD) of  $28.7 \pm 3.3$  ants were used in each assay ( $14.3 \pm 2.0$  ants per colony). Groups of ants were used in assays because preliminary trials showed that, as in other ant species (e.g., Roulston et al. 2003; Sorrells et al. 2011), *P. barbatus* workers were more likely to fight when in groups than when alone. In all assays, colonies were tested once per treatment, and no individual was tested more than once.

We performed 3 behavioral assays to test for differences among task groups in aggression toward non-nest mates.

1) *Exterior–interior worker assays*. We first tested for differences in aggression between exterior workers, which included both foragers and patrollers, and interior workers. Exterior workers leave the nest mound and encounter non-nest mates, whereas interior workers do not. Ants were paired with non-nest mates of the same task group in 2 treatments: (a) exterior workers versus non-nest mate exterior workers and (b) interior workers versus non-nest mate interior workers. There were 12 trials per treatment. The same colonies were paired across treatments with unique colony pairs used in each trial.

2) *Exterior–exterior worker assays*. We next tested for differences in aggression between patrollers and foragers, which

were the only task groups to display significant levels of aggression in exterior–interior worker assays. Ants were paired with the same and different task group in 6 treatments and 3 experimental: (a) forager treatment: foragers versus non-nest mate foragers, (b) patroller treatment: patrollers versus non-nest mate patrollers, (c) patroller–forager treatment: patrollers versus non-nest mate foragers, and 3 control treatments: (d) foragers versus nestmate foragers, (e) patrollers versus nestmate patrollers, and (f) patrollers versus nestmate foragers. There were a total of 12 trials for each treatment. To ascertain the direction of aggression in the patroller–forager treatment, we marked patrollers with a drop of paint (Uni-Paint markers, Bellwood, Illinois). Once dry, the paint does not affect aggression or nestmate recognition (Brown and Gordon 1997). As in exterior–interior worker assays, the same colonies were paired across all treatments with unique colony pairs used in each trial.

3) Neighbor–distant colony assays. Finally, we compared aggression between ants of neighboring and distant colonies in 4 treatments: (a) foragers versus neighboring foragers, (b) foragers versus distant foragers, (c) patrollers versus neighboring patrollers, and (d) patrollers versus distant patrollers. There were a total of 6 trials for each treatment. Two colonies were considered to be neighbors if the foraging trails of the 2 colonies had been observed to meet during the previous day before the collection of ants (Gordon 1992; Gordon and Kulig 1996). The mean ( $\pm$ SD) distance between pairs of neighboring colonies was  $12.6 \pm 2.9$  m. Pairs of colonies were considered to be distant if they were at least 50 m apart and there was at least one other colony in between them, so that the probability of encounter was extremely low (Gordon and Kulig 1996).

Behavioral assays were performed using 10–16 individuals from each colony. The 2 groups of ants from different colonies were placed in a  $192 \times 137 \times 90$  mm plastic nest-box (Tri-State Plastics, Dixon, Kentucky) with fluon coating the inner walls. Once all ants were in the box, which took up to 10 s, they were allowed to acclimate for 1 min. After 1 min, ants were observed once per minute for 5 min. We recorded the total number of workers from both sides that were involved in aggressive encounters, defined as an interaction of 2 or more ants in which at least 1 ant was engaged in either biting or stinging another ant. Since we measured only biting and stinging, which was clearly aggressive behavior, assays were not performed blind with respect to task group. Once an ant was involved in an aggressive encounter, it usually remained engaged in an aggressive interaction with the same ants for the remainder of the 5-min observation period. Each container was wiped down with ethanol after each assay.

The highest recorded number of ants involved in aggressive encounters for each 5-min assay was used in further analysis. For each assay, we calculated the proportion of ants involved in an aggressive encounter out of the total number of ants present. We used a 2-tailed paired *t*-test in which proportions were arcsine transformed (Zar 1998) to compare incidence of aggression in exterior–interior worker assays. We used generalized linear models with binomial errors and contrasts to compare aggression levels among both exterior–exterior worker assays and neighbor–distant colony assays. All statistical analyses were performed using the statistical package R version 2.13.0.

#### Relationship between differences in HC profile and aggression

To evaluate Newey's (2011) hypothesis that the smaller the difference between the individual and colony-specific odors, the greater the aggression toward non–nest mates, we calculated the Euclidean distances between individual HC profiles

and colony-specific HC profiles for each task group and the Euclidean distances between non–nest mates of the same task group for both foragers and patrollers. We used the principal component scores from the PCA of the methyl HC fraction, because the methyl HC fraction was the only profile that was a significant predictor for colony membership. As a measure of each colony's colony-specific HC profile, we used the centroid of all individual HC profiles for that colony. We calculated the average Euclidean distance between the individual and colony-specific HC profiles and between non–nest mate individual HC profiles for both foragers and patrollers for all colonies and compared them using a 1-way Anova and 2-tailed *t*-test, respectively.

#### Relationship between differences in HC profile and nest proximity

To determine whether the similarity of HC profiles is correlated with nest proximity, 25 workers were haphazardly collected from each of 36 mature colonies (ages 6–18 years;  $N = 900$  workers). Five individuals were pooled to create 1 sample, because pooled samples enhance the recognition of patterns in the colony-specific HC profile (Lavine et al. 2011). There were 5 samples per colony for a total of 180 samples. HC extraction and identification were performed as described above.

We used Spearman's rank correlation test to examine the relation between the Euclidean distances of colony HC profiles and the geographical distances between colony pairs. Euclidean distances between colony-specific HC profiles were calculated using discriminant analysis of 5 principal components from the full HC profile (Manova:  $F_{1,178} = 3.7$ ,  $P = 0.003$ ). The number of principal components used in calculating Euclidean distances was determined as above using the Kaiser criterion. We calculated the average Euclidean distance ( $N = 5$  Euclidean distances per colony pairs) for all colony pairs. Using census data that specifies the location of all colonies at the long-term site (methods in Gordon and Kulig 1996), we calculated for each colony the geographical distance to all others in meters.

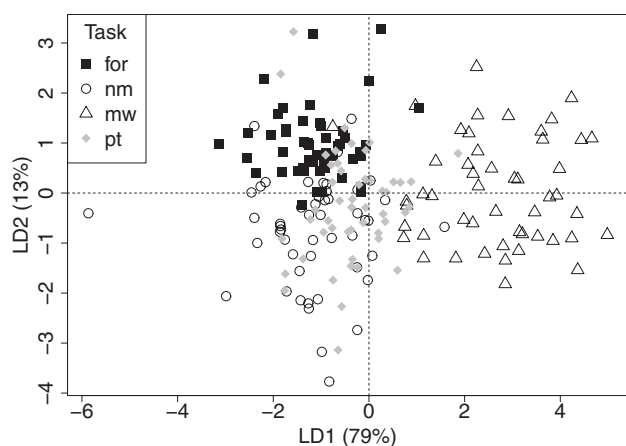
## RESULTS

### Comparison of HC profiles among task groups

Task groups differed in HC profile (Figure 1). Five principal components were chosen for LDA. Midden workers were the first task group to be separated from the 3 other task groups. The first linear discriminant function (LD1) explained 79% of the variance in the data. The HCs with the highest loadings for LD1 in descending order were *n*-hentriacontene, *n*-tricosane, *n*-tetracosane, and *n*-pentacosane. Foragers were the next task group to be separated from the remaining 2 task groups. The second linear discriminant function (LD2) explained 13% of the variance in the data. The HCs with the highest loadings for LD2 were 15,13-,11,9-dimethylhentriacontanes, 7-methylnonacosane, *n*-tetracosane, and *n*-tricosane. Nest maintenance workers and patrollers were the last groups to be separated. The LD3 explained 8% of the variance. The HCs with the highest loadings for LD3 were 7-methylnonacosane, 15,13,11,9-methylhentriacontanes, 17,15,13,11,9,7-methyltrientriacontanes, and 9-methylnonacosane.

### HC profiles as predictors for colony and task group membership

HC profiles were better predictors of task group than colony identity. All combinations of the HC profile were significant predictors for task membership, but only the methyl HC fraction



**Figure 1**  
Discriminant plot of *Pogonomyrmex barbatus* task groups. Linear discriminant analysis was performed on 200 individuals from 4 task groups—foragers, nest maintenance workers, midden workers, and patrollers;  $N = 50$  individuals per task group. The first and second linear discriminant functions, LD1 and LD2, explained 92% of the variance in the data, 79% and 13%, respectively. Black diamonds (for)-foragers, open circles (nm)-nest maintenance workers, open triangles (mw)-midden workers, grey diamonds (pt)-patrollers.

was a significant predictor for colony membership (Table 1). There was no significant interaction between colony and task for any combination of the HC classes (Manova:  $P > 0.05$ ). Moreover, the methyl fraction of the HC profile classified *P. barbatus* workers better by task group than by colony identity. QDA correctly classified 49% of workers to task and 12.5% of workers to colony (Cohen's inter-rater "z" statistic  $>1.96$ ;  $z = 16.9$ ).

### Relationship between task and aggression

#### Exterior and interior worker assays

Exterior workers were significantly more aggressive toward non-nest mate exterior workers than were interior workers toward non-nest mate interior workers (Figure 2; paired  $t$ -test,  $t_{11} = 3.3$ ,  $P = 0.007$ ).

**Table 1**

Comparison of colony and task as grouping variables for all combinations of *Pogonomyrmex barbatus* HC profiles using quadratic discriminant analysis and Cohen's inter-rater agreement

HC profile	Group	Number of HCs <sup>a</sup>	Number of PCs <sup>b</sup>	Manova $P$ values <sup>c</sup>	Percentage CC <sup>d</sup>	Cohen's $z^e$
Full	Colony	21	5	0.29	17%	<b>29.8</b>
	Task	21	5	<b>&lt; 0.001</b>	70.5%	
Alkanes	Colony	6	2	0.39	13.5%	<b>23.2</b>
	Task	6	2	<b>&lt; 0.001</b>	60%	
Methyls	Colony	12	4	<b>0.005</b>	12.5%	<b>16.9</b>
	Task	12	4	<b>&lt; 0.001</b>	49%	
Alkenes	Colony	3	2	0.12	17.5%	<b>2.9</b>
	Task	3	2	<b>&lt; 0.001</b>	35%	
Alkanes + Methyls	Colony	18	4	0.58	17%	<b>32.2</b>
	Task	18	4	<b>&lt; 0.001</b>	73.5%	
Alkanes + Alkenes	Colony	9	3	0.43	11.5%	<b>31.3</b>
	Task	9	3	<b>&lt; 0.001</b>	68%	
Methyls + Alkenes	Colony	15	5	0.14	20.5%	<b>23.6</b>
	Task	15	5	<b>&lt; 0.001</b>	64.5%	

Numbers in bold denote statistical significance.

<sup>a</sup>Number of hydrocarbons in each hydrocarbon profile.

<sup>b</sup>Number of principle components used in the discriminant analysis.

<sup>c</sup>Manova  $P$  values for the principle components for both colony and task.

<sup>d</sup>The percentage of correctly classified workers to colony and task.

<sup>e</sup>Cohen's  $z$  value for comparing classification of colony and task.

#### Forager and patroller assays

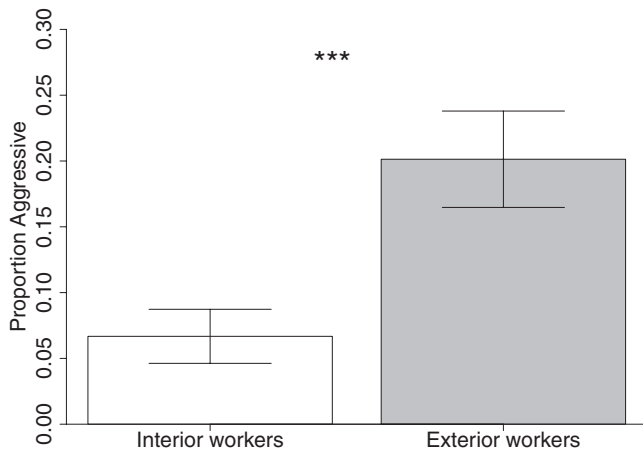
Patrollers were more aggressive toward non-nest mates than were foragers (Figure 3; generalized linear model:  $F_{6,77} = 18.0$ ,  $P < 0.001$ ). There was no significant difference in aggression between non-nestmate foragers (forager treatment), foragers and non-nestmate patrollers (patroller-forager treatment), and forager and nestmate controls (control treatments). The only treatments that differed from controls were the non-nestmate patroller and patroller-forager treatments (contrasts with Bonferroni corrections; patroller and patroller-forager treatments,  $P < 0.001$ ). In the patroller-forager treatment, patrollers were significantly more aggressive to non-nestmate foragers than foragers were to non-nestmate patrollers (contrast with Bonferroni correction;  $P < 0.001$ ).

#### Neighbor and distant colony assays

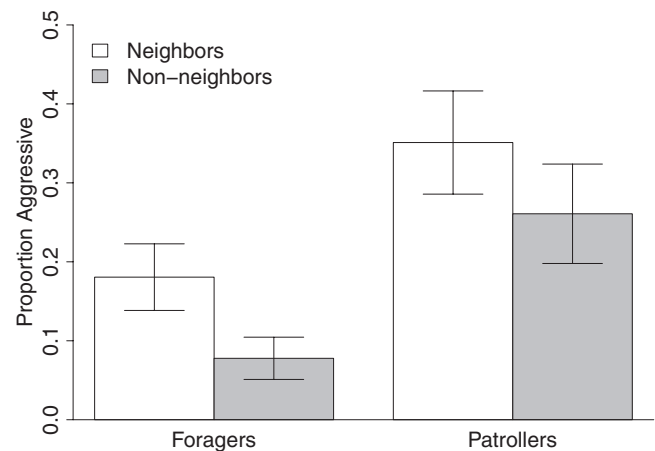
Aggression was more likely between foragers and patrollers of neighboring colonies than between those of distant colonies, but this interaction was not significant (Figure 4; generalized linear model:  $F_{1,20} = 3.4$ ,  $P = 0.07$ ). There was a significant difference between the overall aggression levels of foragers and patrollers (generalized linear model:  $F_{1,20} = 11.7$ ,  $P = 0.002$ ); patrollers were more aggressive. There was no interaction between task and distance between nests (generalized linear model:  $F_{1,20} = 0.01$ ,  $P = 0.90$ ).

### Relationship between differences in HC profile and aggression

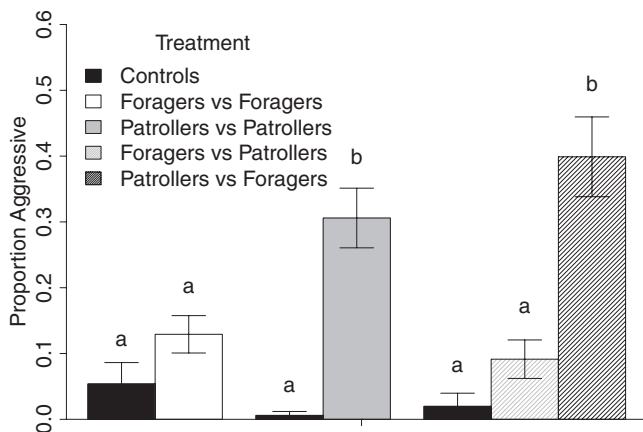
Aggressive behavior was not associated with the distances between individual and colony-specific HC profiles, or with the difference in HC profiles between non-nest mates. Considering only the methyl component of the HC profile, because that component was the best predictor of colony differences, all task groups were similar in the distances between individual and colony-specific HC profiles. The mean ( $\pm$ SD) Euclidean distances were 2.0 ( $\pm 0.2$ ), 2.2 ( $\pm 0.2$ ), 2.5 ( $\pm 0.2$ ), and 2.3 ( $\pm 0.1$ ) for patrollers, foragers, midden, and nest maintenance workers, respectively; these were not significantly different (1-way Anova,  $F_{3,196} = 1.3$ ,  $P = 0.28$ ).



**Figure 2**  
Comparison of aggression toward non-nest mates in exterior and interior workers. Each bar shows the mean proportion of ants engaged in aggressive encounters during nestmate recognition trials;  $n = 12$  trials per treatment. \*\*\* $P < 0.001$ , paired  $t$ -test. Error bars represent the standard error of the mean.



**Figure 4**  
Comparison of aggression between foragers and patrollers of neighboring and distant colonies. Each bar shows the mean proportion of ants engaged in aggressive encounters during nestmate recognition trials;  $n = 6$  trials per treatment. Open bars—neighbors, filled bars—non-neighbors. Error bars represent the standard error of the mean.



**Figure 3**  
Comparison of aggression toward non-nest mates in foragers and patrollers. Each bar shows the mean proportion of ants engaged in aggressive encounters during nestmate recognition trials;  $n = 12$  trials per treatment. Lowercase letters indicate significant differences. Error bars represent the standard error of the mean.

We tested for aggression between non-nest mates in patrollers and foragers. Although patrollers were more aggressive toward non-nest mates than foragers, there was no difference in the distance between individual and colony-specific HC profiles for foragers and patrollers (unpaired  $t$ -test:  $t_{98} = 0.5$ ,  $P = 0.61$ ), and non-nestmate patroller HC profiles were more similar to each other than non-nestmate forager HC profiles (unpaired  $t$ -test:  $t_{2245} = 4.0$ ,  $P < 0.001$ ). The mean ( $\pm$ SD) Euclidean distances from non-nest mates of the same task were 2.8 ( $\pm 0.1$ ) and 3.1 ( $\pm 0.1$ ) for patrollers and foragers, respectively.

#### Relationship between differences in HC profile and nest proximity

Differences in the HC profiles of colony pairs were not related to the geographical distances between nests (Spearman's rank correlation,  $\rho = 0.02$ ,  $N = 630$ ,  $P = 0.56$ ).

#### DISCUSSION

Our results show that aggression is clearly task dependent, but not related to differences in HC profiles. There is no evidence for an effect on aggression of the similarity between an individual's profile and that of its colony, therefore our data does not support Newey's (2011) model. HC profiles are more representative of task, across all colonies, than of colony membership. Differences between non-nest mates in HC profiles were not related to aggression or nest proximity. Aggression toward non-nest mates is more likely in patrollers than in other task groups.

We found clear task-specific HC profiles (Figure 1), as in other studies of ants (Bonavita-Cougourdan et al. 1993; Kaib et al. 2000; Martin and Drijfhout 2009a). Such distinct and conserved differences in HC profiles among task groups reflect the influence of task environment on HCs (see Wagner et al. 2001). The task-specific profiles we found for 3 task groups, foragers, patrollers, and nest maintenance workers, are identical to those previously reported (Wagner et al. 1998). Ants use task-specific HC profiles to assess the task of the ants they meet (Greene and Gordon 2003a), and these cues function in task allocation (Gordon and Mehdiabadi 1999; Greene and Gordon 2007b); for example, contact with patroller HCs initiates foraging each day (Greene and Gordon 2003b). Further work is needed to ascertain whether changes to cuticular HCs are caused by structural chemical changes on the cuticle or physiologically mediated production changes within the body of the ant (Sturgis and Gordon, unpublished data) and how genetic factors contribute to colony-specific HC profiles (Sturgis and Gordon, unpublished data).

Midden workers had the most distinct HC profile of any task group. Midden workers arrange pebbles and other midden material, which contain colony-specific HCs that deter non-nest mates from the nest mound and guide foragers to the nest entrance (Sturgis et al. 2011). Midden workers have a higher relative concentration of alkenes than any other task group. Further work is needed to determine whether the task environment of midden workers influences their HC profiles.

Foragers differed from other task groups mainly in methyl-branched HCs. This probably reflects the lower relative concentration of HCs on the cuticle of foragers as compared with

workers in other task groups. Foragers make long trips outside the nest during which they are exposed to UV light, high desert temperatures, and high relative humidity. They have a higher concentration of cuticular alkanes, the HCs responsible for desiccation prevention (Gibbs 1998; Gibbs et al. 2003), than any other task group, and have fewer methyl-branched HCs, relative to alkanes, than task groups that spend more time inside the nest (Wagner et al. 1998).

Patrollers and nest maintenance workers were similar in HC profiles. Both patrollers and nest maintenance workers spend much of their time inside the nest. Both task groups are most active in the early morning (Gordon 1986). Exposure to similar environmental conditions may lead to similarity in HC profiles.

It is surprising that HC profiles are more similar within task groups, across colonies, than within colonies (Table 1). The low prediction ability of the QDA to correctly predict task membership using methyl HCs is likely due to the fact that other HC classes, including methyls, are involved in discriminating among workers of different task groups. It is also possible that HCs may not be the only chemical cues used in nestmate recognition; environmental cues may be important (Katzav-Gozansky et al. 2004, 2008; Errard et al. 2008). Using the HC profiles of individuals, it was difficult to predict colony membership (as in Lavine et al. 2011). The methyl-alkane fraction of the HC profile was the only significant predictor for colony membership (Table 1). Several social insect species use complex HCs such as methyls as their nestmate recognition cues (Dani et al. 2001; Akino et al. 2004; Dani et al. 2005; Martin et al. 2008). *n*-Alkanes are typically associated with task and social status within colonies (Wagner et al. 2001; van Zweden and d' Ettore 2010). Ants may use methyl-branched HCs to differentiate between nestmates and non-nest mates because these convey information in both the length of the chain of carbon atoms and the position of the methyl group, whereas saturated HCs such as alkanes have only the former (van Zweden and d' Ettore 2010).

Overall, we found that the profiles of individuals, which are what an ant detects when it encounters another, vary greatly. Most previous studies of colony-specific odor have used group samples, and further work is needed to determine the extent of individual variability in HC profiles in nestmates. The gas chromatography methods we used may not have detected some HCs because of the small quantity of HCs in individual samples. However, HCs are collinear in nature and correlate with other HCs within the same class (Martin and Drijfhout 2009b), so even if some peaks were missing, they would probably have been represented by other HCs in our data set.

Aggression toward non-nest mates differed among task groups. Exterior workers were more aggressive to non-nest mates than interior workers (Figure 2), and only patrollers showed significant aggression toward non-nest mates, including encounters with ants from different task groups (Figure 3) and non-neighboring colonies (Figure 4). In assays with non-nestmate foragers, patrollers often initiated aggression. Only patrollers and foragers, who travel away from the nest, encounter ants from other colonies (Gordon 1992; Gordon and Kulig 1996; Adler and Gordon 2003); patrollers as they inspect the foraging areas and foragers as they search for food. Patrollers, which were significantly more aggressive than foragers, are the task group most likely to engage in crucial interactions with ants of other colonies. Foragers avoid confrontation with neighboring foragers (Gordon 1989b), whereas patrollers respond to disturbances (Gordon 1987) and determine forager direction (Gordon 1991), apparently in response to encounters with non-nest mates.

Surprisingly, the extent of aggression was not related to differences in HC profiles or the degree to which an ant's individual profile differs from its colony profile. Although patrollers were more aggressive than any other task group, they were more similar to non-nest mates of the same task than were foragers, which were much less aggressive toward non-nest mates. In addition, there was no difference in the distances between patroller and forager individual profiles and their colony profiles. In interactions between patrollers and foragers, there was no difference in the amount of aggression in patroller-patroller and patroller-forager interactions, even though the HC profile differences were much greater in the patroller-forager treatment than in the patroller treatment.

Newey (2011) hypothesized that the smaller the difference between the individual and colony-specific odors, the greater the aggression toward non-nest mates. Our results do not support this model. Patrollers were significantly more aggressive than foragers to non-nest mates, but there was no difference between these 2 task groups in the distance between the individual and colony HC profiles. Newey's model would predict that foragers and patrollers would be equally aggressive to non-nest mates. However, aggression tests may not reflect discrimination. It appears that foragers do discriminate between nestmates and non-nest mates because they avoid non-nestmate foragers from neighboring colonies on overlapping foraging trails (Gordon 1989b). It may be that an ant's ability to discriminate the odor of non-nest mates does depend on the variance in individual profiles around the colony odor, but that unlike patrollers, foragers of *P. barbatus* do not display discrimination by acting aggressive.

Both patrollers and foragers were slightly more aggressive toward ants from neighboring colonies than from distant ones, but the difference was not significant. Patrollers were significantly more aggressive to both workers from neighboring and distant colonies than were foragers. There may be a slight "nasty neighbor" effect; however, there was no relationship between colony HC profile differences and nest proximity. We may have lacked the power to definitively state whether there is or is not a "nasty neighbor" effect. It would be interesting to examine further the causes of *P. barbatus* workers' reactions to neighbors.

Aggression in *P. barbatus* is task dependent and is determined by factors other than differences in HC profiles. Ants switch tasks according to colony needs, and for some species this may be age dependent (Calabi and Traniello 1989; Seid and Traniello 2006). It is unlikely that age explains task-related differences in aggression in *P. barbatus* because patrollers and foragers are probably similar in age (Gordon 1989a), but differ greatly in aggression. Neurophysiological differences among individuals within a colony, resulting from environmental conditions associated with task, age, and experience, result in behavioral differences among individuals (Gronenberg et al. 1996; Muscedere and Traniello 2012) and may lead to differences in aggression among task groups.

The relation of aggression, task, and HC profiles reflects the ecology of this harvester ant species. Colonies compete with each other for foraging area (Gordon 1992; Adler and Gordon 2003). Patrollers defend the nest and prevent conflict with neighbors, by setting foraging direction and allowing foragers to avoid confrontation with neighboring colonies (Greene and Gordon 2007a). Fighting among neighboring colonies is rare (Gordon and Kulig 1996). It appears that colonies have allocated a particular task group to react aggressively to conspecifics in certain contexts, but not to respond according to the magnitude of difference in HC profiles.

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