

Variation in spatial scale of competing polydomous twig-nesting ants in coffee agroecosystems

K. A. Mathis^{1,2} · S. M. Philpott³ · S. R. Ramirez⁴

Received: 30 November 2015 / Revised: 16 March 2016 / Accepted: 25 April 2016 / Published online: 7 May 2016
© International Union for the Study of Social Insects (IUSSI) 2016

Abstract Arboreal ants are both highly diverse and ecologically dominant in the tropics. This ecologically important group is particularly useful in ongoing efforts to understand processes that regulate species diversity and coexistence. Our study addresses how polydomy can influence patterns of nest occupation in competing arboreal ants. We examined the spatial structure of nest occupation (nest distance, abundance and density) in three polydomous co-occurring twig-nesting ant species (*Pseudomyrmex simplex*, *P. ejectus* and *P. PSW-53*) by mapping twigs occupied by ants from each species within plots in our study site. We then used two colony structure estimators (intraspecific aggression and cuticular hydrocarbon variation) to determine the relative degree of polydomy for each species. All work was conducted in coffee agroforests in Chiapas, Mexico. Our results revealed that the two species with highest abundance and nest density were also highly polydomous, where both species had either single or

multiple non-aggressive colonies occupying nests on a large spatial scale (greater than the hectare level). Our results also indicate that the species with the lowest abundance and density is less polydomous, occupying several overlapping and territorial colonies at the hectare level in which multiple colonies never co-occur on the same host plant. These results contribute evidence that successful coexistence and highly polydomous colony structure may allow ants, through reduced intraspecific aggression, to successfully occupy more nests more densely than ant species that have multiple territorial colonies. Furthermore our study highlights the importance of considering intraspecific interactions when examining community assembly of ants.

Keywords Polydomous ants · Social organization · Intraspecific interactions · *Pseudomyrmex* · Cuticular hydrocarbons · Aggression assays

Electronic supplementary material The online version of this article (doi:10.1007/s00040-016-0489-8) contains supplementary material, which is available to authorized users.

✉ K. A. Mathis
kmathis@email.arizona.edu; kamathis@berkeley.edu

¹ Department of Environmental Science, Policy, and Management, University of California, Berkeley, 130 Mulford Hall, Berkeley, CA 94702-3114, USA

² Present Address: Department of Ecology and Evolutionary Biology, University of Arizona, 1041 East Lowell St, Tucson, AZ 85721, USA

³ Environmental Studies Department, University of California, Santa Cruz, 1165 High St, Santa Cruz, CA 95064, USA

⁴ Department of Evolution and Ecology, University of California, Davis, 2320 Storer Hall, 1 Shields Ave, Davis, CA 95616, USA

Introduction

Arboreal ants are some of the most numerically dominant arthropods in the tropics largely due to their ability to access plant resources in the form of extrafloral nectar and hemipteran honeydew (Hölldobler and Wilson 1990; Davidson 1997; Bluthgen et al. 2000; Davidson et al. 2003). As such they are an ideal group of insects to study patterns of species distribution and community assembly. Moreover, understanding the distribution patterns of ant communities is critical as ants are frequently involved in ecosystem services. For example, ants are common seed dispersers, pollinators, and often engage in plant protection mutualisms that benefit agricultural systems (Dejean et al. 2007; Liere and Larsen 2010; Vandermeer et al. 2010; Mathews et al. 2011, De Vega et al. 2014). Early research on arboreal ants

suggested that the structure of ant assemblages is largely determined by interspecific competitive interactions (Room 1971; Majer 1972; Majer et al. 1994), but this notion is now widely criticized (Ribas and Schoereder 2002; Blüthgen and Stork 2007; Sanders et al. 2007). Recent work has focused on how interactions with resources can shape the structure of ant assemblages (Davidson 1997; Blüthgen et al. 2004; Blüthgen and Stork 2007; Powell et al. 2011; Dejean et al. 2008, 2015; Jiménez-Soto and Philpott 2015). However, particularly in studies with cavity-dwelling ants where polydomy is common, colony scale and intraspecific aggression are infrequently examined.

Ants display high levels of diversity in spatial organization strategies and colony size, ranging on a continuum from establishment of a single nest (monodomy) to colonies that establish multiple nests (polydomy) in a given area (Debout et al. 2007). Polydomy as a nesting strategy has many benefits including increased foraging area, foraging efficiency, and brood rearing capacity when nesting sites are spatially finite (reviewed in Debout et al. 2007), as well as a reduction in costly energy expenditure on territorial fights (Holway 1998; Holway et al. 2002; Sanders et al. 2003). However, having spatially separated nests can impose costs, including queen-worker conflicts over both resource and sex allocation (Herbers et al. 2001) and could also hamper colony recognition among colony members that occupy distant nests (Vander Meer and Morel 1998).

Estimating colony size and structure in polydomous ants is notoriously difficult, particularly when nest sites are ephemeral and often the only method to determine colony size is by observing trails between nesting sites (e.g. Debout et al. 2007; Buczkowski 2011); however such trails are rare or difficult to follow for many twig nesting ants. Social insects use low-volatility chemical cues found on their cuticle (generally hydrocarbons) to distinguish colony members from non-colony members (Vander Meer and Morel 1998; Howard and Blomquist 2005). Nestmates share a common chemical signature through a combination of genetic similarity and the colony members creating an admixture through physical contact, social grooming, and trophallaxis (Crozier and Dix 1979; Foitzik et al. 2007). If an ant's chemical signature deviates from the admixed template (i.e. chemotype), the individual is then recognized as a non-colony member and frequently attacked (Tsutsui 2004). High cuticular hydrocarbon similarity is typically associated with low levels of aggression (e.g. Suarez et al. 2002; Torres et al. 2007). Furthermore, cuticular hydrocarbons are also species and caste specific (Howard and Blomquist 2005). Thus, aggression assays and chemical analysis of cuticular hydrocarbons are useful tools in assessing the colony structure of ants (Vander Meer and Morel 1998; Howard and Blomquist 2005).

In coffee agroecosystems in southern Mexico, a diverse community of arboreal ants nest in hollow twigs on coffee plants (Philpott and Armbrrecht 2006; Livingston and Philpott 2010). The hollow coffee twigs are a naturally occurring, ephemeral and limited resource in the ecosystem (Philpott and Foster 2005; Jiménez-Soto and Philpott 2015). Furthermore, interspecific aggression, while present at varying degrees between species, does not impact the structure of ant assemblages in this system (Livingston and Jackson 2014). Here we investigate the colony spatial structure of three sympatric species of polygynous, polydomous *Pseudomyrmex* ants (*Pseudomyrmex simplex*, *Pseudomyrmex ejectus*, and an un-described *Pseudomyrmex* sp. hereafter referred to as *Pseudomyrmex* PSW-53) that occupy twig-nests and compare the nesting strategies of these species.

This study uses a robust approach, combining spatial mapping of nests, aggression assays and cuticular hydrocarbon analysis of three sympatric polydomous ant species in the genus *Pseudomyrmex* to assess and compare social association between nests. This is the first study to use these approaches to examine the social structure of polydomous twig-nesting ants. Furthermore, the use of multiple approaches to estimate social association also makes it possible to assess the reliability of each method in evaluating colony spatial structure. Our findings highlight the importance of investigating colony spatial structure of polydomous ants to fully understand community dynamics.

Methods

Sites and study system

We sampled ants on Finca Irlanda, a 300 ha, shaded coffee plantation in the Soconusco region of Chiapas, Mexico (15°11'N, 92°20'W). The plantation is located between 950–1150 m elevation and receives approximately 4500 mm of rain each year. The farm contains approximately 100 species of shade trees, the most common being trees in the genus *Inga* (Vandermeer et al. 2008). Canopy cover at the time of ant sampling ranged from 35 to 70 % in the 45 ha area and was around ~90 % in the 7 ha area.

In the study site, there are at least 60 arboreal ant species including 40 that nest in dry, hollow coffee twigs (Philpott and Foster 2005; Livingston and Philpott 2010). Heavy rains, natural processes of decomposition, and farm workers moving through the coffee plantations frequently break these twigs from the coffee plants, making the nesting sites ephemeral. There are nine species of *Pseudomyrmex* that nest in coffee twigs, and the three most common at the study site are *P. simplex* (occupies ~35 % of all occupied coffee twigs), *P. ejectus* (~20 % of twigs), and *P. PSW-53* (~6 % of twigs) (Philpott and Foster 2005). *Pseudomyrmex simplex*

range from Florida to Paraguay and are commonly collected from forest habitats (AntWeb 2015). *Pseudomyrmex simplex* nests are commonly found without queens, suggesting a high level of polydomy (Ward 1985). *Pseudomyrmex ejectus* are also commonly found in forests and coffee plantations, and range from Florida to Costa Rica (Ward 1985; AntWeb 2015). *Pseudomyrmex* PSW-53 have been collected from Baja California to Costa Rica, and commonly inhabit forests and woodlands (AntWeb 2015). The majority of nests collected for this study were queenless, accounting for 76 % of *P. simplex* nests ($n = 143$), 66.1 % of *P. ejectus* nests ($n = 96$), and 65.0 % of the *P. PSW-53* nests ($n = 92$). These three ant species differ in their abilities to gain access to nesting sites. In nesting colonization trial experiments *P. simplex* won 37 % of paired trials with other twig-nesting species, *P. ejectus* won 24 % of trials, and *P. PSW-53* won 70 % of nest competition trials (Yitbarek and Philpott, unpublished data). We chose to work with these three species because of the differences they exhibit in their relative abundance in dry, hollow coffee twigs, and also because of their different apparent success in winning nest sites in competitive encounters.

Twig nesting ant survey

During 2013 we surveyed twig-nesting ants in randomly selected 20×20 m plots within established permanent plots at the study site (Fig. 1). We sampled twig-nesting

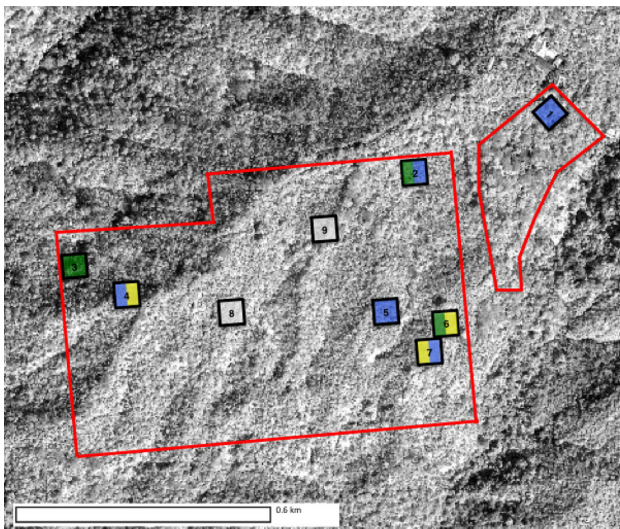


Fig. 1 Satellite image of the study areas. Red boxes are around the larger 45 ha area (left) and smaller 7 ha area (right). Smaller black boxes represent the plots where ants from each of the three species were sampled. Boxes are numbered according to plot number. Plots are shaded according to the species sampled there during the twig nesting ant survey (*P. simplex* = yellow, *P. ejectus* = green, *P. PSW-53* = blue). Ants from plots 8 and 9 were only used in the aggression assays and are therefore not shaded (color figure online)

ants on every coffee plant (~ 100 – 250 coffee plants) within each 20×20 m plot and removed all dry twigs, counted hollow and occupied twigs and identified all occupant ants. The twig nests are most easily sampled destructively by breaking into the twigs to determine their occupants (Philpott and Foster 2005). We mapped the coordinates of each coffee plant within 20×20 m plots, and noted the plot location within each sampled hectare. We took all twigs occupied by *P. simplex*, *P. ejectus*, and *P. PSW-53* back to the lab where we freeze-killed the ants and placed them at -18 °C until extraction.

Cuticular hydrocarbon collection and analysis

For each twig that contained at least three ant workers ($n = 197$), we collected three individuals for CHC extraction (270 ants for *P. simplex*, 207 ants for *P. ejectus* and 114 ants for *P. PSW-53*). Each ant was freeze-killed and submerged in 100–200 μ L of hexane for 10 min. The ants were then removed, the hexane was evaporated, and the samples were stored at -18 °C until they were transported to the lab. Once in the lab, samples were placed at -20 °C until processing. Directly before processing, we resuspended each sample in 200 μ L of hexane. We filtered samples through a 1.5 cm hexane-rinsed silica column to remove non-hydrocarbon material and flushed the column with 300 μ L of hexane to maximize sample recovery. We then used nitrogen gas to reduce our samples to a 60 μ L volume. To analyze the samples, we injected 2 μ L of the 60 μ L into the GC/MS.

For each sample, we acquired electron impact mass spectra (70 eV) with an Agilent 5975 C mass selective detector interfaced to an Agilent 7890A gas chromatograph fitted with a DB-5 column (30 m \times 0.32 mm i.d., Agilent Technologies). Extracts were analyzed in splitless mode, with a temperature program that started at 100 °C for 1 min, then increased by 20 °C min^{-1} until it reached 150 °C, and then increased by 5 °C min^{-1} until it reached 325 °C where it was held for 5 min. Injector and transfer line temperatures were kept at 325 and 280 °C respectively. We used MSD ChemStation Software for Windows (Agilent Technologies, Palo Alto, CA, USA) for data acquisition.

Aggression assays

We collected ants from twig nests for aggression trials during June 2015. As before, we collected ants from coffee plants within 20×20 m plots in different hectares within the permanent plots. Sample plots were separated by a minimum of 100 m (see Table S1 for exact distances between each sampled hectare). We surveyed areas of the farm that had been surveyed in 2013 to locate nests of *P. simplex*, *P. ejectus*, and *P. PSW-53*, but were not able to

locate each of the three species in the same locations, and thus added two additional sample plots (8 and 9, Fig. 1). *P. simplex* and *P. ejectus* were both collected from plots 2, 4, 6, 7, and 8. *P. PSW-53* were collected from plots 6, 8, and 9 (Fig. 1). Occupied twigs were placed individually into ziplock bags and bags were marked with *x*, *y* coordinates within the 45 ha plot. We did not measure the size of each coffee plant sampled, or the height from which ants were collected. However, most coffee plants in this farm that have dry twigs are between 1.0–1.5 m in height. Once back in the lab, twigs (and all ant occupants) were placed into plastic tubs and kept until ant aggression was tested (a maximum of 4 days).

We tested ant aggression by placing two individual ants of the same species in a petri dish (3.5 cm diameter, 1 cm high). We tested aggression in four combinations increasing from small to large scale differences—(1) ants from the same twig, (2) ants from two twigs on the same coffee plant, (3) ants from different coffee plants (but from the same 20 × 20 m plot), and (4) ants from different hectares (>100 m apart). Ant interactions were observed in petri dishes for 5 min. We recorded the highest of the following levels of aggression to take place: touch (i.e. antennation or other non-aggressive contact, scored as 1, avoid (i.e. mandible flair where one ant moves towards the other while opening the mandibles or one or both ants recoil and retreat in the opposite direction, scored as 2, bite (i.e. one or both ants bite each other during a <30 s attack, scored as 3, aggression (i.e. >30 s attack that may involve removing leg or antenna, scored as 4, and kill (i.e. one ant kills another, scored as 5 (modified from Suarez et al. 1999 and Torres et al. 2007). We only observed behaviors 1–4. To minimize observer bias, blinded methods were used when all behavioral data were recorded and analyzed.

Data analysis

For the twig nesting survey, ant abundance in the study area was measured by total number of twigs occupied by the ants and the percentage of occupied twigs occupied by the species. Density of each species was measured as the inverse of the average distances (in meters) between each occupied twig and its closest conspecific neighbor.

All compounds in the chromatograms identified as cuticular hydrocarbons were included in the analysis. Quantitative comparisons of spectra were based on peak integration of total ion chromatograms. For each sample, we only included compounds with >1 % total abundance. When comparing species as a whole, only compounds with an average of >1 % total abundance were included after standard deviation was added. All subsequent analyses were performed after peak areas were standardized to relative proportions. Individual hydrocarbon peaks were identified

through a combination of (1) comparing retention times and mass spectra with those of synthetic standards to determine carbon chain length and calculate Kovat Indices following Katritzky et al. (2000), (2) matching our spectra with those previously published and (3) studying fragmentation patterns. To determine whether species grouped by chemotype, we used Ward's hierarchical cluster analysis, and assessed the uncertainty of clusters using multiscale bootstrap resampling (R Development Core Team 2013, pvclust). For each species, we used the entire data matrix containing all the compounds present in their workers for each hectare. All cuticular hydrocarbon compounds were included in the analysis, as previous experiments show that excluding compounds may increase error (Krasnec and Breed 2013). Chemotypes were divided based on statistically significant clusters (Ward's hierarchical cluster analysis), and individuals were assigned to each chemotype if the majority (two out of three) of the twig-mates were assigned to a particular cluster.

For aggression assay analysis we examined whether variation in aggressive behavior correlated with increasing distance between nests. To this end, we compared the aggression score for worker–worker interactions within twig, within plant, within 20 × 20 m plot, and between hectares. We used a two-way ANOVA with AI as the dependent variable, and ant comparison (within twig, within plant, within 20 × 20 m plot, and between hectares) and ant species (*P. simplex*, *P. ejectus*, and *P. PSW-53*) as factors.

Results

Twig nesting ant survey

Overall, the three species of ants differed in density and abundance within the sampling area. We found that *Pseudomyrmex simplex* occupied the most available nesting sites ($n = 214$ or 36 %), followed by *Pseudomyrmex ejectus* ($n = 104$ or 17 %) and *Pseudomyrmex PSW-53* ($n = 43$ or 7 %). *P. simplex* nests were also in the highest density (1.528), followed by *P. ejectus* (0.814) and *P. PSW-53* (0.164) respectively (Table 1).

Cuticular hydrocarbon identification and analysis

For the three species, *P. simplex*, *P. ejectus*, and *P. PSW-53*, we identified 18, 7, and 12 hydrocarbons (or combinations of co-eluting hydrocarbons) respectively, each representing at least 1 % of the total area of all compounds (Table 2). Compounds consisted of straight chain alkanes, mono-methyl alkanes and some multimethyl alkanes. Compounds had chain lengths ranging from 20 to 36 carbons, with *P. simplex* containing, on average, compounds with longer

Table 1 Summary of abundance, density and competitive ability of each of the three species of *Pseudomyrmex*

Species	Abundance	Density	Competitive ability ^a
<i>P. simplex</i>	215 (36 %)	1.528	37.21 %
<i>P. ejectus</i>	105 (17 %)	0.814	23.94 %
<i>P. PSW-53</i>	43 (7 %)	0.164	69.23 %

Abundance is measured by total number of twigs occupied by this species in our study and in parentheses the percentage of occupied twigs occupied by the species. Density was measured as the inverse of the average distances between each twig and its closest conspecific neighbor. The competition score is percent success in competition trials

^a From Yitbarek and Philpott, in review

Table 2 Summary of compounds present in three species of *Pseudomyrmex* ants with average abundances

No.	Compound ID	<i>P. simplex</i>	<i>P. ejectus</i>	<i>P. PSW-53</i>
1	C20	0.84 ± 0.49	0.93 ± 1.06	
2	mix of 8me and 10me C22		1.16 ± 1.16	
3	mix of 11me and 13me C23			1.22 ± 1.87
4	3me C23			0.90 ± 1.56
5	C25	1.87 ± 6.90	47.13 ± 14.65	8.19 ± 9.46
6	mix of 9me, 11me, 13me, and 15me C25			15.07 ± 11.03
7	3me C25			5.48 ± 4.79
8	mix of 9me and 11me C25		2.69 ± 1.41	
9	C26			1.00 ± 0.44
10	C27	10.73 ± 6.62	30.46 ± 9.41	8.51 ± 4.43
11	mix of 9me, 11me, 15me, and 17me C27	6.83 ± 3.09		
12	Mix of 13me and 15me C27			29.94 ± 16.45
13	3me C27	3.30 ± 2.65	2.67 ± 3.97	5.84 ± 4.39
14	C28	2.06 ± 1.27		
15	(3,7) dime C27			1.03 ± 0.78
16	mix of 10me and 12me C28	2.59 ± 1.23		
17	C29	5.91 ± 5.33	2.05 ± 1.94	
18	mix of 11me, 13me, and 15me C29	21.29 ± 11.98		13.76 ± 10.33
19	mix of (11, 15) dime C29, (7,11) dime C29 and 3me C29	5.63 ± 3.58		
20	(11,13,15) trime C29	1.09 ± 0.86		
21	C30	1.96 ± 1.23		
22	mix of 11me, 13me, 15me, and 17me C31	8.57 ± 3.99		
23	(11, 13) dime C30			1.16 ± 2.30
24	mix of (13, 15) dime C30, 3me C31, and 5me C31	4.75 ± 2.59		
25	mix of 11me, 13me, 15me, and 17me C34	3.58 ± 2.50		
26	(11, 13) dime C34	4.74 ± 4.17		
27	mix of 11me, 13me, 15me, and 17me C36	1.02 ± 1.10		
28	(15,17) dime C36	1.99 ± 2.36		

Percentages indicate the average relative proportion of each compound, as determined by the area under each peak in the chromatogram divided by the sum of all peaks, ± Standard Deviation

carbon chains than *P. ejectus* and *P. PSW-53*. Three compounds (C25, C27, and 3me C27) were found in all three species (Fig. 2).

All three *Pseudomyrmex* species exhibited significantly different cuticular hydrocarbon profiles that clustered independently of one another (Ward's hierarchical cluster

analysis, $P = 0.04$). *P. simplex* and *P. ejectus* did not reliably cluster into chemotypes beyond the species level, either within or between hectares. However, *P. PSW-53* individuals exhibited substantial spatial variation both within and between hectares (Fig. 3). Individual ants of *P. PSW-53* divided initially into two primary clusters ($P < 0.05$) and

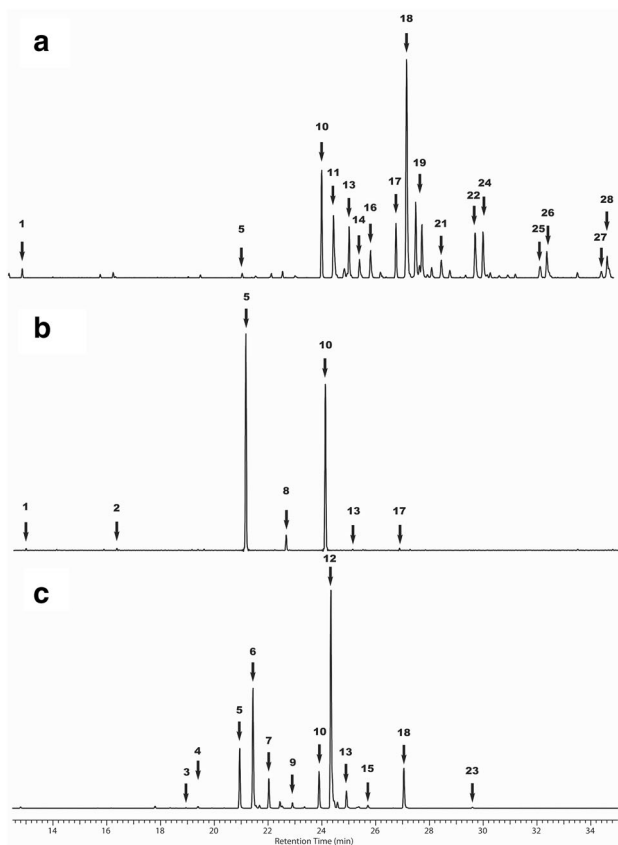


Fig. 2 Representative chromatograms of the three species of *Pseudomyrmex*. **a** *Pseudomyrmex simplex*, **b** *Pseudomyrmex ejectus*, **c** *Pseudomyrmex* PSW-53. Peak numbers refer to compound numbers found in Table 1

further divided into five distinct secondary clusters ($P < 0.05$, Fig. 4). Furthermore, although some coffee plants did contain multiple *P.* PSW-53 nests, individual coffee plants did not support more than one *P.* PSW-53 chemotype. In other words, all ants from twigs on the same

plant shared the same chemotype. Yet, some ants found in twigs on plants within 1 m of one another had different chemotypes (Fig. 4). At the primary cluster level, all *P.* PSW-53 individuals separate accurately into their clusters. Furthermore, individuals separated into their secondary clusters with 91 % accuracy, which is a similar level of accuracy as that obtained with monodominant, monogynous ant species using CHC profiles of ants known to belong to different colonies (see Krasnec and Breed 2013).

Aggression assays

Overall, aggression levels differed by species, and the intensity of aggression increased with spatial scale for *P.* PSW-53, but not for *P. simplex* and *P. ejectus*. Intraspecific aggression (according to the AI) was significantly higher for *P.* PSW-53 overall compared with *P. simplex* and *P. ejectus* ($F_{2, 125} = 12.32$, $P < 0.001$, pairwise comparisons $P < 0.01$ for both *P. simplex* and *P. ejectus*). Aggression between *P. simplex* and *P. ejectus* individuals did not increase with spatial scale (*P. ejectus*, $F_{3, 6} = 11.41$, $P = 0.99$; *P. simplex*, $F_{3, 6} = 11.41$, $P = 0.98$). However, *P.* PSW-53 aggression increased with spatial scale, where ants from the same twigs and plants were significantly less aggressive than ants interacting from within the plots or between hectares ($F_{3, 6} = 11.41$, $P < 0.01$) (Fig. 5). Thus, *P.* PSW-53 engaged in more aggressive behavior than the other two species, especially at greater distances.

Discussion

Our analysis revealed striking differences in the strategies of social organization of the three species of *Pseudomyrmex* ants that we examined, which appears to be regulated by a combination of spatial, chemical and behavioral parameters.

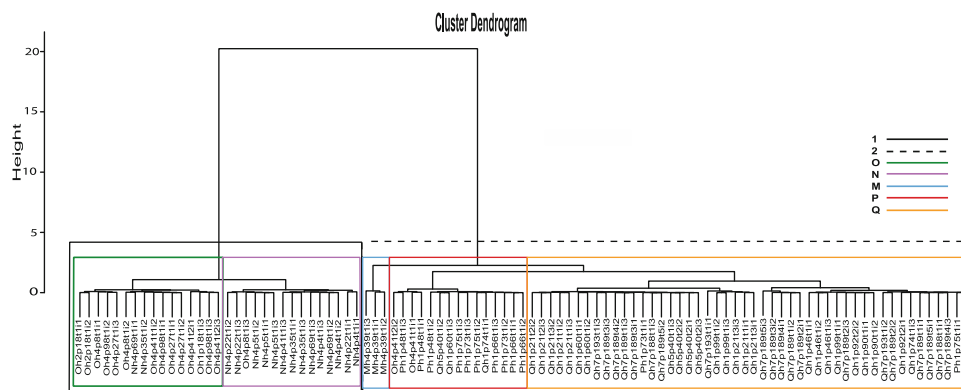


Fig. 3 Ward's hierarchical cluster analysis to assign *Pseudomyrmex* PSW-53 individuals to chemotype using the entire CHC profile. Out of 98 individuals, no individual clustered outside of the first division (clusters 1 or 2). Within all five secondary clusters (O, M, N, P, Q) only

10 individuals clustered outside of the chemotype with their twigmates. Individual specimens are labeled according to (1) secondary cluster (O, M, N, P, Q), (3) plot, (4) plant number, (5) twig number on plant, and (6) individual number within each twig

Fig. 4 Spatial distribution of plants from which ants were sampled in each *plot*. All *plots* are included with the exception of *plot* 5 which only had one plant with *P. PSW-53*. Location of plants occupied by *P. simplex*, *P. ejectus*, and *P. PSW-53* are represented by *circles*, *triangles*, and *squares* respectively. Plot points increase in size based on the number of occupied twigs per plant. Plot points are color-coded based on chemotypes assigned in Fig. 3

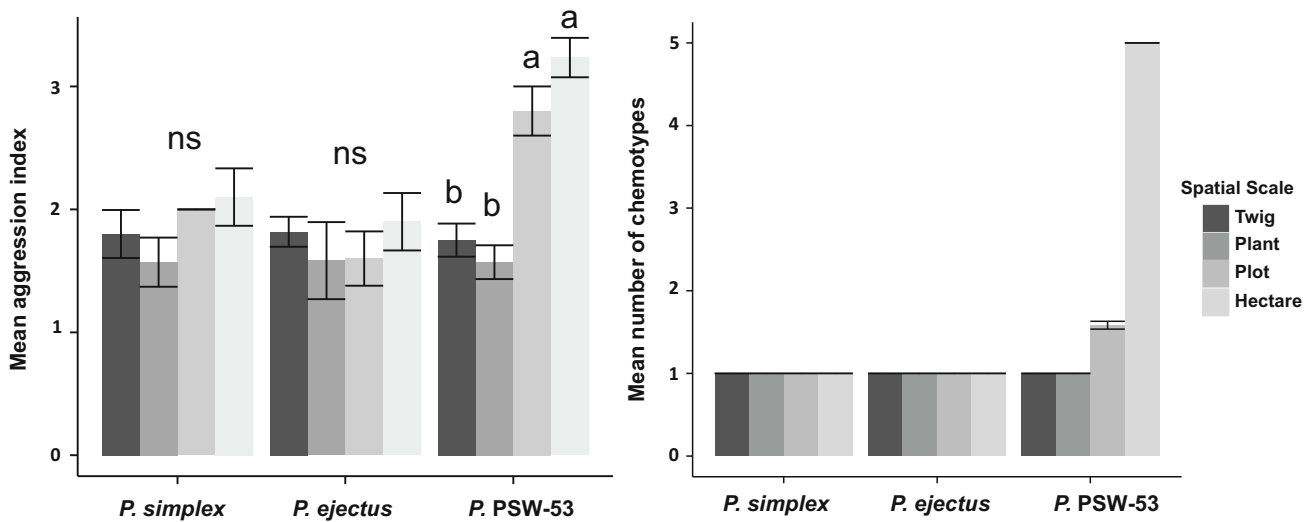
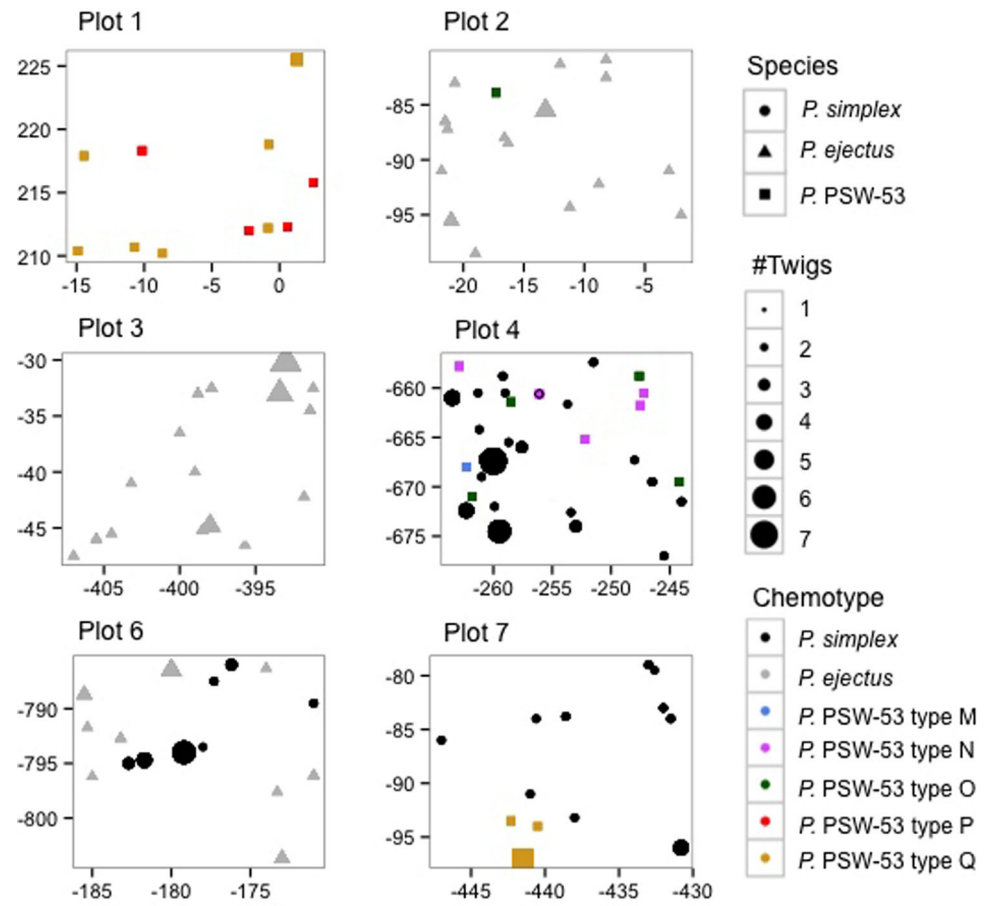


Fig. 5 Barplots of aggression assay results (*left*) and average number of chemotypes per spatial scale (*right*). Spatial scales include: twig (i.e. individuals from the same twig), plant (i.e. individuals from different twigs on the same plant), *plot* (i.e. individuals from different plants in

the *same plot*), and hectare (i.e. individuals from different hectares, >100 m apart). Error bars represent standard error. Small letters show significant differences in aggression between spatial scales ($p < 0.05$)

Ants discriminate between nestmates through antennal contact and perception of cuticular hydrocarbons prior to aggression (Vander Meer and Morel 1998). Both *P. simplex* and *P. ejectus* exhibited cuticular hydrocarbon profiles that remained homogeneous through our entire study area and almost no aggression was observed between ants of these species regardless of distance, thus suggesting that either both species only have one colony within the study area or colonies exhibit non-aggressive behaviors to one another. Further work comparing aggression levels and cuticular hydrocarbon profiles of both *P. simplex* and *P. ejectus* nests from our sites to those at even greater distances would help elucidate whether these species are truly non-aggressive or if they can simply belong to very large colonies.

This is in contrast to the ant *P. PSW-53*, whose worker aggression increases with increasing spatial scale and cuticular hydrocarbon profiles can be broken reliably into five distinct chemotypes with the number of chemotypes increasing with distance. These results indicate that there are multiple territorial colonies of *P. PSW-53* ants present within our study site that overlap and span across hectares. Interestingly, while *P. PSW-53* workers with different chemotypes could be found on plants only 1 m apart, *P. PSW-53* ants on the same plant were always within the same chemotype. This is in accordance with our behavioral data, which showed that *P. PSW-53* ants did not have a higher aggression index at the plant level than ants interacting at the twig level. Additionally, there were no physical attacks (i.e. bites) between workers from the same plant.

Our finding that both *P. ejectus* and *P. simplex* are either one large colony or a group of non-aggressive colonies was unexpected given the size of the study area. A previous study that sampled 77 nests of *P. ejectus* in Florida reported pronounced intercolonial aggression in lab experiments between *P. ejectus* colonies and estimated maximum colony size to be approximately 500 workers (Klein 1987). We sampled 71 twigs containing a total of 1217 *P. ejectus* workers, where workers exhibited low cuticular hydrocarbon variation between nests, and *P. ejectus* workers across our study area exhibited little intraspecific aggression. The contrast between our findings and those of Klein (1987) further highlight that *P. ejectus* is capable of intercolonial aggression in the laboratory and help rule out that our results can be explained merely by *P. ejectus* being a non-aggressive species. Additionally, our results in combination with Klein (1987) indicate that colony size may vary greatly between regions and cannot be generalized.

While *P. simplex* and *P. ejectus* ants from across several hectares have similar hydrocarbons and will not attack their conspecifics, it is possible that workers of these ant species do not regularly travel such distances or exchange food resources across the full extent of this range. A study examining colony boundaries in the polydomous ant *Lasius*

niger found that average area in which ants remained non-aggressive was much larger than the average area in which ants exchanged food resources (Buczowski 2011). Other studies with the invasive argentine ant, *Linepithema humile*, show that these ants have reduced aggression and similar cuticular hydrocarbon profiles over many hundreds of miles in their invasive range (Tsutsui et al. 2003; Torres et al. 2007) yet workers only share food resources within a radius of up to 50 m (Heller et al. 2008).

Although *P. ejectus* and *P. simplex* display interspecific aggression, both species are less successful in winning direct interspecific competitions over nest-sites than *P. PSW-53* (Livingston and Philpott 2010). Despite the success of *P. PSW-53* in competition for nest site colonization, nests of both *P. simplex* and *P. ejectus* are more abundant and more densely aggregated than those of *P. PSW-53* in our study area. Nest sites are a limited resource for the *Pseudomyrmex* species, as hollow, unoccupied twigs are rare and occupation rate of experimentally placed artificial twigs is high (Livingston and Philpott 2010; Jiménez-Soto and Philpott 2015). Most likely, low cuticular hydrocarbon variation and reduced aggression between conspecifics facilitates the success of *P. simplex* and *P. ejectus* regardless of whether these nests are a part of the same colony or multiple non-aggressive colonies. Studies with *Temnothorax* ants report that species with reduced nestmate recognition have higher nest density than those with more efficient nestmate recognition (Foitzik et al. 2007). Additionally, nest site limitation can trigger cooperative colony founding of unrelated queens (i.e. pleometrosis) (Feldhaar et al. 2005), which provides a competitive advantage in colony founding between species that compete for nesting sites (Vasconcelos 1993; Izzo et al. 2009). Cooperative colony founding is a common phenomenon in ants, where several queens might be present at the early stages of colony founding and early workers will admix cuticular hydrocarbons through physical contact, social grooming, and trophallaxis (e.g. Helms and Helms Cahan 2012; Overson et al. 2014). Additionally, the presence of multiple unrelated queens in mature colonies (i.e. oligogyny) also facilitates chemical similarity and reduced aggression between workers. Callow (i.e. freshly pupated) workers often lack colony cuticular hydrocarbons (Lenoir et al. 2001), and can result in unrelated ants ultimately possessing the same cuticular hydrocarbon admixture (Lenoir et al. 1999; Tsutsui 2004). This may also explain the high chemical similarity and reduced aggression in both *P. simplex* and *P. ejectus*.

Regardless of the mechanism that controls social structure, we observed two different strategies for social organization within the three species of *Pseudomyrmex* that may help explain the sharp differences in the nest abundance and density of these species within coffee agroecosystems. Previous research has examined in detail

how both interspecific competition (e.g. Room 1971; Majer 1972; Taylor 1977; Jackson 1984; Majer et al. 1994) and environmental factors (e.g. Sanders et al. 2007; Powell et al. 2011; Jiménez-Soto and Philpott 2015) impact the structure of local arboreal ant assemblages. However, particularly in studies with cavity-dwelling ants where polydomy is common, colony scale and intraspecific aggression are infrequently considered (but see Kautz et al. 2012). Our study highlights the importance of considering intraspecific interactions when examining the community assembly of ants.

Acknowledgments G. Lopez Bautista, G. Domínguez-Martínez, and F. Sánchez-López assisted with field collection and sample processing. We thank Finca Irlanda for allowing us to conduct research on the farm. We thank SEMARNAT (Secretaría de Medio Ambiente y Recursos Naturales) for permission to collect and export samples and J. Rojas and E. Chamé Vasquez for facilitating the process of acquiring permits. N. Tsutsui provided helpful comments on the manuscript. Funding was provided by a Packard Foundation Grant to SRR, National Science Foundation DEB-1262086 to SMP, and National Science Foundation GRFP DGE 1106400 and National Institutes of Health Award Number K12GM000708 to KAM.

References

- AntWeb (2015) Available at <http://www.antweb.org/> (accessed 10 October 2015)
- Bluthgen N, Stork NE (2007) Ant mosaics in a tropical rainforest in Australia and elsewhere: a critical review. *Austral Ecol* 32:93–104
- Bluthgen N, Verhaagh M, Goitia W et al (2000) How plants shape the ant community in the Amazonian rainforest canopy: the key role of extrafloral nectaries and homopteran honeydew. *Oecologia* 125:229–240. doi:10.1007/s004420000449
- Blüthgen N, Stork NE, Fiedler K (2004) Bottom-up control and co-occurrence in complex communities: honeydew and nectar determine a rainforest ant mosaic. *Oikos* 106:344–358
- Buczowski G (2011) Colony spatial structure in polydomous ants: complimentary approaches reveal different patterns. *Insectes Soc* 59:241–250. doi:10.1007/s00040-011-0211-9
- Crozier RH, Dix MW (1979) Analysis of two genetic models for the innate components of colony odor in social Hymenoptera. *Behav Ecol Sociobiol* 4:217–224. doi:10.1007/BF00297645
- Davidson D (1997) The role of resource imbalances in the evolutionary ecology of tropical arboreal ants. *Biol J Linn Soc* 61:153–181. doi:10.1006/bjil.1996.0128
- Davidson DW, Cook SC et al (2003) Explaining the abundance of ants in lowland tropical rainforest canopies. *Science* 300:969–973. doi:10.1126/science.1082074
- De Vega C, Herrera CM, Dötterl S (2014) Floral volatiles play a key role in specialized ant pollination. *Perspect Plant Ecol Evol Syst* 16:32–42. doi:10.1016/j.ppees.2013.11.002
- Debout G, Schatz B, Elias M, Mckey D (2007) Polydomy in ants: what we know, what we think we know, and what remains to be done. *J Linn Soc* 90:319–348
- Dejean A, Corbara B, Orivel J, Leponce M (2007) Rainforest canopy ants: the implications of territoriality and predatory behavior. *Funct Ecosyst Communities* 1:105–120
- Dejean A, Djiéto-Lordon C, Céréghino R, Leponce M (2008) Ontogenetic succession and the ant mosaic: an empirical approach using pioneer trees. *Basic Appl Ecol* 9:316–323. doi:10.1016/j.baae.2007.03.001
- Dejean A, Ryder S, Bolton B et al (2015) How territoriality and host-tree taxa determine the structure of ant mosaics. *Sci Nat*. doi:10.1007/s00114-015-1282-7
- Feldhaar H, Fiala B, Gadau J (2005) A shift in colony founding behaviour in the obligate plant-ant *Crematogaster* (*Decacrema*) morphospecies 2. *Insectes Soc* 52:222–230. doi:10.1007/s00040-004-0797-2
- Foitzik S, Sturm H, Pusch K et al (2007) Nestmate recognition and intraspecific chemical and genetic variation in *Temnothorax* ants. *Anim Behav* 73:999–1007. doi:10.1016/j.anbehav.2006.07.017
- Heller NE, Ingram KK, Gordon DM (2008) Nest connectivity and colony structure in unicolonial Argentine ants. *Insectes Soc* 55:397–403. doi:10.1007/s00040-008-1019-0
- Helms KR, Helms Cahan S (2012) Large-scale regional variation in cooperation and conflict among queens of the desert ant *Messor pergandei*. *Anim Behav* 84:499–507. doi:10.1016/j.anbehav.2012.05.019
- Herbers JM, DeHeer CJ, Foitzik S (2001) Conflict over sex allocation drives conflict over reproductive allocation in perennial social insect colonies. *Am Nat* 158:178–192. doi:10.1086/321312
- Hölldobler B, Wilson EO (1990) *The Ants*. Harvard University, Cambridge
- Holway D (1998) Loss of Intraspecific Aggression in the Success of a Widespread Invasive Social Insect. *Science* 282:949–952. doi:10.1126/science.282.5390.949 (80-)
- Holway D, Lach L, Suarez AV et al (2002) The causes and consequences of ant invasions. *Annu Rev Ecol Syst* 33:181–233. doi:10.1146/annurev.ecolsys.33.010802.150444
- Howard RW, Blomquist GJ (2005) Ecological, behavioral, and biochemical aspects of insect hydrocarbons. *Annu Rev Entomol* 50:371–393. doi:10.1146/annurev.ento.50.071803.130359
- Izzo TJ, Bruna EM, Vasconcelos HL, Inouye BD (2009) Cooperative colony founding alters the outcome of interspecific competition between Amazonian plant-ants. *Insectes Soc* 56:341–345. doi:10.1007/s00040-009-0029-x
- Jackson D (1984) Ant distribution patterns in a Cameroonian cocoa plantation: investigation of the ant mosaic hypothesis. *Oecologia* 62:318–324. doi:10.1007/BF00384263
- Jiménez-Soto E, Philpott SM (2015) Size matters: nest colonization patterns for twig-nesting ants. *Ecol Evol* 5:3288–3298. doi:10.1002/ece3.1555
- Katritzky AR, Chen K, Maran U, Carlson D (2000) QSPR correlation and predictions of GC retention indexes for methyl-branched hydrocarbons produced by insects. *Anal Chem* 72:101–109. doi:10.1021/ac990800w
- Kautz S, Ballhorn DJ, Kroiss J et al (2012) Host plant use by competing acacia-ants: mutualists monopolize while parasites share hosts. *PLoS One* 7:1–10. doi:10.1371/journal.pone.0037691
- Klein R (1987) Colony structures of three species of *Pseudomyrmex* (Hymenoptera: Formicidae: Pseudomyrmecinae) in Florida. In: *Chemistry and biology of social insects*. Velag J. Peperny, Munich, p 107–108
- Krasnec MO, Breed MD (2013) Colony-specific cuticular hydrocarbon profile in *Formica argentea* ants. *J Chem Ecol* 39:59–66. doi:10.1007/s10886-012-0227-2
- Lenoir A, Fresneau D, Errard C, Hefetz A (1999) Individuality and colonial identity in ants: the emergence of the social representation concept. In: *Detrain C, Deneubourg J, Pasteels J (eds) Information Processing in Social Insects*. Birkhauser Verlag Ag, Basel, pp 219–237

- Lenoir A, Hefetz A, Simon T, Soroker V (2001) Comparative dynamics of gestalt odour formation in two ant species *Camponotus fellah* and *Aphaenogaster senilis* (Hymenoptera: Formicidae). *Physiol Entomol* 26:275–283
- Liere H, Larsen A (2010) Cascading trait-mediation: disruption of a trait-mediated mutualism by parasite-induced behavioral modification. *Oikos* 119:1394–1400. doi:10.1111/j.1600-0706.2010.17985.x
- Livingston GF, Jackson D (2014) Spatial clustering of twig nesting ants corresponds with metacommunity assembly processes. *Ecología austral* 24:343–349
- Livingston GF, Philpott SM (2010) A metacommunity approach to co-occurrence patterns and the core-satellite hypothesis in a community of tropical arboreal ants. *Ecol Res* 25:1129–1140. doi:10.1007/s11284-010-0738-7
- Majer JD (1972) The ant mosaic in Ghana cocoa farms. *Bull Entomol Res* 62:151. doi:10.1017/S0007485300047593
- Majer JD, Delabie JHC, Smith MRB (1994) Arboreal ant community patterns in Brazilian cocoa farms. *Biotropica* 26:73–83. doi:10.2307/2389112
- Mathews CR, Bottrell DG, Brown MW (2011) Interactions between extrafloral nectaries, ants (Hymenoptera: Formicidae), and other natural enemies affect biological control of *Grapholita molesta* (Lepidoptera: Tortricidae) on peach (Rosales: Rosaceae). *Environ Entomol* 40:42–51. doi:10.1603/EN10161
- Overson R, Gadau J, Clark RM et al (2014) Behavioral transitions with the evolution of cooperative nest founding by harvester ant queens. *Behav Ecol Sociobiol* 68:21–30. doi:10.1007/s00265-013-1618-2
- Philpott SM, Armbrrecht I (2006) Biodiversity in tropical agroforests and the ecological role of ants and ant diversity in predatory function. *Ecol Entomol* 31:369–377
- Philpott Stacy M, Foster PF (2005) Nest-site limitation in coffee agroecosystems: artificial nests maintain diversity of arboreal ants. *Ecol Appl* 15:1478–1485
- Powell S, Costa AN, Lopes CT, Vasconcelos HL (2011) Canopy connectivity and the availability of diverse nesting resources affect species coexistence in arboreal ants. *J Anim Ecol* 80:352–360. doi:10.1111/j.1365-2656.2010.01779.x
- R Development Core Team (2013) A language and environment for statistical computing. R Development Core Team, Vienna, Austria. Available at <http://www.R-project.org>
- Ribas CR, Schoederer JH (2002) Are all ant mosaics caused by competition? *Oecologia* 131:606–611. doi: 10.1007/s00442-002-0912-x
- Room PM (1971) The relative distributions of ant species in Ghana's Cocoa farms. *J Anim Ecol* 40:735–751
- Sanders NJ, Gotelli NJ, Heller NE, Gordon DM (2003) Community disassembly by an invasive species. *Proc Natl Acad Sci USA* 100:2474–2477. doi:10.1073/pnas.0437913100
- Sanders NJ, Crutsinger GM, Dunn RR et al (2007) An ant mosaic revisited: dominant ant species disassemble arboreal ant communities but co-occur randomly. *Biotropica* 39:422–427. doi:10.1111/j.1744-7429.2007.00263.x
- Suarez A, Tsutsui N, Holway D, Case T (1999) Behavioral and genetic differentiation between native and introduced populations of the Argentine ant. *Biol Invasions* 1:43–53. doi:10.1023/A:1010038413690
- Suarez AV, Holway D, Liang D et al (2002) Spatiotemporal patterns of intraspecific aggression in the invasive Argentine ant. *Anim Behav* 64:697–708. doi:10.1006/anbe.2002.4011
- Taylor B (1977) The ant mosaic on cocoa and other tree crops in Western Nigeria. *Ecol Entomol* 2:245–255. doi:10.1111/j.1365-2311.1977.tb00887.x
- Torres CW, Brandt M, Tsutsui ND (2007) The role of cuticular hydrocarbons as chemical cues for nestmate recognition in the invasive Argentine ant (*Linepithema humile*). *Insectes Soc* 54:363–373. doi:10.1007/s00040-007-0954-5
- Tsutsui ND (2004) Scents of self: the expression component of self/non-self recognition systems. *Ann Zool Fennici* 41:713–727
- Tsutsui ND, Suarez AV, Grosberg RK (2003) Genetic diversity, asymmetrical aggression, and recognition in a widespread invasive species. *Proc Natl Acad Sci USA* 100:1078–1083. doi:10.1073/pnas.0234412100
- Vander Meer R, Morel L (1998) Nestmate Recognition in Ants. In: Vander Meer RK, Breed MD, Espelie KE, Winston ML (eds) *Pheromone communication in social insects: ants, wasps, bees and termites*. Westview Press, Boulder, CO, p 79–103
- Vandermeer J, Perfecto I, Philpott SM (2008) Clusters of ant colonies and robust criticality in a tropical agroecosystem. *Nature* 451:457–459. doi:10.1038/nature06477
- Vandermeer J, Perfecto I, Philpott S (2010) Ecological complexity and pest control in organic coffee production: uncovering an autonomous ecosystem service. *Bioscience* 60:527–537. doi:10.1525/bio.2010.60.7.8
- Vasconcelos HL (1993) Ant colonization of *Maieta guianensis* seedlings, an Amazon ant-plant. *Oecologia* 95:439–443. doi:10.1007/S00442-004-V
- Ward PS (1985) The Nearctic species of the genus *Pseudomyrmex* (Hymenoptera: Formicidae). *Quaest Entomol* 21:209–246
- Yitbarek S, Philpott SM. Dominance hierarchies drive local twig-nesting ant abundance patterns in a tropical agroecosystem (Unpublished data)