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Weak nestmate discrimination behavior in native and invasive populations of a yellowjacket wasp (*Vespula pensylvanica*)

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Abstract In geographic regions with warm winters, invasive yellowjacket wasp colonies (genus *Vespula*) often exhibit polygyny (multiple queens) and persist for multiple years, despite these phenomena being rare in the native range. Here, we test the hypothesis that polygyny, caused by foreign queens being accepted into an existing colony, is the result of relaxed nestmate recognition in the invasive range, as has been observed in some supercolonial invasive ants. In bioassays with wild colonies in the field, we found that nestmate discrimination was weak in both invasive (Hawaii) and native (California) populations of *Vespula pensylvanica,* with significant nestmate discrimination in only ~ 30% of trials. We also found that the diversity and variability of cuticular hydrocarbons, chemical compounds that mediate nestmate recognition, were not reduced in introduced populations, unlike several supercolonial invasive ant species. Our findings suggest that ancestral weak nestmate discrimination behavior of *V. pensylvanica* may make this species pre-adapted to transition to polygyny and extended colony lifespans when introduced into environments with benign winters that facilitate foreign queens joining existing colonies in late season.

Keywords Nestmate recognition · Cuticular hydrocarbons · Supercolonies · Polygyny · Social insects · Hawaii

Introduction

Introduced species often undergo dramatic phenotypic shifts that affect their spread and ecological impact. Classic examples include the novel social and reproductive phenotypes of invasive social insects, including the Argentine ant (*Linepithema humile*; Tsutsui et al. 2000), the little fire ant (*Wasmannia auropunctata*; Errard et al. 2005), the red imported fire ant (*Solenopsis invicta*; Ross et al. 1996), a termite (*Reticulitermes urbis*; Leniaud et al. 2009) and several yellowjacket wasps (*Vespula* spp; Plunkett et al. 1989; Gambino 1991). Many of the most damaging invasive ant species exhibit some form of supercoloniality, which involves a relaxation of colony boundaries and the absence of intraspecific aggression among colonies (Helanterä et al. 2009). This results in free exchange of

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resources and workers between colonies, greatly reducing relatedness and intraspecific competition. The transition to this social phenotype likely exaggerates the ecological effects of these invasions (Holldobler and Wilson 1977; Holway et al. 2002; Holway and Suarez 2004; Huszár et al. 2014). In several cases, supercoloniality appears to be associated with a reduced number of, or low variability in, recognition cues in invasive populations (Errard et al. 2005; Cremer et al. 2008; Brandt et al. 2009), suggesting a possible mechanism for the rise of supercoloniality: low cue variability can result in supercoloniality if individuals cannot distinguish non-nestmates from nestmates, leading to indiscriminate cooperation. Such low variability may precede the introduction (Cremer et al. 2008), or be the result of a bottleneck during introduction (Tsutsui et al. 2000), or of selection against recognition cue diversity in the introduced range (Giraud et al. 2002; Tsutsui et al. 2003).

Like the relatively well-studied invasive ants, yellowjacket wasps (genus *Vespula*) are some of the most damaging invasive species worldwide (Beggs et al. 2011), and these social insects also exhibit a shift in social organization in some introduced populations. Instead of the ancestral single-queen colony and annual colony cycle, some colonies exhibit polygyny (multiple reproducing queens) and a colony lifespan of up to 3 years (Gambino 1991; Goodisman et al. 2001; Hanna et al. 2014; EWR, unpublished data). This shift to polygyny and perennial rather than annual colony lifespan occurs in invasive populations in Hawaii (*Vespula pensylvanica*), parts of Australia and New Zealand (*V. germanica* and *V. vulgaris*; Spradbery 1973; Plunkett et al. 1989), and rarely, at the southern limits of the native range (e.g., *V. squamosa* and *V pensylvanica*, Ross and Matthews 1982; Visscher and Vetter 2003). Polygyny is almost certainly a prerequisite for perennial colonies: the vast majority of reported perennial colonies are polygynous (e.g. Spradbery 1973; Ross and Matthews 1982; Ross and Visscher 1983; Gambino 1991; Ratnieks et al. 1996; Vetter and Visscher 1997; Pickett et al. 2001; Goodisman et al. 2001; Visscher and Vetter 2003; Stewart et al. 2017, though for a possible exception see Thomas 1960). This could be because individual queens have limited lifespans, given their evolutionary history with an exclusively annual colony cycle, though data on queen longevity are lacking. The fitness costs and benefits to joining queens are unclear (see discussion in Ratnieks et al. 1996), but mechanistically, it seems possible that queens searching for hibernating sites sometimes encounter and enter active colonies in the fall, and eventually start to reproduce in them, particularly if environmental conditions do not trigger colony decline. This is plausibly an extension of the behavior exhibited by recently mated *V. pensylvanica* queens in California, which often return to and overwinter in their natal nest (Visscher and Vetter al. 2003). The perennial colony phenotype magnifies the ecological impact of invasive *Vespula* colonies because they often grow to enormous size and consume and compete year-round with a diverse array of arthropods, including native pollinators and honeybees (Wilson et al. 2009; Wilson and Holway 2010).

In a surprising parallel to ant supercoloniality, recent genetic work has shown that at least 20% of polygynous colonies of invasive *V. pensylvanica* in a Hawaiian population contain queens that possess different mitochondrial haplotypes, and are thus not close relatives (Hanna et al. 2014). This, coupled with observations of multi-queen colonies early in the season before new queens are produced, strongly implies that foreign queens have been accepted into existing colonies, resulting in polygyny (Gambino et al. 1990; Gambino 1991). Such foreign joiner queens could effectively be social parasites if they use colony resources to rear unrelated individuals (Gambino 1991). Vespine wasps, like many social insects (Van Zweden and D'Ettorre 2010), have effective nestmate discrimination systems (Ruther et al. 1998, 2002; Steinmetz and Schmolz 2005). Such nestmate discrimination behavior, observed at the nest entrance, presumably evolved to defend the colony against interspecific social parasitism and usurpation by conspecifics from other colonies (Matthews 1982; Greene 1991; Oliveira et al. 2016). *Vespula pensylvanica* colonies are likely the target of both intra- and interspecific usurpation (Akre et al. 1976; Akre and Myhre 1993), and in a closely related *Vespula* species, workers and queens frequently have been observed to kill attempted usurpers (Matthews 1982). Given these observations of nestmate discrimination in similar wasps, in Hawaii, why are foreign queens not rejected by workers of the host colony? We hypothesize that the permissive colony boundaries that allow foreign queen joining in Hawaii are the result of weakened nestmate recognition and discrimination associated with the invasion, perhaps as a result of low recognition cue diversity or variability. Alternatively, nestmate discrimination could be ancestrally weak in this species, making *V. pensylvanica* pre-adapted to queen-joining polygyny under the right environmental conditions.

Our goal in this study was to determine if there has been a relaxation in nestmate discrimination behavior in invasive *V. pensylvanica* colonies in Hawaii, as seen in invasive ant populations. Thus, in bioassays, we compared responses of workers to nestmates and non-nestmates in Hawaii and in two sites in the native range in California. We also analyzed the cuticular hydrocarbons (CHCs) that are typically used by social insects to recognize nestmates and non-nestmates (Van Zweden and D'Ettorre 2010). With these data, we test the hypothesis that a change, either qualitative or quantitative, in the chemical profiles of wasps in the invasive population could explain a change in nestmate discrimination behavior.

Methods

We performed nestmate discrimination assays on 6 colonies in Hawaii Volcanoes National Park, Hawaii, USA from Sept. 29 to Oct. 9, 2015. Between 12 and 24 October, 2016, the same assays were performed on 8 colonies on Santa Cruz Island (SCI), California, and 3 colonies in Descanso, California, USA. Data were collected at the times of year that colonies were producing gynes (female reproductives), and thus when we would expect new queens to possibly be joining existing colonies. Colonies were separated by 627–5570 m (median = 1655 m) in Hawaii, 10–500 m (median 200 m) on SCI, and 23, 74, and 80 m in Descanso. Using worker traffic as an index of colony size (Malham et al. 1991), median worker populations were 420 (range 177–985) on SCI, 743 (range 484–1065) in Descanso, and 922 (range 500–1792) in Hawaii.

Observation tunnels

We modified protocols from previous studies of vespine nestmate discrimination (Ruther et al. 1998; Steinmetz and Schmolz 2005) to perform assays on colonies in the field, rather than on transplanted laboratory colonies. This minimized disruption of colonies and prevented unnecessary colony collection. We selected colonies with nest entrances amenable to placement of artificial nest tunnel extensions (Fig. 1), constructed of 1.25-inch diameter PVC tube, with three 90° junctions: one connecting the tube with the entrance to the nest, and two that formed a "U" bend in the tunnel where observations occurred. Both tunnel arms were 60 cm long, and the width of the "U" was 30 cm. A Perspex window covered with a red filter was placed in a cut in the "U" section (wasps cannot see red light). This window was covered with an opaque cloth when not in use. Tunnels were installed at night so that workers departing in the morning performed orientation flights to the new entrance. Colonies readily accepted the new tunnels, with guards positioned at the new entrance and workers constructing paper in parts of the tunnel.

Behavioral trials

Each colony was given at least 48 h to acclimate to the new entrance tunnel before experimentation. All colonies were assayed with two trials, each with foreign workers from a different foreign colony, except one colony from SCI that was assayed with only one trial. A trial consisted of the presentation of 20 nestmate and 20 non-nestmate workers, in random order. Presented workers were collected approximately 1 h prior to the trial, anesthetized lightly with CO_2, and rendered immobile by removing their legs and wings with microscissors. Wasps were then stored in individual plastic wells for 30–45 min prior to introduction to the test colony. Focal individuals were picked up by an antenna with forceps, and quickly placed into the observation chamber. They were similarly removed, minimizing the time the window was open. We used live wasps because preliminary trials with freeze-killed, thawed workers (as in Steinmetz and Schmolz 2005) reliably resulted in colony alarm and stinging, presumably as a result of alarm pheromone leaking from the glands of the dead wasps. Our method of immobilizing live wasps kept them in the field of view of the tunnel, and still allowed the commonly observed antennation interactions and even trophallaxis with workers in the test colony. We observed no instances of immobilized wasps releasing alarm pheromone and agitating the colony.

Each test worker was placed on the floor of the tunnel below the red Perspex window, and was filmed

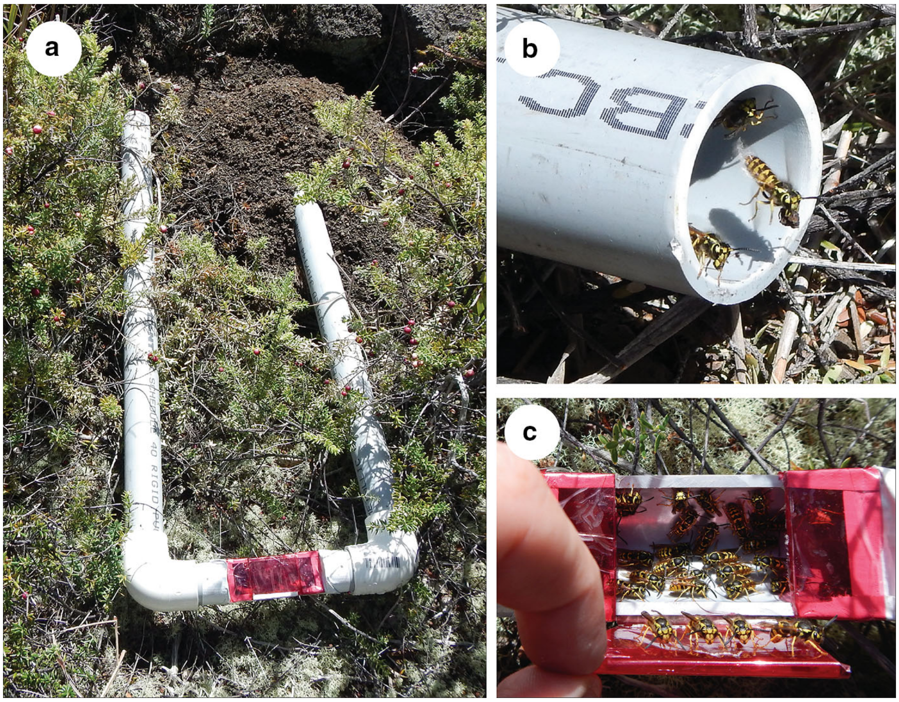

Fig. 1 Nestmate discrimination assay tunnel. **a** One end of the PVC tunnel is buried at the original entrance to the colony, forcing foragers to travel through the tube to reach the new entrance at the other end. **b** Foragers and guards quickly learn to use the new entrance. **c** A hinged window allows access to the tunnel, where non-nestmates and nestmates are presented and observed. Red filters are used on the window because wasps cannot see red light

for 1 min using a Canon Vixia HF200 camcorder, or (rarely) until attacking workers dragged it from view. The test worker was then removed from the tunnel and returned to the plastic well until it was frozen and stored for CHC analysis. We then scored these videos using the program BORIS 5.1.0 (Friard and Gamba 2016) with the observer blind to focal wasp identity. We used the same scoring methodology as Steinmetz and Schmolz (2005): each interaction was scored as a short antennation (any antennal contact), a long antennation (lasting > 1 s), or an attack (any biting or dragging of the test wasp). For each interaction, we also scored whether the interacting worker was departing or arriving based on the direction of travel. Observations of the window and the nest entrance during trials indicated that the direction of travel observed at the window reliably corresponded with an arrival or a departure at the nest entrance; although inactive workers did spend time in the tunnel, they rarely interacted with our test wasps, and thus the vast majority of scored interactions involved foragers.

CHC variability

For all populations, we used the workers that that had been assayed in the behavioral experiments in their colony of origin. Foragers were frozen and kept at − 10 °C until extraction. We extracted the CHCs of 16 workers per colony by individual immersion in 400 µl of heptane. We quantified CHCs using a HP-5890 Series II gas chromatograph with flame ionization detection (Hewlett Packard, Avondale, PA), with a Rxt-5HT column (30 m × 0.25 mm × 0.10 µm film; Restek Corp., Bellefonte, PA) with the following parameters: injection volume = 1.0 µl; injector temperature = 280 °C; detector temperature = 280 °C; oven program: 50 °C for 1 min, 20 °C/min to 150, 5 °C/min to 280 °C, hold at 280 °C for 15 min. A preliminary test programming the oven temperature up to 350 °C revealed no additional quantifiable peaks eluting at temperatures higher than 280 °C. To identify compounds, we ran a representative sample from each population on an Agilent 7820A gas chromatograph with an Agilent DB-5MS column

(30 m × 250 μm × 0.25 μm film), coupled to an Agilent 5977E mass selective detector, using the same temperature program. Concentrated samples were run again on an Agilent 6890 gas chromatograph with an Agilent DB-17 column (30 m × 0.25 mm × 0.25 μm film) coupled to an Agilent 5973 mass selective detector to verify small peak identifications. Compounds were identified using a combination of the molecular ion (when visible), diagnostic fragment ions, and retention times relative to straight chain alkane standards (Carlson et al. 1998). We quantified peak areas from GC-FID chromatograms using Agilent ChemStation software. Within each of the three populations, we identified peaks with greater than 0.2% mean relative abundance (relative to total CHC area). These peaks were integrated in all samples.

Statistical analyses

Behavioral data

All statistical analyses were conducted in R (R Core Team 2017). We used logistic Generalized Linear Mixed Models (GLMMs) to determine the factors that influenced our two response variables: whether an interaction was an attack (*attack*) and whether an interacting wasp attacked or antennated for > 1 s (*interest*). We analyzed "attack" separately from the broader measure of "interest" because attacks were rare, and long antennations could indicate detection of non-nestmates even if those non-nestmates were not aggressed. Another benefit of the "interest" metric (any behavior that was a long antennation or an attack) is that it was also used in a previous study of *Vespula* nestmate recognition behavior, allowing comparison (Steinmetz and Schmolz 2005). We included identity of *presented individual* and *trial* as random effects (colony is encompassed by trial, with two trials per colony). The random effect of identity of presented individual accounts for the fact that each presented focal individual experienced numerous encounters which were not independent. We used corrected Akaike Information Criterion scores to compare nested models with the predictors *population* (Hawaii or California), *inter-colony distance* (the distance in meters between source and test colonies used in a trial), *type* (nestmate or non-nestmate), *direction* (departing or arriving) and the interaction *population × type*, which we predicted to be significant if Hawaiian wasps have relaxed nestmate discrimination relative to California wasps. Because there was a significant interaction between *population* and *type*, we then created similar models independently for each population to look for differences between populations in the effect of *type*. To examine variation in nestmate discrimination across colony pairings, we used Fisher's exact tests to compare the probabilities of attack and interest for nestmates and non-nestmates in each trial, correcting for multiple comparisons with the False Discovery Rate (FDR) method (Benjamini and Hochberg 1995). We also checked for intra-population effects of inter-colony physical distances using linear regressions of physical distance against attack rate and interest rate, at the trial level.

CHC variation

We compared variation in CHC profiles within populations and within colonies using the permutest() in the package 'vegan' (Dixon 2003), using Manhattan distances calculated with the vegdist() function. Permutational MANOVA (perMANOVA) analyses were used to determine how much CHC variation was predicted by colony identity within each population. As another index of colony separability in chemical space, we calculated the average pairwise chemical distance between all pairwise combinations of colonies using the "group centroid" method of van Zweden et al. (2014). In this method, we first found the centroid between all individuals in two colonies, and then calculated the mean of the Manhattan distances from each individual to that centroid using the vegdist() function.

Distinguishing colonies using CHC data

We used random forest (RF) models to determine the degree to which CHCs could be used to distinguish nestmates from non-nestmates. RF analysis is an extremely flexible machine learning tool in which subsets of predictor variables are randomly selected to form each node of many classification trees, and the results of these trees are then weighted by their prediction success (Breiman 2001; Brückner and Heethoff 2017). After constructing the RF model, its predictive ability can be evaluated by applying it to a withheld subset of the data to avoid overfitting. RF models are virtually assumption-free, making them

Table 1 Results of logistic GLMMs of nestmate discrimination behaviors with trial and individual as random effects

Response	Predictor	β (SE)	z	P
interest	***direction*** (departing)	0.36 (0.04)	8.73	< 0.0001
	type (nestmate)	− 0.45 (0.09)	− 4.81	< 0.0001
	population (Hawaii)	0.28 (0.24)	1.17	0.24
	type × ***population*** (nestmate:Hawaii)	0.35 (0.14)	2.54	0.011
attack	***direction*** (departing)	0.68 (0.12)	5.59	< 0.0001
	type (nestmate)	− 0.76 (0.22)	− 3.45	0.0006
	population (Hawaii)	− 0.48 (0.33)	− 1.47	0.14
	type × ***population*** (nestmate:Hawaii)	0.66 (0.33)	1.98	0.048

Significant predictors are in bold. n = 36,237 interactions for each response

amenable to datasets with non-linear relationships, multicollinearity of predictors, non-normal distributions, and with a large number of predictors relative to the number of samples, which is typical of CHC datasets. Such limitations are problematic for other approaches such as principal components analysis and linear discriminant analysis (Martin and Drijfhout 2009). Because our goal was to compare how separable colonies are across populations, we compared the average classification accuracy between pairs of colonies within each population. This avoided the problem of having different numbers of colonies in each population (separating 8 colonies would be more difficult than distinguishing 3 colonies, all else being equal). RF models were created using the package 'randomForest' (Wiener and Liaw 2002), with 10^6 trees and 5 variables sampled at each split. For each model, 12 randomly selected individuals per colony were used in the training set, with the 4 remaining individuals per colony reserved to determine model accuracy. This was repeated 20 times per pair of colonies, and the resulting classification accuracy for the samples reserved for validation was averaged to create a single index of separability for that pair.

Results

Nestmate discrimination behavior

We presented a total of 1313 individuals in 33 trials across 17 colonies (40 individuals per trial, with 7 individuals not presented because they were lost or died before introduction). We observed a total of 36,237 interactions with presented individuals, with a median of 23 per individual (range 2–144). In addition to receiving attack and long antennation behaviors (for videos, see Online Resources 2–4), presented individuals also participated in mutual antennation and trophallaxis with test colony workers (Online Resources 3 and 4), indicating that the manipulated workers were recognized and treated as conspecific social partners.

For both response variables (*attack* and *interest*), the model including direction of travel, population, nestmate/non-nestmate, and an interaction between population and nestmate/non-nestmate, was the preferred model (Table 1). Adding trial inter-colony distance as a predictor did not significantly improve models of either attack or interest (Chi squared test: $P > 0.05$), suggesting that the varying distance between test colonies in different populations did not influence our experiments. The probability that a given interaction indicated interest (i.e., was either a long antennation or an attack) was higher for interactions involving non-nestmates than nestmates, and for interactions involving departing foragers rather than arriving foragers (Table 1). There was no difference in the overall probability of interest between populations, but there was a significant interaction between population and foreign/nestmate status, with a greater effect of being a non-nestmate in California than Hawaii, suggesting more lenient nestmate discrimination in the invasive population. We found the same results when looking at the probability of attack (Table 1). Because the interaction term with *population* was significant, we ran the same GLMMs (but without a *population* predictor) for each population separately. There were greater probabilities of attack and interest directed toward non-nestmates in both SCI and Descanso (Fig. 2; attack: SCI: β (SE) = − 0.55 (0.26), z = − 2.1, $P = 0.034$; Descanso: β (SE) = − 1.86 (0.58), z = − 3.2, $P < 0.001$, interest: SCI: β (SE) = − 0.40 (0.11), z = − 3.8, $P < 0.0002$;

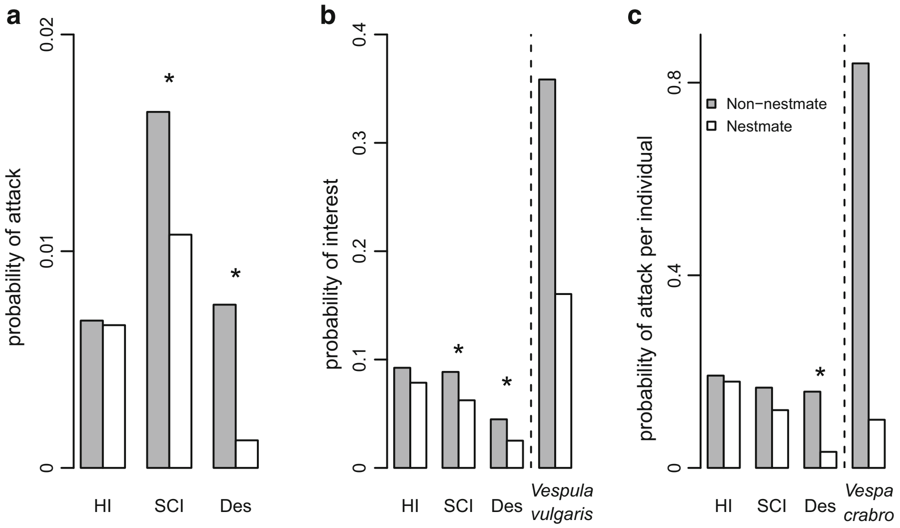

Fig. 2 Nestmate discrimination behavior by population and type. **a** The overall probability of attack (per interaction) in our three study populations. **b** The overall probability of interest (attack + long antennation, per interaction) in our three study populations and the same measure from a study of *V. vulgaris* (calculated from Steinmetz and Shmolz 2005 Fig. 3). **c** The overall probability that a presented individual is attacked at least once in our three study populations, and the same measure from a study of *Vespa crabro* (calculated from Ruther et al. 1998, Figs. 2 and 3). Asterisks indicate a significant difference between nestmate and non-nestmate in population-specific GLMMs (**a**, **b**) or Fisher's exact tests (**c**). We made no comparisons for *V. vulgaris* and *Vespa crabro* data from the literature. Sample sizes for non-nestmate/nestmate interactions in **a** and **b** are 9122/9413 in Hawaii, 5600/6039 on SCI and 2921/3142 in Descanso. Sample sizes for non-nestmate/nestmate individuals in **c** are 240/240 in Hawaii, 300/300 on SCI, 120/120 in Descanso, and 47/37 for *Vespa crabro* (Ruther et al. 1998)

Descanso: β (SE) = − 0.62 (0.22), z = − 2.8, $P < 0.005$), but we observed no difference between non-nestmates and nestmates in Hawaii (attack: $P = 0.70$; interest: $P = 0.30$).

On a per trial basis, 3 of 12 trials in Hawaii exhibited significant nestmate discrimination behavior (interest directed more toward non-nestmates; Fig. 3). On SCI, 5 of 15 trials showed significant nestmate discrimination, as did 2 of 6 trials in Descanso. Only one trial showed a significant difference in attack rate between nestmates and non-nestmates (Fig. 3). There was no effect of the physical distance between colonies on aggression toward non-nestmates across trials within each population ($P > 0.30$ for all 6 linear regression tests).

Qualitative CHC variation

Qualitatively, all populations possessed very similar CHC profiles. We found 62 unique peaks with mean relative abundance > 0.2% in at least one population. All 62 peaks were present in all populations (Fig. 4), and these peaks contained the same compounds in representative samples from each population (Table S1). For a full list of compounds detected, including those in trace abundance, see Online Resource 1.

Quantitative CHC variation

Overall CHC variation was significantly lower in the Descanso population compared to the SCI and Hawaii populations, likely due to the smaller number of colonies sampled (Fig. 5a; Permutation test: $F_{2,269} = 10.29$, $P < 0.0003$; pairwise permuted $P = 0.0001$ for Descanso-Hawaii; $P = 0.007$ for Descanso-SCI; $P = 0.19$ for Hawaii-SCI). Colony level CHC variation (distance to colony centroid) was significantly higher in Hawaii than in Descanso but not SCI (Fig. 5b; GLMM with random effect of colony: Hawaii-Descanso: $t_{14} = -2.42$, $P = 0.03$, Hawaii-SCI: $t_{14} = -1.69$, $P = 0.11$). Colony identity explained a similar amount of CHC variation across the three populations: 17% of CHC variation among Descanso samples (perMANOVA: $F_{2,45} = 4.68$, $P < 0.0001$), 26% of CHC variation in SCI samples

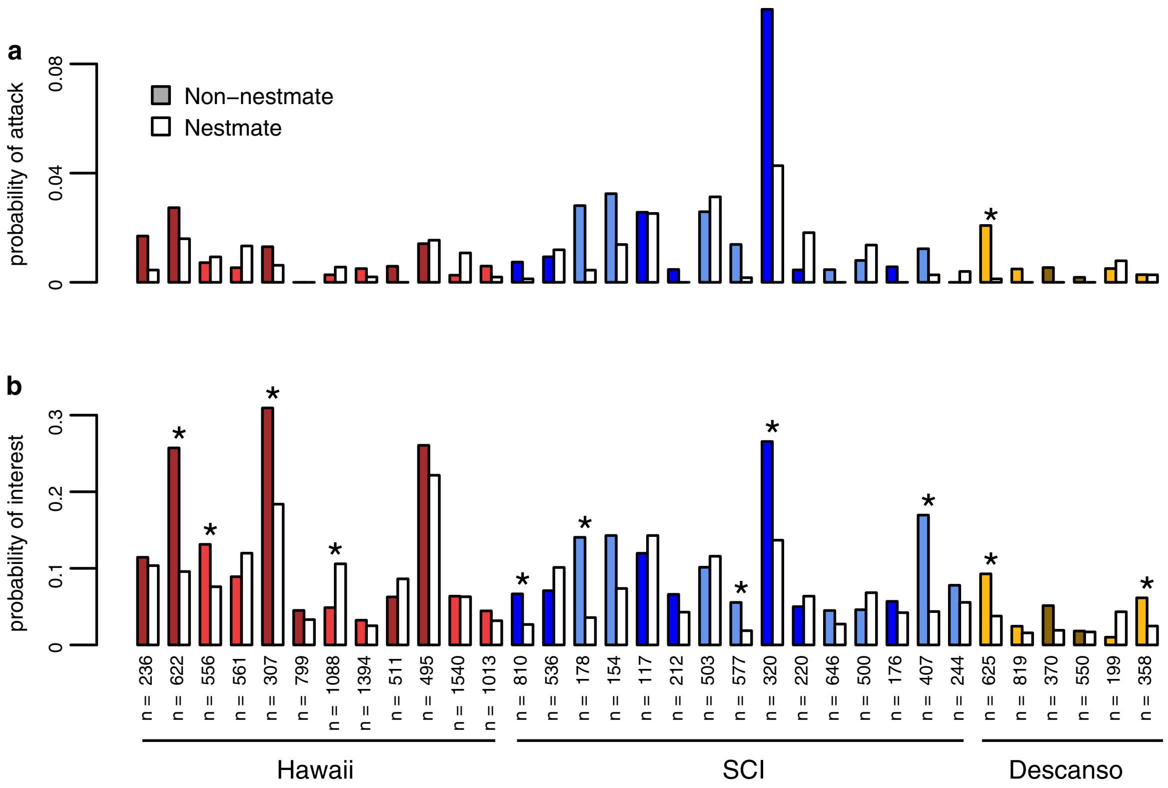

Fig. 3 Probability of **a** attack and **b** interest (per interaction) for each trial. Each pair of bars represents a trial of 20 non-nestmate and 20 nestmate workers presented to a focal test colony. Adjacent pairs of bars with the same shade are trials using the same focal colony and different source colonies for non-nestmates. Bar height is the frequency of attack or interest per interaction. Sample sizes (number of observed interactions) per trial are on the x-axis. Asterisks indicate significant differences between nestmates and non-nestmates (two-tailed Fisher's exact tests, $P < 0.05$ after FDR-corrected for multiple comparisons)

($F_{7,120} = 6.05$, $P < 0.0001$), and 22% of CHC variation in Hawaii samples ($F_{5,90} = 5.18$, $P < 0.0001$).

While the mean pairwise colony chemical distance was significantly higher in Hawaii than California colony pairs (Welch's $t_{31.6} = 3.68$, $P = 0.0008$; Fig. 6a), pairwise discrimination accuracy between colonies in random forest analysis was the same across populations (Fig. 6b). Pairwise colony chemical distance did not predict pairwise random forest discrimination accuracy ($F_{1,44} = 3.26$, $P = 0.08$, adj $R^2 = 0.05$; Fig. 6c).

Neither chemical distance nor classification accuracy based on CHCs between pairs of colonies had a positive relationship with rates of attack or interest toward non-nestmates across behavioral trials. Instead, there were no significant relationships with chemical distance (linear regressions: attack $t_{31} = -1.04$, $P = 0.31$; interest $t_{31} = 1.5$, $P = 0.14$), or RF accuracy (attack: $t_{31} = 1.69$, $P = 0.10$; interest: $t_{31} = 1.01$, $P = 0.32$).

Discussion

Our behavioral and chemical analysis results suggest a scenario quite different from those described for supercolonial invasive ant species, such as the Argentine ant and little fire ant (Tsutsui et al. 2000, 2003; Giraud et al. 2002; Errard et al. 2005). While overall models suggested that Hawaiian wasps, unlike Californian wasps, treated nestmates and non-nestmates indiscriminately, an examination of the trials individually showed that the three populations had similar proportions of trials with aggression or interest significantly directed toward non-nestmates (Fig. 3). Although there may be a slight difference between populations, the more striking result was the apparent lack of nestmate discrimination behavior in the majority of trials in both California and Hawaii. This suggests that *V. pensylvanica* colonies may have ancestrally weak nestmate discrimination, at least in mature, late-season colonies like those we assayed. It

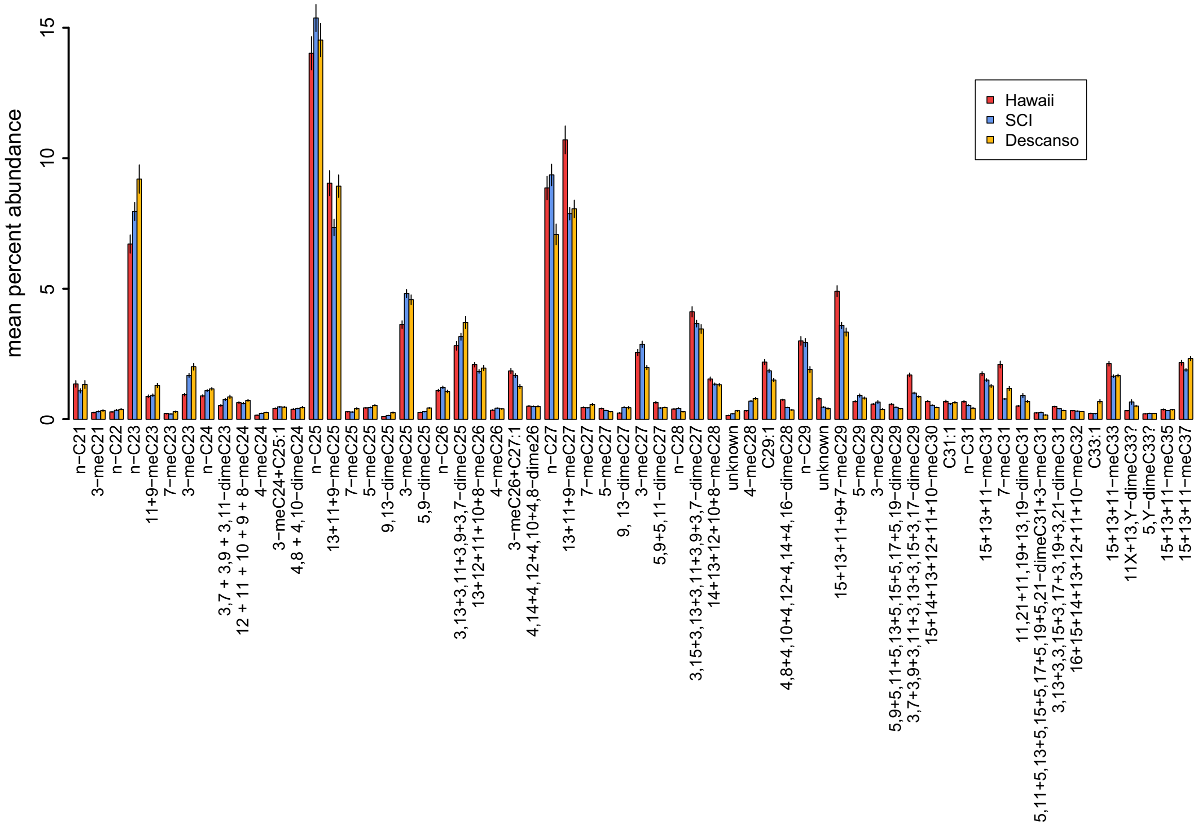

Fig. 4 Mean relative abundance of 62 major cuticular hydrocarbon peaks in workers of each population. Error bars depict SEM. For a full list of compounds detected, see Online Resource 1

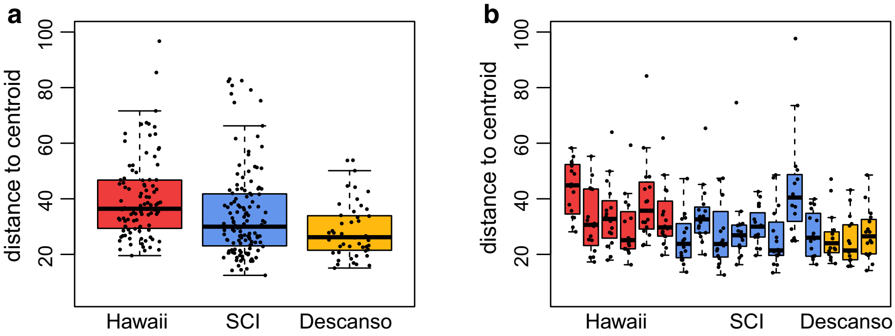

Fig. 5 Cuticular hydrocarbon variation with populations (**a**) and colonies (**b**). Points depict the Manhattan distance between each individual and the group (population or colony) centroid. Boxes show 1st and 3rd quartiles, whiskers are 1.5 times the interquartile range

is not clear from our results whether this weak nestmate discrimination is the result of the inability of workers to recognize non-nestmates because recognition cues do not accurately indicate colony membership, or alternatively, that workers can recognize non-nestmates but do not respond aggressively to them. Random forest analyses suggest CHCs do not perfectly indicate colony membership, although it is possible that workers' discrimination ability is not similarly limited. On the other hand, the observation that the rate of investigation with long antennation was substantially greater than the attack rate suggests that workers detect but tolerate some foreign workers.

If the treatment of foreign queens is similar to that of foreign workers, polygyny via foreign queen joining could result from pre-existing tolerance of

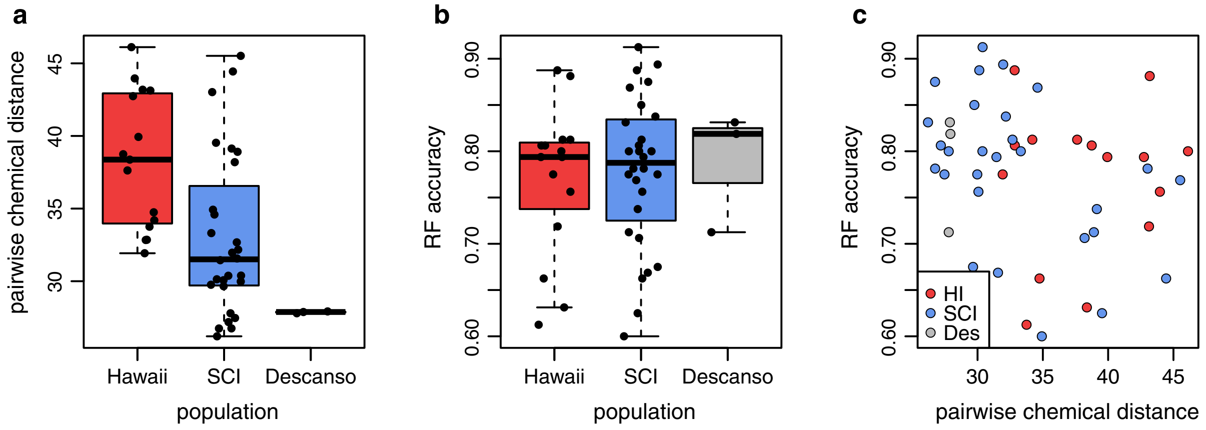

Fig. 6 Pairwise colony chemical distance and classification. **a** Pairwise chemical distance between all pairs of colonies in each population. **b** Mean accuracy of colony classification from random forest analyses on CHC profiles for each colony pair in each population. This is the average fraction of correct colony assignments when validating models with randomly selected, left-out samples from 20 random forest runs for each colony pair (we would expect 0.5 accuracy by chance alone). **c** Colony classification accuracy is not predicted by chemical distance between pairs of colonies

non-nestmates, rather than novel decreased nestmate recognition behavior caused by some aspect of the invasion. Thus, *V. pensylvanica* may be pre-adapted for polygyny regardless of the nature of the introduction, rather than only in contexts with genetic bottlenecks. Such findings are consistent with occasional observations of polygyny in the native range (Ratnieks et al. 1996; Visscher and Vetter 2003), though it is not known whether the additional queens in those colonies were daughters or foreign joiners. It is important to acknowledge that these inferences about foreign queen joining rely on the assumption that nestmate discrimination behavior toward non-nestmate workers is similar to that toward non-nestmate queens. It is possible that foreign queens would be treated differently than workers (e.g., Moore and Liebig 2010), and it would be interesting, though logistically much more difficult, to repeat our experiments with mated queens, to determine if young queens are treated differently when introduced into foreign colonies. We also lack understanding of the fitness consequences of queen joining for both queens and host colonies in both the native and invasive ranges.

We observed numerous "attacks" (biting, mounting) directed toward non-focal workers visible in the video, often preceded by solicitation of trophallaxis, and followed by trophallaxis (Online Resource 2). We also saw such solicitation and aggression toward our presented individuals, suggesting that some "attacks" may be part of dominance or communication systems organizing food transfer among adults (Online Resource 2 and 3). This as yet unexplained nestmate aggression (called "mauling") has been reported for *V. pensylvanica* (Akre et al. 1976), and has been anecdotally observed in other vespine wasp foragers returning to the nest with nectar (KJL, per obs). Such aggressive behaviors could be important in regulating foraging (O'Donnell 2001, 2006) or organizing food distribution within the colony. These non-defense attacks likely add noise to our dataset, and could inflate attack rates for both nestmate and non-nestmate workers.

We observed much lower overall rates of attack and interest toward both nestmates and non-nestmates compared to previous studies on the closely related yellowjacket, *Vespula vulgaris* (Steinmetz and Schmolz 2005), and the hornet *Vespa crabro* (Ruther et al. 1998). This difference could be the result of true differences in behavior between these species, with *V. pensylvanica* having much weaker nestmate discrimination. Alternatively, it could be that presenting live individuals, rather than dead ones as was done in previous studies, reduced the amount of attention they received. In our opinion, interactions with immobilized, live individuals are more likely to be similar to interactions with actual non-nestmate intruders than are interactions with dead dummies, given our live workers' ability to participate in antennation interactions and in trophallaxis (Online Resources 3 and 4). Furthermore, preliminary trials with freeze-killed individuals often resulted in undertaking behavior (i.e., the removal of dead individuals from the nest), which could be confused with attack behavior, thus inflating aggression rates toward dead dummies. This may explain the greater overall interest rate seen in the study on *V. vulgaris* (Steinmetz and Schmolz 2005), but it cannot explain why most of our trials revealed no difference in behavior toward non-nestmates and nestmates, whereas those studies with dead dummies consistently observed significant differences in all (albeit fewer) trials.

Several factors could explain the higher rate of nestmate discrimination observed by Steinmetz and Schmolz (2005). First, the distances between test and foreign worker source colonies were shorter in our experiments (40 m–5 km) than in their study (> 10 km). It is possible that relatedness between colonies reduced non-nestmate rejection, particularly in California where many nests were near to one another. Furthermore, workers from colonies close to one another may also encounter each other more frequently, and this familiarity could influence acceptance behavior (Esponda and Gordon 2015). On the other hand, there was no relationship between intercolony distance and the rate of interest or aggression toward non-nestmates in our dataset, and nest density in these populations was high relative to the likely dispersal distance of queens, suggesting that colonies in close proximity may not have been related. The intercolony distances of our study are likely to be more similar to those of natural foreign queen joining events than the distances used in Steinmetz and Schmolz (2005), because *Vespula* queens often disperse < 1 km from their mother colony (Crosland 1991; Masciocchi and Corley 2013). A second factor that could contribute to our observed weak nestmate discrimination is the stage of colony development. The colonies used in Steinmetz and Schmolz (2005) were probably earlier in their developmental trajectory, given their size. Workers should be under selection to tune nestmate discrimination behavior in response to the relative costs of acceptance and rejection errors (Reeve 1989), which could be associated with colony stage. For example, intrusion by unrelated egg-layers [such as queens (Greene 1991) or reproductive workers (Oliveira et al. 2016)] could be costly earlier in the season, when annual colonies are still growing and allocating resources to reproduction. Later in the season, once most reproductive-destined eggs are laid, the costs of admitting an unrelated egg-layer could be lower, selecting for a more permissive acceptance threshold later in the season. Such tuning of nestmate discrimination has been demonstrated in a variety of contexts (e.g., Starks et al. 1998; Downs and Ratnieks 2000), including in response to colony stage (Gamboa et al. 1991), although in that study the observed effect was an *increase* in aggression later in the season. Regardless of the cause, the observed weak nestmate discrimination in our late-season colonies suggests that colony boundaries are permeable, at least to foreign workers, at the time of year when most queens are produced, and thus when queen joining is most likely to occur. Further studies will be necessary to determine whether colony stage or caste influence nestmate discrimination in this species.

We found no evidence for a change in CHC profiles that could affect nestmate recognition in the introduced population of *V. pensylvanica* in Hawaii. In particular, there was no reduction or increase in the number of compounds present in the invasive population, quantitative CHC variation was not lower, and inter-colony chemical distances and separability (by random forest analysis) were also not reduced in Hawaii compared to California. Counter to our prediction, population-level and inter-colony distances were greater in Hawaii (Figs. 5a, 6a), which could reflect the larger geographic distances between colonies at that site. Unlike in several ant species (Suarez et al. 2002; van Zweden et al. 2014) and a stingless bee (Nunes et al. 2008), we found no evidence that colony-level CHC profile dissimilarity influenced aggression toward non-nestmates. Indeed, we have no conclusive evidence that CHCs are directly involved in nestmate recognition in these wasps. Although CHCs are important in nestmate recognition in the vespine wasp *Vespa crabro* (Ruther et al. 1998, 2002), there is evidence that nestmate recognition cues in *Vespula* may be volatile chemicals (Steinmetz and Schmolz 2005), suggesting that perhaps CHCs are less important in *Vespula* spp., in contrast to the vast majority of other social insect species that have been studied (Van Zweden and D’Ettorre 2010). Regardless, our results indicate that behaviorally and chemically, the *V. pensylvanica* invasion is unlike the Argentine ant (*Linepithema humile*) and the little fire ant (*Wasmannia auropunctata*) invasions, which exhibit reduced non-nestmate aggression and reduced compound number and variability in the invasive range (Tsutsui et al. 2000; Errard et al. 2005; Brandt et al. 2009). Here, we found the *V. pensylvanica* invasion of Hawaii was instead more similar to the invasions of the garden ant *Lasius neglectus* in Europe (Cremer et al. 2008) and the pharaoh ant *Monomorium pharaonis* worldwide (Schmidt et al. 2010), in which the invasive populations exhibit little change in CHC profiles from populations in their native ranges, and native populations may possess weak ancestral non-nestmate rejection. In conclusion, the weak nestmate discrimination behavior of *V. pensylvanica* may make this species "spring-loaded" to transition to polygyny when introduced into environments with benign winters that permit late-season queen joining and extended colony lifespans.

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