Journal of Chemical Ecology
https://doi.org/10.1007/s10886-021-01325-3

Similarities in Recognition Cues Lead to the Infiltration of Non-Nestmates in an Ant Species

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Received: 10 May 2021 / Revised: 12 October 2021 / Accepted: 17 October 2021

Abstract
Chemical cues are among the most important information-sharing mechanisms in insect societies, in which cuticular hydrocarbons play a central role, e.g., from nestmate recognition to queen signaling. The nestmate recognition mechanism usually prevents intruders from taking advantage of the resources stored in the nest. However, nestmate recognition is not unconditionally effective, and foreign individuals can sometimes infiltrate unrelated nests and take advantage of the colony resources. In this study, we investigated the role of overall colony odor profiles on the ability of conspecific workers to drift into unrelated colonies. We hypothesized that drifters would have higher chances of success by infiltrating colonies with the odor profiles most similar to their own nest, avoiding being detected as non-nestmates. By performing a drifting bioassay, we found that workers of the ant *Formica fusca* infiltrated unrelated conspecific colonies at a rate of 2.4%, significantly infiltrating colonies displaying CHC profiles most similar to their natal nests. Notably, methyl branched hydrocarbons seem to play a role as recognition cues in this species. In addition, we show that environmental rather than genetic factors are responsible for most contributions on the CHC phenotype, presenting ca. of 50% and 27.5% of explained variation respectively, and playing a major role in how worker ants detect and prevent the infiltration of non-nestmates in the colony. Hence, relying on cuticular hydrocarbons similarities could be a profitably evolutionary strategy by which workers can identify conspecific colonies, evade detection by guards, and avoid competition with genetic relatives.

Keywords Drifting behavior · Chemical signaling · Nestmate recognition

Introduction

The colonial lifestyle is among the remarkable features of social insects, playing a key role in their extraordinary ecological success (Wilson 1987). Hymenopteran colonies are usually composed of one or more queens that monopolize reproduction, and their worker offspring that takes care of other colony tasks such as foraging and defending the nest against predators and exploiters (Wilson 1971). To effectively organize themselves, it is therefore important that workers carry the ability to differentiate kin from non-kin (Bourke and Franks 1995; Hölldobler and Wilson 1990), which expresses itself mainly in the form of nestmate recognition, assessing if another individual belongs to the same colony and generally responding aggressively if it does not. Nestmate recognition is mediated primarily through the comparison of chemical cues present on the cuticle, comprising mainly hydrocarbons, with a neural template (van Zweden and d'Ettore 2010). The cuticular hydrocarbon (CHC) profile is shaped by an interplay between genetic and environmental factors, in the sense that, despite individual variation in the production of CHC levels, a common colony odor bouquet ("Gestalt") is formed by transfer of odors among nestmates (Dahbi et al. 1999; Lenoir et al. 2001; Soroker et al. 1998; van Zweden and d'Ettore 2010).

Nevertheless, as in most biological mechanisms, nestmate recognition is not perfect (Johnson et al. 2011; van Zweden and d'Ettore 2010) and individuals from the same or different species might infiltrate and exploit the colony resources, a form of social parasitism. Taking advantage of resources from an unrelated nest might end up being a profitable strategy. Indeed, in nature, several different strategies of social parasitism exist, from temporary inquilines to obligatory associations (Brandt et al. 2005; Lenoir et al. 2001; Nash and

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Published online: 11 November 2021

Boomsma 2008). Social parasites are not limited to exploiting the food resources of their hosts. In some cases, the host workforce is also a target, for example, when the offspring of the parasite is reared by the host as in the famous case of the cuckoo and other bird species (Davies et al. 1989; Petrie and Møller 1991).

Although unable to mate, social insect workers retain the ability to develop their ovaries and lay unfertilized male-destined eggs (Wilson 1971), creating a conflict over reproduction between queens and workers, which is usually resolved when either the queen or nestmate workers selectively eat or "police" worker-laid eggs (Ratnieks and Wenseleers 2005; Ratnieks and Visscher 1989; Wenseleers and Ratnieks 2006). Given the slim chances of reproduction in their own colonies, in some cases reproductive workers drift into unrelated colonies and lay their male-destined eggs, avoiding competition with relatives. This kind of intraspecific worker parasitism has recently been described in several social insect species, including bees and wasps (e.g. (Chapman et al. 2009a; Lopez-Vaamonde et al. 2004; Nanork et al. 2007; Oliveira et al. 2016)). Such behavior is in line with the predictions of kin selection theory in the sense that individuals maximize their inclusive fitness by avoiding competition with genetic relatives (Hamilton 1964a; Hamilton 1964b). In fact, it has been hypothesized that policing through egg cannibalism is an adaptation to the occurrence of social parasitism, whereby workers would identify the colony chemical signature in the eggs and police those that do not match the profile (Helanterä and Sundström 2007). As a counter-strategy to this recognition system, cheater individuals sometimes either acquire (chemical camouflage) or synthesize (chemical mimicry) their host chemical profiles, which is normally the case in interspecific parasitism (Akino 2008; Dettner and Liepert 1994; Fiedler et al. 1996). For intraspecific social parasitism, however, it would be more advantageous for cheaters if they could drift into colonies with an overall chemical profile similar to their natal nest. Since genetic relatedness can be often dissociated from chemical similarity (Frizzi et al. 2015; Helanterä et al. 2011; Leonhardt et al. 2013), drifters would still be avoiding competition with relatives by drifting into colonies matching their own CHC profile, with the advantage of increasing the chances of evading detection by host guards. Moreover, even if the host colony has some degree of genetic relatedness with the parasite, the highest genetic relatedness that a worker drifter could encounter is only half of its own colony (if a host colony is headed by a single sister of the focal drifter, $r = 0.375$).

In this study, we investigate the occurrence of drifting behavior on an ant species and tested the prediction that workers would tend to drift into colonies that have their CHC odor most similar to their own colony profile. In order to test this hypothesis, we used the ant *Formica fusca* as a model, which is a facultative polygyne species showing an average relatedness of $r = 0.63$ for monogyne and $r = 0.27$ in polygyne colonies (Bargum et al. 2007). Moreover, we applied variance partitioning on a full factorial cross-fostering experiment in order to determine both the broad-sense heritability (h2) and the environmental component of such chemical cues in the attempt to identify the degree to which genetics and environment play a role in colony cuticular hydrocarbon phenotype.

Material and Methods

Study Species

Twenty-four *F. fusca* ant colonies were collected in the vicinity of Leuven, Belgium, (50.8798° N, 4.7005° E) in early spring and placed in identical plastic nest boxes. Throughout the experiments all colonies were given water and fed ad libitum honey and mealworms. For the purpose of these experiments, colonies were classified as monogyne (with a single queen present) or polygyne (with two or more queens present), since the largest differences in the within-colony average genetic relatedness are between one and two queens, assuming that they are singly-mated (the effective mating frequency is 1.19 in this species (Hannonen et al. 2004)) and matrilines are equally represented (single mated queen $r = 0.75$ vs. double mated queen $r = 0.375$).

Worker Drifting Bioassay

To test whether workers drift to unrelated colonies, we used a setup in which four queenright colonies were connected to a square arena of 60×60 cm (Fig. 1). The vertical sides of the arena were coated with Fluon® to prevent workers from climbing out. The colonies were placed on the exterior four corners of the arena and connected to this central area by a single 2 cm wide entrance bridge. Food was delivered to all colonies inside their nests, so that workers were not forced to forage in the arena and compete for food. Three trials with four colonies each ($N = 12$ colonies) were performed for 15 days each. In each trial, two colonies were monogyne and two were polygyne with approximately the same number of workers per colony ($N = 142 \pm 12$). All workers were paint-marked with different colors per colony and were inspected every three days for the occurrence of drifted workers in order to observe the temporal dynamics of the drifting behavior. Prior to the beginning of every trial, 10 adult workers were collected from the colonies for chemical analyses (see below) to test the influence of cuticular chemical compound variability on the occurrence of drifting behavior.

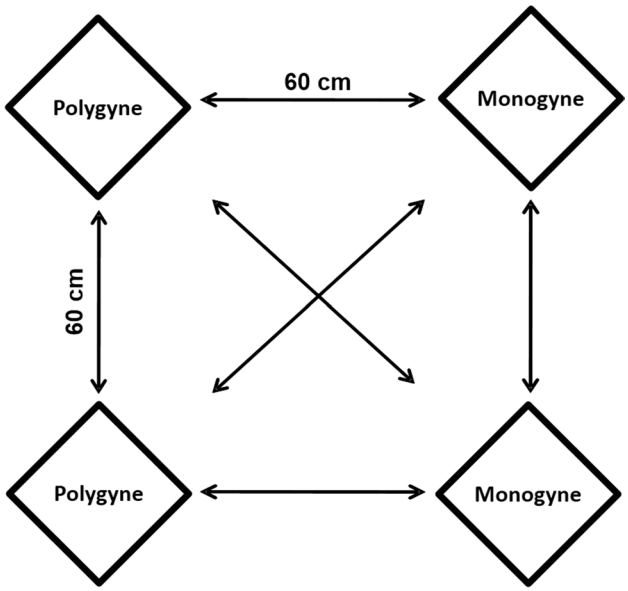

Fig. 1 Scheme of the experimental setup for the drifting bioassay not to scale. Each square represents an ant colony in which two were polygyne and two monogyne colonies and arrows show the drifting possibilities

All drifters present in the colonies at the end of the experiment (N = 41) and a subset of natal workers (N = 10 per colony) were dissected to assess the ovary development levels. Ovaries were considered undeveloped when the oocytes and trophocytes inside the ovaries were in their early stages of development and still undifferentiated and considered developed when the largest oocyte inside the ovaries was clearly larger than the associated trophocyte follicle, or when mature eggs with a chorion were present and the trophocytes were fully degenerated.

Cross-Fostering Experiment

In order to determine the influence of both genetic and environmental factors on the cuticular hydrocarbon composition of *F. fusca* we performed a full factorial cross-fostering experiment with three trials and four queenless mini colonies per trial, of which two were monogyne and two were polygyne (Fig. 2). Per trial, every colony was divided into four queenless foster subcolonies containing fifteen paint-marked workers, then each subcolony received fifteen cocoons of either their own colony (control groups, N = 60 per trial, N = 180 in total) or of one of the other three unrelated colonies (treatment groups, N = 180 per trial, N = 540 in total). The relocated cocoons were allowed to emerge and interact with foster workers for 30 days, after which the transplanted workers were collected and freeze-killed (−20 °C) for cuticular chemical profile analysis.

Chemical Analyses

Individuals were extracted in 800 µl HPLC-grade hexane for 10 min. The samples were evaporated at room temperature under a laminar flow hood and resuspended in 500 µl hexane. Samples were analyzed on a Thermo Fisher Scientific Trace 1300 gas chromatograph coupled with Restek RXi-5sil MS 20 m × 0.18 mm × 0.18 µm column and a Thermo Fisher Scientific ISQ mass spectrometric detector. We injected 1 µL of the sample extracts using splitless injection and an inlet temperature of 290 °C. The initial column temperature of 40 °C was held for 1 min, then increased to 200 °C at a rate of 20 °C/min, and then to 340 °C at a rate of 2 °C/min, which was held for 4 min. Helium was used as a carrier gas at a constant flow rate of 0.9 mL/min. The electron ionization voltage was auto-tuned to enhance the acquisition performance according to the molecular weight of the compounds and the ion source temperature was set

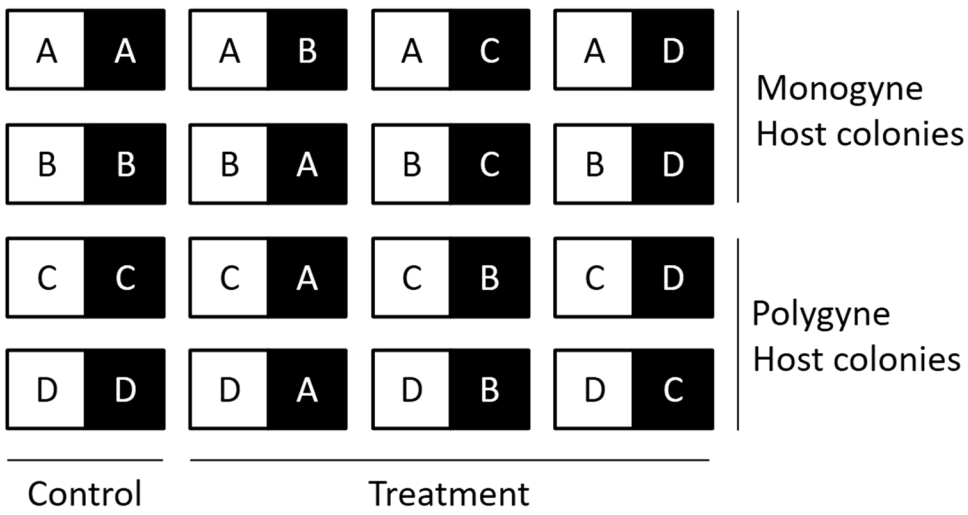

Fig. 2 Experimental setup of a full factorial cross-fostering trial. White squares indicate the colony of origin of adopted workers and the black squares show the identity of the foster colonies. Half of the foster colonies at each trial were monogyne and half polygyne

to 300 °C. Peaks in the chromatogram were aligned and integrated using a custom script in R v3.3.0 software (R Core Team 2020), and compounds were identified based on their retention times and diagnostic ions in the mass spectra, as well as through comparison with known standards and library searches using the NIST 14 mass spectral database. Raw peak areas were transformed according to Aitchison (Aitchison 1982) before further statistical analyses.

Phenotypic Variance Partitioning Analysis

Using the cross-fostering experiment dataset, we estimated the genetic and environmental factors affecting the cuticular hydrocarbon profile by fitting a linear mixed model with restricted maximum likelihood using the package breedR (Muñoz and Sanchez 2019). We calculated the total phenotypic variance, which is given by the sum of the genotypic value or broad-sense heritability, the environmental variance, and the residual variance. The environmental variance was further divided into variance given by the natal nest (factors influencing the phenotype during the larval stage while still in their native nest or maternal effects in the egg stage), the variance caused by the foster host nest, and the interaction between the two nests. Hence the general formula for the phenotypic variance was: $V_p = V_g + V_{natal} + V_{rearing} + V_{colonies\_interaction} + V_r$, where V_p is the total phenotypic variance, V_g is the genotypic value, V_{origin} is the variance given by the natal nest, $V_{rearing}$ is the variance given by the foster host nest, $V_{colonies\_interaction}$ is the variance given by the interaction between the two nest types and V_r is the residual variance. In this analysis, we used a subset of the data with only the monogyne colonies since all workers derived from a single colony were full sisters. All variables were treated as random effects in the model because we were interested in the overall patterns of phenotypic variance in the population (Wilson et al. 2010) with individual CHC abundances coded as response factors. We then produced a table with the relative variance partitioning representing the contribution of each factor affecting the individual CHC phenotypes (Table 1).

Statistical Analyses

All statistical analyses were carried out using R v4.0.0 (R Core Team 2020). Multivariate chemical dissimilarity between pairs of colonies was calculated by log transforming the average colony CHC relative abundance, centering the logarithmic abundances by subtracting the individual CHC means from the given colony value and rescaling it by dividing the centered values by their standard deviation. Pairwise dissimilarity colony Euclidean distances were then calculated using the package vegan (Oksanen et al. 2007). In order to analyze the influence of the queen number and the influence of the overall multivariate chemical dissimilarity on the drifting patterns, we ran a binomial generalized linear mixed model using penalized quasi-likelihood (GLMMPQL, package MASS (Ripley et al. 2013)) with the proportion of workers drifting as response factor and the directionality of the drifting behavior (i.e. from polygyne to polygyne, from polygyne to monogyne, from monogyne to monogyne and from monogyne to polygyne) interacting with time, plus the overall multivariate chemical dissimilarity as a covariate. Trial session, the colony of origin and the host colony were treated as random factors and the temporal autocorrelation was accounted for by using a continuous autoregressive process for a continuous time co-factor (corCAR1). The model was weighted to account for fact that drifters had twice as much chance to encounter a colony from a different breeding system than their own in the bioassay. Ovary development levels were assessed via a binomial generalized linear mixed model whereby ovary development was the response factor, the occurrence of drifting (i.e. whether or not the worker was a drifter) as fixed factor, and colony of origin nested with host colony as a random factor.

To test whether specific classes of cuticular chemical compounds influence the occurrence of drifting behavior, we fitted a model similar to the previous one, but with the multivariate chemical distances calculated per class of CHCs present in *F. fusca* (i.e. linear alkanes, methylalkanes, and dimethylalkanes) as covariates. Furthermore, we calculated the accuracy of different classes of compounds in predicting colony identity using linear discriminant analysis with cross-validation (package MASS (Ripley et al. 2013)) in which the subset of the classes of compounds were the predictors and colonies were the response factors.

In a separate analysis, we assessed the proportion of gain and loss of individual CHCs in the foster environment compared to the control from the same colonies, combining the data from both monogyne and polygyne colonies. To this end, we ran linear mixed models with the relative abundance of a given CHC as a response factor, whether foster ant workers gained, lost, or belonged to control group as fixed factor, and the colony of origin, host colony, and trial coded as random factors, with p values later corrected for multiple comparisons. In addition, we analyzed the variations of individuals on all different rearing conditions against the controls with linear mixed models (LMM, package lme4 (Bates et al. 2016)). The Aitchison transformed abundance of a given CHC was coded as the response factor with the interaction of the condition to which the individual was submitted (i.e. control polygyne; control monogyne; from polygyne to polygyne; from polygyne to monogyne; from monogyne to monogyne and from monogyne to polygyne) was the explanatory factor with trial coded as a random factor. Then, the models were post-hoc tested with multiple comparisons using general

Table 1 Variance estimates of the total phenotypic variability of cuticular hydrocarbons of *F. fusca* workers based on restricted maximum likelihood models

Compounds	V_g	V_{natal}	$V_{rearing}$	$V_{colonies\_interaction}$	V_r
n-C_{23}	0.343	0.321	0.030	0.038	0.268
Mix of 11-;9-MeC_{23}	0.195	0.284	0.156	0.245	0.119
7-MeC_{23}	0.089	0.664	0.149	0.049	0.049
5-MeC_{23}	0.273	0.162	0.218	0.164	0.183
3-MeC_{23}	0.272	0.204	0.176	0.166	0.182
5,11-diMeC_{23}	0.284	0.232	0.003	0.285	0.196
n-C_{24}	0.356	0.313	0.041	0.008	0.282
Mix of 3,11-;3,9-;3,7-diMeC_{23}	0.196	0.018	0.540	0.127	0.119
12-MeC_{24}	0.360	0.293	0.001	0.057	0.290
8-MeC_{24}	0.301	0.332	0.094	0.052	0.221
4-MeC_{24}	0.343	0.121	0.275	0.008	0.253
10,14-diMeC_{24}	0.273	0.255	0.104	0.180	0.187
3-MeC_{24}	0.403	0.045	0.175	0.044	0.334
n-C_{25}	0.484	0.002	0.048	0.001	0.466
Mix of 13-;11-;9-MeC_{25}	0.238	0.468	0.098	0.038	0.158
7-MeC_{25}	0.231	0.164	0.331	0.126	0.148
5-MeC_{25}	0.141	0.632	0.047	0.098	0.082
9,13-diMeC_{25}	0.130	0.526	0.179	0.091	0.074
3-MeC_{25}	0.256	0.472	0.089	0.010	0.174
Mix of 5,17-;5,15-;5,13-;5,11-;5,9-diMeC_{25}	0.120	0.692	0.099	0.021	0.068
n-C_{26}	0.451	0.079	0.044	0.001	0.425
Mix of 3,13-;3,11-;3,19-diMeC_{25}	0.063	0.183	0.650	0.071	0.034
Mix of 13-;12-;11-;10-MeC_{26}	0.237	0.516	0.047	0.040	0.160
6-MeC_{26}	0.388	0.008	0.203	0.080	0.322
4-MeC_{26}	0.199	0.483	0.091	0.099	0.127
3-MeC_{26} and 6,10-diMeC_{26}	0.478	0.000	0.000	0.066	0.455
Mix of 4,10-;4,8-diMeC_{26}	0.447	0.064	0.076	0.002	0.411
n-C_{27}	0.486	0.000	0.037	0.002	0.474
Mix of 13-;11-MeC_{27}	0.279	0.324	0.122	0.080	0.196
5-MeC_{27}	0.329	0.028	0.298	0.100	0.245
11,15-diMeC_{27}	0.252	0.248	0.206	0.130	0.164
Mix of 7,15-;7,13-diMeC_{27}	0.135	0.497	0.263	0.027	0.078
3-MeC_{27}	0.099	0.633	0.078	0.134	0.056
5,15-diMeC_{27}	0.278	0.370	0.144	0.013	0.194
n-C_{28}	0.124	0.764	0.001	0.039	0.071
Mix of 12-;10-MeC_{28}	0.346	0.264	0.004	0.112	0.274
12,16-diMeC_{28}	0.207	0.568	0.001	0.090	0.134
8,y-diMeC_{28}	0.260	0.357	0.010	0.193	0.180
n-C_{29}	0.379	0.252	0.001	0.055	0.313
Mix of 13-;11-MeC_{29}	0.253	0.162	0.002	0.412	0.171
n-C_{30}	0.294	0.289	0.051	0.159	0.206

V_g is the genetic variance, V_{origin} is the variance attained during the larval stages on the natal nest, $V_{rearing}$ is the variance acquired from the rearing nest, $V_{colonies\_interaction}$ the variance derived from the interaction between the two nests and V_r is the residual variance

linear hypotheses test in the multicomp package (Hothorn et al. 2008), and the p values were then corrected for false discovery rate (detailed results for this section presented in the supplemental Table S1).

Results

Worker Drifting Bioassay

Our drifting bioassay showed that a small percentage of workers (2.4% out of 1705 workers) of the ant *F. fusca* indeed drifted to different colonies. These were unlikely to be orientation mistakes since their distribution is different than what was expected by chance, showing clear directionality patterns. Workers were more likely to infiltrate colonies with a different breeding system than their natal nests, i.e. workers from monogyne colonies infiltrated polygyne colonies at higher rates than monogyne colonies (binomial GLMM, t-value = 3.75, $p < 0.001$) while workers from polygyne colonies infiltrated more monogyne colonies (binomial GLMM, t-value = 3.67, $p = 0.001$). Moreover, no statistical difference could be observed between colonies with the same number of queens (from monogyne to monogyne, binomial GLMM, t-value = 1.91, $p = 0.066$; polygyne to polygyne, binomial GLMM, t-value = 0.94, $p = 0.356$) (Fig. 3). The rates of ovary activation of drifter workers were not different from natal workers (drifters 31.7%, natal workers 35%, binomial GLMM, Wald Z score = 0.397, $p = 0.691$).

The observed pattern of worker drifting behavior was correlated to the chemical composition between the pairs of colonies. For all cuticular hydrocarbons combined, we observed that workers drifted more to colonies in which the odor bouquet resembled those of their natal nests (binomial GLMM, t-value = −2.82, $p = 0.016$). When the chemical dissimilarity between pairs of colonies was analyzed per different chemical class of CHCs, the occurrence of drifting behavior was only significantly influenced by the similarity levels of methyl alkanes (binomial GLMM, t-value = −3.48, $p = 0.007$; Fig. 4), whereas linear and dimethyl alkanes presented no significant effect in *F. fusca* worker drifting behavior (Linear alkanes: Poisson GLMM, t-value = −1.26, $p = 0.238$; Dimethyl alkanes: binomial GLMM, t-value = 1.52, $p = 0.163$; Fig. 4). Moreover, a linear discriminant analysis with cross-validation was able to correctly assign colony identity with 94.2% of accuracy for all CHCs combined and with 91.67% accuracy for the subset of methyl-branched alkanes, while dimethyl alkanes showed an accuracy of 87.5% and the abundance of linear alkanes

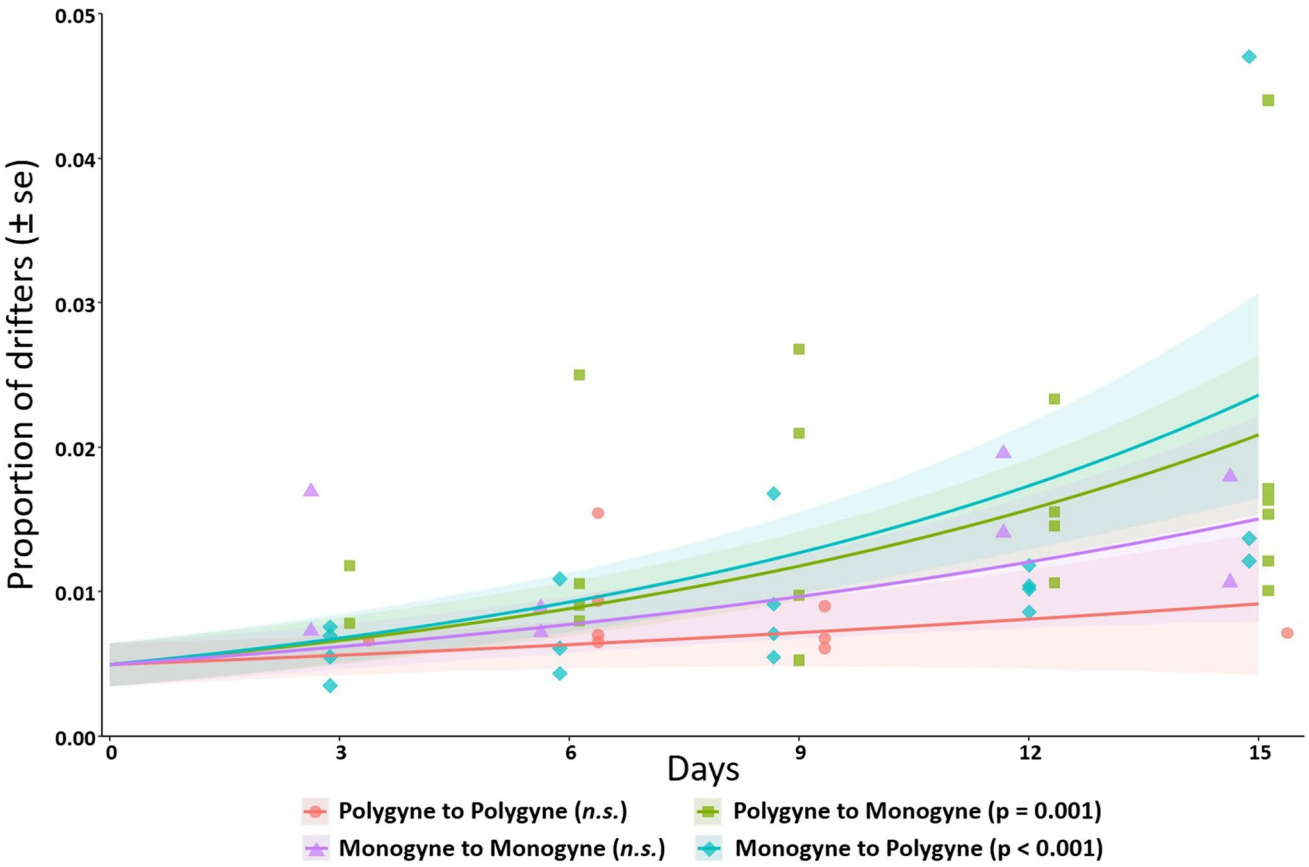

Fig. 3 Workers of *F. fusca* drifting rates over the experimental period. Lines represent the Binomial GLMM model predictions of the proportion of drifting events observed with partial residuals (symbols) over the 15-days experimental period

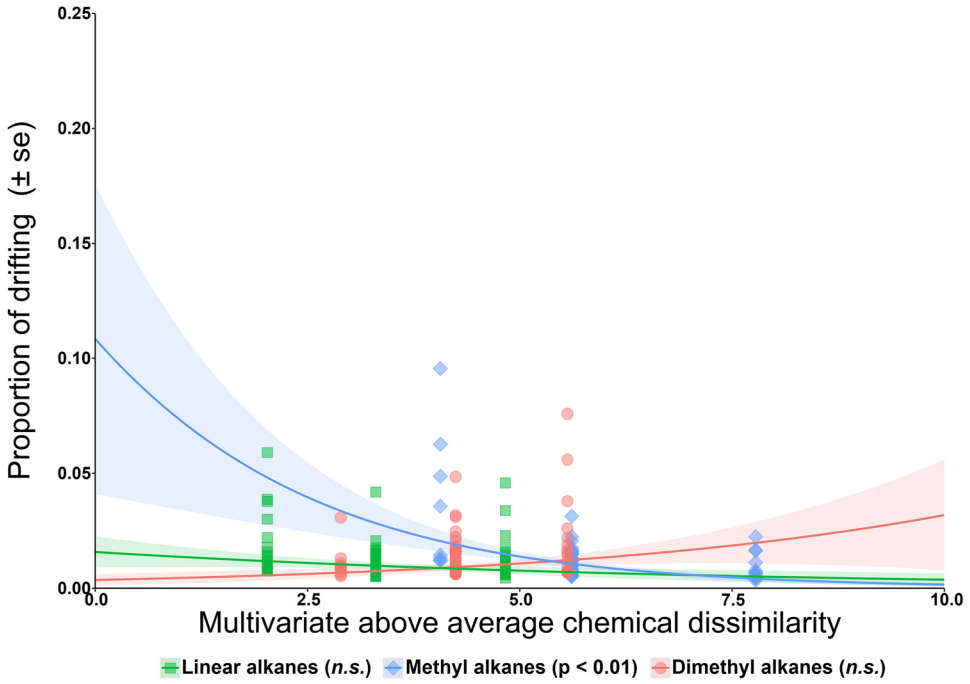

Fig. 4 Binomial GLMM fit of the relationship between the proportion of drifting and the multivariate chemical dissimilarity of cuticular hydrocarbons between pairs of colonies involved on the drifting event. The most similar the overall colony chemical profiles, more drifting was observed. Different symbols represent the partial residuals at the 10th, 50th, and 90th percentiles for each group of chemical compounds

was able to correctly assign colony identity with only 60% accuracy.

Cross-Fostering Experiment

In terms of the genetic and environmental influences on the CHCs phenotype, all individual compounds display a somewhat similar genotypic component while environmental factors, represented by the variance derived from the natal colony (V_{origin}), the rearing colony ($V_{rearing}$), and their interaction ($V_{colonies\_intereaction}$) are responsible for a larger proportion of the cuticular composition phenotypic component (Table 1). On average, environmental factors together were responsible ca. of 50% of the cuticular hydrocarbon variation in *F. fusca*, while the genotypic components account for 27.5% of the variation with the remaining as residual variance. It is interesting to note that when faced with a foster environment where CHC levels were higher than their natal colonies, workers presented up to 8% increase in individual compound abundances, whereas in a foster colony with CHC abundances lower than those of the natal nest the resulting levels of some CHCs decreased by almost 5% (Fig. 5). In fact, the same set of compounds, being mostly methylated (e.g. 3-MeC_{27}) and dimethylated (e.g. 9,13-diMeC_{25} and other mixtures of diMeC_{25}) compounds, are prone to be either acquired from or lost to the new foster environment (Fig. 5). The same set compounds also show high environmental variance with ca. of 15% of the phenotypic variance acquired in the foster colony and ca. of 31% derived from environmental cues acquired from their nest of origin (Table 1). In addition, all linear alkanes with exception of *n*-C_{28} present a higher genetic component, with 40% of explained variance on average, presumably because of the primary function of these compounds in preventing desiccation.

The different experimental groups could be reliably identified based on their overall chemical profile with some compounds being acquired from the host colonies in significant quantities (Table S1). When individuals from polygyne colonies were reared in monogyne colonies, their overall CHC profile significantly differed from the polygyne control group (Permanova, $p = 0.007$), while polygyne workers reared in different polygyne colonies showed no differences in the overall CHC profile (Permanova, $p = 0.672$). Furthermore, when monogyne workers were transferred to both monogyne and polygyne host colonies they significantly changed their CHC profile (Permanova, monogyne, $p = 0.035$; polygyne, $p = 0.005$). The effect of workers of *F. fusca* being fostered in a different environment on individual cuticular compounds abundances is shown in Table S1 in the supplemental material.

Discussion

In this study, we show that workers of the ant *F. fusca* can drift into different colonies at relatively low rates in laboratory conditions. The drifting rates observed in our bioassay (ca. 2.4%) are in line with what is found in the literature for other social insect species, such as the common wasp

Transferred CHCs	Control Colonies	Acquired CHCs	
2.99 ***	5.03	8.56 ***	*n*-C_{23}
0.98	1.34	2.00	Mixture of 11-;9-MeC_{23}
0.36	0.73	3.38 ***	7-MeC_{23}
0.14 ***	0.28	0.42 ***	5-MeC_{23}
1.09	1.58	2.35	3-MeC_{23}
0.08 ***	0.18	0.34 ***	5,11-diMeC_{23}
0.78	1.38	2.18 *	*n*-C_{24}
0.32	0.90	1.21	Mixture of 3,11-;3,9-;3,7-diMeC_{23}
0.88	1.19	1.54 *	12-MeC_{24}
0.09 ***	0.20	0.39 ***	8-MeC_{24}
0.17 ***	0.33	0.57 *	4-MeC_{24}
0.16 ***	0.24	0.49 ***	10,14-diMeC_{24}
0.05	0.28	0.67	3-MeC_{24}
11.74 ***	14.20	20.84 ***	*n*-C_{25}
18.78 ***	23.48	29.93 ***	Mixture of 13-;11-;9-MeC_{25}
2.29 *	3.27	7.35 **	7-MeC_{25}
1.72	2.57	4.01 ***	5-MeC_{25}
1.46 *	2.20	3.84 **	9,13-diMeC_{25}
5.71 ***	6.79	10.32 ***	3-MeC_{25}
2.56	3.66	8.62 *	Mixture of 5,17-;5,15-;5,13-;5,11-;5,9-diMeC_{25}
1.28	1.65	2.04 ***	*n*-C_{26}
1.29 **	3.65	11.75	Mixture of 3,13-;3,11-;3,19-diMeC_{25}
1.50	2.14	2.81 ***	Mixture of 13-;12-;11-;10-MeC_{26}
0.26 **	0.38	0.86 ***	6-MeC_{26}
0.24 **	0.35	0.56 *	4-MeC_{26}
0.28 ***	0.32	0.56 ***	Mixture of 3-MeC_{26} and 6,10-diMeC_{26}
0.03 ***	0.09	0.87 **	Mixture of 4,10-;4,8-diMeC_{26}
2.75	3.46	5.34 ***	*n*-C_{27}
5.36 ***	7.60	9.54 ***	Mixture of 13-;11-MeC_{27}
0.77	1.28	1.94	5-MeC_{27}
0.29 ***	0.40	0.71 ***	11,15-diMeC_{27}
0.91	1.40	2.24	Mixture of 7,15-;7,13-diMeC_{27}
0.87 **	1.87	6.56	3-MeC_{27}
0.61 *	0.85	1.14	5,15-diMeC_{27}
0.27	0.51	0.71	*n*-C_{28}
0.27 ***	0.43	0.53	Mixture of 12-;10-MeC_{28}
0.12	0.34	0.67 ***	12,16-diMeC_{28}
0.09	0.29	0.49 **	8,y-diMeC_{28}
0.42	0.77	1.37	*n*-C_{29}
0.86	1.27	1.30 **	Mixture of 13-;11-MeC_{29}
0.43 **	1.14	1.48	*n*-C_{30}

CHC transfer rate

-8% -5% 0% 5% 8%

◂**Fig. 5** Heatmap with overall cuticular hydrocarbons transfer rates. CHC transfers are shown in terms of differences on average compound relative abundances between ants fostered in a different colony environment and those derived from the same nest, combining the data from both monogyne and polygyne colonies. Values indicate the relative abundance of a given CHC in the control groups and colors indicate the difference in relative abundance observed when ants were raised in a foster colony. LMM FDR corrected significance levels * $P \leq 0.05$ ** $P \leq 0.01$ and *** $P \leq 0.001$

Vespula vulgaris (2.3% (Oliveira et al. 2016)) and honeybees (1–5% (Chapman et al. 2009a; Chapman et al. 2009b; Chapman et al. 2009c; Nanork et al. 2007; Nanork et al. 2005)), nevertheless, clearly lower than the rates observed in bumblebees (2.7–28% (Birmingham and Winston 2004; Takahashi et al. 2010; Zanette et al. 2014)), in *Polistes* paper wasps (8.9–56% (Sumner et al. 2007)) and in the congeneric species *Formica exsecta* (ca. 16% (Katzerke et al. 2006)). Although no differences in the ovary activation levels between drifters and natal workers were observed, our data shows that drifting did not happen by chance (i.e. were no orientation error) since a clear pattern of workers drifting from monogyne to polygyne colonies and from polygyne to monogyne colonies was observed (Fig. 3), suggesting that individual workers are potentially able to assess the queen number of the colonies they drift in, likely making use of cuticular chemical cues.

Colonies of the ant *F. fusca* in nature were shown to be susceptible to interspecific parasitism (Hlaváč et al. 2011; Mori et al. 2000). In fact, a recent global phylogeny of the genus *Formica* shows that social parasitism is widespread within the genus (Borowiec et al. 2021). A hypothesis for the origin of polygyny as a breeding system in Hymenoptera is that it is a response to parasite load in the colonies in such a way that increasing the genetic variability would turn colonies more resilient against parasitism and pathogens (Pérez-Lachaud et al. 2011; Schmid-Hempel and Crozier 1999). Indeed, several studies have shown a positive relationship between parasitic load and the occurrence of polygynic colonies in social insects (Liersch and Schmid-Hempel 1998; Pérez-Lachaud et al. 2011; Schmid-Hempel and Crozier 1999; Shykoff and Schmid-Hempel 1991). From an individual worker perspective, however, increasing the number of breeding queens reduces the overall genetic relatedness within the colony and consequently its inclusive fitness (Hamilton 1964a; Hamilton 1964b). Nonetheless, it has been suggested that workers in polygyne colonies of *F. fusca* can assess the relatedness of close relatives as early as in the brood stages and act nepotistically towards them (Hannonen and Sundström 2003). This demonstrates that a polygynic breeding system would not be necessarily disadvantageous if workers can discriminate kin from non-kin. Such capability could be applied not only from within-colony-level, when workers act nepotistically towards relatives, but also in a broader population-level context in order to avoid competition with genetic related colonies. Additional evidence of social origin discrimination in ants comes from the related *Formica selysi* in which foreign eggs derived from monogyne colonies introduced in the nest had significantly lower chances of survival than those derived from polygyne colonies (Meunier et al. 2011). Moreover, in the red fire ant *Solenopsis invicta,* new colonies can be founded via intraspecific social parasitism when queens derived from monogyne colonies drift and take over unrelated colonies (Tschinkel 1996). Nevertheless, workers of this species have the ability to recognize and execute queens that would otherwise initiate monogyne colonies (Keller and Ross 1998). Whether these two systems are indeed evolutionary counter-strategies to social parasitism still awaits further investigation.

Since all CHCs present generally a lower heritable component (Table 1) when compared to environmental factors, our data suggest that relying on a subset of the total cuticular hydrocarbon profile could be a strategy to identify conspecific unrelated colonies while still avoiding competition with direct kin. From a drifter worker perspective, even if a host nest is headed by a single queen which happens to be her sister, the average relatedness between the drifter and the host individuals is considerably lower than within her natal nest. For example, a drifter would be most related to her host colony when she is derived from a monogyne colony headed by a singly mated queen (average relatedness $r = 0.75$ among workers), and the host colony is headed by one of her sisters. Assuming that the queen in the host colony is mated to an unrelated male, the relatedness between the drifter worker and the workers in the host colony will be only $r = 0.375$. Nevertheless, the fact that workers would rely on a set of chemical compounds with a low heritable and high environmental component seems counterintuitive. A possible explanation is that selective pressure might be relatively low for the evolution of a more refined recognition system and that workers are simply unable to decouple the two components. In addition, relying on a strong (social) environmental component in the CHC profile may be advantageous if workers are generally surrounded by close kin or if it helps to prevent nepotism within the colony. In fact, in *F. fusca*, policing behavior occurs by egg cannibalism instead of aggression towards reproductive individuals and it was suggested to be an adaptation against social parasites, in which workers would be able to discriminate and police eggs not laid by their nestmate queen (Helanterä and Sundström 2007), and therefore, effectively preventing social parasites from reproducing in their colony.

By relying only on methyl branched alkanes drifters might be able to unequivocally identify colony identity, since we show that methyl branched alkanes alone could correctly assign colony identity with more than 90% accuracy, similar to the observed rates of nestmate recognition

accuracy in *Formica* ants (Johnson et al. 2011). A subset of alkenes and dimethyl alkanes were hypothesized to act as nestmate recognition cues in two *Formica* ant species, given that their relative abundance are most variable between colonies (Martin et al. 2008). Our results provide empirical evidence that methylalkanes were implicated in the occurrence of drifting behavior and possibly in recognition itself. If this subset of compounds is indeed acting as nestmate recognition cues in *F. fusca*, by drifting into colonies most similar to their own, foreign workers might evade being detected by the host colony workers, increasing the probability of successfully infiltrating the host colony while still avoiding competition with nestmates. That is because methyl and dimethyl alkanes show a high environmental component, with an observed variation of up to 8% in individuals raised in a foster environment (Fig. 5). Hence, colonies with a similar overall odor in terms of these classes of compounds need not be genetically related, allowing drifters to use this recognition system in their favor. It is important to note, however, that our data do not unequivocally show that drifted workers are social parasites and future studies are still needed to test this hypothesis.

In conclusion, the results of our drifting bioassay demonstrate that workers of the ant *F. fusca* are able to drift into unrelated conspecific colonies, providing insights on how environmental factors affect the phenotypic variation of cuticular hydrocarbons in this species. Moreover, we show that drifting behavior is affected by the chemical similarities between colonies in which workers rely on a subset of methyl branched alkanes, likely linked with nestmate recognition, infiltrating colonies that are most similar to their own chemical profile, and gaining access to the host colony without being detected as non-nestmates.

Supplementary Information The online version contains supplementary material available at https://doi.org/10.1007/s10886-021-01325-3.

Authors' Contributions R.C.O and J.V.Z. had the original idea. R.C.O. performed the experiments. R.C.O, T.W. and J.V.Z. analyzed the data. R.C.O. wrote the first draft of the manuscript. All authors edited and revised the manuscript.

Funding R.C.O. was supported by a grant from CNPq-Brazil (238127/2012–5) and Research Foundation Flanders (Research Grant 1502119 N and Postdoctoral Fellowship 12R9619N). J.V.Z. was supported by a FWO Postdoctoral Fellowship (12Q7615N).

Data Availability The raw datasets and R scripts used in this manuscript are available at Mendeley Data Repository (Oliveira et al. 2021).

Declarations

Conflicts of Interest/Competing Interests The authors declare that they have no competing interests.

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