



Ant genotype, but not genotype of cultivated fungi, predicts queen acceptance in the asexual fungus-farming ant *Mycocepurus smithii* (Hymenoptera: Formicidae)

Brian T. Barrett¹ · Tristan D. Kubik¹ · Peter R. Golightly¹ · Katrin Kellner^{1,2} · Melissa R. Kardish^{1,3} · Ulrich G. Mueller¹

Received: 19 April 2022 / Revised: 26 October 2022 / Accepted: 28 November 2022

© The Author(s), under exclusive licence to Springer-Verlag GmbH Germany, part of Springer Nature 2022

Abstract

Nestmate acceptance in ants can depend on environmental factors (e.g., odors acquired from environment) or on ant genotype. Here, we test whether queen acceptance by workers of the fungus-farming ant *Mycocepurus smithii* can be predicted from the fungal genotypes of fungus gardens and from the ant genotypes of interacting workers and queens. *Mycocepurus smithii* is a clonal (asexual) ant with multi-queen colonies that cultivate clonal fungi. Ant colonies can be switched to new fungi to raise queens in experimental cross-fostered ant-fungus combinations, and ant-genetic factors versus fungus-derived factors modulating worker-queen interactions can therefore be tested in controlled experiments. In a factorial experiment using all six combinations of three ant clones and two distantly related fungal clones, we performed 180 blind observation trials in which we introduced queens to queenless mesocosms (workers and garden) and scored worker aggression toward the introduced queens. We found that aggression toward queens is correlated with ant genotype, and that odor cues that ants may have acquired from their native fungal cultivar do not override the cues correlated with ant genotype during queen acceptance by workers. The acceptance of queens of *M. smithii* is based therefore less on fungal odor cues and more on cues correlated with ant genetics. Because hundreds of queens can be raised in laboratory nests of *M. smithii*, future research can use the queen-adoption protocol developed here to test whether the ant-genetic factors mediating queen acceptance could perhaps be important in kin recognition in *M. smithii*.

Significance statement

Ants benefit in many ways from living together and cooperating in colonies, but these benefits can potentially be exploited by unrelated queens from different colonies that insinuate themselves into an ant society. We use the fungus-farming ant *Mycocepurus smithii* to show that workers are more aggressive towards unrelated queens and more accepting towards queens of the same genotype as resident workers. Queen adoption and possibly nestmate recognition can therefore be predicted from genetic similarity.

Keywords Attina · Aggression · Nestmate recognition · Queen adoption · Queen acceptance · Fungus-farming ants

Communicated by: L. Keller.

Brian T. Barrett and Tristan D. Kubik contributed equally to this work.

✉ Tristan D. Kubik
tristan.kubik@utexas.edu

✉ Ulrich G. Mueller
umueller@austin.utexas.edu

² Department of Biology, University of Texas at Tyler, Tyler, TX 75799, USA

³ Department of Evolution and Ecology, University of California, Davis, CA 95616, USA

¹ Department of Integrative Biology, University of Texas at Austin, Austin, TX 78712, USA

Introduction

Aggressive behaviors of workers guarding nests of social insects can prevent an intrusion by non-nestmates (d'Ettorre and Lenoir 2010; Dobata et al. 2011; Kronauer et al. 2013; Lorenzi and d'Ettorre 2020). In ants, nestmate recognition and acceptance are mediated in part by non-volatile, integumental odors (Wagner et al. 2000; Provost et al. 2008; Brandstaetter et al. 2008; Smith et al. 2009; d'Ettorre and Lenoir 2010; van Wilgenburg et al. 2011; Sprenger and Menzel 2020). When ants antennate one another, they perceive one another's cuticular hydrocarbons, which in many ant species function as a chemical fingerprint suitable for nestmate recognition and possibly kin recognition (Ratnieks 1991; Smith et al. 2009; d'Ettorre and Lenoir 2010; Sprenger and Menzel 2020). Recognition cues such as cuticular hydrocarbons can be shared among individuals in an ant nest, leading to homogenization of cuticular hydrocarbon profiles throughout the colony and development of a colony-specific odor (Dahbi et al. 1999; Soroker et al. 1998; d'Ettorre and Lenoir 2010; van Wilgenburg et al. 2011; Sprenger and Menzel 2020). Individuals that insufficiently match a colony's odor profile are rejected or attacked by resident workers of a colony (Wagner et al. 2000; Lorenzi and d'Ettorre 2020).

The colony odor in some ant species derives primarily from environmental sources such as the nest (Heinze et al. 1996; Soroker et al. 1998), whereas in other ant species the genotype of the ant can be a determinant of colony odor (Ratnieks 1991; Beye et al. 1997; Kronauer et al. 2013; De Gasperin et al. 2021). A third possibility is that a colony-specific odor profile is determined by some combination of both environment and ant genotype (Crozier and Dix 1979; Pirk et al. 2001). Ant genotype is recognized by proxies that correlate with genotype such as cuticular hydrocarbons and other odor cues that depend on ant genotype (see above; Dietemann et al. 2003; Wagner et al. 2000; Smith et al. 2009), and these odor cues blend with environmentally acquired odors such as odors derived from soil, forage material, or ant-cultivated fungi in the case of fungus-growing ant species (Valadares and do Nascimento 2017).

Using the fungus-farming ant *Mycocepurus smithii* and its cultivated fungi, we developed an assay of aggression directed toward an introduced queen to test the relative importance of ant-genetic versus environmental factors (here fungal factors) in queen acceptance. Specifically, we used this assay to test whether odor cues correlated with ant genotype as compared to odors acquired from the fungal garden best predict ant aggression and queen acceptance. The role of fungus-derived odor cues in nestmate acceptance has been documented in several fungus-growing ant species of the leafcutter genera *Atta* and *Acromyrmex*

(Viana et al. 2001; Richard et al. 2007a,b; Larsen et al. 2014; Valadares and do Nascimento 2017), but no studies have been conducted so far with any non-leafcutter fungus-growing ant species such as *M. smithii* studied here.

Mycocepurus smithii is unique among fungus-growing ants because it reproduces asexually through most of its geographic range from northern Argentina to northern Mexico (Rabeling et al. 2009, 2011; Barros et al. 2022). Males of *M. smithii* have not been found in most populations across this range (Fernández-Marín et al. 2005; Himmler et al. 2009; Rabeling et al. 2009, 2011; Kellner et al. 2013; Barros et al. 2022), but some sexual populations with males have been discovered along the Rio Amazonas in the States of Amazonas and Pará in Brazil (Rabeling et al. 2011; Barros et al. 2022). Queens produce diploid offspring through thelytokous parthenogenesis (Rabeling et al. 2009, 2011), and *M. smithii* is the only known fungus-growing ant species that reproduces by thelytokous parthenogenesis (Barros et al. 2022). New nests of *M. smithii* are founded by single or multiple queens that disperse from their natal nest during short dispersal flights (Fernández-Marín et al. 2004). In Puerto Rico, 12% of new nests were observed to be co-founded by more than one queen and 88% founded by single queens (eight multi-queen nests of 63 new nests surveyed). In Panamá, 8% of new nests were observed to be co-founded by more than one queen and 92% of new nests were founded by single queens (1 multi-queen nest of 12 new nests surveyed) (see Table 1 in Fernández-Marín et al. 2004). Because single-queen nests transition over time into multi-queen nests, mature nests found in the field are invariably multi-queen nests (i.e., polygynous nests, Fernández-Marín et al. 2005; Rabeling et al. 2009, 2011; Kellner et al. 2013), and supernumary reproductive females are identical clones of their mothers (Fernández-Marín et al. 2004; Rabeling et al. 2009, 2011; Fang et al. 2020; Barros et al. 2022).

Mycocepurus smithii is furthermore special in that it cultivates an asexually propagated, leucocoprinaceous fungus, and under natural conditions, clonal lineages of ants frequently switch between fungal clone lineages cultivated by the ants (Mueller et al. 1998; Kellner et al. 2013). These fungal clone lineages can be phylogenetically distinct as much as different species of free-living leucocoprinaceous fungi (Mueller et al. 1998; Kellner et al. 2013), whereas most fungus-farming ant species associate typically with only a single fungal type (Mueller et al. 1998, 2004; Mehdiabadi et al. 2006, 2012; Mikheyev et al. 2010; Seal and Mueller 2014; Beigel et al. 2021; but see exceptions summarized in Mueller et al. 2017, 2018). In central Panamá where we collected *M. smithii* in 2010 to establish queenright laboratory colonies for experiments, the local diversities of both ant clones and associated fungal lineages are relatively

high, with at least 11 distinct ant clone lineages and at least 9 fungal lineages distributed across the Isthmus of Panamá, and 7 distinct ant clone lineages and 5 fungal lineages at our main study site in Gamboa (Kellner et al. 2013), the source of the ant clone lineages that we use here in our experiment. In Gamboa, neighboring colonies less than 50 cm apart can be occupied by distinct ant clones that cultivate very distinct species of leucocoprinaceous fungi (Mueller et al. 1998; Kellner et al. 2013; Mueller, unpublished observations). Because of these high local genetic diversities and because queens do not disperse far during mating flights (Fernandez-Marín et al. 2004, 2005), incipient nests can potentially be co-founded by queens belonging to distinct ant clones. Second, any dispersing queen that founds a nest close to an established nest, or perhaps seeks acceptance in an established nest after dispersal, could be of same or different ant genotype as the resident ants. A third possibility where workers may encounter queens of other genotypes is that queens may attempt to migrate between colonies when genetically distinct colonies in the dense nest aggregations of *M. smithii* meet underground during lateral nest expansion.

Because of the unique ant-fungus co-evolution and reproductive biology, asexual clones of *M. smithii* ants can be experimentally cross-fostered in the laboratory with different fungal clones to generate colonies of ant-fungus combinations that allow for experimental disentangling of ant-associated and fungus-associated factors contributing to behavior and colony properties (Kellner et al. 2013, 2015, 2018) such as the queen adoption behaviors investigated here. Our study capitalizes on these unique strengths of the study system of *M. smithii*. We introduced queens of known genotypes, which had been cross-fostered on known fungus-types, into queenless mesocosms of workers with a garden to test whether ant genotype influences aggressive interactions by workers directed at the introduced queen. Queen acceptance or rejection may limit the movement of queens between ant colonies that are in close proximity to one another (Fernandez-Marín et al. 2005; Rabeling et al. 2009, 2011; Kellner et al. 2013). We hypothesized that if environmental cues are determinants for nestmate recognition, then the identity of the resident ants' native fungus type should be a predictor of worker aggression toward introduced queens. In contrast, if ant genotype is a determinant, then worker-queen genetic similarity or correlated proxies such as genotype-dependent cuticular odors should be predictors of worker aggression toward introduced queens.

Methods

Study organisms

For a study on ant-microbiome ecology (Kellner et al. 2013, 2015, 2018), we had collected a large sample of

Mycocepurus smithii colonies with their fungal gardens in 2010 in the Republic of Panamá. These colonies were maintained in a lab at the University of Texas at Austin using the methods in Kellner et al. (2018). Because *M. smithii* is clonal and colonies continuously produce supernumary queens, colonies can be maintained indefinitely in the lab, and we were able to create, through transfer of queens to workerless gardens, combinations of ant and fungal lineages as source colonies for experiments. From our collection of lab-reared colonies, we chose colonies of three ant genotypes (A-ant, G-ant, and J-ant) and two fungal types (fungus-5 and fungus-7, as defined by Kellner et al. 2013) (Supplementary Fig. S1). We refer below to each combination using these ant and fungal lineage designations, such that a colony of ants of the G-ant genotype entrained on fungus type 7 is denoted G7 (G-ant with fungus-7). Using the ant-fungus co-phylogenies established by Kellner et al. (2013) (Supplementary Fig. S1), we selected two relatively closely related ant lineages (genotypes A-ant and J-ant), and genotype G-ant that is distantly related to these two ant lineages. As fungus types, we chose one fungus type from each of the two major fungal clades of the fungal phylogeny (Supplementary Fig. S1). These two fungal types belong to different species of *Leucocoprinus* fungi (Kellner et al. 2013; Mueller et al. 1998, 2017, 2018), whereas all ant genotypes are clone lineages of the same species, *M. smithii* (Kellner et al. 2013).

In the native habitat of *M. smithii* in Panamá, ant genotype G-ant was most frequently collected in association with fungus-7 and rarely with fungus-5 (Kellner et al. 2013), whereas ant genotypes A-ant and J-ant were frequently associated with fungus-5 and rarely with Fungus-7 (Supplementary Fig. S1). In an experiment testing for ant-fungus synergisms contributing to colony growth (Kellner et al. 2015), ants were fungus-switched in the lab and colonies of some of these ant-fungus combinations (i.e., A5=combination of A-ant and Fungus-5 and combinations A7, G5, G7, J5, and J7) were available in sufficient numbers for experimentation in our study. Each of the source colonies had been maintained in the laboratory since 2010 until the experiments were conducted in 2015. Because colonies of ant-fungus combinations A7, G5, and J7 grow slowly under laboratory conditions and do not produce many queens, only workers were drawn from these colonies for experiments but not queens. We used both queens and worker ants from colonies of ant-fungus combinations A5, G7, and J5. The source colonies were all housed in the same temperature-controlled room (25 ± 1 °C), given an identical substrate of polenta and minced oat flakes for fungi culture, and placed in identical nest boxes, as described in Kellner et al. (2015). Although we tested the interactions between workers and young, alate females that had not yet shed wings to mature into queens, for simplicity, we refer here to these alate females as queens.

Experimental design

From each of the six source colonies, we established 30 mesocosms in laboratory nests (see description in Mehdiabadi et al. 2006) for a total of 180 mesocosms. Each mesocosm contained 10 workers and an approximately 1-cm³ fragment of the worker's native fungus garden, placed in the center of a cylindrical container of clear plastic (5.1 cm diameter, 3.7 cm high; model SKU 002c, Pioneer Plastics, Dixon KY, USA) with the bottom three-fourths of the chamber filled with a substratum of UV-sterilized moistened dental plaster (Sure Laboratory Stone, MarJoy Enterprises LLC, Boerne TX, USA). Each cohort of 10 workers had completed their entire development from egg to adult on the fungus type they were cultivating, and they had never been exposed to a different fungus type or ants from different ant genotypes. Each mesocosm was given 14 days to habituate workers to their new nest chamber. The bottom of moistened plaster ensured 100% humidity in the mesocosm during that time without need for watering during the 2 weeks of habituation. Because gardens of *M. smithii* have a slow growth rate, it was possible to keep ants and gardens in a mesocosm without giving the ants a substrate for fungi culture; this minimized the chance of introducing uncontrolled odor cues or pathogens (Kellner et al. 2018) into a mesocosm (i.e., each mesocosm was effectively sealed during the 2 weeks of habituation, except for a very narrow space at each container lid allowing gas exchange).

To ensure blind experimentation (Kardish et al. 2015), each replicate colony was assigned a computer-generated random number, and each mesocosm was labeled by a third party such that the observer (B.T.B.; Barrett 2015) would have no knowledge of which ant-fungus combination was in each mesocosm. For each observation, an alate female from either the A5, G7, or J5 source colony was introduced into a mesocosm by moving the queen with forceps and placing her directly onto the fungal garden. The randomization was performed such that there were ten replicate introductions for each of the eighteen combinations of queens and workers. All tests were performed in a randomly generated order to control for test-order effects and to further obscure to the observer any information on the ant-fungus combination tested.

We video-recorded the first 5 min after we introduced a queen, and the recording was later scored blindly by a naïve observer for aggression directed by workers at the queen. Each 5-min video was broken into 30 10-s intervals. Every queen-worker interaction inside of each 10-s interval was scored according to the following aggression scale modified from Carlin and Hölldobler

(1986): 0: there were no worker-queen interactions during a 10-s interval, but the introduced queen was visible for at least 25% of the time interval; 1: a worker briefly antennated the introduced queen (lasting less than 3 s); 2: a worker antennated the introduced queen for a prolonged period (longer than 3 s); 3: a worker placed two or more legs on top of the introduced queen; 4: a worker bit the introduced queen but did not attempt to drag her; 5: a worker bit the introduced queen, dragged her, or otherwise moved her; 6: a worker dismembered the introduced queen (i.e., removal of antennae, wing, leg, etc.), and the queen remained alive; 7: a worker killed the introduced queen; minus-2: the queen was visible for less than 8 of the total 30 10-s intervals (i.e., less than 25% of all the 30 intervals); this negative dummy score of minus-2 was used to indicate a trial in the full dataset that we had pre-planned to be excluded from analyses, to ensure a minimum number of 10-s interval observations per queen (i.e., each replicate included in analyses was based on at least 8 of the maximum 30 10-s observation intervals per queen); minus-1: workers moved away from the introduced queen, for example, as a response to physical disturbance when the queen was introduced to a mesocosm, or because of worker movement unrelated to queen presence. Because this last category of minus-1 captured disturbance and unrelated worker motion rather than worker aggression toward an introduced queen, the minus-1 scores were dismissed in analyses and treated as zero values by the R code (i.e., treated as no worker-queen interaction, see above).

We recorded the most aggressive interaction exhibited by any workers interacting with the introduced queen during a given time interval. If the observer had any doubt about how to score a time interval, that time interval was noted and then blindly rescored by a second observer.

Behavioral response variables

We calculated five metrics for quantifying aggression for each trial: (a) average aggression, (b) latency to first aggression of at least a score of 3, (c) maximum aggression, quantified on the above aggression scale, (d) maximum number of workers interacting with the queen during a 10-s time interval, and (e) percent duration of aggression during each trial. These five metrics were used in the principal component analysis (Fig. 1) described below. For heatmap visualization of aggression intensities (Fig. 2), we calculated also a sixth metric, total aggression (see below), from the above five metrics, but total aggression was not used as a variable in the principal component analysis, because total aggression already integrates information across the other five aggression metrics.

Computational details of how each of the six metrics was calculated from behavioral observations are recorded in the R-scripts available in the Supplementary Information, and computational methods are summarized here briefly. Average aggression was calculated as the sum of all aggression scores divided by the number of time intervals the queen was visible (i.e., the average was calculated only for those 10-s intervals when a queen was visible). If the queen was not visible for the entirety of a 5-min trial, the average aggression score was set to 0. Latency to aggression was calculated as

the number of 30-s time intervals before the first interaction with an aggression score of at least 3. If the queen was not visible during a 5-min trial, the latency to aggression for that trial was set to 300 s (= 5 min), the maximum length of the recording. Percent duration of aggression was calculated as the number of time intervals with an aggression score of at least 3, divided by the number of time intervals the queen was visible. If the queen was not visible for the entirety of the trial, this percent duration score was set to zero. Lastly, total aggression was calculated as the sum of all interaction scores

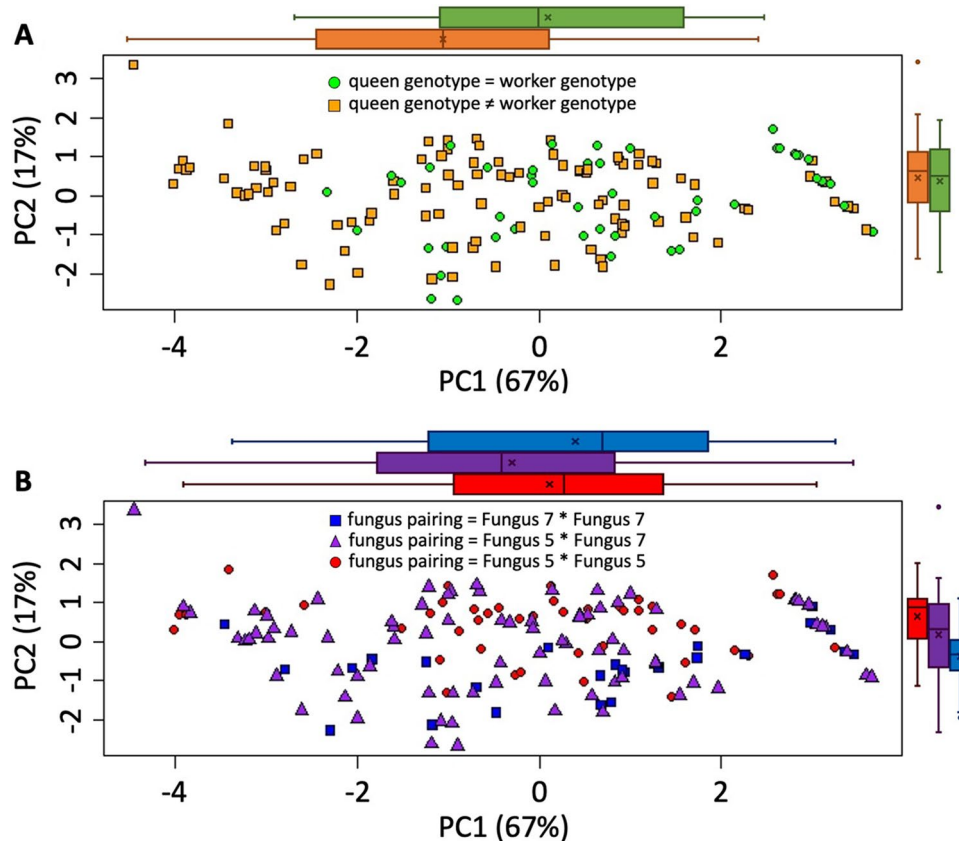


Fig. 1 Principal component analysis (PCA) plots generated from the aggression responses observed in workers of *Mycocepurus smithii* toward queens introduced in mesocosm experiments. Boxplots at the top and right summarize the variation in the PCA plots for the values that load onto the first principal component axis (PC1, corresponding boxplots at the top) and onto the second principal component axis (PC2, corresponding boxplots on the right). The PCA plots in A and B are identical but color-coded differently to highlight the importance of ant-genotype pairings in A and fungus-type pairings in B. **A** PCA plot color-coded according to ant genotype similarity. Aggression responses cluster by worker-queen genetic identity (green circle) versus non-identity (orange square), with separation along PC1 (top boxplots) but not along PC2 (right boxplots). Along PC1, the leftmost datapoints are mostly observations where the queen's genotype is different from the genotype of the workers (mostly orange squares), in the center are observations where both the queen's genotype is the same or different from that of the workers, and the rightmost datapoints are more observations where the queen's genotype is the same

as that of the workers (more green circles). This clustering by ant-genotype pairing along PC1 is not because the interactions differ by fungus-type pairings along PC1, but because all three fungus-type pairings are distributed broadly overlapping along the entire PC1 axis, as visualized in the second PCA plot shown in B. **B** PCA plot color-coded by fungus-type pairing, for observations where the fungus cultivated by the resident workers was the same (fungus-5 * fungus-5, circles; fungus-7 * fungus-7, squares) or different (fungus-5 * fungus-7, triangles) as the fungus that the introduced queen was reared on in her natal nest. Observations cluster somewhat by fungus-type pairing along PC2 but not along PC1. Overall, the PCA plots and corresponding boxplots show that ant-genotype is a more accurate predictor of aggression response variables than fungus cultivar in the queen-acceptance experiments. The preplanned ANOVA of principal component information (Supplementary Table S3) and post-hoc nonparametric statistical analyses (Supplementary Tables S4 and S5) confirm ant genotype as a statistically significant predictor of worker aggression toward introduced queens

A. Average Aggression

		Workers' Genotypes		
		A-worker Fungi 5&7	G-worker Fungi 5&7	J-worker Fungi 5&7
Queen's Genotype	A-queen Fungus 5	3.83 (± 1.86)	6.46 (± 2.76)	6.62 (± 4.16)
	G-queen Fungus 7	3.94 (± 3.03)	2.30 (± 1.49)	4.42 (± 2.45)
	J-queen Fungus 5	4.75 (± 4.62)	4.50 (± 1.98)	4.34 (± 2.05)

B. Latency to Aggression

		Workers' Genotypes		
		A-worker Fungi 5&7	G-worker Fungi 5&7	J-worker Fungi 5&7
Queen's Genotype	A-queen Fungus 5	153.5 (± 109.7)	128.5 (± 59.2)	161.0 (± 95.2)
	G-queen Fungus 7	86.0 (± 102.8)	206.5 (± 110.0)	124.4 (± 75.6)
	J-queen Fungus 5	111.0 (± 104.6)	101.4 (± 78.2)	147.5 (± 77.0)

C. Maximum Aggression

		Workers' Genotypes		
		A-worker Fungi 5&7	G-worker Fungi 5&7	J-worker Fungi 5&7
Queen's Genotype	A-queen Fungus 5	3.65 (± 1.21)	3.95 (± 0.51)	3.81 (± 0.83)
	G-queen Fungus 7	4.35 (± 1.00)	3.00 (± 1.26)	4.00 (± 0.90)
	J-queen Fungus 5	4.15 (± 1.12)	4.48 (± 0.77)	4.15 (± 0.92)

D. Maximum # workers interacting with queen

		Workers' Genotypes		
		A-worker Fungi 5&7	G-worker Fungi 5&7	J-worker Fungi 5&7
Queen's Genotype	A-queen Fungus 5	3.45 (± 1.54)	3.20 (± 1.11)	4.33 (± 1.16)
	G-queen Fungus 7	4.95 (± 1.19)	2.85 (± 0.96)	4.83 (± 1.26)
	J-queen Fungus 5	4.50 (± 1.46)	3.14 (± 1.04)	4.10 (± 1.22)

E. Percent Duration of Aggression

		Workers' Genotypes		
		A-worker Fungi 5&7	G-worker Fungi 5&7	J-worker Fungi 5&7
Queen's Genotype	A-queen Fungus 5	27.6% (± 31.0%)	37.9% (± 31.2%)	26.6% (± 37.4%)
	G-queen Fungus 7	46.6% (± 34.0%)	14.0% (± 23.4%)	26.6% (± 31.0)
	J-queen Fungus 5	51.6% (± 28.0%)	47.5% (± 22.0%)	27.3% (± 22.2%)

F. Total Aggression

		Workers' Genotypes		
		A-worker Fungi 5&7	G-worker Fungi 5&7	J-worker Fungi 5&7
Queen's Genotype	A-queen Fungus 5	92.10 (± 51.67)	173.80 (± 72.41)	163.65 (± 122.29)
	G-queen Fungus 7	112.90 (± 89.44)	57.15 (± 44.64)	118.76 (± 70.74)
	J-queen Fungus 5	117.67 (± 129.20)	121.89 (± 51.11)	102.95 (± 56.19)

over the duration of a trial. In trials where the queen was not visible for at least eight 10-s intervals, those trials were not included in our analyses (see dummy score of minus-2 in the above aggression scale). There were 21 such cases, roughly distributed equally among all the six experimental treatments.

Statistical analyses

We conducted a principal component analysis (PCA) using R statistical software (ver. 3.6.1) based on the aggression response variables (Supplementary Tables S1 and S2). Principal component 1 (PC1) accounted for 67% of the variance in the data and correlated strongly with overall aggression, while principal component 2 (PC2) accounted for 17% of the variance and was correlated with the number of workers interacting with the queen (Supplementary Table S1). We then ran ANOVAs on these principal components. We also conducted a factorial MANOVA on the response variables themselves. The ANOVAs and MANOVA were pre-planned before the start of the

experiments. To address reviewer comments, we conducted post-hoc nonparametric tests (Mann–Whitney U tests, sign test) on the data visualized in the aggression heat maps in Fig. 2 to evaluate whether the aggression averages in cells along the diagonal in each heatmap panel (worker and queen genotypes are identical) are different from the aggression averages in off-diagonal cells (worker and queen genotypes are different), as summarized in Supplementary Tables S4 and S5. To address additional reviewer comments, we used the lme4 package in R 3.6.1 to explore linear mixed-effects models evaluating the importance of ant-genetic and fungus-genetic factors in predicting aggression. All of the five mixed-effects models that we explored (Supplementary Information) had the format Aggression ~ Ant_genotypes_matching * Fungus_genotypes_matching + Random Effects, as recommended by the reviewer. Obtaining *p*-values is not always possible for mixed-effects models, because the complexity and layering of a model can make it difficult to calculate degrees of freedom. However, because the model format suggested by the reviewer is relatively simple, we

Fig. 2 Heat maps of aggression responses observed in workers of *Mycocepurus smithii* toward queens introduced in mesocosm experiments. Each heat map visualizes average responses on a color scale using green for lowest aggression (RBG color values where $G=255$ and $R=0$), yellow for median aggression ($R=255$ and $G=255$), red for the highest aggression ($R=255$ and $G=0$), and light green and orange interpolated between low, median, and high aggression. Because we found no significant effects of fungal genotype on aggression (Supplementary Table S3), we combine here ant-fungus replicates where workers had the same ant genotype but different fungal genotypes (e.g., A-worker fungus-5 and 7 in the top-left label of each sub-panel means: A-workers with either fungus-5 or fungus-7 gardens). Average aggression scores (\pm standard deviation) are also inserted numerically in each cell. Overall, cells off the diagonal tend to indicate higher aggression scores (red, orange) for interactions between queens and workers of *different* genotypes, while cells on the diagonal tend to indicate less aggression (green, yellow) for the interactions between ants of the *same* genotype. This visual impression is supported by Mann–Whitney U tests (Supplementary Table S5) and a global sign test with $p=0.0127$ of the statistical significance of observing less aggression in diagonal cells where worker-queen genotypes are identical, compared to off-diagonal cells where worker-queen genotypes are different (Supplementary Table S4). **A** Maximum aggression level directed by workers toward the introduced queen. The maximum aggression received was by queens of genotype J-ant from workers of genotype G-ant, and by queens of genotype G-ant from workers of genotype A-ant. **B** Average aggression level directed by workers toward the introduced queen. On average, queens of genotype G experienced the most aggression from workers of the other two genotypes than any other combination. Genotype combinations encompassing queens of genotype G-ant with workers of genotype A-ant were observed to have the most workers interacting simultaneously with the queen. **C** Latency (in seconds) to first aggressive interaction between resident workers and the introduced queen. Along the diagonal, ants of the same genotype exhibited longer latencies to first aggression (i.e., less aggression), compared to off-diagonal interactions between ants of different genotypes. The most rapid aggressive response (shortest latency = most severe aggression) was experienced by queens of genotype G-ant from workers of genotype A-ant. **D** Maximum number of workers interacting with the introduced queen during a trial. Genotype combinations encompassing queens of genotype G-ant with either workers of genotype A-ant or genotype J-ant were observed to have the most workers interacting simultaneously with the queen. **E** Percent of observable duration that a queen was being aggressed by workers. Workers with the same genotype as the introduced queen aggressed the queen for less of the observable duration than did workers whose genotypes were different from the introduced queen. **F** Total of all aggression scores for each trial. As in panels A–E, queens whose genotypes did not match the worker genotype received the most aggression throughout each trial than did queens whose genotypes matched the worker genotypes (green colors arranged along the diagonal). The same data presented here as heat maps are also represented in Supplementary Fig. S3 as bar graphs with standard errors. The lowest aggression scores were consistently between workers of genotype G-ant interacting with introduced queens of genotype G-ant

were able to obtain estimates of p -values using Satterthwaite approximations to estimate degrees of freedom (Kuznetsova et al. 2017). We used `anova()` from the `lmerTest` package in R to estimate statistical significance of model parameters (R-codes and detailed explanations of models are in the Supplementary Information).

Table 1 Results of type 3 analysis of variance using Satterthwaite's method (Kuznetsova et al. 2017). This linear mixed-effects model uses genotype pairs of workers and queens as random effects. PC1 is used as a proxy for aggression; `ant_genotypes_matching` is a categorical variable describing whether the worker genotype and queen genotype match; `fungus_genotypes_matching` is also a categorical variable describing whether the worker fungus-genotype of workers matched the fungus-genotype of queens. The model shows that ant genotype, and not fungus genotype, is a significant predictor of aggression in the asexual fungus-farming ant *Mycocepurus smithii*

	Df	Sum Sq	Mean Sq	F-value	P-value
PC1					
ant_genotype_matching	1	20.6144	20.6144	6.37	0.03903
fungus_genotype_matching	1	8.8311	8.8311	2.73	0.10050
ant_genotype_matching:fungus_genotype_matching	1	2.1283	2.1283	0.65	0.41846
Residuals	3				

Results

The ANOVA of the principal components revealed that worker genotypes, queen genotypes, and the interaction between worker-X-queen genotypes were the most important predictors of aggression in our queen-acceptance experiments with *M. smithii*, but we found no statistically significant effect of fungus genotype on aggression (Supplementary Table S3). Both principal component 1 (PC1) and PC2 showed a statistically significant effect of worker genotype ($P < 0.015$) and of queen genotypes ($P < 0.01$) in an ANOVA of the principal components, and PC1 showed a significant interaction effect between worker and queen genotypes ($P < 0.001$) (Supplementary Table S3). The results from the MANOVA mirrored those found in the ANOVA of the principal components, with worker genotype, queen genotype, and their interaction being statistically significant predictors of aggression, but fungal genotype was not a statistically significant predictor (Supplementary Fig. S2).

The exploration of five linear mixed-effects models confirms the ANOVA result that ant genotype, and not fungus genotype, is a significant predictor of aggression in *M. smithii* (Supplementary Information). All the models indicated that fungus-genotype pairing does not contribute to variation in the data. Models that incorporated fungus genotype as a random effect, as suggested by a reviewer, were overfit (lme4 warnings indicated that the fit of the model is near or at singularity), because fungus genotype did not contribute to the variance seen in the fixed effects. Model 4 (ant-genotype pairings as random effects, Supplementary Information) was the best representation of the experimental design and the data, and the type 3 analysis of variance table with Satterthwaite's method indicated that matching of ant

genotypes is a significant predictor of worker aggression toward introduced queens ($P=0.03903$, Table 1).

The consistent finding that ant genotype and not fungus type is an important factor predicting aggressive behavior was driven primarily by two patterns. First, workers of ant genotype G-ant were far less aggressive toward queens of genotype G-ant than they were toward queens of other genotypes (Fig. 2). Second, queens of ant genotype A-ant received aggression more frequently from ant genotypes G-ant and J-ant than these two genotypes received from ant genotype A-ant (Fig. 2). Aggression patterns for the J-ant genotype were similar but not as pronounced (Fig. 2).

For all the three ant genotypes, workers showed less total aggression toward queens of same genotype than they were toward queens of a different genotype (Fig. 2F) irrespective of fungus type (Fig. 2). This trend in total aggression is significant, and the probability of this outcome occurring by chance in Fig. 2F is $P=(1/3)^3=0.037$. In addition, the visual trend of lower aggression scores in cells along the diagonals in all six sub-panels in Fig. 2 (genotypes of interacting workers and queens are identical along the diagonal; proportionally more of these cells are green and yellow) compared to the aggression scores in off-diagonal cells (genotypes of interacting workers and queens are different from each other; proportionally more of these cells are orange and red) is supported by corresponding trends in Mann–Whitney U tests comparing averages in diagonal versus off-diagonal cells (Supplementary Table S5). Using the information from panels Fig. 2A–E, a sign test evaluating whether there is less aggression when queen and worker genotypes are identical (diagonal cells) for the five aggression metrics (i.e., five panels Fig. 2A–E) yielded $P=0.0127$ (Supplementary Table S4). These nonparametric tests therefore provide further statistical support, in addition to the results from the above ANOVAs and mixed-effects models, that workers are less aggressive to introduced queens if genotypes are identical between workers and queens.

While we found evidence that workers were less aggressive toward queens of the same genotype than they were toward queens of the other two genotypes, interestingly, the extent of relatedness between ant clones (close versus distant relatedness) did not correlate perfectly with aggression. For example, genotypes A-ant and J-ant were most closely related to one another (Supplementary Fig. S1), but there was insufficient statistical support to conclude that workers of genotype J-ant were less aggressive toward queens of the more closely related genotype A-ant than toward queens of the more distantly related genotype G-ant (Welch's two-sample t -test; $t=1.91$; $P=0.066$). Furthermore, for genotype J-ants, there was no statistically significant difference between aggression directed toward queens of their own genotype versus queens of genotype G-ants (Welch's two-sample t -test; $t=-0.28$; $P=0.78$). These results should

be considered preliminary, and a more comprehensive experiment testing a larger array of ant genotypes in pairwise combinations will need to be conducted to adequately test for possible effects of genetic distance between ant genotypes in mediating queen adoption in *M. smithii*.

Discussion

Nestmate acceptance by workers of introduced queens in the asexual ant *Mycocepurus smithii* can be predicted from the combination of the genotype of the resident workers and the genotype of the experimentally introduced queens (Fig. 1, Supplementary Tables S3–S5), but we did not find evidence that queen acceptance is also dependent on fungus genotypes that the ants were raised on (Fig. 1, Supplementary Table S3). The importance of ant genotype in nestmate acceptance was shown before for another asexual ant, the clonal raider ant *Ooceraea* (formerly *Cerapachis*) *biroi* (Kronauer et al. 2013), which does not cultivate fungi like *M. smithii*. We found insufficient evidence in support of the hypothesis that *M. smithii* workers use only environmentally acquired cues, such as odors derived from their cultivated fungus type, in nestmate acceptance. This establishes an important foundation for future studies aiming to explore the specific mechanisms underlying genetically based nestmate recognition in *M. smithii*. Because the nest environment has been shown to be an important determinant of nestmate acceptance across many ant species (Heinze et al. 1996; Soroker et al. 1998; d'Ettorre and Lenoir 2010; van Wilgenburg et al. 2011; Sprenger and Menzel 2020), and because the fungus dictates a substantial portion of the odor environment of other fungus-farming ants (Viana et al. 2001; Richard et al. 2007a,b; Larsen et al. 2014; Valadares and do Nascimento 2017), the lack of a significant effect of fungus type relative to ant genotype in mediating queen acceptance in *M. smithii* is surprising.

While queen acceptance was correlated with ant genotype in our experiments, aggression patterns do not appear to be correlated strictly with genetic distance between the ant lineages tested (Supplementary Fig. S1), and aggression did not increase with increasing genetic distance between ant genotypes. This may be an effect of the small genetic distance between the colonies used in our study, because even the colony-pairs with the greatest intraspecific genetic distance used here differ by only a few alleles at a total of 12 microsatellite loci (see Supplementary Fig. S1; Kellner et al. 2013). It is possible that there was simply not enough resolution in the ants' sensory system, so the workers in our experiments were unable to resolve the genetic distance between workers and introduced queens. Consequently, our large sample of 180 tests may not have had enough statistical power to detect such a pattern of graduated aggression

response increasing with genetic distance between interacting ants. A follow-up study with larger sample sizes and a greater assortment of ant genotypes is needed to adequately test whether gradations of ant relatedness modulate recognition and aggression behaviors in *M. smithii*.

It is possible that *M. smithii* workers are not perceiving actual genetic distance to introduced queens, but that they are employing a binary-like Boolean same-versus-different rule of nestmate acceptance. Similar Boolean decision-making systems have been identified in other ants such as ritualized warfare in *Iridomyrmex* (Ettershank and Ettershank 1982). If so, queens of *M. smithii* genotype A-ant, which were strongly rejected by workers of the two other ant genotypes in our experiments (Fig. 2), may produce or reliably acquire genotype-dependent recognition cues such as strong or unique odors that make them more readily identifiable as “different”.

Another possibility is that the ants are not using direct proxies for ant genotypes as the basis of recognition cues such as ant-secreted cuticular hydrocarbons or cuticular glycoproteins, but rather use indirect proxies such as distinct microbiomes that correlate with different ant genotypes (Dietemann et al. 2003; Kovacs et al. 2011; Ishak et al. 2012; Mueller 2012; Kellner et al. 2015), and these microbiomes are specific to ant genotypes and contribute to ant odors. If so, microbiome composition and microbiome-dependent odors are actually ant traits, because microbiome properties are dependent on ant genotype (see box 2 in Mueller and Linksvayer 2022). Other odor-generating factors that could be dependent on ant genotype, such as pathogens, colony size, foraging patterns, or maternal effects other than the cultivated fungus, could play additional roles. If cuticular or other microbiomes are in fact a source of colony odor or even a partial source, it may be possible to cross-foster ant brood of one genotype by workers of a different genotype such that ants of one genotype acquire the microbiomes of the different genotype (Mueller et al. 2010), and then test these cross-fostered, microbiome-switched ants in our aggression assay to disentangle the relative importance of ant-genetic effects from microbiome-dependent effects in nestmate acceptance by *M. smithii*.

Conclusions

Factors correlated with ant genotype, but not with the cultivated fungi, play a role in queen acceptance behaviors between resident workers and experimentally introduced queens in the fungus-farming ant *Mycocepurus smithii*. This finding suggests for *M. smithii* that genome-correlated nestmate recognition and acceptance could potentially be a gateway to the evolution of kin-modulated interactions,

mediated here through worker aggression toward dispersing queens that may try to enter established nests of *M. smithii*, or toward queens that may attempt to migrate between colonies in the dense nest aggregations of *M. smithii* when genetically distinct colonies meet underground during lateral nest expansion. Queens that are readily accepted by a colony (i.e., not aggressed against by resident workers) are consequently able to reap the benefits of the invaded colonies without risking the perils of solitary nest founding (Fernández-Marín et al. 2004, 2005). If so, it is possible that the documented genome-correlated nestmate recognition may constrain the origin of intraspecific parasitism by dispersing queens of *M. smithii*. Such parasitism evolved in the common ancestor of the sister species pair *Mycocepurus goeldii* and its social parasite, *Mycocepurus castrator*, which appears to have originated as intraspecific social parasites following Emery’s rule (Rabeling and Bacci 2010; Rabeling et al. 2014).

Supplementary Information The online version contains supplementary material available at <https://doi.org/10.1007/s00265-022-03276-5>.

Acknowledgements We thank Mark Kirkpatrick, Luke Reding, Jared Cole, three anonymous reviewers, and especially Romain Libbrecht for helpful comments.

Author contribution B.T.B., K.K., K.M.R., and U.G.M. designed the research; B.T.B. performed the experiments; B.T.B., T.D.K., and P.R.G. analyzed the data; T.D.K. designed all the figures; B.T.B., U.G.M., T.D.K., and P.R.G. wrote the manuscript; B.T.B., T.D.K., and U.G.M. procured the funding. All the authors edited and approved the final version of the manuscript.

Funding This work was supported by National Science Foundation Graduate Research Fellowships (to B.T.B. and T.D.K.) and National Science Foundation awards DEB-0919519 and DEB-1911443 (to U.G.M.).

Data availability All the data and analytical codes are available in the Supplementary Information.

Declarations

Competing interests The authors declare no competing interests.

References

- Barrett TB (2015) Ant genotype, not fungus genotype, predicts aggression in the asexual fungus-farming ant, *Mycocepurus smithii*. Master Thesis, The University of Texas at Austin
- Barros LAC, Rabeling C, Teixeira GA, dos Santos Ferreira Mariano C, Delabie JHC, de Aguiar HJAC (2022) Decay of homologous chromosome pairs and discovery of males in the thelytokous fungus-growing ant *Mycocepurus smithii*. *Sci Rep* 12:4860
- Beigel K, Matthews AE, Kellner K, Pawlik CV, Greenwold M, Seal JN (2021) Cophylogenetic analyses of *Trachymyrmex* ant-fungus specificity: “one to one with some exceptions.” *Mol Ecol* 30:5605–5620

- Beye M, Neumann P, Moritz RFA (1997) Nestmate recognition and the genetic gestalt in the mound-building ant *Formica polyctena*. *Insectes Soc* 44:49–58
- Brandstaetter AS, Endler A, Kleineidam CJ (2008) Nestmate recognition in ants is possible without tactile interaction. *Naturwissenschaften* 95:601–608
- Carlin NF, Hölldobler B (1986) The kin recognition system of carpenter ants (*Camponotus* spp.). *Behav Ecol Sociobiol* 19:123–134
- 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
- d'Ettorre P, Lenoir A (2010) Nestmate recognition. In: Lach L, Parr C, Abbot K (eds) *Ant ecology*. Oxford University Press, Oxford, pp 194–209
- Dahbi A, Hefetz A, Cerda X, Lenoir A (1999) Trophallaxis mediates uniformity of colony odor in *Cataglyphis iberica* ants (Hymenoptera, Formicidae). *J Insect Behav* 12:559–567
- De Gasperin O, Blacher P, Chapuisat M (2021) Social insect colonies are more likely to accept unrelated queens when they come with workers. *Behav Ecol* 32:1004–1011
- Dietemann V, Peeters C, Liebig J, Thivet V, Hölldobler B (2003) Cuticular hydrocarbons mediate discrimination of reproductives and nonreproductives in the ant *Myrmecia gulosa*. *Proc Natl Acad Sci USA* 100:10341–10346
- Dobata S, Sasaki T, Mori H, Hasegawa E, Shimada M, Tsuji K (2011) Persistence of the single lineage of transmissible ‘social cancer’ in an asexual ant. *Mol Ecol* 20:441–455
- Ettershank G, Ettershank JA (1982) Ritualised fighting in the meat ant *Iridomyrmex purpureus* (Smith) (Hymenoptera: Formicidae). *Aust J Entomol* 21:97–102
- Fang C-C, Chang F-H DP, Kurian J, Mueller UG (2020) Colony fitness and garden growth in the asexual fungus-growing ant *Mycocepurus smithii* (Attini, Formicidae). *Insectes Soc* 67:35–49
- Fernández-Marín H, Zimmerman JK, Wcislo WT (2004) Ecological traits and evolutionary sequence of nest establishment in fungus-growing ants (Hymenoptera, Formicidae, Attini). *Biol J Linn Soc* 81:39–48
- Fernández-Marín H, Zimmerman JK, Wcislo WT, Rehner SA (2005) Colony foundation, nest architecture and demography of a basal fungus-growing ant, *Mycocepurus smithii* (Hymenoptera, Formicidae). *J Nat Hist* 39:1735–1744
- Heinze J, Foitzik S, Hippert A, Hölldobler B (1996) Apparent dearenemy phenomenon and environment-based recognition cues in the ant *Leptothorax nylanderii*. *Ethology* 102:510–522
- Himler AG, Caldera EJ, Baer BC, Fernández-Marín H, Mueller UG (2009) No sex in fungus-farming ants or their crops. *Proy Soc B-Biol Sci* 276:2611–2616
- Ishak HD, Miller JL, Sen R, Dowd SE, Meyer E, Mueller UG (2012) Microbiomes of ant castes implicate new microbial roles in the fungus-growing ant *Trachymyrmex septentrionalis*. *Sci Rep* 1:204
- Kardish MR, Mueller UG, Amador-Vargas S, Dietrich EI, Ma R, Barrett B, Fang CC (2015) Blind trust in unblinded observation in ecology, evolution, and behavior. *Front Ecol Evol* 3:51
- Kellner K, Fernández-Marín H, Ishak HD, Sen R, Linksvayer TA, Mueller UG (2013) Co-evolutionary patterns and diversification of ant–fungus associations in the asexual fungus-farming ant *Mycocepurus smithii* in Panama. *J Evol Biol* 26:1353–1362
- Kellner K, Ishak HD, Linksvayer TA, Mueller UG (2015) Bacterial community composition and diversity in an ancestral ant fungus symbiosis. *FEMS Microbiol Ecol* 91:fiv073
- Kellner K, Kardish MR, Seal JN, Linksvayer TA, Mueller UG (2018) Symbiont-mediated host-parasite dynamics in a fungus-gardening ant. *Microb Ecol* 76:530–543
- Kovacs A, Ben-Jacob N, Tayem H, Halperin E, Iraqi FA, Gophna U (2011) Genotype is a stronger determinant than sex of the mouse gut microbiota. *Microb Ecol* 61:423–428
- Kronauer DJC, Tsuji K, Pierce NE, Keller L (2013) Non-nest mate discrimination and clonal colony structure in the parthenogenetic ant *Cerapachys biroi*. *Behav Ecol* 24:617–622
- Kuznetsova A, Brockhoff PB, Christensen RHB (2017) lmerTest package: tests in linear mixed effects models. *J Stat Softw* 82:1–26
- Larsen J, Fouks B, Bos N, d'Ettorre P, Nehring V (2014) Variation in nestmate recognition ability among polymorphic leaf-cutting ant workers. *J Insect Physiol* 70:59–66
- Lorenzi MC, d'Ettorre P (2020) Nestmate recognition in social insects: what does it mean to be chemically insignificant? *Front Ecol Evol* 7:488
- Mehdiabadi NJ, Hughes B, Mueller UG (2006) Cooperation, conflict, and coevolution in the attine ant–fungus symbiosis. *Behav Ecol* 17:291–296
- Mehdiabadi NJ, Mueller UG, Brady SG, Schultz TR (2012) Symbiont fidelity and the origin of species in fungus-growing ants. *Nat Commun* 3:840
- Mikheyev AS, Mueller UG, Abbott P (2010) Comparative dating of attine ant and lepiotaceous cultivar phylogenies reveals co-evolutionary synchrony and discord. *Am Nat* 175:E126–E133
- Mueller UG (2012) Symbiont recruitment versus ant-symbiont co-evolution in the attine ant–microbe symbiosis. *Curr Opin Microbiol* 15:269–277
- Mueller UG, Linksvayer TE (2022) Microbiome breeding: conceptual and practical issues. *Trends Microbiol* 30:997–1011. <https://doi.org/10.1016/j.tim.2022.04.003>
- Mueller UG, Rehner SA, Schultz TD (1998) The evolution of agriculture in ants. *Science* 281:2034–2038
- Mueller UG, Poulin J, Adams RMM (2004) Symbiont choice in a fungus-growing ant (Attini, Formicidae). *Behav Ecol* 15:357–364
- Mueller UG, Ishak H, Lee JC, Sen R, Gutell RR (2010) Placement of attine ant-associated *Pseudonocardia* in a global *Pseudonocardia* phylogeny (Pseudonocardiaceae, Actinomycetales): a test of two symbiont-association models. *Antonie Van Leeuwenhoek* 98:195–212
- Mueller UG, Ishak HD, Bruschi SM, Smith CC, Herman JJ, Solomon SE, Mikheyev AS, Rabeling C, Scott JJ, Cooper M, Rodrigues A (2017) Biogeography of mutualistic fungi cultivated by leafcutter ants. *Mol Ecol* 26:6921–6937
- Mueller UG, Kardish MR, Ishak HD, Wright AM, Solomon SE, Bruschi SM, Carlson AL, Bacci M Jr (2018) Phylogenetic patterns of ant–fungus associations indicate that farming strategies, not only a superior fungal cultivar, explain the ecological success of leafcutter ants. *Mol Ecol* 27:2414–2434
- Pirk CWW, Neumann P, Moritz RFA, Pamilo P (2001) Intranest relatedness and nestmate recognition in the meadow ant *Formica pratensis* (R.). *Behav Ecol Sociobiol* 49:366–374
- Provost E, Blight O, Tirard A, Renucci M (2008) Hydrocarbons and insects’ social physiology. In: Maes RP (ed) *Insect physiology: new research*. Nova Science Publishers, Hauppauge, New York, 19–72
- Rabeling C, Bacci M Jr (2010) A new workerless inquiline in the lower attini (Hymenoptera: Formicidae), with a discussion of social parasitism in fungus-growing ants. *Syst Entomol* 35:379–392
- Rabeling C, Lino-Neto J, Cappellari SC, Dos-Santos IA, Mueller UG, Bacci M Jr (2009) Thelytokous parthenogenesis in the fungus-gardening ant *Mycocepurus smithii* (Hymenoptera: Formicidae). *PLoS ONE* 4:e6781
- Rabeling C, Gonzales O, Schultz TR, Bacci Jr M, Garcia MV, Verhaagh M, Ishak HD, Mueller UG (2011) Cryptic sexual populations account for genetic diversity and ecological success in a widely distributed, asexual fungus-growing ant. *Proc Natl Acad Sci USA* 108:12366–12371
- Rabeling C, Schultz TR, Pierce NE, Bacci Jr M (2014) A social parasite evolved reproductive isolation from its fungus-growing ant host in sympatry. *Curr Biol* 24:2047–2052

- Ratnieks FLW (1991) The evolution of genetic odor-cue diversity in social Hymenoptera. *Am Nat* 137:202–226
- Richard F-J, Poulsen M, Drijfhout F, Jones G, Boomsma JJ (2007a) Specificity in chemical profiles of workers, brood and mutualistic fungi in *Atta*, *Acromyrmex*, and *Sericomyrmex* fungus-growing ants. *J Chem Ecol* 33:2281–2292
- Richard F-J, Poulsen M, Hefetz A, Errard C, Nash DR, Boomsma JJ (2007b) The origin of the chemical profiles of fungal symbionts and their significance for nestmate recognition in *Acromyrmex* leaf-cutting ants. *Behav Ecol Sociobiol* 61:1637–1649
- Seal JN, Mueller UG (2014) Instability of novel ant-fungal associations constrains horizontal exchange of fungal symbionts. *Evol Ecol* 28:157–176
- Smith AA, Hölldobler B, Liebig J (2009) Cuticular hydrocarbons reliably identify cheaters and allow enforcement of altruism in a social insect. *Curr Biol* 19:78–81
- Soroker V, Fresneau D, Hefetz A (1998) Formation of colony odor in the ponerine ant *Pachycondyla apicalis*. *J Chem Ecol* 24:1077–1090
- Sprenger PP, Menzel F (2020) Cuticular hydrocarbons in ants (Hymenoptera: Formicidae) and other insects: how and why they differ among individuals, colonies, and species. *Myrmecol News* 30:1–26
- Valadares L, do Nascimento FS (2017) Changes in the cuticular hydrocarbon profile associated with the molting cycle correlate with the hydrocarbon profile of the fungus cultivated by the ant *Atta sexdens*. *Insectes Soc* 64:591–596
- van Wilgenburg E, Symonds MRE, Elgar MA (2011) Evolution of cuticular hydrocarbon diversity in ants. *J Evol Biol* 24:1188–1198
- Viana AMM, Frézard A, Malosse C, Della Lucia TMC, Errard C, Lenoir A (2001) Colonial recognition of fungus in the fungus-growing ant *Acromyrmex subterraneus subterraneus* (Hymenoptera: Formicidae). *Chemoecology* 11:29–36
- Wagner D, Tissot M, Cuevas W, Gordon DM (2000) Harvester ants utilize cuticular hydrocarbons in nestmate recognition. *J Chem Ecol* 26:2245–2257

Publisher's note Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.

Springer Nature or its licensor (e.g. a society or other partner) holds exclusive rights to this article under a publishing agreement with the author(s) or other rightsholder(s); author self-archiving of the accepted manuscript version of this article is solely governed by the terms of such publishing agreement and applicable law.