Co-evolutionary patterns and diversification of ant–fungus associations in the asexual fungus-farming ant *Mycocepurus smithii* in Panama


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**Abstract**
Partner fidelity through vertical symbiont transmission is thought to be the primary mechanism stabilizing cooperation in the mutualism between fungus-farming (attine) ants and their cultivated fungal symbionts. An alternate or additional mechanism could be adaptive partner or symbiont choice mediating horizontal cultivar transmission or de novo domestication of free-living fungi. Using microsatellite genotyping for the attine ant *Mycocepurus smithii* and ITS rDNA sequencing for fungal cultivars, we provide the first detailed population genetic analysis of local ant–fungus associations to test for the relative importance of vertical vs. horizontal transmission in a single attine species. *M. smithii* is the only known asexual attine ant, and it is furthermore exceptional because it cultivates a far greater cultivar diversity than any other attine ant. Cultivar switching could permit the ants to re-acquire cultivars after garden loss, to purge inferior cultivars that are locally mal-adapted or that accumulated deleterious mutations under long-term asexuality. Compared to other attine ants, symbiont choice and local adaptation of ant–fungus combinations may play a more important role than partner-fidelity feedback in the co-evolutionary process of *M. smithii* and its fungal symbionts.

**Introduction**
Mutualisms are ubiquitous cooperative and beneficial interactions among species that can range from diffuse interactions to highly co-evolved associations (Bronstein, 1994; Leigh, 2010). The defining signature of co-evolution is the presence of reciprocal, novel adaptations and novel counteradaptations in co-evolving partners (Mueller, 2012). Tightly interacting species can exhibit phylogenetic congruence (co-phylogenies) (Hafner & Page, 1995; Page, 2003; Light & Hafner, 2007; Refrégier et al., 2008; Scharill et al., 2008) because of co-speciation and co-diversification (Cook & Rasplus, 2003; Quek et al., 2004; Marussich & Machado, 2007).

Fitness of symbionts can be directly linked to host fitness, leading to shared evolutionary interests (Herre et al., 1999), but conflicts can also occur between hosts and symbionts (Mueller, 2002). For example, conflict over the mixing of symbiont types can lead to symbiont–symbiont competition for the same host resources, symbionts may evolve strategies for sole occupancy of a host, and hosts may avoid harbouring more than one symbiont lineage (Frank, 1996). Genetic variation of hosts and symbionts can be further influenced by spatially variable selection and drift (geographic mosaic of co-evolution, Thompson, 1999) leading to geographically varying adaptation or mal-adaptation of host–symbiont combinations (Spoeke et al., 1996; Wilkinson et al., 1996; Bever, 2002).
Fungus-growing ants (Formicidae: Attini) are prominent systems for research on mutualistic interactions. Some attine ants are dominant herbivores of the Neotropics (Hölldobler & Wilson, 2010), owing their ecological success to mutualistic association with co-evolving fungal cultivars (Chapela et al., 1994). The fungus serves as the ants’ main food source and is propagated asexually by the ants (Mueller et al., 2010). This mutualism has been shaped by 40–60 million years of complex ant–fungus re-associations, but specific clades of attine ants associate predominantly with specific clades of fungi in the tribe Leucocoprineae (formerly Leptotaceae, now Agaricaeae, Agaricales, Basidiomycota) (Chapela et al., 1994; Mueller et al., 1998; Munkaci et al., 2004; Schultz & Brady, 2008; Mikheyev et al., 2010).

Ant–fungus associations are maintained primarily through vertical transmission of fungal symbionts from one generation to the next (Chapela et al., 1994; Mueller et al., 1998; Schultz & Brady, 2008; Caldera et al., 2009). As far as known, young queens start gardens in newly founded nests with fungal inocula collected before dispersal from the maternal nests [vertical symbiont transmission; (Huber, 1905)]. In contrast to predictions from a scenario of strict vertical cultivar inheritance, phylogenetic studies have shown that the cultivars are polyphyletic (indicating multiple domestication events) (Chapela et al., 1994; Mueller et al., 1998; Mikheyev et al., 2010), and that ant and fungal phylogenies are not topologically congruent, implicating horizontal transfer of fungal clones between different attine lineages (Mueller et al., 1998, 2011; Green et al., 2002; Mikheyev & Mueller, 2007), occasional gene flow between free-living and ant-cultivated fungal populations (Mueller et al., 1998; Pagnocca et al., 2001; Dentinger et al., 2009; Vo et al., 2009), or both. Some fungal clades originated more recently than the corresponding clades of host ants (Mikheyev et al., 2010), suggesting de novo domestication of free-living fungi.

Host–symbiont re-associations are most common in the ‘lower attines’ (a paraphyletic group including basal attine lineages) because they can import novel strains into the mutualism (Mueller et al., 1998; Vo et al., 2009) and because fungi can be transferred between ant species and lineages through garden stealing and sharing (Adams et al., 2000; Green et al., 2002). Typically, lower-attine ant species tend to associate locally with only one fungal species, but there are exceptions (Mueller et al., 1998; Mikheyev, 2008; Mehdibadi et al., 2012). The most remarkable exception is the ant *Mycocepurus smithii*, which cultivates at least six different fungal lineages in Central Panama (Mueller et al., 1998), with similar cultivar diversity in Amazonian Brazil (Rabeling, 2004).

*Mycocepurus smithii* is unique because it reproduces by thelytokous parthenogenesis in most populations across its vast range from northern Argentina to northern Mexico (Fernández-Marín et al., 2005; Himler et al., 2009; Rabeling et al., 2009, 2011). In thelytokous populations, reproducing queens are unmated, and female offspring are genetically identical to their mothers (Himler et al., 2009; Rabeling et al., 2011). The cultivated fungus is likewise clonally propagated by the ants within nests and between ant generations. This ‘double asexuality’ (Himler et al., 2009) may impose an evolutionary handicap, because adaptive novelty generated by genetic recombination is limited in both mutualistic partners. Additionally, because of clonal propagation, both the clonal ants and the clonal fungi may accumulate deleterious mutations over time [Muller’s ratchet, (Maynard Smith, 1983)], which might result in inferior nutritive quality or greater disease susceptibility of the fungus, as well as inferior gardening abilities of the ants, all of which reduces fitness of the overall mutualism. The ants might mitigate this handicap through frequent fungal switching, thus generating novel ant–fungus combinations adapted to varying environmental conditions (Himler et al., 2009).

Here, we use high-resolution molecular markers to characterize phylogeographic patterns of ant–fungus association in *M. smithii* in the Panama Canal Zone, and to evaluate the potential for ant-fungus co-evolution in *M. smithii*. Previous population genetic studies of *M. smithii* investigated either only the ant diversity (Himler et al., 2009; Rabeling et al., 2009, 2011) or only the fungal diversity (Mueller et al., 1998), whereas the present study is the first detailed co-phylogenetic analysis of ant–fungus associations of a single attine species.

**Materials and methods**

**Sample collection**

Colonies of *M. smithii* were collected in Central Panama in April 2010 by excavating chambers, as previously described (Mueller et al., 1998; Rabeling et al., 2007). Each chamber was processed separately because nest boundaries were often difficult to discern. Ants and fungus gardens were collected with flame-sterilized forceps and preserved separately in 100% ethanol. Material was collected from 11 nest aggregations: East of the Panama Canal in Gamboa at Building 183, Resort, Greenhouse, Harbor, and Pipeline Road entrance; west of the Panama Canal at Casa Verde, Corozales Afuera, Chorrera, Achiote, Gatun, and Piña Beach (See Table S1, Supporting information). These sampling sites differed in their habitats: Open, grassy, meadow-like and disturbed habitats with light shade were found in Achiote, Chorrera, Casa Verde, Corozales Afuera and at the Pipeline road entrance in Gamboa. In contrast, wooded, heavily shaded and disturbed habitats were found at Building 183, Resort, Greenhouse and the Harbors in Gamboa and Gatun. One habitat very distinct from all others was the Piña Beach, were the ants were found under shrubs in sandy soil close to the tide line.
**Molecular methods**

**Ants**

DNA was extracted from three ants per chamber by crushing single, whole ants in liquid nitrogen, incubating in 100-μl 10% Chelex resin (Sigma-Aldrich, St. Louis, MO, USA) for 1.5 h at 60 °C, then 10 min at 99 °C. For microsatellite genotyping, we used 12 specific markers developed by (Rabeling et al., 2011) and one additional marker (A6_2; see Table S1). PCR products were sized on a Capillary Sequencer (ABI PRISM 3100; Applied Biosystems, Foster City, CA, USA) and scored with GeneMarker v1.5 (SoftGenetics, State College, PA, USA).

**Fungi**

DNA was extracted by incubating hyphal clusters (ca. 0.5 mm) in 100-μl 10% Chelex, following the same protocol as for the ants. The internal transcribed spacer (ITS) rDNA region was amplified with ITS4 and ITS5 primers (White et al., 1990; Mueller et al., 1998). PCR reaction, purification and cycle sequencing (ABI BigDye Terminator Kit; ABI PRISM 3100) followed methods in (Vo et al., 2009). Forward and reverse sequences were assembled in Sequencher 4.6 (GeneCodes, AnnArbor, MI, USA). Sequence information is deposited at NCBI GenBank (JQ405665-JQ405735).

**Data analyses**

Previous studies showed that *M. smithii* ants and their cultivated fungi are clonal (Rabeling et al., 2011), with no genetic variation within colonies to be expected. Indeed, chambers collected closely to each other (ca. 10 cm) contained identical ant and fungus genotypes, except in two cases where fungi in two single chambers were found to be different and therefore assumed to be from different colonies. The otherwise clonal identity of samples collected from adjacent chambers justified grouping into putative colonies.

**Ant population genetics and clone-lineage analyses**

We analysed ants from a total of 64 chambers (52 colonies). Analysis of molecular variance (AMOVA) was performed in ARLEQUIN 3.11 (Excoffier et al., 2005): Individual microsatellite genotypes were grouped into colonies within collection sites, and these within the total population. Significance of population structure was tested using a permutation procedure (1000 replicates). Significance for collection site pairwise FST values was tested using permutation tests (1000 replicates) and adjusted with sequential Bonferroni correction.

Departure from Hardy–Weinberg equilibrium was calculated using GENEPOP 4.0 (Raymond & Rousset, 1995), with a global test [Score (U) test] for each population using the Markov chain method (100 batches, 10 000 iterations/batch). Observed and expected heterozygosities were calculated in GDA (Lewis & Zaykin, 2001) for each population and each locus.

Ant genotypes were sorted into clone lineages by 100% allele identity across the 13 microsatellite loci (Table S1). To analyse phylogenetic relationships between clone lineages, we computed a pairwise distance matrix and a neighbour-joining tree based on allele-sharing distances (Chakraborty & Jin, 1993) using POPULATIONS 1.20.30 (Langella, 1999). Support values were obtained by bootstrapping over loci (100 pseudoreplicates). The inferred phylogenetic tree was visualized in MEGA 5 (Tamura et al., 2011).

**Fungus population genetics and fungus clone-lineage analyses**

We analysed fungi from 64 chambers (52 colonies). Population genetic structure was analysed using AMOVA in ARLEQUIN (Excoffier et al., 2005), testing variation among and within collection sites. Pairwise genetic distances between populations were calculated (Kimura2P distances), and significance of these distances was evaluated with 1000 permutations and sequential Bonferroni correction.

**Phylogeny of the fungus cultivars**

Lower-attine fungi are grouped into two distinct clades (previously called Clade 1 and Clade 2) (Mueller et al., 1998). To associate our fungal ITS sequences with the existing Clade 1 and Clade 2, we added them into the original lower-attine fungal cultivar alignment from (Vo et al., 2009) (alignment shown in NCBI GenBank PopSet: 140104520), using McClade 4.08(Maddison & Maddison, 2000). One sequence from each ITS lineage was blasted against NCBI GenBank; sequences with query coverage >94% and maximum identity >85% were added if not already present in the (Vo et al., 2009) alignment. Addition of these new sequences did not cause any changes in the original alignment, that is, gaps and excluded characters were preserved unchanged (complete alignment length 916 bp, 578 bp after excluding ambiguously aligned characters). The total data set consisted of 305 sequences. jModeltest 0.1.1 (Guindon & Gascuel, 2003; Posada, 2008) identified the GTR + I + G model as the most suitable model for phylogenetic analyses. A maximum-likelihood tree was computed using the default settings in GARLI 0.951 (Zwickl, 2006). Ten trees were generated from which the tree with the highest likelihood score was chosen. Bootstrap support was evaluated in 100 pseudoreplicates.

To construct subtrees of Clade 1 and Clade 2, we realigned sequences within each Clade including the new sequences from the *M. smithii* cultivars. For each Clade, a maximum-likelihood tree was generated with GARLI default settings and 1000 pseudoreplicates bootstrap support. *Myrmicocrypta infuscata* G11 (GenBank accession AF07971 6.1) and *Lepiota cf. abruptibulba*
we used EstimateS 8.2.0 (Colwell, 2006) to construct

coded into a presence/absence matrix of ant genotype

was also 

As expected for a clonal organism, all populations

between the two trees. Additionally, ParaFit performs a 

test on each single observed link to assess the signifi-

We found 11 different clone lineages among the ants 

(1) a presence/absence matrix of the 21 observed 

Association links between the two groups has been in-

DEFINING THE CHOOSING AND CHosen PARTNERS IN PARAfit CORRECTLY IS MOST CRITICAL 

inbreeding coefficients (over loci: \( f = -0.14 \), over colo-

We identified nine different cultivar ITS-sequence types 

We found only one ant genotype at each of the seven sites (Gamboa Pipe-

informative for a total of 58 ant–fungus chambers. Fungal 

variation between and within ant host geno-

independent, as inferred from the observed association links 

Parafit performs the two tests: The null hypothesis of the ParaFitGlobal test 

Ant host occurrence was also 


11 different clone lineages among the ants (genotypes A–K; Fig. 1). There was no correlation between the 

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Percentages of identity varied among the clones and were highest (100%) for the clone 

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\( P_{ST} = 0.32, \ P < 0.001 \); see also Table S3). 

Results 

Population genetics of ant hosts 

Eleven of the 13 microsatellite loci were polymorphic (2–5 alleles per locus). Two loci (B4, C2) were mono-


to test for significant phyllogenetic co-

between sample sites. Because attine ants are the choosing partner in the ant–

To investigate possible influences of sampling effort, we used EstimateS 8.2.0 (Colwell, 2006) to construct 

sample-based rarefaction curves (Fig. S1, Supporting information). 

Population genetics of fungal symbionts 

We identified nine different cultivar ITS-sequence types (labelled ITS-types 1–9). Only one ITS-type was found 

at four of the eleven collection sites (Piña Beach, Corozales Afuera, Casa Verde, Gatun), but more than one fungal ITS-type was found at each of the other seven sites (Resort, Greenhouse, Pipeline Road, Gamboa Building 183, Harbor and Achiote: 2 ITS-types each; Chorrera: 3). Only one of the nine ITS-types was exclusive to one collection site (type 6: Corozales Afuera) (Fig. 1). AMOVA results revealed more genetic variation between sites (78.4%) than within sites (21.6%) for the fungi, with significant genetic differentiation between sites ($F_{ST} = 0.77$, $P < 0.001$; see also Table S3).

**Fungal symbiont phylogeny**

The placement of our nine ITS-types within the lower-at-tine cultivar phylogeny is consistent with results from previous analyses (Mueller et al., 1998) (Fig. S3). Fungal types 1–5 were placed into Clade 1 (types 1–3 in Clade 1A, and types 4 & 5 in Clade 1B) and fungus types 6, 7, 8 and 9 cluster together in Clade 2 (Fig. S4a,b).

**Ant–fungus associations**

We did not find a pattern of strict one-to-one association between ant hosts and fungal symbionts (Fig. 2). Seven of the eleven ant genotypes were found in association with only a single fungus type, whereas four ant genotypes (B, C, H, G) were found in association with 2–6 different fungi. ParaFit analyses showed an overall barely significant association between ant and fungus phylogenetic relationships ($ParaFit_{Global} = 0.027$, $P = 0.048$), rejecting therefore the null hypothesis of independence between ant and fungus phylogenies. The permutation test after Hommola et al., 2009.
supported this result (overall pattern of coevolution between the ant and the fungus phylogenies was confirmed; \( P = 0.0047 \)). Twelve of 21 association links emerged as significant links in ParaFit (\( P = 0.014-0.043 \)), whereas nine links were nonsignificant (\( P > 0.05 \)) (Fig. 2). Significant association links between ant clones and fungal types presumably resulted from vertical cultivar transmission between ant generations, whereas the nonsignificant links likely resulted from recent cultivar switching. Some ant clones appear to be specialized on specific fungus Clades: whereas ant genotypes A, B, C, D, H, I and J are found in significant association with Clade 1 fungus. In contrast, associations between ant lineage G and various fungus cultivars from Clade 1 (1, 2, 3, and 4) were nonsignificant, and these associations therefore derived most likely from recent between-Clade switches.

Fungi were significantly differentiated between ant hosts (\( F_{\text{among ant hosts}} = 0.52, P < 0.001 \)); however, overall genetic variation of fungi was partitioned similarly among (51.9%) and within ant hosts (48.2%).

Genetic distance of fungus populations was correlated with geographic distance (Mantel test, \( r = 0.34, P = 0.034 \)), even when controlling for genetic distance of ant populations (partial Mantel test, \( r = 0.34, P = 0.040 \)) and when controlling for ant host occurrence (partial Mantel test, \( r = 0.34, P = 0.042 \)). In contrast, no correlations between genetic and geographic distance were found in the ant populations (\( r = -0.11, P = 0.19 \)). Genetic distances between fungus populations were independent of genetic distances between ant populations (Mantel test, \( r = -0.06, P = 0.317 \)).

**Discussion**

Ant–fungus associations between the thelytokous fungus-farming ant *Mycocepurus smithii* and its asexual fungal cultivars would seem to be more transient than in other fungus-farming ants. For example, ants in the *Cyphomyrmex* wheeleri-complex exhibit cultivar fidelity across millions of years (Mehdiabadi et al., 2012) and *Mycocepurus goeldii* appears to specialize on one of two fungal lineages either north or south of the Amazon River (Rabeling, 2004). Thus, vertical transmission appears to be frequently disrupted in *M. smithii* either by *de novo* domestication of fungi from free-living sources or by horizontal cultivar transmission between nests of *M. smithii* or other sympatric, lower-attine ant species. Compared to other attine ants, therefore, partner-fidelity feedback is probably relatively less important, and symbiont choice relatively more important, in stabilizing the mutualism (preventing the invasion of inferior self-serving symbionts that are of lesser benefit to the ants).

The observed large number of fungal ITS-types cultivated locally by a single attine ant species (nine fungal types from two different clades in Central Panama) is so far unknown for any lower-attine ant and extends the elevated cultivar diversity of *M. smithii* noted in a previous analysis (Mueller et al., 1998). The different cultivar types found in association with *M. smithii* could have been acquired through transfer from other sympatric ant hosts, or through *de novo* domestication of free-living lepiotaceaeus fungi belonging to Clade 1 and Clade 2 cultivars. For example, cultivar type 3 of *M. smithii* in our study was found to be sequence-identical in its ITS sequence to cultivars of *Mycocepurus tardus* (GenBank accession number AF079710) and *Myrmicocrypta ednaella* (AF079715) collected previously in Panama. The sister-cultivars types 7 and 8 are most closely related to a known cultivar of *Mycocepurus curvispinosus* from Costa Rica (AF079709). Cultivar type 6 is nearly sequence-identical in ITS (except for two nucleotides) to a sympatric free-living lepiotaceaeus fungus from Panama (EF527398; see Fig. S3).

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**Fig. 2** Tanglegram phylogenies of *Mycocepurus smithii* ant clones (microsatellite genotypes) and their corresponding fungal cultivars (ITS-types) from Central Panama. Ant–fungus associations observed in natural nests are indicated through lines linking the two trees. The fungal diversity is split into two main clades, corresponding to Clade 1 Cultivar (top clade) and Clade 2 Cultivar (bottom clade). Significance of each link was inferred in ParaFit (Legendre et al. 2002). For complete ParaFit results, see Table S4.
We confirmed thelytokous parthenogenesis (Fernández-Marín et al., 2005; Himler et al., 2009; Rabeling et al., 2009, 2011) as the primary mode of reproduction in Panamanian populations, with a total of eleven sympatric ant genotypes from three distinct groups; Fig. S2). Some ant genotypes in our survey correspond to types that have been found previously in the Panama Canal Zone (Rabeling et al., 2011), and some ant genotypes are closely related to lineages known only from neighbouring countries in Central America, but not from more distant regions across the range of M. smithii. This indicates that the ant genotypes included in our study represent genotypes typical for Panama, that the ant populations in our study are embedded in a viscous population structure across Latin America, and that the ant–fungus associations studied here represent local or regional associations.

We did not find strict one-to-one association patterns between ants and their cultivated fungi. A few fungal types were only associated with one ant lineage (Fig. 2), but such apparent one-to-one associations could be an artefact of insufficient sampling (see rarefaction analysis of ant–fungus combinations in Fig. S1b). More typically, we found ant lineages associated with more than one fungus type, and likewise, fungus types associated with more than one ant genotype (Fig. 2). This finding agrees with our AMOVA analyses, which revealed that genetic variation of fungi was equally partitioned among and within ant hosts, indicating only weak host-cultivar specialization. Permutation tests for co-speciation revealed evidence of statistically significant, global co-phylogenetic associations between ant and fungal types in Panama. This suggests that fungus cultivars are inherited vertically across a limited number of ant generations, but that such inheritance is not indefinite; rather, novel ant–fungus combinations may be frequently generated by (i) horizontal cultivar transmission of fungal cultivars between ant nests, (ii) de novo recruitment of free-living fungal clones into the mutualism, (iii) gene flow between different symbiotic fungi, or between symbiotic and nonsymbiotic fungi, or (iv) some combination of these processes. The lower $F_{ST}$ values and lower isolation-by-distance effect in the ants than in the fungi indicate that gene flow is stronger between ant populations than between fungus populations, possibly because of a higher effective dispersal-potential in the ants than in the fungus (queens might regularly substitute their native cultivar and re-acquire a local fungus after dispersal). The finding that ant genetic variance among colonies within sites is higher than variance between sites, whereas fungal genetic variance is lower within sites than between sites, further supports the conclusion of greater gene flow in the ants than in the fungus.

Horizontal fungal transfer between ant lineages could occur through several mechanisms in M. smithii. First, it is possible that, compared with other attine ants, incipient fungus gardens during colony founding are more frequently lost in M. smithii [many founding queens often lack fungus gardens; (Fernández-Marín et al., 2005)]. Second, colonies may lose their gardens during catastrophes (e.g. flooding, garden diseases), which forces the ants to recruit free-living fungi or steal garden from neighbouring colonies, which has been shown for Cyphomyrmex ants in laboratory experiments (Adams et al., 2000). At sites where diverse ant and fungal types co-occur, such re-association could generate novel ant–fungus combinations. Third, pleometrotic (multiple foundress) nest-founding, which occurs in M. smithii and a few other attine ants (Rissing et al., 1989; Mintzer, 1990; Bekkevold et al., 1999; Fernández-Marín et al., 2005), also facilitates generation of novel combinations whenever fungi are transferred between co-founding queens. In such multiple-foundress nests, gardens may be chimaeric during the early nest-founding stage. Coexistence of different cultivars of the same fungal species has been observed in chimaeric gardens in laboratory experiments of the more derived higher Attini (Sen et al., 2010). In M. smithii it is likely that different fungal species will be vegetatively incompatible and thus compete with each other if they were co-cultivated by M. smithii in a chimaeric garden, and co-founding queens may even bias cultivation in favour of a specific strain in a chimaeric garden. This predicts absence or rapid transience of chimaeric gardens in M. smithii, and indeed, we never observed more than one fungus type in a single chamber in our field survey.

In the M. smithii mutualism, both partners reproduce asexually; in the absence of sexual recombination, creation of novel genotypes in the ants and the fungal symbiont is therefore limited to mutation. Moreover, as in endosymbionts of other insects (Moran, 1996; Pettersson & Berg, 2007), both of the asexual partners might be prone to the inevitable accumulation of deleterious mutations, a process known as Muller’s ratchet operating in all asexual lineages of finite population size (Maynard Smith, 1983). Ant–fungus re-association would alter the combined number of deleterious mutations accumulating within an ant–fungus combination, and such re-associations therefore could function as a recombinatory analogue operating on the level of ant–fungus combinations, potentially purging the ants of degenerate fungi and purging the fungi of degenerate ants.

Symbiont choice could enable M. smithii to choose between cultivar types that are superior or inferior in different habitats (habitats offering different garden substrate, or different environmental conditions such as moisture or temperature, or different biotic challenges such as garden diseases). Symbiont choice could be particularly important if the fungi are adapted to local conditions, and the ants disperse widely between habitats and across ecological gradients. Consistent with this scenario, we found distinct cultivar types and distinct
culivar prevalence between the varying habitats surveyed in Panama (Fig. 1); in addition, isolation-by-distance tests showed that genetic variation (about 30%) correlates significantly with geographic distance in the fungal cultivars, but not in the ants. This suggests that the ants disperse regionally, but they tend to grow local crops because of frequent cultivar replacement after dispersal. Dispersing queens might try to propagate their natal cultivar in newly colonized habitats, which could lead occasionally to ‘local mal-adaptations’, as predicted by the geographic mosaic theory of co-evolution (Thompson, 1999). Alternatively, dispersing queens might lose cultivars and then be forced to switch to new, locally adapted fungal strains. Whether M. smithii selects cultivars optimally adapted to local conditions, is the topic of a future investigation.

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Data accessibility

DNA sequences used in this study are deposited at NCBI GenBank (Accessions JQ405665–JQ405735 for the ITS fungus sequences, and JX000236 for microsatellite A6-2).

Microsatellite loci A5, A6, A9, B1, B4, C2, C6, C104, C119, D8, D11, D117 were developed by Rabeling et al., 2011 and are deposited at GenBank (Accessions JN054745-JN055353).

Fungus ITS sequences from Mueller et al., 1998 are deposited at GenBank (Accessions AF079591-AF079754) and from Vo et al., 2009 at GenBank (EF527400-EF527280), and the original alignment is shown in GenBank PopSet 140104520.

References


Zwickl, D.J., 2006. Genetic algorithm approaches for the phylogenetic analysis of large biological sequence datasets under the maximum likelihood criterion. (Ph.D. dissertation). The University of Texas at Austin, Austin, TX.

**Supporting information**

Additional Supporting Information may be found in the online version of this article:

**Table S1** Multilocus genotypes of *Mycocepurus smithii* samples, GPS data information for sample locations, primer sequence for locus A6_2.

**Table S2** Expected ($H_e$) and observed ($H_o$) heterozygosities of *M. smithii* ants.

**Table S3** Pairwise genetic differentiation ($F_{ST}$ values) between eleven populations of *Mycocepurus smithii* ants and fungus.

**Table S4** Statistics for the significance of ant–fungus associations analysed in ParaFit.

**Figure S1** Sample-based rarefaction curves (lineage-accumulation curves) of (a) fungus types; (b) ant lineages; and (c) ant/fungus combination of *M. smithii* collected in the Republic of Panama in 2010.

**Figure S2** Phylogenetic relationships (neighbour-joining tree) of eleven ant lineages of *M. smithii* in Central Panama.

**Figure S3** Maximum-likelihood phylogeny of 131 lower-attine cultivars and 174 free-living lepiotaceous fungi.

**Figure S4** Maximum-likelihood phylogeny inferred from ITS rDNA sequence information for (a) Clade 1 and (b) Clade 2 fungi cultivated by lower-attine ants.

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