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Phylogeography of post-Pleistocene population expansion in a fungus-gardening ant and its microbial mutualists

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Abstract

Although historical biogeographical forces, such as climate-driven range shifts, greatly influence the present-day population genetic structure of animals and plants, the extent to which they affect microbial communities remains largely unknown. We examined the effect of postglacial expansion on the population structure of the northern fungus-gardening ant *Trachymyrmex septentrionalis* and compared it with that of its two microbial mutualists: a community of lepiotaceous fungal cultivars and associated antibiotic-producing *Pseudonocardia* bacteria. The ant population genetic structure showed signs of population expansion and subdivision into eastern and western phylogroups that likely originated in the Pleistocene — a pattern shared by many other North American taxa found in the same region. Although dispersal limitation was present in all three symbionts, as suggested by genetic isolation increasing with distance, the host's east–west subdivision of population genetic structure was absent from the microbial mutualist populations. While neither the cultivar nor the *Pseudonocardia* genetic structure was correlated with that of the ants, they were significantly correlated with each other. These results show that biogeographical forces may act differently on macro- and microscopic organisms, even in the extreme case where microbial mutualists are vertically transmitted from generation to generation and share the same joint ecological niche. It may be that historical climate change played a larger role in determining the population structure of the ant hosts, whereas present-day environmental forces, such as pathogen pressure, determine the structure of associated microbial populations.

Keywords: actinomycete, coexistence, latitudinal gradients, *Lepiotaceae*, population expansion, refugia

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Introduction

The study of animal and plant distribution patterns has occupied centre stage in biology for centuries. Biogeographical regularities, which were extensively catalogued by naturalists, eventually formed the framework for major conceptual advances, such as the theory of evolution by natural selection, which was intuited by Darwin and Wallace from the patterns they observed in insular faunas (Darwin 1859; Wallace 1876). However, biogeographical observations have been limited to the realm of macroscopic organisms until relatively recently. In contrast to the extensive literature on animal and plant biogeography, much less is known about the nature of ecological and evolutionary forces shaping the geographical structure of microbial populations. The large effective populations size of many microbes, together with the ability of some to form environmentally resistant spores, has lead to the commonly cited and controversial belief that 'everything is everywhere, but the environment selects' (Baas Becking 1934).

Recent evidence suggests geographical isolation does indeed structure microbial communities (Whitaker *et al*. 2003; Papke & Ward 2004). At the same time, some of the prevalent phylogeographical patterns, such as latitudinal species gradients, appear absent in microbial communities, although they are common in many macroscopic taxa (Fierer & Jackson 2006). On the other hand, bacteria do appear to exhibit species-area correlations similar to those of macroscopic animals (Green & Ostling 2003; Horner-Devine *et al*. 2004; Bell *et al*. 2005). Nonetheless, based on our limited understanding of processes driving bacterial community assembly, dynamics generating species-area curves of bacterial communities most likely differ from

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those operating on insular faunas, where stochastic events may play a larger role (Fenchel & Finlay 2005; Woodcock *et al*. 2006). Consequently, the extent to which the well-studied axioms of macroscopic animal phylogeography apply at the 'micro' scale remains unclear.

Extrapolation of well-known biogeographical processes to the microscopic scale is difficult because microbes have ecologies vastly different from those of macroscopic taxa. Extreme variations in bacterial abundance and community composition between microhabitats within any given locale further complicate comparisons between sites. However, the study of obligate mutualistic relationships between macroscopic animals and microbes, which occupy a joint ecological niche, allows us to circumvent the above problems, since the patterns of host distribution should influence microbial presence and community structure (e.g. Fisher *et al*. 2001 and Linz *et al*. 2007). Furthermore, the microbial mutualists should respond similarly to ecological forces important in phylogeographical structuring of host populations, such as range shifts and allopatric isolation, particularly in the case of vertically transmitted mutualists. Thus, the phylogeographical structure of the macroscopic host can serve as a hypothesis for the distribution of their microbial mutualists.

We used the well-studied fungus-gardening ant symbiosis (a 50-million-year-old obligate nutritional mutualism between ants and fungi) to explore phylogeographical concordance between the macroscopic ant host and its microbial symbionts. The attine ants and their cultivar fungi show phylogenetic congruence at the basal levels, but cultivar-sharing dominates at the population level (Chapela *et al*. 1994; Hinkle *et al*. 1994; Mueller *et al*. 2001; Green *et al*. 2002; Mikheyev *et al*. 2007). The most phylogenetically derived attine clades cultivate specialized fungi that have been found only in their nests (Mueller *et al*. 1998; Vo *et al*. in press). *Pseudonocardia* bacteria, which are housed in co-evolved crypts in the ants' cuticle, are thought to protect the garden from the specialized pathogen *Escovopsis* (Currie *et al*. 1999b, 2003a, b, 2006; Gerardo *et al*. 2006). Ant queens take both the *Pseudonocardia* and the fungal cultivar from their native gardens, assuring at least some vertical cotransmission (Weber 1972; Currie *et al*. 1999a). Despite this link, the association between the ants and their mutualists is inherently labile, and both inter- and intraspecific host-switching has been documented for both cultivars and *Pseudonocardia* (Green *et al*. 2002; Cafaro & Currie 2005; Poulsen *et al*. 2005; Mikheyev *et al*. 2006, 2007).

The northern fungus-gardening ant *Trachymyrmex septentrionalis*, which ranges through the Eastern USA, provides a particularly interesting case study. First, *T. septentrionalis* is largely allopatric with congenerics, except at the extreme edges of its range: in central Texas, where it co-occurs with *Trachymyrmex turrifex*, and in the south of Florida, where it may overlap with the extremely rare *Trachymyrmex jamaicensis* (Deyrup *et al*. 1988; Wheeler 1973; Rabeling *et al*. 2007). Although distributions of other fungus-gardener ants in the genera *Atta*, *Cyphomyrmex* and *Mycetosoritis* partially overlap with that of *T. septentrionalis* (Wheeler 1973), they all cultivate genetically distinct fungi (Chapela *et al*. 1994; Mueller *et al*. 1998). Thus, there should be no host–cultivar switching between *T. septentrionalis* and other sympatric attines through most of its range. Second, *T. septentrionalis* has undergone a relatively recent population expansion after the retreat of Pleistocene glaciers that covered the north of its present range (Marshall *et al*. 2002). In general, we would expect its population structure to be similar to those of other temperate North American species, which often show signs of population differentiation in glacial refugia, followed by rapid population expansion during the interglacial periods (Pielou 1991). Thus, we predict that (i) *T. septentrionalis* populations would show signs of glacial retreat and postglacial expansion, and (ii) if phylogeographical processes (i.e. postglacial range extension) act in the same manner on the microbes and on the ant hosts, the microbial mutualists should have congruent population structure.

Materials and methods

Sites and collection

Ant nests were collected in 2001–2006 at the 16 sites listed in Table 1. Ants and fungi were preserved in 95% ethanol and stored at –80 °C until DNA extraction. Only a single worker ant was analysed per colony.

DNA extraction, amplification and sequencing

Protocols for polymerase chain reaction (PCR) purification and sequencing conditions, as well as the procedure for fungal DNA extraction using the Chelex method can be found in Mikheyev *et al*. (2007). Ant and bacterial DNA were extracted simultaneously using DNeasy tissue kits according to the manufacturer's instructions (QIAGEN). The elongation factor 1-α (*EF1α*) of the cultivar was amplified using primers by Mikheyev *et al*. (2006). The phylogenetic clusters identified using *EF1α* were confirmed by sequencing the ribosomal internal transcribed spacers 1 and 2 (*ITS*) for a random subset of the cultivars in each of these groups using primers and reaction conditions by White *et al*. (1990). The cytochrome oxidase 1 (*COI*) mitochondrial gene of the ants was amplified using the primer pair Ben and Jerry (Simon *et al*. 1994; Villesen *et al*. 2004). In addition, we amplified the *ITS* region of ant rDNA, using primers and conditions developed by Ji *et al*. (2003). The *Pseudonocardia* elongation factor Tu (*EF-Tu*) gene was amplified using a nested PCR procedure. The first reaction used the specific primer pair 1F and 920R from Poulsen *et al*. (2005).

The second amplification, intended to improve sensitivity and specificity, was carried out using a 1:10 dilution of the first reaction as a template, and used a custom primer pair (ActinoNF 5′-GACAAGGCGCCGGAAGAG-3′ and ActinoNR 5′-CGTCCTCACGCTGATAAC-3′). One microlitre of the extract (or in the case of nested PCR, the diluted product from the previous reaction) was used as template in 10 μL reaction volumes. The PCR contained 1× reaction buffer, 1 mM dNTPs, 0.5 μM primers, 5 mM $MgCl_2$ with 0.1 U of Bioline *Taq* polymerase. Reaction conditions involved an initial denaturing step of 94 °C for 2 min, followed by 35 cycles of 94 °C for 10 s, 60 °C for 20 s, and 72 °C for 30 s. PCR purification and sequencing protocols were as published earlier by Mikheyev *et al*. (2006). We deposited our sequences in GenBank under Accession nos EU561361–EU561600.

In the ants, but not in the fungi, *ITS* often contained varying numbers of one- and two-nucleotide repeats, making direct sequencing difficult and producing no readable sequence in some individuals at all. Although *ITS* and *COI* partitioned the ant genetic variance into the same phylogroups (see below), *ITS* data contained numerous nucleotide scoring ambiguities and was not used in the quantitative analysis.

Phylogeography of Trachymyrmex septentrionalis

A post-Pleistocene population expansion should result in a unimodal distribution of haplotype mismatches (Harpending *et al*. 1998). We calculated the haplotype mismatch distributions for a spatial expansion model implemented in Arlequin (version 3.1) (Excoffier *et al*. 2005) and used Harpending's (1994) and SSD raggedness index to determine the goodness of fit of observed mismatch distributions to those predicted by the demographic expansion model.

Reconstruction of the cultivar and Pseudonocardia *phylogenies*

For phylogenetic analysis of the cultivar, DNA substitution models for each gene were estimated using ModelTest (Posada & Crandall 1998). Phylogenies for cultivars and *Pseudonocardia* were constructed in a manner similar to those of the ants, using GTR + *G* for both fungal genes and F_{81} + *I* + *G* model for the bacterial gene. Phylogenies were computed using MrBayes (version 3.1; Ronquist & Huelsenbeck 2003). In the case of the cultivars, the analysis was run with separate partitions for the *EF1α* and *ITS* genes, each partition with its own independently estimated set of parameters. The calculation was continued until the average standard deviation of split frequencies between the two runs dropped below 0.01. Afterwards, the first 75% of the generations were discarded as burn-in and a majority-rule consensus tree was computed to estimate posterior probabilities for each node. The Markov chain Monte Carlo simulation was performed several times to assure convergence to the same solution space. To confirm that genetic exchange through recombination does not occur between cultivar types, we conducted a partition homogeneity test on the same samples used to create the phylogeny, but with identical sequences removed, using PAUP's branch-and-bound search algorithm and 1000 pseudoreplicates (keeping one tree per pseudoreplicate; Swofford 1993; Farris *et al*. 1995).

Comparative phylogeography

To separate long-term trends from short–term associations caused by kinship, comparisons between ant and fungal phylogeographical structure were carried out at the population level. F_{ST} distances between *T. septentrionalis* populations were estimated in Arlequin using the Tajima–Nei nucleotide substitution model with a 0.009 Gamma rate heterogeneity value (Excoffier *et al*. 2005). The nucleotide substitution model and Gamma parameter were selected using a ModelTest block (Posada *et al*. 1998), which was reduced to include only models supported by Arlequin, which does not implement many of the models evaluated by ModelTest. Fungal community distances were measured using Jaccard and Morisita's indexes. Both measures of community composition were highly correlated and gave qualitatively identical answers; only the results of computations using Jaccard's index are presented. Cultivar diversity was measured using Shannon's index. The much lower numbers of *Pseudonocardia* symbionts amplified prevented the computation of reliable population-level statistics. Instead, Mantel tests were performed directly on genetic distance matrixes. Because it is not clear how genetic distance between cultivar types should affect *Pseudonocardia* population structure, cultivars types were treated as discrete entities. Consequently, the cultivar distance matrix was coded as ones and zeros (membership in the same cultivar type represented by ones, zero otherwise). Genetic distance matrixes were computed in PAUP (version 4.0 beta 10; Swofford 1993) using nucleotide substitution models selected by ModelTest separately for each data set. Mantel tests of similarity matrixes were carried out using 10^6 permutations of the raw values in ZT (Bonnet & Van de Peer 2002). In addition to Mantel tests, we tested the extent to which *Pseudonocardia* genetic variation was partitioned among cultivar types and ant phylogroups using an AMOVA, carried out in Arlequin.

Results

Trachymyrmex septentrionalis *phylogeography*

Previously, Wheeler (1911) recognized two subspecies of *Trachymyrmex septentrionalis* based on morphological and demographic differences occurring between northern and

State	Locality name	Ants	Fungi	*Pseudonocardia*	Latitude	Longitude
AL	Prattville	5	5	3	32.44	86.47
AR	Devil's Den	5	5	5	35.79	94.25
FL	Tallahassee	5	5	3	30.44	84.48
GA	Waycross	5	4	3	31.16	82.20
IL	Sand Ridge	5	5	0	40.43	89.91
IL	Dixon Springs	5	5	3	37.38	88.66
LA	Reeves	5	4	3	30.52	93.05
MS	Starkville	5	5	1	33.51	88.74
NC	Hoffman	5	5	4	35.02	79.62
NC	Buxton	4	4	2	35.24	75.53
NJ	Lebanon	5	2	3	39.88	74.56
NY	Centereach*	5	10	3	40.86	73.08
TX	Angelina	8	8	7	30.87	94.18
TX	Smithville	5	5	3	30.08	97.17
VA	Pamplin City	2	2	2	37.29	78.69
	Total:	74	74	45		

Table 1 Collection localities and the number of successfully PCR-amplified specimens. Only one ant was sampled per colony. Samples from the Western phylogroup of *Trachymyrmex septentrionalis* are in highlighted in grey; all other samples are from the Eastern phylogroup

*Population was sampled twice, in 2001 and in 2006, 5 colonies each time, to confirm the stability of cultivar community composition through time. AL, Alabama; AR, Arkansas; FL, Florida; GA, Georgia; IL, Illinois; LA, Louisiana; MS, Missouri; NC, North Carolina; NJ, New Jersey; NY, New York; TX, Texas; VA, Virginia.

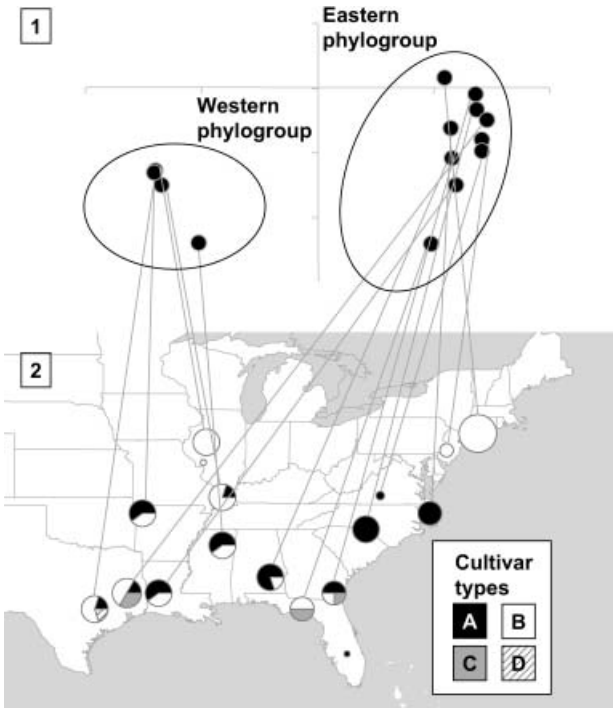

Fig. 1 Phylogeographical structure of ants and their cultivars. (1) Nonmetric multidimensional scaling analysis (NMDS) of the ant populations. The first and second components are plotted on the *x* and *y* axis, respectively. (2) Cultivar community structure. At every sampling site, cultivar community structure is represented by a pie chart depicting relative proportions of the four cultivar types (area proportional to sample size, ranging from 1 to 10). Lines connect cultivar communities' structure to the NMDS plot of ant genetic structure for all samples with more than five collections.

southern populations, although a recent comprehensive revision of the US *Trachymyrmex* by Rabeling *et al*. (2007), based on both morphological and molecular characters, did not support this distinction. Thus, geographical morphological variation noticed by Wheeler probably results from adaptive climate-driven intraspecific differences, such as those noted by Beshers & Traniello 1994), and we treated *T. septentrionalis* as a single species. However, ant populations clustered into two phylogroups, approximately divided by the Mississippi river valley and having only a single mitochondrial haplotype in common (a typically western type found in one individual from north Florida; Table 1, Fig. 1). In both phylogroups, haplotype mismatch frequencies were consistent with a sudden population expansion (SSD and raggedness indexes $P > 0.20$ in both phylogroups).

Ant–cultivar interactions

Trachymyrmex septentrionalis cultivates several genetically distinct types of fungi (Fig. 2). A partition homogeneity test using 181 parsimony-informative characters did not detect recombination of *ITS* and *EF1α* between cultivar types ($P = 0.35$), indicating that there is no gene flow between these types and suggesting that they are either genetically distinct clones or species. The relative frequencies of these types was unequal, approximately 39%, 49%, 10% and 1%, for types A, B, C and D, respectively ($\chi^2 = 44.5$, d.f. = 3, $P = 1.210^{-9}$). Based on our samples, the genetic diversity of the *Trachymyrmex* cultivars appears much greater than that of the leaf-cutting ant cultivars, and is comparable to the diversity present in the entire lower fungus-gardening ant

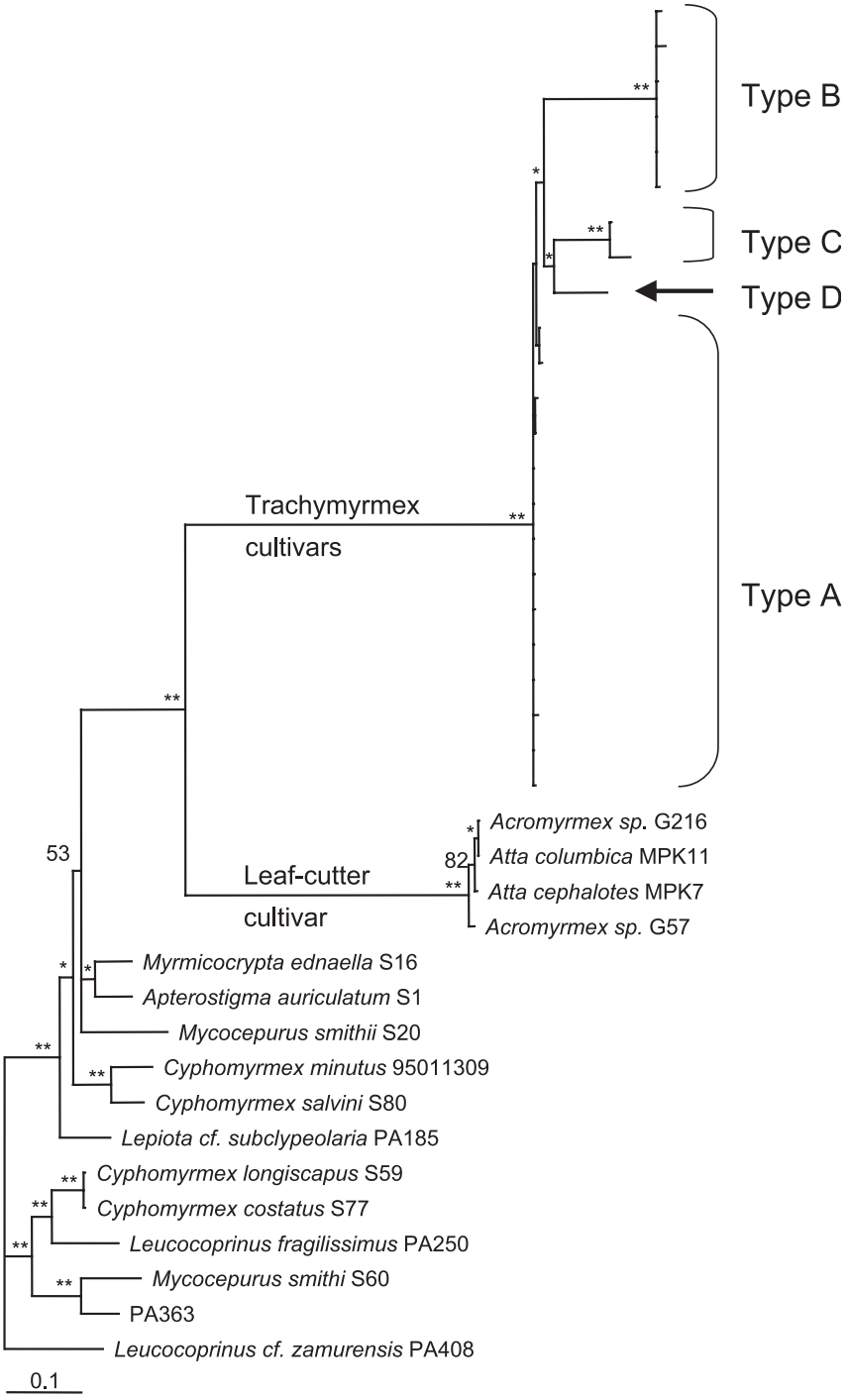

Fig. 2 Bayesian phylogeny of the cultivar fungi based on a combined analysis of *ITS* and *EF1α*. Outgroups are labelled according host ant species and collection code specified in the GenBank title field (codes of free-living relatives begin with the letters 'PA'). Nodes labelled with * and ** have > 90% and 100% posterior probability, respectively. The tree includes 9, 18, 2 and 1 representatives of types A, B, C and D, respectively. The scale bar corresponds to 0.1 substitutions per site.

symbiosis. Genetically divergent fungi often occur in each other's immediate vicinity, sometimes less than a meter apart.

The diversity of *T. septentrionalis*-associated cultivars markedly decreased northward and eastward ($r = –0.78$, $P = 0.001$ and $r = –0.63$, $P = 0.011$, respectively) from a centre of diversity around Texas (Fig. 2). As a result of this spatial structuring, the fungal community composition exhibited significant isolation by distance ($r_{xy} = 0.23$, $P = 0.034$). Likewise, the genetic relatedness between *T. septentrionalis* populations was geographically structured, largely due to the existence of two large phylogroups ($r_{xy} = 0.38$, $P = 0.003$; Fig. 2). However, there was no correlation between the ants' genetic and the fungal community similarity matrixes, even after the effects of geographical distance were factored out ($r_{xyz} = 0.0043$, $P = 0.49$).

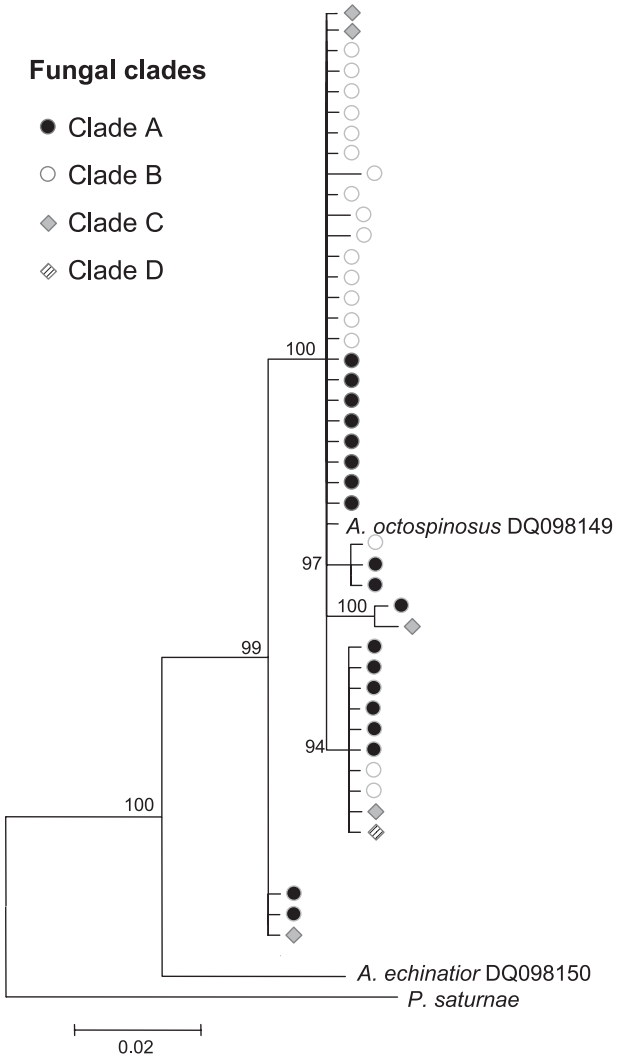

Fig. 3 Phylogeny of *Pseudonocardia* symbionts with outgroups from Poulsen *et al*. (2005). *Pseudonocardia* population structure was uncorrelated with that of the ants. Although there was no clear phylogenetic mapping of *Psedonocardia* phylogeny on cultivar type, there was nonetheless a slight, significant association between the two, suggesting differential association due to environmental factors rather than by vertical cotransmission with the ants. The scale bar corresponds to 0.02 substitution per site.

Ant–cultivar-Pseudonocardia *relationships*

Despite low genetic variability (Fig. 3), like the other two symbionts, *Pseudonocardia* showed slight, although significant, isolation by distance ($N = 45$, $r_{xy} = 0.10$, $P = 0.041$). There was no evidence for association between ant and *Pseudonocardia* lineages, as evidenced by the lack of a correlation of their genetic distance matrixes ($N = 45$, $r_{xyz} = -0.08$, $P = 0.95$). Likewise, the effect of ant phylogroup on *Pseudonocardia* genetic structure was not significant ($P = 0.34$) and explained only 0.5% of the molecular variance in *Pseudonocardia* population structure. By contrast, there was a significant positive association between *Pseudonocardia* genotypes and fungal cultivar type ($N = 43$, $r_{xyz} = 0.092$, $P = 0.029$; cultivar type explained 19% of the molecular variance in *Pseudonocardia* population structure ($P = 0.003$)).

Discussion

The eastern and western phylogroups of *Trachymyrmex septentrionalis* were genetically differentiated at both nuclear and mitochondrial loci. Although the eastern and western populations share few mitochondrial haplotypes, suggesting a long-term differentiation, they are not separated by present–day geographical barriers. Given that *T. septentrionalis* species itself originated recently, approximately within the past million years (Schultz & Brady 2008), it seems overwhelmingly likely that the two phylogroups arose at some point in the Pleistocene. The pattern of *T. septentrionalis* population subdivision parallels that of 'highland' fishes, amphibians, reptiles and mammals in central North America (Blair 1958, 1965; Wiley & Mayden 1985; Robinson 1986; Mayden 1988; Walker *et al*. 1998; Burbrink *et al*. 2000; Near *et al*. 2001; Brant & Orti 2003; Fig. 2). The congruence of phylogeographical patterns across such different taxa suggests shared historical biogeographical influences that are most commonly interpreted as genetic diversification in allopatric Pleistocene refugia, followed by expansion and secondary contact between diverged populations (Avise 2000).

The phylogeographical structure evident in *T. septentrionalis* was completely absent from the population genetic structure of the associated microbes. Since there was evidence of population viscosity in both the fungal cultivars and the *Pseudonocardia*, the lack of congruence was not due to limitless dispersal by the microbes. However, previous work on leaf-cutting ant cultivars has shown that they are better dispersers than the ants, probably because of their ability to form easily dispersed spores (Möller 1893; Mikheyev *et al*. 2006). A similar occasional decoupling from vertical transmission appears to exist in the *T. septentrionalis* symbiosis. Thus, it is possible that the fungal communities were not fully isolated and had appreciable migration between Pleistocene refugia, while the relatively more viscous ant populations underwent genetic differentiation. Much less is known about the ecology of *Pseudonocardia*, but it seems likely that they are either capable of existence outside the symbiosis, or are better dispersers than the ants, which disperse much less than 500 m/year during an annual mating flight, based on flight distances of the much larger and more powerful *Acromyrmex octospinosus* (Mikheyev, 2008).

The long-term existence of multiple species or clones occupying the same niche, in this case supposedly obligate

fungal lineages associated with a single ant host, presents a puzzle. Extensive surveys of free-living lepiotaceous cultivars have thus far failed to find any free-living relative (Mueller *et al*. 1998; Vellinga 2004; Vo *et al*. in press). If a free-living existence is impossible for the cultivars, in the absence of other forces stochastic extinction or competition, this should lead to the fixation of a single cultivar species in the *T. septentrionalis* symbiosis. Conceivably, coexistence may be driven by migration of cultivars at the large diverse population farther south in Latin America, where they form complex associations with several ant species. This hypothesis is difficult to test, although it is supported by the observation that cultivar diversity decreases northward, away from the presumed centre of diversity (Fig. 1). Alternatively, diversity may be maintained by frequency-dependent selection. For example, low-frequency cultivars may be less likely to encounter specialized pathogens, such as locally adapted strains of *Escovopsis* (Currie *et al*. 1999a).

Although showing no correlation with ant population structure, *Pseudonocardia* populations were structured with respect to the cultivar types. These results are consistent with our knowledge of *Pseudonocardia* biology, since they play a role in controlling the garden pathogen *Escovopsis* (Currie *et al*. 1999b) that itself tracks cultivar genotypes (Currie *et al*. 2003b; Gerardo *et al*. 2006). Like cultivars, they are exchanged between sympatric species of ants (Poulsen *et al*. 2005). While ant-*Pseudonocardia* genotype pairings may be vertically propagated by dispersing ant queens (Weber 1972; Currie *et al*. 1999a), these associations appear ephemeral. The existence of cultivar-specific population differentiation in the *Pseudonocardia* predicts that (i) pathogen strains differ between cultivar species, and (ii) different *Pseudonocardia* strains differ in their efficacy against pathogens. Thus, it appears likely that cultivar diversity is maintained by frequency-dependent selection as a result of *Escovopsis* (or some other) disease pressure.

Although attine cultivars were initially believed to be ancient asexual clones (Chapela *et al*. 1994), more recent work has documented the existence of a connection to free-living cultivars in lower attines, and the presence of recombination in leaf-cutting ants (Mueller *et al*. 1998; Mikheyev *et al*. 2006; Vo *et al*. in press). Although no data direct on recombination exists on *Trachymyrmex*-associated fungi, basidiocarp production was observed in laboratory colonies (Mueller 2002) and there is evidence that purifying selection acts on meiosis-specific genes (Mikheyev *et al*. 2006). Thus, it seems reasonable to believe that the genetically distinct types found associated with *T. septentrionalis* represent different reproductively isolated species, although additional sampling may reveal evidence of genetic exchange.

Recognizing the possible existence of several fungal species allows us to make direct comparisons with other well-studied attine systems. For example, earlier it has been suggested that apparent intraspecific cultivar diversity may actually be a case of cryptic ant speciation, with each species specializing on its own cultivar (Mueller *et al*. 1998). Our data show that this is not a general rule and that multiple cultivars can be involved with the same ant species (although see Schultz *et al*. 2002) for a possible exception). The many–to–one association of the *T. septentrionalis*–fungus symbiosis contrasts with that of the leaf-cutting ants, which cultivate largely one fungal species (Mikheyev *et al*. 2006). If the cultivars have no free-living state, there must be intense competition between the *T. septentrionalis* cultivar types for their ant hosts. Given that *T. septentrionalis* fitness remains the same even when experimentally raised on distantly related (Fig. 1) leaf-cutter cultivar (Seal & Tschinkel 2007), it would seem unlikely that the different *Trachymyrmex* cultivars would have substantially different effects on ant fitness. Thus, in the presence of cultivar exchange, a single 'super cultivar' may potentially replace all others if it can markedly increase ant fitness or be preferred by the ants through symbionts choice (Mueller 2002). This may have been the case at some point in leaf-cutting ant evolution, because their sole cultivar has low genetic variation, consistent with a sudden expansion of a single species (Silva-Pinhati *et al*. 2004; Mikheyev *et al*. 2006).

Conclusion

The discordance between the population genetic structure of the ant host and its microbial mutualists illustrates the different ecological forces acting on macro- and microscopic organisms. The Pleistocene climatic changes, which have shaped present-day population genetic structure of the ants, have had little effect on the current structure of their microbial mutualists. This could be due either to the ability of microbes to travel between Pleistocene refugia, which prevented differentiation, or to the more rapid equilibration of their population genetic structure following the retreat of the glaciers. In either case, although microbial populations are not free of geographical structure, the connectivity between their populations is far greater than that of the ants, as has been noted earlier for leaf-cutting ant fungal cultivars (Mikheyev *et al*. 2006). Our data illustrate how fundamental differences between macro- and microscopic organisms, such as differences in dispersal rates, result in markedly different phylogeographical patterns. Historical, rather than present-day co-evolutionary forces, appear to have a greater effect on macroscopic organisms than even on their immediate microbial associates. Thus, perhaps in contrast to macroscopic taxa, knowledge of microbial biogeography may benefit more from the study ecological interactions than from accumulation of geographical patterns.

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