PROCEEDINGS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES

Frontier mutualism: coevolutionary patterns at the northern range limit of the leaf-cutter ant –fungus symbiosis

Ulrich G. Mueller, Alexander S. Mikheyev, Scott E. Solomon and Michael Cooper

Proc. R. Soc. B 2011 **278**, doi: 10.1098/rspb.2011.0125 first published online 9 March 2011

PROCEEDINGS OF THE ROYAL SOCIETY B

Proc. R. Soc. B (2011) **278**, 3050–3059
doi:10.1098/rspb.2011.0125
Published online 9 March 2011

Frontier mutualism: coevolutionary patterns at the northern range limit of the leaf-cutter ant–fungus symbiosis

Ulrich G. Mueller[1,*], Alexander S. Mikheyev[1,2], Scott E. Solomon[1,3] and Michael Cooper[1]

[1]*Section of Integrative Biology, University of Texas at Austin, Austin, TX 78712, USA*
[2]*Okinawa Institute of Science and Technology, 1919-1 Tancha, Onna-son, Kunigami, Okinawa 904-2234, Japan*
[3]*Department of Ecology and Evolutionary Biology, Rice University, Houston, TX 77005, USA*

Tropical leaf-cutter ants cultivate the fungus *Attamyces bromatificus* in a many-to-one, diffuse coevolutionary relationship where ant and fungal partners re-associate frequently over time. To evaluate whether ant–*Attamyces* coevolution is more specific (tighter) in peripheral populations, we characterized the host-specificities of *Attamyces* genotypes at their northern, subtropical range limits (southern USA, Mexico and Cuba). Population-genetic patterns of northern *Attamyces* reveal features that have so far not been observed in the diffusely coevolving, tropical ant–*Attamyces* associations. These unique features include (i) cases of one-to-one ant–*Attamyces* specialization that tighten coevolution at the northern frontier; (ii) distributions of genetically identical *Attamyces* clones over large areas (up to 81 000 km^2, approx. the area of Ireland, Austria or Panama); (iii) admixture rates between *Attamyces* lineages that appear lower in northern than in tropical populations; and (iv) long-distance gene flow of *Attamyces* across a dispersal barrier for leaf-cutter ants (ocean between mainland North America and Cuba). The latter suggests that *Attamyces* fungi may occasionally disperse independently of the ants, contrary to the traditional assumption that *Attamyces* fungi depend entirely on leaf-cutter queens for dispersal. Peripheral populations in Argentina or at mid-elevation sites in the Andes may reveal additional regional variants in ant–*Attamyces* coevolution. Studies of such populations are most likely to inform models of coextinctions of obligate mutualistic partners that are doubly stressed by habitat marginality and by environmental change.

Keywords: *Attamyces*; *Atta texana*; *Acromyrmex versicolor*; coevolution; mutualism; range limit

1. INTRODUCTION

Evolution in peripheral populations can differ markedly from evolution in more central populations within a species range [1–3]. Peripheral populations often exist at lower densities, produce fewer offspring per individual, are more fragmented and are more prone to local extinction [2,3]. These demographic properties leave distinct population-genetic footprints, such as reduced genetic diversity, increased homozygosity and increased levels of unique (private) alleles in peripheral populations compared with central populations [4,5]. A large body of work has confirmed these general population-genetic predictions, although there are exceptions [5–7]. Much less is known about the population-genetic patterns of host–symbiont associations in marginal habitat, yet studies on symbioses near their distributional range limits are needed to inform models of coextinctions of symbiotic partners that are doubly stressed by habitat marginality and by accelerated environmental change [8,9]. To inform such models of host–symbiont coextinction, we elucidate here the genetic diversities and host-specificities of *Attamyces* fungi cultivated by leaf-cutter ants at their northern range limit.

* Author for correspondence (umueller@mail.utexas.edu).

Electronic supplementary material is available at http://dx.doi.org/10.1098/rspb.2011.0125 or via http://rspb.royalsocietypublishing.org.

The obligate mutualism between leaf-cutter ants (genera *Atta* and *Acromyrmex*) and their *Attamyces* fungi originated 8–12 Myr ago in the South American tropics but extends today into temperate regions [10–13]. In the Southern Hemisphere, leaf-cutter ants reach 44° S latitude [14]. In the Northern Hemisphere, *Atta texana* reaches 33° N latitude in north-central Texas and northeastern Louisiana [15; this study] and *Acromyrmex versicolor* reaches almost 36° N latitude in northwestern Arizona [16]. It is unknown whether leaf-cutter ants dispersed into the North American continent before or after the closing of the Isthmus of Panama approximately 1–3 Myr ago [17]. However, leaf-cutter ants must have reached their current latitudinal limit in the southern USA sometime during the past 10 000 years following the most recent Pleistocene glaciation. The leaf-cutter ant *At. texana* was abundant in central and east Texas at the time when European settlers arrived [18,19], whereas a historical presence of *Ac. versicolor* in Northern Arizona is unknown, but likely. Because tropical *Attamyces* fungi are cold-intolerant and grow best at the warm, stable temperatures of tropical soils (between 20°C and 30°C) [20–23], the cold-sensitivity of *Attamyces* symbionts is thought to have been one factor that constrained the expansion of the leaf-cutter ant–*Attamyces* symbiosis from tropical into temperate habitats [14,24].

All leaf-cutter ants depend on symbiotic fungi for food, which grow in gardens tended by the ants in excavated

Received 19 January 2011
Accepted 14 February 2011

subterranean cavities or in thatched shelters [10,12,19,25]. The fungi cultivated by leaf-cutter ants belong to the agaric tribe Leucocoprineae (fungal anamorph *Attamyces bromatificus*, teleomorph *Leucocoprinus gongylophorus*, Agaricales, Basidiomycota; [26,27]). All *Attamyces* strains genotyped so far showed polyploid-like allele patterns with more than two alleles per locus [24,28,29], consistent with cytological studies indicating that *Attamyces* cells are multi-nucleate [30]. The polyploid-like allele patterns could derive from a complex heterokaryon (coexistence of genetically differentiated haploid nuclei in the same cell, which is the typical growth form of basidiomycete mycelia), from duplicated genomes within single nuclei or from a combination of both [29]. Because *Attamyces* fungi have not been found so far growing independently of the ants [31,32], *Attamyces* appears to be on obligate symbiont. However, at least some *Attamyces* fungi are fruiting-competent and can produce spore-bearing mushrooms (table 3 in Mueller [26] summarizes records of fruiting leaf-cutter fungi). *Attamyces* fruiting bodies in the field are so far known mostly from *Acromyrmex* leaf-cutter ants that build thatched gardens at ground level [33,34].

Attamyces fungi are clonally propagated by the ants within and between nests [35,36], suggesting the possibility of strict *Attamyces* asexuality imposed by the ant farmers; however, incongruence between the phylogenetic topologies of segregating genes indicates that clonality of *Attamyces* is occasionally punctuated by recombination events [37,38]. In laboratory experiments, co-cultivated cultivar genotypes may recombine in artificially created chimaeric gardens [28,39], possibly through the exchange of nuclei between cultivar mycelia. A separate study testing for chimaeric gardens in natural leaf-cutter nests revealed no evidence for *Attamyces* polyculture in the leaf-cutter ants *At. texana* and *Atta cephalotes*, suggesting that *Attamyces* is grown by the ants in single-strain monoculture throughout the hundreds of gardens within a single mature leaf-cutter nest [36].

Across the leaf-cutter ant range from Argentina to the USA, the approximately 50 described leaf-cutter species are thought to associate with a single cultivar species (*Attamyces bromatificus* Kreisel) in a many-to-one coevolutionary relationship [37,40]. Whereas the leaf-cutter ant clade is estimated to be about 8–12 Myr old, the corresponding clade of *Attamyces* cultivars is significantly younger (about 2–4 Myr old) [40]. *Attamyces* lineages of recent origin, therefore, may have spread by means of horizontal transfer between leaf-cutter ant lineages (i.e. *Attamyces* sweeps through the range of leaf-cutter ants). Comparison of fast-evolving genes of *Attamyces* strains from geographically distant leaf-cutter species across South America [41] and population-genetic patterns of *Attamyces* cultivated by sympatric leaf-cutter species in Panama [38] confirmed the expected sharing of *Attamyces* lineages between tropical leaf-cutter ant species. Because a local community of tropical leaf-cutter ants shares a corresponding local community of cultivar lineages [38,40,41], *Attamyces* cultivars in the tropics are thought to evolve within a continually shifting landscape of the microhabitats occupied by the diverse leafcutter ants between which cultivars are exchanged.

The most detailed population-genetic study of *Attamyces* symbionts to date was conducted by Mikheyev *et al.* [38] for sympatric populations of three *Atta* species and two *Acromyrmex* species from Panama. Despite vertical transmission of *Attamyces* strains from mother to daughter nests, different leaf-cutter ant species and genera sometimes share identical *Attamyces* genotypes (clones), indicating widespread cultivar exchange across Panama. *Attamyces* genotypes group into six distinct *Attamyces* genotype-clusters in Panama, but these clusters do not correspond to the five ant hosts (only about 10% of the structure in genetic variance of *Attamyces* is attributable to species and generic boundaries among ant hosts; [38]). Frequent exchange of *Attamyces* clones between heterospecific ant nests or other forms of *Attamyces* gene flow between nests, therefore, prevents the long-term persistence of specific ant–*Attamyces* combinations, leading to an overall pattern of diffuse coevolution between leaf-cutter ant hosts and *Attamyces* symbionts in this tropical population.

At the northern range limit of leaf-cutter ants (southern USA), *Attamyces* evolution progresses differently than in the tropics, because here leaf-cutter ants do not exist sympatrically with other leaf-cutter species. For example, the Texas leaf-cutter ant *At. texana* is the only leaf-cutter species within its range, except for a contact zone with *Atta mexicana* just south of the USA–Mexico border [19,42]. Likewise, the desert leaf-cutter *Ac. versicolor* overlaps with *At. mexicana* south of the USA–Mexico border, but *Ac. versicolor* is the only leaf-cutter ant in its northern range in the USA. Ant–fungus coevolution in these northernmost leaf-cutter populations, therefore, is expected to be tighter (i.e. involving a single ant species and its *Attamyces* cultivars) compared with the multi-species, diffuse coevolution resulting from sharing of *Attamyces* strains between sympatric leaf-cutter ant species in the tropics. The expectation of possibly tighter ant–fungus coevolution in the northernmost leaf-cutter populations stimulated our investigations into the population genetics of *Attamyces* at the northern range limit of the leaf-cutter ant distribution.

2. MATERIAL AND METHODS

Attamyces fungi were collected from leaf-cutter gardens excavated from nests throughout the US ranges of the northernmost leaf-cutter ants *At. texana* ($n = 165$ *Attamyces* accessions from an equal number of nests) and *Ac. versicolor* ($n = 35$ *Attamyces*). To place these *Attamyces* collections in a larger population-genetic context of North American *Attamyces*, we also collected garden material from five *Atta insularis* nests from Cuba; from seven *At. mexicana* nests from northeastern and southern Mexico; and from eight *At. cephalotes* nests from southeastern Mexico. The electronic supplementary material, table S1 lists information collected for all 220 *Attamyces* accessions collected from the five leaf-cutter ant species from the USA, Mexico and Cuba.

Attamyces accessions were genotyped with a panel of 12 polymorphic microsatellite loci [29]. Population-genetic patterns were analysed in STRUCTURE v. 2.2 [43,44] by clustering individuals into populations on the basis of the multi-locus genotype information. Because *Attamyces* fungi are multinucleate and exhibit genotype profiles of unknown ploidy [29], we did not calculate standard population-genetic parameters (e.g. heterozygosities; *F*-statistics, etc.), but treated all alleles as dominant markers for genotype clustering and inference of population-genetic structure, as recommended for polyploid organisms by Falush *et al.* [44]. STRUCTURE requires that individuals differ by at least one marker, and we therefore included in our population-genetic analyses only one representative per microsatellite genotype. Because

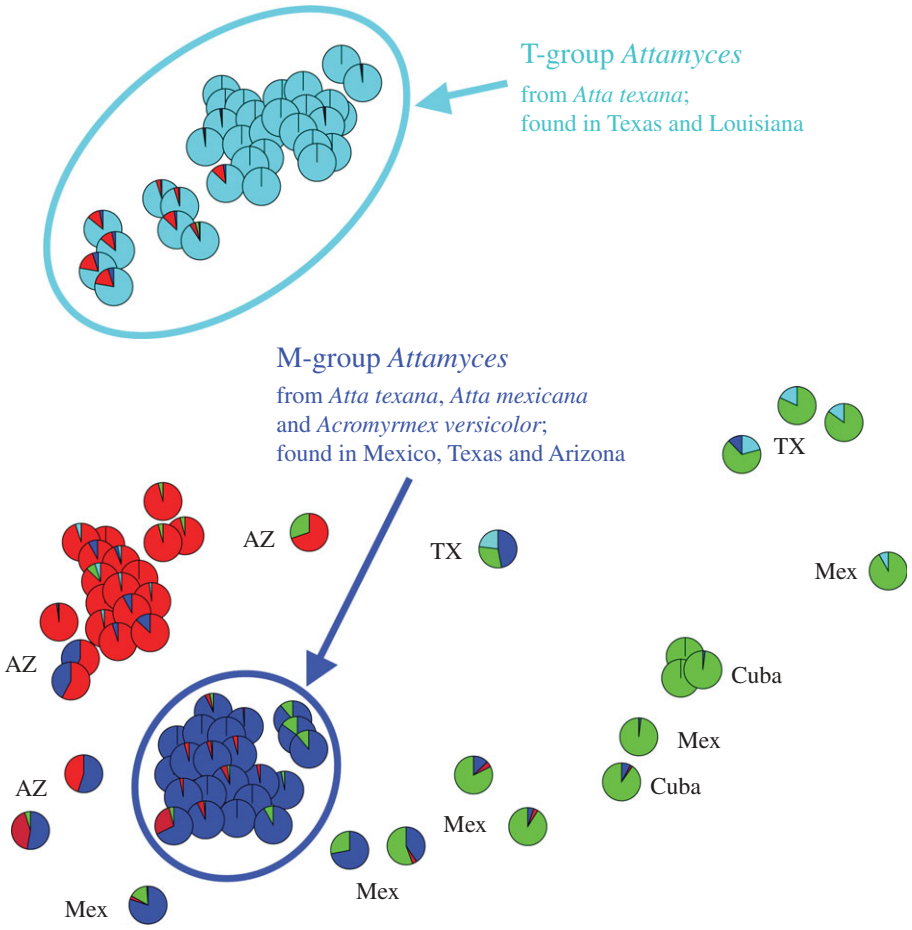

Figure 1. Multi-dimensional-scaling of genetic diversity of *Attamyces* fungi cultivated by five leaf-cutter ant species in North America. STRUCTURE analyses identify four populations of *Attamyces*, shown in different colours. Light blue: *Attamyces* from *At. texana* (T-group *Attamyces*). Dark blue: *Attamyces* from *Ac. versicolor*, *At. texana* and *At. mexicana* in, respectively, Arizona, Texas and Mexico (M-group *Attamyces*). Red: *Attamyces* from *Ac. versicolor* in Arizona and California. Green: diverse *Attamyces* from *At. mexicana* and *At. cephalotes* in Mexico, from *At. insularis* in Cuba and from three unusual collections from *At. texana* (far right); 97.6% of the *Attamyces* from *At. texana* belong to two populations; one population (dark blue) also includes members found in Mexico (hence M-group). The other *Attamyces* population from *At. texana* (light blue) is clearly distinct from the three other populations and is known so far only from Louisiana and Texas (hence T-group). Collection locations: AZ = Arizona, Mex = Mexico, TX = Texas. For most of the genotypes shown, genetically identical duplicates were collected in different leaf-cutter nests; only one representative is shown for each distinct *Attamyces* genotype. The total sample size included 220 *Attamyces* collections, which were grouped by 91 informative microsatellite markers into the 93 unique genotypes shown (listed also in the electronic supplementary material, table S1).

Attamyces is clonally propagated within and between leaf-cutter nests [36], 55.7 per cent of the 220 *Attamyces* accessions possessed a genotype profile that was identical to the profile of at least one other accession (see the electronic supplementary material, table S1). After eliminating duplicates to retain only one representative per genotype, the final dataset analysed in STRUCTURE included 93 unique genotypes (each profiled for 91 variable microsatellite markers). A Markov chain Monte Carlo algorithm implemented in STRUCTURE identified genetically differentiated populations and inferred contributions of ancestry for each allele (likelihood that an allele derived from one of the inferred populations). Admixture (mixed ancestry) was inferred for a particular fungal accession if different alleles of this genotype were assigned with high likelihood to different populations. Such admixture may result from (i) recombination of alleles (as in sexual organisms); or (ii) exchange of nuclei between differentiated *Attamyces* strains, generating re-assortment and novel combinations of nuclei that coexist in the multi-nucleate mycelium of *Attamyces* (see the electronic supplementary material *Admixture* for a detailed description of the diverse mechanism of genetic exchange in multi-nucleate fungi). Separately for the groups of *Attamyces* genotypes from *At. texana* ($n = 165$) and *Ac. versicolor* ($n = 35$), we also estimated the probability of sexual recombination within each group by applying the methods of Bengtsson [45] and De Fine Licht *et al.* [46] in a Bayesian framework implemented in WINBUGS [47]. To visualize the genetic diversity and to validate results obtained from STRUCTURE, we calculated a two-dimensional non-metric multi-dimensional scaling solution of the binary distances between cultivar genotypes (figure 1), which was computed in the programs R and GENALEX (www.anu.edu.au/BoZo/GenAlEx/) [48]. Collection, genotyping and statistical methods are explained in detail in the electronic supplementary material.

3. RESULTS

(a) *Specificity and sharing of* Attamyces *symbionts at the northern range limit*

STRUCTURE analyses inferred four populations of *Attamyces* symbionts cultivated at the northern range limit of

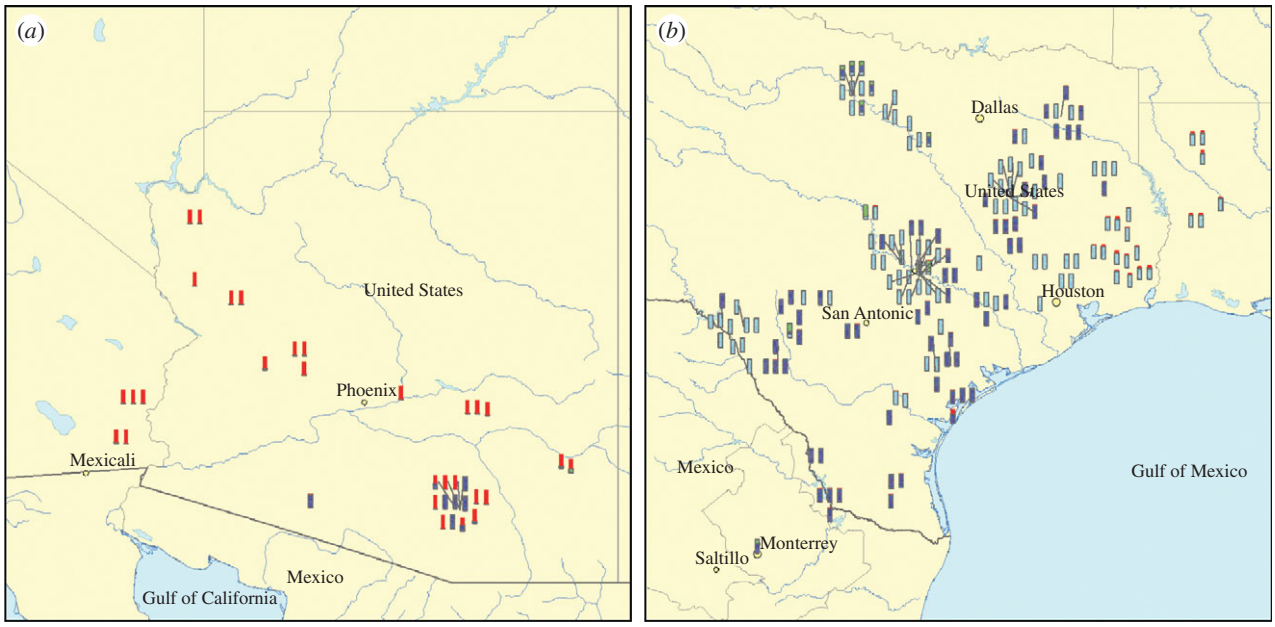

Figure 2. Biogeographic distributions of *Attamyces* groupings inferred in STRUCTURE. (*a*) *Attamyces* from Arizona and California were collected from gardens of the desert leaf-cutter ant, *Ac. versicolor*. (*b*) *Attamyces* from Texas and Louisiana were collected from gardens of the Texas leaf-cutter ant, *At. texana*; the collection from Monterrey, Mexico, was obtained from *At. mexicana*. All other Mexican and Cuban *Attamyces* analysed in figure 1 were collected outside the boundaries of the two maps. Maps in (*a*) and (*b*) are drawn to different scales. Each vertical bar presents the proportional contribution to a particular genotype of markers inferred by STRUCTURE to belong to one of four *Attamyces* populations (light blue, dark blue, red, green). Light blue: *Attamyces* from *At. texana* (T-group *Attamyces*). Dark blue: *Attamyces* from *Ac. versicolor*, *At. texana* and *At. mexicana* in, respectively, Arizona, Texas and Mexico (M-group *Attamyces*). Red: *Attamyces* from *Ac. versicolor* in Arizona and California. Green: rare *Attamyces* from *At. texana* with close affinity to *Attamyces* from *At. insularis* in Cuba and to some *Attomyces* from *At. mexicana* and *At. cephaloes* in Mexico. Ninety four per cent of the *Attamyces* from *At. texana* belong to two populations; one population (dark blue) also includes members found in Mexico (hence M-group *Attamyces*). The other *Attamyces* population from *At. texana* (light blue) is known so far only from Louisiana and Texas (hence T-group *Attamyces*). Only M-group *Attamyces* (dark blue) are currently known from south Texas; only T-group *Attamyces* (light blue) are currently known from east Texas and Louisiana. Admixed genotypes are shown as genotypes that combine significant portions of genetic markers assigned to several *Attamyces* populations (see also the electronic supplementary material, table S1). Because T-group *Attamyces* appear to be distinct types within the greater diversity of North American leaf-cutter fungi (figure 1), and because of the exclusive association of T-group *Attamyces* only with *At. texana*, T-group *Attamyces* may be more tightly coevolved with *At. texana* than M-group *Attamyces* (the latter *Attamyces* are shared also with the leaf-cutter species *At. mexicana*, *At. cephalotes* and *Ac. versicolor*, most likely because M-group *Attamyces* are transferred occasionally in Mexico between sympatric nests of these leaf-cutter species).

the leaf-cutter ant distribution (figure 1). These solutions were convergent between three repeat runs. Modelling more than four populations ($K > 4$) did not significantly improve likelihood scores (see the electronic supplementary material, figure S2). Two of the four inferred populations represent two dominant cultivar types of *At. texana* ('T-group' and 'M-group' *Attamyces*); one of these two *Attamyces* populations from *At. texana* (dark blue, figure 1) also includes *Attamyces* cultivated by other leaf-cutter species in Mexico (hence M-group *Attamyces*) and by *Ac. versicolor* in Arizona. The second *Attamyces* population from *At. texana* (light blue, figure 1) is clearly distinct from the three other populations and was found only in Louisiana and Texas (hence T-group *Attamyces*), but not in Mexico or the western USA. A third population was found only in Arizona and California (cultivated there by *Ac. versicolor*, therefore, called V-group *Attamyces*; red, figure 1). A fourth population includes a more diverse assemblage of *Attamyces* cultivated by *At. insularis* in Cuba (hence C-group *Attamyces*; green, figure 1), by *At. mexicana* and *At. cephalotes* in Mexico and by atypical and rare nests of *At. texana* in Texas (these two rare *Attamyces* genotypes are shown in figure 1 far right centre, and they are listed as genotypes nos. 74 and 75 in the electronic supplementary material, table S1).

Among the 220 collections, therefore, two *Attamyces* populations appear ant–species-specific: V-group cultivars from *Ac. versicolor* and T-group cultivars from *At. texana*. *Attamyces* belonging to the remaining two cultivar populations are shared between several leaf-cutter ant species across North America, paralleling the *Attamyces* sharing observed between sympatric leaf-cutter species in Panama [38]. Additional collections of *Attamyces* from *At. mexicana* in northern Mexico may reveal that V-group and T-group *Attamyces* are less ant–species specific than suggested by the present analysis. This is more likely for the V-group *Attamyces* of *Ac. versicolor* that occur in close proximity to known populations of *At. mexicana* (figure 2*a*), but less likely for T-group *Attamyces* that have so far not been found in south Texas and thus not near the range of *At. mexicana* south of the USA–Mexico border (figure 2*b*).

(b) *Biogeography of* Attamyces *symbionts cultivated by* At. texana

The two dominant *Attamyces* symbiont populations cultivated by *At. texana* together comprise 97.6 per cent of the known *Attamyces* diversity associated with *At. texana* (56.4% T-group *Attamyces* accessions, $n = 93$ of 165 accessions total; 41.2% M-group *Attamyces* accessions,

$n = 68$; see the electronic supplementary material, table S1). The remaining 3.4 per cent of the *Attamyces* accessions ($n = 4$) from *At. texana* includes: (i) one accession (0.6%) that was significantly admixed under the $K = 4$ model (more than 30% of an individual's markers assigned to at least two populations; this single accession is listed as genotype no. 73 in the electronic supplementary material, table S1 and is shown in the centre of figure 1); and (ii) three accessions (1.8%) assigned under the $K = 4$ model to the fourth population of *Attamyces* that is also associated with several *Atta* species in Mexico and Cuba (the two genotypes of these three unusual accessions are listed as genotypes nos 74 and 75 in the electronic supplementary material, table S1 and are shown at the far right of figure 1). Assignment of accessions to either T-group or M-group *Attamyces* does not change when modelling a range of $K = 4$ to $K = 12$ populations (see the electronic supplementary material), and T-group and M-group *Attamyces* populations therefore represent differentiated symbiont types (figure 1) that together dominate the symbiont pool of the leaf-cutter host *At. texana*.

The populations of T-group and M-group *Attamyces* largely overlap across the range of the host *At. texana*, but with two interesting biogeographic differences. T-group *Attamyces* have so far not been collected in southern Texas (figure 2*b* and electronic supplementary material, table S1; all 11 accessions from *At. texana* south of latitude 28.06° N were M-group *Attamyces*). Second, M-group *Attamyces* have so far not been collected in Louisiana and east Texas (figure 2*b* and electronic supplementary material, table S1; all 19 *Attamyces* accessions collected from far east Texas and Louisiana were T-group *Attamyces*). Although our *Attamyces* collections are somewhat limited in the southern range ($n = 11$ *Attamyces* accessions) and the eastern range ($n = 19$ accessions) of *At. texana*, it appears that T-group *Attamyces* are restricted to more northern latitudes and are prevalent in the eastern range of *At. texana* for unknown ecological or historical reasons (e.g. T-group *Attamyces* are the only types that so far expanded with their host into east Texas and Louisiana).

(c) *Biogeography of* Attamyces *symbionts cultivated by* Ac. versicolor

Within *Ac. versicolor* (35 collections total), most *Attamyces* can be assigned to one of two populations; 69.6 per cent of these ($n = 24$) belonged to the V-group *Attamyces* and 11.1 per cent ($n = 6$) to the M-group *Attamyces* population (which are *Attamyces* lineages shared with the leaf-cutter hosts *At. mexicana* and *At. texana*; figure 1 and electronic supplementary material, table S1). Three *Attamyces* accessions from *Ac. versicolor* were inferred to be significantly admixed (defining admixture, as above, as more than 30% of the markers assigned to at least two *Attamyces* populations). M-group *Attamyces* have been found only in southeast Arizona, and *Ac. versicolor* appears to cultivate only V-group *Attamyces* throughout the rest of its US range (figure 2*a* and electronic supplementary material, table S1). Because of the fewer *Attamyces* collections from *Ac. versicolor* ($n = 35$) compared with *At. texana* ($n = 165$), and because *Ac. versicolor* ranges into Mexico where it is sympatric with *At. mexicana*, the apparent specificity of V-group *Attamyces* on *Ac. versicolor* is provisional and will need to be tested through further collection of *Attamyces* in northwest Mexico.

(d) *Biogeography of* Attamyces *symbionts cultivated by* At. insularis *in Cuba and by* At. mexicana *in Mexico*

Despite the marine dispersal barrier between mainland North America and Cuba, and despite the significant evolutionary divergence between the ant *At. mexicana* and the sister-species pair *At. insularis* and *At. texana* [49], we find no evidence for significant genetic differentiation between *Attamyces* from *At. insularis* and some *Attamyces* from *At. mexicana* (i.e. STRUCTURE assigned Cuban and some mainland *Attamyces* to the same population (C-group); figure 1 and electronic supplementary material, table S1).

(e) *Geographical structure of* Attamyces *clones*

Among the 165 accessions from *At. texana*, 12.7 per cent ($n = 21$) represent unique genotypes (collected in only a single *At. texana* nest), and the remaining 87.3 per cent accessions ($n = 144$) belong to genotypes that were collected in at least two different ant nests. Some of these *Attamyces* cultivars with genetically identical marker profiles were collected from nests at surprisingly distant locations. In *At. texana*, the average distance and the average maximum-distance between genotypically identical *Attamyces* accessions measured, respectively, 68.0 km (± 10.64 s.e.) and 125.0 km (± 11.36 s.e.; see the electronic supplementary material, table S1). In the most extreme case, two genetically identical *Attamyces* genotypes were collected from *At. texana* nests 490 km apart (southern to central Texas). The most wide-ranging M-group *Attamyces* genotype ranged over an area of at least 80 948 km^2 (across south and central Texas), and the most wide-ranging T-group *Attamyces* ranged over an area of at least 22 188 km^2 (across central and east Texas; see the electronic supplementary material, table S1). On average, M-group genotypes ranged over significantly larger areas (average = 23 973 km^2, s.e. = 12 121, $n = 7$) compared with T-group genotypes (average = 2636 km^2, s.e. = 1707, $n = 13$; $t = 2.372$, d.f. = 18, two-tailed $p = 0.029$; see the electronic supplementary material, table S1). Despite the enormous ranges of some *Attamyces* genotypes, the geographical clustering of *Attamyces* by genotype (see the electronic supplementary material, table S1) indicates that gene flow of *Attamyces* is viscous. This population viscosity across the range of the host *At. texana* is consistent with the observation that female *At. texana* disperse less than 20 km per mating flight [50]. Overall, the genotype distributions across space confirm the expectation [35,36] that *Attamyces* cultivars of *At. texana* are primarily clonally propagated by dispersing females over many ant generations (long enough for *Attamyces* to spread as clones across areas of thousands of square kilometres).

Among the 35 accessions from *Ac. versicolor*, 57.1 per cent ($n = 20$) represented unique genotypes. The average distance and average maximum-distance between *Attamyces* accessions belonging to the same genotype measured, respectively, 8.9 km (± 7.13 s.e.) and 17.49 km (± 15.58 s.e.; see the electronic supplementary material, table S1). In the most extreme case, two genetically identical

Attamyces genotypes were collected from *Ac. versicolor* nests 57.1 km apart (in southeast California). The *Attamyces* genotype with the largest and second-largest geographical distribution ranged, respectively, over only 2.61 km^2 (in central Arizona) and 0.56 km^2 (in southeast California; see the electronic supplementary material, table S1). As in *Attamyces* from *At. texana*, these patterns are likewise consistent with clonal propagation of *Attamyces* between *Ac. versicolor* nests; however, the comparatively smaller average range over which genotypically identical *Attamyces* were found in *Ac. versicolor* could indicate that, compared with clonal propagation in *At. texana*, strict clonal propagation of *Attamyces* occurs over fewer ant generations in *Ac. versicolor*. For example, clonal propagation in *Ac. versicolor* may be more frequently punctuated by genetic changes because the polygynous nest-founding of *Ac. versicolor* [16] creates frequent opportunities for co-cultivation of genetically differentiated *Attamyces* strains in incipient gardens (i.e. frequent opportunities for genetic recombination). However, because only 35 *Attamyces* were collected from *Ac. versicolor* (compared with 165 *Attamyces* from *At. texana*), *Attamyces* from *Ac. versicolor* may have been collected at insufficient geographical density relative to the dispersal distances of queens. A larger number of *Ac. versicolor* nests therefore will need to be collected across Arizona and California to rule out sampling biases.

(f) *Admixture*

Under all of the models examined in STRUCTURE, a significant fraction of *Attamyces* accessions was inferred to be admixed (i.e. these accessions combined markers assigned by STRUCTURE to at least two different *Attamyces* populations; see the electronic supplementary material, table S1). In the $K = 4$ model and under a relatively stringent definition of admixture (at least 30% of a genotype's markers were assigned to different populations), admixed *Attamyces* genotypes were found at lower frequency in the host *At. texana* (6.1%, $n = 3$ admixed genotypes of 49 total) compared with the host *Ac. versicolor* (11.5%, $n = 3$ admixed genotypes of 26 total) and compared with the tropical leaf-cutter hosts in Mexico and Cuba (average of 22.2%, $n = 4$ admixed genotypes of 18 total; see the electronic supplementary material, table S1). These proportions are not significantly different (all χ^2 comparisons $p > 0.1$). Under a less stringent definition of admixture (at least 10% of a genotype's markers were assigned to different populations), admixed *Attamyces* occurred at comparable levels in *At. texana* (24.5%, $n =$ 12 admixed genotypes) and *Ac. versicolor* (26.9%, $n = 7$ admixed genotypes), but more frequently in the tropical leaf-cutter hosts (average of 66.7%, $n = 12$ admixed genotypes; see the electronic supplementary material, table S1); this proportion of admixed genotypes among the tropical leaf-cutter hosts is significantly higher (χ^2_1 4.749, $p =$ 0.0293) compared with the two northernmost leaf-cutter hosts. A larger collection of *Attamyces* from tropical leaf-cutter ants will need to be genotyped to corroborate this pattern and rule out sampling artefacts as an explanation for the observed difference in admixture between temperate and tropical *Attamyces*. For the *Attamyces* from *At. texana* ($n = 165$) and *Ac. versicolor* ($n = 35$), fitting equation 8 of Bengtsson [45] within a Bayesian framework to the observed genotype distributions implicated very low frequencies of sexual reproduction per generation (0.3% with a 95% highest posterior density confidence interval (HPD-CI) of 0.01–1% for *At. texana*; 0.6% with a 95% HPD-CI of 0.02–3% for *Ac. versicolor*).

4. DISCUSSION

Population-genetic patterns of *Attamyces* cultivated near the northern range limit reveal some unique features of the ant–fungus mutualism that have so far not been observed for *Attamyces* in tropical habitats [37,38,41], but also some parallels between northern and tropical *Attamyces*.

(a) *Parallels between northern and tropical* Attamyces *populations*

Attamyces is largely clonally propagated between parent and offspring nests in both northern and tropical habitats. Clonality is implicated in our study most strongly by the high frequency (87.3%) of *Attamyces* accessions that were collected as genetically identical genotypes in at least two different *At. texana* nests. The most abundant *Attamyces* genotypes were collected across vast ranges (15 000–80 000 km^2, see the electronic supplementary material, table S1). Because our population-genetic markers mutate at rates typical for microsatellite loci [36], the large ranges of some *Attamyces* clones indicate that the spread of *Attamyces* clones across hundreds of kilometres can occur rapidly relative to the rates of endogenous (mutational) change. A second parallel between northern and tropical *Attamyces* populations is that *Attamyces* lineages are shared between leaf-cutter ant species, exemplified most prominently by the sharing of M-group *Attamyces* between four leaf-cutter species in North America. A third parallel is that *Attamyces* lineages occasionally recombine (implicated by the observation of admixed *Attamyces* genotypes); *Attamyces* propagation is, therefore, not strictly clonal across an endless series of host generations as proposed by initial phylogenetic studies of *Attamyces* [51]. These three basic features of *Attamyces* biology had already emerged in previous population-genetic and phylogenetic analyses of much smaller *Attamyces* datasets from Central and South America ([37,38,41]; see also discussions in [31,40]).

(b) *Differences between northern and tropical* Attamyces *populations*

Several features emerged in the northernmost *Attamyces* populations that have so far not been documented for Central and South American populations: (i) cultivation of specific *Attamyces* lineages by only a single ant host (i.e. ant–fungus specializations that tighten ant–fungus coevolution); (ii) regional or ant-specific differences in admixture between *Attamyces* lineages; and (iii) dispersal by *Attamyces* over significant distances and across ocean barriers, indicated most prominently by the surprisingly close genotypic proximity between *Attamyces* representatives from Cuba and Mexico (figure 1). These three differences are discussed in detail in the following sections.

(i) *Host–symbiont specializations tighten coevolution at the northern frontier*

As expected for peripheral populations, genotypically differentiated cultivar lineages were indeed found in the

northernmost leaf-cutter populations, giving rise to specialized host–symbiont combinations. The evidence for host–symbiont specialization is strongest for T-group *Attamyces*, which emerged as the most distinct symbiont type in the present study (figure 1). This implies that, whereas ant–*Attamyces* coevolution is rather diffuse in the tropics (several leaf-cutter species interact with several *Attamyces* lineages in a complex interaction network [38,40,41]), coevolution is tighter for some ant–fungus combinations at the northern range limit. The most prominent cases of coevolutionary tightening were found, first, in Louisiana where *At. texana* associates with only one narrow subgroup of T-group *Attamyces* (figure 2*b*), and second, in California and northern Arizona where *Ac. versicolor* associates with only a narrow subset of V-group *Attamyces* (figure 2*a*). Moreover, among the *Attamyces* cultivated by *At. texana*, T-group *Attamyces* appear to be more tightly coevolving with the host *At. texana* (because of the exclusive association of this symbiont type only with *At. texana*) compared with M-group *Attamyces* (which have close genetic links to *Attamyces* cultivated by other leaf-cutter species (figure 1). A trendin declining genetic diversity of the cultivated fungi towards the northern range limit and a corresponding tightening of coevolutionary interactions had also been observed in the northernmost fungus-growing ant *Trachymyrmex septentrionalis* (a non-leaf-cutter ant), which spread after the Pleistocene northwards through the eastern and central USA [52].

(ii) *Admixture rates appear lower in temperate than in tropical* Attamyces

Given the biology of United States leaf-cutter ants and their *Attamyces* fungi, it is surprising that our survey revealed few significantly admixed *Attamyces* accessions (three in *At. texana* and three in *Ac. versicolor*; see the electronic supplementary material, table S1) and that Bayesian modelling implicated very low frequencies of sexual reproduction per *Attamyces* generation (0.3% for *At. texana* and 0.6% for *Ac. versicolor*). Higher frequencies could be expected because of (i) the known capacity of *Attamyces* for recombination ([37] and this study; see also the electronic supplementary material *Admixture* for a detailed description of the diverse mechanism of genetic exchange in multi-nucleate fungi); (ii) the extensive overlap between T-group and M-group *Attamyces* across the range of *At. texana* (figure 2*b*) and the overlap of V-group and M-group *Attamyces* in southeast Arizona (figure 2*a*); (iii) the frequent proximity of neighbouring nests with *Attamyces* from distinct *Attamyces*-groups (frequently within 100 m of each other; see the electronic supplementary material, table S1); and (iv) polygynous nest-founding and thus possible cultivar mixing in both ant species (5% polygynous nest-founding in *At. texana* [53]; between 0 and 100% in *Ac. versicolor*, depending on the population [16]).

The low levels of observed recombinants between differentiated *Attamyces* groups in the northern range may have several, non-exclusive explanations. First, when *Attamyces* strains are co-propagated by the ants in a common garden or when a novel strain enters the monoculture of an established nest, recombination between differentiated *Attamyces* strains may be prevented by mycelium–mycelium antagonism (i.e. somatic incompatibility *sensu* [54], preventing anastomosis and migration of nuclei between the two mycelia). Second, *Attamyces* types may be maintained as genotypically differentiated, sympatric lineages because of nucleus–nucleus incompatibilities if nuclei are ever exchanged between two *Attamyces* strains (i.e. the two nuclei do not function together when residing in the same cell, leading to cell death; or nuclei may compete with each other, leading to elimination of competitively inferior nuclei). Third, *Attamyces* recombinants may exhibit hybrid inferiority relative to the unrecombined parental types (i.e. nests with hybrid *Attamyces* therefore may have reduced survivorship, reduced growth rates or reduced fecundity). These three explanations are not mutually exclusive, and they can be tested in laboratory experiments with experimental ant–fungus combinations or with artificially created chimaeric gardens [28].

The possible lower level of recombination (admixture) of *Attamyces* from *At. texana* and *Ac. versicolor* compared with *Attamyces* of tropical leafcutter hosts is intriguing and hopefully will stimulate follow-up work to test for this difference with a larger sample of tropical *Attamyces*. Both *Ac. versicolor* and *At. texana* can found their nests polygynously [16,50,53], whereas *At. mexicana* and *At. cephalotes* are strictly monogynous ([42,55]; U. G. Mueller 1991–2010, personal observations). If co-cultivation of *Attamyces* strains during polygynous nest-founding facilitates genetic exchanges, one would expect that the *Attamyces* of *Ac. versicolor* and *At. texana* actually show higher levels of admixture, whereas we found the opposite pattern. This implicates processes other than co-cultivation as primary factors facilitating genetic exchanges between *Attamyces* lineages.

(iii) Attamyces *fungi can disperse independently of the ant hosts across marine barriers*

Although the link between mainland and Cuban *Attamyces* had already emerged in the phylogenetic analysis of Mikheyev *et al.* [37], our analysis documents the population-genetic proximity between mainland and Cuban *Attamyces* populations with greater resolution and for a more comprehensive sample (220 *Attamyces* strains from North America). Cuba, as part of the proto-Greater Antilles, moved through the gap between North and South America at the end of the Cretaceous (over 60 Myr ago) and may have temporarily abutted what is now Southern Mexico [17,56]. Since the early Tertiary, however, Cuba is thought to have remained separate from the mainland and without temporary land-bridges during changes in sea levels [17,56]. In the absence of gene flow across the ocean between mainland and Cuba, the *Attamyces* populations in Cuba, therefore, should have become genetically differentiated from the mainland populations, a prediction that is not supported by the observed population-genetic patterns (figure 1). Because the host *At. insularis* occurs only in Cuba but not on mainland North America, and because *At. insularis* is significantly derived at both the molecular and morphological levels from the mainland *Atta* lineages ([49], suggesting significant evolutionary time to permit divergence of *At. insularis*), it seems unlikely that the current genetic similarity between mainland and Cuban *Attamyces* was maintained during the divergence process of *At. insularis* without gene flow in *Attamyces*.

Consequently, recent or ongoing gene flow in *Attamyces* between the mainland and Cuba is a more likely explanation for the absence of genetic differentiation between mainland and Cuban *Attamyces*. Such gene flow could occur via three possible avenues.

(i) Leaf-cutter queens occasionally disperse across the ocean barrier from the mainland to Cuba (e.g. across the 250 km wide Yucatan Channel between the Yucatan Peninsula in Mexico and the Guanahacabibes Peninsula in Cuba); these queens vector *Attamyces* to Cuba, establish nests long enough to permit *Attamyces* to be transferred to *At. insularis*, then these temporary colonizers become extinct. Although other poorly dispersing ant species managed to colonize many islands throughout the Caribbean [57], we consider this scenario unlikely, primarily because of the rarity of observed leafcutter dispersal across Caribbean islands [58].

(ii) Similar to (i) but assuming human-mediated dispersal, *Attamyces* fungi were recently introduced to Cuba with leaf-cutter ants brought accidentally by humans from the mainland (e.g. in soil of potted plants); once in Cuba, introduced *Attamyces* replaced pre-existing cultivars in *At. insularis* nests. This scenario would receive support if the only other leaf-cutter ant species in Cuba (*Acromyrmex octospinosus*) can be shown to have colonized Cuba recently, which is not supported by available genetic information (i.e. the Cuban *At. octospinosus* populations appear genetically differentiated from mainland populations [58]).

(iii) *Attamyces* is able to disperse independently of the leaf-cutter hosts, either by airborne spore dispersal (spore-bearing mushrooms of *Attamyces* have been observed on rare occasions; table 3 in Mueller [26] summarizes the relevant literature), or by dispersal with the help of unknown vectors (e.g. by one of the many arthropods frequenting leaf-cutter nests, such as mites, beetles or moths; [10,15,59]).

Irrespective of these unresolved dispersal mechanisms, the close population-genetic links between mainland and Cuban *Attamyces* support the view that tropical *Attamyces* cultivars may be able to survive independently of leaf-cutter ants at least for some time (e.g. as spores, or when dispersed by vectors other than ants). Consequently, *Attamyces* fungi may be more than enslaved domesticates tied inescapably to the fate of their particular leaf-cutter host [26,37,38,40]. If so, long-distance dispersal and genetic mixing of *Attamyces*, including oceanic dispersal independent of leaf-cutter ants, may occur throughout the range of *Attamyces*. Such long-distance dispersal ability of *Attamyces* would contrast with the dispersal ability of *Termitomyces* fungi of the termite–*Termitomyces* symbiosis, in which effective long-distance dispersal of *Termitomyces* across oceanic barriers appears to be rare (e.g. between mainland Africa and Madagascar [60]). However, long-distance dispersal and genetic mixing of *Attamyces* could help explain the much younger coalescence and origin of *Attamyces* fungi (about 2–4 Myr old) compared with the corresponding origin of leaf-cutter ants (about 8–12 Myr old) [40].

5. CONCLUSION

Peripheral populations of the leaf-cutter ant–*Attamyces* symbiosis emerge as particularly interesting test cases of coevolution between ant farmers and their cultivated fungi, for two reasons. First, the documented local reduction of ant–species diversity and *Attamyces* diversity at the northern range limit increases local ant–*Attamyces* specificity; increased specificity, in turn, should lead to tighter, local coevolutionary interactions. Second, because novel *Attamyces* variants can sweep through the range of leaf-cutter ants [40], peripheral populations are least likely to be reached by novel *Attamyces* variants. These demographic processes should affect peripheral and island populations alike; however, because of dispersal across oceanic barriers, marine island populations are no longer the most promising candidates for finding unusual *Attamyces* variants. Specifically, if the Cuban populations of *Attamyces* analysed here are representative, we predict that Caribbean island populations of *Attamyces* known from Trinidad [61], Guadeloupe [58] and Curacao [62] will likewise retain strong population-genetic links to continental populations. Instead, populations at the periphery of the leaf-cutter distribution (in the USA, in Argentina, perhaps at mid-elevation sites in the Andes) are most likely to reveal unique and ant–species-specific leaf-cutter ant–*Attamyces* associations. These peripheral populations therefore contribute the most distinct geographical variants in coevolutionary interactions between leaf-cutter ants and their cultivated fungi. Studies of such marginal populations will inform models of coextinctions of obligate symbiotic partners that are doubly stressed by habitat marginality and by environmental change [8,9].

We thank numerous landowners for permission to collect on their properties; Kevin Anderson and John Crutchfield for permission to work at the Hornsby Bend Environmental Research Center and at the Brackenridge Field Laboratory; Kevin Anderson, Don Bass, Family Blackstock, Jason Byrd, Joy Camp, Craig Clark, Rebecca Clark, James Cokendolpher, Kenneth Conaway, Jerry Cook, Stefen Cover, Shawn Dash, Lloyd Davis, Mary Dixson, Diane Dudney, Evan Economo, Tim Fennell, Brush Freeman, Patricia Garlough, Nicole Gerado, Larry Gilbert, Paula Gilleland, Dale Groom, Don Grosman, David Hall, Ray Hampton, Ken Helms, Anna Himler, Rick Knipe, Jean-Nick Jasmin, Bob Johnson, Donald Johnson, Carol Jones, Charles Jones, Joey Jordan, Steve Lanoux, David Long, Malcolm MaCallum, Callen McWilliams, Marcy Marsden, Jack Martin, Mike Merchant, Alex Mintzer, John Moser, Scott Myres, Christian Rabeling, Ed Riley, Justin Rhodes, Andre Rodrigues, Jerod Romine, Ron Schmidt, Jarrod Scott, Carrol Simanek, Gordon Snelling, Roy Snelling, Jeff Sparks, Tracy Villareal, Roy Vogtsberger, Gerald Walls, Debbie Walker, Gordon Walton, Phil Ward, Stuart West, Alex Wild and Royce Wright for collection information; Damian Broglie and Christian Rabeling for drawing electronic supplementary material, figure S1; and Jon Seal, Duur Aanen and two anonymous reviewers for comments on the manuscript. The work was supported by the National Science Foundation (DEB-0920138, DEB-0639879 and DEB-0110073 to U.G.M.; DEB-0949689 to T. R. Schultz, N. J. Mehdiabadi and U.G.M.), the EEB Fellowship Endowment (to A.S.M. and S.E.S.) and the W. M. Wheeler Endowment from The University of Texas at Austin.

REFERENCES

1 Brown, J. H., Stevens, G. C. & Kaufman, D. M. 1996 The geographic range: size, shape, boundaries, and

internal structure. *Annu. Rev. Ecol. Syst.* **27**, 597–623. (doi:10.1146/annurev.ecolsys.27.1.597)
2 Bridle, J. R. & Vines, T. H. 2006 Limits to evolution at range margins: when and why does adaptation fail? *Trends Ecol. Evol.* **22**, 140–147. (doi:10.1016/j.tree.2006.11.002)
3 Kawecki, T. J. 2008 Adaptations to marginal habitats. *Annu. Rev. Ecol. Evol. Syst.* **39**, 321–342. (doi:10.1146/annurev.ecolsys.38.091206.095622)
4 Holt, R. D. & Keitt, T. H. 2005 Species' borders: a unifying theme in ecology. *Oikos* **108**, 3–6. (doi:10.1111/j.0030-1299.2005.13145.x)
5 Sexton, J. P., McIntyre, P. J., Angert, A. L. & Rice, K. J. 2009 Evolution and ecology of species range limits. *Annu. Rev. Ecol. Evol. Syst.* **40**, 415–436. (doi:10.1146/annurev.ecolsys.110308.120317)
6 Vucetich, J. A. & Waite, T. A. 2003 Spatial patterns of demography and genetic processes across the species' range: null hypotheses for landscape conservation genetics. *Conserv. Genet.* **4**, 639–645. (doi:10.1023/A:1025671831349)
7 Eckert, C. G., Samis, K. E. & Lougheed, S. C. 2008 Genetic variation across species' geographical ranges: the central–marginal hypothesis and beyond. *Mol. Ecol.* **17**, 1170–1188. (doi:10.1111/j.1365-294X.2007.03659.x)
8 Dunn, R. R., Harris, N. C., Colwell, R. K., Koh, L. P. & Sodhi, N. S. 2009 The sixth mass coextinction: are most endangered species parasites and mutualists? *Proc. R. Soc. B* **276**, 3037–3045. (doi:10.1098/rspb.2009.0413)
9 Gilman, S. E., Urban, M. C., Tewksbury, J., Gilchrist, G. W. & Holt, R. D. 2010 A framework for community interactions under climate change. *Trends Ecol. Evol.* **25**, 325–331. (doi:10.1016/j.tree.2010.03.002)
10 Weber, N. A. 1972 *Gardening ants: the Attines*. Philadelphia, PA: American Philosophical Society.
11 Schultz, T. R. & Brady, S. G. 2008 Major evolutionary transitions in ant agriculture. *Proc. Natl Acad. Sci. USA* **105**, 5435–5440. (doi:10.1073/pnas.0711024105)
12 Mueller, U. G. & Rabeling, C. 2008 A breakthrough innovation in animal evolution. *Proc. Natl Acad. Sci. USA* **105**, 5287–5288. (doi:10.1073/pnas.0801464105)
13 Solomon, S. E., Bacci, M., Martins, J., Gonçalves Vinha, G. & Mueller, U. G. 2008 Paleodistributions and comparative molecular phylogeography of leafcutter ants (*Atta* spp.) provide new insight into the origins of Amazonian diversity. *PLoS ONE* **3**, e2738. (doi:10.1371/journal.pone.0002738)
14 Farji-Brener, A. G. & Ruggiero, A. 1994 Leaf-cutting ants (*Atta* and *Acromyrmex*) inhabiting Argentina: patterns in species richness and geographical range sizes. *J. Biogeogr.* **21**, 391–399. (doi:10.2307/2845757)
15 Moser, J. C. 2006 Complete excavation and mapping of a Texas leafcutting ant nest. *Ann. Entomol. Soc. Am.* **99**, 891–897. (doi:10.1603/0013-8746(2006)99[891:CEAMOA]2.0.CO;2)
16 Rissing, S. W., Johnson, R. A. & Martin, J. W. 2000 Colony founding behavior of some desert ants: geographic variation in metrosis. *Psyche* **103**, 95–101. (doi:10.1155/2000/20135)
17 Ricklefs, R. & Bermingham, E. 2008 The West Indies as a laboratory of biogeography and evolution. *Phil. Trans. R. Soc. B* **363**, 2393–2413. (doi:10.1098/rstb.2007.2068)
18 Buckley, S. B. 1860 The cutting ant of Texas. *Proc. Acad. Natl Sci. Phila.* **12**, 233–236.
19 Wheeler, W. M. 1907 The fungus-growing ants of North America. *Bull. Am. Mus. Nat. Hist.* **23**, 669–807.
20 Weber, N. A. 1959 Isothermal conditions in tropical soil. *Ecology* **40**, 153–154. (doi:10.2307/1929940)
21 Quinlan, R. J. & Cherrett, J. M. 1978 Aspects of the symbiosis of the leaf-cutting ant *Acromyrmex octospinosus* (Reich) and its food fungus. *Ecol. Entomol.* **3**, 221–230. (doi:10.1111/j.1365-2311.1978.tb00922.x)
22 Powell, R. J. & Stradling, D. J. 1985 Factors influencing the growth of *Attamyces bromatificus*, a symbiont of attine ants. *T. Br. Mycol. Soc.* **87**, 205–213. (doi:10.1016/S0007-1536(86)80022-5)
23 Bollazzi, M., Kronenbitter, J. & Roces, F. 2008 Soil temperature, digging behaviour, and the adaptive value of nest depth in South American species of *Acromyrmex* leaf-cutting ants. *Oecologia* **158**, 165–175. (doi:10.1007/s00442-008-1113-z)
24 Mueller, U. G. *et al.* In press. Evolution of cold-tolerant fungal symbionts permits winter fungiculture by leafcutter ants at the northern frontier of a tropical ant–fungus symbiosis. *Proc. Natl Acad. Sci. USA*. (doi:10.1073/pnas.1015806108)
25 Hölldobler, B. & Wilson, E. O. 2010 *The leafcutter ants: civilization by instinct*. New York, NY: Norton & Company.
26 Mueller, U. G. 2002 Ant versus fungus versus mutualism: ant cultivar conflict and the deconstruction of the attine ant–fungus symbiosis. *Am. Nat.* **160**, S67–S98. (doi:10.1086/342084)
27 Vellinga, E. 2004 Ecology and distribution of lepiotaceous fungi (Agaricaceae): a review. *Nova Hedwigia* **78**, 273–299. (doi:10.1127/0029-5035/2004/0078-0273)
28 Sen, R., Ishak, H. D., Kniffin, T. R. & Mueller, U. G. 2010 Construction of chimaeric gardens through fungal intercropping: a symbiont choice experiment in the leafcutter ant *Atta texana* (Attini, Formicidae). *Behav. Ecol. Sociobiol.* **64**, 1125–1133. (doi:10.1007/s00265-010-0928-x)
29 Scott, J. J., Kweskin, M., Cooper, M. & Mueller, U. G. 2009 Polymorphic microsatellite markers for the symbiotic fungi cultivated by leafcutter ants (Attini, Formicidae). *Mol. Ecol. Resour.* **9**, 1391–1394. (doi:10.1111/j.1755-0998.2009.02684.x)
30 Mohali, S. 1998 Ultrastructural study of the mutualistic fungus of the ant *Atta cephalotes*. *Rev. Ecol. Latinoamericana* **5**, 1–6.
31 Mueller, U. G., Rehner, S. A. & Schultz, T. D. 1998 The evolution of agriculture in ants. *Science* **281**, 2034–2038. (doi:10.1126/science.281.5385.2034)
32 Vo, T. L., Mueller, U. G. & Mikheyev, A. S. 2009 Free-living fungal symbionts (Lepiotaceae) of fungus-growing ants (Attini: Formicidae). *Mycologia* **101**, 206–210. (doi:10.3852/07-055)
33 Möller, A. 1893 *Die Pilzgärten einiger südamerikanischer Ameisen*. Jena, Germany: Verlag Gustav Fisher.
34 Pagnocca, F. C., Bacci, M., Fungaro, M. H., Bueno, O. C., Hebling, M. J., Sant'Anna, A. & Capelari, M. 2001 RAPD analysis of the sexual state and sterile mycelium of the fungus cultivated by the leaf-cutting ant *Acromyrmex hispidus fallax*. *Mycol. Res.* **105**, 173–176. (doi:10.1017/S0953756200003191)
35 Mueller, U. G., Gerardo, N. M., Aanen, D. K., Six, D. L. & Schultz, T. R. 2005 The evolution of agriculture in insects. *Annu. Rev. Ecol. Evol. Syst.* **36**, 563–595. (doi:10.1146/annurev.ecolsys.36.102003.152626)
36 Mueller, U. G., Scott, J. J., Ishak, H. D., Cooper, M. & Rodrigues, A. 2010 Monoculture of leafcutter ant gardens. *PLoS ONE* **5**, e12668. (doi:10.1371/journal.pone.0012668)
37 Mikheyev, A. S., Mueller, U. G. & Abbott, P. 2006 Cryptic sex and many-to-one co-evolution in the fungus-growing ant symbiosis. *Proc. Natl Acad. Sci. USA* **103**, 10 702–10 706. (doi:10.1073/pnas.0601441103)
38 Mikheyev, A. S., Mueller, U. G. & Boomsma, J. J. 2007 Population genetic signatures of diffuse co-evolution between leaf-cutting ants and their cultivar fungi. *Mol. Ecol.* **16**, 209–216. (doi:10.1111/j.1365-294X.2006.03134.x)

39 Mueller, U. G., Poulin, J. & Adams, R. M. M. 2004 Symbiont choice in a fungus-growing ant (Attini, Formicidae). *Behav. Ecol.* **15**, 357–364. (doi:10.1093/beheco/arh020)

40 Mikheyev, A. S., Mueller, U. G. & Abbott, P. 2010 Comparative dating of attine ant and lepiotaceous cultivar phylogenies reveals coevolutionary synchrony and discord. *Am. Nat.* **175**, E126–E133. (doi:10.1086/652472)

41 Silva-Pinhati, A. C. O., Bacci, M., Hinkle, G., Sogin, M. L., Pagnocca, F. C., Martins, V. G., Bueno, O. C. & Hebling, M. J. A. 2004 Low variation in ribosomal DNA and internal transcribed spacers of the symbiotic fungi of leaf-cutting ants (Attini: Formicidae). *Braz. J. Med. Biol. Res.* **37**, 1463–1472. (doi:10.1590/S0100-879X2004001000004)

42 Sanchez-Peña, S. R. 2005 Essays on organismal aspects of the fungus-growing ant symbiosis. PhD thesis, The University of Texas, Austin, USA.

43 Falush, D., Stephens, M. & Pritchard, J. K. 2003 Inference of population structure using multilocus genotype data: linked loci and correlated allele frequencies. *Genetics* **164**, 1567–1587.

44 Falush, D., Stephens, M. & Pritchard, J. K. 2007 Inference of population structure using multilocus genotype data: dominant markers and null alleles. *Mol. Ecol. Notes* **7**, 574–578. (doi:10.1111/j.1471-8286.2007.01758.x)

45 Bengtsson, B. O. 2003 Genetic variation in organisms with sexual and asexual reproduction. *J. Evol. Biol.* **16**, 189–199. (doi:10.1046/j.1420-9101.2003.00523.x)

46 De Fine Licht, H. H., Boomsma, J. J. & Aanen, D. K. 2006 Presumptive horizontal symbiont transmission in the fungus-growing termite *Macrotermes natalensis*. *Mol. Ecol.* **15**, 3131–3138. (doi:10.1111/j.1365-294X.2006.03008.x)

47 Lunn, D. J., Thomas, A., Best, N. & Spiegelhalter, D. 2000 WinBUGS—a Bayesian modelling framework: concepts, structure, and extensibility. *Stat. Comput.* **10**, 325–337. (doi:10.1023/A:1008929526011)

48 Peakall, R. & Smouse, P. E. 2006 GenALEx 6: genetic analysis in Excel. Population genetic software for teaching and research. *Mol. Ecol. Notes* **6**, 288–295. (doi:10.1111/j.1471-8286.2005.01155.x)

49 Bacci, M., Solomon, S. E., Mueller, U. G., Martins, V. G., Carvalho, A. O. R., Vieira, L. G. E. & Silva-Pinhati, A. C. O. 2009 Phylogeny of leafcutter ants in the genus *Atta* Fabricius (Formicidae: Attini) based on mitochondrial and nuclear DNA sequences. *Mol. Phylogenet. Evol.* **51**, 427–437. (doi:10.1016/j.ympev.2008.11.005)

50 Moser, J. C. 1967 Mating activities of *Atta texana* (Hymenoptera, Formicidae). *Insectes Soc.* **14**, 295–312. (doi:10.1007/BF02252831)

51 Chapela, I. H., Rehner, S. A., Schultz, T. R. & Mueller, U. G. 1994 Evolutionary history of the symbiosis between fungus-growing ants and their fungi. *Science* **266**, 1691–1694. (doi:10.1126/science.266.5191.1691)

52 Mikheyev, A. S., Vo, T. & Mueller, U. G. 2008 Phylogeography of post-Pleistocene population expansion in a fungus-gardening ant and its microbial mutualists. *Mol. Ecol.* **17**, 4480–4488. (doi:10.1111/j.1365-294X.2008.03940.x)

53 Mintzer, A. C. 1987 Primary polygyny in the ant *Atta texana*: number and weight of females and colony foundation success in the laboratory. *Insectes Soc.* **34**, 108–117. (doi:10.1007/BF02223829)

54 Worrall, J. J. 1997 Somatic incompatibility in basidiomycetes. *Mycologia* **89**, 24–36. (doi:10.2307/3761169)

55 Weber, N. A. 1937 The biology of the fungus-growing ants. II. Nesting habits of the bachac (*Atta cephalotes* L.). *Trop. Agr.* (*Trinidad*) **14**, 223–226.

56 Iturralde-Vinent, M. A. 2006 Meso-Cenozoic Caribbean paleogeography: implications for the historical biogeography of the region. *Int. Geol. Rev.* **48**, 791–827. (doi:10.2747/0020-6814.48.9.791)

57 Seal, J. N., Kellner, K., Trindl, A. & Heinze, J. In press. Phylogeography of the parthenogenic ant, *Platythyrea punctata*: highly successful colonization of the West Indies by a poor disperser. *J. Biogeogr.* (doi:10.1111/j.1365-2699.2010.02447.x)

58 Mikheyev, A. S. 2008 History, genetics and pathology of a leaf-cutting ant introduction: a case study of the Guadeloupe invasion. *Biol. Invasions* **10**, 467–473. (doi:10.1007/s10530-007-9144-7)

59 Sanchez-Peña, S., Davis, D. & Mueller, U. G. 2003 A gregarious, mycophagous, myrmecophilous moth, *Amydria anceps* Walsingham (Lepidoptera: Acrolophidae), living in *Atta mexicana* (F. Smith) (Hymenoptera: Formicidae) spent fungal culture accumulations. *P. Entomol. Soc. Wash.* **105**, 186–194.

60 Nobre, T., Eggleton, P. & Aanen, D. K. 2009 Vertical transmission as the key to the colonization of Madagascar by fungus-growing termites? *Proc. R. Soc. B* **277**, 359–365. (doi:10.1098/rspb.2009.1373)

61 Weber, N. A. 1945 The biology of the fungus-growing ants. Part VIII. The Trinidad, B.W.I. species. *Rev. Entomologia* **16**, 1–88.

62 Cherrett, J. M. 1968 Some aspects of the distribution of pest species of leaf-cutting ants in the Caribbean. *Proc. Am. Soc. Hortic. Sci.* **12**, 295–310.