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Diversification and phylogeographic structure in widespread *Azteca* plant-ants from the northern Neotropics

ELIZABETH G. PRINGLE,*‡ SANTIAGO R. RAMÍREZ,† TIMOTHY C. BONEBRAKE,*§ DEBORAH M. GORDON* and RODOLFO DIRZO*

**Department of Biology, Stanford University, Stanford, CA 94305, USA, †Department of Environmental Science, Policy, and Management, University of California, Berkeley, CA 94720, USA*

Abstract

The Neotropical myrmecophytic tree *Cordia alliodora* hosts symbiotic *Azteca* ants in most of its widespread range. The taxonomy of the genus *Azteca* is notoriously difficult, which has frequently obscured species identity in ecological studies. We used sequence data from one mitochondrial and four nuclear loci to infer phylogenetic relationships, patterns of geographic distribution, and timing of diversification for 182 colonies of five *C. alliodora*-dwelling *Azteca* species from Mexico to Colombia. All morphological species were recovered as monophyletic, but we identified at least five distinct genetic lineages within the most abundant and specialized species, *Azteca pittieri*. Mitochondrial and nuclear data were concordant at the species level, but not within species. Divergence time analyses estimated that *C. alliodora*-dwelling *Azteca* shared a common ancestor approximately 10–22 million years ago, prior to the proposed arrival of the host tree in Middle America. Diversification in *A. pittieri* occurred in the Pleistocene and was not correlated with geographic distance, which suggests limited historical gene flow among geographically restricted populations. This contrasts with the previously reported lack of phylogeographic structure at this spatial scale in the host tree. Climatic niches, and particularly precipitation-related variables, did not overlap between the sites occupied by northern and southern lineages of *A. pittieri*. Together, these results suggest that restricted gene flow among ant populations may facilitate local adaptation to environmental heterogeneity. Differences in population structure between the ants and their host trees may profoundly affect the evolutionary dynamics of this widespread ant–plant mutualism.

Keywords: ant–plant mutualism, *Cordia alliodora*, gene trees, Middle America, phylogeography, Pleistocene climate changes, seasonally dry tropical forests

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Introduction

The ecological and evolutionary dynamics of mutualisms depend on the genetic diversity of the mutualistic partners at both the species and population levels. In symbiotic ant–plant mutualisms, host plants provide space and food for nesting ant colonies, and ants provide protection against herbivores and encroaching vegetation (Heil & McKey 2003). The dynamics of these interactions may be affected by asymmetries in rates of evolution between host plants and symbiotic ants at both small (Palmer *et al.* 2010; Orivel *et al.* 2011) and large (Quek *et al.* 2007; Léotard *et al.* 2009) geographic scales.

Azteca (Dolichoderinae) is an exclusively Neotropical genus of arboreal ants, perhaps best known for the symbiotic associations that some species form with

Correspondence: Elizabeth G. Pringle, Fax: (734) 763 0544; E-mail: epringle@umich.edu
‡Present address: Michigan Society of Fellows, University of Michigan, Ann Arbor, MI 48109, USA.
§Present address: Department of Biology, University of California, Riverside, CA 92521, USA.

Cecropia (Urticaceae) trees (Longino 1991). Other species of *Azteca* build carton nests or ant gardens, and some are specialized symbionts of other myrmecophytic plants, including two in the genus *Cordia* (Boraginaceae). There may be considerable unrecognized genetic diversity in *Azteca*; high species diversity [approximately 100 extant species (Bolton *et al.* 2007)] and intraspecific morphological variation have hindered taxonomy and systematics of the genus (Longino 1991, 1996, 2007). To date, there is one molecular phylogeny of *Azteca* with eight species, six of which are *Cecropia* specialists (Ayala *et al.* 1996).

The myrmecophytic tree *Cordia alliodora* is widely distributed in Neotropical forests from Mexico to Argentina. The trees host *Azteca* ant colonies in hollow stem nodes, known as 'domatia,' in which ants also tend honeydew-producing scale insects (Wheeler 1942). The ants, in turn, can defend the trees from leaf herbivory (Tillberg 2004; Trager & Bruna 2006; Pringle *et al.* 2011). Of the 29 species included in a full revision of Costa Rican *Azteca* (Longino 2007), at least five species can inhabit *C. alliodora* stem domatia; these include three generalist stem nesters (*Azteca beltii* Emery 1893, *Azteca nigricans* Forel 1899, *Azteca velox* Forel 1899) and two specialists that are only known to nest in *C. alliodora* (*Azteca oecocordia* Longino 2007 and *Azteca pittieri* Forel 1899). In Middle America, *C. alliodora* trees can also host generalist stem-nesting species of *Crematogaster*, *Camponotus*, and *Pseudomyrmex*, as well as the specialist *Cephalotes setulifer* Emery 1894 (Longino 1996). Of the non-specialists, *A. beltii* is the most common in Costa Rica (Longino 1996). Throughout Mexico and Central America, the specialized *A. pittieri* are the most common ants to occupy *C. alliodora* trees (Longino 1996). The range of *A. pittieri* extends from Mexico to Panama (Longino 2007).

Within *A. pittieri*, there is geographic variation in both morphological (Longino 1996) and behavioural (Pringle *et al.* 2011) traits. Morphological variation in queen head size in *A. pittieri* has been detected within Costa Rica (Longino 1996, 2007), but variation that is restricted to head size may result from different local selective pressures without representing reproductive isolation (Longino 1996; Léotard *et al.* 2009). Longino (1996, 2007) thus described *A. pittieri* as a single taxonomic unit, but recognized the potential for cryptic species. In addition to morphological variation, our studies of the mutualism between *C. alliodora* and *A. pittieri* from Mexico to Costa Rica have revealed considerable geographic variation in traits of ants, including colony size and defensive behaviour, that affect how well ants defend the host tree (Pringle *et al.* 2011).

The coevolutionary dynamics of mutualisms across their geographic distributions depend on the evolutionary history and biogeography of the interacting clades, as well as on levels of gene flow across heterogeneous landscapes within species (Thompson 2005). Because tropical ants often belong to diverse genera that display cryptic morphological variation (e.g., Ross *et al.* 2010) and discordance between morphospecies and gene trees (Feldhaar *et al.* 2003; Quek *et al.* 2004), it has been challenging to investigate patterns of codiversification between mutualistic ant and plant partners (but see, e.g., Chenuil & McKey 1996; Quek *et al.* 2004; Gómez-Acevedo *et al.* 2010). Within pairs of interacting species, asymmetries in gene flow may cause important asymmetries in local adaptation (Hoeksema & Thompson 2007), but it is difficult to predict a priori the phylogeographic patterns of any given species (Smith *et al.* 2011; Thompson & Rich 2011). It remains unclear whether asymmetries in population structure between mutualistic partners are typical, and what the consequences of the presence or absence of such asymmetries may be for geographically widespread mutualisms.

Due in part to its importance to agroforestry, there have been several informative studies of the population genetics and phylogeography of *C. alliodora* across its broad range (Boshier *et al.* 1995; Chase *et al.* 1995; Rymer *et al.* in press). Although *C. alliodora* can be found in rainforests and disturbed habitats, it is most common in seasonally dry tropical forests (Gottschling *et al.* 2005) and may have originated in the dry forests of South America (Rymer *et al.* in press). In dry forests, the highly seasonal, limited annual rainfall (approximately 800–1600 mm) creates a favourably sparse canopy for the shade-intolerant *C. alliodora* (Menalled *et al.* 1998). Seeds of *C. alliodora* are wind-dispersed, and a recent phylogeographic study of populations across the tree's entire range found very little genetic structure, indicating gene flow between populations as widely separated as Mexico and Brazil (Rymer *et al.* in press). Rymer *et al.* (in press) propose that *C. alliodora* may have dispersed to Central America as recently as 3 million years ago, subsequent to the uplift of the tropical Andes and the formation of the Panama Isthmus.

In this study, we aim to elucidate the geographic patterns and timing of diversification of the *Azteca* associates of *C. alliodora* in the northern Neotropics, as well as to investigate the population structure of *A. pittieri*, the most common mutualistic symbiont in Middle America. We collected *Azteca* ants from *C. alliodora* trees between Jalisco, Mexico and Colombia, covering the entire known range of *A. pittieri.* We reconstructed the relationships among *Azteca* lineages using molecular phylogenetic analyses based on one mitochondrial and four nuclear loci, estimated the timing of diversification of well-supported lineages, and investigated the population genetic structure of *A. pittieri* from Mexico to Costa Rica. We then compared these results to what is known about the

host tree. We asked: (i) Does the phylogenetic tree support monophyly of morphological species? (ii) Do nuclear and mitochondrial markers reconstruct similar relationships among and within species? (iii) Does the timing of diversification in Middle American *Azteca* coincide with the arrival of *C. alliodora*, with known lowland biogeographic barriers, or with Pleistocene climate changes? (iv) Does the common mutualist *A. pittieri* show phylogeographic structure, and, if so, does this pattern reflect barriers to gene flow or isolation by distance? (v) Do *A. pittieri* lineages segregate across current climatic regimes in Middle American dry forests?

Materials and methods

Collection of samples

We collected *Azteca* ants from 182 colonies in *Cordia alliodora* trees from 33 localities (Fig. 1; Appendix S1, Supporting information). Host trees were located in forests, farms, pastures, and roadsides. For each tree, we noted the locality using a hand-held GPS unit. Ants were collected by trimming 1–3 subterminal domatia from tree branches and immediately placing domatia in collecting vials. The domatia were placed in a freezer for 1–3 h and then dissected; ants were stored in 95% ethanol until subsequent DNA extraction. Ants were collected without regard to species identity; our data thus reflect the relative abundances of different *Azteca* species. Voucher specimens are currently held in research collections of E.G. Pringle and J.T. Longino; ultimately, they will be deposited in major museum collections.

DNA sequence data

We sequenced DNA fragments from five loci (approximately 4 kb), using both nuclear and mitochondrial DNA. These loci comprised four nuclear regions: approximately 0.6 kb of Elongation Factor 1-alpha F1 copy (*EF1αF1*), approximately 0.55 kb of Long-Wavelength Rhodopsin (*LWRh*), and approximately 0.6 kb Wingless (*wg*); approximately 1.2 kb of the nuclear ribosomal internal transcribed spacer region 2 (*ITS-2*); and one mitochondrial gene, approximately 1 kb of Cytochrome Oxidase 1 (*CO1*). All three single-copy nuclear genes (*EF1αF1*, *LWRh*, *wg*) included one intron.

Genomic DNA was isolated from individual worker ants using a QIAGEN DNeasy kit with the standard protocol for animal tissues. Primers for each fragment are listed in Table S1 (Supporting information). PCRs were conducted using a 25-μL mix of 10 × buffer, 25 mM MgCl, 2 mM dNTPs, 10 μM primers, 1 unit of Taq DNA polymerase, and approximately 50 ng of DNA. PCR amplification for each fragment began with initial denaturation at 94 °C for 2 min, followed by 35–40 cycles of: denaturation at 95 °C for 30–60 s; annealing at 45 °C (*CO1*), 59 °C (*wg*), 60 °C (*ITS-2*, *EF1αF1*), or 62 °C (*LWRh*) for 30–60 s; and extension at 72 °C for 1–2 min; followed by final elongation at 72 °C for 2–6 min. PCR products were checked by electrophoresis on a 1% low-melting point agarose gel. Products were then purified using exonuclease and shrimp phosphatase and sequenced directly on an Applied Biosystem Genetic Analyzer Model 3730xl. All fragments were sequenced in both directions, and additional internal sequencing primers were used for the two longest fragments, *CO1* and *ITS-2* (Table S1, Supporting information). All sequences were deposited in GenBank with the following accession numbers (JQ867506–JQ868413).

Sequences were aligned and manually edited using the software package GENEIOUS v5.4 (Drummond *et al.* 2011). A *Cecropia*-dwelling outgroup, *Azteca ovaticeps* (voucher code B224) was also collected in Guanacaste, Costa Rica, and sequenced as indicated above. Additional outgroup taxa, identified from the literature, included *Azteca instabilis*, *A. ovaticeps*, *Azteca schimperi*, *Gracilidris pombero*, and *Linepithema humile* from Ward *et al.* (2010) and an unidentified *Azteca* species from Moreau *et al.* (2006). The corresponding sequences were downloaded from GenBank. All sequences were aligned in GENEIOUS v5.4 using the MUSCLE alignment function with default settings (Drummond *et al.* 2011). We manually edited this alignment and added intron/exon and codon position information in MACCLADE v4.06 (Maddison & Maddison 2000). Alignments are available at TREEBASE (#S12472) and Dryad (doi:10.5061/dryad.p8n5kb15).

Phylogenetic analyses

For the 182 *Azteca* colonies and all outgroups, we used tree-based methods to reconstruct relationships among species. Genetic distances were calculated separately for nuclear and mitochondrial sequence data using the GTR model of sequence evolution in PAUP* v4.0b10 (Swofford 2002). Parsimony and maximum-likelihood methods recovered relationships similar to those reconstructed by Bayesian methods and are not discussed further here. Prior to Bayesian analyses, we determined the appropriate models of sequence evolution for our data using the Akaike Information Criterion (AIC) (Posada & Buckley 2004), implemented in MrModelTest2.3 (Nylander 2004). Results are summarized in Table S2 (Supporting information). All phylogenetic analyses were run on the freely available computer cluster Bioportal (http://www.bioportal.uio.no).

Bayesian analyses were conducted on concatenated sequence matrices of all five markers, of the mitochondrial *CO1* marker, and of the four nuclear markers

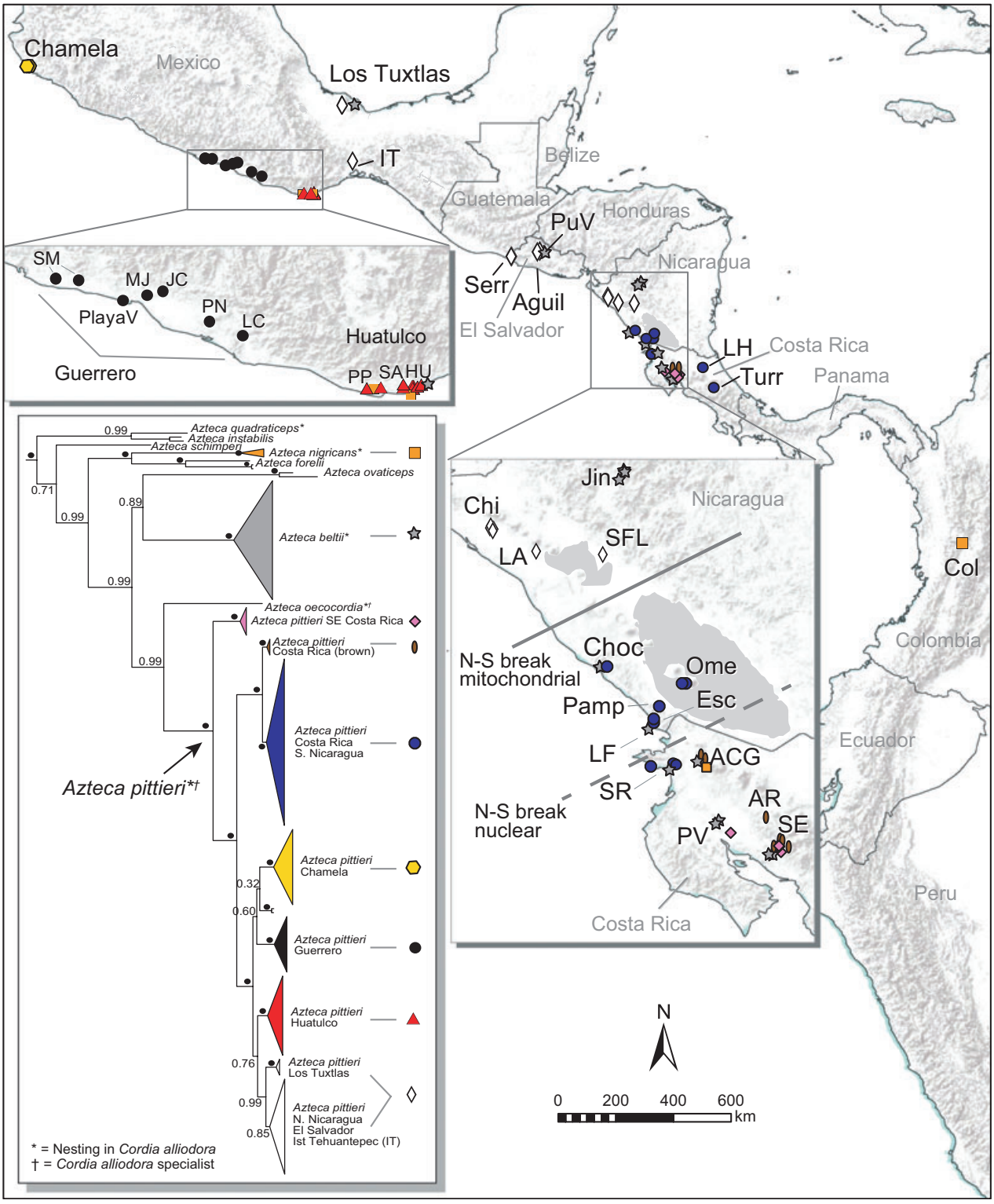

Fig. 1 Bayesian phylogram of *Azteca* ants collected from *Cordia alliodora* trees and outgroups based on all five loci, and the geographic distribution of the supported lineages. Tree nodes marked with black dots indicate posterior probabilities of 1; numbers indicate posterior probabilities <1. Lineages are named according to species identity or, within *Azteca pittieri*, to geographic location. Coloured shapes on the map correspond to collection localities and are identified on the phylogeny. Collection localities are identified by abbreviations (see Appendix S1, Supporting information). Lines identified by 'N-S break' indicate where the split between northern and southern lineages occurred in the mitochondrial and nuclear data sets, respectively.

using MRBAYES v3.1 (Ronquist & Huelsenbeck 2003). In each of these analyses, the mitochondrial locus *CO1* was partitioned by codon position, and the nuclear markers were partitioned by coding and noncoding regions (Table S2, Supporting information). For all analyses, we conducted two independent runs with four chains. For *CO1*, we set heated chain temperature = 0.25, to run for 2×10^7 generations, sampling every 2000 generations. For analysis of all five markers and of only the four nuclear markers, we set heated chain temperature = 0.20, to run for 3×10^7 generations, sampling every 3000 generations. When the analyses reached completion, log files were verified for convergence between both runs, and trees were summarized in TREEANNOTATOR v1.6.1 from the BEAST v1.6.1 package (Drummond & Rambaut 2007) with 10% burnin.

For each resulting lineage in the phylogenetic tree, JT Longino helped us to identify the species morphologically using alate females or queens from colonies where they had been collected in combination with workers.

Divergence time analysis

To estimate divergence times, we used concatenated sequences from all five markers, with separate partitions for *CO1*, nuclear coding sequences, and nuclear noncoding sequences. We performed the dating analysis using Bayesian MCMC methods in BEAST v1.6.1 (Drummond & Rambaut 2007) and ran analyses on the Bioportal computer cluster (http://www.bioportal.uio.no). We constrained lineages that were recovered with posterior probabilities ≥0.99 in Bayesian analyses to be monophyletic, defined a monophyletic ingroup excluding *L. humile*, and used the model of sequence evolution GTR + I + G with estimated base frequencies for all three partitions (Table S2, Supporting information). We used an uncorrelated lognormal relaxed-clock model with two calibration points based on the fossil-calibrated tree of Ward *et al.* (2010). We calibrated the root age (the divergence between *L. humile* and the ingroup) to 47 ± 8 million years (Myr) and the time to most recent common ancestor of the ingroup, including *G. pombero* and all *Azteca*, to 43 ± 9 Myr. Age calibrations were assigned LaPlace distributions (BEAST priors: mean = 47 or 43, respectively; scale = 3 or 3.5, respectively). The analysis was run for 8×10^7 generations, sampling every 8000 generations, with the mean of the branch rates (ucld.mean) set to a uniform prior distribution of initial value 0.001 (lower = 0; upper = 1). We used a coalescent tree prior for populations of constant size. When the analysis reached completion, we checked the trace files in TRACER v1.5 (Rambaut & Drummond 2009) for convergence and verified that Effective Sample Size (ESS) for all parameters was ≥200. All parameters reached ESS above 200 with these settings except for the parameter describing the partition for nuclear noncoding sequences, which reached only 154. We ran the analysis again with 1×10^8 generations, sampling every 10 000 generations, and although there was no change in estimated node ages, the ESS for nuclear noncoding sequence did not improve substantially, so here we present the results from the first run. The 10 000 resulting trees from each run were summarized in TREEANNOTATOR v1.6.1 (Drummond & Rambaut 2007) with 10% burnin.

Population genetic analyses

Our phylogenetic analyses recovered *Azteca pittieri* as a monophyletic clade composed of 144 individual colonies with several strongly supported, geographically restricted lineages. We investigated the geographic population structure in these 144 colonies. To generate haplotypes from our sequence data for each of the four nuclear markers, single nucleotide polymorphisms were coded as ambiguities, extensive gaps with ambiguous alignments were removed, and haplotypes were reconstructed using the PHASE algorithm (Stephens *et al.* 2001), as implemented in the software package DNASP v5 (Librado & Rozas 2009).

To determine how many distinct population clusters were supported by the data, we examined population structure in the software STRUCTURE v2.3.3 (Pritchard *et al.* 2000), run on the Bioportal computer cluster (http://www.bioportal.uio.no). The program uses a Bayesian method to predict the number of distinct genetic clusters (K) in the data. We collapsed sequence data for each of the five loci into unique haplotype codes by importing DNA sequences to TCS v1.21 (Clement *et al.* 2000). From the resulting parsimony network, each distinct group of haplotypes was manually assigned a unique number. Numbers were then assigned to the two alleles of each individual. For mitochondrial *CO1*, the second allele was coded as missing data. Because of the presence of some rare alleles, we estimated the parameter λ in a preliminary run with $K = 1$ and then set λ to this estimated value (0.959) for subsequent runs. All other parameters were set to program defaults for unlinked loci and correlated allele frequencies (Falush *et al.* 2003). For each proposed K, we conducted 20 runs of 1×10^7 steps with 10% burnin. Preliminary runs suggested that these run lengths were sufficient to reach stationarity. The results were imported into Structure Harvester v0.6.8 (Earl & vonHoldt 2011), which allowed us to select the number of clusters by simultaneously evaluating the estimated posterior probability of the data and the ΔK statistic of Evanno *et al.* (2005). Finally, population and individual

output files were summarized in CLUMPP v1.1.2 (Jakobsson & Rosenberg 2007), using the Greedy algorithm with 10 000 random input orders. Graphical output was produced using DISTRUCT v1.1 (Rosenberg 2004).

Estimates of nucleotide diversity (π), population differentiation (Φ_{ST}s), and molecular variance (AMOVA) were determined separately for nuclear and mitochondrial markers using ARLEQUIN v3.5 (Excoffier & Lischer 2010). The four nuclear genes were concatenated for all analyses. The 144 *A. pittieri* individuals were divided into nine populations for estimates of π and pairwise Φ_{ST} based on geographically and genetically homogenous areas (Fig. 1; Table 1). Significance of pairwise Φ_{ST} values was based on 110 permutations. For AMOVA, we separated the five Costa Rican individuals whose nuclear haplotypes were distinct from the rest of the Costa Rican individuals into a separate 'population' to group populations by genetic clusters defined in STRUCTURE. AMOVAs were conducted under a Kimura 2-parameter model of sequence evolution with 1000 permutations.

We tested whether populations of *A. pittieri* experienced demographic expansion by examining neutrality of mitochondrial sequences under Tajima's D (Tajima 1989a,b) and Fu's *F*-test (Fu 1997). For populations that were significantly non-neutral under one or both of these tests, we looked for evidence of sudden population expansion using mismatch distribution analysis (Slatkin & Hudson 1991; Rogers & Harpending 1992). The mismatch distribution tests whether the distribution of the observed number of pairwise differences differs significantly from the unimodal distribution expected under a model of sudden expansion. Significance of the deviation of the observed pattern from the expected pattern was determined by 5000 bootstrap replicates (Schneider & Excoffier 1999). All demographic analyses were conducted using ARLEQUIN v3.5 (Excoffier & Lischer 2010).

To test for isolation by distance in *A. pittieri*, we performed a Mantel Test between Φ_{ST} values among the nine populations and geographic distances, calculated using the average latitude and longitude coordinates for the geographic area and geodesic distances in km for the WGS84 ellipsoid (Karney 2011) using the online tool (http://geographiclib.sourceforge.net/cgi-bin/Geod). Mantel tests were performed using a Monte-Carlo test with 9999 replicates in the *ade4* package (Dray & Dufour 2007) of R v2.14.0 (R Development Core Team R 2011).

Climatic niche analysis

To investigate whether climatic niches were distinct for different genetic lineages of *A. pittieri*, we downloaded data on 19 bioclimatic variables for the years approximately 1950–2000, interpolated to 1-km resolution for the entire study area, from WorldClim.org (Hijmans *et al.* 2005a). We extracted the values for each of these 19 variables from our GPS locations of each of our collection sites in DIVA-GIS v7.4 (Hijmans *et al.* 2005b). We extracted the principal components of these 19 variables using JMP 8.0.2 (SAS Institute 2009) and visually examined the overlap of climatic niche space among the genetic lineages of *A. pittieri*.

Results

Sequence data

The final matrix included individuals from 182 colonies collected from *Cordia alliodora*, a second individual worker from one of those colonies (B054), and seven outgroup taxa for a total of 190 tips. Within individuals sequenced for this study, there were no completely missing sequences for *CO1* or *ITS-2*, one missing sequence each for *wg* and *LWRh*, and only four missing sequences for *EF1αF1*. The entire matrix consisted of 4432 characters including gaps because of indels; 607 of the variable characters in the matrix were parsimony informative. Characteristics for all sequenced fragments are listed in Table S2 (Supporting information).

Pairwise distances of both nuclear markers and *CO1* reflected previous taxonomic hypotheses, with the most

Table 1 Pairwise Φ_{ST} values for the nine geographically defined populations of *Azteca pittieri*. Nuclear indices are above the diagonal; mitochondrial indices are below the diagonal

	Chamela	Guerrero	Huatulco	Ist Tehuantepec	Los Tuxtlas	El Salvador	N Nicaragua	S Nicaragua	Costa Rica
Chamela		0.0756	0.3900	0.6525	0.6565	0.6515	0.5355	0.4863	0.6667
Guerrero	0.7512		0.3050	0.6749	0.6963	0.6780	0.5776	0.5444	0.7159
Huatulco	0.8435	0.7688		0.7228	0.7829	0.7111	0.6126	0.5659	0.7696
Ist Tehuantepec	0.8388	0.6494	0.8721		0.9553	0.4727	0.7401	0.5831	0.8930
Los Tuxtlas	0.8446	0.6670	0.8920	0.9404		0.7176	0.6806	0.4619	0.6242
El Salvador	0.8281	0.6714	0.8367	0.3303	0.6846		0.4671	0.3864	0.7922
N Nicaragua	0.8637	0.7063	0.8898	0.7261	0.9074	0.2214		0.3447	0.7202
S Nicaragua	0.9326	0.8767	0.9577	0.9779	0.9839	0.9498	0.9782		0.4727
Costa Rica	0.6668	0.6315	0.6918	0.5715	0.5986	0.6132	0.6276	0.1416	

divergence between *Azteca* and the outgroup species *Linepithema humile*, and the least divergence among lineages in the *Azteca pittieri* complex (Table S3, Supporting information).

Phylogenetic reconstruction

All currently recognized morphological species, including *A. pittieri*, were recovered as monophyletic in concatenated, mitochondrial, and nuclear trees (Figs 1 and 2). At least nine lineages of *C. alliodora*-dwelling *Azteca* were recovered with high support, with at least five lineages hypothesized to form the *A. pittieri* complex (Figs 1 and 2). A deep split was recovered between *A. pittieri* lineages in the northern and southern parts of their Middle American range.

Among lineages that fall outside the *A. pittieri* group, there were four *C. alliodora*-dwelling species, morphologically identified as: (i) *Azteca quadraticeps* Longino 2007; a recently described species known only from queens, collected as a newly colonized queen without workers in Costa Rica; (ii) *Azteca nigricans* Forel 1899, a generalist live-stem dweller, collected in Costa Rica and Colombia (orange lineage); (iii) *Azteca beltii* Emery 1893, another generalist live-stem dweller and the second most common ant symbiont after *A. pittieri*, whose distribution ranged from Costa Rica to southern and eastern Mexico (grey lineage); and (iv) *Azteca oecocordia* Longino 2007; which was recently described as another *C. alliodora* specialist (Longino 2007), and of which we discovered only one individual colony in the Santa Elena area around the Monteverde Cloud Forest in Costa Rica, similar to the restricted distributional pattern reported by Longino (2007). *Azteca forelii* Emery 1893 was collected in Nicaragua (voucher EGP160) and was recovered as sister to *A. nigricans*, but it was collected in a broken *C. alliodora* domatium. Thus, we cannot be sure that the colony was actually nesting in *C. alliodora*, rather than forming carton nests on the trees, which is believed to be its usual nesting habit (Longino 2007). Sister to *A. forelii*, we collected two individuals from Oaxaca, Mexico, that are probably at least one, if not two, additional species (vouchers EGP91 and EGP121; Fig. S1; Appendix S1, Supporting information), but no female reproductives were collected from these colonies, and their identification remains uncertain.

Within *A. pittieri*, there was strong support for a split between northern and southern lineages that occurred near Southern Nicaragua in concatenated, mitochondrial, and nuclear trees (Figs 1 and 2). In the concatenated and mitochondrial trees (Fig. 1), individuals collected from the north (Mexico to Northern Nicaragua) formed four well-supported lineages, each of which was associated with distinct geographic ranges. These were: (i) a widespread lineage of *A. pittieri* that was collected from Northern Nicaragua, El Salvador, the Isthmus of Tehuantepec in Mexico, and Los Tuxtlas in eastern Mexico (white lineage); (ii) *A. pittieri* collected in Huatulco National Park, Oaxaca, Mexico (red lineage); (iii) *A. pittieri* collected in southern Guerrero and northwestern Oaxaca, Mexico (black lineage); and (iv) *A. pittieri* collected in the Chamela-Cuixmala Biosphere Reserve in Jalisco, Mexico (yellow lineage). In contrast, the nuclear tree exhibited strong support for a lineage that did not differentiate between individuals from Chamela and Guerrero (Fig. 2). In addition, in the nuclear tree, individuals from the Isthmus of Tehuantepec grouped with Huatulco individuals (Fig. 2), rather than grouping with the widespread, white lineage (Fig. 1).

Individuals collected from the south (Southern Nicaragua to Costa Rica) appeared to form at least two well-supported lineages (Figs 1 and 2), but the relationships recovered among individuals in this part of the range depended on whether the sequence data used in the analysis were mitochondrial or nuclear (Fig. 2). In the concatenated and mitochondrial trees, the split between northern and southern lineages occurred between Southern and Northern Nicaraguan populations, whereas in the nuclear tree, the split occurred between Nicaragua and Costa Rica (Figs 1 and 2). In addition, the mitochondrial data recovered a lineage with strong support that was sister to the rest of *A. pittieri*, composed of eight individuals from Santa Elena and Palo Verde, Costa Rica (purple lineage; Fig. 1, Fig. S1, Appendix S1, Supporting information). In contrast, the nuclear tree recovered these individuals together with those from Northwestern Costa Rica (Fig. 2). The nuclear tree recovered a distinct lineage of five individuals from Santa Elena, Arenal, and ACG, Costa Rica (brown lineage; Fig. 1, Fig. S1; Appendix S1, Supporting information), which received only weak support in the mitochondrial tree (Fig. 2).

When mitochondrial and nuclear sequence data were considered separately, posterior support values for the monophyly of lineages were much higher than support values for relationships among lineages (Fig. 2). The nuclear tree, in particular, exhibited extremely low support values, illustrative of the smaller proportion of parsimony informative variable sites found in nuclear genes in comparison with mitochondrial *CO1* (Table S2, Supporting information).

Divergence times

Azteca symbiotic ants began to diversify in the Neogene, but much of the intraspecific diversification in *A. pittieri* occurred in the Pleistocene. The results from our relaxed molecular clock analysis conducted in BEAST

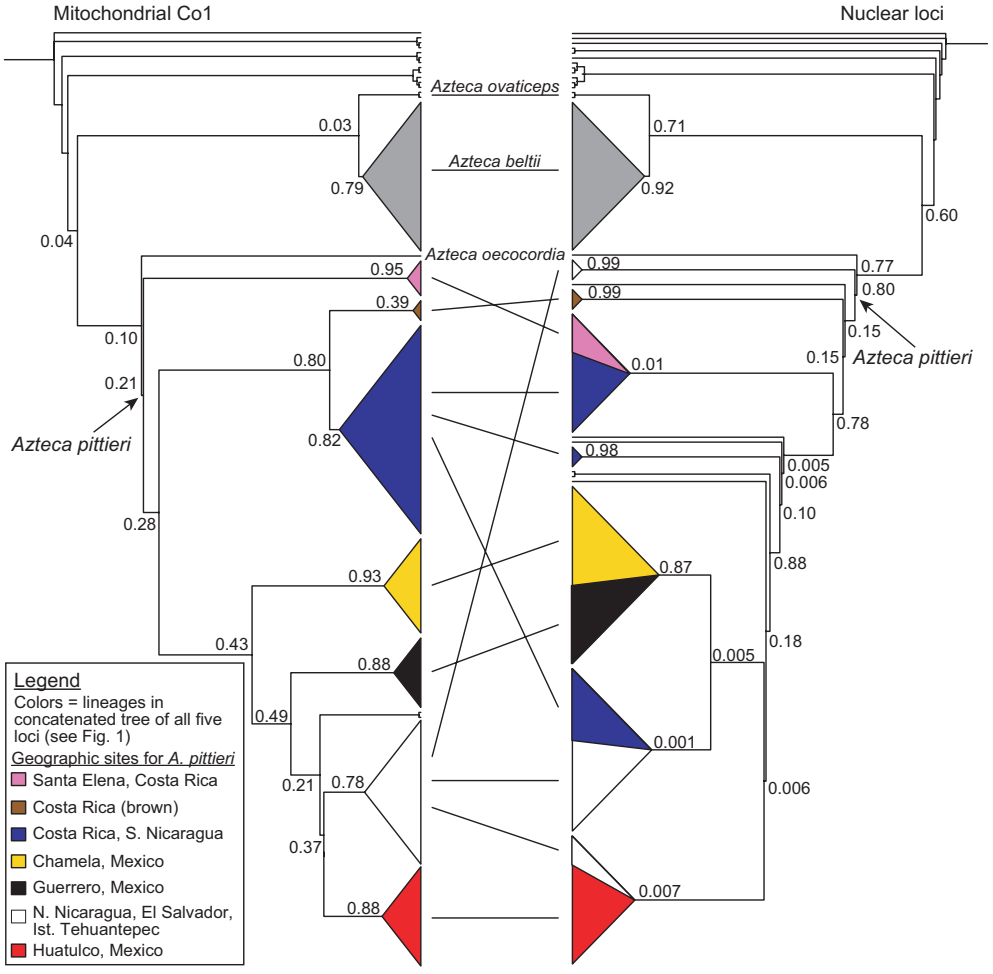

Fig. 2 Bayesian tree derived from mitochondrial DNA [Cytochrome Oxidase 1 (*CO1*)] compared with that derived from nuclear DNA [*Ef1αF1*, *ITS-2*, Long-Wavelength Rhodopsin (*LWRh*), *wg*]. Lines connect corresponding lineages. Colours are the same as those in the concatenated tree shown in Fig. 1; split-colour lineages in the nuclear tree indicate proportional numbers of individuals from the like-coloured monophyletic mitochondrial lineage. Numbers at nodes indicate Bayesian posterior probabilities.

indicated that the *C. alliodora*-dwelling *Azteca* shared a most recent common ancestor that lived during the Miocene (approximately 10–22 Ma), and *A. pittieri* shared a most recent common ancestor during the late Miocene or Pliocene (approximately 2.8–6.6 Ma) (Fig. 3). Diversification within *A. pittieri* lineages in Middle America was much more recent, with most recent common ancestors for Mexican and Northern Central-American lineages extending to the early Pleistocene (approximately 2 Ma), and for Costa Rican lineages extending to the late Pleistocene (approximately 0.2–0.7 Ma) (Table S4, Supporting information).

Population genetics of Azteca pittieri

Population genetic analyses supported five genetically differentiated groups of *A. pittieri* with relatively stable demographic histories and nonsignificant isolation by distance among groups. There was strong support for distinct genetic clusters within the *A. pittieri* clade based on the analyses conducted in STRUCTURE. The Δ*K* statistic of Evanno *et al.* (2005) suggested that there were between three and five clusters (Δ*K* = 4.27, 4.03, 2.10, and 0.15 for *K* = 3–6, respectively). The posterior probabilities of the data suggested that improvement in the model began to approach its asymptote at *K* = 6, providing some support for the interpretation that there were in fact five genetic clusters (Pritchard *et al.* 2000, 2007). Because Δ*K* is strongly influenced by the standard deviation of the posterior probability of all runs for each inferred value of *K*, and standard deviations may increase in complex data sets, we investigated which individuals in the data set were separated into distinct clusters when we set *K* = 4 or 5 and reran simplified subsets of the data including these individuals through STRUCTURE. In both cases, the additional clusters,

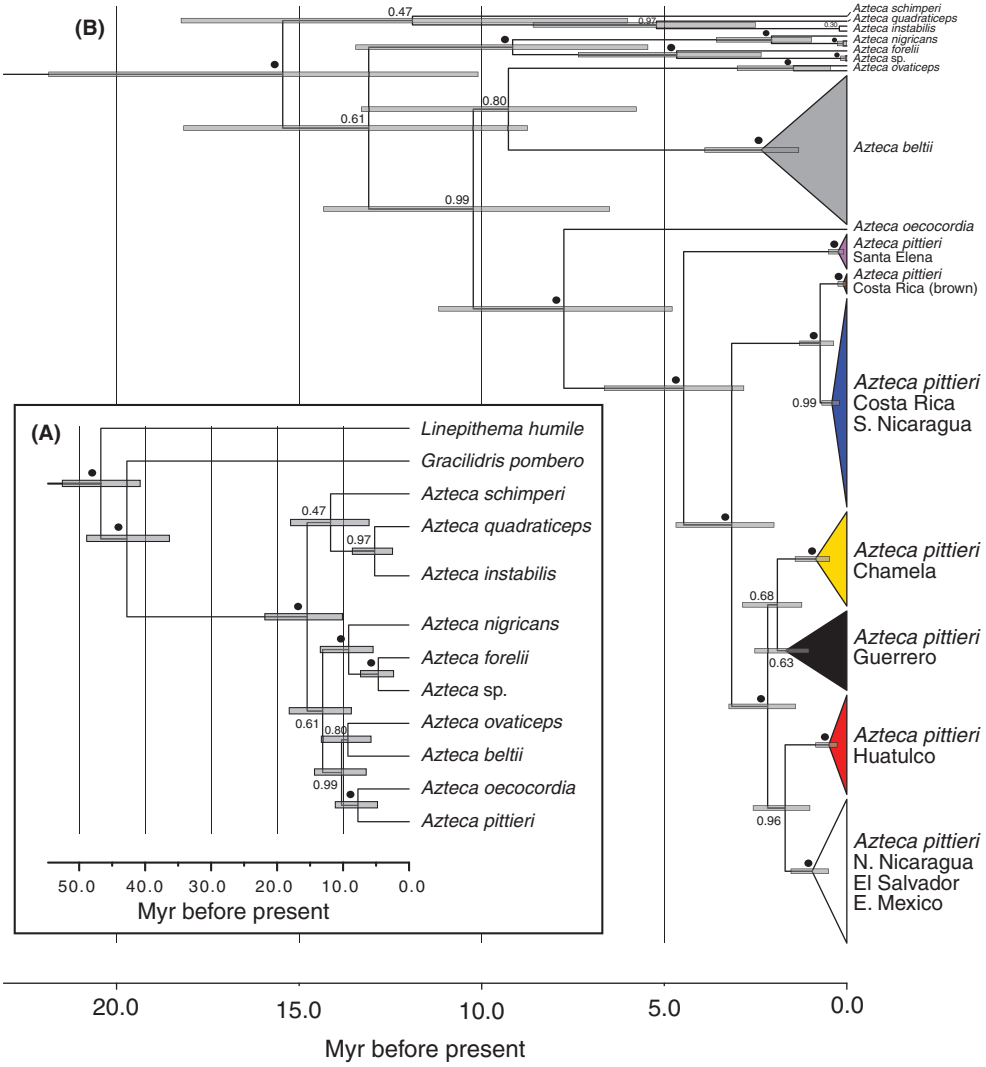

Fig. 3 Chronogram of (A) all species included in this study and (B) the *Cordia alliodora*-dwelling *Azteca* lineages based on a partitioned, uncorrelated lognormal relaxed-clock analysis of the four nuclear loci and mitochondrial Cytochrome Oxidase 1 (*CO1*) in BEAST. The root age and most recent common ancestor of the ingroup, including *Gracilidris pombero*, were calibrated according to dates in Ward *et al*. (2010). Horizontal grey bars represent the 95%-confidence limits for node ages, in units of million years (Myr) before present. Numbers at nodes indicate Bayesian posterior probabilities (PP); black dots represent PP = 1. For *Azteca pittieri* lineages, geographic locations where specimens were collected are listed under the species name.

which separated the individuals from Huatulco ($K = 4$) and those from Los Tuxtlas and five of the 74 individuals from Costa Rica ($K = 5$), were strongly supported by these additional runs. Thus, our data appear to support a value of $K = 5$ (Fig. 4); these five groups approximately corresponded to five of the six primary lineages in the Bayesian nuclear tree (Fig. 2).

Coinciding with the strong support values for these lineages in the tree-based analyses, most of the individuals from these five groups showed very low levels of admixture (Fig. 4). Only individuals from the Isthmus of Tehuantepec were not given a majority assignment to one of the five clusters. There was evidence for one potential migrant from Costa Rica to Southern Nicaragua (blue line in S. Nicaragua cluster in Fig. 4).

Nucleotide diversity (π) averaged 0.229% for nuclear loci and 0.834% for mitochondrial *CO1* for all *A. pittieri* samples (Table 2). For nuclear loci, pairwise Φ_{ST} values ranged from 0.0756 between Chamela and Guerrero to 0.9553 between Los Tuxtlas and the Isthmus of Tehuantepec (Table 1). For mitochondrial *CO1*, pairwise Φ_{ST} values ranged from 0.1416 between South Nicaragua and Costa Rica to 0.9839 between South Nicaragua and Los Tuxtlas (Table 1). All pairwise Φ_{ST} values were significant at the $P < 0.05$ level. The molecular variance analysis of the four nuclear loci showed strong and

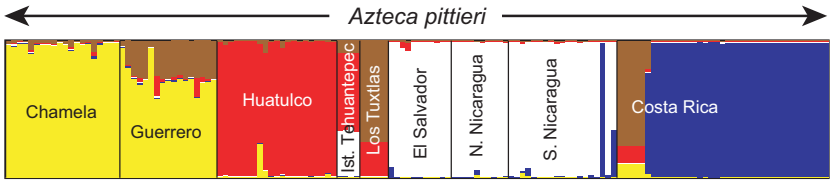

Fig. 4 Membership of *Azteca pittieri* individuals in distinct genetic clusters based on STRUCTURE analyses of the four nuclear loci and mitochondrial Cytochrome Oxidase 1 (*CO1*). Each of the 144 individuals is represented by a vertical bar divided into parts proportional to the presence of haplotypes from each of the five clusters, coded by colour. The nine proposed geographic populations are separated by black lines and identified by name.

Table 2 Sample size, nucleotide diversity, and tests of neutrality for *Azteca pittieri* populations. Note that the 5% critical significance value of Fu's *Fs* is 0.02

Population	*N*	Nucleotide diversity (π) (Nuclear/*C01*)	Tajima's D	Fu's *Fs* test
Chamela	20	3.31e-3 ± 1.79e-3/8.23e-3 ± 4.46e-3	−0.44 (0.37)	−0.88 (0.32)
Guerrero	17	2.91e-3 ± 1.61e-3/1.74e-2 ± 0.91e-2	−0.38 (0.36)	−3.65 (0.06)
Huatulco	21	1.91e-3 ± 1.11e-3/4.25e-3 ± 2.43e-3	−1.08 (0.14)	−3.89 (0.04)
Ist Tehuantepec	4	2.09e-3 ± 1.26e-3/1.70e-3 ± 1.45e-3	1.09 (0.83)	0.006 (0.29)
Los Tuxtlas	5	3.14e-4 ± 2.65e-4/0.00 ± 0.00	–	–
El Salvador	11	3.01e-3 ± 1.61e-3/6.18e-3 ± 3.57e-3	−1.36 (0.09)	−0.78 (0.31)
N Nicaragua	10	1.92e-3 ± 1.08e-3/1.51e-3 ± 1.10e-3	−0.97 (0.20)	**−3.99 (0.002)**
S Nicaragua	19	4.13e-3 ± 2.19e-3/1.35e-3 ± 0.96e-3	−1.52 (0.05)	**−3.56 (0.005)**
Costa Rica	37	1.05e-3 ± 0.61e-3/3.44e-2 ± 1.70e-2	1.42 (0.94)	14.48 (1.00)

P values for each test are indicated in parentheses. Dash indicates that there were no pairwise differences within the sample. Significant values are highlighted in bold.

significant genetic variation among all nine geographic populations (Φ_{ST} = 0.63, $P < 0.0001$) and among the five groups defined by STRUCTURE (Φ_{CT} = 0.32, $P < 0.0001$). In contrast, molecular variance of the mitochondrial locus showed strong and significant genetic variation among populations (Φ_{ST} = 0.76, $P < 0.0001$), but no additional variation among groups (Φ_{CT} = 0.00, P = 0.5). Consistent with this result, the percentage of variation among populations within groups was lower for nuclear (31.54%) than for mitochondrial data (75.25%), whereas the percentage of variation among groups was higher for nuclear (31.77%) than for mitochondrial data (0.38%). Both nuclear and mitochondrial data exhibited substantially less variation within populations (nuclear: 36.69%; mitochondrial: 24.37%) than among populations and groups (nuclear: 63.31%; mitochondrial: 75.63%).

Neutrality tests for mitochondrial sequences were all nonsignificant for Tajima's D and showed that only two of the populations, North Nicaragua and South Nicaragua, differed significantly from a neutral model by Fu's F_s test (Table 2). Mismatch distributions showed that South Nicaragua in particular carried a signature of sudden population expansion (Table 3).

Mantel tests for isolation by distance revealed nonsignificant correlations between geographic and genetic distances for both nuclear (r = 0.046, P = 0.4) and mitochondrial loci (r = 0.227, P = 0.1).

Table 3 Mismatch distribution statistics for sudden population expansion for North and South Nicaragua. The observed distribution differs significantly from the unimodal distribution expected under population expansion when $P < 0.05$

Population	SSD	*P* (SSD)	τ	θ0	θ1
N Nicaragua	0.054	0.108	1.896	0.000	99999.000
S Nicaragua	0.00078	0.940	1.500	0.000	407.525

Climatic niches of Azteca pittieri

Climatic niches, and particularly the precipitation niches, of sites occupied by northern and southern lineages were distinct. The first three principal components of the 19 bioclimatic variables associated with each of the 144 collection points for *A. pittieri* described 84.5% of the data. The first principal component was composed of both temperature and precipitation variables; the second principal component was defined mostly by temperature, particularly the temperatures of the driest and coldest periods; the third principal component was defined mostly by precipitation, particularly the precipitation of the driest and warmest periods (Table S5, Supporting information). All six *A. pittieri* lineages overlapped substantially in temperature niche (PC1 v. PC2; Fig. 5A); however, northern and southern lineages showed strong partitioning by precipitation niche (PC1 v. PC3; Fig. 5B).

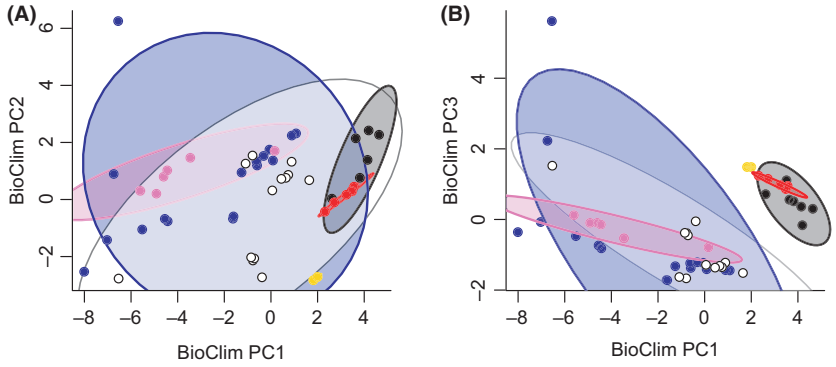

Fig. 5 Distribution of geographical occurrences of *Azteca pittieri* lineages relative to the first three principal components of 19 bioclimatic variables describing temperature and precipitation of the respective location (see Table S5, Supporting information). BioClim PC1 described a mix of temperature- and precipitation-related variables; BioClim PC2 described mostly temperature variables (A), and BioClim PC3 described mostly precipitation variables, particularly precipitation patterns in the driest times of year (B). Distinct colours of points and ellipses represent distinct lineages; colours of lineages are as in Fig. 1. Ellipses represent 95%-confidence levels of the distribution of data points.

Discussion

Here, we investigated the phylogeography of *Azteca* ants that are obligate symbionts of live plant stems of the tree *Cordia alliodora* in the northern Neotropics. We recovered all current morphological species as monophyletic and found higher diversity of ant symbionts at lower latitudes, closer to the equator. Mitochondrial and nuclear gene trees agreed at the species level, but there was substantial disagreement within species, perhaps caused by the different modes of inheritance of mitochondrial and nuclear genomes, or by incomplete lineage sorting within the nuclear genome. The timing of diversification of *Azteca* corresponds with other studies of lowland, dry-forest taxa from Middle America, suggesting important biogeographic roles for the origin of Middle American dry forests, marine incursions across lowland areas, and Pleistocene climate changes. Within the obligate and most common symbiont, *Azteca pittieri*, we found strong phylogeographic structure. Most of the diversification within *A. pittieri* occurred subsequent to the proposed arrival of the host tree, approximately 3 Ma, to Middle America (Rymer *et al.* in press). In contrast to the host tree, there are apparently low levels of gene flow between geographic populations of *A. pittieri*. This may facilitate local adaptation of symbiotic plant-ants to the distinct climatic niches of the northern and southern parts of their Middle American distribution.

Phylogenetic reconstruction

Our results showed substantial divergence within the *Azteca* ant symbionts. We identified at least nine monophyletic lineages of *C. alliodora*-dwelling *Azteca*; all morphological species reported by Longino (2007) were recovered as monophyletic. The two most abundant symbionts were the generalist *Azteca beltii*, distributed from Costa Rica to Southern and Eastern Mexico, and the specialist *A. pittieri*, distributed throughout Middle America. We identified two principal, geographically disjunct lineages within the *A. pittieri* complex, one in the south, including Costa Rica and Southern Nicaragua (the latter only when considering the mitochondrial data), and the other in the north, from Mexico to Nicaragua. Within the northern lineage, there was additional genetic structure, including distinct lineages from Nicaragua through El Salvador, Southeastern Mexico (Los Tuxtlas), Southern Oaxaca, Mexico (Huatulco), and Western Mexico.

Gene tree discordance

Nuclear and mitochondrial markers independently recovered morphological species as monophyletic. Within *A. pittieri*, however, there was substantial disagreement in the placement of lineages between nuclear and mitochondrial markers. First, the split between northern and southern lineages was different: occurring between Northern and Southern Nicaragua in the mitochondrial data and between Nicaragua and Costa Rica in the nuclear data. Our data indicate that Nicaraguan populations, and particularly the population from Southern Nicaragua, underwent sudden population expansion in the mitochondrial genome, unlike the other populations of *A. pittieri*. Mitochondrial sequences evolve more rapidly than nuclear sequences in *Azteca* (Table 2), as in most other taxa (Palumbi *et al.* 2001), and have the tendency to introgress more readily than nuclear regions between populations in close proximity (Bachtrog *et al.* 2006). Thus, we suggest that the

discordance between gene trees was caused by Nicaraguan populations that dispersed from the north to the south and came into secondary contact with Costa Rican individuals, which resulted in rapid mitochondrial introgression. A similar scenario could be imagined for why the Southeastern Mexico (Los Tuxtlas) lineage and Isthmus of Tehuantepec lineage are more similar to southern lineages in the mitochondrial tree than in the nuclear tree.

Second, there was greater divergence between the Western Mexico populations, Chamela and Guerrero, in the mitochondrial tree than in the nuclear tree. Because mitochondrial sequences are evolving faster than nuclear sequences, we suggest that this could be due to a relatively recent barrier to gene flow between these populations, and incomplete lineage sorting in the nuclear markers. Interestingly, divergence between Chamela and Guerrero populations has also been observed in lowland iguanas (Zarza *et al.* 2008), indicating that there may have been an important historical biogeographic barrier for lowland, seasonally dry-forest-dwelling taxa between these geographically proximate areas.

Finally, there were interesting and comparatively inexplicable patterns of gene tree discordance within the Costa Rican population. Two distinct, but small, sets of individuals from the area near the Monteverde Cloud Forest (Santa Elena), Costa Rica, were very divergent and placed as sister to the rest of *A. pittieri* in the mitochondrial and nuclear trees, respectively. Possible explanations for these unusual patterns include mitochondrial genome capture related to infection by the bacterial endosymbiont *Wolbachia* (Xiao *et al.* 2012) and/or hybridization between *A. pittieri* and other species (Feldhaar *et al.* 2008). However, we note that this same region of Costa Rica is also the only place where the apparent *C. alliodora* specialist *Azteca oecocordia* has been found (Longino 2007), indicating that there may be an unusual history of *Azteca* ants in this area.

Divergence times

The origin of *C. alliodora* ant symbionts occurred approximately 15.4 Ma, which approximately corresponds to mid-Miocene-cooling scenarios for the expansion of tropical dry forests (Dick & Wright 2005; Graham 2010; De-Nova *et al.* 2012). Interestingly, despite having only two calibration points connected to *Azteca* by long branches, this date is highly concordant with the 14 Ma estimated previously for the divergence of *Azteca* in a different analysis with six internal calibration points (Ward *et al.* 2010). This concordance lends credence to the dates we recovered internal to *Azteca*.

Higher genetic diversity in *C. alliodora* in South America than in Middle America (Rymer *et al.* in press) and the presence of the sister species, *Cordia trichoma*, in South America (Gottschling *et al.* 2005) indicate that the tree may have originated in South America and dispersed north to Central America and Mexico when conditions favored dry-forest expansion in the Quarternary (Rymer *et al.* in press). The higher diversity of *C. alliodora*-dwelling *Azteca* lineages in South Middle America suggests that *Azteca* may also have dispersed from south to north (Fig. 1). However, sampling of *Azteca* in South America has been more limited than in Middle America, and additional studies will be required to completely reconstruct the historical biogeography of the genus. If *C. alliodora* arrived in Middle America in the Quarternary, approximately 3 Ma, unaccompanied by its ant symbionts from South America, then much of the diversification in *Azteca* symbionts, including the origin approximately 7.7 Ma of the *C. alliodora* specialists, *A. oecocordia* and *A. pittieri*, occurred prior to the tree's arrival. This suggests a pattern of host-switching in the history of this mutualism, similar to patterns revealed in other ant–plant symbioses (Ayala *et al.* 1996; Chenuil & McKey 1996; Feldhaar *et al.* 2003).

Our results suggest that the *A. pittieri* species complex shared a most recent common ancestor during the Pliocene, approximately 4.5 Ma. Within the *A. pittieri* species complex, a stem lineage of approximately 2 Myr leads to the subsequent diversification (approximately 2 Ma) of northern lineages (Mexico to N. Nicaragua), and a stem lineage of approximately 3.5 Myr leads to the recent diversification (approximately 0.7 Ma) of southern lineages (S. Nicaragua to Costa Rica). The combination of significant tectonic activity (Barrier *et al.* 1998) and climate changes (Pennington *et al.* 2000) in Middle America since the Miocene have contributed to complex biogeographic patterns for many species in this area (Daza *et al.* 2010). Our data support the suggestion that there were important biogeographic barriers in two lowland areas—the Isthmus of Tehuantepec and the Nicaragua depression—that influenced the diversification of lowland taxa (Mulcahy *et al.* 2006; Zarza *et al.* 2008; Daza *et al.* 2010). These lowland areas may have been crossed by marine seaways in the Pliocene (Coates & Obando 1996; Barrier *et al.* 1998; Daza *et al.* 2010).

Although the MRCA of *A. pittieri* lived in the Pliocene, much of the diversification in *A. pittieri* occurred during the Pleistocene, corresponding with patterns of diversification of other dry-forest, lowland taxa in Middle America, including plants (Pennington *et al.* 2004) and iguanas (Zarza *et al.* 2008). This diversification also corresponds to dates when *C. alliodora* was probably already present in the Middle American dry forests. The frequent cycles of glacial and interglacial periods in the Pleistocene probably contributed to expansions and

contractions of the seasonally dry tropical forests (Pennington *et al.* 2000), which may have led to interglacial dry-forest refugia shaping genetic diversity in dry-forest species.

Population genetics and climatic niches of Azteca pittieri

There was strong support in the STRUCTURE analyses for at least five distinct genetic clusters in *A. pittieri*, mostly corresponding to distinct geographic areas. Within these clusters, there was little admixture from neighbouring populations, an observation that was supported by large, significant Φ_{ST} values among populations. The fifth cluster (brown in Fig. 4), which comprised Los Tuxtlas individuals, the five distinct Costa Rican individuals, and apparent admixture in Guerrero individuals, probably reflected unique variation in each of these geographic areas that would have separated into distinct clusters, as in the phylogenetic tree, with increased sampling and more statistical power. We note that, for our data, the ΔK statistic of Evanno *et al.* (2005) provided a very conservative estimate of the number of genetic clusters, possibly because standard deviations of the posterior probabilities increased with larger numbers of clusters in our complex data set. We also note that the clusters recovered in STRUCTURE using haplotypes from all five markers primarily reflected variation in the four-locus nuclear data set. This contrasts with the concatenated phylogenetic tree, which was more heavily influenced by the mitochondrial data set. In the STRUCTURE analysis, sequences were collapsed to unique haplotypes, and the four-locus nuclear data set had more degrees of freedom than the one-locus mitochondrial data set. Conversely, the presence of more informative polymorphisms in mtDNA sequence data relative to nuclear loci resulted in higher degrees of freedom for mtDNA in tree-based methods.

Despite the evidently strong barriers to gene flow among populations, our data did not show that populations of *A. pittieri* are reproductively isolated, and *A. pittieri* may still constitute a single biological species. Ross *et al.* (2010) found that lack of morphological resolution in South American *Solenopsis saevissima* ants obscured substantial genetic variation that indicated possible species boundaries between evolutionarily independent lineages. In *A. pittieri*, all of the observed genetic disjunctions occurred in parapatry, with the exception of the individuals from the Santa Elena region of Costa Rica. However, additional sampling of *Azteca* from other non-*C. alliodora* nesting sites may reveal that *A. pittieri* is paraphyletic if other *Azteca* species fall within the highly divergent lineages herein defined as *A. pittieri*. For example, in a widespread species of Neotropical tree, *Cedrela odorata*, an initial pattern of strong phylogeographic breaks among populations (Cavers *et al.* 2003) was later shown to be several species upon increased sampling of the genus (Muellner *et al.* 2010).

The geographic population structure we observed in *A. pittieri* across its Middle American range does not appear to arise primarily from isolation by distance. The lack of significant correlation between genetic and geographic distances for both mitochondrial and nuclear loci strongly suggests that historical geologic or climatic conditions created barriers to gene flow between neighbouring populations. Such historical barriers may be reinforced in the past or present by ecologically mediated adaptations driven by the local environment. Divergent selection on organisms between distinct environments may be a common path to speciation (Schluter 2009; Sobel *et al.* 2009), even in the absence of complete geographical isolation (Nosil 2008). There is a gradient of increasing precipitation with decreasing latitude in the Middle American dry forests (Stotz *et al.* 1996), and we found nonoverlapping climatic envelopes between northern and southern lineages of *A. pittieri*. Phylogenetic niche conservatism in tropical-dry-forest woody plants suggests that certain adaptations are necessary for success in seasonal tropical environments (Pennington *et al.* 2009). Individuals of *C. alliodora* are smaller and thinner and display different phenology in dry habitats (Boshier & Lamb 1997). Life-history traits of *A. pittieri* related to seasonality directly or to seasonality-induced changes in the host plant may thus be subject to different selective pressures between the northern and southern edges of its Middle American range.

Conclusions

Here, we have shown that the levels of gene flow among populations of *Azteca pittieri* plant-ants appear to be substantially lower than those previously shown in the host plant *C. alliodora* (Chase *et al.* 1995; Rymer *et al.* in press). The shorter generation times and dispersal distances of *Azteca* ants (Bruna *et al.* 2011; Orivel *et al.* 2011) relative to those of *C. alliodora* trees (Boshier *et al.* 1995; Boshier 2002) could mean that locally adaptive mutations fix more rapidly in *Azteca* than in their host trees, especially in the presence of natural barriers to gene flow, such as those existing between dry-forest interglacial refugia. Although studies directly comparing the levels of gene flow among populations of two or more mutualists are still rare, evidence to date from yucca–moth mutualisms (Godsoe *et al.* 2010; Smith *et al.* 2011), ant–plant mutualisms (Quek *et al.* 2007;

Guicking *et al.* 2011), and plant–fungal mutualisms (Hoeksema & Thompson 2007) suggests that asymmetries between mutualistic partners in the spatial scale of either gene flow or local adaptation may be quite common. In the case of the mutualism between *C. alliodora* and its *Azteca* ant symbionts, variations in traits of both mutualists have been identified over their broad geographic ranges (Longino 1996; Boshier & Lamb 1997; Pringle *et al.* 2011). Given the differences in population structure between trees and ants, the extent of local adaptation may be asymmetric, with important consequences for mutualistic coevolution.

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This work represents part of E.G.P.'s PhD dissertation, co-supervised by D.M.G. and R.D. E.G.P. studies the ecology and evolution of species interactions, with a particular focus on mutualisms. S.R.R. studies the evolutionary biology, chemical ecology, and genomics of insects, including their associations with host plants. T.C.B. has broad interests in global change and tropical ecology. D.M.G. studies the evolutionary ecology of collective behaviour in ants. R.D. is interested in the evolutionary ecology of species interactions in tropical ecosystems, as well as the effects of anthropogenic impact on biodiversity and processes, particularly plant-animal interactions, in the tropics.

Author contributions

E.G.P., D.M.G., and R.D. conceived the study and designed research. E.G.P., S.R.R., and T.C.B. performed research. EGP and SRR analyzed the data. E.G.P., S.R.R., D.M.G., and R.D. wrote the article.

Data accessibility

DNA sequences: GenBank accessions JQ867506–JQ868413.

Phylogenetic data: TreeBASE URL: http://purl.org/phylo/treebase/phylows/study/TB2:S12472.

Sequence alignments, haplotypes, distance matrices, bioclimatic variables, Arlequin input files: DRYAD entry doi:10.5061/dryad.p8n5kb15.

Supporting information

Additional supporting information may be found in the online version of this article.

Appendix S1 Collection and voucher data for all samples.

Table S1 Primers used for PCR amplification and sequencing.

Table S2 Sequence characteristics and models of evolution.

Table S3 Nuclear and mitochondrial genetic distances among *Azteca* lineages.

Table S4 Estimated ages of highly supported clades.

Table S5 Principal components of bioclimatic variables from *A. pittieri* collection sites.

Fig. S1 Phylogram of *Azteca* collected from *C. alliodora* with voucher codes.

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