



Recurrent breakdowns of mutualisms with ants in the neotropical ant-plant genus *Cecropia* (Urticaceae)

Juanita Gutiérrez-Valencia, Guillaume Chomicki*, Susanne S. Renner

Systematic Botany and Mycology, University of Munich (LMU), Menzinger Str. 67, 80638 Munich, Germany



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ABSTRACT

Mutualisms could be evolutionarily unstable, with changes in partner abundances or in the spatial context of interactions potentially promoting their dissolution. We test this prediction using the defense mutualisms between species of the Neotropical genus *Cecropia* and *Azteca* ants. A new, multigene phylogeny with representatives of all five genera of Cecropieae (most of them from the Neotropics) and half of the 61 species of *Cecropia* shows the West African endemic *Musanga* (2 spp.) as sister to *Cecropia*, implying dispersal from the Neotropics to Africa, with a molecular clock suggesting that this occurred about 23 Mya. *Cecropia*, a genus of neotropical pioneer trees, started diversifying ca. 8 Mya. We infer a single origin of specialized symbiosis with *Azteca* within *Cecropia*, eight complete losses of this symbiosis, and a potential partner shift involving the replacement of *Azteca* by *Neoponera luteola* ants. Niche space modeling based on geo-referenced occurrences of over 9000 collections representing 58 of the 61 species of *Cecropia*, together with several comparative analyses, implies that mutualism loss is concentrated at high altitudes and on Caribbean islands, with the surprisingly frequent breakdowns potentially facilitated by low species-specificity of interacting *Cecropia* and *Azteca* mutualists.

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1. Introduction

Mutualisms are interactions between species in which associated partners obtain benefits from each other; their net outcome is highly context-dependent (Chamberlain et al., 2014). Mutualistic systems are therefore thought to be evolutionarily unstable with two main predicted outcomes. First, transition to parasitism should be frequent because ‘cheaters’ should have a higher fitness by obtaining the benefits without paying the costs (Axelrod and Hamilton, 1981). Available phylogenetic and ecological data, however, suggest that species evolving from being mutualists to parasites are rare (Sachs and Simms, 2006) and that unrelated exploiters (either generalist or specialized) may be a more important selective pressure on mutualisms (Sachs and Simms, 2006; Frederickson, 2013; Chomicki et al., 2015, 2016). The second predicted outcome is a complete loss of the mutualism when partners become rare or even absent (Chomicki and Renner, 2017). A better understanding of such evolutionary transitions requires large clades in which several transitions occurred and the ability to relate them to intrinsic or extrinsic factors.

* Corresponding author.

E-mail address: guillaume.chomicki@gmail.com (G. Chomicki).

The defense of plants by ants has been at the core of studies aiming to understand the stability of mutualisms (Bronstein, 1998). In exchange for protection from herbivores and the elimination of surrounding competitors, plants reward ants with nesting places (domatia) or/and carbon-based food sources (O'Dowd, 1982; Davidson and McKey, 1993; Chomicki et al., 2016). Plant structures meant for ant housing and feeding have been gained and lost hundreds of times (Weber and Keeler, 2013; Chomicki and Renner, 2015; Chomicki et al., 2017), making ant/plant interactions suitable for investigating convergent evolutionary transitions in mutualisms.

The protective mutualisms between the Neotropical plant genus *Cecropia* and ants of the genus *Azteca* have been the subject of numerous ecological studies (Bequaert, 1922; Wheeler, 1942; Janzen, 1973; Schupp, 1986; Longino, 1989; Folgarait and Davidson, 1995; Vasconcelos and Casimiro, 1997; Latteman et al., 2014). Such symbioses occur in ca. 46 of the 61 species of *Cecropia* (whether they are also present in *C. chlorostachya* and *C. velutinella* is unknown; Berg and Franco-Rosselli, 2005) that interact with species of *Azteca*, with each *Cecropia* species being occupied by one or more *Azteca* species throughout its range. At least six species of *Cecropia* harbor ants from other genera, such as *Neoponera*, *Camponotus* and *Crematogaster*, but whether these ants are mutualists that defend their hosts or parasites that only take advantage

of the domatium and food rewards remains to be investigated in the field. The *Azteca*-occupied *Cecropia* harbor 13 species of that genus, with little species-specificity (Longino, 1991; Yu and Davidson, 1997). The 13 *Cecropia*-inhabiting *Azteca* species apparently are not monophyletic, indicating independent colonization or independent abandonment of *Cecropia* trees (Longino, 1991; Ayala et al., 1996).

In myrmecophytic *Cecropia* species, ants nest in the internodes of the trunk and branches into which several competing founder queens enter by biting a small hole through a preformed weakened region, called the prostoma; the queen and her workers then remove the softened parenchyma (pith) to enlarge the domatium space and connect the hollow internodes (Wheeler, 1942; Davidson, 2005; Valverde and Hanson, 2011). The ants harvest glycogen-rich multicellular bodies (Müllerian bodies) produced on pads of trichome-covered tissue (trichilia) at the petiole-stem interface (Rickson, 1971, 1976a, 1976b; Bischof et al., 2013; Fig. 1) to feed their larvae (V. Mayer, pers. comm. to G.C., February 2017). Eleven species of *Cecropia* are non-myrmecophytic (Longino, 1989, 1991; Berg and Franco-Rosselli, 2005), and Janzen (1973) suggested that colonization of Caribbean islands or the occupation of high-altitude habitats might correlate with loss of ant mutualisms. Two of the non-ant associated species, *C. sciadophylla* and *C. hololeuca*, however, inhabit lowlands (<1000 m alt., Global Biodiversity Information Facility, GBIF: <http://www.gbif.org>), arguing against a consistent correlation between high-altitude occurrence and loss of ant occupation.

Another open question about the evolution of the ant mutualisms within the *Cecropia* clade derives from the position of the

African genus *Musanga*. The latter comprises two tree species occurring in West and Central Africa that resemble *Cecropia* in habit and ecology, except for never housing ants (de Ruiter, 1976). It has been hypothesized that the common ancestor of these African species lost its interactions with *Azteca* ants when dispersing to Africa (Janzen and McKey, 1977). However, molecular phylogenetic analysis of the tribe Cecropieae (Treiber et al., 2016), which comprises three Neotropical genera (*Cecropia* Loebl., *Coussapoa* Aubl., and *Pourouma* Aubl.) and two African genera (*Musanga* R. Br., and *Myrianthus* P. Beauv.), did not support this hypothesis. Treiber et al. (2016) retrieved *Musanga cecropioides* (the only *Musanga* sampled) as sister to the non-myrmecophytic *C. sciadophylla*, and both sister to the rest of *Cecropia*, but their sampling was limited to seven of the 46 *Cecropia* species associated with *Azteca* ants, and three of the 11 non-myrmecophytic *Cecropia* species (Treiber et al., 2016).

In the present study we use a denser phylogenetic sampling of *Cecropia* species to answer three questions, (i) Is the West African genus *Musanga* indeed embedded among neotropical *Cecropia*? (ii) Given that *Azteca* phylogenetic relationships imply repeated colonization or independent abandonment of *Cecropia* trees by these ants (Longino, 1991; Ayala et al., 1996), are there phylogenetic or morphological clues pointing to repeated origins or losses of the *Azteca/Cecropia* symbioses? And (iii) are there common environmental or intrinsic factors favoring the persistence or breakdown of *Azteca/Cecropia* mutualisms as Janzen (1973) hypothesized? We also use molecular clock-dating to infer a geological time frame for the *Cecropia* and *Azteca* mutualisms and for the dispersal between South America and Africa. Prior work

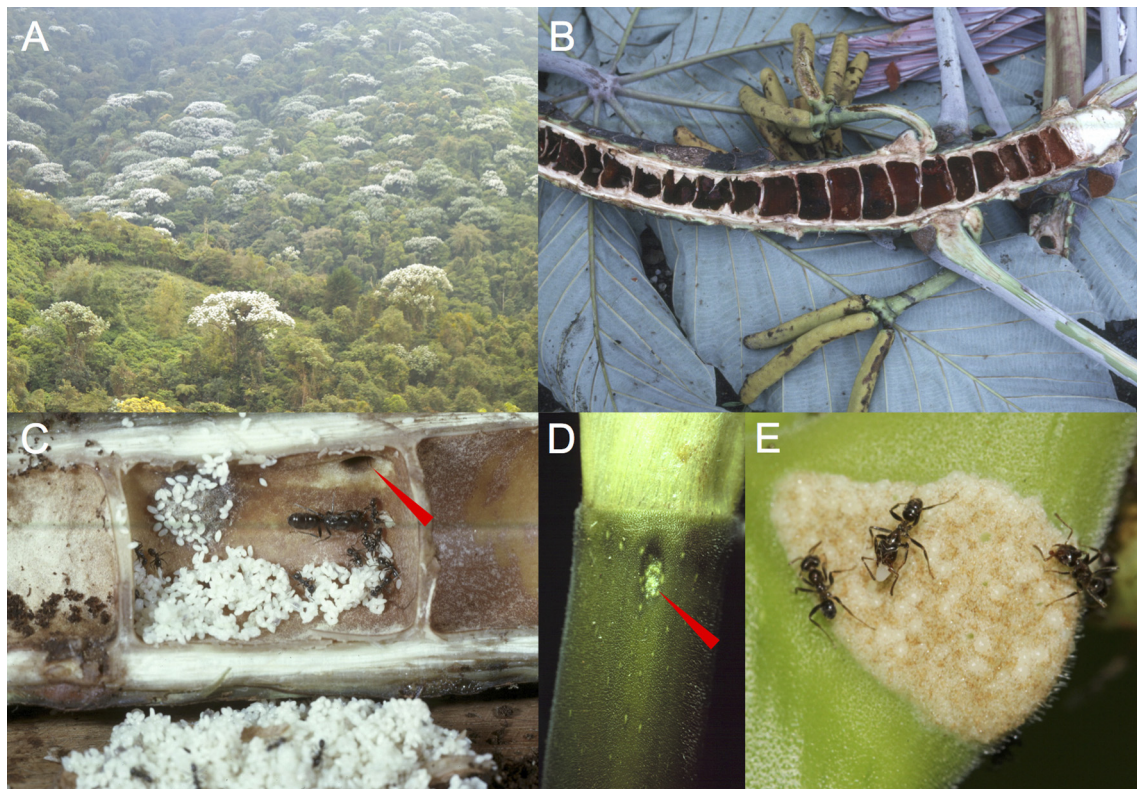


Fig. 1. *Cecropia/Azteca* mutualism. (A) *Cecropia* contains the most abundant pioneer trees in the Neotropics. (B, C) At least 46 species are engaged in regular mutualistic interactions with *Azteca* ants that nest in the plants' hollow stems and protect their host plants against herbivores. (C–D) Founder queens colonize the stems by cutting a hole at a preformed site, called prostomata. Red arrows point to the internal view of a perforated prostomata (C) and the external view of a young prostomata. (E) Mutualistic ants are also rewarded with Müllerian bodies, lipid- and protein-rich trichomes produced in trichilia pads; these bodies are removed by *Azteca* workers. Photographic credit: A: Samuelsohl; B–D: J. Longino; E: P. Marting. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

has dated the most-recent common ancestor (MRCA) of *Azteca* to 14 (7–22 95% CI) Mya (Ward et al., 2010), and the most recent common ancestor of *Cecropia* to 29 Mya (Zerega et al., 2013) or 4.78–11.92 Mya (Chomicki and Renner, 2015; Fig. S3).

2. Materials and methods

2.1. Taxon sampling, DNA sequencing, alignment, and phylogenetic analyses

Our sampling of Cecropieae includes the following representation of its five genera: *Cecropia*, 33 of its 61 species (Berg and Franco-Rosselli, 2005); *Coussapoa*, eight of its ca. 50 species (Berg and Ulloa Ulloa, 2014); *Pourouma*, seven of its 29 species (Gaglioti and Neto, 2014); both species of *Musanga* (de Ruiter, 1976), and three of the seven *Myrianthus* species (de Ruiter, 1976). Overall, this sample represents ca. 36% of the named species in the tribe. Trees were rooted on *Leucosyke* (*L. quadrinervia*, *L. capitellata*, *L. australis*) and *Moutia* (*M. puya*, *M. setosa*) based on Wu et al. (2013). We sampled twenty *Cecropia* species that are always occupied by *Azteca* ants, eleven species that are not associated with ants, one that is associated with *Azteca* in the mainland but not in the Caribbean Islands (*C. peltata*; Janzen, 1973; Berg and Franco-Rosselli, 2005), and one, namely *C. membranacea*, that is facultatively associated with *Neoponera luteola* (Table S1).

We generated 172 new sequences of four chloroplast (*rbcl*, *matK*, *trnL-trnF*, and *ndhF*) and three nuclear regions (ITS-I, ITS-II, and 26S) from specimens deposited in the herbaria of Aarhus (AAU), Bergen (BG), La Paz (LPB), Munich (M), New York (NY), and Paris (P). DNA extraction, targeted-region amplification, and sequencing followed standard procedures. Total genomic DNA was extracted from 20 to 25 mg of leaf tissue, using plant DNA extraction kits (NucleoSpin, Macherey-Nagel, Düren, Germany) according to manufacturers' instructions. Polymerase chain reactions (PCR) followed standard protocols, using Taq DNA polymerase (New England Biolabs, Ipswich, MA, USA) and a set of primers listed in Table S2. PCR products were purified with the ExoSap clean-up kit (Fermentas, St. Leon-Rot, Germany), and Sanger sequencing was performed with the Big Dye Terminator kits (Applied Biosystems, Foster City, CA, USA) in an ABI 3130 automated sequencer. All newly generated sequences were BLAST-searched with default parameters settings (<https://blast.ncbi.nlm.nih.gov/Blast.cgi>) to confirm the identity of the amplified region and the taxonomic affinity of the specimens. Table S3 lists species names and taxonomic authors, voucher numbers, geographic origin, and GenBank accession numbers for sequences used in this study.

Sequences of each marker were aligned separately using the iterated refinement method G-INS-i implemented in MAFFT v7 (Katoh and Standley, 2013). The resulting alignments were concatenated in SeaView v4.6.1 (Gouy et al., 2010). The final matrix consisted of 4559 aligned nucleotides and 58 species, with 53 sequences of the *rbcl* gene (269 aligned nucleotides), 24 sequences of the *matK* gene (243 aligned nucleotides), 18 sequences of the *trnL-trnF* intergenic spacer (398 aligned nucleotides), 33 sequences of the *ndhF* gene (1986 aligned nucleotides), 42 sequences of the ITS-I region (317 aligned nucleotides), 36 sequences of ITS-II (349 aligned nucleotides), and 43 species of the 26S rDNA region (998 aligned nucleotides).

Phylogenetic trees were inferred using Maximum Likelihood (ML) optimization as implemented in RAxML v. 8.0.26 (Stamatakis, 2014), with the GTR + Γ substitution model and 100 bootstrap replicates under the same model. Trees were visualized using FigTree v1.4.3 (<http://tree.bio.ed.ac.uk/software/figtree/>; Rambaut, 2007). We conducted a second analysis using a reduced

matrix that included only the 28 species represented in the alignment by five or more sequences, including both ITS regions, these being the most informative. The 28-species matrix included two species of *Pourouma*, four of *Coussapoa*, two of *Myrianthus*, one of *Musanga*, 17 of *Cecropia*, and *Leucosyke quadrinervia* to root the tree.

2.2. Molecular clock dating

Dating relied on Bayesian optimization in BEAST v1.8.2 (Drummond et al., 2012) and a reduced matrix with 47 of the 58 species, after removing accessions with very short or zero-length branches and *Myrianthus*, which showed unsupported and conflicting positions. Zero-length branches are known to cause problems for Bayesian time estimation algorithms (and may introduce a bias) because flat topological priors prohibit them (Lewis et al., 2005; Lemmon et al., 2009). This matrix was subsequently used for all ancestral state reconstruction analyses. Genetic branch lengths in the Bayesian tree were converted into absolute times by applying alternative rate calibrations and secondary calibrations since well-placed Cecropieae-like fossils are not available (Collinson, 1989). First, we applied a rate of 0.0011 subst./site/myr to the nuclear data partition (from Savard et al., 1993; *Alnus*), leaving the plastid partition unlinked. Second, we applied a rate of 0.00056 subst./site/myr to the plastid (from Palmer, 1991), leaving the nuclear partition unlinked. Lastly, we used two secondary constraints from Zerega et al. (2005) who calibrated their analysis of Moraceae and Urticaeae (including four species of *Cecropia*) with four Moraceae fossils and a root node constraint of 132 myrs based on the oldest known angiosperm fossil. Specifically, we set the divergence between Cecropieae and the clade to which *Leucosyke* belongs to 64 ± 1 Myr and the MRCA of Cecropieae to 44 ± 1 Myr. Each calibration approach was used in strict and relaxed clock models, but since the coefficients of variance estimated for the substitution rates were >0.5 in all the cases, subsequent analyses focused on relaxed clock models. We used pure-birth (Yule) tree priors, the GTR + Γ substitution model, and Monte Carlo Markov chains (MCMC) of 1 billion generations, with parameters sampled every 1000th generation. Tracer v1.6 (part of the BEAST package) was used to assess effective sample sizes (ESS > 200) for all estimated parameters and TreeAnnotator v1.8.2 (part of the BEAST package) to discard the first 10% of the trees as burn-in and to combine trees. Maximum clade credibility trees with mean node heights were visualized using FigTree.

2.3. Inference of ancestral trait states and the evolution of interactions

Because a few species were excluded from molecular-clock dating (previous section), representations in the chronogram used for ancestral state reconstructions (ASRs) were as follows: (i) 17 *Azteca*-occupied *Cecropia*, (ii) nine non-ant-associated species, and (iii) one species (*C. membranacea*) engaged in facultative symbioses with *Neoponera luteola* (Table S1). Because we only had one facultative non-*Azteca* mutualist, we grouped it with non-ant associated *Cecropia*, so that we were left with only two categories: ant associated (18 species) and not ant associated (9 species). For the coding of states in *Pourouma* and *Coussapoa* we relied on the monographs of these genera by Berg et al. (1990). The coding of myrmecophytic strategy was based on ecological literature and herbarium labels (Table S3), where ant occupation has been directly observed and not inferred from traits (which would be circular). The coding of morphological traits was based on specimens studied by the first author and descriptions in Berg and Franco-Rosselli (2005). We coded three traits having two or three states: Stem pith (absent; present [when the stem is filled as, for instance, in *C. andina*, or woody as in *C. sciadophylla*]; or variable); prostomata (indistinct

or distinct); and trichilia (absent, present, or variable). We decided not to code the presence or absence of Müllerian bodies because (i) it is uncertain whether some non-mymecophyte species also produce these modified trichomes, which can be removed by insects others than ants (Andrade, 1984), and (ii) they have not been observed in all trichilia-bearing *Cecropia* because ants remove them as soon as they are produced (Berg and Franco-Rosselli, 2005).

For the ASRs we used the ML approach implemented in Ape (Paradis et al., 2004) and the stochastic mapping approach (Bollback, 2006) as implemented in phytools v. 04-60 (Revell, 2012), in each case using the 47-species chronogram. For both approaches, we compared three models of trait change, (i) an equal rates (ER) model, wherein all rates are equal; (ii) a symmetrical rate model (SYM), wherein the backward and forward character state transition rates are equal for each combination of character states, but distinct state combinations can have distinct rates; and (iii) an all rates different (ARD) model, wherein all state transition rates are allowed to vary. The goodness of fit (log-likelihood) of each model was estimated using Akaike Information Criterion (AIC) values as implemented in Ape. The selection of the best model relied on the comparison of AICc scores using chi square tests, and if equally fitting, the simplest model was preferred. For the stochastic character mapping, we focused on the stem pith, prostomata, and trichilium traits. We simulated 1000 character histories on the maximum clade credibility tree. Similarly to the Ape approach, we used three models (ER, SYM and ARD) and selected the best model, using logLikelihood values obtained with the function fitMk in phytools followed by the estimation and comparison of their AIC values as described before.

2.4. Bayesian correlation between ant occupation and morphological traits

To ask whether the coded traits (pith, prostomata, trichilia) are correlated with each other, and which of them have an effect on regular *Azteca* occupation, we fitted the different Bayesian trait models implemented in the program BayesTraits v. 2 (Pagel and Meade, 2013). To simultaneously account for phylogenetic uncertainty, we used a set of 5000 trees from the BEAST analysis of the 47-species matrix. The models require all characters to be binary. We thus coded *Azteca*-specialized *Cecropia* species as “1” and the facultative ant-mutualistic species (*C. membranacea*) together with the non-ant-occupied species as “0”; trichilia present (“1”) or absent (“0”; *C. peltata*, *C. telenitida*, *C. gabrielis* and *C. tacuna*, which exhibit intraspecific variation, were coded as “0”); prostomata absent (“0”) or distinct (“1”); and stem filled with pith (“0”) or with reduced or no pith (“1”). Two models were tested, namely one of independent trait evolution (M_1) estimating the four transition rate parameters α_1 , α_2 , β_1 , β_2 , wherein double transitions from state 0,0 to 1,1 or from 0,1 to 1,0 are set to zero, and one of dependent trait evolution (M_2) with eight parameters (q12, q13, q21, q24, q31, q34, q42, q43). To compare these non-nested models, we calculated the Bayes Factor (BF) score. The evolution of these binary traits was inferred from 1000 simulations of character histories using the function densityMap or describe.simmmap (also in phytools).

2.5. Comparing niche space across mutualistic strategies

To test whether *Azteca*-inhabited *Cecropia* species and non-ant associated *Cecropia* have separate niches, we generated a list of 9416 occurrence data for the 58 recognised species represented in GBIF and then cleaned the data by checking that species' geographic and altitudinal ranges matched information in Berg and Franco-Rosselli (2005). We next downloaded all 19 bioclim variables (plus

altitude) at 30" resolution on WorldClim (<http://www.worldclim.org>). For each data point, we extracted the 19 bioclim climate variables plus altitude using the function 'extract' from the R package 'raster' (Hijmans and Van Etten, 2013). We then generated a file with a single average value per bioclim variable for each species. Since correlated variables can result in spurious results, we determined the Pearson's correlation coefficients between the bioclim variables and altitude and then included only variables with a Pearson's correlation coefficient <0.5. The analyses were thus performed with altitude, bio_7 (temperature annual range), bio_16 (precipitation of wettest quarter), bio_17 (precipitation of driest quarter), and bio_18 (precipitation of warmest quarter). We used the R package vegan (Oksanen et al., 2013) to perform non-metric multidimensional scaling (NMDS) and used either (i) the whole dataset of 58 *Cecropia* species or (ii) only the 27 *Cecropia* species represented in our chronogram. For the latter analysis, we extracted the NMDS1 and NMDS2 values for each species and then matched the data to the phylogeny tips as input for a phylomorphospace that showed the ordination, phylogeny, and mutualism strategies, using the function 'phylomorphospace' of the R package phytools (Revell, 2012). Finally, we performed a multinomial logistic regression model to estimate the probability of the occurrence of a given mutualistic strategy (*Azteca*-specialist; facultative ant occupied [used only in the logistic regressions since four facultative species are represented, Supplementary Dataset S1]; not ant occupied) along the altitudinal gradient by linking each specimen record to the strategy of the species it represents.

The colonization of Andean high-altitude habitats has been previously identified as a possible cause of the dissolution of ant mutualisms in *Cecropia* (Janzen, 1973). To test this, we performed another ASR based on the mean altitude at which each species occurs, and compared the mapping of this continuous trait against the binary-coded ASRs (above). We used 7863 records of *Cecropia* and 6606 records of the other *Cecropieae* included as outgroups in the tree (Supplementary Dataset S1) by extracting elevation from georeferenced records from GBIF as described before. *Coussapoa panamensis* has been synonymized with *C. villosa* (Berg et al., 1990), but specimens representing these species do not cluster together in our ML tree (Fig. 2A); we used the altitude of 500 m from a specimen of *C. panamensis* deposited in Munich (P. Döbberler 771, Costa Rica, 1987). The continuous trait 'elevation' was mapped on the *Cecropia* chronogram using the ML approach implemented in the functions fastAnc and contMap in phytools (Revell, 2012).

3. Results

3.1. Phylogenetic relationships and divergence times

The Maximum Likelihood tree from the 58-species matrix resolved the five genera of *Cecropieae* as monophyletic, but left most within-*Cecropia* species relationships unresolved (Fig. 2A, Fig. S1). The African genus *Musanga*, however, was always supported as sister to *Cecropia* (Fig. 2A, Figs. S1 and S2). A ML tree with 32 (ca. 50%) of the species of *Cecropia* shows *C. sciadophylla*, a non-mymecophytic species, as sister to the remaining species (Fig. 2A), followed by *C. hololeuca*, also not associated with ants. The next divergence involves a polytomy that includes *C. hispidissima*, housing *Neoponera* ants, and the *Azteca*-inhabited *C. litoralis*, as well as all remaining species, most of them housing *Azteca*, with the exception of the Caribbean non myrmecophytic *C. schreberiana* (Fig. 2A, *Cecropia* clade I), and a group of mostly non-mymecophytes (*Cecropia* clade II). *Cecropia* clade I is supported by a non-synonymous substitution in *rbcl* (a threonine or serine replaces the asparagine in the residue 95 as marked in Fig. 2A).

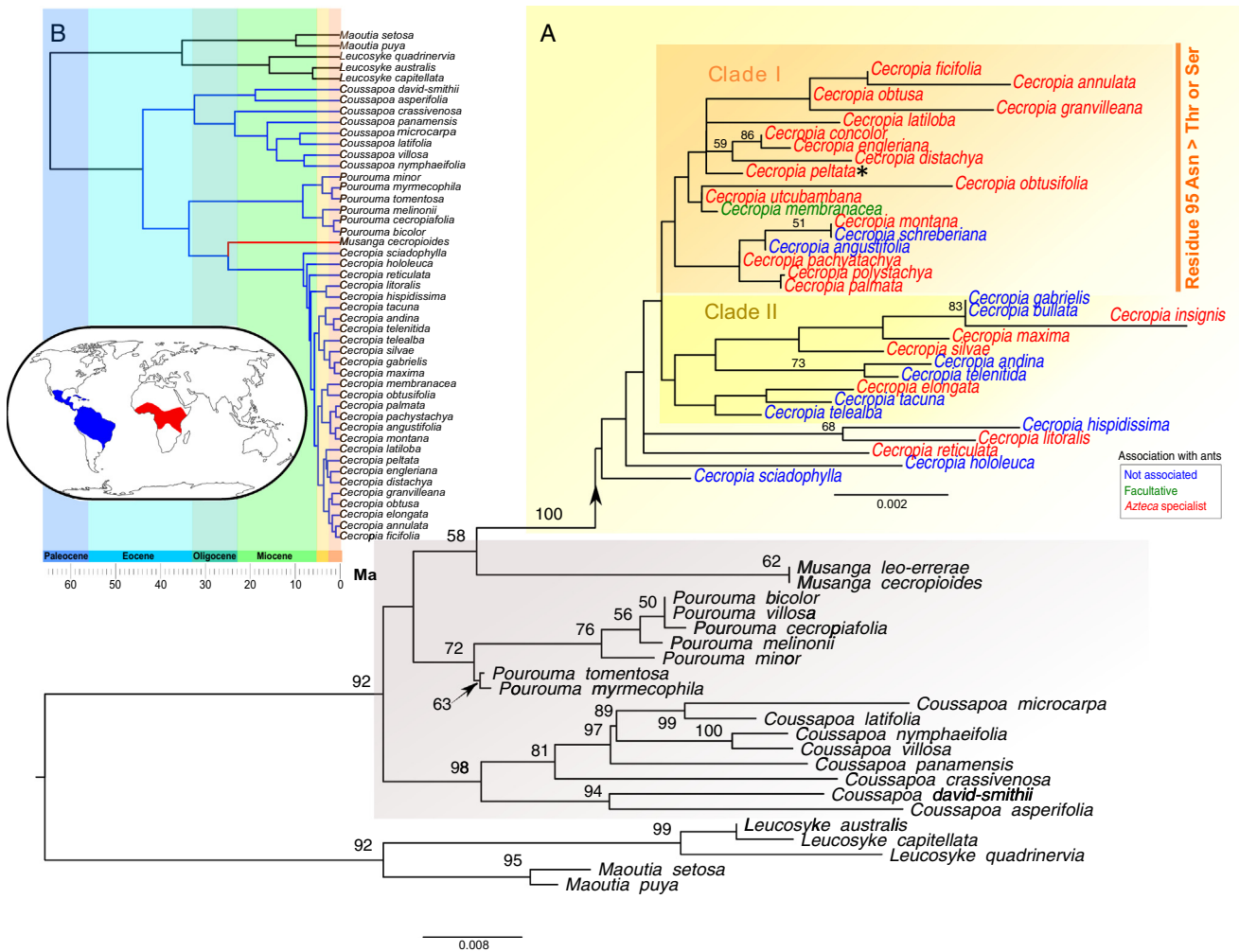


Fig. 2. (A) Phylogenetic relationships in Cecropieae inferred from nuclear and plastid sequences. Values above branches show the Maximum Likelihood bootstrap support (>50) from 100 replicates. Clade I is supported by a non-synonymous substitution in residue 95 of the *rbcl* gene (labeled Residue 95 Asn > Thr or Ser). (B) Dated phylogeny of Cecropieae obtained under a nuclear substitution rate-calibrated relaxed clock model (see Table 1 for other calibration schemes). The outgroups *Maoutia* and *Leucosyke* occur in South East Asia.

Table 1

Node ages for selected divergence events under a relaxed clock model using different calibration approaches. Ages are given in million years, and values in brackets are the 95% posterior probability intervals.

Estimation approach	<i>Cecropia</i> / <i>Musanga</i> divergence	<i>Cecropia</i> crown
Plastid substitution	18.60	6.03
rate-based ^a	(12.70–23.77)	(4.26–7.79)
Nuclear substitution	22.97	8.38
rate-based ^b	(13.73–32.15)	(5.37–11.89)
Node calibration ^c	22.88	11.13
	(14.41–32.23)	(6.42–16.70)

^a 0.00056 subs./site/My (Palmer, 1991).
^b 0.0011 subst./site/My (Savard et al., 1993).
^c Based on the fossil-calibrated chronogram of Zerega et al. (2005), we set the divergence between Cecropieae/*Leucosyke* to 64 ± 1 Myr and the MRCA of Cecropieae to 44 ± 1 Myr.

The divergence times obtained with the three calibration approaches are shown in Table 1, and Fig. 2B shows a chronogram for the Cecropieae resulting from the nuclear rate calibration. *Musanga* and *Cecropia* diverged from each other at the Oligocene/Miocene boundary, about 23 (13.73–32.15) Mya, and the crown group of *Cecropia* started to diversify 8.38 (5.37–11.89) Mya, that is, during the late Miocene.

3.2. Ancestral state reconstruction of mutualistic traits in *Cecropia*

The ancestral state reconstructions yielded consistent results with both the ML and stochastic mapping approaches (Fig. 3, Fig. S3, Table S4) and imply that the Most Recent Common Ancestor (MRCA) of the living species of *Cecropia* formed a specialized symbiosis with *Azteca*, had reduced pith, and prostomata but no trichilia (Fig. 3, Table S4). We identified eight evolutionary losses of *Azteca* occupation (counting the non-myrmecophyte *C. schreberiana* which, although absent in the chronogram, is embedded in the mainly-myrmecophytic clade I in the full ML tree (Figs. 1B and 3A)). There appears to have been one partner shift from obligate *Azteca* occupation to facultative interactions with *Neoponera luteola* (namely in *Cecropia membranacea*).

Stems with reduced parenchyma pith (Fig. 1B) were inferred as the ancestral state in *Cecropia* (Fig. 3B) and as possibly present since the *Cecropia*/*Musanga* divergence in the ML analysis (Fig. S3E). Under the all rates different (ARD) model, prostomata were inferred as already present in the *Cecropia* MRCA, followed by five independent losses in *C. hololeuca*, and the Andean species *C. andina*, *C. tacuna*, *C. telealba* and *C. gabrielis* (Fig. 3C). Under the ARD model, trichilia originated after the divergence of *C. hololeuca* (Fig. 3D) with three independent transitions to a variable state in *C. peltata*, *C. gabrielis* and the MRCA of *C. tacuna*, *C. telenitida* and *C. andina*, with a subsequent complete loss in *C. andina* (Fig. 3D).

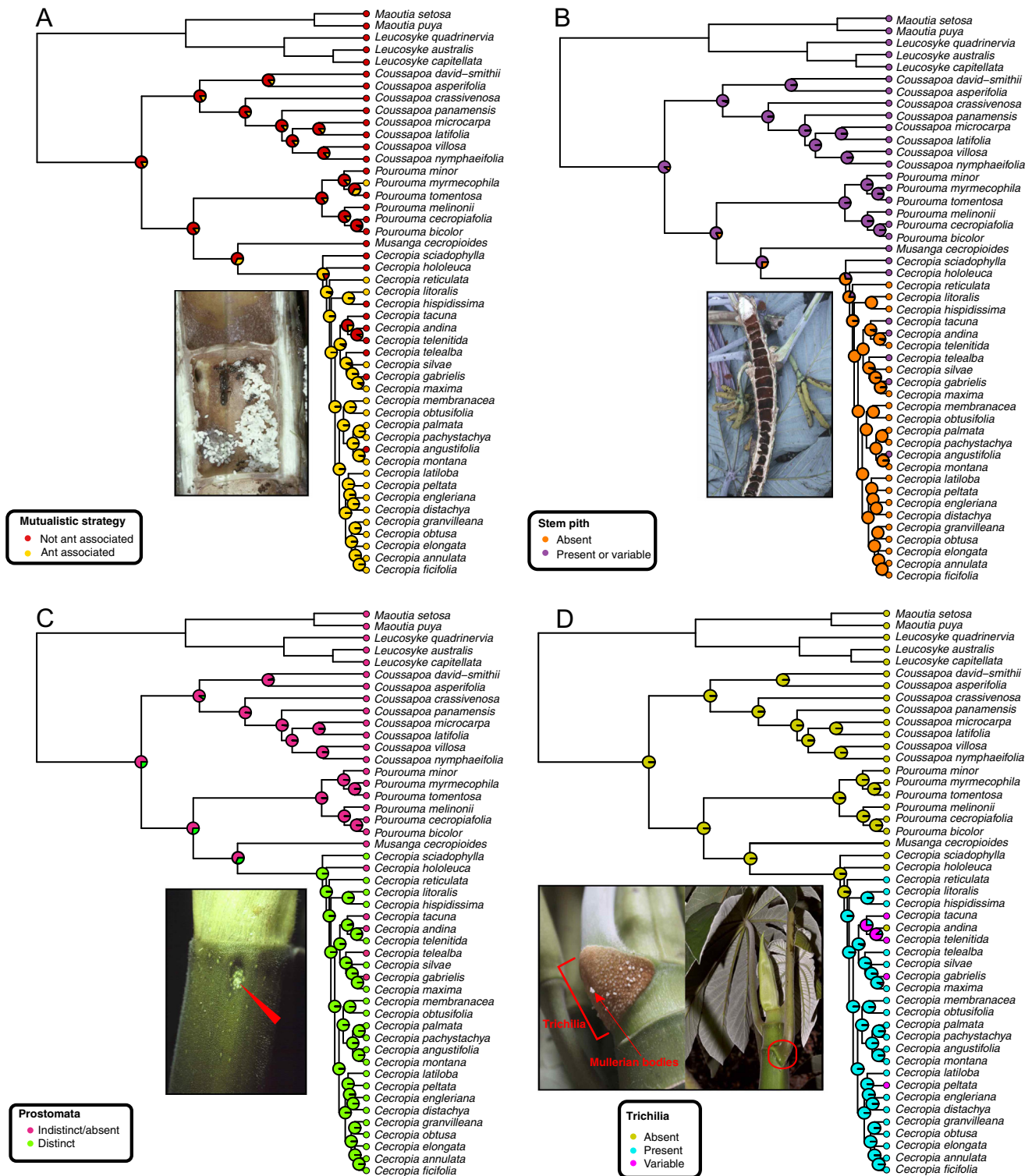


Fig. 3. Ancestral state reconstructions for *Cecropia* mutualistic traits. (A) Myrmecophytic state (Maximum Likelihood, all rates different [ARD] model). (B) Stem pith (Stochastic mapping, ARD model). (C) Prostomata (Stochastic mapping, ARD model). (D) Trichilia (Maximum Likelihood, ARD model). The inset photos show an *Azteca* ant colony inside a *Cecropia* domatium (A); a transection of an *Azteca*-specialized *Cecropia* stem, showing the reduced pith (B); a yet-to-be-perforated prostomata on a young *Cecropia* stem; a trichilia pad with Müllerian bodies on a petiole base (D, left), and a *Cecropia* without trichilia (D, right). The codings for A were not based on the traits analyzed in C–D. See also Fig. S3.

3.3. Altitude and mutualism breakdown

All six pairwise correlations between the coded traits were strong ($BF > 10$) (Fig. 4A). The absence of association with ants and occurrence at elevations >1500 m are correlated (this is driven

by *C. andina*, *C. angustifolia*, *C. gabrielis*, *C. tacuna*, *C. telealba*, and *C. telenitida*; Fig. 4B). To further investigate the altitudinal range of each mutualistic *Cecropia* species, we performed a multinomial logistic regression analysis, which showed that the probability of being *Azteca*-occupied is highest at low altitude and decreases as

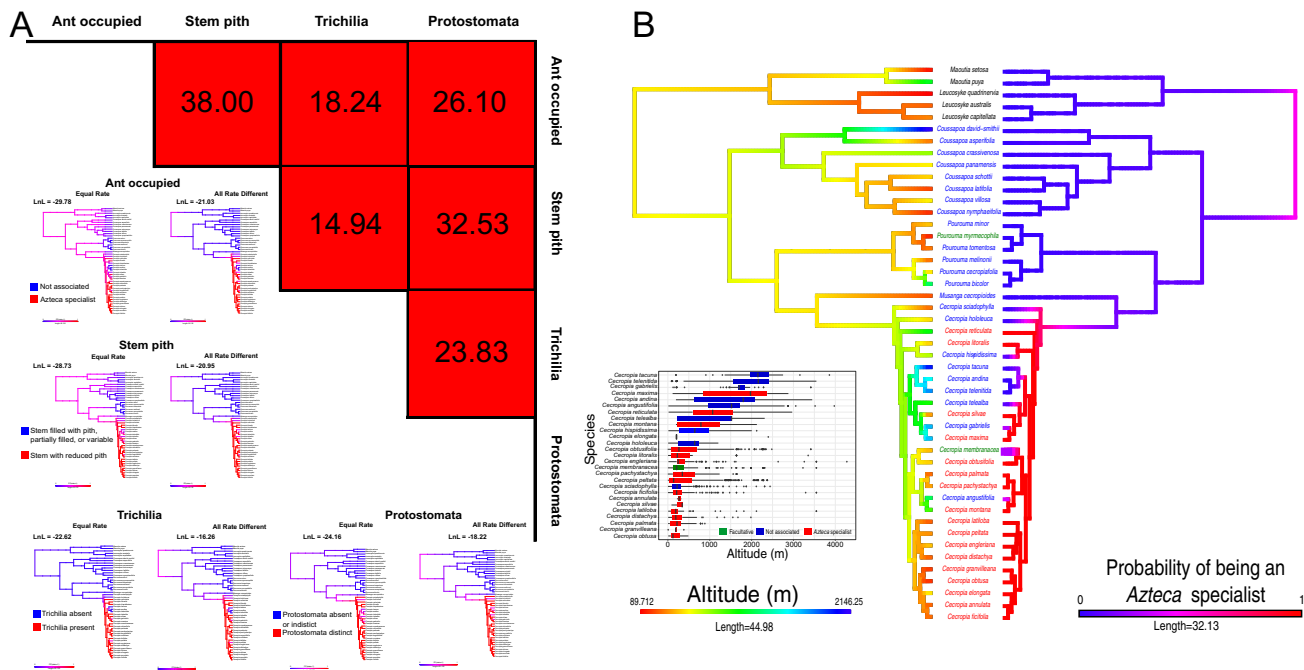


Fig. 4. Correlated evolution of ant occupation and morphological traits in *Cecropia*. (A) Pairwise comparisons for the Bayesian correlations between presence or absence of specialized symbiosis with *Azteca* ants (based on ecological observations), reduced vs. abundant pith in the internodes, presence vs. absence of prostomata, and presence vs. absence of trichilia. Bayes Factor (BF) > 10 are considered very strong evidence for correlations. Stochastic mapping ancestral state reconstructions of binary-coded traits are shown. (B) Ancestral state reconstruction of the mean elevation of species occurrence (left) compared with the stochastic mapping of specialized mutualism with *Azteca* presence or absence (right). The inset shows the distribution of altitudinal records for each *Cecropia* species sampled in the chronogram.

altitude increases; while the opposite is true for non-ant associated *Cecropia* (Fig. 5A). A non-metric dimensional scaling analysis (NMDS) of all 9416 *Cecropia* occurrences (Supplementary Dataset 1) revealed a weak differentiation of the three mutualistic strategies, with the specialized *Azteca*-occupied *Cecropia* being distinct from non-ant associated species, but overlapping with the

facultatively ant-associated *Cecropia* (Fig. S4). We repeated this analysis, using the same bioclim variables and mean elevation for all species sampled in our tree and plotted the results in a phylo-morphospace (Materials and Methods; Fig. 5B), which yielded a clearer differentiation between non-ant-associated and *Azteca*-occupied *Cecropia*.

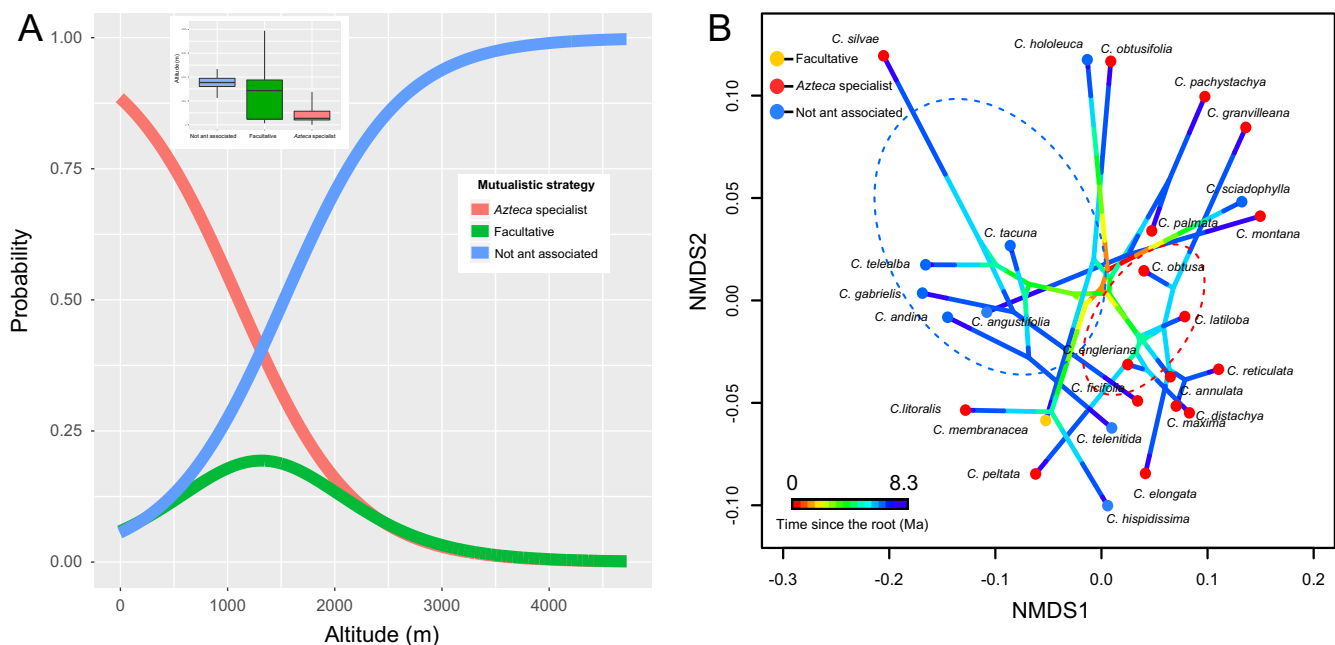


Fig. 5. Mutualistic strategy and altitudinal range in the genus *Cecropia*. (A) Multinomial logistic regression showing the probability of a species belonging to a particular mutualistic strategy as a function of altitude (based on over 9000 collections representing 58 of the 61 species of *Cecropia*). The inset shows a boxplot of altitudinal range for each strategy. (B) Phylomorphospace showing a non-metric multidimensional scaling (NMDS) analysis of niche space. Dashed ellipses show the standard deviation for each mutualistic strategy.

4. Discussion

4.1. Trans-Atlantic disjunction of *Cecropia* and *Musanga*

One of the goals of this study was to test the relationship between the West African genus *Musanga* and the neotropical genus *Cecropia*, given that a previous study had unexpectedly found *Musanga* embedded in *Cecropia* (Treiber et al., 2016). Our wider gene and taxon sampling showed *Musanga* as sister to *Cecropia* (Fig. 2A, Figs. S1, S2). The inferred direction of the transatlantic dispersal events in Cecropieae depends on the placement of *Myrianthus*, the only other African genus of Cecropieae. *Myrianthus* has seven species, all in tropical Africa. While our data failed to confidently place this genus (Figs. S1, S2), RAD seq data indicate that *Myrianthus* is sister to *Pourouma* (G. Weiblen, pers. comm. to S.S.R., 3 Jan. 2017), implying two dispersals to Africa, one by the ancestor of *Musanga*, one by that of *Myrianthus*. The Oligocene/Miocene age that we inferred for the dispersal of the ancestor of *Musanga* to Africa implies that vegetative plant parts or diaspores on a floating island were able to establish a population there. Similar events have been inferred in numerous plant clades (reviewed in Renner, 2004), often dating to the Oligocene/Miocene boundary, the time of the establishment of the northern trans-Atlantic currents, which appear to have facilitated South American/African dispersal (in both directions). Janzen and McKey's (1977, p. 57) suggestion that “*Musanga* is nothing more than a *Cecropia* that made a long hop (via seed) to the Azteca-free island of Africa” is difficult to reject by reconstructions that have to rely on the states found in today's species. However, our results suggest that the common ancestor of *Cecropia* and *Musanga* may not yet have been an ant-plant and that Azteca occupation first evolved in the MRCA of *Cecropia*.

4.2. The origin of the *Cecropia*/Azteca mutualism

Less densely sampled studies dated the crown age of *Cecropia* to 29 Mya (Zerega et al., 2013) or 4.78–11.92 Mya (Chomicki and Renner, 2015; Fig. S3); we inferred 8.38 (5.37–11.89) Mya, completely overlapping Chomicki and Renner (2015) estimate. The diversification of Azteca, a genus of >100 species of which only three were included in a clock-dated phylogeny, has been dated to 14 (7–22) Mya (Ward et al., 2010), and the divergence of the *Cecropia*-dwelling *A. ovaticeps* from its closest relative to ca. 8 Mya (Pringle et al., 2012). A denser sampling of *Cecropia*-inhabiting Azteca would be required to test whether Azteca codiversified with some of its *Cecropia* hosts. The diversification of *Cecropia* interestingly coincides with the evolution of leaf-cutter ants (genus *Atta*), 8–12 Mya (Schultz and Brady, 2008), which are dominant herbivores in South America. Azteca *alfari*, for instance, has been observed patrolling *Cecropia* trees against the activity of the leaf-cutting ant *Atta laevigata* (Vasconcelos and Casimiro, 1997), and leaf-cutting ants are also key herbivores on other species of *Cecropia* (Schimper, 1888; Wheeler, 1942; Bailey, 1922). Thus, the evolution and dominance of these ants may have been a selective pressure in the evolution of the Azteca/*Cecropia* defense mutualism.

4.3. Evolution of mutualistic traits in *Cecropia*

Another result of this study is that the ancestor of today's species of *Cecropia* likely was already engaged in mutualistic interactions with ants, with reduced pith in stems and/or branches that were accessible for ants by preformed entry holes (prostomata), but probably still without trichilia (and thus without Müllerian bodies; Fig. 3). Our Bayesian analysis revealed the lock-step evolu-

tion of trichilia and the Azteca/*Cecropia* symbiosis (Fig. 4A), suggesting that the stability of these symbioses is enhanced when both mutualistic services (domatia and food) are provided. A similar ‘bed-and-breakfast’ strategy is present in other insect/plant symbioses (Heil et al., 2009; Weber et al., 2012). Thus, the basal *C. sciadophylla* and *C. hololeuca* may well represent the demise of early stage unstable mutualisms, as suggested by our analyses, although we cannot exclude that stable *Cecropia*/Azteca symbioses first evolved after these species diverged. Some *Cecropia* species that lost ant mutualism also lost trichilia or have trichilia with variable development (Fig. 3D). Others, such as *C. angustifolia*, have dense hairs up to 8 mm long, which likely prevent ant foraging (Janzen, 1973). Our trait reconstructions (Fig. 3) suggest that the presence of prostomata in *C. sciadophylla* is a holdover from previous ant occupation rather than a preadaptation as suggested by Janzen (1973), the latter would imply that the *Cecropia* MRCA lacked symbiosis with ants. Notably, this species occasionally harbors ants of arboreal genera other than Azteca (Wheeler, 1942). An alternative explanation for the presences of prostomata in this species was provided by Bailey (1922) who found that its prostomata are more developed than those in other species obligately inhabited by Azteca. He therefore suggested that prostomata might be a by-product of the development of an axial bud. This explanation remains to be examined by comparing bud structure across myrmecophytic and non-myrmecophytic *Cecropia*.

4.4. Breakdown of specialized *Cecropia*/Azteca mutualisms correlates with shifts to high altitude environments and isolated islands

We inferred recurrent shifts from specialized Azteca occupation to ant-free autonomy (Fig. 3A). Species that represent the earliest diverging lineages (*C. sciadophylla* and *C. hololeuca*), species that colonized the Andes (*C. andina*, *C. angustifolia*, *C. gabrielis*, *C. tacuna*, *C. telealba*, and *C. telenitida* and *C. bullata*), and species that dispersed to the Caribbean (*C. schreberiana* and some populations of *C. peltata*) all lost ant symbiosis.

Most specialized *Cecropia* myrmecophytes grow in the lowlands, perhaps constrained by the altitudinal range of *Cecropia*-dwelling Azteca, which usually occur below 1400 m (Longino, 1989). In general, tropical ant abundances decrease with increasing altitude (Longino et al., 2014; Gillette et al., 2015). Transitions away from ant symbiosis could also have been favored if the herbivore community attacking *Cecropia* in a new habitat was less abundant or diverse, making ant defense less valuable. This could have been the case both at high altitudes and on the Caribbean islands. Recurrent mutualism breakdown correlating with migration to ant-depleted high altitudes also occurred in the epiphytic ant-plants Hydnophytinae (Rubiaceae) associated with generalist ants (Chomicki and Renner, 2017). In the *Cecropia* system, the breakdowns occurred in a much more specialized mutualism involving not ants from various genera, but a single obligately plant-nesting genus (Azteca) and several ant-associated traits (trichilia, pith reduction, prostomata). This suggests that even in specialized mutualisms, return to the autonomous state is possible. In many specialized species interactions, morphological or chemical traits that are compensated for by the interaction are lost (Ellers et al., 2012; e.g., plant defense traits compensated by Azteca ant protection). Reversion to autonomy is thus perhaps still possible when genetic redundancy allows to compensate for trait loss or when trait reversion can readily occur. The facultative association between *C. membranacea* and *Neoponera* could well represent an instance of partner replacement as has been inferred for other Neotropical ant/plant mutualisms (Chomicki et al., 2015). While obligate mutualisms in which partners (by definition) cannot exist without each other may be evolutionary ‘dead ends’ (Vandermeer and Boucher, 1978; Briand and Yodanis, 1982), the Azteca/*Cecropia*

mutualisms, which apparently do not involve species-specific interdependencies, clearly have been lost repeatedly.

5. Conclusions

We inferred that *Musanga* arose following a dispersal event to Africa in the Miocene and that *Cecropia* started diversifying about 8 Mya, probably in symbiosis with ants. It is noteworthy that studies of *Azteca* have inferred that obligate *Cecropia*-nesting *Azteca* species may have evolved several times (Longino, 1991; Ayala et al., 1996), while we inferred a single origin of *Cecropia*/*Azteca* symbiosis. This discrepancy may arise from the *Azteca* clade having diversified onto other plant hosts, such as *Cordia* (Pringle et al., 2012), and then having formed new *Cecropia*-inhabiting species. Some *Azteca* are generalist twig nesters (Ayala et al., 1996), and parallel evolution is known in other Neotropical plant-ants (Chomicki et al., 2015). Our results might change with further sampling, but drastic changes seem unlikely, given that we sampled 17 of the 44 *Cecropia*-species regularly occupied by *Azteca*, nine of the 11 species never interacting with ants, and two of four species with facultative ant associations (with *Azteca* or other ant species). Further field work on population-level changes along altitudinal gradients should address the costs and benefits of glycogen-fed ant symbionts in different environmental contexts.

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Appendix A. Supplementary material

Supplementary data associated with this article can be found, in the online version, at <http://dx.doi.org/10.1016/j.ympev.2017.04.009>.

References

- Andrade, J.C., 1984. Observações preliminares sobre a eco-etologia de quatro coleópteros (Chrysomelidae, Tenebrionidae, Curculionidae) que dependem da embaúba (*Cecropia lyratiloba* var. *nana* - Cecropiaceae), na restinga do Recreio dos Bandeirantes. Rio de Janeiro. Rev. Bras. Entomol. 28, 99–108.
- Ayala, F.J., Wetterer, J.K., Longino, J.T., Hartl, D.L., 1996. Molecular phylogeny of *Azteca* ants (Hymenoptera: Formicidae) and the colonization of *Cecropia* trees. Mol. Phylogenet. Evol. 5, 423–428.
- Axelrod, R., Hamilton, W.D., 1981. The evolution of cooperation. Science 211, 1390–1396.
- Bailey, I.W., 1922. Notes on neotropical ant-plants. I. *Cecropia angulata* sp. nov. Bot. Gaz. 74, 585–621.
- Bequaert, J., 1922. Ants of the American Museum Congo expedition. IV. Ants in their diverse relations to the plant world. Bull. Am. Mus. Nat. Hist. 45, 333–583.
- Berg, C.C., Franco-Rosselli, P., 2005. *Cecropia*. Flora Neotrop. 94, 1–230.
- Berg, C.C., Akkermans, R.W.A.P., van Heusden, E.C.H., 1990. Cecropiaceae: *Coussapoa* and *Pourouma*, with an introduction to the Family. Flora Neotrop. 51, 1–208.
- Berg, C.C., Ulloa Ulloa, C., 2014. Two new species of *Coussapoa* (Urticaceae, Cecropiaceae). Novon A. J. Bot. Nomencl. 23, 14–17.
- Bischof, S., Umhang, M., Eicke, S., Streb, S., Qi, W., Zeeman, S.C., 2013. *Cecropia peltata* accumulates starch or soluble glycogen by differentially regulating starch biosynthetic genes. Plant Cell 25, 1400–1415.
- Bollback, J.P., 2006. SIMMAP: stochastic character mapping of discrete traits on phylogenies. BMC Bioinform. 7, 88.
- Briand, F.P., Yodanis, P., 1982. The phylogenetic distribution of obligate mutualism: evidence of limiting similarity and global instability. Oikos 39, 273–274.
- Bronstein, J.L., 1998. The contribution of ant-plant protection studies to our understanding of mutualism. Biotropica 30, 150–161.
- Chamberlain, S.A., Bronstein, J.L., Rudgers, J.A., 2014. How context dependent are species interactions? Ecol. Lett. 17, 881–890.
- Chomicki, G., Renner, S.S., 2015. Phylogenetics and molecular clocks reveal the repeated evolution of ant-plants after the late Miocene in Africa and the early Miocene in Australasia and the Neotropics. New Phytol. 207, 411–424.
- Chomicki, G., Ward, P.S., Renner, S.S., 2015. Macroevolutionary assembly of ant/plant symbioses: *Pseudomyrmex* ants and their ant-housing plants in the Neotropics. Proc. Royal Soc. B 282, 20152200.
- Chomicki, G., Staedler, Y.M., Schönenberg, J., Renner, S.S., 2016. Partner choice through concealed floral sugar rewards evolved with the specialization of ant-plant mutualisms. New Phytol. 211, 1358–1370.
- Chomicki, G., Janda, M., Renner, S.S., 2017. The assembly of ant-farmed gardens: mutualism specialization following host broadening. Proc. Royal Soc. B 284, 20161759.
- Chomicki, G., Renner, S.S., 2017. Partner abundance controls mutualism stability and the pace of morphological change over geologic time. Proc. Nat. Acad. Sci. USA. <http://dx.doi.org/10.1073/pnas.1616837114> (in press).
- Collinson, M.E., 1989. The fossil history of the Moraceae, Urticaceae (including Cecropiaceae), and Cannabaceae. In: Crane, P.R., Blackmore, S. (Eds.), Evolution, systematics, and fossil history of the Hamamelidaceae, “Higher” Hamamelidaceae, vol. 2. Clarendon Press, Oxford, UK, pp. 319–339.
- Davidson, D.W., McKey, D., 1993. The evolutionary ecology of symbiotic ant-plant relationships. J. Hymenopt. Res. 2, 13–83.
- Davidson, D.W., 2005. *Cecropia* and its biotic defenses. In: Berg, C.C., Franco Rosselli, P. (Eds.), *Cecropia*. Flora Neotropica Monograph Bronx New York: The New York Botanical Garden, pp. 214–226.
- De Ruiter, G., 1976. Revision of the genera *Myrianthus* and *Musanga* (Moraceae). Bull. du Jard. Bot. Natl. Belgique 46, 471–510.
- Drummond, A.J., Suchard, M.A., Xie, D., Rambaut, A., 2012. Bayesian phylogenetics with BEAUti and the BEAST 1.7. Mol. Biol. Evol. 29, 1969–1973.
- Ellers, J., Kiers, T.E., Currie, C.R., McDonald, B.R., Visser, B., 2012. Ecological interactions drive evolutionary loss of traits. Ecol. Lett. 15, 1071–1082.
- Folgarait, P.J., Davidson, D.W., 1995. Myrmecophytic *Cecropia*: ant herbivore defenses under different nutrient treatments. Oecologia 104, 189–206.
- Frederickson, M.E., 2013. Rethinking mutualism stability: cheaters and the evolution of sanctions. Q. Rev. Biol. 88, 269–295.
- Gaglioti, A.L., Neto, S.R., 2014. *Pourouma amacayacuensis* (Urticaceae), a new species from Colombia. Syst. Bot. 39, 902–905.
- Gillette, P.N., Ennis, K.K., Domínguez-Martínez, G., Philpott, S.M., 2015. Changes in species richness, abundance, and composition of arboreal twig-nesting ants along an elevational gradient in coffee landscapes. Biotropica 47, 712–722.
- Gouy, M., Guindon, S., Gascuel, O., 2010. SeaView version 4: a multiplatform graphical user interface for sequence alignment and phylogenetic tree building. Mol. Biol. Evol. 27, 221–224.
- Heil, M., González-Teuber, M., Clement, L.W., Kautz, S., Verhaagh, M., Bueno, J.C.S., 2009. Divergent investment strategies of *Acacia* myrmecophytes and the coexistence of mutualists and exploiters. Proc. Nat. Acad. Sci. USA 106, 18091–18096.
- Hijmans, R.J., Van Etten, J., 2013. Raster: geographic data analysis and modeling. R package version 2.1–49.
- Janzen, D.H., 1973. Dissolution of mutualism between *Cecropia* and its *Azteca* Ants. Biotropica 5, 15–28.
- Janzen, D.H., McKey, D., 1977. *Musanga cecropiodes* is a *Cecropia* without its Ants. Biotropica 9, 57.
- Katoh, K., Standley, D.M., 2013. MAFFT multiple sequence alignment software version 7: improvements in performance and usability. Mol. Biol. Evol. 30, 772–780.
- Latteman, T.A., Mead, J.E., Duvall, M.A., Bunting, C.C., Bevington, J.M., 2014. Differences in anti-herbivore defenses in non-myrmecophyte and myrmecophyte *Cecropia* trees. Biotropica 46, 652–656.
- Lemmon, A.R., Brown, J.M., Stanger-Hall, K., Lemmon, E.M., 2009. The effect of ambiguous data on phylogenetic estimates obtained by maximum likelihood and Bayesian inference. Syst. Biol. 58, 130–145.
- Lewis, P.O., Holder, M.T., Holsinger, K.E., 2005. Polytomies and Bayesian phylogenetic inference. Syst. Biol. 54, 241–253.
- Longino, J.T., 1989. Geographic variation and community structure in an ant-plant mutualism: *Azteca* and *Cecropia* in Costa Rica. Biotropica 21, 126–132.
- Longino, J.T., 1991. Taxonomy of the *Cecropia*-inhabiting *Azteca* ants. J. Nat. Hist. 25, 1571–1602.
- Longino, J.T., Branstetter, M.G., Colwell, R.K., 2014. How ants drop out: ant abundance on tropical mountains. PLoS One 9, e104030.
- O'Dowd, D.J., 1982. Pearl bodies as ant food: an ecological role for some leaf emergences of tropical plants. Biotropica 14, 40–49.
- Oksanen, J., Blanchet, F.G., Kindt, R., Legendre, P., Minchin, P.R., O'Hara, R.B., Simpson, G.L., Solymos, P., Stevens, M.H.H., Wagner, H., Oksanen, M.J., 2013. Package ‘vegan’. Community Ecology Package, Version, 2.
- Palmer, J.D., 1991. Plastid chromosomes: structure and evolution. In: Bogorad, L., Vasil, I.K. (Eds.), Cell Culture and Somatic Genetics of Plant. Academic Press, San Diego, pp. 5–53. Molecular Biology of plastids.
- Pagel, M., Meade, A., 2013. Bayes Traits V2. Computer Program and Documentation. Available at: <http://www.evolution.rdg.ac.uk/BayesTraits.html>.
- Paradis, E., Claude, J., Strimmer, K., 2004. APE: analyses of phylogenetics and evolution in R language. Bioinformatics 20, 289–290.
- Pringle, E.G., Ramírez, S.R., Bonebrake, T.C., Gordon, D.M., Dirzo, R., 2012. Diversification and phylogeographic structure in widespread *Azteca* plant-ants from the northern Neotropics. Mol. Ecol. 21, 3576–3592.
- Rambaut, A., 2007. FigTree [WWW Document]. Mol. Evol. phylogenetics Epidemiol. <http://tree.bio.ed.ac.uk/software/figtree/> (accessed 4.16.16).

- Renner, S.S., 2004. Plant dispersal across the tropical Atlantic by wind and sea currents. *Int. J. Plant Sci.* 165, S23–S33.
- Revell, L.J., 2012. Phytools: an R package for phylogenetic comparative biology (and other things). *Methods Ecol. Evol.* 3, 217–223.
- Rickson, F.R., 1971. Glycogen plastids in Müllerian body cells of *Cecropia peltata* - a higher green plant. *Science* 173, 344–347.
- Rickson, F.R., 1976a. Anatomical development of leaf trichilium and Müllerian bodies of *Cecropia peltata* L. *Am. J. Bot.* 63, 1266–1271.
- Rickson, F.R., 1976b. Ultrastructural differentiation of Müllerian body glycogen plastid of *Cecropia peltata* L. *Am. J. Bot.* 63, 1272–1279.
- Sachs, J., Simms, E., 2006. Pathways to mutualism breakdown. *Trends Ecol. Evol.* 21, 585–592.
- Savard, L., Michaud, M., Bousquet, J., 1993. Genetic diversity and phylogenetic relationships between birches and alders using ITS, 18S rRNA, and *rbcl* gene sequences. *Mol. Phylogenet. Evol.* 2, 112–118.
- Schimper, A.F.W., 1888. Die Wechselbeziehungen zwischen Pflanzen und Ameisen im tropischen Amerika. *Bot. Mitteil. aus den Tropen. Jena I*, 1–95.
- Schultz, T.R., Brady, S.G., 2008. Major evolutionary transitions in ant agriculture. *Proc. Natl. Acad. Sci. USA* 105, 5435–5440.
- Schupp, E.W., 1986. *Azteca* protection of *Cecropia*: ant occupation benefits juvenile trees. *Oecologia* 70, 379–385.
- Stamatakis, A., 2014. RAxML version 8: a tool for phylogenetic analysis and post-analysis of large phylogenies. *Bioinformatics* 30, 1312–1313.
- Treiber, E.L., Gaglioti, A.L., Romaniuc-Neto, S., Madriñán, S., Weiblen, G.D., 2016. Phylogeny of the Cecropieae (Urticaceae) and the evolution of an ant-plant mutualism. *Syst. Bot.* 41, 56–66.
- Valverde, J.P., Hanson, P., 2011. Parenchyma: a neglected plant tissue in the *Cecropia*/ant mutualism. *Symbiosis* 55, 47–51.
- Vandermeer, J.H., Boucher, D.H., 1978. Varieties of mutualistic interactions in population models. *J. Theo. Biol.* 74, 549–558.
- Vasconcelos, H.L., Casimiro, A.B., 1997. Influence of *Azteca alfari* ants on the exploitation of *Cecropia* trees by a leaf-cutting ant. *Biotropica* 29, 84–92.
- Ward, P.S., Brady, S.G., Fisher, B.L., Schultz, T.R., 2010. Phylogeny and biogeography of dolichoderine ants: effects of data partitioning and relict taxa on historical inference. *Syst. Biol.* 59, 342–362.
- Weber, M.G., Clement, W.L., Donoghue, M.J., Agrawal, A.A., 2012. Phylogenetic and experimental tests of interactions among mutualistic plant defense traits in *Viburnum* (Adoxaceae). *Am. Nat.* 180, 450–463.
- Weber, M.G., Keeler, K.H., 2013. The phylogenetic distribution of extrafloral nectaries in plants. *Ann. Bot.* 111, 1251–1261.
- Wheeler, W.M., 1942. Studies of neotropical ant-plants and their ants. *Bull. Mus. Comp. Zool. Harvard* 90, 1–262.
- Wu, Z., Monro, A.K., Milne, R.I., Wang, H., Yi, T., Liu, J., Li, D., 2013. Molecular phylogeny of the nettle family (Urticaceae) inferred from multiple loci of three genomes and extensive generic sampling. *Mol. Phylogenet. Evol.* 69, 814–827.
- Yu, D.W., Davidson, D.W., 1997. Experimental studies of species-specificity in *Cecropia*-ant relationships. *Ecol. Monogr.* 67, 273–294.
- Zerega, N.J.C., Clement, W.L., Datwyler, S.L., Weiblen, G.D., 2005. Biogeography and divergence times in the mulberry family (Moraceae). *Mol. Phylogenet. Evol.* 37, 402–416.