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Molecular Phylogeny of *Azteca* Ants (Hymenoptera: Formicidae) and the Colonization of *Cecropia* Trees

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Despite the prominence of the *Azteca–Cecropia* interaction as the focus of extensive ecological investigation, a reliable phylogeny of the *Azteca* ants has been lacking, primarily because many of the morphological and behavioral characters are phylogenetically uninformative or conflicting. A phylogenetic analysis of a select set of *Azteca* ants, including six *Cecropia* inhabitants and two non-*Cecropia* inhabitants, plus an outgroup taxon, is presented on the basis of mitochondrial DNA sequences. The evolutionary relationships deduced from the molecular data are analyzed with reference to ecological and morphological studies, specifically addressing the phylogenetic relationship of structurally and behaviorally ambiguous taxa, species complex groupings, and the colonization of *Cecropia* trees. According to the molecular phylogeny, the *Cecropia*-inhabiting *Azteca* do not form a monophyletic clade, indicating multiple independent colonization or abandonment of *Cecropia* trees by the *Azteca*.

INTRODUCTION

The *Azteca* ants (subfamily Dolichoderinae) comprise approximately 150 described species, all of which are found exclusively in the Neotropics (Wheeler, 1942; Kempf, 1972; Shattuck, 1994). Because of their obligate association with myrmecophytic trees, the *Azteca* have received considerable attention from evolutionary ecologists (Bequaert, 1922; Wheeler, 1942; Buckley, 1982; Davidson and Fisher, 1991; Yu and Davidson, submitted for publication). Many *Azteca* species have specialized to live in particular plant lineages; 13 known species have evolved an obligate relationship with *Cecropia* trees (Urticaceae) (Hölldobler and Wilson, 1990; Longino, 1991b). Despite the prominence of the *Azteca–Cecropia* interaction as the subject of much ecological research, evolutionary and ecological studies of this system have suffered from the lack of a precise phylogeny. Although the extensive morphological and behavioral investigations have produced reliable taxonomies of the *Azteca* (Longino, 1989; 1991a,b; Shattuck, 1994), they have not yielded a reliable phylogeny, primarily because many character states are homoplasious and conflicting (Harada and Benson, 1988; Longino, 1991b).

Benson (1985) and Longino (1991b) have proposed that at least two independent lines of *Azteca* have colonized *Cecropia:* The *alfari* complex, comprising *Azteca alfari* and *A. ovaticeps,* share the following characters: hairless appendages, small queen size, deep ventral lobe on the queen petiole, polydomous nests, and the lack of standing setae on the tibiae and scapes (Longino, 1989). The *muelleri* complex, comprising *A. aragua, A. australis, A. isthmica, A. merida, A. muelleri, A. petalocephala, A. salti,* and *A. xanthocroa* share mottled orange to pure orange queens, large subpyramidal petioles, densely pilose tibae, and the formation of a central carton in the tree bole (Longino, 1991b). The two complexes also differ in colonization behavior (Yu and Davidson, submitted for publication). Species belonging to the *alfari* complex most likely evolved from an ancestor that nested in live stems. *Alfari* species exhibit strong habitat specialization but weak host species specialization, exclusively colonizing linear and contiguous riverine environments. Yu and Davidson hypothesized that the streamlined thoraces of the queens in this group are adaptive for entering small holes (such as live stems) but render queens unable to fly the long distances needed to colonize patchily distributed forest gaps where many *Cecropia* species establish. In contrast, *muelleri* complex species most likely evolved from an ancestor that nested in carton nests. Members of this species group exhibit weak habitat specialization, colonizing both riverine and forest gap habitats, but strong host species specialization, preferring *Cecropia* species with high levels of pearl body production. The placement of carton nests on host trees producing some form of ant reward is a common behavior in many carton-nesting ants.

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The phylogenetic associations of the three remaining species of *Cecropia*-inhabiting *Azteca* are less clear (Longino, 1991b). *A. lattke* and *A. constructor* appear to be allied with the *muelleri* complex, on the basis of their short heads, dense pilosities, and central carton nests in tree boles. However, both *A. lattke* and *A. constructor* have black queens, unlike the *muelleri* complex species. Furthermore, *A. constructor* has a petiole unlike any *muelleri* species, but more like that of *A. gnava,* which nests in ant gardens. The third species, *A. coeruleipennis,* has polydomous nests like *alfari* species, pilosity like *muelleri* species, and petiole shape similar to stem-nesting *Azteca* such as *A. patruelis.*

In this report we supplement the reconstruction of the evolutionary history of the *Azteca* with molecular data. We present a phylogenetic analysis of a select group of *Azteca* species on the basis of mitochondrial DNA sequences, including portions of cytochrome c oxidase subunits I and II, and the $tRNA^{Leu}$ locus. We analyze the evolutionary relationships deduced from the molecular data with reference to ecological and morphological studies, specifically addressing the independent colonization of *Cecropia,* species complex groupings, and the phylogenetic relationship of structurally and behaviorally ambiguous taxa.

MATERIALS AND METHODS

Specimens of *A. alfari, A. constructor, A. instabilis, A. ovaticeps, A. patruelis,* and *A. xanthocroa* were collected by J.K.W. at La Selva, Costa Rica, in April 1993 and stored at 0°C; specimens of *A. coeruleipennis* and *A. merida* were collected by J.T.L. at Puntarenas, Costa Rica, in May 1989 and Estado Lara, Venezuela, in August 1987, respectively, and stored at room temperature in 95% ethanol. *Froggattella kirbii,* the outgroup taxon, was collected in Paluma, Australia, by Naomi Pierce in January 1988 and stored at 0° C.

DNA extraction was performed by incubating individual ants in 500 μl of 5% Chelex 100 resin (Bio-Rad) at 95°C for 15 min, followed by vortexing for 15 s. Immediately prior to amplification, the Chelex solution was spun for 1 min to pellet the resin, and 1 μl was used for PCR (Wash *et al.,* 1991; Christián Orrego, personal communication).

Two partially overlapping regions of mtDNA, comprising a total segment of ~650 bp, including the 3′ end of the cyctochrome c oxidase subunit I (COI) locus, the $tRNA^{Leu}$ locus, and the 5′ end of the cytochrome c oxidase subunit II (COII) locus, were PCR-amplified using the following primer pairs: George III, 5′-TAG GTI TAG CIG GAA TAC CTC G-3′ (sense) and Pat, 5′-TCC AAT GCA CTA ATC TGC CAT ATT A-3′ (antisense); Ollie, 5′-GCY YTA TCA TCW AAA CG-3′ (sense) and Marilyn, 5′-TCA TAA GTT CAR TAT CAT TG-3′ (antisense).

The combined amplified region extends from bases 2759 to 3408, numbered according to the *Drosophila yakuba* sequence of Clary and Wolstenholme (1985). The Marilyn primer, as well as numerous others we attempted, failed to amplify in *A. paturelis* and *A. merida* and thus the sequences from these two species only extend to sites 3037 and 3033, respectively. The sequences for primers George III, Pat, and Marilyn were obtained courtesy of the laboratory of Professor Richard Harrison (personal communication). The sequence for Ollie, as well as those of several other primers used for sequencing, were determined with a primer-walking approach on the basis of knowledge of the nucleotide sequences from all of the ants.

Following amplification (Saiki *et al.,* 1988), the PCR products were purified by diluting the 30-μl reaction volume to a final volume of 100 μl with H_2O, followed by phenol extraction, precipitation in the presence of 2.5 M NH_4OAc and 1 vol of 100% ethanol, and washing with 70% ethanol (Sambrook *et al.,* 1989, pp. E.10–E.13). PCR products were sequenced either by direct sequencing of the purified reactions or by sequencing cloned products using the TA Cloning system (Invitrogen). Sequencing was performed with an Applied Biosystems Model 373A DNA sequencing system (Halloran *et al.,* 1993; recommendations of the manufacturer) using the PRISM Ready Reaction sequencing kits. Every nucleotide was sequenced at least twice, each time from a different amplification.

DNA sequences were aligned using the CLUSTAL V program (Higgins *et al.,* 1992), using the default alignment parameters. Phylogenetic analyses were performed by parsimony methods with PAUP 3.1.1 (Swofford, 1991) and by neighbor-joining methods with the MEGA program (Kumar *et al.,* 1993).

RESULTS

Variable sites from the aligned DNA sequences are shown in Fig. 1. The alignment yielded 168 variable sites, 85 of which were phylogenetically informative. A small region, between sites 3059 and 3062 of the published sequence of Clary and Wolstenholme (1985), contained several insertion/deletions which could not be unambiguously aligned and were therefore not used for the phylogenetic analyses. The results of the phylogenetic reconstruction of the *Azteca* species, based on the DNA sequences, are shown in Fig. 2. *F. kirbii* (subfamily Dolichoderinae) was used as an outgroup for all analyses (Shattuck, 1992; Shattuck, submitted for publication). Two phylogenetic techniques were employed to test the evolutionary relationships. First, an exhaustive search of all possible topologies was employed to determine the most parsimonious tree (that requiring the fewest evolutionary steps), assuming equal weighting of all characters. Five hundred bootstrap replicates were performed with the branch-and-bound search algorithm to test the robustness of the parsi-

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                    2222222222222222222222222222222222222222222222222222222222
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                    1470369245789403469258124734690891739235681789136957894 7

A. xanthocroa       AATACCATCCACTATCACTATACCTCTATATTTATCATCATATAAAAATTTACTCT
A. alfari           AACATCATCCACTACTATCATAATTTTATTTTTATTGCTATAACTTAGCTTACCTT
A. instabilis       CATACTTTCTACCCTTGATAAGACTTTGTCTTTATCATCCTATTAACTCTTACCTT
A. ovaticeps        AACTTCATCAACTACTATCATAACTTTATTTTTATTACTATAACCTAACTTACCTT
A. patruelis        AATATCATCTATTATTATTAAACCCCTATTCTTATCGCTATAATATAACTCACCTT
A. constructor      ATTATTATCATCTATTACTTAXACTXTATATTCAXCGACATATAAAAATCTATTTT
A. merida           CACATTATCCTCCACTACATAAACTCAATATTTACTATCATTTAAAAAATTATTTT
A. coeruleipennis   AAXACCTCCTATCACTACCAATCCTATATACTTATCGACATATTAAAACATATTCT
Froggattella        CATATCATACTCTTTTACACTCTAATTAAATACTTTATCAGATTAAATTATTCTTA


                    2222222222222222222222222233333333333333333333333333333333
                    9999999999999999999999999000000000000000000001111111111
                    4444455555666667778888999902333455566668899999000000 1112
                    0136905678014783692368145706479412834565814567134569358 1

A. xanthocroa       TXAXATTAGAACTTGTTTTAAATTTATTAATGATG--TCTTAAATATTAGATATTT
A. alfari           TTAAACTTATATTTATTATAGATCTTTTAATGATG--TCCCAXACXCAAATAGTAT
A. instabilis       CTAGGCTAACTCTTATCATGGGCTTATTAATGTTGATTCTTCAATACTAGCAATTC
A. ovaticeps        CTAAACTTATATTTATTATAGATTTTTTAATGATG--TCCCAGACCCAAATAGTAT
A. patruelis        CTAAATCTCAATGTATTATAGATTTCTTTGXXXXXXXXXXXXXXXXXXXXXXXXXX
A. constructor      TCTAATTAGAACTTACCACAGACCTACTAATAATGTCCCTTAAATATAAGACACTC
A. merida           CCXAATCAGAACTTATCATAGACAXACTXXXXXXXXXXXXXXXXXXXXXXXXXX
A. coeruleipennis   CTAAATCAAAATTTATTATGGACATTTTAATGACG--TCCCAAACATAAATAGTTC
Froggattella        TTAAATTTTTACTAATTATAGATAGATCAACAATATTTTCTCATTATATCTTATTT


                    33333333333         33                  333333333333
                    11111111111         11                  222222222222
                    22233334455         88                  334444445566
                    24702365814         57                  681345780403

A. xanthocroa       TCTTACTTTCTTGTCCTTCCTTAATTATTTTCTTCCAATATATCGTCTTCTCTCAC
A. alfari           TCATATTTCTCTGTTTTTTCTTAATTATTCTCTTCAAATCTATTATATTTGCTAAT
A. instabilis       TCACATTTCTTTGCTCTTTTTTAATATATATCTTCTAATTTATTGTATTCTAAAAT
A. ovaticeps        TCATATTTCTTTGTTCTTTCTTAATTATTCTCTTCAAATTTATTGTATCTACTAAT
A. patruelis        XXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXX
A. constructor      TCTCACTTCCTCTTACTTTTTCAACTATTTTCTTCAAATATGACGTATTCTCTGAT
A. merida           XXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXX
A. coeruleipennis   TTACATTCTTTTGTTTCTTTTTAGCCTTACTCTCCTAATTTATTGTAATCTCTTTT
Froggattella        ATACTTCTTCCTATATTATTAATATAXTATAAAATTTCAAAATTACATTTATATAT
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FIG. 1. Sequence alignment of variable sites from eight *Azteca* species and the outgroup taxon, *Froggattella kirbii*. Sites are numbered according to the *D. yakuba* sequence of Clary and Wolstenholme (1985). Sites 2765 to 3006 are from the COI locus; sites 3012 to 3077 are from the $tRNA^{Leu}$ locus; sites 3083 to 3266 are from the COII locus; and the remaining sites are intragenic regions. Gaps in the numbering indicate regions not clearly homologous to the published sequence. Alignment gaps are indicated by "–"; undetermined nucleotides are indicated by "X."

mony tree (Felsenstein, 1985), using equal weighting of all characters. An additional 500 bootstrap replicates were performed with the branch-and-bound search algorithm using parsimony and a "4–10–1" weighting scheme, in which first codon positions were weighted by 4, second codon positions by 10, and third codon positions, as well as intragenic sequences and the $tRNA^{Leu}$ regions, were weighted by 1 (Brower, 1994). These weighting values approximate the inverse of the frequency of informative sites in these regions among the aligned *Azteca* sequences. Second, a neighbor-joining tree was generated with the Kimura two-parameter correction for multiple substitutions (Kimura, 1980).

Both phylogenetic methods yielded topologies which were identical. However, the precise placement of the *A. constructor*/*A. merida* cluster is not supported by either bootstrap analysis. Both the exhaustive parsimony search and the neighbor-joining methods placed this cluster as a sister clade to the *A. alfari*/*A. ovaticeps*/*A. patruelis*/*A. coeruleipennis*/*A. instabilis* cluster. The bootstrap with parsimony using unweighted characters placed the *A. constructor*/*A. merida* cluster as a sister clade to *A. xanthocroa*. Finally, the bootstrap with parsimony using the "4–10–1" weighting scheme placed the *A. constructor*/*A. merida* cluster as an outgroup to all the other *Azteca*. Neither

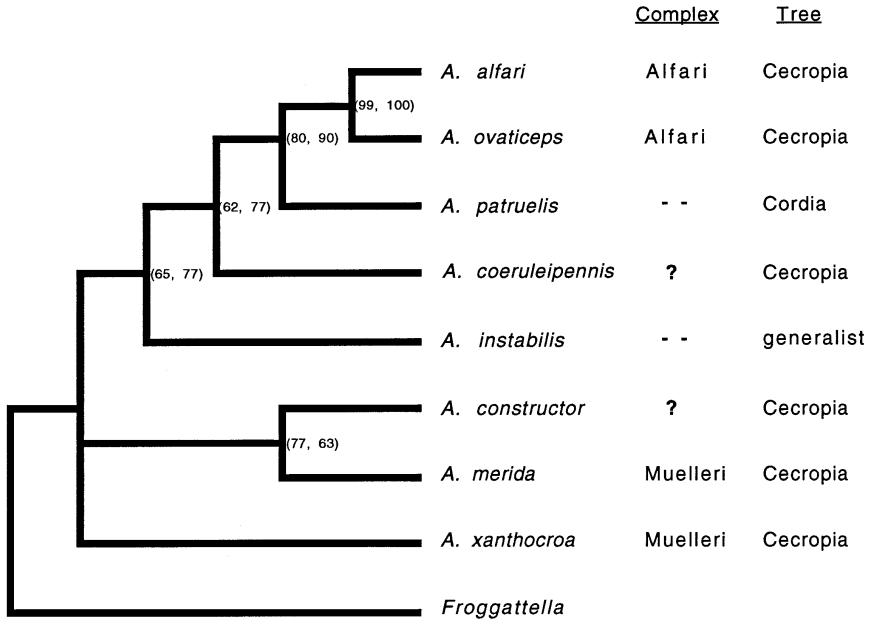

FIG. 2. Molecular phylogeny of *Azteca* ants, with *F. kirbii* as outgroup. Numbers in parentheses represent bootstrap confidence intervals from the unweighted search and the "4–10–1" weighting scheme, respectively (see text). Listed under the heading "Complex" is the species complex to which each *Cecropia*- inhabiting *Azteca* species belongs; species with uncertain affinity are listed as "?"; species not assigned to either the *alfari* or *muelleri* complexes are listed as "—." Listed under the heading "Tree" is the ant–plant genus which each *Azteca* species inhabits.

bootstrap method ascribed high confidence to the node which gave rise to the *A. constructor / A. merida* cluster: the unweighted method yielded a bootstrap value of 52, while the "4–10–1" method yielded a bootstrap value of 54. The ambiguous placement of the *A. constructor / A. merida* cluster is reflected as a three-way polytomy in Fig. 2. All remaining nodes were well supported by both bootstrap analyses.

DISCUSSION

Previous hypotheses concerning the evolutionary history of the *Azteca* were speculative, relying on ecological and morphological characters that were of limited phylogenetic utility and often contradictory. The molecular phylogeny presented here allows an independent analysis of the evolutionary relationships and a synthesis of the molecular, behavioral, and structural evidence, although we caution that gene trees are not always precise reflections of species trees (Goodman *et al.*, 1979; Pamilo and Nei, 1988).

The ambiguity in the placement of the *A. constructor / A. merida* cluster on the basis of the molecular data may reflect very rapid branching events leading to the three descendant lineages. However, as molecular data have resolved ambiguities in ecological and morphological data, so too may the converse be helpful. On the basis of several structural characters, including orange queens, large subpyramidal petioles, densely pilose tibae, and central cartons in tree boles, *A. merida* is likely to be closely related to *A. xanthocroa* (Longino, 1991b). *A. constructor* also shares many features with *A. merida* and *A. xanthocroa*, including short heads, dense pilosities, and central carton nests, although the former has black queens, which may represent an autapomorphy. On the basis of the ecological data, it appears likely that *A. xanthocroa* and the *A. constructor / A. merida* cluster are sister clades, and the cluster of all three taxa is a sister clade to the remaining *Azteca* shown in Fig. 2.

Figure 2 also shows the species complex to which each *Cecropia*-inhabiting *Azteca* species was assigned by Longino (1991b) on the basis of morphological and ecological criteria. The two members of the *muelleri* complex analyzed here, *A. merida* and *A. xanthocroa*, are linked in the phylogeny, although the uncertain placement of the *A. constructor / A. merida* cluster precludes a definitive assignment to a monophyletic clade on the basis of the molecular data alone. Nevertheless, the molecular data indeed place *A. constructor* within the *muelleri* complex and as a close relative of *A. merida*.

Longino's grouping of *A. alfari* and *A. ovaticeps* into the *alfari* complex is also supported by the molecular phylogeny, according to which the two species together form a monophyletic clade. Interestingly, however, one of these two species might be paraphyletic or even polyphyletic with respect to the other; Longino (1989) has proposed that the numerous sibling species and their patterns of geographic variation suggest *A. ovaticeps*

may have evolved directly from an *A. alfari* ancestor, or possibly that selection for increased pilosity in some habitats has driven two independent *A. alfari* lineages to convergence as *A. ovaticeps*. Intraspecific sequencing surveys of these species from throughout the Neotropics would likely resolve their mode of origin.

Also of previously uncertain affinity was *A. coeruleipennis*, which shares ecological and morphological characters with the *alfari* complex, the *muelleri* complex, and *A. patruelis*. The molecular data align *A. coeruleipennis* with neither complex nor directly with *A. patruelis*. The structural characters which *A. coeruleipennis* shares with the other groups are almost certainly homoplasious.

Also shown in Fig. 2 is the tree genus in which each *Azteca* species inhabits. Consistent with the hypotheses of Benson (1985) and Longino (1991b), the *Cecropia*-inhabiting *Azteca* clearly do not form a monophyletic group, indicating either independent colonization or independent abandonment of *Cecropia* trees. Several evolutionary scenarios may be invoked to explain the scattered distribution of *Cecropia* inhabitants along the topology. For example, *Cecropia* may have been colonized in a single event by the common ancestor to all eight *Azteca* species and then abandoned independently by *A. instabilis* and *A. patruelis*. Conversely, *Cecropia* may have been colonized independently by the *alfari* complex, by *A. coeruleipennis*, and by the *muelleri* complex (assuming the *A. constructor / A. merida* clade is most closely related to *A. xanthocroa*). No single, most-parsimonious explanation is possible from the molecular data alone, as all scenarios require at least three independent evolutionary steps. Furthermore, the reconstruction of evolutionary events along any phylogeny is very sensitive to sampling of taxa (Wilson *et al.*, 1991); thus, additional *Azteca* species, as yet unsequenced, may fall within the present topology and alter the evolutionary scenarios inferred from the present analysis. Addition of any of the seven remaining *Cecropia*-inhabiting *Azteca* would most likely not affect the analysis, since *A. lattke* is closely allied with *A. constructor* (Longino, 1991b), and the other six species are in the *muelleri* complex. Their inclusion in the phylogeny would therefore not likely lead to additional inferred evolutionary events in the reconstruction of the colonization of *Cecropia*. However, the addition of non-*Cecropia* inhabitants could alter the number of inferred colonizations or abandonments.

The phylogeny proposed here is consistent with the phylogenetic considerations and evolution of nesting habits proposed by Longino (1991a,b). The ancestral *Azteca* was proposed to have a highly defended central carton nest where all of the brood is concentrated. *A. constructor*, *A. merida*, and *A. muelleri* are known to have very similar nesting habits, in which a spindle-shaped carton nest is placed in the bole of a *Cecropia* tree, deforming the bole at that point, and numerous exit holes are maintained around the nest. Worker response to disturbance is immediate and fierce. Longino interpreted this as a rather crude use of *Cecropia* trees, retaining much of the plesiomorphic nesting behavior. In contrast, *A. xanthochroa* showed a more sophisticated use of *Cecropia* trees. Although there is still a centralized carton nest, the nest is cylindrical, with no deformation of the *Cecropia* bole, and exit holes are placed far below and far above the nest. Thus, the location of the nest is difficult to discern from outside (and thus perhaps less susceptible to discovery by woodpeckers and other predators). The molecular phylogeny reported here places *A. constructor* well within the *muelleri* complex, a result which was not apparent in Longino's revision. The deep branches of *A. xanthochroa* and the *A. constructor / A. merida* cluster suggest that these lineages have been distinct for a long time. If these two lineages share their use of *Cecropia* through common ancestry, *Cecropia* use is ancient in the genus. Alternatively, they could represent two independent colonizations. The deep branch for *A. xanthochroa* is consistent with the proposal that *A. xanthochroa* has had a long association with *Cecropia*, long enough to evolve a highly specialized use of the plant.

Longino proposed that having dispersed polydomous nests in live stems of plants, with distributed brood and a reduced use of carton, was derived relative to central carton nests. Among the taxa analyzed in this report, polydomy in live stems is exhibited by members of the monophyletic group (*A. coeruleipennis* (*A. patruelis* (*A. alfari, A. ovaticeps*))) and so may be a synapomorphy.

There are many additional species of *Azteca* that share live stem polydomy, and among these only *A. alfari, A. ovaticeps*, and *A. coeruleipennis* are obligate inhabitants of *Cecropia*. In the phylogeny produced here, the sister taxon of the *A. alfari / A. ovaticeps* lineage is not a *Cecropia* ant, and the sister taxon of the entire polydomous clade is not a *Cecropia* ant. If the phylogeny is accurate, *Cecropia* use by *Azteca* most likely arose independently in the *A. alfari / A. ovaticeps* lineage and the *A. coeruleipennis* lineage. The alternative is that *Cecropia* use is plesiomorphic, either for the genus as whole or as an independently derived condition of the polydomous clade, and has been secondarily lost in several *Azteca* species.

Multiple evolutionary colonizations of ant–plants appears to be common (Davidson and McKey, 1993). Evidence for this at the generic level is obvious: many ant–plant systems have obligate inhabitants from different ant genera. For example, obligate *Cecropia* ants include not only species of *Azteca*, but also species of *Pachycondyla, Camponotus*, and *Crematogaster* (Davidson and McKey, 1993; Davidson and Fisher, 1991). Increasingly there is also evidence for multiple colonizations within genera. For example, the genus *Pseudomyrmex* shows multiple colonizations of the specialized ant acacias and a variety of other ant–plant

systems (Ward, 1993). The genus *Azteca* is now suggesting a similar pattern of multiple independent colonizations of ant–plants. Currently there is no known case of long-term, parallel cladogenesis of ants and plants.

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REFERENCES

Benson, W. W. (1985). Amazon ant plants. *In* "Amazonia" (G. T. Prance and T. T. Lovejoy, Eds.), pp. 239–266, Pergamon, Oxford.

Bequaert, J. (1922). Ants of the American Museum Congo expedition. IV. Ants in their diverse realtions to the plant world. *Bull. Am. Museum Nat. Hist.* **45:** 333–583.

Brower, A. V. Z. (1994). Phylogeny of *Heliconius* butterflies inferred from mitochondrial DNA sequences (Lepidoptera: Nymphalidae). *Mol. Phylogenet. Evol.* **3:** 159–174.

Buckley, R. C. (1982). Ant–plant interactions: A world review. *In* "Ant–Plant Interactions in Australia" (R. C. Buckley, Ed.), pp. 111–162, Junk, The Hague.

Clary, D. O., and Wolstenholme, D. R. (1985). The mitochondrial DNA molecule of *Drosophila yakuba:* Nucleotide sequence, gene organization, and genetic code. *J. Mol. Evol.* **22:** 252–271.

Davidson, D. W., and McKey, D. (1993). The evolutionary ecology of sybiotic ant-plant relationships. *J. Hymen. Res.* **2:** 13–83.

Davidson, D. W., and Fisher, B. L. (1991). Symbiosis of ants with *Cecropia* as a function of light regime. *In* "Ant–Plant Interactions," pp. 289–309, Oxford Univ. Press, Oxford.

DeBry, R. W. (1992). The consistency of several phylogeny-inference methods under varying evolutionary rates. *Mol. Biol. Evol.* **9:** 537–551.

Felsenstein, J. (1985). Confidence limits on phylogenies: An approach using the bootstrap. *Evolution* **39:** 783–791.

Goodman, M., Czelusniak, J., Moore, G. W., Romero-Herrera, A. E., and Matsuda, G. (1979). Fitting the gene lineage into its species lineage, a parsimony strategy illustrated by cladograms constructed from globin sequences. *Syst. Zool.* **28:** 132–163.

Halloran, N., Du, Z., and Wilson, R. (1993). Sequencing reactions for the Applied Biosystems 373A automated sequencer. *In* "DNA Sequencing: Laboratory Protocols" (H. G. Griffin and A. M. Griffin, Eds.), Vol. 23, Humana, Totowa, NJ.

Harada, A. Y., and Benson, W. W. (1988). Espécies de *Azteca* (Hymenoptera, Formicidae) especializadas em *Cecropia* spp. (Moraceae): Distribuição geográfica e considerações ecológicas. *Revta bras. Ent.* **32:** 423–435.

Hendy, M. D., and Penny, D. (1989). A framework for the quantitative study of evolutionary trees. *Syst. Zool.* **38:** 297–309.

Higgins, D. G., Bleasy, A. J., and Fuchs, R. (1992). CLUSTAL V: Improved software for multiple sequence alignment. *CABIOS* **8:** 189–191.

Hölldobler, B., and Wilson, E. O. (1990). "The Ants," Harvard Univ. Press, Cambridge, MA.

Kempf, W. W. (1972). Catálogo abreviado das formigas da Região Neotropical. *Stud. Entomol.* **15:** 3–344.

Kimura, M. (1980). A simple method for estimating evolutionary rate of base substitutions through comparative studies of nucleotide sequences. *J. Mol. Evol.* **16:** 111–120.

Kumar, S., Tamura, K., and Nei, M. (1993). MEGA: Molecular Evolutionary Genetics Analysis, The Pennsylvania State University.

Longino, J. T. (1989). Taxonomy of the *Cecropia*-inhabiting ants in the *Azteca alfari* species group (Hymenoptera: Formicidae): Evidence for two broadly sympatric species. *Contributions Sci. Nat. History Museum Los Angeles County* **412:** 1–16.

Longino, J. T. (1991a), *Azteca* ants in *Cecropia* trees: Taxonomy, colony structure, and behaviour. *In* "Ant–Plant Interactions" (C. R. Huxley and D. F. Cutler, Eds.), pp. 271–288, Oxford Univ. Press, Oxford.

Longino, J. T. (1991b). Taxonomy of the *Cecropia*-inhabiting *Azteca* ants. *J. Nat. Hist.* **25:** 1571–1602.

Pamilo, P., and Nei, M. (1988). Relationships between gene trees and species trees. *Mol. Biol. Evol.* **5:** 568–583.

Saiki, R. K., Gelfand, D. H., Stoffel, S., Scharf, S. J., Higuchi, R. G., Horn, G. T., Mullis, K. B., and Erlich, H. A. (1988). Primer-directed enzymatic amplification of DNA with a thermostable DNA polymerase. *Science* **239:** 487–491.

Sambrook, J., Fritsch, E. F., and Maniatis, T. (1989). "Molecular Cloning: A Laboratory Manual," Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY.

Shattuck, S. O. (1992). Generic revision of the ant subfamily Dolichoderinae (Hymenoptera: Formicidae). *Sociobiology* **21:** 1–181.

Shattuck, S. O. (1994). Taxonomic catalog of the ant subfamilies Aneuretinae and Dolichoderinae (Hymenoptera: Formicidae). *Entomology* **112:** i–xix+241.

Shattuck, S. O. Generic-level relationships within the ant subfamily Dolichoderinae, submitted for publication.

Swofford, D. L. (1991). PAUP: Phylogenetic Analysis Using Parsimony, Version 3.0s. Computer program distributed by the Illinois Natural History Survey, Champaign, IL.

Walsh, P. S., Metzger, D. A., and Higuchi, R. (1991). Chelex 100 as a medium for simple extraction of DNA for PCR-based typing from forensic material. *BioTechniques* **10:** 506–513.

Ward, P. S. (1993). Systematic studies on *Pseudomyrmex* acacia-ants (Hymenoptera: Formicidae: Pseudomyrmecinae). *J. Hymen. Res.* **2:** 117–168.

Wheeler, W. M. (1942). Studies of neotropical ant-plants and their ants. *Bull. Museum Comp. Zool.* 1–262.

Wilson, A. C., Stoneking, M., and Cann, R. L. (1991). Ancestral geographic states and the peril of parsimony. *Syst. Zool.* **40:** 363–365.

Yu, D. W., and Davidson, D. W. The colonization of *Cecropia* by symbiotic ants: Factors leading to specificity in species interactions, submitted for publication.