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## Mutualism within a parasitism within a mutualism: the bees and coccids that inhabit *Cecropia* ant-plants

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Thomas Belt (“The Naturalist in Nicaragua” 1874) recorded scale insects (Hemiptera) and biting ants, *Azteca*, in trees of *Cecropia* (Urticaceae), but no one has reported bees. Discovery of stingless bees (Meliponini) nesting high in Amazonian *Cecropia* (Fig. 1; Appendix S1: Fig. S1), with obligatory coccid scale insects, led me to recast the evolution of an ant-plant. Because I found two pioneer trees with bees and scales, and they constitute the most primitive and most derived of the entire *Cecropia* clade of over 60 species, their ecology offers a glimpse of chaotic first steps toward an intricate modern mutualism. Without bees in the system, *Cecropia* and ants may not have coevolved.

One of the two *Cecropia* clades both feeds and houses ants (Appendix S1: Fig. S2), usually *Azteca* or similarly aggressive species. The sister to those *Cecropia* represents ancestral ant-plants and is a large and common pioneer tree in western Amazonia (Wheeler 1942, Berg et al. 2005, Pérez et al. 2014, Gutiérrez-Valencia et al. 2017): *C. sciadophylla*. An early adaptation among *Cecropia* was the formation of small pits that could be chewed open by the winged, dispersing queen ants. This benefitted *Cecropia* because certain ants could rid their host of competitors and herbivores, thus would be most effective when nesting within the hollow branches that *Cecropia* possess. Near

the close of the Miocene, a new adaptation allowed glyco-gen to form near leaf bases on *Cecropia*. Ant foragers that dominated the minute food bodies as they were dispensed evolved obligate mutualisms, which I suggest occurred while ineffectual ants and stingless bees were outcompeted for host trees. However tight that mutualism appears, there remains a mutualism within a parasitism within a mutualism, examined here.

In the middle canopy, 20–30 m aboveground, the denizens of *Cecropia* were largely unexplored. Windfallen trees investigated with a Waorani resident at Yasuní Biosphere in eastern Ecuador contained the first bee colony I noted, followed with three years and 55 field days cutting and examining the largest *Cecropia* at forest edges and in wet lowlands. Only *Cecropia sciadophylla* and *C. ficifolia* contained bees and ants, although I focused most on those species. They were conspicuous by having several individuals per hectare in the forest (Pérez et al. 2014) and many more at forest edges. A tiny (2 mm long) social bee, *Plebeia* (Meliponini, *P. minima* group), and both soft and mealy scales (Coccidae: mainly *Cryptostigma*) along with different ants (*Camponotus*, *Crematogaster*, *Daceton*, *Azteca*, *Cephalotes*) lived within the branches, often in neighboring internodes. Almost all the two *Cecropia*, 91% of 30 examined, had bees, an average of six to eight colonies per tree species. Biogeography and taxonomy of most the species involved are scarcely known. Their Latin names are given here at the generic level.

The *Cryptostigma* scale insects offered honeydew and provided wax to *Plebeia* for nest construction and, as is common among stingless bees (Roubik 2006), wax is combined with collected resin to make clay-like cerumen. This cerumen material was piled upon brood cells, but nest walls were occupied by scale insects (Fig. 1B). I kept colonies in severed branches for up to eight weeks that died without the coccids, which died first. Studied in the laboratory, scales excreted 13.5% sugar liquid, measured with a refractometer, when stimulated by a slight touch (Fig. 1). The honeydew was taken by worker bees and is deep red, while white wax also was taken from scales of all sizes except “crawlers,” the mobile first-instar nymphs (Fig. 1A; Appendix S1: Fig. S1). Red bee nests took their color from red scale secretions turned to wax by bees. I deduced the lack of stored honey in *Cecropia*-bee nests was related to scale insect symbionts. Coccids were anchored in all the 186 nests of *Plebeia* that were found. These immobile and parthenogenetic females must avoid fungal infection (Kondo and Gullan 2004). The *Plebeia* that I examined over multiple seasons and years basically stored no honey (see also Appendix S1: Table S1), which creates suitable dry space for coccids. Since nectar contains water, honey ripening raises humidity. *Plebeia* can

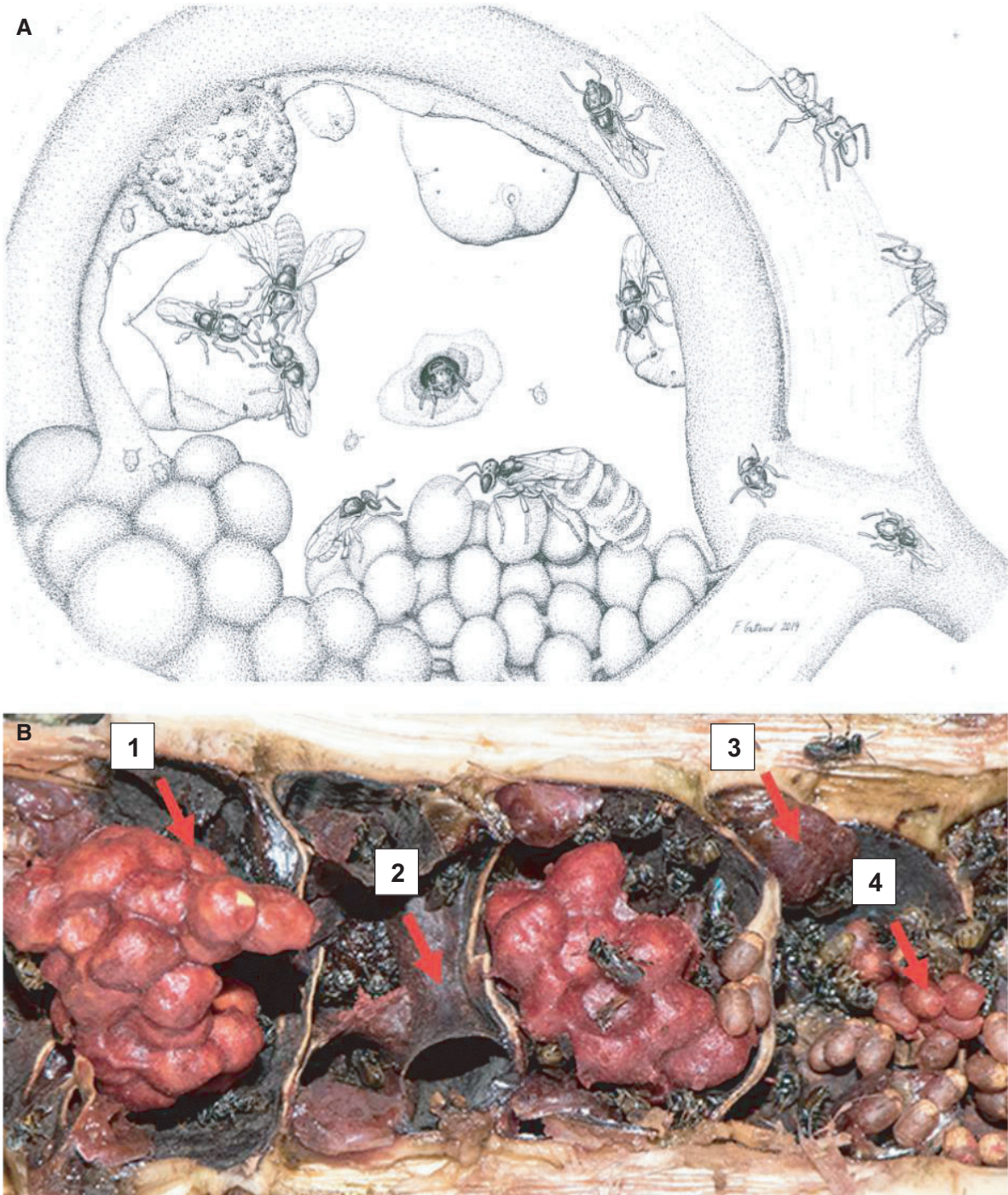


FIG. 1. *Cecropia* bees (*Plebeia* sp.) and coccids of different life stages living in *Cecropia* stems. (A) General schema in nest section in *C. sciadophylla*, with bees entering, leaving entrance tube with trash pellet, and nest pollen pots, brood and queen, resin storage deposit (upper left), three bees feeding at coccid anal pore exudate, worker passing through hole between *Cecropia* internodes, coccid "crawler" first-instar larvae in nest; ants on exterior bark, one with pseudococcid in mandibles. (B) *Plebeia* nest in section of *C. ficifolia*; light red pollen pots (1), internal ring entrance tube (2), dark red scale insect *Cryptostigma* (3), young and mature *Plebeia* brood (4). Panel A drawing credit: F. Gattesco; panel B photo credit: D. W. Roubik.

further dehumidify nests by wing fanning that moves air through nest entrances and vents over former ant holes (Fig. 1; Appendix S1: Fig. S1). The pollen stored in nests was notably dry, and colonies harbored dozens of large female *Cryptostigma* (means 20–31, in the two trees). Therefore, the removal of honeydew and also excreta benefited both the scale and bee colony. Wax is produced by honey-making bees by metabolizing seven or more times the wax weight in honey. Thus, coccids save bees space and energy in return for protection but, at the same time, bees lose space for brood or pollen when occupied by the large, female *Cryptostigma*. A further trade-off is evident. Tiny recruiting tropical social bees are nuisances for collecting human sweat. Among the 40 smallest stingless bees that inhabit Yasuní (Roubik 2018), the two *Plebeia* (2 mm long) that I studied predominate on sweaty skin (Appendix S1: Fig. S1). Sap, pollen, and nectar contain negligible sodium yet all animals require it (Xiao et al. 2010). Hemipterans provide honeydew, further remove salt for their needs, and salt-seeking behavior thus had particular selective advantages for obligate coccidophile bees. Obligate coccidophiles were first discovered in Amazonian meliponines of another genus (Camargo and Pedro 2002, Kondo 2010), which also may forage sweat and sodium more often than other bees.

Because ants open holes to occupy *Cecropia*, and the bees have no means of doing this, bees depend upon myrmecophyte plants and the ants themselves. Now, Ecuadorian *Cecropia*, ranging the western Amazon to the Guiana Shield, are the first known melittophytes. In French Guiana, the relatively unaggressive *Crematogaster*, *Camponotus*, *Daceton*, or *Cephalotes* ants were found, but no bees lived in the latter region's *C. sciadophylla* and no coccids (Appendix S1). In Ecuador, one individual *C. sciadophylla* had *Azteca* nesting in all 11 branches, with *Cryptostigma* in their nests, and no bees in the tree. Several meters away, two *Plebeia* species dominated the same tree species. *Cryptostigma* inhabited all their 22 nests while other ants but no *Azteca* were present. Thus the relationship of *Cecropia*, ants, bees, and coccids varied. As aggressive ants eliminate competitors, and bees may vie with ants for *Cecropia* internodes, their strategies differ. Bees seem unequipped to displace ants; the *Plebeia* guards have only one small mandibular tooth and do not pursue intruders outside nests, although they are well defended by resinous bee nest entrance tubes that can be elongated. The longest was 11 cm, is a chemical and physical barrier, and can be closed by guard bees (Roubik 1989, 2006). The queen cannot fly once mated, and when a *Cecropia* was opened, she often concealed herself in the hard resin internal nest tube (Fig 1).

The question arises of how *C. ficifolia* in Ecuador was generally dominated by *Azteca* while *C. sciadophylla* had bees and other ants, but both had one *Plebeia* species living with them, sometimes as neighbors. No clues

have emerged. My evolutionary argument is that a fitness penalty for harboring opportunists might have prevented further evolution in the ant plants. Instead, food was established as a supplement to an ant nest site, and those ants that increased *Cecropia* fitness predominated. However, bees and coccids, which provide nothing to their host trees, are still abundant. They seem to remain beyond the reach of natural selection but are not simply commensals.

Due to proximity, aggressive *Azteca* should protect successfully cohabiting bee colonies from predators, a collateral advantage to bees. Common bee and ant nest predators were hammerhead woodpeckers, *Dryocopus*, which made furrows through *Cecropia* (Appendix S1: Fig. S1). There is no reciprocation by bees for protection. Bee colonies in *Cecropia* would fail to pollinate the unisexual trees, pollinated by wind (Pérez et al. 2014); flowers have no nectar. Moreover, all bees were unaggressive. Bees and *Cryptostigma* were not mutualists of *Cecropia* or ants.

Scale crawlers may be dispersed to bee nests on virgin queens, which are larger than workers (Fig. 1), or upon worker bees (Camargo and Pedro 2002), but this has not been verified. However, in nests I studied in the laboratory, no interactions occurred between bees and crawlers. Visits to trees by animals foraging at *Cecropia* are other possible dispersal routes for coccids, but colony reproduction by meliponines requires nest construction before dispersal, and should include the nest symbionts, which avidly seek transport on bees (Roubik and Wheeler 1982, Roubik 2006).

*Plebeia* dates to the Miocene (Rasmussen and Cameron 2010) as do *Azteca* and *Cecropia* (Gutiérrez-Valencia et al. 2017). In the Miocene, as now, ant colonies that die or move up the stem as trees grow provide unoccupied cavities that bees, ants, coccids and others may exploit. Predators like *Dryocopus* also facilitate access to living *Cecropia* stems. Together their interactions affected the *Cecropia* ant-plant system, with perhaps only one bee group that has consistently exploited it as a third party (Bronstein 2015), aided by a special mutualist and now living within a mutualism that it helped to forge.

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