

Biology of *Lissoderes* Champion (Coleoptera, Curculionidae) in *Cecropia* saplings inhabited by *Azteca* ants

JU-LIN WENG^{1,2}, KENJI NISHIDA¹, PAUL HANSON¹ & LOUIS LAPIERRE³

¹Escuela de Biología, Universidad de Costa Rica, San José, Costa Rica, ²Department of Entomology, Kansas State University, Manhattan, KS, USA, and ³Department of Biology, Lower Columbia College, Longview, WA, USA

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Abstract

This paper provides an account of the biology of *Lissoderes* (Coleoptera, Curculionidae, Conoderinae) focusing on *L. pusillus* Hesperheide and *L. subnudus* Champion. The eggs, larvae, and pupae live inside the hollow stems of *Cecropia* saplings. Adult weevils chew through the stem and deposit eggs on the inner surface of the internode. The larvae feed on the parenchyma lining the hollow internodes and pupate inside the internode. Emerging adults chew their way out of the stem. Two hymenopteran parasitoids were reared from larvae and prepupae of *L. pusillus*: *Neocatolaccus* sp. (Pteromalidae) and *Heterospilus* sp. (Braconidae). *Menozziola* sp. (Diptera, Phoridae) and *Conoaxima* sp. (Hymenoptera, Eurytomidae) were observed parasitizing *Azteca* queens. Parasitism by these species may explain part of the high mortality observed in colonizing *Azteca* queens. Direct competition with *L. pusillus* and *L. subnudus* appears not to be a major cause of queen mortality, although possible indirect effects of the weevils remain unknown.

Keywords: *Azteca*, *Cecropia*, *Conoderinae*, *Costa Rica*, *Lissoderes*, *parasitoids*

Introduction

Species of *Cecropia* Loefling (Cecropiaceae) are some of the most common and characteristic trees of early successional vegetation in the Neotropics (Berg and Franco Rosselli 2005). Moreover, the mutualism between *Cecropia* and certain species of *Azteca* ants (Hymenoptera, Formicidae) is one of the best known ant–plant associations (Wheeler 1942; Benson 1985; Davidson and Fisher 1991; Longino 1991a; Folgarait and Davidson 1995; Yu and Davidson 1997; Davidson 2005), whereas other insects associated with *Cecropia* are diverse yet less well understood (Jolivet 1991; Jordal and Kirkendall 1998; Hesperheide and LaPierre 2002, forthcoming; LaPierre 2002; H. A. Hesperheide and L. M. LaPierre, unpublished). The ants nest in the hollow stems and harvest Müllerian bodies

Correspondence: Louis M. LaPierre, Department of Biology, Lower Columbia College, 1600 Maple St., Longview, WA 98632, USA. Email: llapierre@lowercolumbia.edu

from the dense pad of trichomes (the trichilium) located at the base of the petioles and pearl bodies from the undersides of the leaves; in turn the worker ants cut encroaching vegetation (Janzen 1969), remove insect herbivores (Schupp 1986; Agrawal 1998), and provide the plant with nitrogen (Sagers et al. 2000). The hollow stems of *Cecropia* are divided into a series of closed chambers (internodes) which are separated from one another by solid partitions (septa) at each node. In young stems the upper part of each internode develops an oval depression (prostoma) where the wall is thinner and through which the colonizing *Azteca* queen chews a hole in order to enter. Once inside the internode the queen scrapes plant material over the entrance hole, which eventually closes even more tightly as callus tissue grows over it. The queen thus remains sealed inside the internode and apparently relies primarily on her own body reserves to rear the first brood of workers.

Cecropia saplings are colonized by numerous *Azteca* queens, although mature trees are usually dominated by just a single colony of ants (Longino 1989, 1991a). This results in competition for nesting sites among queens, especially since they often prefer the upper internodes, and different species of *Azteca* vary in their colonization strategy. For example, queens of *A. constructor* Emery are more successful when they found colonies together (pleometrosis) in the same internode, whereas queens of *A. xanthochroa* Roger survive equally well in both haplometrotic (founding alone) and pleometrotic associations, including those containing both species (Choe and Perlman 1997). Once the first workers emerge and reopen the prostoma, they begin foraging for Müllerian bodies, storing them in the internodes presumably as food for larvae. In pleometrotic associations each queen produces workers; in *A. xanthochroa* only a single queen survives past the emergence of the first workers whereas in *A. constructor* cofoundresses may cooperate for a year or more (Choe and Perlman 1997). As the colony grows the workers chew holes in the septa that separate the internodes. Saplings frequently contain numerous incipient colonies in separate internodes and at some point the strongest colony presumably eliminates all others (either directly and/or indirectly by harvesting most of the Müllerian bodies; Longino 1991a).

In Costa Rica there are four species of *Cecropia* (excluding a species restricted to Cocos Island), three of which are colonized by *Azteca* ants: *C. insignis* Liebmann, *C. obtusifolia* Bertoloni, and *C. peltata* L. (primarily on the Pacific side); the fourth species, *C. angustifolia* Trécul [formerly *C. polyphlebia* Donnell Smith (Berg and Franco Rosselli, 2005)], is a higher elevation (1400–2400 m) species devoid of *Azteca* colonies, although founding queens often attempt colonization (Burger 1977; Longino 1991a). There are more than 20 species of *Azteca* in Costa Rica, five of which are associated with *Cecropia*: *A. alfari* Emery, *A. ovateiceps* Emery, *A. coeruleipennis* Emery (primarily in *C. peltata* on the Pacific side), *A. constructor*, and *A. xanthochroa* (Longino 1991b). The only other ant that is a regular associate of *Cecropia* in Costa Rica is an undescribed species of *Pachycondyla*. Unlike the *Azteca* species mentioned above, queens of this species keep the prostoma open (they do not seal themselves inside the internode) and their colonies, which are much smaller, mature and reproduce very quickly before *Azteca* take over the tree. Other ants in various genera (e.g. *Camponotus*, *Crematogaster*, and *Pseudomyrmex*; L. M. LaPierre, personal observation) occasionally colonize young *Cecropia* stems, but in Costa Rica none of these is known to be a *Cecropia* specialist.

Even before they begin producing workers, *Azteca* queens suffer a high rate of mortality (Longino 1989). Because the causes of this mortality are largely unknown, more knowledge of the other inhabitants of young *Cecropia* stems may be relevant to a better understanding of the colonization process. In general, these other inhabitants have received very little

attention. In his revision of the genus *Lissoderes* Champion (Curculionidae, Conoderinae, formerly Zygopinae), Hespenseide (1987) noted that the larvae and adults of these weevils have been observed inside the internodes of *Cecropia* saplings, an observation that was confirmed by LaPierre (2002). However, as far as we are aware no further details are known of their biology. Indeed, the biology of Neotropical Conoderinae is in general poorly known (Lyal 1986; Jordal and Kirkendall 1998; Hespenseide 2005 and references cited therein; Hespenseide and LaPierre forthcoming; H. A. Hespenseide and L. M. LaPierre, unpublished). The genus *Lissoderes* currently comprises five described species, all of which are confined to the Neotropical region, and four of which occur in Costa Rica. Here we describe the biology of *Lissoderes*, based on data collected from *L. pusillus* Hespenseide and *L. subnudus* Champion, and compare its colonization of *Cecropia* saplings with that of *Azteca* queens. We also discuss to what extent the weevils are a parasite of the mutualism between *Cecropia* and *Azteca* and whether it contributes to the high mortality rate observed in colonizing *Azteca* queens. Finally, we provide some previously unreported observations on other sources of this high mortality rate.

Study sites and methods

Alberto Manuel Brenes Biological Reserve

Most of the research was carried out by Weng, Nishida, and Hanson in Alberto Manuel Brenes Biological Reserve (AMBBR; 10°13'12"N, 84°36'6"W; 850 m altitude) located on the Caribbean side of the Tilarán mountains, in the San Ramón protected zone, Alajuela Province, Costa Rica. The site is in premontane rainforest, with an average daytime temperature of 21°C and yearly precipitation of 4000–5000 mm (Ortiz 1996). Three visits were made to the field site: March 2002, October 2002, and April 2003. Although more than one *Lissoderes* spp. can occur at a given site (Hespenseide 1987), only one species of *Lissoderes*, *L. pusillus*, was observed and reared at this site during the three visits. Two species of *Cecropia* grow at the site, *C. insignis* (the dominant species in the area) and *C. obtusifolia* (which reaches the upper altitudinal limits of its distribution in this area). Saplings (<1.5 m) of both species, which were growing along the dirt road leading into the station, were cut at the base and brought back to the laboratory. These plants all lacked patrolling ants. Samples for the quantitative studies of colonization were taken in October and included 35 saplings of *C. insignis* and 44 of *C. obtusifolia* (two very small saplings of the latter species were excluded because they lacked internodes).

At AMBBR, stems were cut transversely at each node; each internode was dissected longitudinally and examined under a dissecting microscope. The number of internodes occupied by ants, weevils (including oviposition holes), and other insects was recorded. Photographs of each life stage were taken with a digital camera (Nikon Coolpix 990; Nikon Corp., Tokyo, Japan). Some larvae were boiled and preserved in 75% ethanol. At the entomological laboratory of the University of Costa Rica (UCR; average room temperature 23–24°C), the remaining larvae and pupae were reared in freshly cut internodes, which were placed in plastic bags for further observation. Larvae and pupae of weevil parasitoids were also reared in the internodes under well-ventilated conditions to avoid infestation by fungi. Voucher specimens of all insects are deposited in the Museo de Zoología, Escuela de Biología, Universidad de Costa Rica.

To compare resource quantity between the two *Cecropia* species, the length of internodes occupied by larvae and the thickness of its parenchyma (at the midpoint of the internode)

were measured. Interactions between weevil larvae and ants were observed by splitting open internodes containing one or more queens, placing a weevil larva within, and then either watching the ensuing interaction under the dissecting microscope, or attaching the two halves of the internode with masking tape and reopening it about an hour later.

The Mann–Whitney *U* test was used to compare the frequency of living and dead *Azteca* queens found in internodes harboring solely dead and living queens in each *Cecropia* species. Pearson correlation analyses were used to determine the relation between number of internodes occupied by weevils and *Azteca* ants (live and dead ants) of each plant species. Simple linear regression analyses were used to determine the effect of total number of internodes of each plant type (independent variable) on the number of internodes colonized by *Azteca* ants and those occupied by weevils, excluding saplings that harbored neither *Azteca* ants nor weevils. The Student's *t* test was used to compare the average length and parenchymal thickness of occupied internodes between two *Cecropia* species.

La Selva Biological Station

Data from a second study site, La Selva Biological Station (“La Selva”), visited by LaPierre during September 1996 are included in order to make comparisons with some of the data gathered from AMBBR. La Selva (10°26'N, 83°59'W; 50–150 m altitude) is located on the Caribbean slope of the Cordillera Central at the confluence of the Río Puerto Viejo and Río Sarapiquí, Heredia Province, Costa Rica. La Selva is in lowland rainforest with an average daytime temperature of 26°C and yearly precipitation of 3962 mm (McDade et al. 1994). Two *Lissoderes* species, *L. pusillus* and *L. subnudus*, are known to occur at La Selva (H. A. Hespeneheide, personal communication). Similar to AMBBR, only *C. insignis* and *C. obtusifolia* occur at this site but with the latter species being more abundant at La Selva. Twenty-two *C. obtusifolia* and 12 *C. insignis* saplings (0.5–2.0 m) lacking patrolling ants were harvested and brought into the laboratory for dissection. The data recorded from the La Selva samples were limited to the numbers of ants, weevils, and other insects encountered in each internode.

Two-by-two contingency table analyses applying *G* tests were used to compare the internode contents between the two *Cecropia* species at La Selva.

Results

Colonization of Cecropia saplings by L. pusillus and ants at AMBBR

Seventy-nine of 81 *Cecropia* saplings examined had 2–18 well-developed hollow internodes (mean \pm SD = 8.1 \pm 3.8 internodes). Most saplings (71/79 = 90%) showed evidence of various insects (Table I), primarily *L. pusillus* and queens (or incipient colonies) of *Azteca constructor* and *A. xanthochroa*. The eight saplings that lacked insects ranged between 11 and 44 cm in length.

About 40% of the 639 internodes examined were used by *Lissoderes* or *Azteca*: 19.2 and 20.4%, respectively. In only about 1% of the internodes did we find evidence of *Lissoderes* and *Azteca* living together. In the laboratory, the results of placing *Lissoderes* larvae in the same internode as *Azteca* queens were variable; the ants ignored the weevil larva, cared for it (one queen repeatedly carried the weevil larva back to her pile of eggs as it attempted to crawl away), or killed it. In contrast *Pachycondyla* queens and *Azteca* workers always killed the weevil larva.

Table I. Number of hollow internodes examined and the percentage of internodes occupied by *Lissoderes*, *Azteca* ants, and other insects, in two species of *Cecropia* saplings occurring at two locations in Costa Rica, the Alberto Manuel Brenes Biological Reserve (AMBBR) and La Selva Biological Station (La Selva).

<i>Cecropia</i> sp. (number of plants)	Internodes (% occupied)	<i>Lissoderes</i>			<i>Azteca</i> ants				Others	
		Live	Dead	Parasitoids	Queens		Incipient colonies	Parasitoids	Other ants ^a	Moth larvae ^b
					Live	Dead				
<i>C. insignis</i>										
AMBBR (<i>n</i> =35)	317 (32.45)	12.60%	6.90%	0.90%	3.15%	5.40%	No data	No data	2.20%	1.30%
La Selva (<i>n</i> =12)	186 (70.97)	No data	No data	No data	5.91%	16.13%	5.38%	5.38%	9.14%	No data
<i>C. obtusifolia</i>										
AMBBR (<i>n</i> =44)	322 (53.35)	16.15%	1.24%	0.62%	9.63%	22.05%	No data	No data	2.73%	0.93%
La Selva (<i>n</i> =22)	236 (48.31)	No data	No data	No data	5.51%	9.75%	15.25%	2.54%	0.85%	No data

See text for study site descriptions. ^aAMBBR: *Pachycondyla* sp. undet., *Pheidole* sp. undet; La Selva: *Pseudomyrmex* sp. undet., *Camponotus* sp. undet; ^bGlyphipterygidae sp. undet.

Lissoderes colonization of *Cecropia* saplings begins very early, when the plant has only three or four available internodes (Figure 1). In contrast, colonization by *Azteca* ants differs between the two species of *Cecropia*—in *C. obtusifolia* colonization begins simultaneously with that of the weevils (when the plant has four internodes), but in *C. insignis* it does not begin until the plant has eight internodes (Figure 1). Thus the ants colonize a greater range of sapling sizes in *C. obtusifolia* than in *C. insignis*, and in the former species ants occasionally colonize every available internode (e.g. one plant with eight internodes), which never occurred in *C. insignis*. As a result, ants occupied a greater proportion of internodes in *C. obtusifolia* than in *C. insignis* (Table I), and more *C. obtusifolia* saplings (27.3%) contained only ants, compared with *C. insignis* (5.7%). In contrast, the proportion of internodes occupied by live weevils in the two *Cecropia* species was quite similar (Table I). Fewer *C. insignis* saplings (33%) contained weevils and ants, compared with 73% for *C. obtusifolia*. A higher percentage of *C. insignis* saplings (28.6%) contained only weevils compared with *C. obtusifolia* (15.9%). Despite these differences between *Cecropia* species, we found no evidence of any particular sequence of colonization by weevils and ants; weevils were found just as often in internodes above and below those harboring ants.

Azteca queens were the principal colonizers of the 44 *C. obtusifolia* saplings' 322 internodes; they occupied 32% of the internodes, whereas the weevils occupied only 18%. In contrast, *Azteca* queens occupied only 9% of the 317 internodes in *C. insignis*, while the weevils occupied 20% (Table I). However, *C. obtusifolia* also had more internodes harboring dead *Azteca* queens—twice as many internodes as those with living queens (Table I). The number of dead queens found per internode ranged from 1 to 17 (mean \pm SD = 3.99 ± 4.13 queens) but most of the internodes (69.8%, *n*=63) contained one to three individuals. The maximum number of living queens found within internodes of *C. obtusifolia* was three (mean \pm SD = 1.37 ± 0.63 queens), but about 70% of 27 internodes harbored only one queen. Mortality seemed to be related to a larger number of queens in each internode ($U_{(63,27)}=521.5$, $Z=2.90$, $P<0.004$) in *C. obtusifolia* saplings. In *C. insignis* saplings, there were almost twice as many internodes with dead queens (mean \pm SD = 1.07 ± 0.26 queens) as with live queens (mean \pm SD = 1.12 ± 0.35 queens).

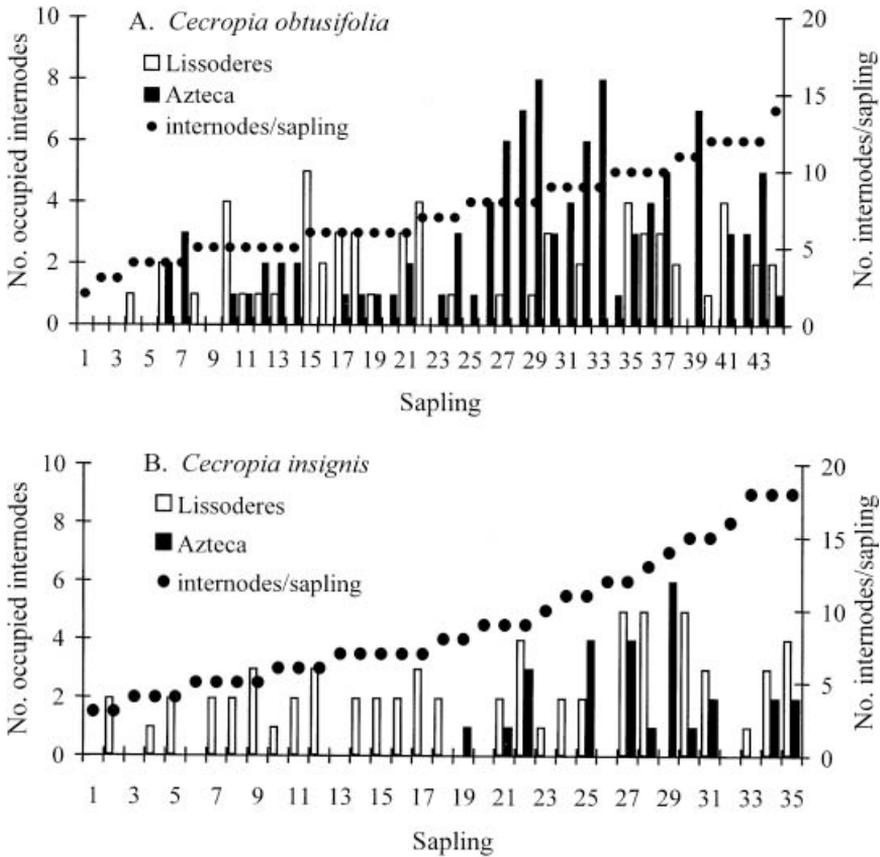


Figure 1. Number of internodes per sapling occupied by *Azteca* ants and *Lissoderes pusillus* in *Cecropia obtusifolia* ($n=44$) (A) and *C. insignis* ($n=35$) (B). Saplings are ranked by increasing number of internodes.

Here, the frequency of dead queens per internode is similar to that of living queens ($U_{(15,8)} = 56.5$, $Z = -0.22$, $P = 0.82$).

Among saplings colonized by ants, weevils, or both, the number of internodes colonized by weevils was negatively related to the number of internodes occupied by *Azteca* ants in *C. obtusifolia* ($r = -0.34$, $P < 0.05$, $n = 39$), but did not show any relation in *C. insignis* ($r = -0.25$, $P > 0.05$, $n = 28$). The weevils did not show any pattern of abundance in relation to the size of the sapling in either *C. insignis* ($r = 0.29$, $r^2 = 0.05$, $F(1,33) = 2.95$, $P = 0.10$) or *C. obtusifolia* ($r = 0.20$, $r^2 = 0.02$, $F(1,42) = 1.85$, $P = 0.18$). In contrast, *Azteca* occupied more internodes in larger saplings of *C. insignis* ($r = 0.47$, $r^2 = 0.20$, $F(1,33) = 9.46$, $P = 0.004$), which is not surprising since colonization does not begin until the sapling has eight or more internodes (as mentioned above). Similarly, the frequency of internodes containing *Azteca* queens increased with size of *C. obtusifolia* saplings ($r = 0.43$, $r^2 = 0.16$, $F(1,42) = 9.51$, $P = 0.003$).

Colonization of Cecropia saplings by ants and Lissoderes spp. at La Selva

A total of 422 internodes from 34 saplings were dissected (236 *C. obtusifolia* and 186 *C. insignis*). Although all of the saplings showed evidence of colonization by *Azteca*, *Lissoderes*,

or other ants, more *C. obtusifolia* internodes were empty (51.7% compared to 29.0% for *C. insignis*; *G* test $P < 0.0001$; Table I). Weevils colonized twice as many internodes of *C. insignis* as *C. obtusifolia*, and of the occupied internodes none contained ants and weevil larvae together. Ant species other than obligate *Azteca* species were relatively common in *C. insignis* internodes (Table I). Live *Azteca* queens were encountered in the same proportion of internodes between the two *Cecropia* species, but success in founding a colony was higher in *C. obtusifolia* by a factor of nearly three while the number of *C. insignis* internodes with dead queens was nearly double that of *C. obtusifolia* (Table I). So while the number of internodes with *Azteca* queens did not differ significantly between *C. insignis* and *C. obtusifolia* (27.4% compared to 30.5%, respectively; *G* test $P = 0.49$), the outcome for the queens did differ with the majority of these internodes containing dead queens in *C. insignis* and incipient colonies in *C. obtusifolia* (Table I; *G* test $P = 0.05$).

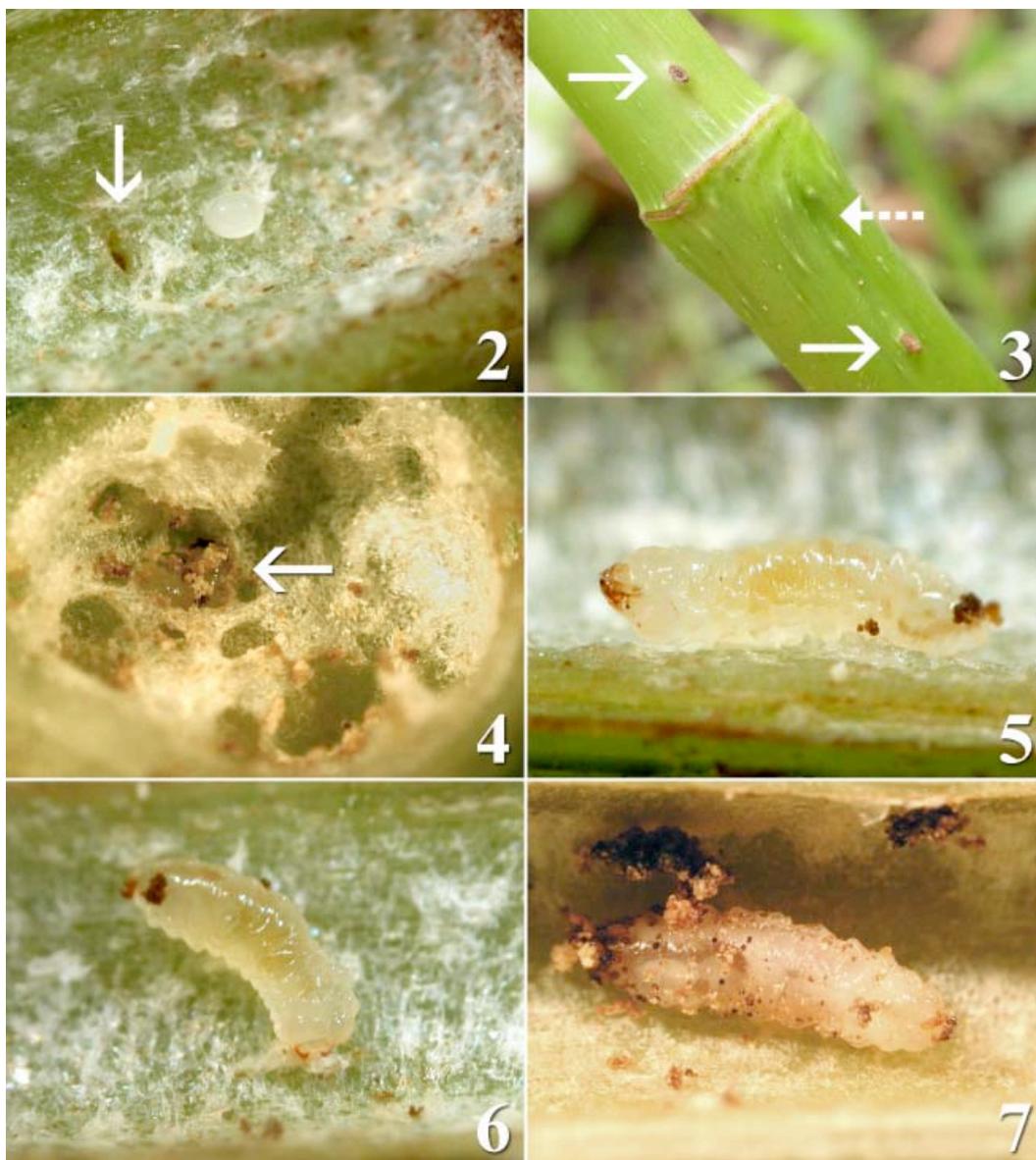
Life history of L. pusillus

Observations of *L. pusillus* at AMBBR suggest that the approximate duration of each life stage is as follows: egg—12 days; first instar larva—4 days; second instar—6 days; third instar—10 days; prepupa—3 days; pupa—14–16 days (mean \pm SD = 15.12 ± 0.35 days, $n = 8$); and teneral adult—3 days. Thus the total duration from oviposition to adult emergence is approximately 7 weeks.

The egg (Figure 2) is white, ovoid, and measures 0.49 ± 0.02 mm \times 0.63 ± 0.03 mm ($n = 4$; mean \pm SD). Under the dissecting microscope the chorion appears to be smooth and shiny. Sixteen eggs were observed in dissected internodes (seven in *C. insignis* and nine in *C. obtusifolia*). The egg is laid singly per internode; however, in three cases we observed two eggs in the same internode of *C. insignis*; in two of these the eggs were laid adjacent to each other and only one perforation scar was found (suggesting that they were laid by the same female), and in the third case, two perforations were observed about 6 mm apart. The eggs were nearly always located on the inner wall of the internode chamber, next to the ovipositional perforation (mean \pm SD = 1.18 ± 1.07 mm from the hole; Figure 2). These perforations are visible from the outside of the stem as a light brown scar measuring approximately 1 mm in diameter (Figure 3), and usually located in the upper one-third of the internode (50%), infrequently in the lower third (31%) and middle third (19%). About a third of the ovipositional perforations located in the upper third were in the prostoma (Figure 3).

Although oviposition behavior was not observed, the female weevil probably utilizes her mandibles to perforate the stem and oviposit through this hole, as has been reported in other weevils (Howden 1995). In one instance we observed an egg that was located inside an incomplete perforation and in this case the stem wall appeared to be thicker than the mean length of the female rostrum. The rostrum of female *L. pusillus* (Figure 13) is about 2 mm long and 0.4 mm wide at its broadest point, the apex.

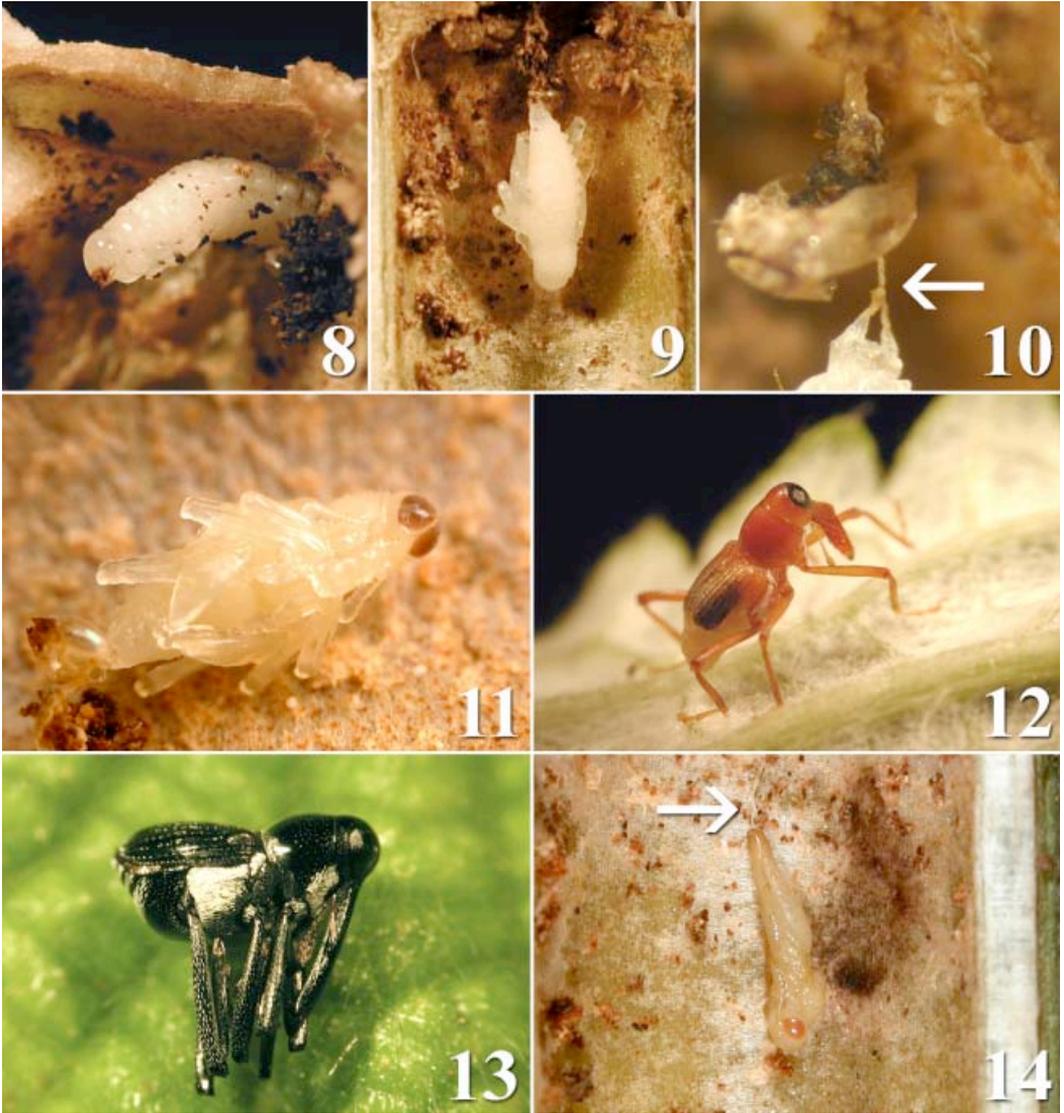
No internode contained more than one living weevil larva, but one larva was found in an internode together with a head capsule of another larva of the same age. When two larvae were placed in the same chamber for 24 h, only one survived ($n = 7$ pairs). When two eggs were placed together, the larva that hatched first killed the other larva upon hatching ($n = 3$ pairs). In both cases the live larva had not eaten the dead larva after 42 h (at which point it was beginning to decompose). Thus they do not appear to be cannibalistic, but rather very aggressive toward competitors.



Figures 2–7. Biology of *Lissoderes pusillus*. (2) Egg *in situ*. Arrow pointing at ovipositional perforation. (3) Ovipositional perforation (solid arrows) and prostoma (dashed arrow). (4) First instar larva feeding on underside of upper node. Grooves or holes indicate where the larva has consumed tissue (arrow pointing at the larva). (5) Second instar larva, lateral view (note the upside-down position). (6) Second instar larva, ventral view, feeding on spongy parenchyma tissue. (7) Mature third instar larva, ventral view (connected fecal pellets are seen on and near the larva).

The body of the larva (Figure 4–7) is subcylindrical and fleshy, with dorsal plicae (enlarged swellings or folds) that bear a few setae. The thoracic segments have a greater diameter than the abdominal segments and the lateral swellings distinguish them from abdominal segments. The first instar larva (Figure 4) is creamy white, 1.86–2.46 mm in length. The head is pale yellowish brown with reddish brown mouth parts, 0.28–0.32 mm

in width, and retracted up to two-thirds of its length into the prothorax ($n=4$). The second instar (Figures 5, 6) is 3.12–3.54 mm, with a head width of 0.40–0.46 mm ($n=4$). The third instar (Figure 7) is 4.02–5.76 mm, head width 0.58–0.64 mm ($n=6$). The prepupa (Figure 8) is creamy white and the head is protruded. The pupa (Figures 9–11) is white with dark red eyes and moveable abdominal segments.



Figures 8–14. Biology of *Lissoderes pusillus*. (8) Prepupa *in situ*, attached to upper node (note head is completely exposed). (9) Pupa *in situ*, hanging freely inside the internode. (10) Close-up view of shed exuvia of last larval instar and last abdominal segments of pupa (arrow pointing at the connection). (11) Pupa (several days old), ventral view. (12) Teneral adult male (note shape of male rostrum). (13) Adult female displaying thanatosis (note shape of female rostrum). (14) Pupa of parasitoid wasp, *Neocatolaccus* sp. *in situ*, hanging from internode chamber wall (arrow pointing at the connection).

The weevil larvae feed on the parenchyma (Figures 4–6) that lines the inner wall of the internode. First instar larvae (Figure 4) were usually found close to or on the upper septum. Some of the sites where first instar larvae had been feeding on the septum developed callus tissue, but larvae were never observed feeding on this tissue. Second and third instar larvae (Figures 5–7) were observed feeding on the spongy white parenchyma that lines the inner walls of the hollow internode. Often, especially when the parenchyma is very spongy, the first and sometimes the second instar larvae burrowed into the tissue upon which they were feeding. Places where the larvae had been feeding on the internode wall (and surrounding tissues) exhibited a light brownish (oxidized) colour.

In the case of 14 larvae in 10 saplings of *C. insignis* (having 5–18 internodes), the larvae had chewed through the septum (which is still tender in young internodes) and were found feeding on the parenchyma of an adjacent internode (number of internodes utilized by a single larva varied from two to six). In 64% of these cases (nine of 14) the larvae had moved to internodes above the original one (younger internodes, with more succulent parenchyma); four larvae moved to lower internodes, and one larva had perforated the septa on both sides and was found in a middle internode. This behavior of penetrating adjacent internodes was observed in only one larva in *C. obtusifolia* (in a plant having seven internodes). This difference in larval behavior in the two species of *Cecropia* could be a result of differences in the parenchymal thickness of occupied internodes in *C. insignis* (mean \pm SD = 7.0 ± 2.0 mm) and *C. obtusifolia* (mean \pm SD = 8.4 ± 2.8 mm) ($t = -2.78$, 86 df, $P = 0.007$). The mean length of occupied internodes did not differ between *Cecropia* species ($t = -1.02$, 86 df, $P = 0.31$). Nonetheless, among all the larvae observed (including those that had crossed over to another internode), the vast majority consumed less than half of the available parenchyma present in the internode. The maximum amount consumed was about 80% of the available surface area in an internode of *C. obtusifolia* harboring a second instar larva.

All larval stages were always observed in an “upside-down” position, that is, in contact with the plant surface with their dorsum (Figures 5–7, 15a). Larvae appeared to move

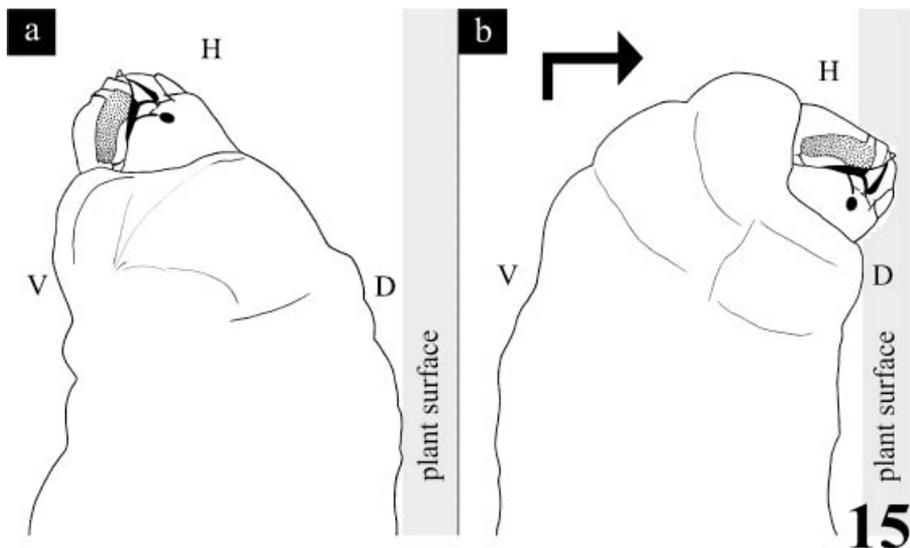


Figure 15. (a) Diagram of larva in normal position (head and thorax area); (b) diagram of larva in feeding position (arrow showing direction of head movement). H, head; V, ventral side; D, dorsal side.

about by means of peristaltic motion of the fleshy swellings located on the dorsum, perhaps helped by setae located on those swellings. Feeding also occurs in an “upside-down” position (Figures 6, 15b) and is accomplished by curving the head backwards and pushing it against the parenchyma. Larvae feed in one location and then crawl to another location in which to feed. Feeding scars were more or less scattered, but were more concentrated in the upper portion of the internode. Larvae in all instars appear to adhere to the vertical surface of the internode wall by means of their dorsal projections (setae) and by surface tension (sometimes they were coated with a liquid that appears to be secreted from the posterior end). When disturbed with forceps, second and third instar larvae regurgitated a translucent brown liquid. Larvae were seen defecating a few minutes after feeding; the fecal pellets (see Figures 4–11) were often attached to the larvae, connected together like a pearl necklace extruding from the anus that opens ventrally. Strings of fecal pellets were found scattered throughout the inner walls of the hollow internode, usually around feeding scars.

The larva pupates in the upper end of the internode by hanging itself freely inside, attaching either to the vertical internal walls of the internode or to the upper septum (Figures 8, 9). The last segment of the pupa is bifurcate and attached to the exuvia of the last instar larva (Figure 10), which in turn adheres to parenchyma.

General adult weevils that had recently eclosed (Figure 12) were occasionally found in the dissected internodes (never more than one per chamber). About 2–3 days ($n=3$) after eclosing, the hardened adult chews through the wall of the stem, making a circular to oval-shaped hole, 1.3–1.5 mm in diameter. We observed two exit holes and in both cases the hole was in the middle portion of the internode.

In the field, adult weevils were observed resting or walking on undersides of *Cecropia* leaves. Most were observed on the upper leaves of saplings, usually on plants with no or few worker ants on the surface (which react aggressively toward adult weevils). Upon disturbance, weevils displayed thanatosis (feigned death; Figure 13). Hespeneide (1987) also noted the occurrence of adult *Lissoderes* on the undersides of leaves and commented that this is rather unusual in this subfamily. He also noted sexual dimorphism in the rostrum (Figures 12, 13) and that in *Lissoderes* the males are larger than females, which is the reverse of most other Conoderinae.

Parasitoids of Lissoderes and Azteca

Two species of parasitoids were found associated with larvae of *L. pusillus* at AMBBR: *Neocatolaccus* sp. (Hymenoptera: Pteromalidae) (Figure 14) and *Heterospilus* sp. (Hymenoptera: Braconidae), both probably undescribed species. These parasitoids presumably oviposit from the outer surface of the stem (there was no evidence that they entered the internode). Both species appear to feed as ectoparasitoids of mature weevil larvae or prepupae, and the adult female probably paralyzes the host prior to oviposition (based on the known behavior of closely related species; Hanson and Gauld 1995). We were unable to determine the range of host stages that are attacked or the site of egg deposition on the body of the host. The mature larva of *Neocatolaccus* sp. has enlarged dorsal projections and several of the abdominal projections are setose. The pupa hangs from the inner wall of the internode (Figure 14), apparently using its larval skin to attach itself, whereas the pupa of *Heterospilus* is enclosed in a cocoon. *Neocatolaccus* was only found in *C. obtusifolia* and *Heterospilus* only in *C. insignis*, although this may be an artifact of the small sample size. Comparing the numbers of parasitoids with the numbers of available hosts (excluding eggs and first instar larvae) in each of these plants, the rates of parasitism

were 8% for *Neocatolaccus* and 14% for *Heterospilus*. No parasitoids were observed associated with *Lissoderes* at La Selva Biological Station.

Among the internodes that harbored *Azteca* ants, 69% contained dead queens at AMBBR and 43% contained dead queens at La Selva. The only parasitoid of *Azteca* queens that we found at AMBBR was an undescribed species of *Menozziola* (Diptera, Phoridae), which was observed attacking both *A. constructor* and *A. xanthochroa*, and in both species of *Cecropia* (but primarily in *C. obtusifolia*). The larvae were found in the gaster of live (but dying) queens, and in several instances we observed dead adult flies in the internodes. The latter presumably died because they cannot chew their way out and are dependent upon the prostoma remaining open or the workers reopening it (one internode of *C. obtusifolia* with an open prostoma contained a dead queen with phorid larvae and a live worker). In total, 16% of the internodes with dead *Azteca* queens contained phorids. At La Selva the only parasitoid of *Azteca* queens observed was *Conoaxima affinis* Brues (Hymenoptera, Eurytomidae; Figure 16). Here 13% of the internodes with dead *Azteca* queens contained *C. affinis*. These percentages are probably underestimated since we did not dissect queens in order to detect earlier stages of the parasitoids. Several of the dead queens were covered with nematodes and infected with entomophagous fungi, although we did not quantify their presence or determine whether the nematodes are parasites or scavengers.

Discussion

Interactions among Lissoderes, Azteca, and Cecropia

Two principal questions regarding the biology of *Lissoderes* are: to what extent is it a parasite of the mutualism between *Cecropia* and *Azteca*, and whether these weevils



Figure 16. *Conoaxima affinis* ovipositing through a prostoma into an *Azteca* queen.

contribute to the high mortality rate observed among colonizing *Azteca* queens. There are at least three plant characteristics/resources that are utilized by *Azteca* ants: (1) the prostoma, a soft spot in the stem, where the queens enter; (2) the hollow internodes, where the ants nest, together with the spongy parenchyma that lines the inner walls of the internodes (see below); and (3) the Müllerian bodies and pearl bodies which the worker ants harvest. The results of our study suggest that *L. pusillus* depends only upon the second of these. Adult females of *L. pusillus* do not seem to be dependent upon the prostoma for oviposition, although they do sometimes utilize this spot for making their ovipositional puncture. The weevil larvae never come into contact with the Müllerian bodies or pearl bodies, but adults have been observed feeding on these food bodies in the field along with other weevils including *Pseudolechriops* spp. (Conoderinae; Hespeneide and LaPierre forthcoming). More observations are required to determine whether or not adult *Lissoderes* are dependent on the food bodies.

The larvae of *Lissoderes* feed almost exclusively upon the spongy parenchyma that lines the inner walls of the internodes; the only exception is that first instar larvae appear to occasionally feed on the upper septum, which in young saplings is soft and spongy. Previous observations (Benson 1985), as well as those from the present study, suggest that *Azteca* queens also feed on this spongy parenchyma. Internodes occupied by queens show large areas where the parenchyma has been scraped away and the detritus at the bottom of the internode appears to consist of chewed parenchyma. We were unable to determine whether the ants simply scrape away this parenchyma without ingesting it (many stem-nesting ants scrape away the inner walls of the stems in which they nest; J. Longino, personal communication), or whether they actually ingest it. Nor are we aware of any previous studies that have demonstrated that *Azteca* queens actually ingest parenchyma tissue, thus further research is required to resolve this question. Nonetheless, it seems likely that the parenchyma layer is being utilized by *Azteca* queens, at least to some extent. Because they seal themselves inside the internode, their only other food resource is the stored reserves in their bodies.

It has been noted previously that among the species of *Cecropia* that harbor ants, the parenchyma tends to be best developed in those species that regularly grow under conditions of high light intensity (Davidson and Fisher 1991). Of the two species included in this study, *C. obtusifolia* is more common in secondary vegetation (Burger 1977), and indeed we noted that the parenchyma of this species is thicker, whiter, and more spongy than that found in *C. insignis*. Our results also provide evidence that these differences in the parenchyma affect both ants and weevils. *Azteca* queens begin colonizing *C. obtusifolia* at an earlier stage of plant development than they do in *C. insignis* (Figure 1), which might be due to an earlier development of parenchyma in the former than in the latter. However, this remains to be demonstrated and the stage at which the prostomata and trichilia begin developing also needs to be considered. Larvae of *L. pusillus* are more likely to cross over into an adjacent internode in *C. insignis* than in *C. obtusifolia*, which could be due to the poorer quality parenchyma present in the former. Although not included in this study, preliminary observations suggest that the ant-free, cloud forest species, *C. angustifolia*, has even thinner parenchyma than *C. insignis*. *Lissoderes cecropiae* Hespeneide larvae were found in the stems of this *Cecropia*, and here the larvae appear to regularly cross over into several adjacent internodes. Thus at least one congener of *Lissoderes* is not dependent upon the *Cecropia*-*Azteca* mutualism (i.e. the well-developed parenchyma present in *Cecropia* species that are inhabited by ants). It would be interesting to compare the chemical composition of the parenchyma in these three species of *Cecropia*.

Assuming that *Azteca* queens ingest parenchyma, *Lissoderes* is utilizing a resource that is presumably being provided by the plant for its mutualistic ant. In this sense it is a parasite of the mutualism. However, there appears to be relatively little direct competition between the weevils and the ants. Approximately 20% of all the *Cecropia* internodes we examined at AMBBR contained only weevils, another 20% contained only *Azteca* queens, and the other 60% were empty. Only once did we observe a live weevil larva in the same internode as live *Azteca* queens. Preliminary results from placing weevil larvae in the same internode as ants suggest that the former is often killed. It is likely that female weevils avoid ovipositing in internodes that contain other weevils or live ants, although more research is required to confirm this and determine if female weevils distinguish between occupied and unoccupied internodes through substrate vibrations by active larvae or ants, or through pheromone signals deposited by conspecifics (Ferguson et al. 1999). One would expect selection to favor female weevils that avoid ovipositing in occupied internodes, since there appears to be a high probability that the offspring will be killed if the internode contains another weevil or live ants. It is less clear whether there is an advantage for *Azteca* queens to avoid colonizing internodes previously occupied by *L. pusillus*, i.e. whether an internode that has had much of its parenchyma removed is deleterious to the queen(s). Once they become sealed inside the internode, *Azteca* queens are apparently unable to cut through the septum and move to an adjacent internode, as occurs in *Lissoderes*. Although we found little evidence to suggest that *Azteca* queens (live or dead) occupied internodes previously occupied by *Lissoderes*, this subject requires further investigation. In addition, studies are required to determine whether feeding by weevil larvae has any indirect effects on *Azteca* queens (e.g. effects on the parenchyma in other internodes or on the production of Müllerian bodies).

When *Azteca* workers emerge, they chew their way out of the stem, begin patrolling the plant, and eventually chew through the septa that separate adjacent internodes. Thus it is likely that *Cecropia* plants become increasingly difficult for *Lissoderes* to colonize as the worker population grows. We did find weevil larvae in the lower internodes of two saplings of *C. obtusifolia* in which the upper internodes were inhabited by queens and workers of *A. constructor*, although the workers had not yet begun chewing through the lower septa. We also found *L. pusillus* larvae in unoccupied internodes of mature trees, although this has not been observed in lowland sites (L. M. LaPierre, personal observation) where *Azteca* colonies more completely dominate mature trees (at higher altitudes, such as in AMBBR, *Azteca* colonies are more feeble; J. Longino, personal communication). Nonetheless, this study found larvae of *Lissoderes* to be slightly more common in saplings of the same two *Cecropia* species in lowland sites (Table I).

Azteca mortality

In agreement with previous studies (Longino 1989), we observed a high incidence of internodes containing dead queens, although the causes of this mortality have never been explained. The results of our study suggest that neither direct competition with weevil larvae nor hymenopteran parasitoids are major causes of this mortality. *Azteca* queens are known to be parasitized by species of *Conoaxima* (Brues 1922; Wheeler 1942), but we observed none in the stems that we dissected at AMBBR and few from stems at La Selva, and it is generally quite uncommon (J. Longino, personal communication). The only parasitoid of *Azteca* queens that we found at AMBBR was a phorid fly, *Menoziola* sp., which appears to account for at least 16% of the mortality. The only previous host records

for this genus are from queens of *Camponotus* and *Crematogaster* in Europe (Brown et al. 1991; B. Brown, personal communication).

Other possible causes of mortality include quality and quantity of parenchyma in the internode (which in turn could be affected by the presence of other queens in the same internode) and non-insect parasites (e.g. nematodes and fungi). In *C. obtusifolia* we found greater mortality in internodes containing numerous queens. At this early stage of colonization the queens apparently do not kill each other, but it is possible that they compete for parenchyma, although this remains to be shown and is contradicted by the increased success of pleometrotic associations of *A. constructor* (Choe and Perlman 1997). In *C. insignis*, where the queens colonized fewer internodes, the aggregation of numerous queens in a single internode was less pronounced; nonetheless, the majority of internodes that were colonized contained dead queens. Because *C. insignis* has a thinner layer of parenchyma it is tempting to speculate that queens require a larger internode than they do in *C. obtusifolia*, but data are currently lacking. The role of nematodes and/or fungi in queen mortality, if any, is also unknown. In South America Jolivet (1991) observed that egg masses of a galerucine chrysomelid oviposited into the internodes of *Cecropia* had a lethal affect upon ant queens inhabiting the same internodes. However, we did not encounter any egg masses in our samples.

Although there is a high mortality rate among colonizing *Azteca* queens, this probably has little effect on the mutualism between *Azteca* and *Cecropia* (assuming that mortality is never 100%) since mature trees are inhabited by one colony. In mature colonies it has been shown that nitrogen from ant residues becomes incorporated into the plant (Sagers et al. 2000). Thus, it is possible that the high rate of colonization and mortality benefit a young plant by supplementing nitrogen.

Lissoderes larvae

In addition to its association with *Cecropia* saplings that also harbor mutualistic ants, another notable aspect of the biology of *Lissoderes* is the unusual position of the larva, which crawls about on its back and feeds by moving its head backwards. Although we have not undertaken an exhaustive review of the literature, this position appears to be unusual in weevils. The only reference to back-crawling weevil larvae that we are aware of is in the Nemonychidae (May 1994; Kuschel and May 1997). The larvae of *Lissoderes* have fleshy swellings and setae on the dorsum, which appear to be used in moving about in the interior of the internode (in nemonychids these structures are termed “ambulatory ampulae”). Setose dorsal swellings have been reported in the final instar larva of *Conoaxima*, a parasitoid of *Azteca* queens (Figure 16; Brues 1922), and we observed them in the final instar larva of *Neocatolaccus*, a weevil parasitoid. More study is required to determine the extent and functional significance of this apparent convergence among the inhabitants of *Cecropia* stems.

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References

- Agrawal AA. 1998. Leaf damage and associated cues induce aggressive ant recruitment in a neotropical ant-plant. *Ecology* 79:2100–2112.
- Benson WW. 1985. Amazon ant-plants. In: Prance GT, Lovejoy TE, editors. Key environments: Amazonia. London: Pergamon Press. p 239–266.
- Berg CC, Franco Rosselli P. 2005. *Cecropia*. New York: New York Botanical Garden. 230 p. (Flora Neotropica; 94).
- Brown BV, Francoeur A, Gibson RL. 1991. Review of the genus *Styletta* (Diptera: Phoridae). *Entomologica Scandinavica* 22:241–250.
- Brues CT. 1922. *Conoaxima*, a new genus of the hymenopterous family Eurytomidae, with a description of its larva and pupa. *Psyche* 29:153–158.
- Burger W. 1977. Flora Costaricensis, family #52, Moraceae. *Fieldiana, Botany* 40:94–215.
- Choe JC, Perlman DL. 1997. Social conflict and cooperation among founding queens in ants (Hymenoptera: Formicidae). In: Choe JC, Crespi J, editors. The evolution of social behavior in insects and arachnids. Cambridge: Cambridge University Press. p 392–406.
- Davidson DW. 2005. *Cecropia* and its biotic defenses. In: Berg CC, Franco Rosselli P, editors. *Cecropia*. New York: New York Botanical Garden. p 214–226. (Flora Neotropica; 94).
- Davidson DE, Fisher BL. 1991. Symbiosis of ants with *Cecropia* as a function of light regime. In: Huxley CR, Cutler DF, editors. Ant-plant interactions. Oxford: Oxford University Press. p 289–309.
- Ferguson AW, Ziesmann J, Blight MM, Williams IH, Wadhams LJ, Clark SJ, Woodcock CM, Mudd A. 1999. Perception of oviposition-detering pheromone by cabbage seed weevil (*Ceutorhynchus assimilis*). *Journal of Chemical Ecology* 25:1655–1670.
- Folgarait PJ, Davidson DW. 1995. Myrmecophytic *Cecropia*: antiherbivore defenses under different nutrient treatments. *Oecologia* 104:189–206.
- Hanson PE, Gauld ID, editors. 1995. The Hymenoptera of Costa Rica. Oxford: Oxford University Press. 920 p.
- Hespenheide HA. 1987. A revision of *Lissoderes* Champion (Coleoptera: Curculionidae: Zygopinae). *Coleopterists Bulletin* 41:p 41–55.
- Hespenheide HA. 2005. Weevils of the genera *Archocopturus* Heller and *Zygopsella* Champion, sibling species, and mimetic homoplasy (Coleoptera: Curculionidae: Conoderinae). *Proceedings of the Entomological Society of Washington* 107:671–685.
- Hespenheide HA, LaPierre LM. 2002. A review of *Ptous* (Coleoptera: Curculionidae: Cryptorhynchinae). *Proceedings of the Entomological Society of Washington* 104:856–862.
- Hespenheide HA, LaPierre LM. A review of *Pseudolechriops* Champion (Coleoptera: Curculionidae: Conoderinae). *Zootaxa* 1384:1–39.
- Howden AT. 1995. Structures related to oviposition in Curculionoidea. *Memoirs of the Entomological Society of Washington* 14:53–100.
- Janzen DH. 1969. Allelopathy by myrmecophytes: the ant *Azteca* as an allelopathic agent of *Cecropia*. *Ecology* 50:147–153.
- Jolivet P. 1991. Ants, plants, and beetles; a triangular relationship. In: Huxley CR, Cutler DF, editors. Ant-plant interactions. New York: Oxford University Press. p 397–406.
- Jordal BH, Kirkendall LR. 1998. Ecological relationships of a guild of tropical beetles breeding in *Cecropia* petioles in Costa Rica. *Journal of Tropical Ecology* 14:153–176.
- Kuschel G, May BM. 1997. A new genus and species of Nemonychidae (Coleoptera) associated with *Araucaria angustifolia* in Brazil. *New Zealand Entomologist* 20:15–22.
- LaPierre LM. 2002. Diversity and ecology of insect herbivores associated with *Cecropia* (Cecropiaceae) in Costa Rica and their relationship with its *Azteca* (Hymenoptera: Formicidae) mutualists [dissertation]. Los Angeles: University of California. 177 p.
- Longino JT. 1989. Geographic variation and community structure in an ant-plant mutualism: *Azteca* and *Cecropia* in Costa Rica. *Biotropica* 21:126–132.
- Longino JT. 1991a. *Azteca* ants in *Cecropia* trees: taxonomy, colony structure, and behaviour. In: Huxley CR, Cutler DF, editors. Ant-plant interactions. Oxford: Oxford University Press. p 271–288.

- Longino JT. 1991b. Taxonomy of the *Cecropia*-inhabiting *Azteca* ants. *Journal of Natural History* 25:1571–1602.
- Lyal CHC. 1986. Observations on zygotine weevil behaviour (Coleoptera: Curculionidae: Zygopinae). *Journal of Natural History* 20:789–798.
- May BM. 1994. An introduction to the immature stages of Australian Curculionoidea. In: Zimmerman EC, editor. *Australian weevils*. Volume 2. Victoria, Australia: CSIRO Publishing. p 365–728.
- McDade LA, Bawa KS, Hespdenheide HA, Hartshorn GS, editors. 1994. *La Selva: ecology and natural history of a neotropical rainforest*. Chicago: University of Chicago Press. 486 p.
- Ortiz R, editor. 1996. Memoria investigación Reserva Biológica Alberto Ml. Brenes, San Ramón. *Revista Pensamiento Actual (Universidad de Costa Rica, Sede de Occidente)* 2:1–134.
- Sagers CL, Ginger SM, Evans RD. 2000. Carbon and nitrogen isotopes trace nutrient exchange in an ant–plant mutualism. *Oecologia* 123:582–586.
- Schupp EW. 1986. *Azteca* and protection of *Cecropia*: ant occupation benefits juvenile trees. *Oecologia* 70:379–385.
- Wheeler WM. 1942. Studies of neotropical ant-plants and their ants. *Bulletin of the Museum of Comparative Zoology, Harvard* 90:1–262.
- Yu DW, Davidson DW. 1997. Experimental studies of species-specificity in *Cecropia*–ant relationships. *Ecological Monographs* 67:273–294.