

Field behavior of parasitic *Coelioxys chichimeca* (Hymenoptera: Megachilidae) toward the host bee *Centris bicornuta* (Hymenoptera: Apidae)*

S. Bradleigh VINSON¹, Gordon FRANKIE², Asha RAO¹

¹ Department Entomology, Texas A&M University, College Station, TX 77843, USA

² Division of Insect Biology, College of Natural Resources, University of California, Berkeley, CA 94720, USA

Received 29 April 2009 – Revised 25 March 2010 – Accepted 28 March 2010

Abstract – *Coelioxys chichimeca* is a cleptoparasite and an important cause of mortality for several wood-hole nesting pollinator bees in Costa Rica. We studied the behavior of this parasitic bee towards one of the more common host bee, *Centris (Heterocentris) bicornuta*. The female parasitic *C. chichimeca* were attracted to host bee, *C. bicornuta* nest cells that contained pollen, but only exhibited interest in entering and ovipositing when these host cells contained nectar. This interest was exhibited in three different ways: (1) the female parasite landed near the host bee's nest entrance and waited for the host bee to exit her nest cell before entering; (2) the female parasite entered briefly, re-emerged and re-entered with abdomen first; and (3) the female parasite entered the host bee nest as in (2), but did not exit and re-enter due to larger nest holes, where she could presumably turn around in any direction to lay the egg. We also found that female parasites would briefly hover in front of vials that contained pollen, but would land nearby when the vial contained nectar. Additionally, eggs of the female parasite were inserted horizontally in the wall of the host bee cell, primarily in the upper half. Larvae of these parasites hatched before the host bee egg and with their sickle shaped mandibles, killed any host bee egg or larva present and consumed the nest provisions. Host bees returning to a parasitized cell often removed the nectar, presumably in an attempt to remove the parasite egg.

host location / oviposition / odour / wood nesting bee / solitary bee

1. INTRODUCTION

Bees of the genus *Coelioxys* are cleptoparasitic. The cleptoparasitic bees are an important factor in the mortality of most solitary bee species in Costa Rica. Although cleptoparasitic bees are found worldwide (Bohart, 1970) and are abundant, little is known about their biology (Rosen and Kamel, 2008) or behavior because their populations are often small (Cane, 1983; Rosenheim 1987). Cleptoparasitic bees lay their eggs in cells of host bees and upon hatching, the parasitic larva kills the host bee egg or larvae and consumes the provisions (Graenicher, 1905; Rozen, 1991).

Some cleptoparasitic species enter an open, partially provisioned cell and lay an egg (Graenicher, 1927; Bohart, 1970; Dansforth and Visscher, 1993). However in some cleptoparasitic species, it is the adult female that kills the host egg before depositing her egg. In yet other species, the parasitic female introduces the egg into the cell only after the cell has been closed by the host female (Rozen, 2003).

The best studied cleptoparasitic bees so far are in the genus *Coelioxys* (Baker, 1971; Scott et al., 2000). In regards to host location and attack behavior, several authors have noted that female *Coelioxys* wait until the female host bee leaves her nest before entering and laying an egg (Cane, 1983; Dansforth and Visscher,

Corresponding author: A. Rao, asha@tamu.edu

* Manuscript editor: Marla Spivak

1993; Michener, 2000; Scott et al., 2000). In this study, we examined host location behavior and behavior leading to oviposition in the host bee nest by the cleptoparasitic bee, *Coelioxys* (Cyrtocoelioxys) *chichimeca* Cresson (Hymenoptera: Megachilidae). We also examined factors that may play a role in making oviposition decisions. Finally, we investigated the response of the provisioning host bee, *Centris* (*Heterocentris*) *bicornuta* Mocsary (Hymenoptera: Apidae: Centridini), to the cleptoparasitic bee activity.

2. MATERIALS AND METHODS

The first 2 authors have conducted studies since 1995 to evaluate nest mortality of wood-hole nesting *Centris* spp. (Hymenoptera: Apidae: Centridine) in Guanacaste Province, Costa Rica. Here, we focus on nests of *C. bicornuta*—one of the common species in the area (Frankie et al., 1988, 1993), and its parasite, *C. chichimeca*, a commonly attacking cleptoparasite of *C. bicornuta*. The studies reported here were conducted each year during a three-week period between Feb. 1 through March 31 from 1997 until 2001.

Some studies involved sitting in the field from sun up to sun set (about 12 h) while other studies involved observations for 6 to 8 h per day. Each study required 3 to 6 consecutive days that were repeated 2 or 3 times in a year.

2.1. Fields site

The research and observations were conducted at two field sites located about half a kilometer apart. Both were in riparian areas of the dry forest along small rivers in Guanacaste Province of Costa Rica about 8.5 km northwest of the town of Bagaces in an area known as Hacienda Monteverde.

2.2. Field host location behavior

Twelve nest blocks were used, consisting of 6 wooden sticks (2 × 2 cm by 11.5 cm) each with a 8.0 mm diameter hole drilled to within 2 cm of the other end of the stick. The sticks were grouped together so that their entrance holes were offset between rows (similar to those described in Frankie et al., 1993). We used sticks with 8.0 mm holes in

this study for two reasons. One was that *C. bicornuta* preferred this diameter of the hole (Frankie et al., 1988), and second was, in earlier studies of bee nesting, we had observed that *C. chichimeca* would enter a nest with a hole ranging from 4.8 mm to 8.0 mm, return to the nest entrance, and then would back into the hole to oviposit. *C. chichimeca* would not return to the nest entrance to back into the hole for oviposition when the diameter of the holes was larger than 8.0 mm (presumably because they could turn around within the hole).

These nest blocks were placed on two or three large trees that could be viewed from one sitting location about 1 m away. Nest blocks on a single tree were separated 30 to 90 cm from each other. The nest blocks were also placed about 10 to 20 m away from our bee nest monitoring area that consisted of a diversity of nest blocks maintained since 1985 (see Frankie et al., 1988, 1993), which have been active with bees and parasites. Once placed, nest blocks were left in the field for 2 days to allow for nest initiation. On the third day we would examine each block and mark any nests that were in progress, with a small numbered white sticky dot (Source: Avery®, Azusa, CA 91702). We then selected 6 to 9 of the active nests on which to focus our attention. These nests were selected based on ease of viewing the entrance from a one-meter location. The white dots were removed from all non-selected nests to reduce confusion while following the behavior of the bee. We recorded bee behavior using a small battery operated cassette tape recorder controlled with a hand held microphone with an “on/off” switch. We recorded the number of the nest, time and bee (host or parasite) and described the behavior (entering the area, entering the nest and how, leaving the nest, flying away, resting nearby or interacting with another bee). Each evening the recordings were transcribed to a notebook. This was repeated following each field day. The focus of these studies from 1995 to 1999 was on the parasite and the focus from 2000–2001 was on the host bee. During the study any nest sticks that were full of nest cells were replaced.

In 1999, the senior author recorded the behavior of *C. chichimeca* in the nest sticks. For this, 10 nest sticks were cut lengthwise and a piece of clear plastic was placed over the cut revealing the lengthwise cavity. These were placed in the field and hung on trees, as described above, so that the plastic covered side was visible from the recording location. These were accepted as nest sites by *C. bicornuta*. When *C. chichimeca* entered, the observer would walk up

and could see into the cavity and record the behavior of the parasite in the nest. This was repeated for 4 days.

2.3. Differences in nest contents when accepted and/or rejected by *C. chichimeca*

For this study we set out twelve nest blocks as described above in February of 1999 and 2000. After two days in the field to allow nest initiation by *C. bicornuta*, we followed *C. chichimeca* females and depending on one of their following three behaviors, we immediately plugged the nest entrance. (A) We plugged any nest with a green rubber stopper in which a female *C. chichimeca* hovered in front of or touched but then continued to search. (B) We plugged any nest with a black stopper in which a female hovered in front of or touched the host nest and then flew nearby and landed to wait (these females left due to our disturbance). (C) During this study several *C. chichimeca* entered the nest, emerged and after turning around re-entered abdomen first indicating initiation of oviposition behavior. When they emerged for the second time from the nests, the nests were plugged with a light brown stopper. After eight to ten nests were marked (about all we could observe and collect on a particular day), we collected and returned them to our field laboratory. They were split open lengthwise, and we recorded the provisions as empty, having only pollen, having both pollen and nectar, and having a host bee or *C. chichimeca* egg. We measured the nectar by sucking it up in several calibrated 10 μ L capillary tubes. We repeated this study until we had about 25 nests each with green plugs and black plugs. We were only able to obtain six nests from situation C with light brown plugs.

2.4. Role of odors from nest contents on host location by *C. chichimeca*

In February of 2001 and 2002, we set out to determine if chemicals are involved in attracting and orientating the *C. chichimeca* to the nests. To do so, we took glass vials of approximately 8.0 mm internal diameter and covered them with a heavy black paper to form a jacket. The sealed end of the tube was painted black so the inside of the tube was dark when the jacket was added, simulating a dark nest hole. The entrance to the tube consisted of a 5 cm

section cut from a 8.0 mm hole nest stick in which we widened the hole part way down the stick to accommodate the vial and jacket to form a square wooden cap (simulating the entrance to a nesting stick) that had a 8.0 mm hole leading to the dark glass lined cavity (vial nests).

We then added to these vial nests (A) 1 mL of pollen from a partially provisioned *C. bicornuta* nest (containing pollen only), (B) 1 mL of nectar from *C. bicornuta* nests, or (C) nothing which served as a control. Following tube preparation a string was tied around the jacket and at the back of the tube and then around the tube near the cap forming a string loop so they could be hung in the field and remain horizontal to the ground. To provide a blind test a small sticky label was applied to the side of each wooden cap and randomly numbered. Five of each type of vial nest was hung in the field each study day among a few active host bee nests so the numbers could be seen as described above. We then recorded the response of all approaching *C. chichimeca* to these vial nests. Following recording sessions for 6 to 8 h the vial nests were collected and the contents of each tube was recorded according to the random number to allow us to relate the recorded behavior of the *Coelioxys* to the vial tube nests with the various contents. This was repeated over 3 consecutive days and again a week later.

2.5. Oviposition location within nest

In 2005, to determine the location of the eggs of *C. chichimeca*, which are always embedded in the host bee cell wall, the nest sticks were set out as described in Section 2.2 and the top of each stick was marked. These were observed, and once *C. chichimeca* entered and left, exhibiting the typical oviposition behavior of backing into the hole, as described above, they were plugged and collected. These nests were brought back to the field laboratory and both ends of each stick were marked into eight equal quadrants with the first 2 marks dividing the sides and top and bottom in half (Fig. 1). The nest was then split at the sides in half and under a microscope we noted the location of the eggs in each quadrant. The eggs of *C. chichimeca* are easy to identify as they are always in a pit in the cell wall of the host bee with the top of the egg visible or covered with pollen. The location of each egg was recorded with respect to the quadrant they were in and as to their location within the cell wall either under the nectar, pollen or in the area of the

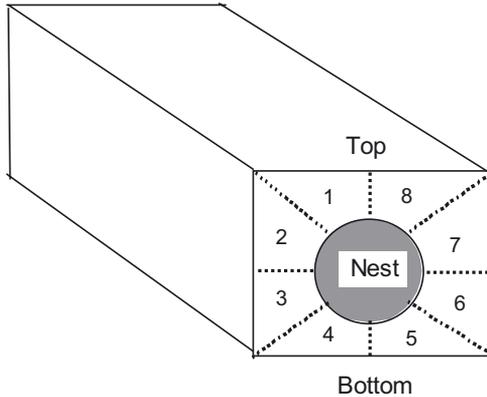


Figure 1. The wooden block showing the circular host bee nest and the quadrants used to quantify the location of eggs inside the nest.

host bee cell that had not yet been provisioned. In cases where the egg was laid in the cell wall beneath the pollen, it was revealed by the disturbance of the smooth surface of the pollen caused by *C. chichimeca* oviposition. Since no differences were found between the left and right quadrants of the cavity the data from the equivalent left and right quadrants were pooled.

2.6. Female host bee response to *C. chichimeca*

A study initiated in 2000–2001 focused on the activity of returning female host bees to their nests in progress and to nests that had just been visited by a female *C. chichimeca* that had entered, returned to the nest entrance, and backed into the nest suggesting that oviposition had occurred. When the *C. chichimeca* left, we marked the nest with a white numbered sticker (Source: Avery®, Azusa, CA 91702) on the side of the nest and recorded the behavior of the returning female bee as described for the “Field Host Location Behavior” (Sect. 2.2) except here the focus was on the female host bee from the time she entered her nest stick until she left the area. When the female had been gone for 2–4 min the nest was collected and later dissected to see if the *C. chichimeca* had oviposited and if an egg remained. We continually recorded any activity of the nests under view. The host bee usually takes only a few hours to place the nectar in the cell, and *C. chichimeca* only oviposits once nectar is present.

This was repeated 2 times each year for six consecutive days.

We also repeated this design in 2007 with one change that involved allowing the female bee to begin to re-provision or seal and start a new cell. In this case, after the host bees had returned to their nest to sleep we left the nest in the field and collected it the next day. These were designated as “in progress” as the cavity had room for more cells. This was repeated for 5 days. We compared these nests to nests collected nearby that were complete as evidenced by an entrance plug typical of completed nests.

3. RESULTS AND DISCUSSION

3.1. Field host location behavior

During our study period in Costa Rica dry forest, the wind (Westerlies) was constant and from the east. Based on this, we observed that female *C. chichimeca* would fly from down-wind to trees where we had our nest blocks located. Once in the area, they flew to within 10 to 16 cm facing the tree trunk or the nest blocks. They would hover very briefly in front of any small holes (or black spots on the tree surface they encountered), going from one hole or spot to another. No particular pattern could be discerned as we saw some females circle the tree, others tended to fly up or down following the vertical trunk of the tree, and others went from tree to tree.

The adults observed on any given day varied in size, and 2 or 3 different sized *C. chichimeca* would often appear every 1 or 1.5 h and pass the observation area checking each active bee entrance hole in the area. This gave the impression that they were “trap lining” as has been reported for some other insects (Janzen, 1971; Gilbert, 1972, 1975). Several authors have suggested that cleptoparasitic bees learn the location of individual bee nests (Linsley and MacSwain, 1955; Thorpe, 1969; Rozen et al., 1978; Dansforth and Visscher, 1993). Although only *C. chichimeca* were identified emerging from *C. bicornuta* bee nest cells that were collected and maintained in the laboratory, they varied in size. While it may have been possible that another species were present in the area we could not

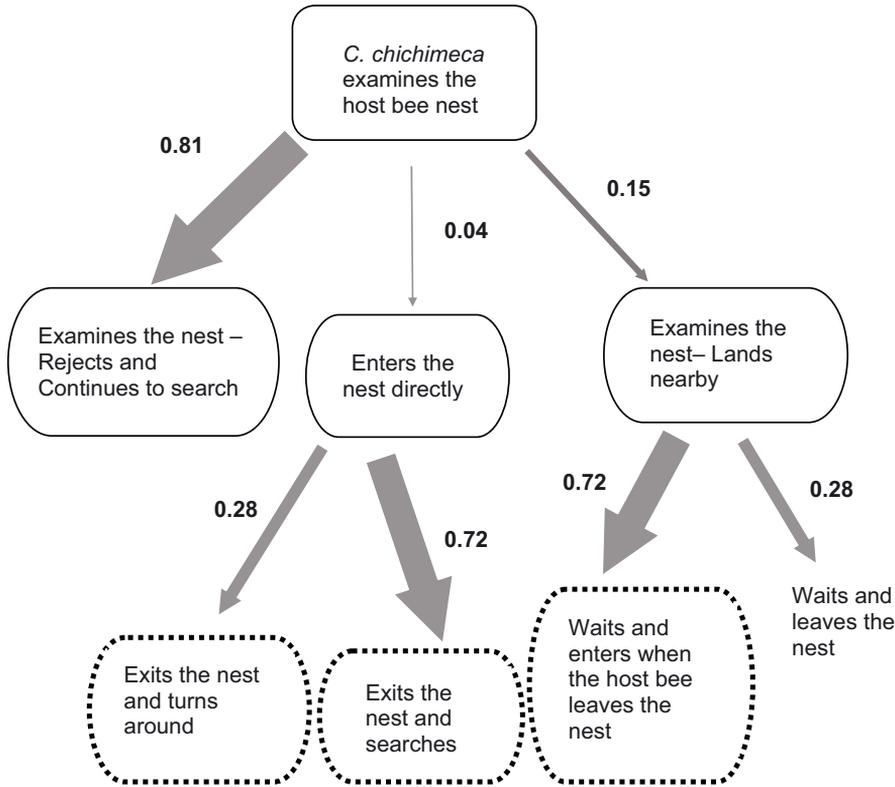


Figure 2. Sequence of behaviors observed in *C. chichimeca*. Solid circles represent the three main behaviors and dotted circles represent the sequential behaviors ($\chi^2 = 174.32$, $df = 2$, $P < 0.001$). The numbers represent the proportion of time that a particular “leading behavior” is followed by a “following behavior” for each pair of behavioral elements. The arrows indicate the direction of the behavior and their thickness indicates the transitional probability for each pair of behavior.

distinguish these in flight and collecting after each observation was not an option.

On occasion during the flights, the *C. chichimeca* would hover in front of, touch, or land at the entrance to a bee nest at which time they exhibited several different behaviors. This initiated our first study that shows the behavior of female *C. chichimeca* as they encountered 168 nests. The initial behaviors were either A) to hover in front of a hole briefly and continue to search (81%) or B) to touch the entrance and either enter or land near-by (15%) or C) enter the host bee nest directly without examining it first (4%) (Fig. 2). As the data indicate, most females rejected the nests after examining them and continued to search. Of the females that directly entered the nest, 28% of them exited, turned around and re-

entered the nest within 12 to 38 s (Fig. 2). The remaining 72% of the females landed within 8 to 31 cm (Ave = 12 cm) of the nest entrance, and all oriented with their heads directed toward the nest entrance in a waiting position (Figs. 2, 3). Of these 72%, a few left after 6 to 9 min although one left after 18 min. The rest flew to the nest they had touched, but only after a female host bee had entered and left. There was no movement on the part of the waiting *C. chichimeca* to a female host entering her nest or to other female host bees nesting nearby (Fig. 2). We did not observe defensive behavior by the host in our study as seen with *Centris trigonoides* (*Hemisiella*) Lepeletier, studied by Aguiar et al. (2006).

Waiting time of the *C. chichimeca* depended on the female host bee’s time to return.



Figure 3. A female *Coelioxys chichimeca* waiting for a potential host bee to leave her nest.

In unpublished studies (Vinson and Frankie) concerning the foraging behavior of the host bee, the last 4 to 6 resource collecting trips were for nectar whose collecting times ranged from 3 to 8 min (Mean = 5 min, 31 s, N = 16). The bees stayed in the nest between collecting trips for about 1 min. In each case, 7 to 14 s (Mean = 10.8, N = 14) after the female bee left, the *C. chichimeca* entered the nest head first for 9 to 29 s (Mean = 16.6, N = 14). She would then reappear backing out to turn around and re-enter abdomen first for another 15 to 112 s (Mean = 52.59, N = 14) where upon she exited again and re-entered head first for another 7 to 21 s (Mean 12.8, N = 14), exited and resumed searching. We found this behavior identical in 6.4 mm bee nest holes, but apparently *C. chichimeca* females did not need to back out of the 9.5 mm bee nest holes, as this behavior was not observed during the 4 observations of *C. chichimeca* attacking *C. bicornuta* that occasionally use these nest hole sizes (Frankie et al., 1988).

Five observations of oviposition in nests that we had split lengthwise and covered the cut with clear plastic, revealed that all 5 *C.*

chichimeca placed their egg in the cell wall roof. In 3 cases, the female thrust her abdomen upward and through the small amount of nectar and into the pollen near the pollen – nectar interface. This left a pit in the pollen. The female would leave and re-enter the nest and covered the pit with pollen appearing to use her front legs. However, in two cells the egg was laid in the roof at the edge of the nectar and while the females returned to examine the pit no attempt was made to cover it. The only difference between the two situations was that the amount of nectar was greater in the latter 2 cells.

Overall, we found the percent parasitism of cells to be low, with nest parasitism being higher than cell parasitism (Tab. I). The dissection of a random sample of 55 *C. bicornuta* nests a few weeks after their completion from a number of sites over the 5-year period revealed that about 22% of the nests were successfully parasitized. Cells averaged 1.96 with a range of 1 to 6 with 6 being about the limit the hole in the stick could accommodate. Successful parasitism of cells was 12% (Tab. I) indicating that the unsuccessful parasitism of

Table I. Parasitism of *C. bicornuta* by *C. chichimeca* in Guanacaste, Costa Rica.

Sample	N	Number parasitized	% parasitized	Number of cells/nest
Number of nests	55	12	21.8	—
Number of cells	108 ¹	13	12	1.96 (1–6)

¹ Cells taken from 55 nests.

Table II. Condition of *C. bicornuta* nest cells in which *C. chichimeca* continued to search or rejected the nest, but landed nearby to wait. Means are compared across columns and rows ($\chi^2 = 39.706$, df 1, $P < 0.001$); means followed by different letters are significantly different.

	Female <i>C. chichimeca</i>		<i>Centris</i> nest condition*	
	N	Pollen only	With nectar	Cell wall
1. Rejected and continued to search	28	22a	2 ¹ b	4b
2. Landed and waited	23	1b	22 ² a	
3. Re-entered with abdomen first to oviposit	6		6 ² b	

¹ The amount of nectar was less than 10 μ L, but coated with pollen.

² The amount of nectar was between 20 and 30 μ L.

cells is about 1 cell per nest even though more than one cell is attacked.

3.2. Differences in nests accepted and rejected by *C. chichimeca*

We followed hundreds of individual female *C. chichimeca* and plugged 28 nests that female *C. chichimeca* briefly hovered in front of and continued to search. We also plugged 23 nests that the female examined and landed nearby to wait. We found in both cases that a host female bee was present in 25% of the nests. The major difference was the contents of nests from both situations. Most nests that the female *C. chichimeca* hovered in front of and resumed searching contained only pollen that was packed down to form a hard concave pollen mass at the back of the cell. Two exceptions were cells that had less than 10 μ L of nectar or just enough to moisten the pollen mass (Tab. II). In contrast, the cells that prompted the *C. chichimeca* to land nearby and wait had over 30 μ L or more of nectar. The complete cells of *C. bicornuta* usually contain about 75–100 μ L of nectar (Vinson and Frankie, unpubl. data). None of these latter nests had been previously parasitized. However, during this

study we collected 6 nests where the female *C. chichimeca* had waited and entered and re-entered the nest abdomen first after the bee left the nest. In these 6 cases the cell was provisioned with pollen and at least 20 μ L of nectar which was parasitized.

Oviposition location was generally not obvious and was usually found probing the cell wall at the nectar-pollen interface where the pollen was softer. In each case an egg was embedded in the cell wall just at the edge of the nectar-pollen interface, but under the pollen. The pollen at this point had been disturbed and had been scraped from surrounding pollen mass and piled over the hole in the cell wall.

A few *C. chichimeca* females did hover in front of nests with no pollen or nectar but with some cell wall material present, suggesting some recognition. There was however, a significant difference in the presence of or absence of nectar in nest cells which *C. chichimeca* either rejected or landed near by and waited ($\chi^2 = 39.706$, df = 1, $P < 0.001$). The results suggest that the presence of nectar is important to whether the *C. chichimeca* enters the nest and oviposits. If only pollen is present they appear to check the entrance to the cell and leave, but if there is some nectar

in the cell making the pollen softer and a female bee is not present, they enter and parasitize (Tab. II).

3.3. Role of odors from nest contents on host location by *C. chichimeca*

In 6–8 h of observation / day, most important behaviors were observed in 6 h. We observed 23 *C. chichimeca* females responding to the glass vials. Of these, 16 briefly hovered near or touched the entrance to the vial containing pollen taken from a host bee nest and then they continued to search. In contrast, only 7 came to the tubes containing host bee nectar but all 7 hovered very briefly and then landed nearby. In these cases, the waiting females left after 4 to 6 min and did not return to the nest entrance to check again. We had no response to the control tubes. The results suggest that the pollen is more attractive, but the nectar elicits a “land nearby and wait” behavior. Unfortunately we did not have a pollen and nectar combination vial that may have attracted more and then initiated a land and wait response.

3.4. Oviposition location within nest

C. chichimeca eggs were embedded in the cell wall of *C. bicornuta* ($\chi^2 = 16.54$, $df = 3$, $P < 0.005$) (Tab. III). As shown in Table III, significantly more oviposition occurred in the upper 1/4th (quadrant 1+8) of the cell with 54% of the eggs occurring in this quadrant followed by 36% of the eggs in quadrant 2+7 and only 9.1% of the eggs in the 3 + 6 quadrant (Tab. III). No eggs were found in the lower quadrant. We also noted that eight of the eggs were under the pollen near the pollen-nectar interface. Ten were under the nectar and four were in the cell wall not yet covered by nectar.

3.5. Returning female host bee response to *C. chichimeca*

During recordings of *C. chichimeca* that had oviposited, we noticed that female host bees returning to their nest displayed a unique

Table III. Location of *C. chichimeca* eggs in the various quadrants of the cell wall of *C. bicornuta* relative to the nest entrance as shown in Figure 1. Means with the same letters are not significantly different. $\chi^2 = 16.54$, $df = 3$, $P < 0.005$.

Quadrant	N	% Total
1+8 (Upper part of nest)	12	54.5a
2+7 (Upper middle)	8	36.4b
3+6 (Lower middle)	2	9.1b
4+5 (Lower part of nest)	0	0.0c

behavior. The returning female would enter her nest and within 15 to 20 s she would exit and fly around making 2 to 5 roughly circular patterns that were 50 to 80 cm across. These females (N = 21) would repeat the process 3 to 5 times. During the circular flights the females made a distinct “sputtering” sound that lasted 4 to 8 s with the circular flights lasting 8 to 12 s. During the sputtering activity the glasses of the senior author on several occasions was covered with many small sticky spots. If the angle of the sun was right, we noticed that a mist was discharged from the mouth of the female host bee. We collected some of this mist on a glass plate and it was sticky and often these spots contained some pollen. Following this activity the female left and then resumed nesting activities.

As soon as females left following this activity, their nests (21) were collected and dissected to determine what had happened to these cells in preparation. These cells were compared to completed cells that were in the same nest (65). We did not know if the completed nests were parasitized or not, but we found four that were sealed without any nectar or pollen. These four were parasitized as evidenced by a pit in the cell wall and some dead tissue in the pit. The results are shown in Table IV and confirm that the bees that entered the parasitized cells and subsequently flew around making a sputtering sound had removed the nectar and possibly some of the pollen. However, in only one case there was there an indication, as evidenced by a hole in the cell wall, that a parasite egg had been laid and possibly removed by the female bee’s activity. It is also clear that cleaned cells do

Table IV. Nectar provisions inside nest cells of *C. bicornuta* in preparation or complete. *Means were compared across columns and rows and means with same letter are not significantly different. $\chi^2 = 39.706$, $df = 1$, $P < 0.001$.

Bee cell condition	N	Number without nectar	Number with nectar	% without nectar
Completed	65	4a	61b	6.2
In preparation	21	19b	2a	90.5

not allow a parasitoid egg to develop. However, this sputtering behavior as a host bee's response requires further study.

To examine the potential that the female bees could remove the *C. chichimeca* eggs, we dissected 107 completed nests from our nearby solitary bee biology study. Forty-seven of these had evidence that at least one cell within the nest was attacked by at least one *C. chichimeca* (43.9% parasitized nests). We should note here that these nests were collected from two areas where nests were concentrated, which could have concentrated the parasites as well. Regardless, we found that of the 167 cells (2 to 4 cells/nest) in these parasitized nests, 62 or 37.1% were parasitized. Of these 51.2% (N = 32) were in cells with pollen and nectar and had a larva that were developing. In another 46.8% (N = 29) of the cells the parasites were dead or not developing and these cells had no nectar. We also found 11.4% (N = 19) of the cells had no bee, parasite, or nectar. Of these 19 cells, 36.8% (N = 7) had a pit in the cell wall, suggesting that female host bees may sometimes remove the parasite egg or one was not laid. A lack of nectar, bee or parasite in the remaining 12 cells may be due to a number of factors, such as the lack of nectar resources, the female for some reason was unable to oviposit or was killed. In this latter case, the nest may be usurped by another bee (Vinson and Frankie, 2000) and the unfinished cell sealed.

The results suggest that the female host bee is sometimes, but not always successful in removing the parasite egg. It is not clear if the nectar had been removed and the female re-added nectar and laid her egg or if the female failed to recognize the cell had been visited by a *C. chichimeca*. We did record that some female bees re-stocked the cells from which they

had removed nectar following a *C. chichimeca* visit. However, the female bees more often do not complete the cells that have been visited by the *C. chichimeca* even though they have invested in the pollen. Further, they always sealed the cell with the pollen inside.

In conclusion, we have determined that the parasitic bee, *C. chichimeca* examines small holes or dark spots as it flies through the forest using pollen and nectar as indicators of a host bee nest. Depending on which one is present, the parasitic bee exhibits certain pre-egg laying behavior and actual egg laying in the nest of the host bee. We also determined that the parasitic bee usually lays an egg in the upper half of the host-bee cell wall, either in the nectar or nectar-pollen interface. We also observed that occasionally, the host bee responds to the parasitic bee intrusion by spitting out the nectar that they had provisioned, thus depriving the resources the parasitic bee would need to develop.

Since the cleptoparasitic bees are an important mortality factor for many of the solitary bee species in Costa Rica, our study provides insights into an intricate relationship between the host and parasitic bee and the latter's parasitizing behavior which is one of the causes of the disappearance of the solitary bee species.

ACKNOWLEDGEMENTS

We thank Texas Agrilife, Texas A&M University and the California Agricultural Experiment Station for partial support. We thank D.A.S. Stewart for allowing us to study bees on his property and Luis Sandoval in placing and collecting many of the nest blocks and monitoring emergence data. We acknowledge the help of Dr. Terry Griswold in identifying the species of *C. chichimeca* reared from nests of *C. bicornuta*, Dr. Jerry Rozen for comments on

an earlier draft and Rollin Coville for contribution of a picture.

Comportement, observé sur le terrain, du parasite *Coelioxys chichimeca* (Hym. : Megachilidae) envers son hôte *Centris bicornuta* (Hym. : Apidae).

Recherche de l'hôte / relation hôte parasite / oviposition / odeur / abeille nichant dans le bois mort / abeille solitaire / Costa Rica

Zusammenfassung – Verhalten der parasitischen *Coelioxys chichimeca* (Hymenoptera: Megachilidae) gegenüber der Wirtsbiene *Centris bicornuta* (Hymenoptera: Apidae) im Freiland. *Coelioxys chichimeca* Cresson ist ein Kleptoparasit und eine wichtige Ursache für die Mortalität von mehreren in Baumlöchern nistenden bestäubenden Bienen in Costa Rica. Wir untersuchten das Verhalten dieser parasitischen Biene gegenüber einer der häufiger vorkommenden Wirtsbienen, *Centris (Heterocentris) bicornuta* Mocsary. Wir untersuchten das Wirtsfinderverhalten durch Beobachten von Nistblöcken im Gelände, und beschreiben es. Wir beobachteten, dass weibliche *C. chichimeca* mit dem Wind zu den Bäumen fliegen, wo unsere Nistblöcke aufgestellt waren und dass sie entweder (1) kurz vor einem Loch schwebten und dann weitersuchten, oder (2) den Eingang berührten und hineinfliegen oder in der Nähe landeten, oder (3) direkt in das Nest der Wirtsbiene eindringen ohne es vorher zu untersuchen (Abb. 2). Diejenigen, die in der Nähe landeten, wendeten ihre Köpfe dem Nest zu und verharrten in einer Wartestellung (Abb. 3).

Wir untersuchten auch Faktoren, die möglicherweise bei der Entscheidung zur Eiablage eine Rolle spielen. Die Präparation einer Zufallsprobe von Nestern von *C. bicornuta* zeigte, dass die prozentuale Parasitierung von Zellen niedriger war als die Gesamtparasitierung der Nester, die viel höher lag (Tab. I). Um die Komponenten des Nestinhalts, die vom Parasiten angenommen oder abgelehnt wurden, näher zu untersuchen, wurden den parasitischen Bienen künstliche Nester mit verschiedenen Inhalten angeboten und ihr Verhalten dokumentiert. Das Nest wurde später der Länge nach aufgeschnitten und der Inhalt nach den Kategorien leer, nur Pollen enthaltend, Pollen und Nektar enthaltend, sowie ein Ei der Wirtsbiene oder ein Ei von *C. chichimeca* enthaltend, sortiert. Die Ergebnisse deuten an, dass das Vorhandensein von Nektar entscheidend dafür ist, ob *C. chichimeca* nach der Landung und dem Verharren in das Nest eindringt und ein Ei legt, nachdem die Wirtsbiene das Nest verlassen hat (Tab. II). Wenn nur Pollen vorhanden ist, scheinen sie den Eingang zu inspizieren und dann weiterzufliegen. Daraufhin

untersuchten wir, ob chemische Stoffe bei der Anlockung und Orientierung von *C. chichimeca* zu den Nestern eine Rolle spielen. Wir dokumentierten das Antwortverhalten von allen sich nähernden *C. chichimeca* zu den künstlichen Nestern, die entweder nur Pollen, nur Nektar, beides, oder gar nichts enthielten. Die Ergebnisse zeigen, dass Pollen attraktiver ist, jedoch Nektar das Verhalten „in der Nähe landen und warten“ auslöst.

Um die Platzierung der Eier von *C. chichimeca* zu untersuchen, wurden die Nester in acht gleiche Sektoren eingeteilt (Abb. 1). Wie in Tabelle III gezeigt wird, erfolgten im oberen Viertel (Sektor 1+8) signifikant mehr Eiablagen, während im unteren Viertel (Sektor 4+5) keine Eier gefunden wurden. Schließlich untersuchten wir noch das Antwortverhalten der Wirtsbiene *C. bicornuta* gegenüber der Aktivität der Kleptoparasiten. Das zurückkehrende Weibchen betrat ihr Nest, verließ es wieder und flog herum, wobei es ein charakteristisches „Stottergeräusch“ produzierte. Danach entfernte es sich. Sobald die weiblichen *C. bicornuta* fortgeflogen waren, wurden ihre Nester eingesammelt und präpariert, um herauszufinden, was im Vergleich zu verschlossenen Zellen mit den noch im Aufbau befindlichen Zellen im selben Nest geschehen war. Die Ergebnisse in Tab. IV bestätigen, dass die Bienen, die in die parasitierten Zellen eingedrungen und danach unter stotternden Geräuschen fortgeflogen waren, den Nektar und möglicherweise auch einen Teil des Pollens entfernt hatten, vermutlich bei dem Versuch, das Ei des Parasiten zu entfernen.

Wirtsfindung / Eiablage / Geruch / in Baumlöchern nistende Biene / Solitärbiene

REFERENCES

- Aguiar C.M.L., Garófalo C.A., Almeida G.S. (2006) Nesting biology of *Centris* (Hemisiella) *trigonoides* Lapeletier (Hymenoptera, Apidae, Centridini), Rev. Bras. Sool. 323–330.
- Baker J.R. (1971) Development and sexual dimorphism of larvae of the bee genus *Coelioxys*, J. Kans. Entomol. Soc. 44, 225–235.
- Batschelet E. (1965) Statistical methods for the analysis of problems in animal orientation and certain biological rhythms, American Institute of Biological Sciences.
- Bohart G.E. (1970) The evolution of parasitism among bees, Utah State University, Logan.
- Cane J.H. (1983) Olfactory evaluation of *Andrena* host nest suitability by kleptoparasitic *Nomada* bees (Hymenoptera: Apoidea), Anim. Behav. 31, 138–144.
- Dansforth B.N., Visscher P.K. (1993) Dynamics of a host - cleptoparasite relationship: *Holcopasites*

- ruthae* as a parasite of *Calliopsis pugionis* (Hymenoptera: Anthophoridae), Ann. Entomol. Soc. Am. 86, 833–840.
- Frankie G.W., Newstrom L., Vinson S.B., Barthell J.F. (1993) Nesting-habitat preferences of selected *Centris* bee species in Costa Rican dry forest, Biotropica 25, 322–333.
- Frankie G.W., Vinson S.B., Newstrom L., Barthell J.F. (1988) Nest site and habitat preferences of *Centris* bees in the Costa Rican dry forest, Biotropica 22, 301–310.
- Gilbert L.E. (1972) Pollen feeding and reproductive biology of *Heliconius* butterflies, Proc. Natl. Acad. Sci. USA 69, 1403–1407.
- Gilbert L.E. (1975) Ecological consequences of a co-evolved mutualism between butterflies and plants, in: Gilbert L.E., Raven P.H. (Eds.), Coevolution of Animals and Plants, Univ. Texas Press, Austin TX, pp. 210–240.
- Graenicher S. (1905) Some observations on the life history and habits of parasitic bees, Bull. Wisconsin Nat. Hist. Soc. 3, 153–167.
- Graenicher S. (1927) On the biology of the parasitic bees of the genus *Coelioxys*. (Hymenoptera: Megachilidae), Entomol. News 38, 231–235.
- Janzen D.H. (1971) Euglossine bees as long-distance pollinators of tropical plants, Science 171, 203–205.
- Linsley E.G., MacSwain J.W. (1955) The habits of *Normada opecella* Timberlake with notes on other species (Hymenoptera: Anthophoridae), Wassmann, J. Biol. 13, 253–276.
- Michener C.D. (2000) The Bees of the World, The Johns Hopkins University Press, Baltimore, MD.
- Rosenheim J.A. (1987) Density-dependant parasitism and the evolution of aggregated nesting in the solitary Hymenoptera, Ann. Entomol. Soc. Am. 83, 277–286.
- Rozen J.G. Jr. (2003) Eggs, ovariole numbers, and modes of parasitism of cleptoparasitic bees, with emphasis on Neotropical species (Hymenoptera: Apoidea), Am. Mus. Novitates 3413, 1–36.
- Rozen J.G. Jr., Eickwort K.R., Eickwort G.C. (1978) The binomics and immature stages of the cleptoparasitic bee genus *Protepeolus* (Anthophoridae: Nomadinae), Am. Mus. Novitates 3038, 1–24.
- Rozen J.G. Jr., Kamel S.K. (2007) Investigations on the biologies and immature stages of the cleptoparasitic bee genera *Radoszkowskiana* and *Coelioxys* and their Megachile hosts (Hymenoptera: Apoidea: Megachilidae: Megachilini), Am. Mus. Novitates 3573, 1–43.
- Scott V.L., Kelley S.T., Strickler, K. (2000) Reproductive biology of two *Coelioxys* cleptoparasites in relation to their Megachile hosts (Hymenoptera: Megachilidae), Ann. Entomol. Soc. Am. 93, 941–948.
- Thorpe R.W. (1969) Ecology and behavior of *Melecta separata callura* (Hymenoptera: Anthophoridae), Am. Mid Nat. 82, 338–345.
- Vinson S.B., Frankie, G.W. (2000) Nest selection, usurpation, and a function for the nest entrance plug of *Centris bicornuta* (Hymenoptera: Apidae), Ann. Entomol. Soc. Am. 93, 254–260.