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Observations on the Behavior of Brazilian Halictid Bees (Hymenoptera, Apoidea) IV. *Augochloropsis*, with Notes on Extralimital Forms[1]

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The purpose of the present paper is to record observations on the nesting behavior of certain species of *Augochloropsis*, a very large American genus of usually brilliantly metallic halictine bees. Observations on *A. sparsilis* and *A. diversipennis* were made over most of a year (July, 1955, to July, 1956), while those on other species mentioned were much more fragmentary. Except for the observations on *A. cleopatra*, all data were gathered at various localities on the southern Brazilian plateau in the state of Paraná. This is a region of strongly seasonal climate, with rather cold winters.

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A. *AUGOCHLOROPSIS SPARSILIS* (VACHAL)

HABITAT

Except for some abandoned nests probably of this species found in banks at Araucaria, Paraná, all nests studied were in the Bariquí roadside banks near Curitiba, Paraná. In these vertical banks the nests were localized in a few zones of only a few square meters, where over 200 nests were found in decomposed gneiss, as described by Michener, Lange, Bigarella, and Salamuni (1958).

Each nest, as described in detail below, consists of a burrow extending into the bank and one or sometimes two or three clusters of adjacent cells. Each is usually occupied by several female bees. Our data were mostly gathered by excavations of nests at intervals during the season, combined with prolonged observations of activity around the nests, aided by some marked bees. All female bees dug from the nests, together with samples obtained outside at various times, were dissected to determine the condition of the ovaries and spermathecae, and examined to determine the extent of mandibular and wing wear, with the use of techniques described by Michener, Cross, Daly, Rettenmeyer, and Wille (1955).

SEASONAL CYCLE

We first found nest aggregations of this species in spring, before the bees had begun spring activities. On September 16 and 18, 1955, the open burrows were observed with no evidence of recent activity. Some of the nests contained no bees, but most were occupied by two to 15 adult females. No males or immature forms were present. Most of the females were fresh in appearance, although two of 30 examined were considered to have slightly worn mandibles, and one had a nick in the margin of one wing. Of 20 of which the spermathecae were examined, all contained sperm cells. These data are summarized in table 1. Although one bee was found near its nest entrance, most were in cell clusters or in burrows beneath the cell clusters or elsewhere far from the entrances.

On September 19, a warm sunny day, a few females were seen at the nest entrances, and one flew about 3 inches, but none left for any distance in spite of an abundance of spring flowers nearby. All disappeared into their nests a few seconds after coming out. The site was observed again on September 25, when earth was seen falling down the bank from the entrances of some nests, which indicated activity within, but no bees were seen outside.

TABLE 1

NEST STATISTICS AND CONDITIONS OF FEMALE OCCUPANTS, *Augochloropsis sparsilis*
(Only data from nests with cells in which all the nest occupants were captured are included.[a])

	Spring					Summer				Autumn		
	Sept. 16, 18	Oct. 2, 9	Oct. 14–18	Nov. 4–17	Dec. 4, 9	Dec. 21, 31	Jan. 15, 29	Feb. 9, 16	Feb. 26	Mar. 11, 24	Apr. 6, 8	May 13
1 No. of nests excavated	5	4	11	10	8	10	10	2	8	7	5	4
2 No. of adult females per nest	7.8	4.2	3.7	2.3	2.2	1.5	1.9	4	3.6	3.1	2.8	5.8
3 No. of formed cells in nests	112	55	214	52	91	114	57	23	59	49	25	54
4 Percentage of cells with pollen or young	0	1.8	12.2	26.9	36.5	48.2	49.1	65.2	81.4	79.6	36.0	5.5
5 Percentage of cells with eggs	0	0	7.5	19.2	9.3	4.4	10.6	30.4	27.1	2.0	0	0
6 Percentage of cells with larvae	0	0	0	5.8	25.0	15.8	1.8	13.0	27.1	30.6	0	0
7 Percentage of cells with male pupae or unemerged males	0	0	0	0	0	12.3	10.5	13.0	3.4	32.6	8.0	1.9
8 Percentage of cells with female pupae or unemerged females	0	0	0	0	1.0	15.8	22.9	0	13.6	20.4	28.0	9.3
9 No. of females with sperms	20	11	22	7	15	11	14	5	17	11	10	20
10 No. of females without sperms	0	0	0	0	0	1	5	3	8	0	1	3

TABLE 1—(*Continued*)

	Spring					Summer					Autumn	
	Sept. 16, 18	Oct. 2, 9	Oct. 14–18	Nov. 4–17	Dec. 4, 9	Dec. 21, 31	Jan. 15, 29	Feb. 9, 16	Feb. 26	Mar. 11, 24	Apr. 6, 8	May 13
11 No. of records of ovary size	20	11	56	25	23	22	23	8	29	21	12	23
12 Percentage of females with ovaries very slender	85.0	36.4	32.3	8.0	4.4	9.1	8.9	25.0	34.5	9.5	41.7	73.9
13 Percentage of females with ovaries slender	15.0	27.3	26.9	44.0	26.1	31.9	43.5	12.5	31.0	42.9	50.0	26.1
14 Percentage of females with ovaries little swollen	0	18.2	21.4	28.0	39.1	31.9	21.8	12.5	17.2	42.9	0	0
15 Percentage of females with ovaries much swollen	0	18.2	19.6	20.0	30.5	27.3	26.1	50.0	17.2	4.8	8.3	0
16 No. of records of mandibular and wing wear	30	20	49	25	22	22	23	8	29	21	13	23
17 Percentage of females with mandibles unworn	93.3	80.0	49.0	40.0	22.7	4.5	26.1	50.0	20.7	38.1	46.1	78.3
18 Percentage of females with mandibles slightly worn	6.6	15.0	49.0	40.0	59.1	45.5	43.5	37.5	51.7	28.6	30.8	21.7
19 Percentage of females with mandibles well worn	0	5.0	2.0	20.0	18.1	45.5	26.1	12.5	27.6	33.3	23.1	0

TABLE 1—(*Continued*)

	Spring					Summer				Autumn		
	Sept. 16, 18	Oct. 2, 9	Oct. 14–18	Nov. 4–17	Dec. 4, 9	Dec. 21, 31	Jan. 15, 29	Feb. 9, 16	Feb. 26	Mar, 11, 24	Apr. 6, 8	May 13
20 Percentage of females with mandibles much worn	0	0	0	0	0	4.5	4.4	0	0	0	0	0
21 Percentage of females with wings unworn	96.6	75.0	73.6	56.0	31.8	45.5	52.2	75.0	55.2	71.4	61.5	78.3
22 Percentage of females with 1–5 marginal nicks in wings	3.3	25.0	22.4	40.0	68.2	45.5	43.5	25.0	37.9	23.8	23.1	21.7
23 Percentage of females with more than 5 marginal nicks in wings	0	0	4.1	4.0	0	4.5	4.4	0	6.9	0	15.4	0
24 Percentage of females with margins of wings largely gone	0	0	0	0	0	4.5	0	0	0	4.8	0	0

[a] Except as otherwise stated, females had emerged and were free in the nests. The entries in line 4 do not equal the sums of entries in lines 5 to 8 because of the inclusion in line 4 of cells that were partially or wholly provisioned but lacked eggs, and in lines 7 and 8 of a few unemerged adults.

The overwintering bees, regardless of their ages or activities the previous fall, are called "spring bees" in the subsequent pages.

Examination of table 1 shows that excavations of nests made on October 2 and 9 did not show very marked differences from those made in September. However, one or more cells in some nests were newly lined with wax, and on October 9 one cell was found in which pollen was being stored. As might be expected, a higher percentage of individuals showed mandibular and wing wear than in September, and more ovary enlargement was noted, to the point that two of 11 individuals dissected contained an oocyte nearly large enough to be laid.

On October 11 considerable activity was noted about the nests, and a very few bees were seen entering with pollen loads on their legs.

As noted in table 1, numerous nests were opened during the period October 14 to 18. The changes observed in early October had progressed considerably. Several cells in each nest were freshly lined with wax, and 16 cells out of 214 examined contained eggs, the first of which was found on October 16. Ovary enlargement, wing wear, and mandibular wear were also progressing, although various individuals in each nest were still unworn and with very slender ovaries.

During November and December continued progress in the same direction was evident, more and more of the cells being renovated, rewaxed, and used for brood rearing. The first larva was found on November 15, the first pupa on December 7, the first new adult, still in its cell, on December 31. Meanwhile, as is evident from table 1, the number of fresh bees with slender or very slender ovaries decreased progressively as more and more bees became active. The number of bees per nest dropped during October, November, and December (from an average of 4.2 to 1.5). As there was no establishment of new nests after the middle of October, the reduction in nest populations after that time was presumably due to the death of certain bees. Indeed in December (as well as January) an occasional nest contained no surviving adults, even though the cells contained a brood of growing young of the next generation.

Several females were found during early October in simple burrows in the bank. We are not able to be sure that these burrows were newly excavated; the bees could have overwintered in them. However, as we saw no evidence of construction of such burrows in the fall, we believe that they were dug in the spring, probably by bees that left the overwintering groups. This conclusion is supported by the fact that *A. diversipennis* builds new nests at the same season. The reduction in the number of bees per nest between mid-September and early October (as shown in table 1) could be explained by the departure of bees to establish new nests. The bees from simple burrows are not included in table 1. All of them had very slender ovaries and showed little mandibular and wing wear.

Progress in provisioning cells seems remarkably slow, as is shown in more detail in the next section. As the great majority of the nests studied were constructed in previous years, the total number of existing shaped cells did not increase much from September to December, or even through the rest of the season. The average number of cells per nest therefore remained about the same, in spite of the construction of a few small new cell clusters. The progressively increasing percentages of cells in use (line 4, table 1) during the spring and summer months is therefore a reasonable index of the number of cells in use and results largely from re-use of cells made in previous seasons.

Although egg production apparently decreased during December (line 5, table 1), some eggs were laid at all times. Adults from eggs laid by spring bees appeared over a long period, from the latter half of December, when young adults were first observed, until February or later.

In January unusually little outside activity was noted. For example, in watching a group of 100 or more nests for an hour in good weather on January 8, we saw only two bees carrying pollen, and two or three others outside their nests. Similar inactivity continued through much of January. At the same group of nests, watched for four hours on January 29, only three bees were seen bringing pollen to their nests. There was already by that date an increase in the number of bees flying in and out (but not carrying pollen). Presumably this was owing to the increasing numbers of young bees that had not yet started provisioning activities. On February 9, however, many more pollen-gathering bees were noted than during the preceding month. Certainly most of the spring bees disappeared by the end of January, but some lasted much longer, as shown by a female marked at the last of November or early December, 1955, found still alive in a nest on February 26, 1956. Because of the long survival of some of the spring bees and emergence of some individuals of the new generation, few nests were found without any adult bees.

The new generation consisted of both sexes, the males being the first adults of that sex seen during the year. On the average the male pupae and unemerged males seemed less numerous than females, as can be seen by a comparison of line 7 and line 8, table 1. Among 106 pupae found during the study, 42.5 per cent were males. The males do not long remain in the nests; they were rarely found there. Males were never seen flying about the nesting places, as with *A. diversipennis*. Presumably mating occurs away from the nests, possibly near flowers.

As shown in line 2, table 1, the average number of females per nest was higher in late summer (February) than earlier because of the emergence of the new generation. The result of the increased nest populations, with young mated females first recorded in the nests in the last half of January, was a sharp increase in egg production, producing a peak in February considerably higher than that of November (line 5, table 1). The corresponding peak in maturation of adults occurred in March and early April (lines 7 and 8, table 1).

Because adults were produced continuously from the beginning of January until the end of the season in May (although less abundantly in February than before or after because of the slump in egg production resulting from senility or depletion of the spring bees), there were naturally unworn females with slender ovaries among the samples taken from nests in late summer and fall. On the other hand, individuals with worn mandibles and wings and with enlarged ovaries continued to be present in the nests; presumably the latter condition resulted from wear and ovarian development of individuals of the new generation, replacing similar spring bees as they died.

On February 26 a good many bees were still collecting pollen, but subsequent observations on March 4, 8, and 16 revealed no pollen-collecting activity. The last egg found in a cell was on March 11. Early in March an occasional female was seen in flight about the nests, but in an hour of observation on March 16, only one was seen in spite of warm weather. In April none was seen, but on May 13 several females were seen at the nest entrances, and a few were seen to leave and return after long absences. Few observations were made in winter, but on June 28, near midwinter, a female was seen entering a nest. Otherwise, no activity was observed.

It is noteworthy that as nesting activity diminished in the fall, nest populations rose sharply (line 2, table 1), no doubt owing to continued emergence at a time when predation and aging were reduced by inactivity.

The overwintering bees were all adult females and apparently consisted of any unworn or little-worn bees in the nests at the time cool weather set in. Thus many of them were the bees that emerged in the fall (March and April). Others, however, were contemporaries of the presumed parents of those bees. For example, one unworn bee found in a nest with several others in May had been marked in the last half of January. Indeed the possibility exists, although it is improbable, that an occasional inactive spring bee may pass through the entire summer and a second winter. This possibility is suggested by the fact that the spring bee already mentioned which had been marked between November 24 and December 3 was fresh looking and unworn, with slender ovaries when found in a nest on February 26, nearly at the end of the active season.

The duration of the various immature stages was not determined, but the period from egg to adult must be about two and one-half months. During the season of observation, among the nests that were fully excavated, 70 cells were found containing eggs, 80 containing larvae, 91 containing pupae, and 15 containing unemerged adults. As there is little evidence of mortality during the immature stages, we judge that the duration of the egg to the larva to the pupa is about as 7/8/9 and that after emergence from the pupa, adults remain in the cells for about one-sixth as long as the duration of the pupal stage.

Individual and Social Behavior

Aggregations: As mentioned above, nests of *Augochloropsis sparsilis* occur in aggregations, so that the entrance of one nest is usually only a few centimeters from entrances of others. This is presumably a result of some social phenomenon; at least Michener, Lange, Bigarella, and Salamuni (1958) were unable to find any physical differences between areas inhabited by this bee and adjacent areas that were uninhabited.

As explained above, not only are the nests in groups, but most nests studied contain more than one female bee. The nest populations were highest during the winter (up to 15 bees in one nest, although occasional lone individuals were found) and considerably lower during the summer months of December and January, when the most populous nest found contained five females, many contained but one, and a few lacked adult bees completely. Average nest populations for various seasons of the year can be seen in line 2, table 1.

The question arises as to how the groups of females in a nest become associated. In all probability they are often sisters that stay in the nest of their birth. We know from two nests that were removed to the laboratory and provided with tubes for the escape of the emerging young that, while the males left the nests and disappeared, the females came and went, at least two females remaining associated in one nest from the time that they emerged in January until winter when we abandoned the study. On the other hand, the bees occupying a nest are not necessarily those born in it. We have several times found a young nest which had not yet produced its first brood, or even a mere burrow with no cells whatever, inhabited by two females. In all probability such bees are not sisters but had diverse origins. In *Pseudagapostemon divaricatus* also, we found that the inhabitants of a nest were not necessarily sisters (Michener and Lange, 1958a).

GUARDING: Nests inhabited by more than one female had a guard at the entrance much of the time during the warm hours of the day. Such guards were first noted in early October and were last seen in mid May. Guards were less in evidence than those of *Pseudagapostemon divaricatus* (Vachal) (see Michener and Lange, 1958a), and timidly withdrew into the nests at the near approach of a person, stick, or other large object. Also, the guarding was not continuous. Yet it appears to be of some importance, for we saw a guard turn a mutillid (apparently a species which we found to parasitize *Augochloropsis*) away from the burrow, and several times we have seen a guard exclude a "lost" female *Augochloropsis*. Once, instead of retreating, such an *Augochloropsis* attempted to enter in spite of the guard. They bit at one another and apparently attempted to sting; finally the guard pushed out of the entrance and both bees fell to the ground beneath the bank, then separated and flew off.

Guards were observed in detail on December 9, and it was found that each time a bee left her burrow she spent from two to 15 minutes at the entrance, her head barely to completely exposed before flying off (usually without a noticeable orientation flight). There is no reason to believe that this long wait was due to the presence of an observer. Inside, another bee could be seen at a depth of about a centimeter. Later this or another bee might come to the entrance for a time and then leave. Returning bees (which usually flew quickly and directly to their nests) were usually allowed to enter promptly, for a guard at the entrance retreated to a point where the burrow was large enough for bees to pass one another. As with other halictids, guards were not seen in nests containing but one bee. From this it can be seen that the guards were largely whatever bees were coming and going; the bees that remain indefinitely in the nest were not involved in guarding. As is shown below, this means that the egg-laying bees rarely guarded the nests, while the foragers regularly did so. It should be noted that guards did not always fly out of the nest; often they remained at the entrance for a time and then disappeared inside.

In nests containing only one adult female bee, that individual must perform as a solitary bee and make the nest and cells, wax the latter, provision them, and lay eggs if the nest is to succeed in producing offspring. We found few nests in which young were being produced by a lone female. One such nest excavated on October 16 contained a female with much swollen ovaries. Her mandibles and wings were worn, as were those of many others at the season. From the cold weather at the time of excavation it was judged very unlikely that any other females were in the field. The nest contained a large cell cluster made the preceding year and already had three provisioned cells with eggs. We do not believe it likely that this lone female could have provisioned three cells by this time and believe that one or more sisters or other bees had been associated with the egg layer in this

TABLE 2

Correlation of Total Number of Nicks on Forewings with Mandibular Wear

(The numbers in the table represent females of *Augochloropsis sparsilis.*)

	Wings				
	Unworn	With 1–3 Nicks	With 4–6 Nicks	With 7–9 Nicks	With More Than 9 Nicks
Mandibles					
Unworn	101	8	1	—	—
Slightly worn	63	39	9	3	2
Well worn	11	20	9	6	7
Much worn	—	—	1	1	2

nest, but probably succumbed, perhaps to predation, before we opened the nest. On December 31 a nest containing three prepupae and four pupae was excavated and found inhabited by a single female. Again it is almost certain that her former associates had died. (We have no evidence that bees shift about from nest to nest.)

On the other hand, two nests opened on December 4, inhabited by one female each, contained small cell clusters with only one or two completed cells. In each case one of the cells was ready to receive pollen, and one of the bees was captured entering her nest with a pollen load on her legs; obviously she was starting to provision a cell. Both females had much-swollen ovaries, with an oocyte nearly ready to be laid. Clearly we cannot know that other females were not associated with these nests earlier, but it seems improbable, especially as the cell cluster of each was apparently constructed that year, for it contained no old wax indicating an earlier usage. Thus we believe that on occasion a lone female can construct a nest and may produce a very few offspring.

There is ample evidence, however, that females alone in nests, even though in a bank with hundreds of other nests, may simply remain inactive. For example, the nest of a bee marked at the end of November was opened on January 15. The burrow was 12 cm. deep and contained no cells, and indeed the entrance was partially closed, which indicated a lack of recent use, but the bee was alive in the burrow.

Division of Labor: Certainly it is normal for nests to be inhabited by several bees. As the most active nests were inactive compared to those of many other species of bees, and one would often wait for hours to see a bee leave or enter a given nest,[1] it was difficult to investigate the interrelationships among the occupants of a nest. However, some significant data were obtained. Guarding is mentioned above as an activity involving primarily one group of bees, namely, those that were coming and going from the nests.

We know from the preceding section that females of various ages went into the winter (and presumably passed thorugh it). Although the badly worn and tattered bees seemed to disappear in the fall, some relatively old but little-worn bees overwintered. In the spring, therefore, all the bees started their activity with relatively unworn mandibles and wings. Very soon, however, some became worn, and from that time on through the season the population consisted of a mixture of tattered and fresh individuals in varying proportions, as shown in table 1. Not only was this true of the population as a whole, as shown in that table, but among the inhabitants of each nest there were usually, after early spring, individuals that showed considerable mandibular and wing wear and others that still appeared quite fresh, as well as those in intermediate conditions. At least until the end of December, when young adults started to emerge, these

[1] We saw one unusually active marked bee bring in four pollen loads during a day, the first at 9:30 A.M., the last at 12:45 P.M. Most bees did much less. One left the nest at 10:00 A.M., returned with pollen at 10:16, left at 10:24, returned without pollen and disappeared for the day at 1:21 P.M. Another left the nest at 10:12 A.M., returned at 10:30 with pollen, left at 12:54 and returned at 2:25. These observations were all made on December 9, a day of fair weather except for a cloudy early morning. The air temperature at noon in the shade near the nests was 27.5° C.; the soil temperature 6 cm. into the bank was 19° C. Observations terminated at 3:15 P.M.

variations indicated diversity in the activities of the occupants. As most of the females were found to be fertilized, the following discussion is limited to such bees.

Mandibular wear must result from work in the nests, presumably largely the constructing and applying of the earth lining of the cells. We believe this to be the case, because lone females in quite deep burrows which they had doubtless dug often showed very little mandibular wear, while if several cells had been constructed and lined, the mandibles were usually distinctly worn. Mandibles of each female were examined and recorded as unworn, slightly worn, well worn, or much worn. Wing wear, on the other hand, occurs outside the nest, probably in flying about flowers, when the wings strike objects, and bits of the margins are broken out. Young individuals kept in nests and not allowed to fly about never exhibited any wing damage whatever. Wings of each female were examined and recorded as unworn, or, if worn, a record of the number of marginal breaks on each forewing was made, or, if (as rarely occurred) most of the margin was gone, a record of that fact was made. Although wing wear was quite erratic and probably largely depends on chance, it is in general correlated with mandibular wear, as shown in table 2. (This table is based on all females of which the wings and mandibles were studied, not merely on those included in complete nest populations, as is table 1.) This correlation shows that the bees that work ordinarily engage in both outside and inside activity. In almost any nest inhabited by several females, however, there were individuals that showed little or no signs of work (table 1, rows 17 and 21). If nests of several bees were watched for periods of six to eight hours, and the bees going in and out were marked or captured, excavation of the nests always revealed certain bees inside that were never seen to leave the nests. The egg layers were usually among these, as were certain individuals that seemed to remain in the overwintering condition, with slender ovaries.

To shed further light on the matter, a study was made of the relation of ovary development to wear. An index of wear was devised by the assignment of numbers 1 to 4 to the various stages of mandibular wear and 1 to 5 to the stages of wing wear shown in table 2. Then the number for mandibular wear and that for wing wear were merely added to give the index, so that unworn individuals have an index of 2, the most worn, of 9. Table 3 shows the relations of wear to ovary width. Ovaries 0.15 to 0.25 mm. in maximum width were classified as very slender; 0.26 to 0.45, as slender; 0.46 to 0.85, as slightly swollen; and 0.86 to 1.20, as much swollen. When right and left ovaries were

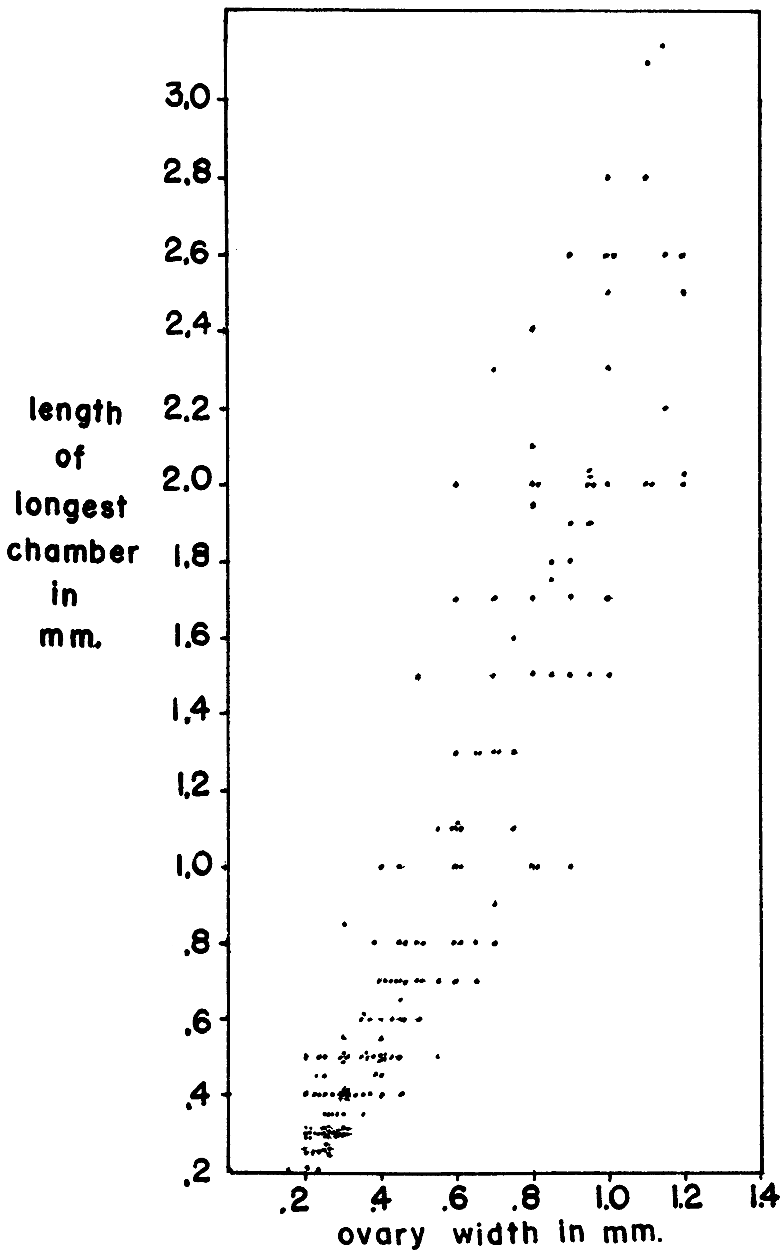


FIG. 1. Diagram showing, for *Augochloropsis sparsilis,* correlation between average ovary width of any individual and length of the longest ovarian chamber.

TABLE 3

CORRELATION OF INDEX OF WEAR WITH OVARY WIDTH

(The numbers in the table represent females of *Augochloropsis sparsilis*, unmated females excluded. The numbers in parentheses represent females captured with pollen loads on their legs, unmated ones excluded.)

	Index of Wear								Totals
	2	3	4	5	6	7	8	9	
Ovaries									
Very slender	31 (2)	12 (2)	5 (1)	1	2	—	—	—	51 (5)
Slender	35 (1)	21 (4)	20 (5)	5 (1)	5 (1)	2 (1)	2	—	90 (13)
Slightly swollen	16	18	15	8	3 (1)	2	1	1	64 (1)
Much swollen	3	12	11 (1)	3	2	2	3	—	36 (1)
Totals	85 (3)	63 (6)	51 (7)	17 (1)	12 (2)	6 (1)	6	1	241 (20)
Percentage of total with much-swollen ovaries	2.4	19.0	21.6	17.7	16.7	33.3	50.0	0	14.9

unequal, the average of the two was used.

Because in this species the development of the three ovarioles of each ovary is usually more or less coordinated, ovary width is a fair measure of general ovary size. Of course the six ovarioles do not mature oocytes simultaneously, but if one ovariole is enlarged, the others usually are also. Figure 1 shows the relationship between ovary width and the length of the longest chamber visible in the fixed ovary under a binocular dissecting microscope. In the smaller ovaries the longest chamber was always a group of nutritive cells. Such chambers increase in length a little more rapidly than ovary width, as shown in the lower part of figure 1. In the larger ovaries, the longest chamber was always

TABLE 4

MEASUREMENTS (MEANS AND THEIR STANDARD ERRORS, IN MILLIMETERS) OF OVARIES OF *Augochloropsis sparsilis* COLLECTED FROM OCTOBER TO FEBRUARY

	No. of Specimens	Ovary Width	Length of Longest Oocyte
Pollen collectors[a]	19	0.2953±0.0259	0.348±0.035
Presumed egg layers	30	1.0183±0.0223	2.262±0.080
Bees entering nests without pollen	21	0.464±0.054	0.869±0.152

[a] One pollen collector that was alone in its nest and had large ovaries is omitted.

an oocyte. Oocytes grow much more rapidly than ovary width, as shown by the steeply inclined band of dots in the greater part of figure 1 (above about 0.8 mm.).

The increasing breadth of the band of dots as one progresses upward in figure 1 is probably partly due to the fact that oocyte length cannot be an ideal measure of ovary size. When an oocyte is nearly ready to be laid, it is very large. After the egg is laid (or the oocyte resorbed), the largest oocyte is very much smaller, and only after an interval of time does it grow to nearly the size of a laid egg (about 3.4 mm. long). Therefore oocyte length varies considerably according to the particular time when the bee was killed. We believe ovary width to be less subject to such short term variation.

We think that all bees classified as much swollen in table 3 were in egg-laying condition. A few bees reach that state while still unworn or virtually so (index of wear: 2). Most bees have done some work by the time the ovaries become "much swollen," and there is a perhaps significant increase in the percentage of individuals with such ovaries among the more badly worn bees, so that 50 per cent of the six bees with an index of wear of 8 had much swollen ovaries. In any event it is clear that work inside and outside the nest is often associated with egg laying. This is true even in nests inhabited by numerous females. The results would not be altered if mandibular and wing wear were considered separately, instead of together by means of an index of wear.

As pointed out above, females with much swollen ovaries from nests occupied by several bees were not commonly found outside the nests. We judge that usually a bee does some work inside and outside the nest before becoming an egg layer, but that if she becomes an egg layer, such work diminishes, unless, of course, the egg layer is alone and acting as a solitary bee. As is explained below and as is suggested by table 3, not all bees become egg layers; some become badly worn, while the ovaries remain slender.

Pollen Collecting: The remaining aspect of the diversity of behavior or division of labor among individuals in a nest involves pollen collecting. Observations of nests in which bees returning with pollen on the legs were marked and released revealed that often, in a nest found on excavation to contain three or four bees, only one was collecting pollen. Or, especially in larger nests, two or three of the nest occupants were sometimes found collecting pollen simultaneously. In such cases, when the nest was opened, there was never found more than one cell being provisioned. The pollen gatherers clearly cooperate in the provisioning of cells.

Examination of the numbers in parentheses in table 3 shows that pollen collecting may begin before there is any appreciable wing or mandibular wear. Both wing and mandibular wear, however, characterized many pollen collectors. Tables 3 and 4 show that pollen collectors as a rule have slender ovaries. The one with much-swollen ovaries shown in table 3 was alone in her nest, working as a solitary bee. This individual was omitted from the data used in table 4.

From these evidences that many pollen collectors are worn, yet have slender ovaries, we think it very probable that some females develop as pollen collectors and wear out without ever becoming egg layers. This surmise is supported by data from marked bees. A total of 92 females was marked (with the use of colored Dope paints) during November and January. Bees for marking were obtained by our blocking the nest entrances and then capturing the returning bees. About half of the bees marked were returning with pollen loads on the legs; the remainder lacked pollen, although when held in the fingers to be marked they often regurgitated nectar, which indicated that they had been visiting flowers. After a series of such bees had been marked and allowed to re-enter their nests, it was usual to see the pollen collectors, especially, for two or three weeks, usually continuing in that activity, but gradually most of them disappeared. We believe that they died. Sometimes their remains were found in nests, but probably they usually died or were killed in the field. Probably loss of such bees is a major factor in diminution of nest populations during October, November, and December (line 2, table 1). However, at least one bee marked as a pollen collector remained alive for as long as two and one-half months. It was not observed collecting pollen during the last two months of this period. The bees marked when returning to the nests without pollen also gradually disappeared, but less rapidly than those marked as pollen collectors. The records are few and therefore inconclusive, but we think that the more rapid disappearance of the pollen collectors means that as these bees die, they are replaced by other, previously relatively inactive bees.

Imaginal Development Among Fertilized Females: Probably all females leave the nests from time to time to feed. Especially in the spring many of them had pollen, doubtless obtained from flowers, in the crop.[1] Bees that become pollen collectors must leave the nest more often, while those that become egg layers perhaps do so less often. As bees develop in one or the other of these directions (or sometimes in a combination of the two), they show the results of wear, but, as indicated above, a number of unworn bees with slender ovaries always remain in the nests. Until the emergence of new adults in early January, the percentage of such bees decreased (lines 12, 17, and 21, table 1) regularly, which shows that bees leave this inactive state to become active as foragers or egg layers.

That the bees die after considerable wear is evident from the decreasing totals for the various indices of wear shown in table 3. Mortality is more evident in the fall, when unworn bees no longer become active and replace the ranks of worn bees, egg laying and pollen collecting cease, the worn bees vanish (die?), and the overwintering bees are all unworn or nearly so (indices of wear: mostly 2, a few 3). As

[1] No positive correlation between pollen in the crop and large ovaries was evident in this species, although such a correlation is evident in various other halictines studied by Michener.

indicated above, their unworn condition does not mean that the overwintering bees are all young; considerable age variation exists. They are probably all physiologically young, however; their differences in actual age may account in part for the variation in time of becoming active among the spring bees.

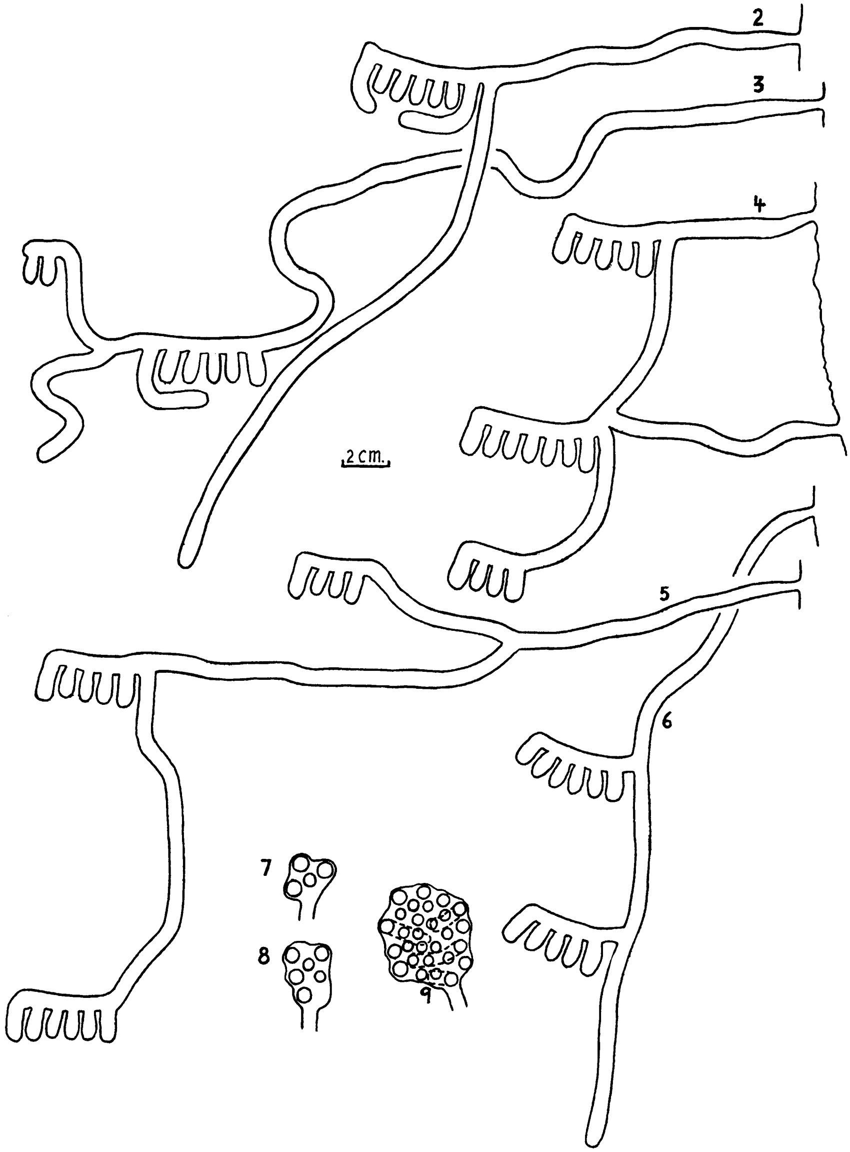
The division of labor described above exists among fertilized bees of approximately the same size. [Mean wing length for pollen-collectors, 7.07 ± 0.042 mm. ($n = 18$); extremes, 6.8 and 7.3 mm.; mean wing length for bees with much swollen ovaries, 7.14 ± 0.039 mm. ($n = 33$); extremes, 6.6 and 7.7 mm.; mean wing length for all bees, 7.061 ± 0.0141 mm. ($n = 270$); extremes, 6.0 to 7.7 mm.] The mean wing length of egg layers is greater than that of pollen collectors, and, as shown below, this is in turn greater than that of unfertilized worker-like bees. An analysis of variance shows that these differences are not statistically significant. These differences are suggestive, and more material should be obtained to show if they are real. However, there are clearly no morphologically distinct female castes.

UNFERTILIZED FEMALES: Before the appearance of young females in January, only one unfertilized female was found among bees of which the spermathecae were examined (lines 9 and 10, table 1). After that time a certain number of unfertilized individuals was found each month. Some of these, to judge by unworn mandibles and wings and sometimes by recently opened cells in the nests from which they came, were merely young females that had not yet had an opportunity to mate. It is the bees that never mate that are of interest to us here.

The one spring female (among 86 the spermathecae of which were examined before January) that had not mated was captured carrying pollen into a nest on December 21. It had been marked in early November, at which time it was carrying pollen. Evidently it had been carrying pollen for a considerable period. It was by far the most worn bee seen up to that time. The mandibles were much worn, and the wings were so worn that the margins were almost entirely gone. Another almost equally worn unfertilized bee was found among the occupants of a nest excavated in March.

These are not isolated cases, although the amount of wear that they displayed was extreme. Of 12 pollen collectors captured entering nests inhabited by more than one bee during January and February, seven were unfertilized, three were fertilized, and two are unknown. Among the seven with empty spermathecae, various degrees of wing and mandibular wear were found, the indices of wear ranging from 2 to 6.

In all the unfertilized bees, the ovaries were very slender or slender,



FIGS. 2–9. Diagrams of nests of *Augochloropsis sparsilis*. 2, 3. Nests in overwintering condition, with marginal burrows from cell clusters curling under cells. 4–6. Nests in summer; nest 4 is one of two that we examined showing connections between nests. 7, 8. Top views of beginning clusters of cells, showing central shaped cells (small circles) and marginal burrows (large circles). 9. Top view of cell cluster of moderate size, showing cells (small circles) and marginal burrows, some of which have been prolonged downward and beneath the cells, as shown in broken lines.

the longest visible chamber being rarely as much as 0.50 mm. long, usually 0.25 to 0.30 mm. In size the unfertilized bees (excluding fresh ones which probably had done no work and might soon have mated) were about the same as the population as a whole [mean wing length, 7.02 ± 0.059 mm. ($n = 18$); extremes, 6.5 and 7.3 mm.].

Clearly a certain percentage of the females did not mate. Among overwintering bees examined in spring, this group was perhaps not more than 1 or 2 per cent; during January and February it was probably 15 to 20 per cent. Such bees lay no eggs, and most or all of them become pollen collectors. Worn mandibles indicate their activity also in cell construction. These bees work considerably more (at least become more worn) than the average mated bees. Only rarely was more than one such bee found in a nest, and often none was present among the occupants of a nest.

It is easy to see that the presence of such active unfertilized bees might be an advantage to a colony, even though they are non-reproductive. We may conjecture that the habit of extensive work on the part of unmated females was a preadaptation that permitted selection for the regular occurrence of such individuals (workers) and thus provided for the establishment of a worker caste among various halictine and other bees.

Nest Structure

Burrows: The nests are 6 to 36 cm. deep (average of 15, 15 cm. deep). The burrows, which are round in cross section and 6 to 9 mm. in diameter (average of 20 measurements, 7.25 mm.), usually start into the bank more or less horizontally (see figs. 2–5) but often make sharp bends and may be very winding. They may bend in any direction, but long downward sections are common. Occasionally (as in fig. 5) the burrow branches well before any cell cluster. More commonly (figs. 2, 4–6) there is a sharply descending burrow from near the point where the main burrow reaches the first cell cluster. This burrow often ends without cells (fig. 2) but sometimes leads to another cell cluster (figs. 4, 5). Rarely such a descending burrow connects with another nest (upper part of fig. 4).

The entrances of the burrows are narrowed to 3 or 4 mm. in diameter; it is this narrowed part that is closed by heads of guarding bees. If the constricted entrances of nests are removed by the slicing off of the outer surface of the bank, the bees in a day or so bring more earth to the nest entrances and once more narrow them by means of a thick lining of dirt. The rest of the burrow is probably lined with soil brought from other parts of the nest and used to form a thin, rather smooth coating, as is clearly the case in nests of *Augochloropsis diversipennis.*

CELL CLUSTERS: Many nests have but one cell cluster. A single cell cluster may occur, even at the end of the summer, in nests that were certainly constructed at least the preceding year, so that their minimum age must have been about 20 months. Nests with two cell clusters are rather common, and others with three exist (figs. 3–6).

Because of the extensive and probably long-continued re-use of old cell clusters, we have few data on the construction of new ones. However, scattered small and probably new clusters were found in the spring. It would seem from these small clusters that a cluster begins as a small horizontal expansion of the end of a burrow. In the floor of this expansion, short vertical burrows are constructed and then lined with earth to form cells. Small cell clusters, in early stages of their development, are shown in top view in figures 7 and 8. It can be seen that in general it is the central cells that are first lined and shaped, while the outer ones remain as mere burrows. This arrangement is not so regular as in *Augochloropsis cleopatra,* discussed below. The cell clusters are enlarged by being expanded laterally in all directions. As this expansion is done, new short vertical burrows are constructed around the margins, and the more central burrows are lined and shaped to form cells. Thus at all times there are burrows of large diameter around the periphery of the cluster, and cells in the center. Figure 18 shows clusters with shaped central cells and large lateral burrows. The lining of the cells and use for rearing young also progress in an irregular way from the center towards the margin of the cluster. Figure 18 shows a rather large cluster in the spring, after three cells have been provisioned and closed. Although the closed cells are not strictly central, they tend to be, as in all such clusters.

The largest clusters reach a diameter of 6 cm., although 4 cm. is more usual, and occasionally a small cluster with only two or three completed cells is found, even in the fall when clearly no new construction is under way. The rate of growth of a cell cluster is unknown in the field. However, a single bee in an artificial nest in the laboratory constructed 10 cells in only about three days. Thereafter she did not increase the size of the cluster. Whether such a cluster is ever further enlarged we do not know.

Even at the height of reproductive activity, every cell cluster always has short marginal burrows which seem as though they might be made into cells. However, this development does not occur, and, especially in the fall, young females lengthen some of the marginal burrows. Occasionally such a burrow is continued straight down, as is one of those in figure 19. Usually, however, they curl under the cluster. Figure 19 shows a section of a cluster with these burrows beneath the cells, while figure 9 shows a vertical plan of such burrows. In winter, hibernating bees are often found in these burrows beneath the cells, although they may also be present in cells or especially near the ends of simple burrows. Sometimes they close themselves into the burrows by means of a partition of loose soil. As shown by figure 9, some of the short marginal burrows are never elongated.

Among 43 clusters studied, the number of cells and short marginal burrows ranged from two to 31, the mean number and its standard error being 13.5 ± 1.12. Among 42 clusters (mostly the same ones as those of which the cells were counted), the number of elongated marginal burrows, usually curled under the cells, ranged from none to nine, the mean number and its standard error being 1.9 ± 0.34. That this average is small is owing to the general absence or small number of elongated lateral burrows during the active summer season. Such burrows are largely filled with earth at this time. Among 25 nests examined during the spring (September and October), before the filling of these burrows occurred, the average number of elongated marginal burrows per nest was 2.5. Some clusters, particularly smaller ones, may, however, have no elongated marginal burrows, even in winter. There is considerable evidence that cell clusters are used year after year, until erosion brings them too near to the bank surface. Details are presented in the section on cells. Although, as indicated above, the cell clusters are basically horizontal, with the cells themselves vertical, in reality the surface on which the cells open is often slightly concave (fig. 19) and usually slopes upward a little from the bank face (see fig. 5).

CELLS: The cells, as indicated above, start as short vertical burrows. They tend to diverge slightly downward (fig. 19), and occasionally a marginal one slopes strongly. When first made the walls of these burrows are rough (fig. 19), often showing the marks made by the mandibles of the bee (shown for *Augochloropsis rufisetis* in fig. 19). Such roughed-out cells are then lined with soil, which gives them their characteristic shape. Sometimes the earth for lining cells comes from other parts of the nest. The banks at Bariguí contain patches of different colored earth (as described by Michener, Lange, Bigarella, and Salamuni, 1958). Sometimes cells in red matrix were lined with white, and sometimes the reverse situation was observed. Figures 18, 19, and 20 show the shape of finished cells. Such a finished cell is relatively flat on one side, more strongly concave on the other. As can be seen from figures 18 and 19, the flat surface of a cell is usually the side towards the center of the cluster, which is logical because, on comparison with halictids that make horizontal cells, it can be seen that the flat surface is morphologically ventral. The cells of *Augochloropsis sparsilis* tend to slope outward from the central part of the cluster; therefore the surface that is most nearly ventral is usually the one towards the central part of the cluster.

After a cell is shaped and smooth on the inside, it is lined with a thin layer of a nearly clear, wax-like material. The wax layer retains about the same appearance until sometime during the prepupal or pupal period of the occupant of the cell. At that time it rather suddenly becomes dark.

The cells are regularly re-used. In early spring they all are lined with dark wax. Later, one of the central cells in the cluster is refurbished, which is done by scraping off with the mandibles (to judge by the marks) the layer of dark wax and replacing it with a new coating of clear wax. Commonly the removal of the old wax is incomplete, so that dark patches show through the fresh lining. Very rarely a very thin layer of earth (perhaps 0.25 mm. thick) is placed over the old wax, and new wax is deposited on it. Commonly two cells are renovated before the provisioning of the first begins, and the refurbishing continues well ahead of provisioning, so that usually there are one or two cells in a nest that seem to be ready to be provisioned but have not yet received any pollen.

Unlike *Neocorynura* and *Pseudaugochlora* (Michener and Lange, in press), there is never any evident major change in the position of a cell when it is re-used. We know (from position, dark wax, and contents when opened) that a given cell may be used at least twice in one season of activity, and we have seen apparently identical cells used in the spring after obviously having been used (as shown by dark wax) the preceding summer. There is no evidence that cells may not be used repeatedly for years, and we presume such re-use to be the case.

Each cell, after the provisioning and egg laying, is closed by an earth plug about 1 mm. thick (figs. 18, 20). This is rather smooth on the outside, which is flush with the upper surface of the cell cluster as a whole, and rougher inside but without evident spiral pattern.

Measurements of 14 cells are summarized below as extremes, means, and standard errors of means: length (to surface, not to inner surface of plug), 11.5 to 14.0 mm.; mean, 12.69 ± 0.198 mm., maximum width, 5.5 to 6.8 mm., mean 6.14±0.092 mm. By contrast, diameters of marginal burrows, either elongated or short ones that might be made into cells, ranged from 7 to 8 mm.

PROVISIONS: Each cell, after having been waxed, is provided with a pollen mass. Sometimes cells examined during the provisioning process contained some loose pollen on the bottom of the cell. At other times part or all of the pollen in such a cell was stuck together to form an irregular mass (fig. 19). Ultimately, however, the pollen is formed, probably with admixture of nectar, into a firm, rectangular, pollen mass (fig. 20) placed near the bottom of the flat surface of the cell. The egg is placed on the pollen mass away from the flat cell surface. The position of the egg and pollen mass is exactly comparable to that of other halictids that build their cells in the presumably more primitive horizontal position. Crude measurements are as follows: pollen mass: height, 4.0 to 5.5 mm.; width, 4.0 to 5.0 mm.; thickness, 2.3 to 3.0 mm.; egg: 3.3 to 3.5 mm. long; greatest width, 0.78 to 0.80 mm.; width near posterior end, about 0.5 mm.

B. *AUGOCHLOROPSIS DIVERSIPENNIS* (LEPELETIER)[1]

HABITAT

Except for one nest found in a roadside bank at Araucaria, Paraná, all the nests of this species that were studied were in the Bariquí roadside banks near Curitiba. Their distribution in these banks is described by Michener, Lange, Bigarella, and Salamuni (1958). In brief, it may be said that widely scattered nests were found in many places, especially in vertical banks facing in a generally northerly direction, but that one aggregation of over 450 nests occurred in that part of a bank consisting of particularly soft soil. A few were also found at the foot of a bank in soil sloping about 45 degrees from the horizontal. In basic features of structure the nests are similar to those of *A. sparsilis*.

Methods of study were the same as those used for *A. sparsilis*, except that little marking of bees was undertaken.

SEASONAL CYCLE

We first found nests of this bee on September 18, 1955. There was already loose dirt at the entrances, at least of freshly made burrows

[1] The "*Halictus diversipennis* Lep." of Janvier (1955) is certainly a misidentification, if Moure's identification which we have followed, is correct. Janvier's description of the nest indicates a species of some genus other than *Augochloropsis*.

that lacked cells. From this time through October 23, no outside activity except occasional flights was observed. However, on and after October 2, cells thought to be freshly constructed were found. As with *A. sparsilis,* no males or immature forms were present in early spring, and the females appeared fresh, so that only three of 28 collected between September 18 and October 23 had slightly worn mandibles and only one had a small nick in one wing. All 28 females had been fertilized and had slender ovaries. (Very slender ovaries such as characterize most overwintering *A. sparsilis* as well as freshly emerged females at any season do not occur in *A. diversipennis.*)

Excavations of nests on November 15 revealed several eggs and one very young larva. From this we judge that rearing of young starts later than in *A. sparsilis.* From this time through January every female dissected (18 in all) had slightly or usually much-swollen ovaries, with one or more large oocytes (over 1 mm. in length). A total of 46 overwintered females dissected had all been fertilized. Mandibular and wing wear, of course, became more noticable as the season advanced, although a few females with unworn mandibles were removed from nests in late December.

Two mature larvae (i.e., larvae with their food supply exhausted) and one prepupa were found on January 3. Our data for January and early February are meager, but on February 15 males were seen, and fresh females were seen carrying pollen. Fom this we judge that the first bees of the new generation must have emerged some time before February 15.

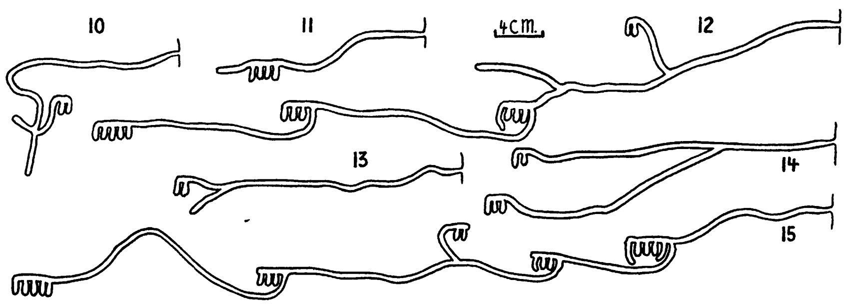
Only four male pupae and five female pupae were found during the summer, which suggests that the sexes are produced in roughly equal numbers. Unlike males of *A. sparsilis,* those of *A. diversipennis* spend a great deal of time in rapid zigzag flight around the banks where the nests occur. They were seen, often in great numbers, at the principal concentration of nests on every warm day when observations were made from mid February until April 8, and on May 13 a few males were still about.

During February there is a considerable increase in the activity of pollen-collecting bees, as judged by hurried observations of the principal nesting area. We last noted pollen collecting on March 11. Emergence of new adults, of course, must have continued into May, if not June. The males must die in the fall; at least none was present when our studies were begun in the spring. We believe that a few females leave their nests to fly about on any warm winter day, for on June 24 one was seen to enter a burrow.

The duration of the immature stages was not determined, but from egg to adult probably requires about two and one-half months.

Individual and Social Behavior

Nests of this species were widely scattered, except for one large aggregation which could be explained by softness of the soil. Therefore, in contrast to *A. sparsilis,* we have no evidence of an aggregative tendency in the behavior of the species. However, the isolated nests may



FIGS. 10–15. Diagrams of nests of *Augochloropsis diversipennis.* 10, 11, 13. Nests in their first season occupied by single females. 12, 15. Old nests, each occupied by two females using the two cell clusters at left in each nest; other cell clusters abandoned. 14. Nest presumably in its first season occupied by two females.

be relatively unsuccessful. All 10 that we opened were constructed, or at least inhabited, by a single female working alone, and none seemed to be producing more than two or three offspring. Simple burrows (i.e., nests being started) and new nests which we found in the aggregation were also inhabited by one female each. It may be, therefore, that *A. diversipennis* lacks the tendency, evident in *sparsilis,* for more than one female to join forces in making a single nest (a possible exception, suggested by figure 11, is discussed below under Nest Structure). The frequency of newly started nests in spring (October) is in contrast to nest making by *A. sparsilis.*

In early October two nests were found with 13 bees in each, although others had but one, and various intermediate populations were recorded. Presumably bees that have left such communal overwintering quarters are responsible for the numerous new nests seen in October. Two or three females often use a single nest burrow, even in summer, but in all such cases that we studied, old cells in the nest indicated that it had been used previously. The occupants, therefore, may have been sisters. Guarding similar to that of *A. sparsilis* but perhaps less consistent was noted at entrances of nests inhabited by several females.

As all the females of the spring generation seem to develop enlarged ovaries, there is no evidence of division of labor among them. The existence of some still unworn bees as late as December may suggest division of labor, but the two such bees found were both alone in nests that had old cells in them. Presumably these bees emerged there, passed the winter, and in spring worked slowly or late, for each had provisioned only one cell when the nests were opened in late December.

Nests, presumably constructed and provisioned by a lone female and containing but two or three provisioned cells and but one adult, or no surviving adult at all, are common in this species. In a considerable number of nests, the bees of the spring generation die before emergence of their progeny.

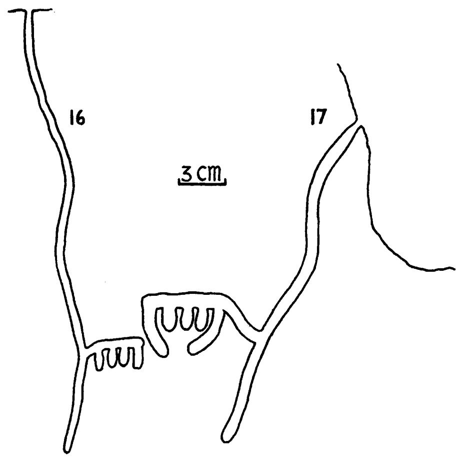
There is good evidence that the majority of cell clusters are constructed and provisioned by single females. In the 26 nests that we excavated from October 23 to April 8, the number of females in a nest was always equal to or sometimes less than the number of cell clusters being used, if unfertilized young females were ignored. From this we judge that each cell cluster is ordinarily made by one female, which was often suggested also by the locations of the occupants of a nest, one in or near each cell cluster, when we opened the nest. Apparently if two or more females remain in a nest, each makes a separate continuation or branch burrow and cluster of cells. We judge that social organization among females in a nest ordinarily involves only the use of a common burrow. This is as in *Augochloropsis humeralis* (Patton) of North America (Smith, 1901).

However, during late summer there is meager evidence indicating division of labor similar to that which occurs in *A. sparsilis.* Thus a bee carrying a full pollen load on her legs when taken on February 15 was fertilized but had slender ovaries (longest oocyte, 0.34 mm.). Presumably she would not have laid an egg in the cell she was provisioning. On March 7 a female captured while carrying a pollen load was found to be unmated, with slender ovaries. She was unworn or nearly so. At the same season, other pollen-collecting females were fertilized and had large ovaries, and doubtless laid eggs in the cells they were provisioning. Among 28 females dissected during February and March, only one belonged to the worker-like class of worn bees with slender ovaries, found in *A. sparsilis*. That one, found on March 11, obviously represented an abnormal situation. She was alone in a very small, shallow (8 cm. deep) nest with a single cell (no cell cluster)! The bee was more worn than any other *A. diversipennis* seen (mandibles very much worn; wing margins completely gone). She was fertilized, and her ovaries were slender but rather long and yellowish, indicating, we believe, regression in size in an old bee. The bee could not have become so worn making this small nest and one cell. She must have worked elsewhere and later made this nest, possibly as an activity of senility. However, she did provision the cell, for it contained a female pupa when we opened the nest. She must have remained with the nest during the entire period of growth of the one young.

In spite of the above suggestions of division of labor, there is strong evidence that this is not the usual state of affairs. Its occurrence, or joint work by two or more egg-laying females, may account for the large cell clusters sometimes found.

Nest Structure

Burrows: Nests were found 14 to 93 cm. deep (average of 22, 52.2 cm. deep). The burrows are round in cross section, 5 to 6 mm. in diameter, and essentially horizontal. A nest occupied by a single bee and showing no evidence of previous occupancy usually resembles figure 11 or figure 13. Figure 10 is a basically similar but curiously formed nest. Such burrows are usually less than 25 cm. in depth. Rarely, much deeper and apparently new nests were found, such as one that was 65 cm. deep, but it now seems probable that these were actually old nest burrows, with the connections to abandoned clusters closed with soil. In one nest, diagrammed in figure 14, a burrow with two active and apparently new clusters forked well before either cluster, which may suggest that after one bee started a burrow, a second entered and constructed a branch and a second cell cluster. This example is the only evidence that we have for such behavior. Otherwise, all nests in which more than one bee was working were like figures 12 and 15 in structure. That is, there was one or more old clusters, often partially filled with earth and often closed off by a wall of earth from the burrow. It seems probable that such a nest starts as a cluster near the bank surface, and in its first season resembled figure 11. One (or more) of the daughter bees, instead of leaving and starting a new nest, continued into the bank to make one (or more) additional cell clusters. Usually the continuation is made as a prolongation of a marginal burrow from the cell cluster, descending from the cell cluster on the side near the bank surface. Thus it is homologous to the descending burrow common in nests of *A. sparsilis*. In subsequent seasons the deepening of the nest may continue, each continuation being roughly comparable to an independent nest. As the nests illustrated in figures 12 and 15, as well as various others studied, had two cell clusters simultaneously occupied, it is quite possible that the burrows leading to both of these clusters were made in the same season. The great depth that the nests attain makes them difficult to study and is one reason for our fragmentary knowledge of this species.



FIGS. 16, 17. Diagrams of nests. 16. *Augochloropsis iris*. 17. *Augochloropsis cleopatra*.

Burrows of *A. diversipennis* are lined throughout with earth taken from elsewhere in the nest, probably from the vicinity of the cells. This fact was especially clear because in some parts of the aggregation the earth bank was brown, but red earth was present at a depth of 65 cm. from the surface. Burrows that penetrated the red were lined to the surface of the bank with red earth, and indeed a small turret 1 to 2 mm. high was often made of similar earth. The burrow lining was especially evident where it walled off old, and currently unused, parts of the nest, or where our excavations covered a burrow entrance with loose soil. In the latter case the bees continued the lining around clods and through the interspaces of the loose dirt to the surface. The same lining material is used to constrict the burrow entrance to a diameter of 2.5 to 4.0 mm.

CELL CLUSTERS: As is explained above, many nests have but one cell cluster, and the number varies up to five. However, we found no nest with more than two clusters occupied at the time of excavation. In contrast to the situation in *A. sparsilis,* we did not find evidence of re-use of cell clusters. Although the entrance burrow is often re-used, new cell clusters appear to be made by each generation of females.

The initiation and growth of cell custers, and their basic structure, are as described for *A. sparsilis.* Figures 7 and 8 could as well have been made for *A. diversipennis,* and we saw many more such small clusters in *diversipennis* nests than in those of *sparsilis.* Clusters are smaller than in *sparsilis,* so that among 38 clusters, the number of shaped cells ranged from one to 17, mean 4.3; the number of short marginal burrows or incompleted cells ranged from none to five, mean 2.5. Or, to use figures comparable to those for *A. sparsilis,* the numbers of cells and short marginal burrows combined ranged from two to 21, with an average of 6.8. The number of elongated marginal burrows ranged from none to seven. There were usually no such burrows during the active season, but they were built in the fall. The average number per cluster in clusters excavated in the spring was 3.7. Unlike those of *A. sparsilis,* the elongated marginal burrows of *A. diversipennis* often extend down vertically or curve but little and do not curve strongly under the cell cluster.

CELLS: The cells have the same positions and shape as in *A. sparsilis,* and the same stages in their construction can be seen. We have no evidence that they are re-used as in that species. Measurements of seven cells are as follows: length (to surface, not to inner surface of plug), 10 to 12 mm.; mean, 10.6 mm.; maximum width, 4.5 to 5 mm.; mean, 4.9 mm.; width of opening of cell, 3 mm.

PROVISIONS: The pollen mass is shaped and located as in *A. sparsilis,* and the egg is placed on it as in that species. Measurements are as follows: pollen mass: height, 4.1 and 4.2 mm.; width, 3.6 and 3.9 mm.; thickness, 2.5 mm.; egg: 2.2 mm. long; greatest width, 0.65 mm.; width near posterior end, 0.45 mm.

C. *AUGOCHLOROPSIS IRIS* (SCHROTTKY)

This species was studied in Xaxim, a suburb of Curitiba, Paraná, and nests were found scattered in the same flat ground where *Paroxystoglossa jocasta* was found (Michener and Lange, 1958b). However, it was much less common than that species. A few females were found on flowers as early in the spring as August 15, 1955. Only five nests were found, all from September 20 to 25. One was merely a burrow being dug by a single female. The others all contained one cell cluster. One contained no bees, two contained one female each, and one contained three females. In these three inhabited nests, a central cell in each cluster was being provisioned, or had been provisioned and provided with an egg. The lone females had slightly worn wings and mandibles and enlarged ovaries. In the nest containing three females, one had well-

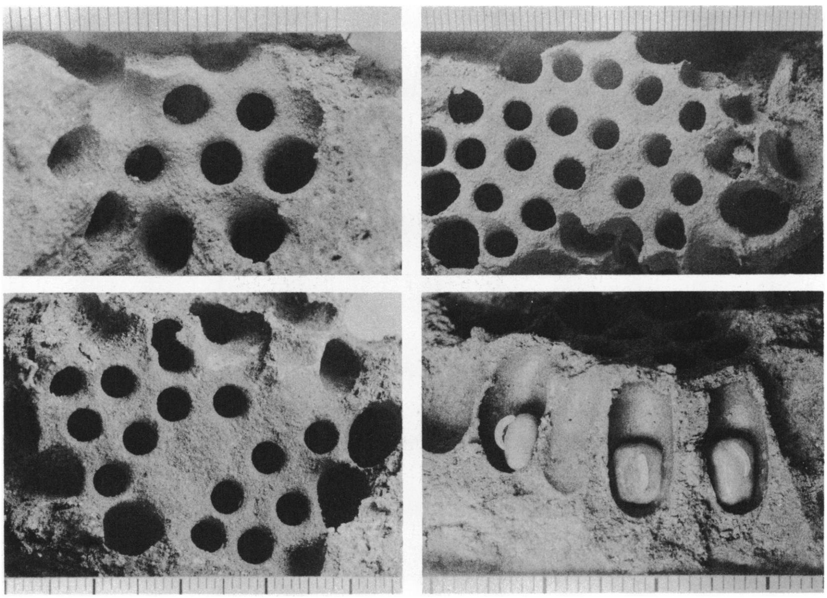


FIG. 18. Cell clusters of *Augochloropsis sparsilis*. *Upper left:* Top view of rather small cluster in overwintering condition (September 16, 1955). Note that two of the large marginal burrows have been broken open above. The irregular lateral margin of the cavity above the cells shows clearly above and to the right of the cell cluster. *Upper right:* Top view of large cluster in overwintering condition (September 16, 1955). Note the large marginal burrows, broken open, above and below. *Lower left:* Top view of a cluster that had passed the winter and was being re-used the following spring (October 16, 1955). Note three cells, two near the center, the other above and to the right of the center, that have been provisioned and sealed. *Lower right:* Sectional view of nest shown at lower left, showing the three closed cells with their pollen masses and eggs. Scales shown in centimeters and millimeters.

worn mandibles and wings with the margins almost completely worn away. The others had mandibles and wings unworn, or nearly so, and slender ovaries. All had mated. From this scant evidence we judge that females of various ages overwinter and in the spring start rearing young either individually or in small groups, much as in the case of

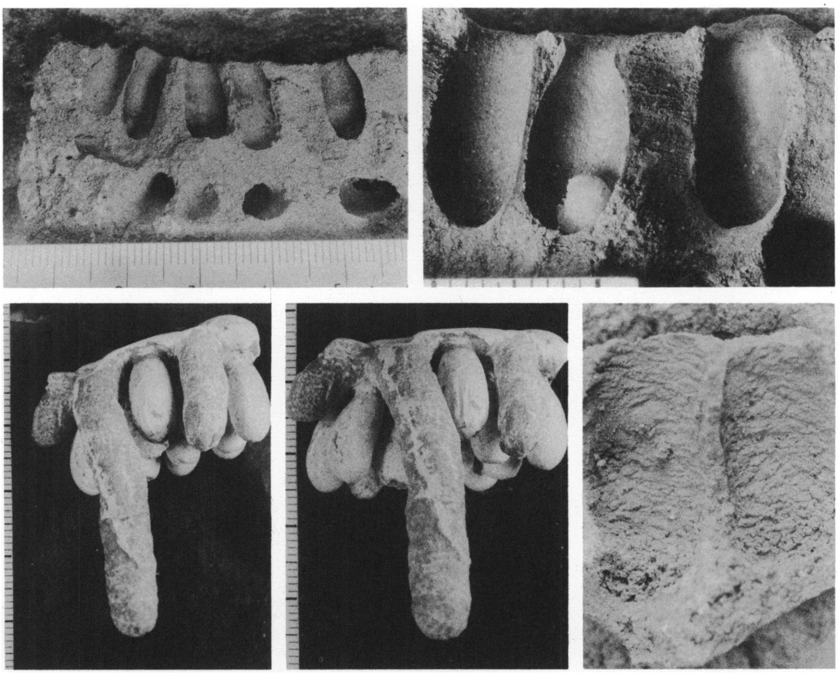


FIG. 19. Cell clusters of *Augochloropsis*. *Upper left:* Cluster of *A. sparsilis* in overwintering condition, September 16, 1955, showing the concave upper surface of the cluster, the cells diverging below with their flat sides towards the center of the cluster. The cells have been lined with wax which has become dark and has been partly removed, especially in the cell just to the left of the center, perhaps in preparation for re-use of the cell. The holes beneath the cells are continuations of the lateral burrows that curve beneath the rest of the cell cluster. These holes usually end blindly, as does one just to the left of the center of the photograph. *Upper right:* Part of cell cluster of *A. sparsilis*, showing, from left to right, a well-formed but unwaxed cell, a waxed cell with pollen store being formed, a cell with dark wax from the previous year, and (partially cut off) a marginal burrow curving under the cells. *Lower left and center:* Side views of a plaster of Paris cast of a cell cluster of *A. sparsilis* in winter condition. Note three rough-walled marginal burrows, one rather long. This particular cluster did not have such burrows curling under the cells, as is often the case. Note the smooth walls of the cells, which are strongly convex on the sides away from the center of the cluster. *Lower right:* Inner views of two freshly excavated, roughed-out cells of *Augochloropsis rufisetis*, showing rough surfaces presumably resulting from mandibular action. Scales shown in centimeters and millimeters.

A. sparsilis, except that in that species well-worn females do not seem to overwinter.

On December 14 in the same area both sexes were common on flowers. The females collected were unworn and mostly unfertilized. Obviously by this date a new generation had emerged.

The nest structure is superficially quite different from that of previously discussed species because the nests are in flat ground. Each consists of an irregularly vertical burrow (figs. 16, 20) 22 to 28 cm. deep, 5 to 6 mm. in diameter, and narrowed at the surface to 3.5 or 4 mm. in diameter. There is sometimes a tumulus of loose soil at the surface closing the entrance. At a depth of 18 to 23 cm. a very short lateral burrow leads to the cell cluster, which is irregularly round and 2 to 5 cm. in diameter, with eight to 30 cells in the nests studied. The cell cluster is roughly horizontal, but sometimes part of it slants (fig. 20). Generally it is the central cells in a cluster that are shaped and marginal cells that remain as short vertical burrows. No deep marginal burrows such as occur in *A. sparsilis* and *diversipennis* were found in the few nests of *iris* that we found. Completed cells were 11.7 to 13 mm. deep, 5 to 5.8 mm. in maximum diameter, and 3.7 to 4 mm. in diameter of the entrance. The shape is as described for cells of *A. sparsilis.* Provisions are in a subrectangular mass as in *A. sparsilis.* One such was 5.25 mm. high and 4.25 mm. wide. The pollen mass is located as in *A. sparsilis,* and the egg is placed upon it as in that species. The one measured egg was 2.5 mm. long, its greatest diameter was 0.8 mm., and its diameter near the posterior end was 0.6 mm.

D. *AUGOCHLOROPSIS CLEOPATRA* (SCHROTTKY)

Our observations on this species were all made near Guaruva, Santa Catarina. This locality is at about sea level, thus quite different from localities for all other species discussed in the present paper. Three nests were found on October 30, 1955, in a small, nearly vertical earth bank about 20 cm. high. Each was an irregular slanting burrow 15 to 20 cm. deep, 9 mm. in diameter, narrowed to 7 mm. in diameter at the entrance, and provided with a short lateral branch slanting upward (fig. 17). In one nest under construction this branch ended, and the nest contained no cells. In the other two nests, the branch ended in a small cell cluster. One consisted of a central cell (shaped but not yet waxed) surrounded by five short burrows not yet lined to form cells (fig. 21). The other consisted of a central group with several longer marginal burrows around them curved in under the cells (fig. 17). Each of these nests contained a single, not or scarcely worn, fertilized bee with slightly enlarged ovaries.

The completed cells are shaped as in *A. sparsilis.* Measurements were as follows: depth, 13 to 15 mm.; maximum diameter, 6.8 to 7 mm.; diameter of entrance, 4.4 to 4.5 mm.

E. *AUGOCHLOROPSIS RUFISETIS* (VACHAL)

One nest of this species was found at Guarapuava, Paraná, September 8, 1955, by one of us (Michener) in company with Father J. S. Moure. It was a burrow entering horizontally into a low earth bank

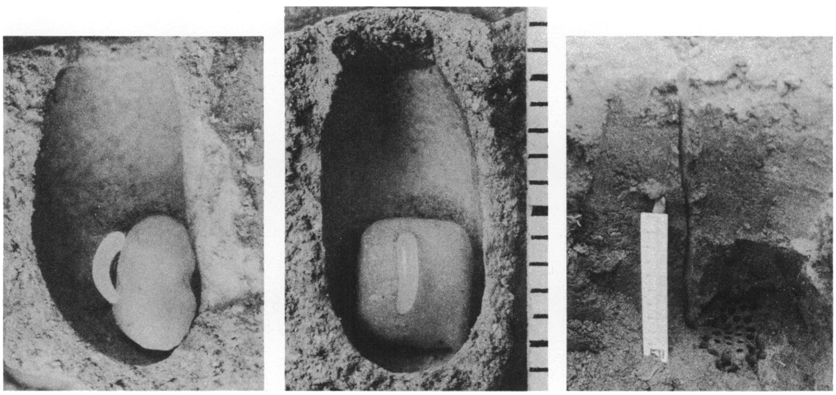


FIG. 20. Nests of *Augochloropsis. Left and center:* Sections of single cells of *A. sparsilis* opened October 16, 1955, showing provisions and eggs. The shape of the pollen mass at the left is unusual, possibly because of damage in opening the cell. The rule on the center photograph, in millimeters, applies also to the left-hand photograph. *Right:* Nest of *A. iris* opened down to the level of the cell cluster. The cell cluster was in winter condition (September 26, 1955). The total height of the rule is about 10.5 cm.

for a distance of about 10 cm. There was a small turret of fresh earth at the entrance. Inside, a cluster of five cells was being constructed. Owing to damage in excavation, the exact details of the cluster cannot be reconstructed, but the cells were close together (walls between them 0.75 to 1.0 mm. thick), and the bee had excavated around the cells so that the cluster was partially free, the earth wall of the cluster being about 2 mm. thick. The situation is probably the same as that described by Smith (1901) for *A. humeralis,* in which the marginal burrows are large, close together, and the female deepens and curves them under the cells until they nearly meet, so that there is an almost continuous space all around the brood cells, only enough earth being left to support them. None of the cells of *rufisetis* was shaped or smoothed on the inside. Figure 19 shows the details of roughening, probably due to mandibular action, on the inner walls.

F. *AUGOCHLOROPSIS HUMERALIS* (PATTON)

Smith (1901) has described the biology of this species. The species is listed here principally in order to call attention to Smith's excellent account, and to provide the species with a letter for comparative purposes in the discussion.

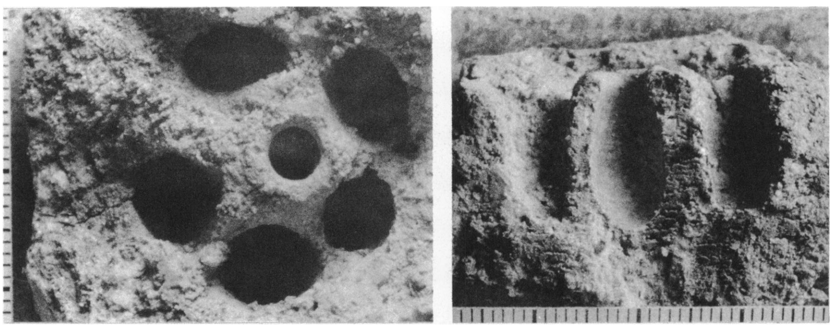


FIG. 21. Cell cluster of *Augochloropsis cleopatra*. *Left:* Top view, with central formed cell surrounded by roughed-out burrows. *Right:* Section of same cell cluster. Scale shown in millimeters.

However, it may be noted here that an isolated nest of this species was opened by A. Wille and H. V. Daly on August 20, 1953. The nest was located on a dry hill of hard stony soil near Lawrence, Kansas. Thus the substrate was entirely different from the sandy soil in which Smith studied the species. The following are points of difference from Smith's account: There was an earthen turret at the entrance nearly 2 cm. high. The total depth was 50 cm. The cells were empty except that one, in one of the two clusters, contained a pollen mass and egg. The pollen mass was rectangular, as in the species described above, and was located as in those species, with the egg placed as in those species. Approximate measurements of the one pollen mass are as follows: 5 mm. high, 4 mm. wide, and 3 mm. thick. The egg was about 3.5 mm. long. Smith's description and figure of rounded pollen masses filling the bottoms of the cells can only be explained as a softening of the pollen masses, so that they slumped down in the cells, as a result of excessive moisture from his plaster of Paris.

DISCUSSION AND CONCLUSIONS

To save space, the species are referred to by the letters A to F, as indicated in the preceding sections.

Seasonal Cycle

All species (species D and E little known) pass the winter only as adult fecundated females (but see *A. brachycephala* Moure; Michener and Seabra, in press). In species F. there is but one generation per year in New Jersey. Limited observations in Kansas (one nest) suggest at least a partial second generation. In A, B, and probably C there are two generations per year. One survives the winter and provisions nests in the spring (called the spring generation in the above account). Another generation emerges from these nests in midsummer and provisions nests. Their progeny emerge in the fall and then hibernate. At least in A some members of the summer generation also survive the winter. In species A and B the overwintering females are all of about the same degree of wear, much-worn individuals disappearing in the fall. In species C much-worn as well as fresh and little-worn females survive the winter.

Nest Structure

Nests of species A, B, D, and E are horizontal or slanting in banks of soil, while those of species C and F are vertical shafts in flat ground. In all species the burrows open into broad, irregularly round horizontal expansions. In C, D, and F these expansions are connected by short lateral burrows to the main burrow; in A some expansions are so connected, while in B the expansions are usually part of the main burrow. In all species (except perhaps E) a burrow (often the main burrow) commonly descends from near one margin of each expansion. The floor of such an expansion is perforated by short vertical burrows. The central one is made first, and followed by others around it in a regular pattern (in D and F) or a somewhat irregular pattern (other species). These short vertical burrows are lined with soil from elsewhere in the nest to form cells. The cells are beautifully smoothed. They are lined with a thin layer of wax. The provisions are formed into a rectangular mass attached to the flatter side of the cell near its lower end, and the egg is placed on the exposed side of the pollen mass. The cell is closed with an earth plug. All these activities start first with a centrally located cell and progress towards the margins of the cell cluster. Marginal burrows that are never formed into cells remain in all species and except in C are often prolonged downward and curved inward beneath the cells. In A cells are used repeatedly, while in other species re-use is not known; in B and F it clearly does not normally occur.

The uniform nest structure here described is in contrast to the varying social levels attained by the species. As similar nest structures among species with different social behavior are also observed in other bees (e.g., *Chloralictus*), it seems probable that evolution in social behavior progresses relatively easily, whereas nest structure is a conservative feature.

Social Behavior

Species A and perhaps F and others seem to have an innate tendency to nest in aggregations. Species B may do so, although isolated nests are also common; the aggregation studied could be explained by edaphic factors. It is suggested by Michener (in press) that aggregations of simple nests are a preliminary step (a) in the direction of complex societies among bees. The next step (b) involves the use of a common burrow by two or more females, each of which makes her own cells and provisions them. The paper cited above suggests reasons for believing that this step is most likely to occur in aggregations of nests rather than in isolated nests, and species B supports this surmise, for nests inhabited by more than one working female were found only in the aggregation, where, probably owing to soil factors, nests were close together. Among rather numerous isolated nests, none was found to contain more than one female. Species F also probably exhibits this kind of organization, in which some but not all of the nests are inhabited by more than one female.

Several females may pass the winter together in most or all of the species. At least in A and B this association is probably usually a matter of their remaining for the winter in the nests in which they were born; F constructs special overwintering burrows. At least in A and F there is good reason to believe that the females working in a common nest are not necessarily sisters. Apparently a tendency exists for females to enter nests already started and to establish themselves there.

Species A, unlike others that have been sufficiently studied (B and F), exhibits a further step (c) in social evolution. Division of labor and cooperation among several females working in a nest occur, as previously shown (Michener and Lange, 1958c). Lone females in nests become both pollen collectors and egg layers, i.e., they function as any solitary bee. However, most nests contain more than one female. Among such individuals there exists division of labor. Some, the ovaries of which remain slender, do most or all of the pollen collecting and wear out and die without laying eggs. Others, usually not over one per nest or per cell cluster, develop enlarged ovaries and presumably lay most or all of the eggs. When there are two or three pollen collectors at the same time in a nest, they cooperate in provisioning cells and probably also in other activities, so that ordinarily but one cell is being provisioned at any one time.

In species A a very small percentage of the spring generation and perhaps 10 to 15 per cent of the females of the summer generation remain unmated. Probably all such bees become pollen collectors. They may become more worn (both wings and mandibles) than any fertilized bees of the same age.

It is easy to see that the presence of such active unfertilized bees may be an advantage to a colony, even though they are non-reproductive. We have suggested elsewhere that the habit of extensive work on the part of unmated females served as a preadaptation that permitted selection for the regular occurrence of such individuals (workers), thus providing for the establishment of the female castes.

APPENDIX

This seems to be the appropriate place to publish a note on the nests of *Augochloropsis ignita* (Smith). The observations were made in Costa Rica by Howell V. Daly and Alvaro Wille, both of the University of Kansas, March 3 to 8, 1954, and we wish to thank these observers for the data here presented.

Five nests were found in the vertical bank of sandy alluvium along the Rio Tempisque, 7 miles south west of Filadelfia, Provincia de Guanacaste, Costa Rica. The bank faced northward. The nests contained from one to three cell clusters each. Four of the nests contained only one or two female bees, but the fifth contained five. Bees with much-worn mandibles as well as unworn ones and all intermediates were present. In no nest was there more than one bee with sperm cells in the spermatheca, and among the bees in a nest, this was always the one with the most enlarged ovaries and most mandibular wear. The other individuals were generally not, or little, worn, although in one case a bee with well-worn mandibles and slender ovaries was in a nest with a bee having much-worn mandibles and swollen ovaries.

This evidence from only five nests means little, but at least it is clear that more than one female may occupy a nest. As a number of them were unfertilized, unmated workers or worker-like bees may occur as a rule in this species. This surmise is supported by size differences which probably exist: the three "queens" with strongly swollen ovaries, sperm cells in the spermathecae, and more worn mandibles than others in their nests had forewings averaging 5.83 mm. long (extremes, 5.60 and 5.95 mm.). Four unfertilized, slightly worn "worker" bees with slender ovaries had forewings averaging 5.37 mm. long (extremes, 4.90 and 5.60 mm.). Unworn females were excluded from consideration, as they might develop into either queens or workers. It must be remembered that this interpretation is rather hypothetical, but, if true, the social relationships in this species of *Augochloropsis* are different from those of the species discussed in the body of this paper.

The nests, to judge by the notes and diagrams provided by Wille and Daly, are essentially horizontal or somewhat slanting, extending from 35 to 93 cm. into the bank. The burrows are about 4 mm. in diameter at the entrances. On the main burrow or usually at the end of short side branches are one to three cell clusters. As in most other species, the cells are vertical and excavated into the floors of broad flat chambers, similar to those of *A. sparsilis*. The number of cells per cluster, in 10 clusters, varied from four to 20, with an average of 8.5. In the one large cluster (20 cells) there were additional marginal burrows curving under the cells, just as often occurs with *A. sparsilis* and others. These burrows were separated from the lower ends of the cells by about 3 mm. of soil. Cells were about 8 mm. deep, 5 mm. in maximum diameter, with the entrances about 2.5 mm. in width. The position and shape of the pollen masses and eggs were as in other species of *Augochloropsis*. The pollen masses were about 4 mm. high, 3 mm. wide, and 2 mm. thick; the eggs were about 2 mm. long.

Immature individuals of all stages were present in the five nests. In any one cluster, all immature stages were of about the same age. For example, one cluster had only eggs and young larvae; another, only prepupae and pupae. This situation is in contrast to that of *A. sparsilis*, in which all stages are often found in a single cell cluster, and suggests rapid provisioning and egg laying in all the cells in a cluster, which may be possible because in *A. ignita* there may be differentiated workers which can provision numerous cells in a short time.

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