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The biology of a subtropical population of *Halictus ligatus* Say (Hymenoptera: Halictidae)

I. Phenology and social organisation

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Summary. A large population of *Halictus ligatus* was studied in the subtropical climate of Knights Key, Monroe County, Florida. The dissection of 858 female bees caught on flowers and 420 bees from completely excavated nests gives the following picture of phenology, colony development and social organisation. In the Florida keys, *H. ligatus* is continuously brooded and multivoltine. However, towards the coldest time of year young gynes may rest in their natal nests rather than found a new colony. This may result in a partial synchronisation of nest initiation when warm weather returns after a particularly cold spell. Most nests are started by a single foundress that usually survives until near the end of the production of reproductives. The first brood is very variable in size and males average 11% of the bees produced at this stage. This figure increases to 56% when the first brood workers begin provisioning. Queens are produced some time after the rise in male production and colony longevity is extended by the presence of some worker brood during this phase. Queens average 16% larger than their workers but appear to exert little inhibition of worker reproductivity: 57% of worker bees mate and 68% show ovarian development. This population is unique amongst social halictines in being continuously brooded, multivoltine and in having such weak physiological caste differentiation. It seems to represent an intermediate stage between the primitively eusocial colonies of *H. ligatus* found in temperate regions and the communal-like ones of the tropics.

Introduction

From Lake Superior in the north to the tropical climes of Colombia in the south, *Halictus ligatus* spans a wider range of latitude than any other primitively eusocial insect. No other social insect species has populations adapted to such a diversity of climates. Therefore, this species is an excellent candidate for studies of intraspecific variation in social organisation. Such studies are important because they can show how various social parameters vary under a wide range of environmental conditions. Furthermore, a study of these ecological-behavioural variants could throw light upon the origin and further elaboration of social behaviour.

Field studies conducted in Indiana (Chandler 1955), Ontario (Knerer 1965, 1980; Knerer and Plateaux-Quénu 1966; Packer, in preparation) and New York (Litte 1977) indicate that in north temperate regions, *H. ligatus* has an annual colony cycle similar to that found in many primitively eusocial halictines. However, Michener and Bennett (1977) report that in the tropics, *H. ligatus* has communal-like colonies which "do not correspond to any of the named types of social organisations". This conclusion was based upon monthly samples of bees from Trinidad and the contents of eleven nests from Trinidad, Costa Rica and Colombia. In discussing the scanty data from the warm temperate climates of Arizona and southern Florida these authors conclude that in these regions "colonies like those of the tropics develop".

This paper is an account of our investigations into the phenology and social organisation of *H. ligatus* in the sub-tropical climate of the Florida keys. This area was chosen because it is the southernmost part of eastern North America and has a climate allowing bee activity throughout the year. Furthermore, it is known that *H. ligatus* is active throughout the year in the extreme south of Florida (Kirkton 1968; Atwood, personal communication). Analyses of variation in reproductive success, male behaviour, associated organisms and the existence of a cuckoo-like sub-caste at this locality will be presented elsewhere.

Methods

A large population of *Halictus ligatus* was discovered at the Knights Key camp ground, Knights Key, Monroe County, Florida – 24.42°N 81.24°W, in February 1981. Collections of bees from flowers were made in the following months: February and November 1981, February, March, April, June, October, November and December 1982 and February 1984. Sampling was carried out between 11:00 am and 12:30 pm and only bees that were seen on the flowers of *Bidens pilosa* over a restricted portion of the study site were captured and preserved in Kahle's solution.

Nests were excavated during November 1981; January, March, April, November and December 1982 and February 1984. Most nests were observed for at least several days before excavation. Foragers were marked with Testors PLA enamel paint so that individual bees could be recognised and their activity patterns recorded. Other nests were excavated soon after their initial discovery. Paprika was blown down the entrances to make the burrows easier to trace and the nests were excavated from an adjacent trench, which was deepened as excavation continued. Drawings of the nests were made and their contents preserved in Kahle's solution.

The head widths and wing lengths of female bees collected from flowers and excavated from nests were measured and the number of nicks in the wing margin and the relative amount of mandibular wear were recorded. An index of total wear was obtained by adding the number of nicks in the wing to the score for mandibular wear. However, some bees had their complete wing margins frayed making it impossible to count nicks. It was estimated that a wing could sustain about 14 nicks before the entire margin was eroded. Therefore, bees which had the edge of the wings frayed were given a score of 15 and those with the wing worn away to the apex of the radial cell a score of 20. The bees were dissected to assess ovarian development and to see if they had mated. Ovarian development was assessed as by Litte (1977) and Michener and Bennett (1977): bees with thread-like ovaries were assigned to ovarian class D, those with one or two enlarged ovarioles class C, three or four developed ovarioles class B and five or six developed ovarioles class A.

Male and female pupae could be distinguished by the longer antennae and smaller heads of the former. Pupae were aged by the sequence of colour changes, first of the compound eyes and then of the general body pigmentation. Pupal head widths provide an accurate indicator of the head width of the adult (Packer, unpublished).

We use the terms queen and worker in our analysis despite the fact that most of the worker bees were mated and many of them appeared to have the ability to lay eggs. We feel justified in this usage because most of the nests contained one female which was larger and had better developed ovaries than the other females in the nest. These large bees also appeared to be older than the others. Their greater age was not readily apparent from the indices of wear because worker bees will forage until they die and the foundresses forage only until the first brood is complete. Rather, their greater age was shown by the almost complete lack of thoracic and abdominal pile in individuals from nests which were at a later stage of colony development. This wear is presumably the result of intranidal locomotion, particularly movements in and out of the brood cells which have comparatively very narrow necks. In most cases these bees were almost certainly the nest foundresses. Furthermore, most nests lacking a queen did contain one bee which seemed to have become dominant over the rest. Such replacement queens are not uncommon in primitively eusocial halictines. We will return to the problems of the applicability of this terminology later.

Nests were classified according to their stage in colony development:

Stage 1: nests containing a foundress and juveniles and/or adults of her first brood, i.e. all adults in the nest except the foundress(es) have resulted from the foraging activities of the foundress(es).

Stage 2: nests with more than first brood workers (i.e. some adult workers have been produced from pollen balls provisioned by earlier emerging workers), but before queen production could be detected – as shown by the absence of any female pupae similar in size to the foundress in the nest.

Stage 3: nests with some pupae that are of similar size to the queen in the nest (or, if the nest has become queenless, some pupae are much larger than the worker bees and replacement queen), but no adult, young, queen-sized bees are present.

Stage 4: nests with a much reduced worker population and with several new queens leaving the nest or "resting" within it.

Queenless nests: nests in which the queen had disappeared. Because the majority of these nests appeared to contain first brood offspring it is likely that most were orphaned as a result of the foundress being caught in the monthly collections.

Clearly, this is a partially artificial categorisation of a continuous process. Nonetheless, it was possible to place all completely excavated nests into one of the above stages with little doubt. The categorisation of female pupae as queen or worker sized presented some problems, however, most stage three nests showed a bimodal size distribution of female pupae, some being approximately the same size as the adult workers, others being similar in size to the queen (nest 132E, Fig. 3 is an example). Others (such as nest O, Fig. 3) showed a wide range of pupal sizes, with intermediates between queen and worker nestmates. In both cases, it is unlikely that the large pupae would have produced workers, as very few workers approached their queen in size (Fig. 4).

Not one callow queen-sized female was found in any of the stage 3 nests, furthermore, large female pupae which were about to emerge were also usually lacking from these nests. Thus, the possibility that some stage 4 nests were accidentally classified as stage 3 seems unlikely.

Many nests were incompletely excavated as a result of difficulties in following a 6 mm wide burrow through several feet of pulverised coral; these nests were not included in the analyses except where stated.

Results

Monthly samples of bees from flowers

Monthly variation in head width, ovarian development, proportion of females mated and total wear are shown in Fig. 1. Analysis of variance of monthly mean head widths reveals that bees in the February 1981 sample are significantly larger than those of November 1981 and June and October 1982 and those caught in December 1982 are larger than those of October 1982, all differences being significant at the 0.05 level. Monthly variation in mating frequency, ovarian development and age was tested by means of the *G*-test for homogeneity of replicates (Sokal and Rohlf 1981). The variables tested were the frequency of unmated bees, the proportion of bees with no ovarian development

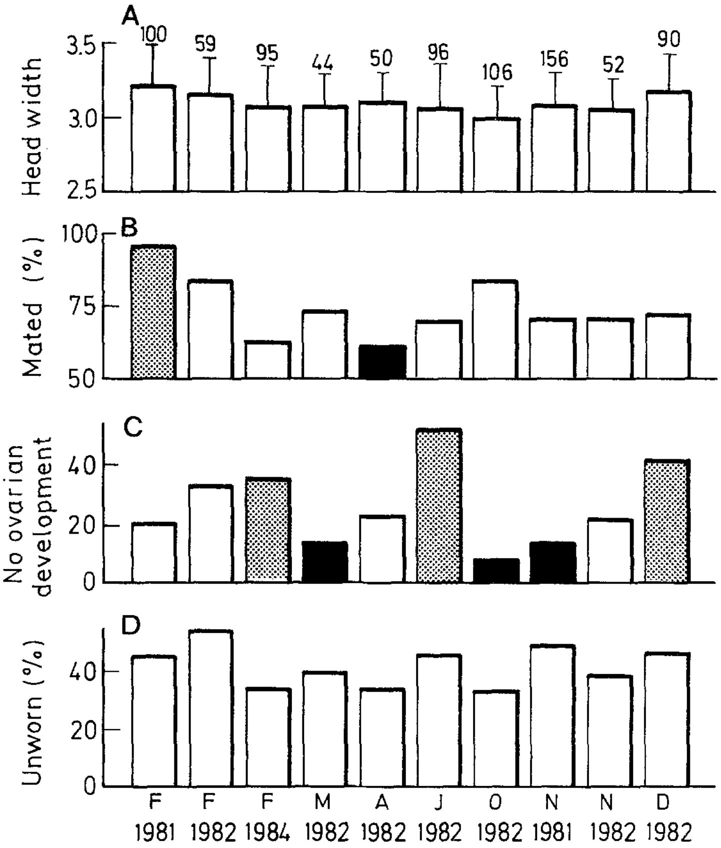

Fig. 1A–D. Monthly samples of female *H. ligatus* collected from flowers at Knights Key Florida. **A** Mean and standard deviation in head width, the numbers refer to the sample sizes. **B** Percent of bees that were inseminated. The dotted and shaded bars represent the highest and lowest frequencies respectively, these cause the significant heterogeneity in the samples as a whole as revealed by a *G*-test ($P < 0.05$). **C** Percentage of bees with no ovarian development (ovarian class D). The dotted and shaded bars represent the samples that cause heterogeneity in the collection as a whole ($P < 0.05$). **D** Percent of bees with no wing or mandibular wear. This variable shows no significant heterogeneity amongst the monthly samples

(Class D) and the frequency of bees with no wing or mandibular wear. As can be seen from Fig. 1 there is no significant heterogeneity amongst the samples for the proportion of unworn bees but there is significant variation in the frequency of unmated and ovarially undeveloped bees. Removal of either of the extreme values for the proportion of unmated bees causes the samples as a whole to become statistically homogeneous. Thus, heterogeneity in the samples as a whole is caused by the high frequency of mating in the February 1981 bees and a low frequency in those of April 1982. For lack of ovarian development it is the highest or lowest three values that cause the heterogeneity; the highest three were June and December 1982 and February 1984, the lowest were November 1981 and March and October 1982.

The February 1981 sample has the largest mean head width and the highest frequency of mated bees. This is consistent with the hypothesis that there are more queen-like bees in this sample than the others. We shall return to the unusual nature of the February 1981 sample later. With this exception, there is little evidence for any meaningful seasonal variation amongst these samples.

The size distribution of all females caught on flowers is shown in Fig. 2. A distinct second mode of 3.6 mm can be seen. This suggests that these larger bees may be foraging gynes. This interpretation is supported by the congruence of this part of the histogram with the size distribution of queens excavated from nests (Fig. 2). These large foraging bees were found in all of the monthly samples.

Colony development

Young gynes have been observed carefully examining suitable nesting substrate every month from October to April (no observations which would detect such behaviour were made in June 1982). These searching gynes were not significantly different in size from queens excavated from nests with brood (searching young gynes – mean head width $= 3.74$ mm, $\mathrm{SD} = 0.15$, $n = 11$; queens from nests – mean $= 3.64$ mm, $\mathrm{SD} = 0.20$, $n = 40$; $t = 1.78$, $df = 59$, $P > 0.05$). As is to be expected of gynes that have yet to excavate a nest, none of the searching females had any ovarian development; however, four out of the eleven had not mated.

Colonies pass through four stages as indicated above. Of the 56 completely excavated nests, 18 (32%) were in stage 1; containing the foundress

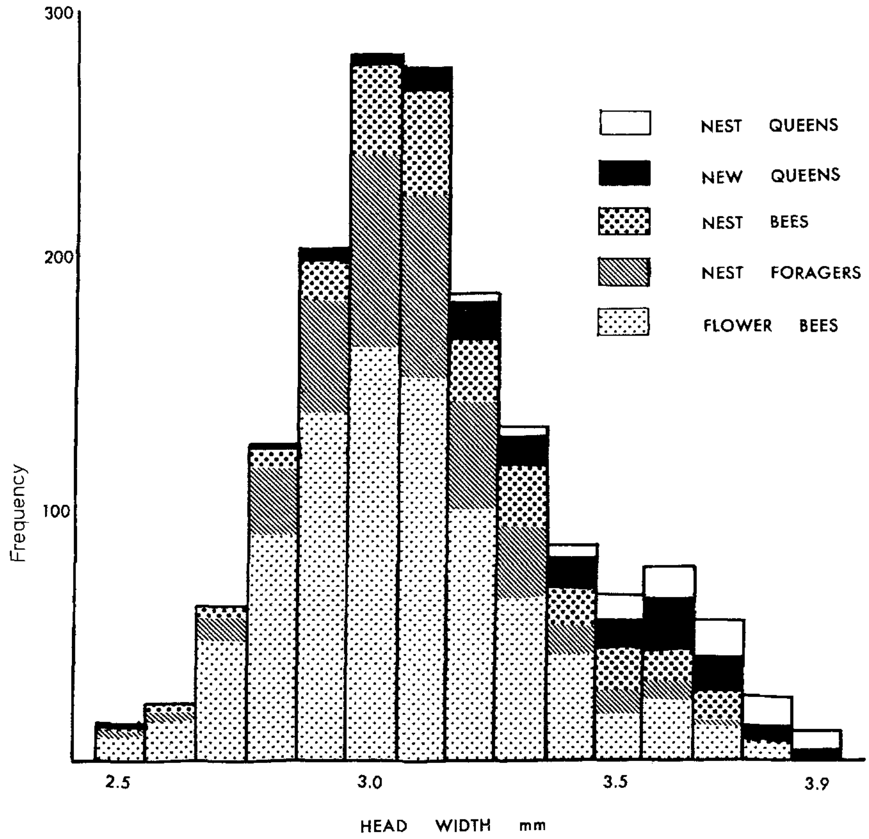

Fig. 2. Histogram showing the head width frequencies of female bees from five different categories. As can be seen, the majority of bees with head widths in excess of 3.5 mm and above probably represent queens

and her first brood in various stages of development. The number of offspring produced in this first brood ranged from 2 to 12 (mean = 6.83, SD = 3.29, $n = 12$; in six nests the gyne was still provisioning). The one pleometrotic (multi-foundress) nest that was excavated contained, in addition to the queen and auxiliary, four young adult workers plus 12 pupae that probably resulted from the activities of the auxiliary, thus giving a first brood size of at least 16 individuals. One solitary foundress had produced 11 offspring and was still provisioning at the time of excavation.

11% of the first brood consisted of males. The sex and relative age of all pupae could be established in three nests. In each of them there were female pupae both older and younger than the male pupae. In one case two male pupae were much older than the youngest female pupae. Even allowing for a faster developmental rate for the smaller males, it is unlikely that the sequence of pupal ages observed in this nest was the result of the haploid eggs being laid last. Fig. 3 shows some examples of nests of all stages.

It is clear that more than one worker brood is interpolated between nest founding and the production of new gynes. Evidence for this comes from the great diversity of age of foragers, the duration of worker activity in some marked nests and the absence of large queen-sized pupae in nests with many small female pupae and a large, active and ageing worker population. Seven of these stage 2 nests were completely excavated, making 12.5% of the total. Although the maximum number of workers found in these nests was 19, an incompletely excavated nest contained 19 foragers and approximately 25 adult workers in total. This is probably close to the maximum colony size reached by *H. ligatus* at this locality.

Nests in which queen-sized pupae were found often contained a wide range of female pupal sizes. This indicates that queen and worker production can occur simultaneously for at least some time. Two of the eleven stage 3 nests had no female pupae that were of worker size. Of the remaining nine nests, six had at least one worker-sized pupa that was as young or younger than the youngest queen-sized pupa. In ten nests there was at least one male pupa that was less well developed than the youngest queen-sized female pupa, indicating that male production continues well into the queen production phase. One stage 4 nest contained a worker that was fractionally larger than the queen

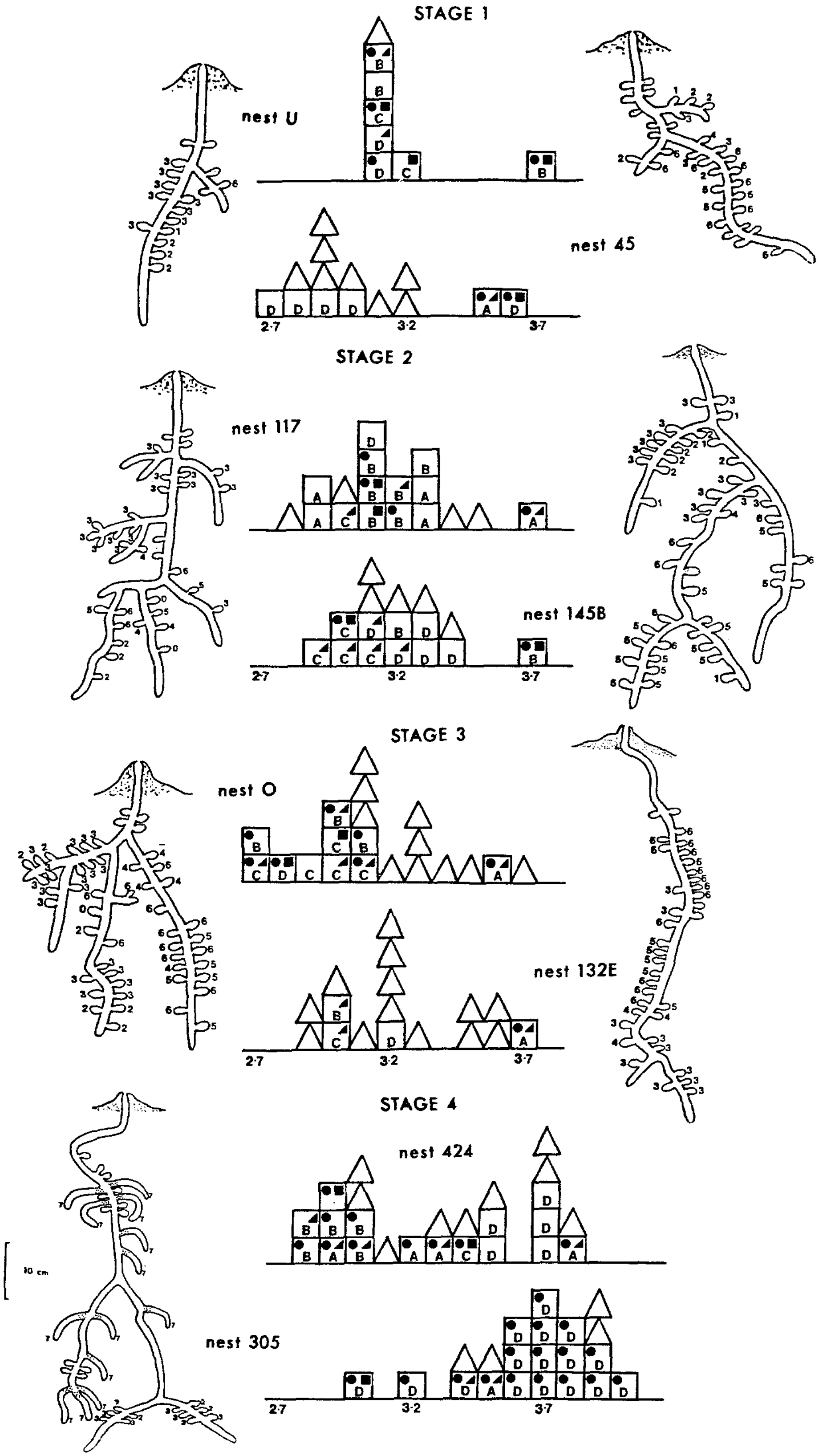

Fig. 3. Examples of nests from different stages in the colony cycle of *H. ligatus* at Knights Key. The symbols in the histograms are as follows – the large squares indicate the head width of one female, the large triangles represent the head width of a female pupa, the letter is the ovarian class of the bee, a solid circle represents an inseminated individual, a small triangle represents a female with some wing or mandibular wear, a square represents a female with a considerable amount of wear, the lack of either of the last two symbols represents a fresh-unworn individual. The numbers on the nest drawings refer to different developmental stages within the cells, thus: 1 = an unfinished pollen ball; 2 = a pollen ball with or without an egg; 3 = a larva; 4 = a prepupa; 5 = a male pupa; 6 = a female pupa; 7 = a young gyne "resting" within its natal nest. The dotted areas in these diagrams represent excavated material, which is expelled at the entrance to form a tumulus, pushed into vacated cells or separating the "resting" burrow of the young gynes from the main burrow. The number of filled in cells in the diagrams is not likely to be accurate

and as a pupa would have been classified as queen-sized. Stage 3 nests make up 20% of those that were successfully excavated.

Stage 4 nests are those in which the number of workers is greatly diminished and gynes are emerging in large numbers. Ten such nests were excavated making 18% of the total. Half of these nests contained no dominant queen. These five nests are considered here and not among the queenless nests because the death of the foundress towards the end of colony life is probably normal. In November through to February, stage 4 nests often contained mated, young gynes with large amounts of nectar in their crops. These females were located in short laterals which were sealed from the main branches of the nest by thin plugs of soil (Fig. 3, nest 305). Upon excavation, these young gynes proved to be quite active and were capable of flight. Thus they are not in a true hibernal diapause but merely "resting" in their natal nest. The few stage 4 nests excavated in March and April did not contain "resting" young gynes.

Some stage 4 nests were observed all day, for several consecutive days. Large, fresh, young gynes were observed to leave in the late morning or afternoon. Usually, they did not return and, presumably, they initiated their own nests. Young gynes that did try to return to their natal nest were repulsed by the few remaining worker bees whose foraging activities were greatly reduced by these interactions. The break up of stage 4 nests following the emergence of new gynes is facilitated by the behaviour of these remaining workers. However, it is possible that one or more young gynes successfully take over some old nests. It is probable that the pleometrotic association was established when two females remained in their natal nest.

Young gynes in stage 4 nests are significantly smaller in size than established queens with brood (mean head width young gynes $= 3.44$ mm, SD $= 0.26$, $n = 121$ established queens, mean $= 3.63$ mm, SD $= 0.19$, $n = 50$; $t = 4.60$, $df = 169$, $P < 0.001$) and significantly larger than worker bees in nests (mean size workers $= 3.11$ mm, SD $= 0.21$, $n = 228$, $t = 16.12$, $df = 276$, $P < 0.001$) (Fig. 2). As is to be expected, none of these young gynes showed any ovarian development or any measurable wing or mandibular wear; however, 12% of them were not mated. These young gynes proved to be extremely attractive to males: forceps used to remove these females from the nest would still attract males several minutes later.

No foundress-like bees were found in 10 of the completely excavated nests. In all but one of these queenless nests, one bee clearly had more ovarian development than the others and was considered to be a "replacement queen". In the remaining nest one bee did have marginally more ovarian development than the others and was also considered a replacement queen. These replacement queens are significantly smaller than foundresses from queenright colonies (mean head width of replacement queens $= 3.16$ mm, SD $= 0.22$, $n = 10$, $t = 2.8$, $P < 0.01$), but there is no significant difference in size between the replacement queens and their adult female nest-mates (mean head width workers $= 3.07$ mm, SD $= 0.27$, $n = 36$, $t = 0.467$, $P > 0.5$). In two nests, the replacement queen was the largest bee and in one she was the smallest of six individuals. In two nests she was the most worn bee (oldest) but in three had no wing or mandibular wear. Three of the replacement queens were unmated. All but two of these nests were equivalent to stage 1 nests, one was stage 2 and one stage 3. The preponderance of stage 1 nests suggests that many of their foundresses had died during foraging activity. It is likely that some of them had been caught during the monthly samples – most queenless nests were excavated at those times when monthly samples had been made comparatively recently.

Summary statistics for nests of the four different stages and for queenless nests are given in Table 1.

Table 2 shows the frequency of nests at different stages of colony development for the five months for which adequate sample sizes were obtained. Although some monthly variation may be apparent from the data (particularly the absence of early stages in the December sample), it seems likely that larger samples would show the simultaneous presence of all stages of colony development throughout the year.

No one nest was observed throughout the entire colony cycle. However, transitions from one stage of colony development to another were observed in the field, particularly during the period February to April 1982. From these observations, plus a consideration of the general pattern of colony development, it appears that the colony cycle rarely exceeds four months.

In six nests, the mandibular wear of the bee that appeared to be the queen was lower than is to be expected in a bee that had excavated a nest. Two of these females were dominant in stage 4 nests and it is possible that they had taken over after the death of the original foundress rather than initiating their own nest. Three of the four remaining nests with comparatively "young" gynes were substantially more productive than

Table 1. Summary statistics for nests of different stages of colony development and for queenless nests. Means above, standard deviations below

Stage	*n*	Queen size	Worker number	Worker size	% Size difference	No. of larvae	No. of pupae	No. of queen pupae	No. of male pupae	Pupa size	Queen wear	Worker wear
1	18	3.57	2.50	3.06	15.86	5.39	1.17	0.00	0.55	3.04	8.65	3.13
		0.25	2.58	0.17	5.47	4.95	1.95	0.00	0.98	0.13	6.54	6.20
2	7	3.71	11.57	3.10	17.87	19.20	6.00	0.00	7.60	3.07	6.00	3.72
		0.19	6.02	0.22	2.79	7.92	2.55	0.00	2.88	0.27	4.47	7.00
3	11	3.68	7.18	3.08	16.77	14.50	4.75	2.87	6.62	3.41	8.30	4.32
		0.14	3.63	0.18	3.57	7.91	2.82	1.96	2.61	0.25	6.21	6.62
4	12	3.66	3.50	3.21	10.14	4.11	1.00	2.67	2.89	3.43	4.60	4.46
		0.10	2.54	0.26	6.49	3.55	1.94	3.50	5.51	0.29	5.03	6.31
5[a]	10	3.16	5.44	3.08	2.41	12.00	2.44	0.11	3.00	3.05	3.67	2.02
		0.12	1.50	0.12	3.96	7.04	3.20	0.31	6.80	0.22	1.31	4.05

[a] Queenless nests

Table 2. Monthly variation in frequency of nests in different stages of colony development. Figures in parentheses indicate estimated stages of the colony cycle for the queenless nests that are otherwise excluded from the Table

Stage	February 84	March 82	April 82	November 82	December 82	Total
1	2	8(1)	8(7)	4	0	22(30)
2	1	4(1)	4	1	1	11(12)
3	0	3	8(1)	0	1	12(13)
4	5	0	4	0	5	13

nests at a similar stage of colony development. This is consistent with the hypothesis that these nests had been pleometrotic earlier in the colony cycle. The remaining nest showed no such enhanced productivity. Thus, the possibility that the foundress in some nests had been usurped after nest establishment cannot be excluded.

Social organisation

Firstly, it is necessary to demonstrate that *H. ligatus* colonies at Knights Key really are matrifilial and that discrete castes are discernible. Towards the end of most nest excavations a large bee that was almost devoid of pilosity was discovered at the bottom of the burrow. Invariably this bee was larger and had better developed ovaries than its nest-mates. This bee was considered to be the queen. Figure 4 indicates that this assessment is correct. As can be seen, only one worker overlaps in size with its queen (the auxiliary from the pleometrotic nest was actually slightly larger than the queen but these two were probably sisters not mother and daughter as is the case with all of the bees considered here). This unusually large worker was found in a stage 4 nest where large queen-sized bees are to be expected. Queens in half of the stage 4 nests had disappeared before the time of excavation and it is possible that this large bee had been recruited into the work force by an unusually resilient queen. Ten bees showed a total ovarian development equal to or greater than their queen. All of these bees were from the same three stage 3 nests. Because most queens appear to die between nest stages 3 and 4, it is possible that those in these three nests were losing what little dominance they had; they may have been close to death.

Non-foundress bees in nests were divisible into three categories: young gynes, foraging workers and nest workers. The distinctions between young gynes and workers have been made above; foraging workers and nest workers were separated on the basis of pre-excavation observations. Summary statistics for all four categories of bees found in nests and searching foundresses are shown in Table 3.

A detailed treatment of variation in female reproductivity will be presented elsewhere; here we restrict ourselves to analyses similar to those of Michener and Bennett (1977) to facilitate comparison of the tropical and subtropical populations of *H. ligatus*.

Foraging workers are significantly larger than nest workers ($t = 3.14$, $P < 0.005$) (Fig. 2), have a higher index of total wear ($t = 4.05$, $P < 0.0001$), are more likely to have an oocyte ready to lay ($\chi^2 = 6.19$, $P < 0.05$), but no more likely to have mated ($\chi^2 = 0.25$, $P > 0.05$). However, because some nest workers are likely to be young bees that have not yet left the nest, these analyses were repeated with unworn bees (those with an index of wear of zero) excluded from the samples: the dif-

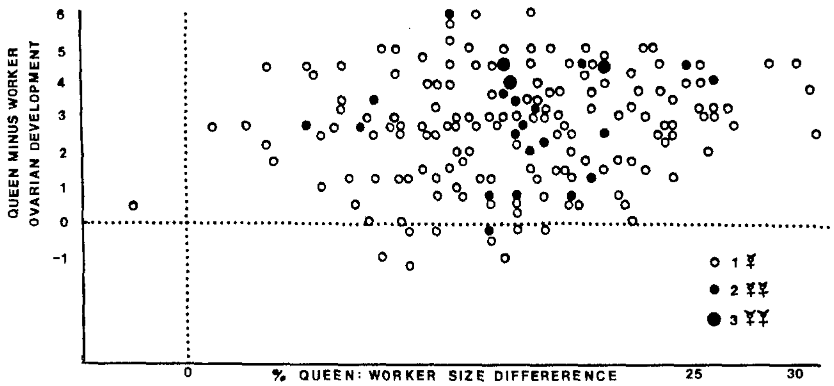

Fig. 4. Caste differentiation in *H. ligatus*. The horizontal axis is the percentage size difference between an individual worker and its respective queen. The vertical axis is the difference in ovarian development between the worker and its queen. This last variable was assessed in the following manner. The sum total of fractions of developing oocytes were summed for all six ovarioles in each bee. The score on the vertical axis represents the score for the queen minus that of the worker. Thus the vertical dotted line separates workers that are larger than their queen (on the left) from those that are smaller. The horizontal dotted line separates the workers with more ovarian development than the queen (below the line) from those with less

Table 3. Summary statistics for bees of different categories (see text for further explanation of categories). Means are shown above, standard deviations below. Numbers in parentheses are the ovarian categories expressed as percentages

Bee status	*n*	Head width	Total wear	Mated (%)	A	B	C	D
Nest queens	40	3.64 0.20	7.56 5.94	100	25(62.5)	11(27.5)	4(10.0)	0(0.0)
New queens	105	3.45 0.27	0 0.0	85.7	0(0.0)	0(0.0)	0(0.0)	105(100.0)
Searching queens	11	3.74 0.15	1.45 2.16	63.6	0(0.0)	0(0.0)	0(0.0)	11(100.0)
Foraging workers	171	3.08 0.20	4.76 7.30	50.3	14(8.7)	48(29.8)	60(37.2)	39(24.0)
Nest workers	64	3.18 0.22	1.80 3.67	53.2	1(1.6)	13(20.3)	17(26.6)	33(51.0)

ferences in size and index of wear between foraging and nest workers remained ($t = 2.21$, $P < 0.05$ and $t = 3.2$, $P < 0.005$ respectively) and the association between foraging and mating stayed non-significant ($\chi^2 = 0.43$, $P > 0.5$). However, worn foraging bees were no more likely than worn non-foragers to have a fully developed oocyte ($\chi^2 = 1.49$, $P > 0.1$). Thus, the significant association between foraging and presence of a complete oocyte is probably a result of a higher proportion of the nest workers being too young to have developed one.

Perhaps the most remarkable fact about the social organisation of this population is the high degree of reproductivity exhibited by the worker bees within the matrifilial colonies. 51.6% of all workers were mated and 68% had some ovarian development. 6.7% had ovaries in class A, 27.1% class B, 34.2% class C and 32.0% class D. There are no significant differences in ovarian development between workers in nests of different stages ($\chi^2 = 6.41$, $P > 0.1$).

Workers with a mature oocyte are no different in size or age from workers with less or undeveloped ovaries (Table 4). Mated workers are significantly larger but no more worn and no more likely to contain a developed oocyte than unmated ones (Table 4).

Workers from the 8 out of 10 queenless nests that represented stage 1 in colony development were compared with workers from queenright nests of the same stage. With the replacement queen removed from the queenless nest sample the following results were obtained: there is no significant difference in the frequency of worker mating, size or index of wear between workers in queenright and queenless nests ($\chi^2 = 0.32$, $P > 0.5$; $t = 2.39$,

Table 4. Summary statistics for workers with or without a developed oocyte and for inseminated versus uninseminated individuals. Figures in brackets are standard deviations

	Complete oocyte		Insemination	
	Present	Absent	Mated	Unmated
Head width (mm)	3.13	3.10	3.15	3.07
	(0.20)	(0.21)	(0.22)	(0.19)
	$t=0.79$, $P>0.1$		$t=2.82$, $P<0.005$	
Wear	2.85	4.15	4.54	3.26
	(5.30)	(6.84)	(6.89)	(6.29)
	$t=1.15$, $P>0.1$		$t=1.49$, $P>0.1$	
Mated	25	87		
Unmated	15	99		
	$\chi^2=3.28$, $P>0.05$			

$P>0.5$; $t=1.35$, $P>0.1$ respectively). Similarly, there is no significant difference between the two types of nest with respect to ovarian development ($\chi^2=4.67$, $P>0.05$). However, if the replacement queens are included in the analysis, then females in queenless nests show significantly more ovarian development than those in queenright colonies ($\chi^2=10.69$, $P<0.005$). This indicates that the queens do have some inhibiting effect upon worker ovarian development and that replacement queens were able to continue this inhibition. But, increased competition for oviposition is indicated in queenless colonies by the significant increase in frequency of workers that contain a complete oocyte when replacement queens are removed from the sample ($\chi^2=5.09$, $P<0.05$). The continued presence of the foundress makes no difference to the proportion of workers that are active foragers ($\chi^2=3.07$, $P>0.05$).

Discussion

Seasonal variation

In north temperate regions, the annual colony cycle exhibited by *H. ligatus* results in marked changes in the size and reproductive condition of females caught at different times of year. Thus, in spring, all females are mated and most have ovarian development whereas summer workers are usually unmated and show little ovarian development (Litte 1977; Knerer 1980; Packer in preparation). Summer workers are smaller than the queens and although the mean size difference between queens and workers varies between localities it is always significant (Michener and Bennett 1977). The index of wear also varies during the season: gynes during the nest founding stage are unworn but gradually become more worn as the spring provisioning phase progresses. Auxiliaries in pleometrotic nests usually continue foraging until they are very worn. With the emergence of the workers, the mean age of bees caught on the wing decreases dramatically and then gradually increases again as these individuals get older. Towards the end of summer next year's foundresses emerge to mate with the males, these gynes are larger and fresher than the workers and have no ovarian development.

No variation in female size, ovarian development, frequency of insemination or age similar to that shown during the flight season in north temperate regions was observable in the bees caught on flowers at Knights Key. Large queen-sized bees were caught in each of the monthly samples from Knights Key, foundresses were seen looking for suitable nest sites every month that detailed field work was done and males were always abundant. Data from excavations indicate that nests of all stages of colony development could be found at any time of year.

Colony development

Nest excavation data provide the following picture of colony development in this subtropical population. Most, but not all, colonies are begun by a solitary foundress. These lone females provision from two to twelve cells and lay mostly female-producing eggs such that 11% of the first brood is composed of males in these stage 1 nests. There is no perceptibly non-random sequence to the laying of haploid and diploid eggs. Workers average 16% smaller than their mothers. Once provisioning of the first brood is complete the nest entrance is plugged until the first offspring emerge, although in pleometric nests activity may be continuous as is the case in multi-foundress nests in north temperate regions (Litte 1977; Knerer 1980). The worker offspring enlarge the nest and provision cells which produce both more workers and an augmented proportion of males. Fifty-six percent of the offspring produced during stage 2 of colony development are males and the worker population may increase up to a maximum of about 25 individuals. The maximum colony size attained seems to vary greatly among nests. Stage 3 nests are those in which queen production has begun, this occurring after the great increase in male production. At this time the nests are at the peak of colony development. Colonies do not switch from worker and male production to queen and male production. Rather, worker and male production continue well into the queen production phase. This "graded control" increases the length of colony life. These late workers aid in the survival and dispersal of the female reproductives as a result of their defensive behaviour at the nest entrances, which includes repulsing returning young gynes. The great majority of foundresses survive well into the phase of reproductive production.

New foundresses may leave the stage 4 nest and begin looking for a suitable nest site soon after they emerge from their natal cells. However, in the majority of nests that are producing female reproductives towards the coldest months of the year (January and February), these young gynes return to their natal nests. Most of these females are mated, their crops full of nectar and they remain in short laterals to the main nest burrow. The length of time these females spend resting in their natal nest probably depends upon the extent and intensity of inclement weather, but it is unlikely that this period ever exceeds six weeks. A comparison of the February 1981 sample of flower caught bees with the other samples supports this hypothesis. The mean size of females in the February 1981 sample is significantly larger than those caught in November 1981 and June and October 1982 and it has the highest proportion of mated bees. The large mean size and high rate of insemination of bees in this sample is probably a result of there being more foundresses in this month's collection than in any other. The unusually cold weather in the two months leading up to February 1981 was probably responsible for these anomalies. It seems likely that the cold weather had delayed the nest founding activities of the young gynes resting in the stage 4 nests. This resulted in some degree of synchronisation of nest initiation by the young foundresses with the arrival of warmer weather. Additionally, those foundresses that had initiated nests during the previous two months would have been prevented from completing their first brood because of poor weather and this too would increase the proportion of mated foundress bees with developed ovaries in the February 1981 sample.

In the subtropical climate of the Florida keys, weather suitable for bee activity occurs throughout the year with only brief interruptions in January or February of insufficient duration to halt colony development. Although young gynes in some nests become quiescent with the onset of cooler weather, the reduced seasonality is insufficient to select for an annual colony cycle. Colony life is quite short (probably no longer than in the north temperate populations in Ontario and New York). This short colony cycle may increase the chances of the queen production phase being reached before predators destroy the nest. The phenomenon of a brief nonreproductive colony phase is well known in social wasps (Jeanne 1982). Ants appear to be the main nest mortality factor at Knights Key (*Paratrechina* sp. being particularly important). The high early male production may be a bet-hedging strategy ensuring some chance of genetic contribution to the next generation even if the queen production phase is not reached. In addition, the high frequency of worker mating and oviposition may make early male production more advantageous than is normally the case.

From a consideration of all these data we make the following conclusion: in the sub-tropical climate of the Florida keys *H. ligatus* is continuously brooded with several complete colony cycles taking place during one year. During the warmer months young gynes initiate a new nest soon after they emerge from their natal cells but from late November until the end of February they may return to their natal nest and excavate a short lateral burrow. The length of time the foundresses spend in this "resting" chamber will depend upon the duration and intensity of cold weather. Thus, some degree of synchronisation is possible within the continuously brooded, multivoltine annual cycle.

That unmated gynes may begin nesting and/or rest in their natal nests during cold weather is most unusual. This is not found in temperate social halictines where gynes have to mate before entering diapause if they are to mate at all.

Social organisation

In north temperate regions, *H. ligatus* has a social organisation typical of many primitively eusocial halictines in which there is a well defined reproductive division of labour. Morphological caste differentiation varies from 4% in California (Eickwort 1985) to 14.4% in New York (Litte 1977). Michener and Bennett concluded that the Trinidad population of *H. ligatus* exhibited a social organisation more similar to the communal than to any other named category. Our studies of a subtropical population indicate that the colonies are truly matrifilial with the founding female living until the end of the colony cycle. These queens average 16% larger than their workers but despite this large morphological caste differentiation they seem to exert little inhibition on either worker ovarian development or mating. 18% of the worker bees had an oocyte ready to lay at the time of nest excavation compared to 36% for queens. The ratio of oocytes ready to be laid in queens and workers may not accurately reflect the actual ratio of queen to worker produced offspring. This is because of the likelihood of egg consumption. Oophagy has been observed in laboratory colonies of *Lasioglossum (Dialictus) zephyrum* (Batra 1964) and is probably a widespread phenomenon in halictine species which have a moderate or high degree of ovarian development in workers. Nonetheless the high worker fecundity in this population is remarkable, particularly considering the large queen-worker size dimorphism.

The only workers that had more ovarian development than their queen were found in late stage nests in which the queen may have been close to death. However, there are no significant differences in ovarian development between the workers in nests of different stages. This indicates that worker ovarian development is not merely the result of the queen losing dominance towards the end of the colony cycle. The fact that a distinct replacement queen emerges in queenless nests and that functional workers from these nests are more likely to develop a complete oocyte than those in queenright nests, indicates that queens do exert some inhibiting effect upon worker ovarian development.

The morphological caste differentiation between queens and workers at Knights Key is unusually high: only *Lasioglossum (Evylaeus) malachurum*, *L.(E.) lineare*, (Packer and Knerer 1985), *L. (Dialictus) umbripenne* (Wille and Orozco 1970), *L. (D.) exiguum* (Packer 1985), *Halictus (Seladonia) hesperus* (Brooks and Roubik 1983; Packer 1985) and *H.(H.) latisignatus* (Sakagami and Wain 1966) exceed this figure. Polygynous spring nesting associations of Ontario *H. ligatus* studied in the laboratory show that interactions between nest-mates is frequently vicious. Several times a larger bee grabbed a smaller nest-mate by the "neck" with its mandibles and either pummeled it into the ground at the bottom of a burrow or knocked it from side to side against the burrow walls. Larger bees would certainly be at an advantage in encounters like these! Selection may have favoured queens that produce proportionately smaller workers because the greater the size differences between the castes the easier it is for queens to win in aggressive interactions.

Table 5. Contingency tables comparing data from subtropical and tropical populations. Data from the tropical populations comes from Michener and Bennett 1977

Locality	"Nest bees"					
	Ovarian class				Mating	
	A	B	C	D	Mated	Unmated
Knights Key	65	157	139	326	415	270
Trinidad	0	32	24	6	36	26

$\chi^2 = 51.44$, $df = 3$, $P < 0.001$ $\chi^2 = 0.14$, $df = 1$, $P > 0.5$

Locality	"Flower bees"					
	Ovarian class				Mating	
	A	B	C	D	Mated	Unmated
Knights Key	94	323	474	310	826	346
Trinidad	12	191	174	6	213	170

$\chi^2 = 128.33$, $df = 3$, $P < 0.001$ $\chi^2 = 28.77$, $df = 1$, $P < 0.001$

Locality	Queen	
	Present	Absent
Knights Key	43	15
Tropics	2	7

Fisher's Exact Test, $P < 0.0043$

Is the social organisation of the subtropical population of *H. ligatus* in the Florida keys intermediate between the primitively eusocial colonies of the temperate regions and the "communal-like" ones of the tropics? In a more communal society there would be less difference in reproductive potential between the bees. Thus we would expect a higher frequency of worker mating and a more even spread of ovarian development amongst all bees in the tropical population. Additionally, we would expect a higher proportion of the nests excavated at the Knights Key locality to contain a queen bee. A comparison of the data presented by Michener and Bennett and that of the present study is made in Table 5. We include in the category "nest bees" all of the queens, young gynes, foraging workers and nest workers from 48 completely excavated queenright nests plus bees from the 10 queenless colonies. This sample should roughly correspond to the 62 bees from 6 completely excavated nests studied by Michener and Bennett (1977). Similarly we have pooled the 849 bees caught on flowers during the monthly samples with 324 foraging workers from known nests for comparisons of "flower bees" of the tropical and subtropical populations. Additionally, we have added data from the observations of Raw (1975) in Jamaica to those of Michener and Bennett (1977) for an analysis of the proportion of nests that contain a queen, or queen-like bee.

As can be seen from Table 5, there is no significant difference between the localities in the proportion of "nest bees" that are inseminated. However, when flower bees are considered, significantly more bees of the Knights Key population are mated. The Trinidad population has comparatively more bees of intermediate ovarian development than those from Knights Key, this being true for both "nest bees" and foragers. Similarly, there is a significantly higher proportion of queenright nests in the Knights Key population than in the various nests that have been excavated in the tropics. Thus, it does seem likely that there is a decrease in the social level of *H. ligatus* from the temperate regions through to the tropics.

How can we explain this apparent progressive breakdown in reproductive division of labour from north temperate regions to the tropics? Most tropical social halictines exhibit low levels of social organisation: many species are communal whereas a few are semi-social. Several species of tropical halictine exhibit primitively eusocial behaviour with well defined castes. Of these – *H.(S.) hesperus* (Brooks and Roubik 1983), *L.(D.) umbripenne* (Wille and Orozco 1970; Eickwort and Eickwort 1971) and *L.(D.) exiguum* (Packer 1985) live in distinctly seasonal habitats and the young gynes diapause during the wet season (although the Turrialba population of *L. (D.) umbripenne* is thought to be an exception). Some species of *Augochlora (Oxystoglosella)* are eusocial with well defined castes but live in comparatively aseasonal environments (Eickwort and Eickwort 1972). However, this is a purely tropical taxon and it can be assumed that sociality originated in a tropical environment in this group whereas the aforementioned species (including *H. ligatus*) almost certainly had temperate ancestors that were social. Some degree of synchronisation in colony development may thus be necessary to maintain a well defined caste system in halictine bees in which sociality originated in temperate regions. Nonetheless, it should be pointed out that a seasonal environment does not necessarily result in well defined castes! Many eusocial, temperate halictines have poorly differentiated castes, another Nearctic *Halictus* species – *H. farinosus* is one example (Eickwort 1985).

Cold spells in the Florida Keys may cause the retention of an ancestral diapause-like stage: a true diapause being found in populations with an annual colony cycle on the Florida mainland at least as far south as Tampa (Packer in preparation). However, the ancestry of the Trinidad population must have included phases in less seasonal habitats, where cold weather was no longer a cue for diapause. It is possible that the dry-season aestivation in this population is a recent adaptation occurring after all traces of seasonal response in the colony cycle had been lost. Thus the breakdown in physiological caste differentiation in *H. ligatus* populations towards the tropics may be related to the loss of environmental cues that enforce synchronous periods in colony development.

The subtropical population of *H. ligatus* is more similar to the tropical ones studied by Michener and Bennett (1977) than any other population of this, or any other halictine species. The high proportion of bees that are mated and have ovarian development, the lack of seasonal variation in these, and other variables etc. indicate close similarity. However, in the tropics, the colonies remain active for a long time after the death of the foundress. Conversely, in the subtropics, the nest population diminishes quite rapidly as a result of reproductive production; returning young gynes are repelled by the few remaining workers after the death of the queen. Thus, the colony life does not greatly exceed the longevity of the foundress. It is possible that the differences found between the tropical and subtropical populations stem from this.

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