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Diapause and Non-delayed Eusociality in a Univoltine
and Basically Solitary Bee, *Ceratina japonica*
(Hymenoptera, Anthophoridae)

II. Non-delayed Eusociality Induced by Juvenile Hormone
Analog Treatment

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Abstract *Ceratina japonica* is a univoltine and basically solitary bee, but sometimes produces semisocial or delayed eusocial colonies. This study aims at the induction of non-delayed eusociality in this bee, which is adopted by most temperate social insects but is incompatible with the univoltine life cycle. The second brood rearing by the mother was induced by removing her brood of immatures. Juvenile hormone analog was topically applied to pupae and prepupae removed from cells and their growth was accelerated by heating. Daughters who newly emerged were returned to the mother's nests. Diapause termination was confirmed in 6 out of 20 colonies and non-delayed eusociality was formed in 4 colonies. In 2 of these colonies daughters acted as workers, but in the other 2 as queens, showing an epigenetic caste flexibility. Observed behaviors of key tasks were described and discussed.

Key words: *Ceratina japonica*; non-delayed eusociality; juvenile hormone analog; epigenetic caste flexibility.

Introduction

Most temperate social insects adopt the non-delayed eusociality, *i.e.*, the association of the post-hibernating mother as the queen and her non-hibernating daughters as workers. *Ceratina japonica* is a univoltine and normally solitary bee, but the nests in the brood rearing period occasionally contain 2 or more females. These colonies are mostly semisocial in which the castes are formed between or among post-hibernating sisters, but some are delayed-eusocial, the association of the mother who hibernated twice and her post-hibernating daughters, where the mother functions as the queen and the daughters as workers (SAKAGAMI & MAETA,

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1982–1987 b). The absence of non-delayed eusociality in this species is easily understood, because its univoltine life cycle normally can not accept insertion of another brood rearing period.

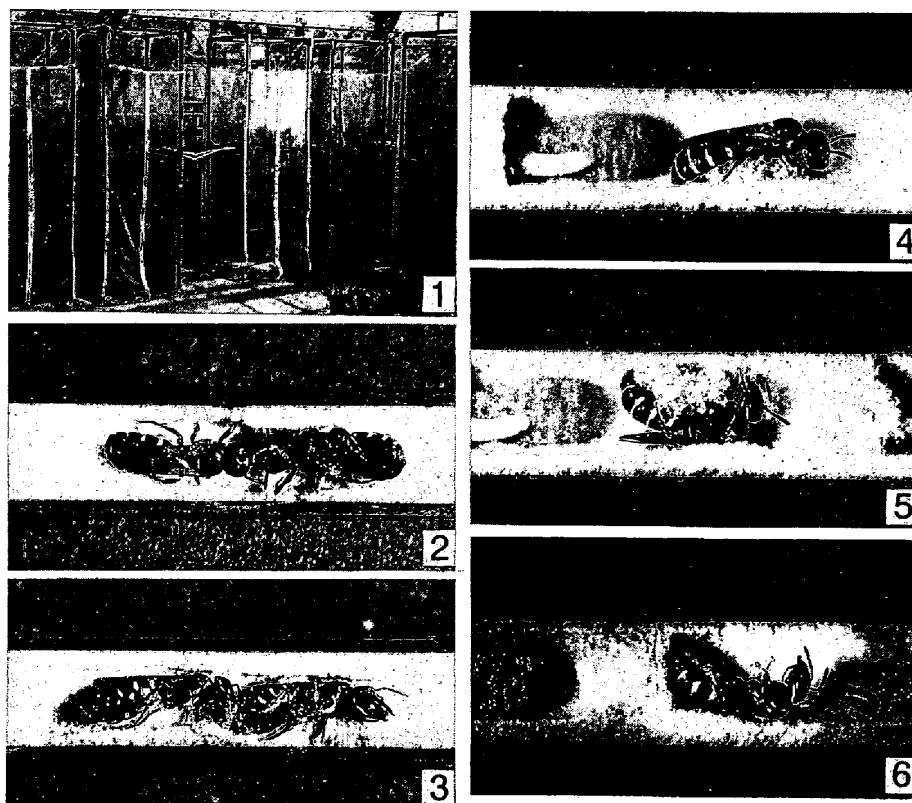
However, females of this species can start their second oviposition when immatures are removed from the nests (SAKAGAMI & MAETA, 1989). Utilizing this trait, we could induce 5 stepmother/stepdaughter colonies and 5 colonies each consisting of the mother and her real daughters. The growth of immatures was accelerated by warming them. Daughters who newly emerged were returned into the natal (or alien) nests after the start of the mother's (or stepmother's) second ovipositions. These experiments were futile, because the daughters (or stepdaughters) did not forage for larval food as do non-hibernating daughters (=workers) of all real social insects. Similar experiments were made with the multivoltine congener *C. okinawana*. Here we could easily induce eusociality within the same year (SAKAGAMI & MAETA, 1989). We thought that our failure in *C. japonica* was due to that prehibernating adult daughters who newly emerged were in reproductive diapause, which made the activation of foraging behavior impossible, and we tried to terminate the diapause artificially before winter. As mentioned in MAETA *et al.* (1993), the reproductive diapause of pre-hibernating daughters can be terminated either by cooling or by applying juvenile hormone analog (JHA). Diapause is completely terminated by cooling them for 3 months, but this duration is too long to permit insertion at the end of the active phase of the normal life cycle (the end of August) to induce the non-delayed eusociality. Thus, we tried to terminate the diapause by topical JHA-application, despite the low termination rate by this method (MAETA *et al.*, 1993).

This second report describes behaviors of JHA-treated daughters in artificial nests with emphasis on the females which were assumed to have entered the post-diapause state. The term 'colony' is applied below to the cohabitation of 2 or more females, irrespective of the presence or absence of brood rearing.

Materials and Methods

Artificial nests: Bees were reared since spring in glass tube nests. The pithy cores of *Kerria japonica* were inserted in the tubes, each core having received a guide furrow to be followed by the bee in excavating. Further details are given in SAKAGAMI & MAETA (1985, 1987).

JHA-treatment: All immatures were removed from the nests and only prepupae and pupae were treated with JHA (S-31183, made by the SUMITOMO Chemical Co, Ltd., Takarazuka) in May and June, 1989 and 1990 by the method detailed in MAETA *et al.*, (1993). JHA was smeared once (1989) or twice (1990) on each individual, but this gave no significant difference in the rate of diapause termination. JHA-treated immatures were kept under the 28°C and 16L 8D regime and females who emerged were fed with honey and pollen for 2 weeks.



Figs. 1-6. Rearing cage and some intranidal behaviors in *Ceratina japonica*. — 1, Cage and nest tubes used for *C. japonica*; 2, mother fed daughter with nectar by regurgitation; 3, mother fed daughter with pollen by unloading in front of her; 4-6, daughter entered the closed cells to clean the contents by opening and reclosing the cell partitions.

Induction of colonies consisting of mother and JHA-treated daughters: After the removal of prepupae and pupae from the nests, mothers continued ovipositions. All immatures involved in nests were removed prior to the insertion of JHA-treated females. JHA-treated females were returned each to its natal nest, always from the bottom to avoid possible rejection by the mother which guarded at the nest entrance.

Rearing cages and colonies induced: Several vinyl-roofed net cages (50 (l) × 50 (w) × 180 (h) cm) were used for rearing colonies. Each cage was constantly supplied with 1-2 pots of *Phacelia tanacetifolia*, *Melilotus officinalis*, *Lythrum anceps* and *Cosmos sulphureus*, from which bees could forage nectar and pollen freely. To each cage, 1 (1990) or 3 (1989) glass tube nests were connected (Fig. 1). In 1989 some originally matrifilial colonies, each consisting of the mother and her own JHA-treated daughter(s), changed to non-kin colonies due to internidal drifting as shown in foot-notes in Table 1. When a real mother-daughter colony lost one of the partners, the substitute partner was introduced in the nest in both years. As post-diapause males were not released in this experiment, JHA-treated daughters

never mated. All their eggs should thus have produced males.

Among 30 matrifilial colonies which were artificially induced, intranidal behaviors were rather constantly traced in 20 colonies cited in Table 1.

Criteria for diapause termination: The termination was confirmed by 2 behaviors, oviposition and pollen foraging (followed by unloading in the brood cell). Unless either or both of these behaviors were confirmed, such daughters were not regarded as in the post-diapause state.

Criteria for social status: Following SAKAGAMI & MAETA (1987 b), 4 tasks were used: Pollen foraging (followed by unloading in the cell) (P_1 =active, P_0 =inactive, p =rare or absent), feeding to the partner(s) with nectar (F =present, f =absent), oviposition (O_1 =principal, O_0 =subsidiary, o =rare or absent), and guarding (G_1 =constantly, G_0 =occasionally, g =rarely). The task allocation formula is expressed as $P_1 f O_1 G_1$ (or simply $P f O G$) in a solitary female, $p f O_1 G_1$ (queen-like)+ $P_1 F o g$ (workerlike) in a colony with the clearest caste differentiation and $p f O_1 g$ (queen)+ $P_1 F o G_1$ (worker) in most advanced social insects. The life cycle of *C. japonica* is divided into 7 periods (P_1 - P_7), following SAKAGAMI & MAETA (1985).

Results and Discussion

Table 1 gives a synopsis of 20 well traced colonies. They were divided in 2 (A, B) types according to whether diapause termination was confirmed or not, and further classified into several types.

Criteria for diapause termination

It is unnecessary to explain the adoption of oviposition as a criterion of diapause termination. Another criterion, pollen foraging (followed by unloading in the brood cell), might be unfamiliar for non-hymenopterists. In Aculeata, foraging for larval food should occur before oviposition as an indispensable element of the brood rearing sequence. Although critical studies are scarce, ovarian development, foraging and oviposition must be activated only after diapause termination in the univoltine and adult-hibernating wasps and bees including *C. japonica*. The same is true for the post-hibernating queens of temperate social insects, but only until the emergence of workers who can start, without passing diapause, foraging for larval food (and often also some ovipositions) just as do non-diapausing females of multivoltine species. Thus, pollen foraging is a reliable and convenient criterion of diapause termination (=post-diapause activation). It can be more frequently and more easily (in the daytime) observed than oviposition. A third reliable criterion, the preparation of the basal partition (=the bottom of the first cell) as a necessary step to start brood rearing, was not used because whether the mother or the JHA-treated daughter built it was not always known.

Table 1. Synopsis of induced and well traced colonies shown with colony codes.

Colony type	Diapause termination (a, b/c) ¹⁾	
	Not confirmed (Type A)	Confirmed (Type B)
A ₁ Incipient matrifilial	89- 1-2 ²⁾ (38*, 12/3), 90-16-1 (44*, 18/2), 90-25-1 (61, 9/3),	90-11-1 ³⁾ (38, 30/1) 90-24-1 (58, 51/3) 90-27-1 (34*, 29/3)
A ₂ Advanced matrifilial	90- 2-1 (129* 127/8), 90- 5-1 (84*, 82/7), 90-18-1 (64, 62/5),	90- 3-1 (85, 83/9) 90-15-1 (48, 47/6) 90-22-1 (41, 39/6)
B ₁ Reversed matrifilial		90-12-1 (29, 23/4)
B ₂ Eusocial		90-1-1 (64, 59/6) 90-8-1 (121, 118/9)
B ₃ Reversed eusocial		90-14-1 (16, 6/3) 89-9-3-1 ^{2), 3)} (47, 13/4)
A ₃ and B ₄ Sororifilial	90- 4-1 ⁴⁾ (83*, -/0) 90-13-1 ⁴⁾ (40*, -/0)	89-8-3 ^{2), 4)} (38*, -/4)

¹⁾ Duration in days from the start of coexistence (a) or from the preparation of basal partition (b) to the end of coexistence (or to the termination of observation*), and the number of cells produced during coexistence (c).

²⁾ Cohabitants are non-kin.

³⁾ Colonies involving 2 or more daughters.

⁴⁾ Colonies involving only daughters.

Rate of diapause termination

Among 28 JHA-treated females, oviposition was confirmed only in 6 bees (21.4%), approximately the level confirmed by dissection of ovaries (MAETA *et al.*, 1993, 11–50% in the most effective concentration range). The rate was not high. Even in successfully activated bees, brood rearing was less intense and less consistent than that observed in the normal life cycle. This was obviously caused in part by the inefficiency of our JHA-application, but probably in part by the complicated situation into which we forced our JHA-treated pre-hibernating daughters. Our purpose was not the mere termination of diapause, but the induction of non-delayed eusociality, a situation hitherto unknown in the univoltine and normally solitary life of *C. japonica*. Thus, our results are numerically poor, but may involve some suggestive information. Below various colony types are explained, in some cases with examples. The mothers and JHA-treated daughters are abbreviated as M and D (MM and DD in case of plural).

A. Colonies in which diapause termination was not confirmed (types A₁ and A₂)

At first 2 types of colonies are described in which diapause termination (=post-diapause activation) by JHA-treatment was not confirmed.

A₁ Incipient matrifilial colonies (6 cases)

DD participated in no tasks, even in guarding. MM monopolized all nest

maintenance and brood rearing tasks. They also fed DD with nectar by regurgitation (Fig. 2) or pollen by unloading in front of DD (Fig. 3). Task allocation was $P_1 F O_1 G_1 (M) + p f o g (D)$. Coexistence continued 38–61 days (Table 1).

A_2 Advanced matrifilial colonies (6 cases)

As in A_1 but DD guarded at the nest entrance, kneaded pollen deposited by MM in the cell to be oviposited, and entered the closed cells to clean the contents by opening and reclosing the cell partitions (Figs. 4–6). Task formula = $P_1 F O_1 G_1 (M) + p f o G_0 (D)$. In some colonies the A_2 state was preceded by the A_1 state.

Colony 90–3–1: Seven cells were provisioned and oviposited exclusively by M during 16 days. She also fed D with nectar (and pollen). D participated in guarding and cleaning of cell contents. After entering the mother-juvenile cohabiting period (P_4), D fed seven new adults (3 ♀ + 4 ♂) and also M.

Colony 90–18–1: As in 90–3–1, all eight cells were prepared by M alone in the laying period (P_2) of 13 days. Cell VII was provisioned and oviposited after the emergence of new adults, cell VIII was also provisioned, but not oviposited (probably because of the mechanical disturbance by new bees). Three new adults (2 ♀ + 1 ♂) were produced and fed only by M.

A_1 colonies are comparable to our previous futile results, using the pre-hibernating daughters (SAKAGAMI & MAETA, 1989). These also correspond to the coexistence at P_4 in the normal life cycle (SAKAGAMI & MAETA, 1985), and the second brood rearing by mothers in some colonies.

The elaborate and prolonged parental care of the offspring that emerged in both diapausing and non-diapausing generations characterizes the life cycle of the xylocopine bees to which *Ceratina* belongs (MICHENER, 1974, 1985, 1990). It is likely that this behavior favored the further evolution of social steps (ANZENBERGER, 1977; VELTHUIS & GERLING, 1983), but this inference should not be extended to the groups that developed sociality without such care, e.g., the halictine bees. A_2 suggests that some behaviors, e.g., kneading of pollen and cleaning of cell contents, are likely to be released in pre-hibernating daughters without diapause termination. These behaviors were occasionally observed also in our previous experiments using pre-hibernating, not JHA-treated females. It is uncertain whether A_1 and A_2 reflect any difference in JHA-effect.

A_3 Sororifilial colonies without brood rearing

Similar to A_1 but the colonies consisted of daughters alone. Colonies 90–4–1 and 90–13–1 each contained 2 real sisters after the loss of the mother. In both the earlier emerged (but larger) sister fed the younger one with pollen (confirmed twice in both colonies) and with nectar (twice in 90–4–1).

B. Colonies in which diapause termination was confirmed (types B_1 , B_2 , B_3)

B_1 Reversed matrifilial colony

Colony 90–12–1 was similar to A_2 type, but the relative status of M and D was reversed (size $D > M$). All 4 cells were exclusively provisioned during 8 days by D, who also fed M 3 times with nectar. M frequently guarded but did not clean the

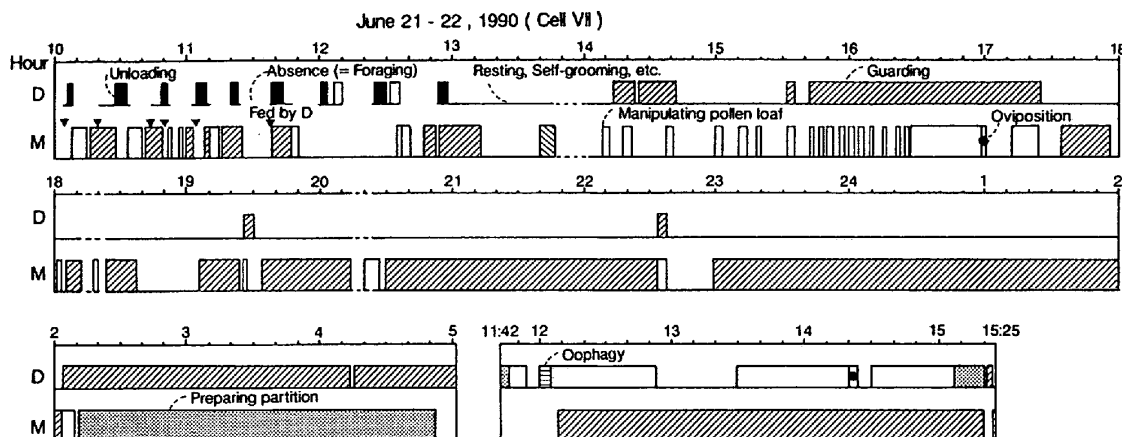


Fig. 7. Representative behavior record of a eusocial colony (90-8-1) observed for 22 hours.

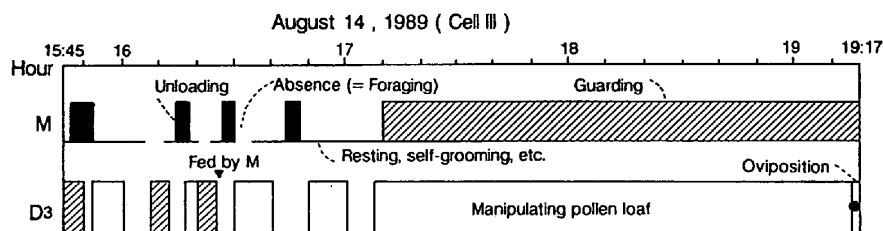


Fig. 8. Representative behavior record of a reversed eusocial colony (89-9-3-1) observed for 3.5 hours.

cell contents. Task formula is $p f o G_1 (M) + P_1 F O_1 g (D)$.

Certainly D was diapause-terminated, but it is unknown why M did not forage for pollen which she could do judging from the previous study (SAKAGAMI & MAETA, 1989). That she was fed by D might have inhibited her pollen foraging.

B₂ Eusocial colonies

Induction of this colony type was the target of our experiment. Observations on the 2 induced eusocial colonies are summarized in Table 2. Frequencies of each task shown in Table 2 were recorded occasionally through the observation periods. Cells completed under the eusocial phase were not many, but the sequence is qualitatively the same as that in the induced semisocial or the delayed eusocial colonies (SAKAGAMI & MAETA, 1985, 1987 b). In the 2 colonies, pre-hibernating daughters were brought by JHA-treatment to the post-hibernating condition, with the post-diapause activation of foraging and oviposition, which never happens in the normal life cycle. In both colonies, the eusocial phase was preceded by the advanced matrifilial "phase" (=A₂ colonies) with the virtual monopolization of most tasks by M. The body size was $M > D$ in both colonies.

Colony 90-1-1: D participated in guarding and cleaning of cell contents before the start of foraging. Six cells were made during 16 days and cell VII was abandoned

Table 2. Task allocation shown by frequencies of observed behaviors in 2 non-delayed eusocial colonies in *Ceratina japonica* formed by the mother (M=queen) and her JHA-treated daughter (D=worker).

Colony codes		90-1-1		90-8-1	
Observation period (Duration in days)		Earlier 27 v-10 vi (15)	Later 11 vi-7 vii (27)	Earlier 30 v-17 vi (19)	Later 18 v-25 vi (8)
Completed cells (Codes and numbers)		I-VI 5	VI-VII 1	I-VI 6	VI-IX 4
Foraging	M	20	1	12	0
	D	0	12	0	34
Oviposition	M	5	1	7	4
	D	0	0	0	3
	?	0	0	2	0
Oophagy	M	0	0	0	1
	D	0	0	0	2
	?	0	0	1	1
Feeding with nectar					
M to D		2	0	1	0
D to M		0	3	0	20
Cleaning of cell contents	M	0	2	2	1
	D	3	11	2	3
Main guard	M	M, D		M, D	
Task formula	M	$P_1 F O_1 g$		$P_1 F O_1 G_1$	
	D	$p f o G_0$		$p f o G_0$	

without completion. D participated in foraging for cell VI, but M oviposited in all cells. After D started foraging, M was fed by D and monopolized guarding. Four juvenile females emerged from this nest, D fed them (=younger sisters). The eusocial phase lasted 48 days until the disappearance of D. Juveniles were later fed by M.

Colony 90-8-1: The eusocial phase lasted for 100 days until observations were ceased. Figure 7 shows a representative daily record in the eusocial phase. In this colony M prepared the basal partition 3 days after the coexistence when D was still near the bottom of the burrow, but she was able to arrive at the entrance of the burrow by opening and reclosing partitions. Cells I-V were exclusively provisioned and oviposited by M. D started her foraging from cell VI. The behavior sequence between M and D was the same as in colony 90-1-1. In cells VII and VIII M laid first, followed by oophagy-oviposition by D, and in cell VI the order was reversed. Newly emerged adults (3 ♀, 5 ♂) were exclusively fed by D with pollen and nectar. M was also fed by D with nectar.

The sequence of matrifilial and eusocial phases in the 2 colonies is exactly same as that in artificially induced non-delayed eusocial colonies of *C. okinawana*, which consisted of the post-hibernating generation (SAKAGAMI & MAETA, 1989). This sequence is also comparable to the well known specialization in oviposition by the

Table 3. Task allocation shown by frequencies of observed behaviors in 2 reversed non-delayed eusocial colonies in *Ceratina japonica* formed by the mother (M=worker) and her JHA-treated daughter (D=queen).

Colony codes		90-14-1	89-9-3-1*	
Observation period (Duration in days)		25-31 vii (7)	Earlier 9-12 viii (4)	Later 13-16 viii (4)
Completed cells (Codes and numbers)		I-II 2	I-II 2	III-IV 2
Foraging	M	5	0	14
	D	5	7	3
Oviposition	M	0	0	0
	D	3	2	2
Oophagy	M	0	0	0
	D	1	0	0
Feeding with nectar				
M to D		0	0	5
D to M		0	0	0
Main guard		M, D	D	D
Task formula	M	P ₁ F o G ₀	p ? o g**	P ₁ F o g
	D	P ₁ f O ₁ G ₀	P ₁ ? O ₁ G ₁	P ₀ f O ₁ G ₁

* M disappeared on 17 August and the remainder D added more 4 cells solitarily.

** Feeding the partner not recorded.

foundress after the start of foraging by her newly emerged non-diapausing daughters in normal eusocial groups such as bumblebees, vespid wasps and social halictine bees. Their eusocial phase was simulated in the 2 colonies of the univoltine and normally solitary bee *C. japonica*.

B₃ Reversed eusocial colonies

The eusocial state was induced also in 2 other colonies, but the status of M and D was somewhat reversed. M functioned as the worker and D as the queen. The results are summarized in Table 3. Frequencies of each task shown in Table 3 were recorded by the same procedure as in Table 2.

Colony 90-14-1: The size was D>M. The basal partition was prepared on day 9 after coexistence by either M or D and the first provisioned by D. On day 10 both M and D foraged so that the nest entrance occasionally remained unguarded. D oviposited in cells I and II on days 11 and 12. Curiously the latter egg was eaten by herself on day 2 after oviposition and another egg was laid by her, an incident known very rare in *Ceratina* bees. On day 15 D disappeared. M continued to store pollen in the 3rd cell, but oviposition did not occur in this cell. On day 28 M swept away the contents of all three cells. On day 12 D begged M for food, but it was not given. The task formula shows that the colony was eusocial for oviposition, but was eosocial (=communal cell use between M and D without caste formation, SAKAGAMI & MAETA, 1977) for pollen

foraging.

Colony 89-9-3-1: First the colony consisted of $M+D_1+D_2$, all non-kin. The membership changed to $M+D_2+D_3$ by the death of D_1 and drifting of D_3 into the nest on day 26. D_3 was smaller than M , but prepared the basal partition on the same day. On day 37 both M and D_3 foraged and prepared a small pollen loaf. The inactive D_2 and small pollen loaf in cell I were artificially removed from the nest. The number of pollen loads brought into the first cell on day 37 is not included in Table 3. The subsequent task allocation was typically reversed-eusocial (Table 3). A representative behavior record is given in Fig. 8.

Reversed eusocial colonies are rare in natural populations. Based on the ovarian state, this type was confirmed only once each among delayed eusocial colonies of *C. japonica* (out of 10 cases, SAKAGAMI & MAETA, 1985), and *C. okinawana* (out of 44 cases, SAKAGAMI & MAETA, 1989). In normally social species the reversed eusociality may appear only as exceptional case, because the eusociality is basically a system started only by the long-lived mother as the queen, who produces daughters functioning as workers. Nevertheless, the produced reversed eusociality is implicit, when viewed synoptically with the normal eusociality (B_2). The occurrence of these 2 types shows an ambivalent or optional state of the JHA-treated daughters after diapause termination. Founding the nest solitarily, she may function as the foundress capable of both food provisioning and oviposition. Cohabiting with the mother, the daughter may usually behave as the worker specializing to food foraging, but occasionally as the queen. Then the mother becomes the worker. Such optional and epigenetic division of labor (WEST-EBERHARD, 1987 a, b) does not exist in the queens of most advanced social insects. In some groups, however, the early solitary phase of the colony life cycle is often replaced by a polygynic phase (polistine wasps, PARDI, 1948; WEST-EBERHARD, 1969. ITÔ, 1985, etc.; halictine bees, KNERER & PLATEAUX-QUÉNU, 1966; SAKAGAMI, 1974, etc.). Under this state the association of cohabiting gynes might be often started from an optional condition.

B_4 Sororifilial colony with brood rearing

Colony 89-8-3 first consisted of $M+D_1+D_2$ (all non-kin from each other.) On the 18th day after the coexistence, M disappeared and D_3 and D_4 invaded from other nests on days 19 and 20, respectively (sizes $D_2>D_3>D_4>D_1$). Four cells were provisioned and oviposited during 8 days from the invasion. Food provisioning and cleaning the cell contents were monopolized by D_4 who fed all partners. The sequence of oviposition and oophagy in these cell is as follows: D_4 oviposited in cells I-IV. D_2 replaced the D_4 's egg in cell IV through the oophagy-oviposition process, but this egg was again replaced by D_4 on the same day. The task formula is $P_1 F O_1 G_1 (D_4)$, $p f O_0 G_1 (D_2)$, and $p f o g (D_1, D_3)$. This colony was virtually a solitary nest of D_4 with 3 hangers-on, although D_2 was in the post-diapause condition.

The above examples clearly show the most incipient caste differentiation so

far confirmed in Hymenoptera. WEST-EBERHARD (1987 a, b) inferred that such state in *Ceratina* might be vestiges of an ancestral social state. The atavism from 'social to solitary' was suggested in *Exoneurella lawsoni*, a solitary relative of the social genus *Exoneura* (MICHENER, 1964), and might occur in some halictine bees, but probably not in *Ceratina*. Except for *C. japonica*, *C. okinawana* mentioned above and also *C. (Ceratina) iwatai* (MAETA, 1993), *C. (Ct.) megastigmata*, and *C. (Neoceratina) dentipes* (MAETA *et al.*, unpub.), multifemale nests in the brood rearing condition are very rare in the genus *Ceratina* (MICHENER, 1985). This genus does not exhibit the extremely low male ratio as seen in *E. lawsoni* and common among social insects. Serially prepared brood cells in a tubular unbranched stems and twigs do not favor the previous occurrence of sociality. Except for the above mentioned 5 Japanese *Ceratina* species, caste-differentiated colonies were difficult to induce in the following species so far studied (Provenance in parentheses): *C. (Ceratinidia) flavipes* (SAKAGAMI & MAETA, 1987 b), *C. (Ct.) satoi* (Japan), *C. (C.) cucurbitina* (Spain), *C. (Euceratina) callosa* (Spain), *C. (E.) cyanea* (Spain), *C. (E.) chalcites* (Spain), *C. (Zadontomerus) neomexicana* (N. America) and *Pithitis smaragdula* (India) (MAETA *et al.*, unpub.). These facts suggest that, unlike their principally social sister group, Allodapini (MICHENER, 1990; MAETA *et al.*, 1992), the tribe Ceratinini is basically solitary, and the observed caste-linked behaviors are rudimentary, not vestigial.

Behavioral Accounts

We cited some key tasks that characterize each caste. Relevant comments are given on behavioral aspects of these tasks and another task, cleaning nest contents.

Foraging and feeding: The 2 key tasks of workers relate from each other in a complicated way. These tasks share the extranidal foraging of foods for different finalities. Foraging of the larval food may be elicited by the presence of the cell to be provisioned and is followed by unloading of pollen on the floor of the cell or on the partly heaped pollen already there. Later, the mass is kneaded with admixing of nectar. This task is performed only by post-diapause females during the brood rearing period (in this study in B colonies).

Foraging of the adult food is released also by soliciting by juveniles, which extend the glossae (for nectar) or dash forward with up-down head movement (for pollen, Figs. 2-3). The returning forager feeds the solicitants by regurgitation (nectar) and by unloading in front of them (pollen). Feeding to juveniles is mainly by the mother (SAKAGAMI & MAETA, 1985), that is, again by a post-diapausing female. However, sometimes (6 out of 36 cases, SAKAGAMI & MAETA, 1989), this role is taken over by the oldest daughter. Then, even the mother ceases foraging and is fed by this diapausing daughter. We presented 2 hypotheses, behavioral diapause termination by foraging and absence of behavior linkage between foraging and preparation of the food loaf. Also, not conclusively, we are now thinking the

above 2 behavior-chains are of different natures, although they share the same behavior chain: The first is elicited only by post-diapaused females, while the other is also elicited by diapausing females. Another interesting fact is that these feeders are smaller than the other daughters, as if they are simulating the worker role, although during reproductive diapause. MAETA *et al.* (1992) surmised that such smaller daughters result from the mother's manipulation so as to produce a replacement female who will feed sibs.

VELTHUIS and GERLING (1983) regarded guarding by juvenile adults of *Xylocopa pubescens* at the nest entrance as a mere result of food competition, and feeding juveniles by the mother with pollen as merely a part of her cell provisioning behavior. These interpretations are clearly valid in a bivoltine *C. iwatai*, which has no soliciting behavior for pollen (MAETA, 1993), but the situation seems different in *C. japonica*. In this species there is no definite relation between food solicitation and guarding by juveniles, and pollen unloading in the brood cell and in front of an adult solicitant are 2 clearly different behaviors. Behavioral homology between the 2 sister groups, *Xylocopa* and *Ceratina*, should be further clarified.

Oviposition: This is the main and often only key task of the queens in advanced social groups. In artificially induced colonies of *C. japonica*, the provisioned cell is first oviposited by the 'queen' in most cases, but this is often followed by oophagy and oviposition by the 'worker' (2 out of 3 cases in this study). In some semisocial or delayed eusocial colonies, most eggs laid by queens were replaced by workers' eggs (35/56, SAKAGAMI & MAETA, 1987 b). In many social Hymenoptera, such worker ovipositions are suppressed by inhibition by the queen, either behaviorally or chemically. In part this is also suggested for *C. japonica* and *C. okinawana* by the occurrence of ovarially undeveloped females in semisocial or eusocial natural colonies (SAKAGAMI & MAETA, 1985, 1987 b). However, there seems to occur another route. In some (not all) groups of stingless bees, workers lay eggs which are eaten by the queen before her own ovipositions (SAKAGAMI, 1982). The evolutionary (not necessarily phylogenetic) precursor of this behavior is known in the orchid bee, *Euglossa cordata* (GARÓFALO, 1985). In this species a subordinate female (daughter or younger sister) first lay an egg in a provisioned cell. The egg is eaten by the dominant female (mother or elder sister) before her own oviposition. The oviposition sequence is usually the opposite in *C. japonica* (cf. Fig. 7) and in the other congeners studied. A narrow slit opened to the sociality for *Ceratina* may be widened in either of the above 2 ways, advanced queen inhibition or reversed laying order, first the worker then the queen.

Guarding: This is the key task of the 'queen' of *C. japonica* (and also in other congeners studied, and *Braunsapis hewitti* and some other allodapine bees, MAETA *et al.*, 1992). However, guarding is also seen in solitary nests even in non-brood-rearing seasons. Guarding in *Ceratina* is behaviorally nothing other than resting at the nest entrance, facing toward the nest bottom and closing the narrow entrance hole with the well sclerotized metasomal tip. Thus, the 'queen' of *C. japonica* is

characterized by 2 negative key tasks (continuous resting and abandonment of foraging) and 1 non-exclusive behavior (oviposition). All other observed behaviors appear during both diapause and post-diapause states, but one behavior must briefly be commented on for its singularity.

Cleaning nest contents: This behavior chain consists of removing the cell partition by shifting the material for a short distance — entering the cell and checking walls and contents — removing something from the surface of eggs and young larvae and feces from older larvae (these debris are admixed to the partition when it is rebuilt) — reshifting and rebuilding the partition. The last requires only about 10 minutes, despite the fact that preparation of a new partition after oviposition lasts for 2–5 hours (Figs. 6–7). The juvenile females in reproductive diapause only rarely participate in cleaning the contents of 1 or several cells, while the mother frequently cleans all the cells. After cleaning the lowermost cell by repeating the above sequence, she returns toward the entrance of the nest, repeating the shiftings of partitions. Food is not added to the cells during this sequence, but the immatures are occasionally moved with the face and fore-legs for a short distance, although far less skillfully than in allodapine bees (MAETA *et al.*, 1992).

Certainly, cleaning cells in *Ceratina* has arisen from the post-emergence behavior of juveniles to reach the entrance side of the nest, but has not appeared in all groups; it is so far confirmed in *C. (Ceratinidia) japonica*, *C. (Cd.) flavipes*, *C. (Cd.) okinawana* and *C. (Ceratina) cucurbitina*, but is absent in *C. (Ct.) megastigmata*, *C. (Ct.) iwatai*, *C. (Ct.) satoi*, *C. (Neoceratina) dentipes*, *C. (Euceratina) cyanea*, *C. (E.) callosa*, *C. (E.) chalcites*, *C. (Zadontomerus) neomexicana* (SAKAGAMI & MAETA, 1986; unpub.). In *C. (Ct.) megastigmata*, all partitions are swept away at one time; thereafter all immatures are exposed in the burrow (MAETA & SAKAGAMI, 1977; MAETA & KATAYAMA, 1978). This state was seen in *C. (Neoceratina) australensis* (MICHENER, 1962), *C. (Rhynoceratina) vollitans* (SAKAGAMI & LAROCA, 1971), *C. (Ct.) unicolor* (MAETA, unpub.), suggesting the same habit.

It is likely that cleaning cell contents is an apomorphic trait and possibly increases the larval survival. However, this behavior also increases egg replacement in colonial nests. Actually replacement is common in *C. (Cd.) japonica* and *C. (Cd.) okinawana*, while rare in *C. (Ct.) iwatai* and *C. (N.) dentipes*, whose 'workers' replace eggs only before cells are closed by the 'queen'.

In connection with the relationship between diapause termination and social behavior, 2 seemingly contradictory observations on bumblebees are cited and interpreted here. RÖSELER (1976) found that the JHA-level of queens of *Bombus hyponorum* and *B. terrestris* who newly emerged was low. He induced some worker behaviors in them JHA-injection. KATAYAMA (1988) found that some new pre-hibernating queens of *B. ardens* performed worker tasks. The former result may indicate that the JHA-injected gynes were not behaving as workers, but as post-diapausing queens which can perform both worker and queen behaviors until the emergence of non-diapausing workers. In the latter case, the observed gynes were

as large as others, but were modified to non-diapausing workers probably by the differential inhibition by the mother queen.

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