

The Role of Handicapped Juveniles for Founding Multifemale
Nests in Basically Solitary Small Carpenter Bees, *Ceratina* (*Ceratinidia*)
okinawana MATSUMURA *et* UCHIDA (Hymenoptera, Apidae, Xylocopinae)

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Abstract *Ceratina* (*Ceratinidia*) *okinawana* is a basically solitary bee, but it occasionally forms multifemale nests (semisocial, eusocial, delayedly eusocial and reversed eusocial). These have a combination of a single large-sized female (queenlike) and usually one small-sized female (workerlike) (SAKAGAMI & MAETA, 1989). The function of size difference among daughters of *C. okinawana* in founding the multifemale nests was studied for 3 years from 1999 to 2001. The body size of both female and male adults (head width used) divided into 5 classes (L, M, MS, S and SS). We reared them in 3 different sized cages. Plenty of flowering plants were supplied in these cages. The first to third adults that emerged were small females and usually the smallest remained in maternal nests as eusocial workers. At first, the mother fed juveniles on pollen and honey, but soon the mother and other juveniles were fed by one of the smaller daughters. These smaller daughters seem to be manipulatively produced by mothers (MAETA *et al.*, 1992). The individuals of the smallest group (SS) had the following handicaps in reproduction: 1) A tendency that decreased the number of eggs laid/female was obvious, according to the decrease of head widths. 2) Sex ratio of juveniles was extremely male-skewed in SS (0.75), while it was female-skewed in L (0.30), suggesting that smaller females invest more for production of male offspring than female offspring. 3) The wet weight of both sexes of juveniles was the lightest in SS. 4) The rate of insemination was extremely low in SS, suggesting that assortative mating with larger males was difficult in SS, due to inharmonious courtship display and presumably other physiological handicaps. 5) The rate of nesting was the lowest in SS, and it was amplified by a shortage of nest stems. Those nests of SS were usurped by larger individuals. SS have various handicaps in their reproduction. These handicaps allow them to form multifemale nests by remaining in their maternal nests.

Key words: Sociality; multifemale nests; handicap; body size; mating success; reproductive success; nesting success; manipulation; *Ceratina*; small carpenter bees.

Introduction

Small carpenter bees of the genus *Ceratina* are basically solitary, but they may have precursory social traits, with rudimentary castes (SAKAGAMI & MAETA, 1977, 1985). Life cycle patterns of ceratinid bees are quite different from those of other solitary bees, consisting of 7 periods: Pre-laying (P₁); Laying (P₂); Post-laying (P₃); Mother-juvenile cohabiting (P₄); Dispersal (P₅); Hibernating (P₆) and Post-hibernating (P₇). Inter-adult behaviors observed in social nests are all derived from those in P₄, none of the new behaviors is elaborated (SAKAGAMI & MAETA, 1985, 1986). Multifemale nests are occasionally found in the field. The major factors that enable

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the induction of sociality are 1) applicable inter-adult behaviors occurring in P₄ (SAKAGAMI & MAETA, 1986), 2) reuse of maternal nests and overwintering as adults in a common burrow (SAKAGAMI & MAETA, 1977, 1985, 1987, 1989), 3) conspicuous size differences among females (SAKAGAMI & MAETA, 1989), 4) prolonged longevity of females, which survived up to 4 years in *C. japonica* COCKERELL (SAKAGAMI & MAETA, 1977, 1985, 1989; KATAYAMA & MAETA, 1979), 5) multiple brooding periods in multivoltine species, and resuming oviposition when immatures were removed from normal nests (SAKAGAMI & MAETA, 1989) and 6) some species are multivoltine. In univoltine species, *C. japonica* and *C. megastigmata* YASUMATSU et HIRASHIMA form semisocial and delayedly eusocial colonies (SAKAGAMI & MAETA, 1977, 1985, 1989; KATAYAMA & MAETA, 1979; YOSHIDA, 1986), while in multivoltine species, *C. iwatai* YASUMATSU, *C. okinawana* (including 2 subspecies, *o. okinawana* and *o. sakishimensis*), *C. nigrolateralis* COCKERELL, *C. boninensis* YASUMATSU and *C. dentipes* FRIES (MAETA, 1993; SAKAGAMI & MAETA, 1989, 1995; MAETA & SAKAGAMI, 1995; MAETA & OKAJIMA, unpubl. DAIMARU, 1998; ADACHI, 2001; MAETA, unpubl.) form eusocial and reversed eusocial (the dominance order reversed between mothers and daughters), as well as the above mentioned semisocial and delayedly eusocial.

These various socialities are compatible with solitary within the same population and place (SAKAGAMI & MAETA, 1989). Types of task allocation between queenlike (Q) and workerlike (W) bees in multifemale nests is formulated by a combination of 4 major tasks: Pollen foraging (P-active; p-not observed), feeding partner (F: observed, f: not observed), oviposition (O-frequent; o-less or absent), guarding (G-principal, g: less frequent or absent). It can be expressed as follows. Q: p f O G and W: P F O (or o) g, showing a clear caste differentiation between them (SAKAGAMI & MAETA, 1995). In social nests, oviposition is always initiated by queenlike bees, but later frequently her eggs are replaced with those of workerlike bees after oophagy of the queenlike bees' eggs, if workerlike bees were fertile (MAETA & SAKAGAMI, 1995). The loss of foragers, resulting in a rapid foraging by queenlike bees was observed in *C. japonica* and *C. okinawana* (SAKAGAMI & MAETA, 1985, 1989). Solitary nesting by other females are confirmed by artificially excluding either queenlike or workerlike bees (MAETA *et al.* unpubl.). Switching to solitary life shows that these species are initially social. Unlike *C. japonica* and *C. okinawana*, the reversibility from social to solitary is rather difficult in *C. nigrolateralis*, which have the most developed sociality among so far studied ceratinid bees (MAETA & OKAJIMA, unpubl.).

In the field, the occurrence of multifemale nests differs, depending on the species (SAKAGAMI & MAETA, 1985, 1989). Multifemale nests are also artificially inducible in those species in which multifemale nests are found in the field, but rather difficult in some ceratinid species that are usually solitary, such as *C. satoi* YASUMATSU, *C. flavipes* SMITH, *C. smaragdula* FABRICIUS, *C. cucurbitina* (ROSSI), *C. callosa* (FABRICIUS), *C. chalcites* GERMAR, *C. cyanea* (KIRBY) and *C. neomexicana* COCKERELL (HYODO, 1987; SAKAGAMI & MAETA, 1987; MAETA *et al.*, 1997a,b, unpubl.).

Insemination rate and ovarian condition in relation to female body sizes are mentioned by SAKAGAMI & MAETA (1985, 1989) in multifemale nests of *C. japonica* and *C. okinawana*

collected from the field. These reports show a distinct correlation between body size and reproductive condition. Uninseminated or ovarially undeveloped females are predominant among small individuals, and play an important role in forming the multifemale nests.

In this paper, how to produce smaller daughters and why such handicapped females remain in maternal nests were experimentally studied in *C. okinawana*. Terminology of socialites used in this paper followed SAKAGAMI & MAETA (1995).

Materials and Methods

Adults of *C. okinawana sakishimensis* SHIOKAWA used for the present study were descendants of the population which had been reared in the greenhouse of Shimane University, Matsue (N 35° 25') since 1995. Original stock of adults was collected in Iriomote Is., the southernmost archipelago of Japan (N 24° 20') in June of 1995.

For experiments, 3 types of cages were used: 1) Large-sized cage (L: 300 cm; W: 200 cm; H: 180 cm), 2) Medium-sized cage (L: 200 cm; W: 150 cm and H: 180cm) and 3) Small-sized cage (L and W: 50 cm; H: 180 cm). Plenty amount of the 9 flowering plants, *Brassica campestris* (Cruciferae), *B. juncea* (ditto), *Phacelia tanacetifolia* (Hydrophyllaceae), *Centaurea cyanus* (Compositae), *Melilotus officinalis* (Leguminosae), *Leonurus sibiricus* (Labiatae), *Lythrum anceps* (Lythraceae), *Cosmos sulphureus* (Compositae) and *Bidens pilosa* (ditto) were successively supplied as floral resources in cages, according to the seasonal flowering sequence.

1. Factors to determine remaining in the maternal nests

Which of the daughters remained as workerlike bee (eusocial) or queenlike bee (reversed eusocial) in her maternal nest was examined in 1998, 1999 and 2000. Overwintered and inseminated new females were liberated in 3 years every April in small cages in which artificial nests (pithy cores of *Kerria japonica* inserted into glass tubes) were set. One nest was set in each small cage. We could directly observe in-nest behaviors of bees (SAKAGAMI & MAETA, 1986). In *C. okinawana*, the number of daughters that remained in their maternal nests is basically a single per nest (SAKAGAMI & MAETA, 1989). In this paper, only those individuals that stayed in maternal nests until the end of the brooding period were regarded as the remainders, and others dispersed from the nest.

To induce normal dispersion of newly emerged females, new artificial nests were added in the cage, but the number of nests set/cage was limited as 1 less than the total number of daughters produced in a nest, so that at least one extra individual remained. Factors that determined a remainder seem to be 1) order of eclosion in a nest, 2) size of body (represented by the head width), 3) age in days from eclosion to start of nesting (on the day when the queenlike bee began oviposition). Eclosed dates and head widths of daughters in all nests were recorded.

2. Reproductive success and relevant matters in relation to the body sizes

Fecundity was represented by the number of eggs laid (=provisioned cells)/nest and the value was examined depending on the different sized females. Sizes and sex ratio of juveniles were also

examined in 2000. The head width of females liberated in the cages were divided into 5 classes, L: > 2.50 mm; M: 2.30–2.49 mm; MS: 2.10–2.29 mm; S: 1.90–2.09 mm; SS: < 1.89 mm. These size classes were also adopted for female and male juveniles. The size classes of bees were easily recognized by the naked eye, but confirmed by measuring their head widths at examination.

Five different sized groups of females were seen (L, M, MS, S and SS), each size involving newly emerged females of 30 in L, 40 in M, 60 in MS, 40 in S and 40 SS with the ratio of one of each sized female to 2 newly emerged MS males, were liberated together at the same time in 5 large cages on July 4 in 2000. Males were excluded on day 7 after liberation. As nesting materials, twice the number of *Rubus palmatus* var. *coptophyllus* (L: 40 cm, Diameter: 8–10 mm) stems than the number of released females were provided in each cage. The nests were dissected after the oviposition period was over and immatures were incubated at 28°C until they developed into adults. The male ratio (males/males+females) and size (head width and wet weight) of female and male juveniles were examined. Weight of adults, who had defecated, were measured within 24 hrs of their emergence without giving food. These items were analyzed in relation to the size of mother bees.

Temporal changes in the head widths of juveniles, which were produced in a nest by 5 different sized groups of mother bees, were examined.

3. Mating success in relation to the body sizes

To study mating completion in relation to the the body sizes in virgin females, the following 3 experiments were carried out by using newly emerged adults on July 20 in 2000. As nesting materials, stems of *Solidago altissima* (Compositae) were supplied with a ratio of one female per 2 stems.

1) To confirm successful mating in the large space, 5 different sized groups of females (L, M, MS, S and SS), each involving 28, 40, 60, 43 and 40 females with same number of MS sized males, were liberated separately in 5 large cages.

2) To confirm occurrence of mating competition among different sized females, 3 different sized groups (L, MS and SS), each involving 20, 20 and 17 females, were liberated together with the same number of MS males in a large cage.

3) To confirm the influence on mating success in relation to the size of males, 3 different sized groups (L, MS and SS), each involving 15, 15 and 17 females with the same number of the same sized males, were liberated separately in 3 small cages.

All males used for the 3 experiments were removed on day 7 after liberation. Mating success was determined by the subsequent presence of female juveniles in nests (only males are produced by unmated bees).

4. Nesting success in relation to the body sizes and availability of nest stems

To study competition among different sized females when they select nest stems, the following 5 experiments were conducted in July of 1999 and in July to August of 2000. Newly emerged L and S females were used, and stems of *R. palmatus* var. *coptophyllus* were supplied as nesting materials. The following experiments of 1)–4) were carried out by using the medium-sized cage

in 1999 and 5) by using the large-sized cage in 2000.

1) To confirm competition to select nest stems between 2 groups of different sized females, L and S, each involving the same number of 50 females, were liberated at the same time in the same large cage. Nest stems were given in the cage with a ratio of one female to one nest stem.

2) To confirm the effect of the number of nest stems, the same experiment as in 1) was conducted, but nest stems were given in the cage with a ratio of 2 females to one nest stem.

3) To confirm the expelling of small females by larger females, 10 L females were liberated 2 weeks after the liberation of 10 S females, and half the number of nest stems as of total females were given in the cage.

4) To confirm the effect of interference behavior between L and S females, observation was carried out in a large cage used for the above experiments of 1) and 2). The rate of expelling success was judged by the replacement of nestowners. Owners of the nests were examined by dissecting all nests in the midst of the experiments of 1)–4).

5) To confirm the effect of delaying release on the nesting success of M and S females, S females were released first and then the same number of M females done on the same day (=day 1), day 2, 4, 6, 8 and 16 after liberation of S females. Nest stems were given in the cage with a ratio of 2 females to each nest stem. Final owners were examined by dissecting nests 2 weeks after each liberation of S females. The size combination of nest-mates was examined when multifemale nests were found in experiment 5.

Results

1. General life history

The important biological features of *C. okinawana*, concerning this study, are mentioned below.

Two new subspecies of *C. okinawana* MATSUMURA *et* UCHIDA are described as *C. okinawana okinawana* and *C. okinawana sakishimensis* SHIOKAWA (SHIOKAWA, 1999). Both subspecies are closely related and distributed in the subtropical region of Japan. The former subspecies is recorded from Okinawa Islands and Amami Islands, and the latter from Miyako Islands and Yaeyama Islands. No clear life history and sociality differs between 2 subspecies, therefore the following descriptions are common to both the subspecies. They choose pithy stems of *Miscanthus sinensis* as nests, growing along the road-side and flat-land, in relatively sunny places. The life cycle pattern of *C. okinawana* is complicated, as shown in Fig. 2 in SAKAGAMI & MAETA (1989). There are 3 generations per year (G_1 – G_3 , OKAZAKI, 1987) and each generation has 2 brood rearing periods ($G_{1.a}$, $G_{1.b}$, etc.) and G_2 has 3 ($G_{2.1.a}$, $G_{2.1.b}$ and $G_{2.2.a}$). Consequently, G_1 consists of descendants of 4 different cohorts, mainly $G_{3.a}$ but partly also of $G_{2.1.b}$, $G_{2.2.a}$ and $G_{3.b}$. This species is basically solitary, but occasionally forms various types of social nests. An egg is deposited on the posterior surface of each pollen loaf, and is not cemented with gluelike material on the burrow floor (MAETA *et al.*, 1997a,b). The pollen loaf and immature in each brood cell are movable at cleaning by both of queenlike and workerlike bees (behavior chain, “cleaning brood cells”, MAETA *et al.*, 1997a,b).

A nest-founding bee usually deposits all her eggs in only one nest burrow, as in other ceratinid bee species. Her brood is composed of several continuous series of sub-broods, each with female eggs preceding male eggs ($\frac{\text{♀}}{\text{♂}}$ type sex distribution, MAETA & SUGIURA, 1990) as in most megachilid bees (SUGIURA & MAETA, 1989). Sex of eggs is voluntarily determined at oviposition, the mother spending a longer time for female eggs (diploid) for fertilization than male eggs (haploid). Prior to oviposition, adequate provisions are stored, according to the size (and sex) of offspring (MAETA & OKAJIMA, 2008).

Life cycle patterns of univoltine ceratinid bees are composed of 7 periods (P_1 – P_7), as mentioned above. These periods are also applicable to *C. okinawana*, but P_6 – P_7 are absent in the first generation (G_1). Adults of the overwintering generation (G_3) do not diapause, just in a quiescent state during the winter (MAETA *et al.*, unpubl.). In P_3 , juveniles are fed first by their mother, later replaced by one of the elder sisters. The mother bee is also fed by these daughters (behavior chain, “feeding adults”, MAETA *et al.*, 1997a, b).

2. Factors that determine bee that remain in their maternal nests

Newly emerged female adults in a nest, arranged from the smallest to largest (H_1 – H_5), and their emergence dates from the earliest to latest (E_1 – E_6) were recorded. Males were removed from the cage after their head widths were measured, because they become an obstacle for starting the nesting. Two types of societies were formed by a mother bee and her remained daughter(s), eusociality and reversed eusociality. The dominance order among eusocial bees became reversed in the latter sociality (SAKAGAMI & MAETA, 1995).

Table 1 shows that the orders of emergence and head width of daughters who remained in their maternal nests as workerlike bees in 33 eusocial nests, or became a queen in 5 reversed eusocial nests. Reversed eusocial generally occurred when the body size of daughters was larger than that of their mothers (SAKAGAMI & MAETA, 1995). Henceforth, we mention the dispersion of daughters and relevant matters without discriminating between the socialities. On the resuming of oviposition by mother bees, nests considered reversed eusocial were excluded.

In the present study, there were some eusocial and reversed eusocial nests in which more than one daughter remained while queenlike bees engaged in successive brooding, but others had dispersed to nest, as described below. Here, the individuals who had stayed as a workerlike bee until the end of brooding in nests regarded as a remainder. The rate of daughters that remained in maternal nests was the highest in the first brood (34.2%, 13/38) and followed by the second brood (23.7%, 9/38). Combined firstly to thirdly emerged daughters became 73.7% (28/38). These facts show that mother bees manipulate to produce smaller daughters at early parts of their laying periods, as described below (see 3.4.). The cause of production of smaller daughters is not due to floral resource, because good flowering condition was maintained by us throughout the experiment periods in all 3 years. These smaller individuals are most apt to remain in their maternal nests. Larger daughters tend to disperse.

As mentioned above, if the number of daughters produced/nest was within 2, the smallest one remained in the nest. However, the other individuals who eclosed later also remained, when more than 3 daughters were produced (Fig. 1). This may imply that earlier eclosed daughters

gradually dispersed from the nests. Interestingly, the last 2 eclosed daughters in 3 nests, which produced a total of 6 daughters, dispersed (Fig. 1). The latest emerged daughters seem to oblige dispersion. In those nests, the pre-laying period (from the eclosion of the first daughter to the commencement of oviposition by the mother) of mothers was extended due to an increase of the number of daughters produced in a nest. During that time, ovaries of those early eclosed daughters developed, and they dispersed from their maternal nests. The resuming of egg-laying activity by the mother was inhibited by daughters who remained longer in their maternal nest. The pre-laying period was gradually extended, according to an increase of the number of daughters produced in a nest, because the duration needed to feed juveniles was obliged to be extended. Eclosion period of daughters (expressed from the date of the first to last eclosed females) was also naturally extended, according to the increase of the number of female broods (Fig. 2). Delaying of mother's oviposition affected the dispersion of those daughters who had eclosed earlier, and later eclosed ones were apt to remain in their maternal nests (Fig. 1).

Feeding juveniles was performed first by the mother bee and later replaced by one of the elder daughters. Table 2 shows the feeding experience and age of daughters at dispersion (age

Table 1. Order of eclosion and head width of daughters who remained in maternal nests (1999 and 2000).

Order of eclosion (E ₁ -E ₆)	Order of head width (H ₁ -H ₅) ¹⁾	Number of nests		
		Eusocial	Reversed eusocial	Total (%)
E ₁	H ₁	10	1	11 (28.9)
	H ₂	1		1 (2.6)
	H ₃	1		1 (2.6)
	Total	12	1	13 (34.2)
E ₂	H ₁	2	1	3 (7.9)
	H ₂	3	1	4 (10.5)
	H ₄	2		2 (5.3)
	Total	7	2	9 (5.3)
E ₃	H ₁	2		2 (5.3)
	H ₂	2	1	3 (7.9)
	H ₃	1		1 (2.6)
	Total	5	1	6 (15.8)
E ₄	H ₂	1		1 (2.6)
	H ₃	1		1 (2.6)
	H ₄	2		2 (5.3)
	H ₅	1		1 (2.6)
	Total	5		5 (13.2)
E ₅	H ₄	1		1 (2.6)
	H ₅	1		1 (2.6)
	Total	2		2 (5.3)
E ₆	H ₃	1		1 (2.6)
	H ₄	1	1	2 (5.3)
	Total	2	1	3 (7.9)
Total		37	5	38 (100)

¹⁾ Head width of daughters is arranged from the smallest to largest.

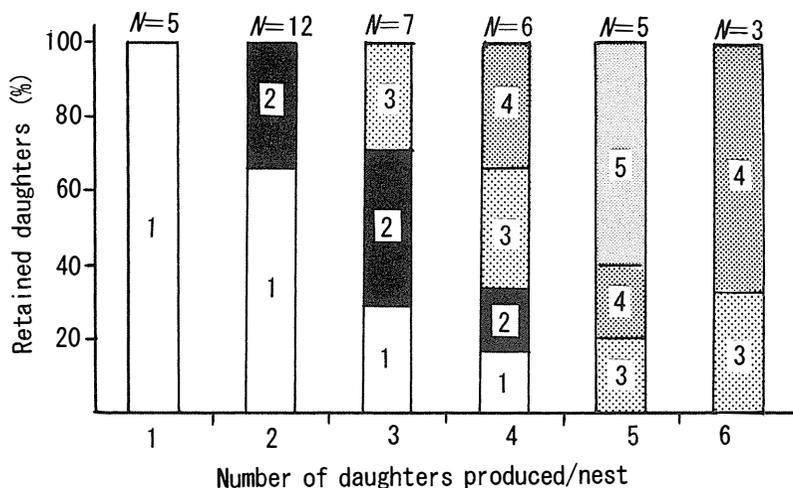


Fig. 1. Percentage of retained daughters, arranged by the order of head widths from the smallest to largest in a brood (shown by 1-5 in each histogram), in relation to the number of daughters produced in a solitary nest (1999-2000). Numerals on the top of each histogram show the number of nests examined, combining 33 eusocial and 5 reversed eusocial nests.

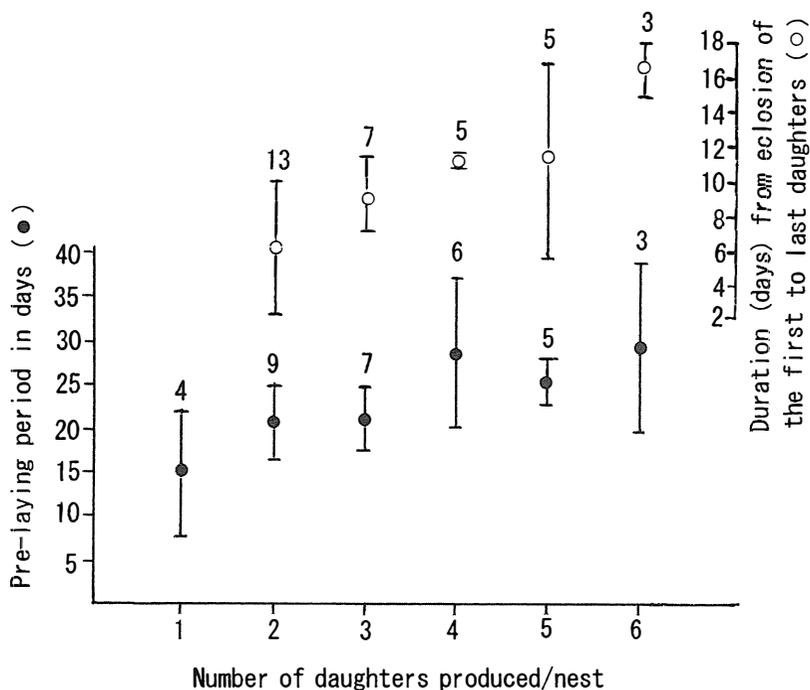


Fig. 2. Relationship between the number of daughters produced in a solitary nest and resuming of oviposition by the mother (1998-2000). Pre-laying period is shown by days from when the first daughter eclosed to the resuming of oviposition by the mother.

Table 2. Age of daughters when they dispersed from maternal nests in relation to the order of eclosion (1999–2000).

Order of eclosion (E ₁ –E ₅)	Presence (+) or absence (–) of feeding	Age in days at dispersion ¹⁾		N
		Range	Mean ± SD	
E ₁	+	11–29	16.5 ± 4.7 ^a	17
	–	10–12	11.0 ± 1.0 ^a	3
	To	11–29	15.7 ± 4.8	20
E ₂	+	14–25	20.8 ± 5.0 ^a	6
	–	4–16	10.4 ± 3.7 ^b	10
	To	14–25	14.3 ± 6.6	16
E ₃	+	12–20	15.3 ± 3.4 ^a	4
	–	9–22	13.9 ± 4.3 ^a	10
	To	19–22	14.3 ± 4.0	14
E ₄	+	10–28	18.0 ± 7.6 ^a	4
	–	5–18	11.4 ± 4.9 ^a	5
	To	10–28	14.3 ± 6.8	9
E ₅	+	13–15	14.0 ± 1.4 ^a	2
	–	12–18	14.3 ± 3.2 ^a	3
	To	13–18	14.2 ± 2.4	5

¹⁾ Shown by days from eclosion. Significant difference is indicated by different letters (a, b) between presence and absence of feeding sibs in the same order of eclosion ($p < 0.05$, BONFERRONI's multiple comparison).

is expressed by days from eclosion to dispersion). There was a tendency that daughters who had experience feeding sibs were delayed dispersal from maternal nests more than those had no feeding experience. There was no significant difference between both daughters, except for the case of secondly eclosed daughters in nests ($p < 0.05$, BONFERRONI's multiple comparison). No relation was recognized between the order of eclosion and days for staying in nests until dispersion. Daughters dispersed 4–29 days after their eclosions and most of them stayed their maternal nests for 14–16 days on average. These facts show that feeding of sisters and brothers is not a major factor to inhibit dispersion from their nests. A single daughter always participated in feeding nest mates in the nest at a given time, and it was usually the first eclosed and the smallest daughter.

Mother bees sometimes resumed their oviposition in spite of more than 2 daughters remaining in the nests, however, dispersion of these daughters continued. Such cases were found in 11 out of 38 nests (28.9%) examined in 1999 and 2000. Sixteen out of a total of 64 dispersed daughters (25%) were left in maternal nests since mother bees had resumed their oviposition. Thirteen of them (81.3 %, 13/16) were major sib feeders. Finally, a stable combination of 2 mother and daughter females was established.

As an important factor to enhance dispersion of daughters, availability of nest stems, is pointed out. If we supplied plenty of nest materials in the cages, some of the smaller females might have also dispersed. However, it is known that some individuals remained in maternal nests in *Ceratina iwatai* YASUMATSU, even though abundant nest materials were supplied (MAETA, 1993).

3. Reproductive success and relevant matters in relation to body size

3.1. Depths of burrow

Burrows became deeper, according to an increase of the head widths of females. The depths of burrows excavated by SS and S females were significantly shallower than those made by MS, M and L females, but with no significant difference among the latter 3 groups (MS, M and L) (Table 3). It may suggest that smaller females make fewer brood cells/nest than larger females do. This occurs in *C. flavipes*, which use the same nest materials (GOUBARA, 1989).

3.2. Oviposition numbers

Reproductive success in relation of maternal body size was studied in many hymenopteran insects (e.g., COWAN, 1981; FREEMAN, 1981; TEPEDINO & TORCHIO, 1982; TORCHIO & TEPEDINO, 1980; SUGIURA & MAETA, 1989).

The tendency for an increase of the number of eggs laid/female was obvious, according to an increase of head widths (size), but no significant difference was obtained among 5 different sized females (Table 3). MAETA *et al.* (1992) compared the number of brood cells/nest between medium- (M, head width: 2.10–2.27 mm) and large-sized females (L, > 2.27 mm) of *C. flavipes*, which were reared in a cage with plenty of floral plants. No significant difference between M and L females was seen. A similar result was reported in *C. calcarata* (JOHNSON, 1990). Number of eggs laid by ceratinid bees seems to be stable and does not differ within the same species depending on the body sizes. However, a clear significant difference was proved among different sized females in a mason bee, *Osmia cornifrons* (RADOSZKOWSKI)(SUGIURA & MAETA, 1989).

3.3. Male ratio and wet weight of juveniles

Male ratio was 0.30 in juveniles produced by L mothers, showing an extremely female-skewed sex ratio. On the other hand, the sex ratio of juveniles produced by SS mothers was extremely male-skewed (0.75). A clear significant difference of male ratio was obtained from juveniles produced by different sized mothers as follows: L/MS, L/S, L/SS, M/S, M/SS, MS/SS and S/SS (Table 3). Figure 3 shows the relationship between head width of mother bees and male ratio of their juveniles. A very clear negative correlation was obtained between them with one percent level of correlation coefficient. Larger mothers tend to invest for production of daughters, while smaller ones for production of sons. Such tactics were well known in mason bees, e.g., *Osmia lignaria* Say and *O. cornifrons* (TEPEDINO & TORCHIO, 1982; SUGIURA & MAETA, 1989).

Wet weights (mg) of juveniles decreased in both sexes, according to the size decrease of their mothers. A significant difference among juveniles produced by different sized mothers is as follows: L/S, L/SS, M/S, M/SS, MS/SS and S/SS in female juveniles. L/M, L/MS, L/S, L/SS, M/MS, M/S, M/SS, MS/SS, and S/SS in male juveniles (Table 3). The heavier female juveniles were derived from larger mothers. This seems to be phenotypic variability resulting from higher foraging ability of large mothers as suggested in *C. calcarata* (JOHNSON, 1990).

These weight differences among juveniles produced by different sized mothers are due to the amount of pollen loaf stored in a brood cell by them. It may relate to the difference in amount of pollen load carried into brood cells by a single foraging trip, depending on the size of mother bees. Larger mother bees can increase their investment to their offspring and result in producing large-sized juveniles. On the other hand, smaller mother bees tend to invest in her male offspring

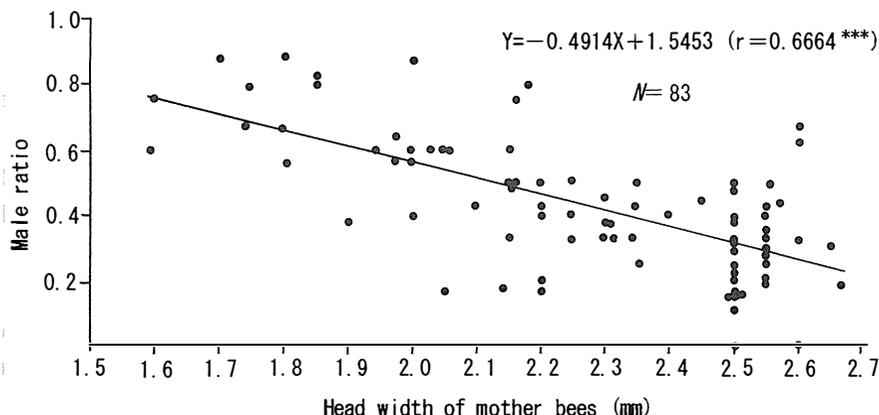


Fig. 3. Relationship between the head width of mother bees and the male ratio of juveniles produced in solitary nests (2000).

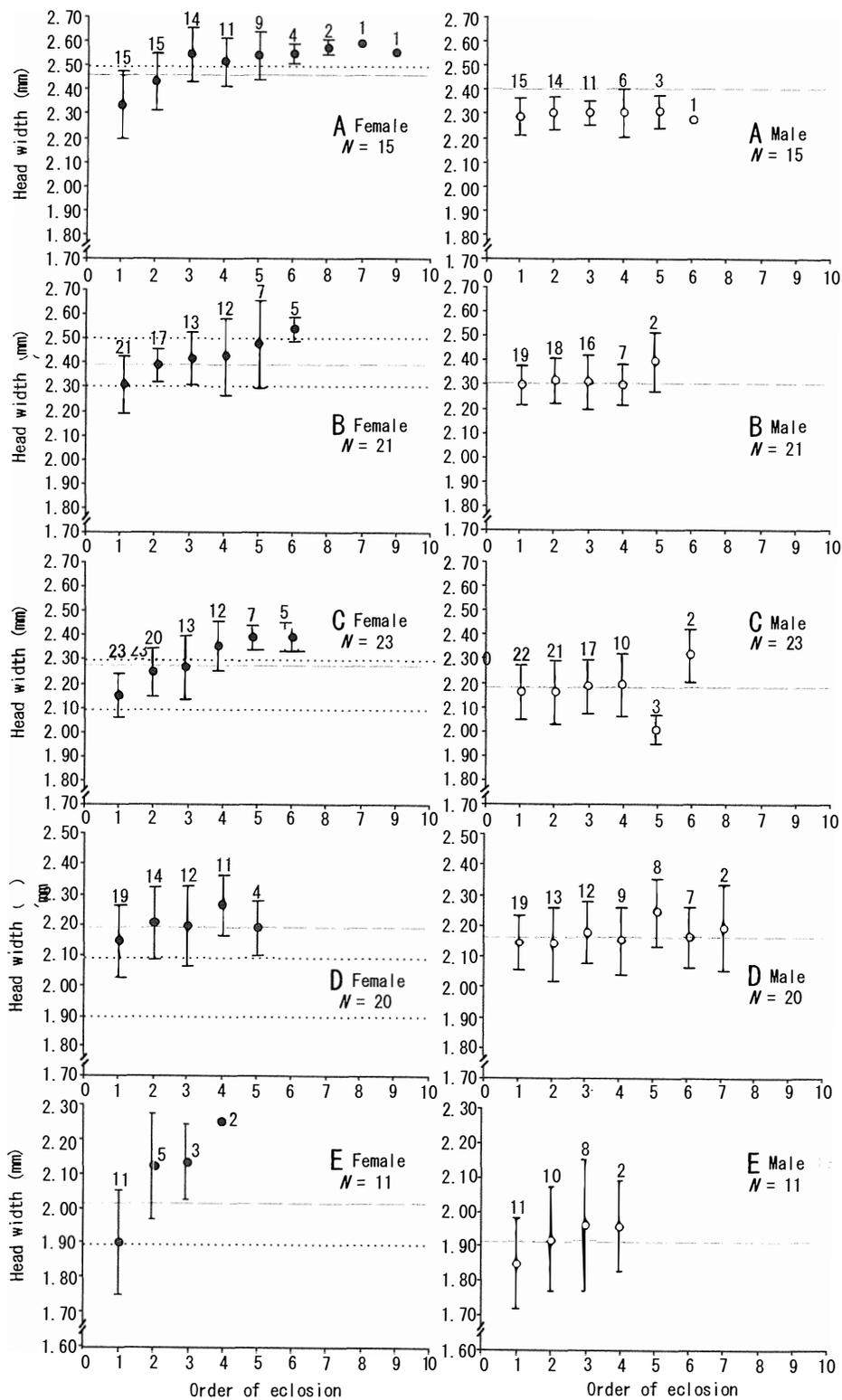
who can mature on smaller provisions, without reducing their oviposition numbers. A similar phenomenon, in an increase of the male ratio and reduction of wet weight of juveniles is reported in *C. flavipes* (MAETA *et al.*, 1992).

3.4. Temporal changes in the head widths of juveniles that emerged borrows

Figure 4 depicts the head widths of juveniles produced by 5 different size groups of mother bees. Female and male juveniles are arranged separately, according to the order of eclosion in each nest burrow. Dead immatures were skipped and the next living juvenile was moved up to its expected eclosion order, however, the mortality of immatures was low in 5 different sized mother bees (7.6% in L; 25.5% in M; 15.8% in MS; 12.8% in S; 14.8% in SS). Moreover, those nests nidificated by unseminated mother bees, producing only males, were excluded from analysis. The first brood cell contained female offspring in the great majority of nests in 5 different sized groups of mother bees (92.9% in L; 93.8% in M; 89.5% in MS; 100.0% in S; 100.0% in SS), as are well known in *C. calcarata* and other congeneric species (JOHNSON, 1988; MAETA *et al.*, 1992, unpubl.).

Sex distribution in a burrow of ceratinid bee species, including *C. okinawana*, is composed of several continuous series of sub-broods, each with a female egg preceding ($\text{♀}\text{♂}$ type, MAETA & SUGIURA, 1990), as in nests of most megachilid bees (SUGIURA & MAETA, 1989). However, founding bees of ceratinid species anticipate the sex of eggs to be laid, and store an adequate amount of provision, depending on the sexes of the juveniles. Time spent for laying eggs differed by the sex of eggs. A longer time was spent laying female eggs than males. Surprising, unseminated females behaved as if laying female eggs, if they were inseminated (MAETA & SAKAGAMI, 1995; MAETA *et al.*, 1997a, b). Thus, females evidently do not know whether they are inseminated or not at the moment of oviposition (MAETA & OKAJIMA, 2008). These facts suggest that anticipated investment patterns for offspring did not differ between inseminated and unseminated mother bees.

The head widths of the first 2 or 3 emerged female juveniles were smaller than those that emerged later in the same nests, irrespective of the sizes of their mother bees. A similar trait



was reported in other ceratinid bee species, e.g., *C. flavipes* SMITH and *C. calcarata* ROBERTSON (MAETA *et al*, 1992; JOHNSON, 1988). Possible causes that produce the smaller-sized juveniles at the beginning period of oviposition, 2 factors might be estimated. 1) Flower dearth at this corresponding period and 2) manipulation by mother bees. The first estimation can be denied, because plenty of flowering plants had been supplied in the cages throughout the oviposition period. The second one was very possible, as described in the discussion.

The head widths of female juveniles that emerged after the third ones were nearly the same sizes of those of the mother bees, as in the mothers' size classes of L and M. However, they were larger than those of their mother bees in the size classes of MS, S and SS. It is a very remarkably interesting phenomenon. Mother bees who were smaller than those of L and M are capable to compensate their juveniles' body sizes to normal sizes. Figure 5 depicts the mean ratio of head widths between daughters and mothers. The ratio in 4 sized groups of mother bees (SS, S, MS and M) exceeded 1.0, while it was nearly 1.0 in the L group of mother bees. Such a phenomenon was very clearly shown in the much smaller mother bees of the groups of S and SS.

Compensation for body size in daughters produced by smaller-sized mother bees is well known in a mason bee, *Osmia cornifrons*. In this species, the ratio of mean wet weight between daughters and mothers was 1.06 in L females, 1.43 in M females and 1.58 in S females. In S females, fecundity was inferior to that of L and M females, but there was no difference of nesting periods among 3 size groups of females (S, M and L). They can possibly compensate by increasing the number of foraging trips to make each pollen loaf (SUGIURA & MAETA, 1989).

4. Mating success in relation to body sizes

In the first experiment, the percentage rate of insemination (number of inseminated females/total number of liberated females) was examined in those females, belonging to the 5 sized groups of L, M, MS, S and SS, each group liberated separately in 5 different large cages. The insemination rate in 5 groups of females very gradually decreased, according to the size reduction of females. However, the insemination rate was normally high in L, M, MS and S, but extremely low in SS. Significant difference was obtained between SS/L, SS/M, SS/MS and SS/S ($p < 0.001$, χ^2 -test). As to the percentage rate of nesting (number of females that nested in stems/total number of females liberated), high insemination rates were also recognized in 4 groups of L, M, MS and S, and the same significant difference was recognized as in that of insemination (Table 4).

In the second experiment, females of 3 different sized groups (L, MS and SS) were liberated together in the same large cage. The percentage rate of insemination was high in L and MS females, but extremely low in SS females. The nesting rate was a similar trait as in the insemination rate. In both rates there was a significant difference between SS/L and SS/MS

←

Fig. 4. Temporal changes in the head widths of juveniles produced in solitary nests of 5 different sized mother bees (A–E, 2000). Both sexes of juveniles are separately arranged, according to the order of eclosion from the inner to outer in a burrow nest. A: L (> 2.50 mm, N=15); B: M (2.30–2.49 mm, N=21); C: MS (2.10–2.29 mm, N=23); D: S (1.90–2.09 mm, N=20); E: SS (< 1.89 mm, N=11). Solid horizontal line in each figure shows the mean value of head width of female and male juveniles. The range of head widths of each size classes of mother bees are also shown by dotted horizontal lines in the figure for female juveniles.

Table 3. Comparison of reproductivity and relevant matters in relation to maternal body sizes (2000).¹⁾

Size group of females (range of head width in mm)	Depth of burrow (cm)		No. of eggs laid		Male ratio (N)	Wet weight of juveniles ²⁾	
	Range	Mean ± SD (N)	Range	Mean ± SD (N)		Female (N)	Male (N)
L (>2.50)	6.9–19.9	12.6 ± 3.1(20) ^b	6–17	9.1 ± 2.5(20) ^a	0.30 ± 0.16(34) ^c	24.9 ± 3.7(70) ^c	24.2 ± 2.9(65) ^d
M (2.30–2.49)	8.2–19.6	11.6 ± 2.6(29) ^b	5–17	8.9 ± 2.4(29) ^a	0.38 ± 0.07(11) ^{d,c}	22.5 ± 3.5(76) ^b	19.7 ± 3.1(88) ^c
MS (2.10–2.29)	6.0–20.9	11.5 ± 3.5(32) ^b	4–18	8.5 ± 3.1(32) ^a	0.45 ± 0.17(18) ^{b,c}	19.2 ± 3.2(78) ^a	17.1 ± 3.3(115) ^b
S (1.90–2.09)	3.5–20.2	10.6 ± 3.4(30) ^a	3–15	7.9 ± 3.0(30) ^a	0.56 ± 0.15(17) ^b	17.4 ± 3.1(58) ^a	16.6 ± 2.7(149) ^b
SS (<1.89)	5.5–12.5	8.7 ± 1.9(17) ^a	5–12	7.6 ± 2.0(17) ^a	0.75 ± 0.12(10) ^a	15.7 ± 5.1(2) ^a	12.1 ± 2.5(94) ^a

¹⁾ Significant difference is indicated by different letters (a, b, c, d) among different sizes of founding bees in each item ($p < 0.05$, BONFERRONI's multiple comparison). ²⁾ Measured within 24 hrs of eclosion without giving food.

Table 4. Percentage rate of inseminated and nesting females in 5 different size groups of bees who were liberated separately in the large cages with the same number of MS males (2000).¹⁾

Size group of females (range of head width in mm)	Inseminated females (%)	Nesting females (%)	Number of females liberated
L (>2.50)	96.0 ^a	89.3 ^a	28
M (2.30–2.49)	89.2 ^a	92.5 ^a	40
MS (2.10–2.29)	84.2 ^a	96.7 ^a	60
S (1.90–2.09)	73.7 ^a	88.4 ^a	43
SS (<1.89)	27.8 ^b	45.0 ^b	40

¹⁾ The same number of nest stems as that of liberated females was supplied. Significant difference was obtained between different letters (a, b) in the same items ($p < 0.01$, χ^2 -test).

Table 5. Percentage rate of inseminated and nesting females in 3 different size groups of bees who were liberated together in the same large cage with the same number of MS sized-males (2000).¹⁾

Size group of females (range of head width in mm)	Inseminated females (%)	Nesting females (%)	Number of females liberated
L (>2.50)	94.7 ^a	95.0 ^a	20
MS (2.10–2.29)	82.4 ^a	85.0 ^a	20
SS (<1.89)	30.0 ^b	58.8 ^b	17

¹⁾ The same number of nest stems as that of liberated females was supplied. Significant difference was obtained between different letters (a, b) in the same items ($p < 0.01$, χ^2 -test).

($p < 0.001$, χ^2 -test) (Table 5).

From these 2 experiments, the mating and nesting successes may relate to the body sizes of females, but all the females were paired with MS sized-males. The results indicated that extremely small individuals were not only apt to fail in mating but also in nesting, irrespective of the situations where bees were liberated. Those SS females, who did not nest, were flying around in corners of the cages without searching for the nest stems.

In the third experiment, to study the space effect on mating success in 3 groups of different sized females (L, MS and SS), with respectively same sized males (L, MS, SS) were liberated separately in 3 small cages (capacity is 1/24 of the large cage). The percentage rate of insemination was extremely low in all 3 groups of females, although the nesting rate was high (Table 6). Comparing those of L and MS females reared separately in the large and small cages (Tables, 4 and 6), a clear significant difference of insemination rates in both L and MS females was obtained between the 2 experiments ($p < 0.001$, χ^2 -test). The size of males paired with L females was MS in the above 2 experiments (Tables 4 and 5), but the rate of insemination did not significantly differ between L/MS in both experiments. Moreover, a high rate of insemination in MS females paired with the same MS males was obtained (Table 6). The cause of low insemination rate in L females paired with L males is not due to the pairings with the same sized individuals (Table 6). It is apparent that small cage space becomes an obstacle to induce normally mating behavior. However, the insemination rate in SS females was always very low, irrespective of the size of cages in which they were liberated.

Figure 6 depicts the relationship between the percentage rate of insemination and the head width of SS females (> 1.89 mm), precisely dividing them from 1.55 to 1.89 mm. Twenty eight SS females nesting in cages were used for the present analysis (Tables 4 and 5). As shown in the figure, those females ($N=10$) whose head width was less than 1.70 mm could not mate, but the percentage rate of insemination gradually increased in those females whose head width was above 1.79 mm, according to an increase of their head widths.

The size difference (%) of head width ($100 \times (H_m - H_f) / H_m$) between MS males (H_m : 2.10–2.29 mm) used for mating partners and the smallest female (H_f : 1.70 mm) was 24 to 35%, and between MS males and L females (> 2.50 mm) was –21 to –40%. Successful mating was accomplished when size variation between males females was within the above mentioned ranges. However, there may be a clear assortative mating in ceratinid bees, as is in reported in other insect taxa.

Mating behavior of *C. okinawana* was not precisely observed in the present study. However, males patrolled to search for females in cages without expelling other males. Males pounced on the females and began to perform courtship displays. They repeated stretching the wings upward, together with rubbing both sides of the female's metasoma with the hind legs. It seems to be difficult to perform a formal mating display between pairs whose body sizes are extremely different.

A supplemental experiment by liberating 13 SS females together with the same number of similar sized males, who were matched for female sizes, was carried out in a large cage in 2000. However, the rate of insemination scarcely attained 53.8% ($N=7$). Presumably, other factors may prevent normal mating, other than size difference, may have intervened.

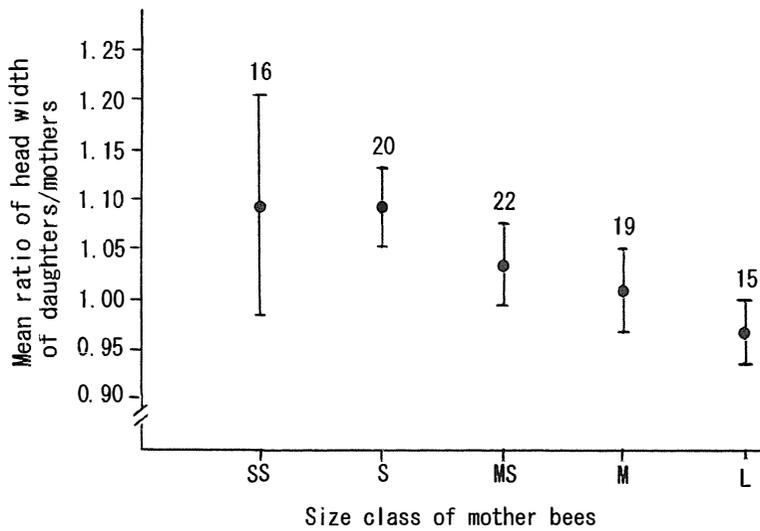


Fig. 5. Size relationship between mother bees and their daughters (2000). Numerals in the figure shows the number of nests examined. Head widths of 5 classes (L, M, MS, S and SS) see in Fig. 4.

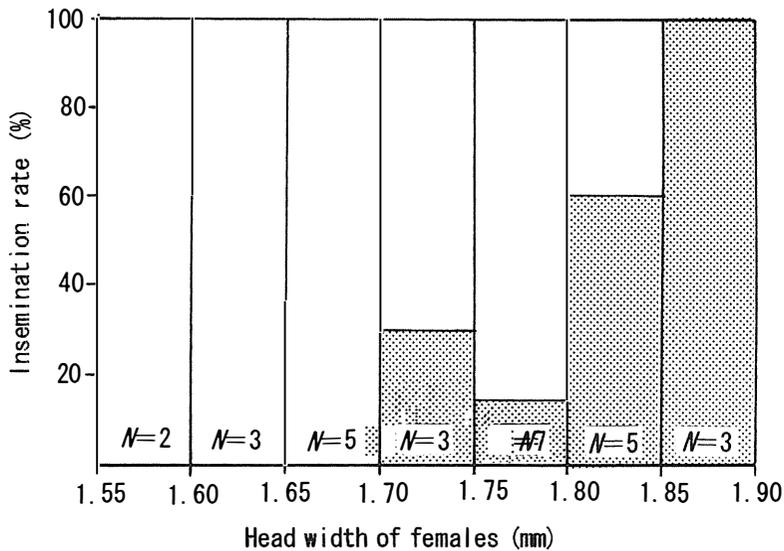


Fig. 6. Insemination rate (%) of 28 SS-sized females liberated together with the same number of MS-sized females was analyzed precisely in relating to their head widths (2000). Based on females in Tables 4 and 5.

5. Nesting success in relation to body sizes and availability of nest stems

In previous section (4.), the nesting success is also mentioned, but not dealt with in relation to the availability of nest stems.

In the experiment I, in which the same number of nest stems as of L and S females (50 stems:

Table 6. Percentage rate of inseminated and nesting females in 3 different sized groups of bees who were liberated separately in the large cages with the same number and the same sized males (2000).¹⁾

Size group of females (range of head width in mm)	Inseminated females (%)	Nesting females (%)	Number of females liberated
L (>2.50)	20.0	100	15
MS (2.10–2.29)	26.7	100	15
SS (<1.89)	28.6	82.3	17

¹⁾ The same number of nest stems as that of liberated females was supplied. No significant difference among 3 groups of L, MS and SS ($p > 0.01$, χ^2 -test).

50 females) was supplied, the nesting rate in L and S females was very high, and no significant difference was obtained between them. On the other hand, in experiment II, in which there were 1/2 as many as total number of L and S females, only 5% of S females nested. In experiment III, in which L females were liberated after all of the S females had selected nest stems, S females were expelled from their nests and these nests were usurped by L females. Finally, the rate of nesting declined to 20.0% (Table 7). Three experiments proved that size competition conspicuously occurred between L and S females when there was a shortage of nest stems.

An interfering behavior was observed between L and S females, and usually actors were L females and actees were S females. Actor interfered with the actee by attacking with her body. This behavior was frequently observed soon after liberation of bees when they were excavating stems. Bees were apt to abandon their excavation by the interference, because they had no strong persistence when first excavating nests. Usurpation by the large actor occurred, when the actee had excavated the burrow deeper and egg-laying had commenced in some nests. Actor pulled actee out of the nest by biting either the actee's hind legs ("pulling out", MAETA *et al.*, 1997a). Finally, the owner of the nest was replaced by an actor's usurpation. Frequency of interference was higher in L against S than in S against L ($p < 0.05$, BONFERRONI's multiple comparison test). Moreover, L succeeded in expelling S at high rate ($p < 0.05$, ditto), but S did not expel L successfully. Successful percentage rate of the expelling between the same sized females usually resulted in half and half. Usurpation ended similarly between L and S as in the cases of interference between them. The successful rate of the both interference and usurpation between L and S was similar, irrespective of bee density (number of bees liberated/number of nest stems supplied) (Table 8).

In experiment 5, in which M females were liberated 6 times, from the same day to 16 days after small females had been liberated, the final nest owners were examined (Table 9). The percentage rate of nesting in M females was significantly higher than that of S females which were liberated on the same day as in other cases mentioned above ($p < 0.05$, χ^2 -test). However, the rate was decreased gradually in M females, according to the delay of liberation, while it was gradually increased in S females. The rate of nesting in populations of M females liberated on the same day

Table 7. Percentage rate of stems used as nests in relation to 3 different combinations of 2 different sized females liberated in cages (1999).¹⁾

Experiment	Date of liberation		No. of bees L/S	Bee density ³⁾	Rate of nesting		No. of stems supplied
	L	S			L	S	
I	July 13	July 13	25/25	1.0	92.0 ^a	84.0 ^a	50
II	July 21	July 21	20/20	0.5	90.0 ^a	5.0 ^b	20
III ²⁾	Aug. 11	July 28	10/10	0.5	80.0 ^a	20.0 ^b	10

¹⁾ Significant different was indicated by different letters (a, b) between L and S in the same experiments ($p < 0.05$, FISHER's exact probability test).

²⁾ L females were liberated after confirmation that all S females had nested.

³⁾ Total number of L and S males/total number of stems.

Table 8. Frequency of the occurrence of expelling behavior and the rate (%) of success in selection of nest stems among L- and S- sized females (1999).¹⁾

Actor and actee ²⁾	Expelling ³⁾						Usurpation ⁴⁾					
	25 L and 25 S liberated with 50 nest stems			20 L and 20 S liberated with 20 nest stems			25 L and 25 S liberated with 50 nest stems			20 L and 20 S liberated with 20 nest stems		
	Success	Failure	Total	Success	Failure	Total	Success	Failure	Total	Success	Failure	Total
L → S	25 (75.8) ^a	8 (24.2)	33 (100)	9 (100) ^a	0 (0)	9 (100)	8 (88.9) ^a	1 (11.1)	9 (100)	6 (85.7) ^a	1 (14.3)	7 (100)
L → L	—	—	—	9 (56.3)	7 (43.7)	16 (100)	—	—	—	5 (26.3)	14 (73.7)	19 (100)
S → L	3 (35.7) ^b	5 (62.5)	8 (100)	5 (33.3) ^b	10 (66.7)	15 (100)	0 (0)	11 (100)	11 (100)	0 (0)	4 (100)	4 (100)
S → S	—	—	—	3 (50.0)	3 (50.0)	6 (100)	3 (33.3) ^b	6 (66.7)	9 (100)	1 (25.0) ^b	3 (75.0)	4 (100)

¹⁾ Observation was made for one week. Significant difference was indicated by different letters (a, b) between L → S and S → L in the same items ($p < 0.05$, FISHER's exact probability test).

²⁾ Left female is actor and right actee.

³⁾ Success means that actor expelled actee and fail means that actor could not expel actee.

⁴⁾ Success means that actor usurped actee's nest by pulling previous owner out. Fail means that actor could not usurp actee's nest.

to 4 days after liberation of S females was significantly higher than those of S females ($p < 0.05$, χ^2 -test), but no difference was recognized between them after 6 days delay ($p > 0.05$, χ^2 -test) (Table 9).

Increase of nesting rates in S females may relate to the persistency of their nests. At the beginning of excavation, bees are apt to easily abandon their nests by interference of the larger individuals, later however, they seem to protect their nests from invaders. In those nests of S females when more than 6 days after liberation elapsed, the building of brood cells was recognized (Table 10). The rate of nests, in which foraging activity had commenced, was 53.3% on day 4, but it finally attained 100% on day 6 after liberation. In such nests, bees persistently guarded their nest entrances. Nevertheless, almost 40% of nests of S females were usurped by M females (Table 9).

In Japanese ceratinid bees, variable nesting environments are reported and differ by species. They use broken twigs and stems of various pithy plant species (MAETA *et al.*, 1994). *Ceratina flavipes* select stems of *Miscanthus sinensis* with appropriate diameters, depending to their body sizes (GOUBARA, 1989; NEGORO, 1980). Various factors, such as habitat segregation, selection of the nest materials and their diameters, may function to reduce the competition among congeneric species and individuals within the same species (MAETA *et al.*, 1994). However, competition over

Table 9. Effect of delaying release of M-sized females on the rate of nesting of S-sized females liberated in 6 large cages (2000).

Days from liberation of S sized-females ¹⁾	No. of females liberated		No. of nest stems supplied	Owners in nests (%) ²⁾					
	S	M		M	M+M	M+S	M+S+S	S	Absent
0	41	41	41	78.0	2.4	0	0	17.1	2.4
2	40	40	40	70.0	7.5	2.5	2.5	17.5	0
4	40	40	40	86.5	0	2.7	0	10.8	0
6	30	30	30	50.0	0	13.3	0	36.7	0
8	40	40	40	55.0	0	5.0	0	40.0	0
16	20	20	20	35.0	0	5.0	0	60.0	0

¹⁾ The day on which M-sized females were liberated.

²⁾ Nests were dissected at night 2 weeks later, after each liberation of M-sized females.

Table 10. Progress of nesting in S-sized females (2000).

Days after liberation	Depth of burrows (cm) ¹⁾		Number of brood cells		N
	Range	Mean ± SD	Range	Mean ± SD	
2	1.2–12.2	8.2 ± 3.4	0–0	0	26
4	1.6–16.7	10.9 ± 4.3	0–2	0.8 ± 0.9	15
6	5.6–12.4	10.0 ± 2.4	1–1	0.8 ± 0.5	8
8	5.7–12.2	9.4 ± 1.7	1–4	1.9 ± 0.8	13
16	9.3–15.5	12.4 ± 2.0	1–4	2.3 ± 0.8	15

¹⁾ Significant difference was not obtained in the depth of burrows and number of brood cells among different dates on which S-sized females were liberated ($p > 0.05$, BONFERRONI'S multiple comparison).

the selection of nest stems is unavoidable under the shortage of nest materials. VELTHUIS (1987) mentioned the advantage of sociality in *Xylocopa sulcatipes* under the circumstance of shortages of nest materials and floral resources.

Discussion

1. Patterns of offspring production and sex allocation

In ceratinid bees, including *C. okinawana*, sex distribution in a single burrow is composed of several continuous series of ♀♂ type (initiated with females and ended with males). The size of each sub-brood was regularly reduced, toward the nest entrance, according to its order. On the other hand, male ratio within each sub-brood gradually decreased. Conspicuously small-sized female juveniles, who are produced at the early part of the egg-laying period, seem to have resulted from the mother's manipulation, so as to produce replacement females who feed their sibs, if the mother should disappear prior to the mother-juvenile cohabiting period (MAETA *et al.*, 1992). A female-skewed sex ratio is prominent among ceratinid bees. Arrangement of several series of sub-broods with ♀♂ type sex distribution and a female-skewed sex ratio enables replacement of feeders, and to keep the later emerged females in maternal nests as workerlike bees, even though elder sisters had dispersed.

2. Social significance of producing smaller daughters

In multifemale nests of ceratinid bees, the number of inhabitants is usually 2, sometimes up to 4, depending on species (SAKAGAMI & MAETA, 1977, 1985). However, 2 females are stable, because they make a single burrow in the nest stems, and the number of pollen loaves (=eggs) to be laid eggs is very limited, only one in a day at the maximum, irrespective of the number of inhabitants. This may also relate to their oviposition time, which is concentrated at late afternoon to early evening in most species (MAETA & SAKAGAMI, 1996; DAIMARU, 1998; MAETA *et al.*, unpubl.). The size variation is remarkable among nest-mates in multifemale nests (SAKAGAMI & MAETA, 1989, 1995). Moreover, it is suggested that the occurrence rate of multifemale nests is higher in those species of which size variation is prominent (SAKAGAMI & MAETA, 1977). These facts imply that size difference in females functions as one of the most important factors to induce multifemale nests.

As mentioned above, smaller juveniles have several handicaps as compared with larger ones, 1) a tendency to less fecundity, 2) extremely male-skewed sex ratio in offspring, 3) lower mating success and 4) lower nesting success. It is rather difficult for those handicapped females independently to make their own nests. Especially, 4) is prominently enhanced to induce the multifemale nests under such circumstances where nesting stems are in shortage. Smaller females are apt to remain in their maternal nests as workerlike bees. Production of smaller female juveniles at the early part of the egg-laying period (the first to third broods) seems to commonly occur in ceratinid bee species, even in an "almost" solitary species, *C. flavipes* (MAETA *et al.*, 1992; SAKAGAMI & MAETA, 1987). This must be one of the precursory social traits manipulated by mother bees, so as to let them remain in maternal nests and form multifemale nests.

Although caste differentiation is clear in multifemale nests, queenlikes always first lay eggs (primary oviposition) in most ceratinid bee species, but those eggs laid by them are occasionally replaced with those of workerlike bees by their secondary oviposition (after partner's primary oviposition, preceded by oophagy, MAETA & SAKAGAMI, 1995), irrespective of social types (MAETA & SAKAGAMI, 1995). Egg replacement is frequently repeated throughout the egg-laying period, so far as workerlike bees have the capability to lay eggs. On the other hand, oviposition is virtually monopolized by queenlike bees in those multifemale nests of which workerlike bees are infertile, showing that the castes function normally as in other social bees. As mentioned above, some workerlike bees are belonging to the smallest-sized group are usually unseminated and infertile. Cell partition is always made by the individual who laid an egg, soon after her oviposition. Two behavior chains, "oviposition" and "preparation of cell partition", successively proceeds without pausing, and are performed by the individual who laid an egg (SAKAGAMI & MAETA, 1995; MAETA & SAKAGAMI, 1995; MAETA *et al.*, 1997a).

Ovarian development of workerlike bees seem to be inhibited by queenlike bees through the size difference between them. No overt agonistic behavior between nest-mates is recognized in ceratinid bee species of which in-nest behaviors are so far studied up to now. MAETA & OKAJIMA (unpubl.) analyzed the ovarian inhibition by queenlike bees in 4 ceratinid species. They used 2 following formulae to express ovarian inhibition by $100 \times (EQ - EW / EQ)$ (EQ and EW: Total number of eggs laid by queenlike and workerlike bees, respectively) and the size difference by $100 \times (HQ - HW / HW)$ (HW and HQ: Head width of workerlike and queenlike bees, respectively). Ovarian inhibition was basically determined by the size difference between nest-mates, but its effect differed by species. In multivoltine species of *C. okinawana*, ovarian inhibition gradually increases, according to the size difference, and infertile workerlike bees occurred when the size difference exceeds at 20%, while in *C. nigrolateralis* complete ovarian inhibition is recognized even if there is no distinct size difference between nest-mates. On the other hand, in univoltine species of *C. flavipes* and *C. japonica*, ovarian inhibition does not occur with less than 20% of size difference. A tendency to gradual ovarian inhibition is exhibited in those pairs whose size difference exceeds 20% in *C. japonica* (MAETA & OKAJIMA, unpubl.).

In addition to the size difference, kinship also affects the ovarian inhibition of workerlike bees, but age does not. Some of the infertile workerlike bees of *C. okinawana* and *C. nigrolateralis* occasionally participated in making the cell partitions after oviposition by queenlike bees (MAETA & SAKAGAMI, 1995; MAETA & OKAJIMA, unpubl.), suggesting that caste differentiation is more advanced.

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