Comparison of the Prepupal Diapause in Two Sibling Megachilid Species, *Megachile* (*Chelostomoda*) *spissula* COCKERELL and *Megachile* (*Chelostomoda*) *esakii* YASUMATSU, Occurring in Different Climatic Zones (Hymenoptera, Megachilidae)

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Abstract In 2 sibling species of megachild bees, *Megachile spissula* and *M. esakii*, the breaking of prepupal diapause was studied. Both species are univoltine. The former occurs in temperate zone and the latter in subtropical zone. The low temperatures of 14℃–18℃ for 3 months was effective subjecting prepupae in *M. spissula* to break diapause. In *M. esakii*, similar low temperatures (15℃–18℃) and also a little higher low temperature (21℃) were effective but very shorter duration (about a month or less). The developmental threshold temperatures in both non-dormant prepupae and subsequent pupae were a little lower in *M. spissula* than *M. esakii* which were 0.9℃ in females and 2.2℃ in males of prepupae, and 1.9℃ in females and 3.1℃ in males of pupae. These differences of thermal response between 2 species may have close relation with their distributional ranges. *Megachile esakii* seems to adapt inhabiting in the subtropical zone, where low temperatures are for short period of time.

Key words: Prepupal diapause; subject to low temperatures; sibling species; temperate and subtropical zones; developmental threshold temperature; *Megachile*.

Introduction

A group of bee species, occurring in temperate zone, overwinter either at prepupal or adult stages, except for those species are perennial (HOSHIKAWA *et al.*, 1992). The diapause of these 2 dormant stages is capable to break by subjecting them to low temperatures in various taxa of bees, including *Andrena, Osmia*, *Megachile* and *Ceratina* (BOSCH & KEMP, 2001, 2004; GÔUKON *et al.*, 1996; KITAMURA *et al.*, 2001; MAETA, 1978a, 2000, 2007, unpubl.; SASAKI & MAETA, 1994; MAETA *et al.*, 1993).

In Japan, closely related 2 sibling species of megachilid bees, *Megachile* (*Chelostomoda*) *spissula* COCKERELL and *M.* (*C.*) *esakii* YASUMATSU are occurring in different climatic zones. The former is distributed in temperate zone from Yaku Is. to southern Hokkaido (ca. 30°30′N–41°55′N), and the latter is in subtropical zone from Iriomote Is. to Amami Is. (ca. 24°15′–28°30′N) (HIRASHIMA, 1989; YAMANE *et al.*, 1999). *Megachile spissula* is also known to occur from Korean Peninsula, Taiwan and China (WU, 2600; MAETA, unpubl.). The difference of distributional ranges and subsequent flying periods in both megachilid species seems to be determined mainly by a state of dormancy of prepupae. In this report, we have described the thermal response of prepupae in both megachilid bees, which were essential to break their prepupal diapause.

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Materials and Methods

For each treatment 50 prepupae of *M. spissula* were kept in a petri dish (Diameter: 90 mm; Depth: 25 mm) were used in all experiments conducted between1973 and 1976. Whereas in *M. esakii* 25 prepupae in 2004, 27 in 2007, 23 in 2008 were kept individually in a small vial (Diameter: 7 mm; length 30 mm) plugged with cotton, so as to prevent reinfestation by *Melittobia sosui* DAHMS, which is a serious parasitoid of the species. The exact number of prepupae of both species used for each experiment is indicated in relevant Tables 2–5. In the treatment of cooling by low temperatures and incubation of prepupae were done under dark condition. "Low temperatures" basically mean those that are lower than that of usual commencement of nesting activity of adult bees. *Megachile spissula* begin their nesting activities above 24℃ on sunny days (SASAKI & MAETA, 1994; MAETA, 2005), and presumably a little higher temperature is needed for normal nesting activity for *M. esakii*.

The low temperatures, which function to break prepupal diapause almost completely (above 95 %) or completely (100%), were regarded as "effective temperatures". The shortest developmental duration (in days) within the effective temperatures, which were required from prepuape to adults after cool treatment, was called as "optium temperature". It was ca. 50 days or less at optimum temperature in both species. However, total developmental duration of prepupae and pupae were calculated from the commencement of incubation to eclosion of adults (A) in *M. esakii*, but from the commencement of incubation to emergence of adults from their cocoons (B) in *M. spissula*. A is several days shorter than B, because the eclosed bees required equivalent days until emergence from their cocoons. Moreover, temperatures used for incubation after cool treatments differed between 2 species, 27℃ for *M. esakii* and 30℃ for *M. spissula*. In *M. esakii*, total developmental duration incubated at 27℃ was ca. 6 days longer than that at 30℃ (Table 7). Therefore, the total duration required from the commencement of incubation to adult eclosion after cooling at the optimum temperature is actually shorter in *M. esakii* than that in *M. spissula*, if they were incubated at the same temperature of 30℃.

We were obliged to perform supplemental experiments repeatedly for several years, because of shortage of materials obtained from trap nests for both species.

1. Rearing and setting trap nests of bees

To obtain dormant prepupae of bees. The rearing of *M. spissula* was conducted in 1973, 1974 and 1975 at the campus of Tohoku National Agricultural Experiment Station (current name: Tohoku Agricultural Research Center) in Morioka (39°45′N). Additional prepupae were obtained from trap nests (reed tubes) set at Oshoji, Nagano City (36°40′N) in 1975 and 1976. The prepupae of *M. esakii* was obtained from trap nests set under the eaves of the houses at several places in Iriomote Is. (24°20′N) in 2003–2007. Trap nests were renewed every time of the withdrawal performed in summer. However, experiments of 2005 and 2006 were not done with present report due to poor materials.

2. Period of adult emergence from cocoons and termination of prepupal diapause in the field

To study 1) the period of adult emergence in the field and 2) the period of termination of prepupal diapause at natural conditions in *M. spissula*, prepupae in cocoons were left in the field. For study 1), the emergence date of adults was recorded every day in 1973. For 2), the following 7 groups of prepupae were withdrawn periodically from the field once a month from October 1, 1974 to March 31, 1975 (7 times), and incubated them at 30℃. No experiment was performed on these matters for *M. esakii*.

3. Incubation of dormant prepupae without and with subjecting to low temperatures

Temperature regimes for the following 2 experiments are summarized in Table 1. Withdrawal of nests from the field was performed soon after ceasing the nesting activity. However, in *M. spissula* those prepupae in the nests encountered more or less low temperatures before the following treatment.

Experiment I: To prove low temperatures are essential to break diapause, the dormant prepupae of *M. spissula* were continuously exposed to higher temperatures, 20℃, 22℃, 26℃ and 30℃ without cooling. Prepupae were subjected to 20℃, 22℃ and 26℃ on September 10, 1974, and to 30℃ on September 3, 1973 and September 20, 1976. In *M. esakii*, prepupae were continuously subjected to 27℃ in 2004 and to 24℃, 27℃ and 30℃ in 2008. Those prepupae were withdrawn from the field on September 10 in 2004 and on July 23 in 2007, and kept them at a temperature-uncontrolled room (in 2004) and constant high temperature of 30℃ (in 2007) in Matsue until the commencement of incubation. Incubation was done on October 20 in 2004 and on August 18 in 2007. In 2007 withdrawal of trap nests and incubation was performed earlier than 2004, so as to prevent them from encountering to low temperatures.

Experiment II; To determine the effective low temperatures to break diapause, dormant prepupae of *M. spissula* were subjected to 6 different low temperatures (5℃, 8℃, 11℃, 14℃, 18℃ and 20℃) for one to 6 months between 1973 and 1976. Incubation was done at 30℃ after each cooling. The detailed dates on the commencement of cooling and incubation were shown in Tables 1 and 5. In *M. esakii*, prepupae which were withdrawn on September 10, 2004 and left them in a temperature-uncontrolled room in Matsue. These prepupae were subjected to 9℃, 12℃, 15℃, 18℃, 21℃ and 24℃ for one to 4 months on October 20, 2004, and then incubated at 27℃ after each cooling. Additional experiments were performed in 2008, used only prepupae which did not encounter low temperatures. Prepupae were withdrawn on July 24–25, 2008 and then kept them at 27℃ until the commencement of cool treatments for one and 2 months by 3 low temperatures (15℃, 18℃ and 21℃) on September 19, 2008. Incubation at 27℃ was done after each cooling.

The percentage of prepupae of which diapause was broken in both species was calculated by A + B/A + B + C (A: number of emerged adults from their cocoons (or eclosed within their cocoons); B: number of dead pupae; C: number of living prepupae remained in dormant state).

4. Developmental duration and threshold temperature of free-diapaused prepupae

Developmental durations of non-dormant prepupae and pupae were examined. Prepupae of *M. spissula* obtained from trap nests set at Oshoji, Nagano Pref. in 1972 were incubated at 4 different temperatures (22℃, 26℃, 30℃ and 34℃) on March 2, 1973. Prepupae of *M. esakii* withdrawn on July 25–26, 2008 were incubated at 4 different temperatures (21℃, 24℃, 27℃ and 30℃). Until the commencement of incubation by these temperatures on November 19, 2008, they were kept at 27℃ for 56 days and then subjected to 15℃ for 2 months, so as to break prepupal diapause. Subsequent pupal duration was also examined at the above mentioned temperatures in both species.

The values of the developmental threshold temperatures in prepupal and pupal stages are calculated from the regression lines obtained by the relationship between incubation temperatures and developmental rate (100/duration in days).

Table 1. Temperature regimes for the experiments to break prepupal diapause in *Megachile spissula* and *M. esakii*.[1]

Exper-iment	Species	Temper-ature (℃)	Starting date of treatment	Duration (months) for treatment	Place where prepupae obtained
I	*spissula*	20	Sept. 10, 1974	Leaving at this temp.	Morioka
		22	〃	〃	〃
		26	〃	〃	〃
		30	Sept. 3, 1973	〃	〃
		30	Sept. 20, 1976	〃	Oshoji, Nagano
	esakii	24	Oct. 20, 2004[2]	〃	Iriomote Is.
		24	Aug. 18, 2007[3]	〃	〃
		27	〃	〃	〃
		30	〃	〃	〃
II	*spissula*	5	Sept. 3, 1973	1, 2, 3, 4, 5	Morioka
		8	〃	3, 4	〃
		8	Oct. 20, 1975	5	Oshoji, Nagano
		8	Sept. 20, 1976	1, 2, 6	〃
		11	Sept. 3, 1973	3, 4	Morioka
		11	Sept. 20, 1976	1, 2, 6	Oshoji, Nagano
		14	Sept. 10, 1974	1, 2, 3, 4	〃
		14	Oct. 1, 1975	5	〃
		14	Sept. 20, 1976	6	〃
		18	Sept. 10, 1974	2, 3, 4, 5	Morioka
		18	Sept. 20. 1976	1	Oshoji, Nagano
		20	〃	1, 2, 3, 4, 6	〃
	esakii	9	Oct. 20, 2004[4]	1, 2, 3	Iriomote Is.
		12	〃	1, 2, 3	〃
		15	〃	1, 2, 3	〃
		18	〃	1, 2, 3, 4	〃
		21	〃	1, 2, 3	〃
		24	〃	1, 2, 3	〃
		15	Sept. 19, 2008[5]	1, 2	〃
		18	〃	1, 2	〃
		21	〃	1, 2	〃

1) Fifty prepupae of *Megachile spissula* and 22–27 of *M. esakii* were used for each experiment.

2) Withdrawal of nests was made on September 10, 2004 and kept them in a temperature- uncontrolled room until the commencement of incubation.

3) Withdrawal of nests was made on July 23, 2007 and kept them at 30℃ until the commencement of incubation.

4) Withdrawal of nests was made on September 10, 2004 and kept them in a temperature- uncontrolled room until the commencement of cooling.

5) Withdrawal of nests was made on July 25–26, 2008 and kept them at 27℃ until the commencement of cooling.

Results and Discussion

1. General life history of bees

Both *M. spissula* and *M. esakii* are univoltine and overwinter at prepupal stage. They use pre-existing cavities such as reed and bamboo tubes, deserted holes of the long-horn beetles, etc. as nest materials. Provisioned cells are partitioned by the walls, which are adhered with partially masticated irregular-shaped leaf pieces. Nest architectures resemble with each other. However, the number of brood cells per completed nest was fewer in *M. spissula* than that in *M. esakii*, and intercalary cells were often laid between brood cells in the former, but was rare in the latter species. Flying period of *M. spissula* is for ca. 2 months from late July to mid September in Morioka, northern Japan (MAETA & SASAKI, 2005), and ca. 2 months from early July to early September in Matsue (35°30'N, south-western Japan (MAETA, unpubl.), showing that the start of flying period is nearly one month late in northern district. On the other hand, the flying period for *M. esakii* is ca. 2 and half months from late April to early July in Iriomote Is. (MAETA, unpubl.).

Major floral resource plants for *M. spissula* are *Lespedeza bicolor* and other *Lespedeza* species (Leguminosae) in Morioka. For *M. esakii*, various plants such as *Pemphis acidula* (Lythraceae), *Verbena litoralis* (Verbenaceae), *Premus serratifolia* (Ditto) and *Vitex bicolor* (Ditto) are used in Iriomote Is. (MAETA, unpubl.). The number of eggs laid by a single female of *M. spissula* is relatively small (11–23 eggs during 41–65 days, reared in the greenhouse with plenty of *Melilotus officinalis*, MAETA, 2005), as compared with other megachilid bees, the genus *Megachile sensu lato*. For instances, one individual females of *M. subalbuta* laid 49 eggs during 55 days, and of *M. rotundata* 77 eggs during 57 days (MAETA, 1999; MAETA & KITAMUA, 2005). No data on the oviposition numbers/female was available in *M. esakii*. So far *Zonitis japonica* PIC, 3 species of the anthracine flies (*Anthrax aygula* FABRICIUS, *A. distigma* WIDEMANN and *A. jezoensis* MATSUMURA), *Zodion vsevolodi* ZIMINA and *Melittobia acasta* (WALKER) were found as natural enemies of *M. spissula* (MAETA & SASAKI, 2005; MAETA *et al*., 2007; MAETA & MACFARLANE, 1993; MAETA, 1978b). And *A. aygula* and *Me. sosui* are known for *M. easkii* (MAETA *et al*., 2009).

2. Period of adult emergence from cocoons and termination of prepupal diapause in the field

2.1. Period of adult emergence

In a group of 45 prepupae of *M. spissula* left in the field in 1973, 25 female adults emerged from July 22 to August 27, 1974 (36 days, 96% of them emerged within 10 days) and 12 males from July 15 to July 24, 1974 (8 days). Among other prepupae one and 7 were killed by *A. jezoensis* and fungus, respectively. Flying period of *M. spissula* seems to be commenced from the middle to late July in Morioka.

2.2. Period of prepupal diapause break in the field

The prepupae of *M. spissula*, which had been left in the field, were incubated periodically at 30℃ for 7 times. A very low percentage of prepupae of which diapause was broken was recognized early October, henceforth, the rate was gradually increased up to the end of March (Table 2). It may be concluded here that the prepupal diapause in *M. spissula* was almost completely broken after March in the field of Morioka. These prepupae withdrawn at the end of March encountered thoroughly low temperatures which functioned effectively to break their diapause, as described below.

3. Incubation of dormant prepupae without and with subjecting to low temperatures

3.1. Incubation of dormant prepupae without subjecting to low temperatures

None of prepupae of *M. spissula*, which were incubated at 22℃, 26℃ and 30℃ without cooling, developed into adults. This fact shows that cooling is essential to break diapause. On the other hand, prepupae incubated at 20℃, which is lower than the temperature (24℃) that bees can begin nesting activity, the diapause of 24.5% of prepupae was broken until 239 days since the incubation has commenced. However, some living pupae and prepupae were found at the final checking made in the next year of July 9, 1975 (Table 3). This fact indicates that the development of prepupae is gradually proceeded during the incubation period at 20℃. In 1973 as much as 85.4% of adults emerged from cocoons which were incubated at 30℃ (Table 3). However, the mean duration from the commencement of incubation to the emergence of adults was enormously long, 365 days in females and 355 days in males, suggesting that prepupal diapause at this temperature was not normally broken. The nesting activity of *M. spissula* in 1973 was ceased earlier than that in other years in Morioka (MAETA & SASAKI, 2005), due to a cool summer. Probably, those prepupae used for the experiment had encountered more or less low temperatures, which function to break diapause, before the commencement of incubation.

Prepupae of *M. esakii* incubated at 27℃ on October 20, 2004 developed into adults, and some were dead at pupal stage (Table 4). Diapause was broken completely, but seemed to be abnormal. Because, they spent much more time (more than 75 days), as compared to those subjected to low temperatures (Table 6). Such high rate of broken-diapause seemed to be because of exposed low temperatures for the prepupae in the laboratory before the start of incubation. On the other hand, those prepupae kept at 30℃ to prevent encountering to low temperatures until the commencement of incubation at 24℃, 27℃ and 30℃ on August 18, 2007 were used for the experiments. About 35 % of prepupae whose diapause was broken during subjection to the temperature at 24℃ for 170 days. It suggests that 24℃ partially and less effectively functioned to break prepupal diapause. Other 2 higher temperatures (27℃ and 30℃) did not function to break diapause at all within the same incubating duration (Table 4). It is apparent that encountering the temperatures lower than 24℃ is essential to break diapause in *M. esakii.*

3.2. Incubation of dormant prepupae with subjecting to low temperatures

A clear tendency was not obvious in these experiments of *M. spissula* in which prepupae were subjected to low temperatures at 8℃ and 11℃ as the materials obtained in different years (Tables 1 and 5). We valued the lowest rate of broken-diapause among different months within the same subjected temperatures. As shown in Table 5, diapause of prepupae was partially or almost completely broken at any low temperature. No remarkable increase of the percentage of diapause broken was occurred at 5℃, 8℃, 11℃ and 20℃, according to an increase of duration subjected to low temperatures. For instance, in the experiments of 1973, prepupae were subjected to 5℃ for one to 5 months, no increase of the rate of broken-diapause and the reduction of duration from the commencement to the emergence of adults was recognized. No clear tendency in the rate of

Table 2. Percentage of prepupae of *Megachile spissula* of which diapause was broken in the field of Morioka.

Date of withdrawal of prepupae from field[1)]	% of adults emerged from cocoons[A]	% of dead[2)]		% of *Anthrax jezoensis*	% of living prepupae[C]	% of diapause broken[3)]	Days from incubation to emergence of adults		N
		prepupa	pupa[B]				Female (N)	Male (N)	
Oct. 1, 1974	8.7	8.7	0	2.1	80.4	9.8	68.0±4.0 (2)	71.0±0.0 (2)	46
Nov. 1, 1974	66.0	14.0	6.0	2.0	12.0	85.7	62.5±5.3 (24)	57.3±6.1 (8)	50
Nov. 30, 1974	81.6	4.1	0	0	14.3	85.1	60.7±6.0 (28)	52.8±2.9 (12)	49
Dec. 30, 1974	86.0	4.0	0	2.0	8.0	91.5	58.0±6.6 (29)	46.6±2.8 (14)	50
Jan. 30, 1975	86.0	8.0	2.0	2.0	2.0	97.8	58.3±8.2 (24)	47.3±5.8 (19)	50
Feb. 28, 1975	94.0	4.0	0	0	2.0	97.9	54.0±4.8 (26)	50.6±6.3 (20)	50
Mar. 31, 1975	84.5	11.3	4.1	0	0	100	–	–	97

1) Incubation was begun at 30℃ soon after withdrawal. Final check by dissecting cocoons was made on May 7, 1975.
2) Presumably, deceased by fungus. 3) Obtained by A + B/A + B + C.

Table 3. Percentage of prepupae of *Megachile spissula* of which diapause was broken without subjecting to low temperatures.

Incubated temper-ature (℃)	Date of incuba-tion	% of adults emerged from cocoons[A]	% of dead[1)]		% of *Anthrax jezsoenis*	% of living prepupae[C]	% of diapause broken[2)]	Days from incubation to emergence of adults		N	Date of final checking[3)]
			prepupa	pupa[B]				Female (N)	Male (N)		
20	Sept. 10, 1974	24.0	0	0	2.0	74.0	24.5	213.8±11.2 (4)	201.8±22.0 (8)	50	May 9, 1975
22	〃	0	2.0	0	0	98.0	0	– (0)	– (0)	50	May 8, 1975
26	〃	0	0	0	6.0	94.0	0	– (0)	– (0)	50	〃
30	Sept. 3, 1973	70.0	18.0	0	0	12.0	85.4	364.7±59.3 (28)	355.1±43.6 (7)	50	Mar. 9, 1975
30	Sept. 20. 1976	0	6.7	0	0	93.3	0	– (0)	– (0)	60	Jun. 25, 1977

1) Presumably, deceased by fungus. 2) Obtained by A + B/A + B + C. 3) Cocoons were dissected to check contents.

Table 4. Percentage of prepupae of *Megachile esakii* of which diapause was broken without subjecting to low temperatures.

Incubated temper-ature (℃)	Date of incuba-tion	% of adults eclosed in cocoons[A]	% of dead[1)]		% of living prepupae[C]	% of diapause broken[2)]	Days from incubation to eclosion of adults		N	Date of final checking[3)]
			prepupa	pupa[B]			Female (N)	Male (N)		
27	Oct. 20, 2004	52.0	44.0	4.0	0	100	76.8±1.6 (5)	75.1±2.4 (8)	25	Feb. 28, 2005
24	Aug. 18, 2007	29.6	14.8	0	55.6	34.8	177.2±10.4 (6)	157.5±2.1 (2)	27	Feb. 15, 2008
27	〃	3.7	14.8	0	81.5	4.3	– (0)	169 – (1)	27	〃
30	〃	0	3.7	0	96.3	0	– (0)	– (0)	27	〃

1) Infested by *Melittobia sosui* and presumably deceased by fungus. 2) Obtained by A + B/A + B + C. 3) Cocoons were dissected to check contents.

diapause broken and the duration of post-diapause development of prepupae among they were treated for different months at the same low temperatures (*e.g.*, 8℃ and 11℃) is apparently due to that cooling was done in different years. In these prepupae the encountered low temperatures before cool treatments differed by years. However, only at 14℃ and 18℃ prepupal diapause was almost completely or completely broken, and the rates of broken-diapause increased quickly, depending on the increase of duration subjected to these temperatures. Effective low temperatures in *M. spissula* were 14–18℃ (optimum is as same as 14–18℃), requiring for 3 months at the minimum. About 50 days for both females and males are required from the commencement to adult emergence (Table 5).

In *M. esakii*, diapause was broken in all treatments by subjecting to 6 different low temperatures on October 20, 2004. Moreover, duration in days from the commencement of incubation to the eclosion of adults was shorter than 50 days. These prepupae obviously encountered low temperatures when they were kept in a temperature-uncontrolled room for 39 days (from Septemberl l to October 19, 2004) before incubation was made at 27℃. The mean temperature was 20.8℃ during 39 days (based on the data by Matsue Observatory, 2004). As described below, cooling at 21℃ for one month was enough to break diapause almost completely (results in 2008, Table 6). Moreover, the duration from the commencement of incubation to adult eclosion in dormant prepupae used for the experiments of 2004 without cooling was longer than those subjected to low temperatures (Table 4). These facts suggest that subjection to low temperatures was also essential to break prepupal diapause in *M. esakii* as was in *M. spissula*.

Similar experiments were performed again in 2008 in *M. esakii*, but prepupae were kept at 27℃ until the commencement of subjection to low temperatures. As shown in Table 6, 3 low temperatures (15℃, 18℃ and 21℃) functioned effectively to break prepupal diapause. Though 20℃ was not wholly functional to break prepupal diapause in temperate *M. spissula* (Table 5). This could be the adaptive feature of subtropical *M. esakii*. However, the optimum temperature was quite similar to that of *M. spissula*, 14–18℃ (Table 5), but the subjecting durations were remarkably shorter than those of *M. spissula*. Only one month was enough to break diapause completely, and it required less than 50 days from the commencement of incubation to adult eclosion in *M. esakii* (Table 6). Presumably, prepupal diapause could be broken completely by more shorter subjecting duration. Critical duration should be cleared by further experiments.

4. Developmental duration and threshold temperature of free-diapaused prepupae

Developmental durations of the free-dormant prepupae and subsequent pupae are summarized in Table 7. The regression lines are drawn based on Table 7 (Fig. 1). A clear relationship was recognized between developmental rate of prepupae and incubated temperatures in both species. However, the development of prepupae was conspicuously inhibited at 34℃ in *M. spissula*. A similar trait might be occurred in *M. esakii*, if the same temperature were used for incubation. The value of developmental threshold temperature of prepupae was calculated by excluding that of 34℃. The developmental threshold temperature of prepupae and pupae was 14.5℃ in females and 13.6℃ in males, and 14.3℃ in females and 13.2℃ in males, respectively. On the other hand, in *M. esakii* the respective values of prepupae and pupae were 15.3℃ in females and 15.8℃ in

Table 5. Percentage of prepupae of *Megachile spissula* of which diapause was broken subjecting to six different low temperatures.

Low temperature (℃)(Year examined)	Duration (months) of subjection	% of adults emerged from cocoons[A]	% of dead[1)] prepupa	% of dead[1)] pupa[B]	% of *Anthrax jezoensis*	% of living prepupae[C]	% of diapause broken[2)]	Days from incubation to emergence of adults: Female (*N*)	Days from incubation to emergence of adults: Male (*N*)	*N*	Date of final checking[3)]
5 (1973)	1	55.1	36.7	0	0	8.2	87.1	345.7±43.6 (20)	330.6±38.6 (7)	49	May 9, 1975
(〃)	2	53.2	36.2	0	0	10.6	83.3	353.1±31.7 (15)	340.8±48.2 (10)	47	
(〃)	3	47.9	29.2	2.1	0	20.8	70.6	375.5±57.9 (22)	359 (1)	48	〃
(〃)	4	57.4	23.4	8.5	0	10.6	86.1	339.1±57.6 (18)	364.8±61.9 (9)	47	〃
(〃)	5	28.0	42.0	2.0	2.0	26.0	53.6	391.5±24.9 (11)	336.3±42.5 (3)	50	〃
8 (1976)	1	0	10.0	0	0	90.0	0	– (0)	– (0)	50	Jun. 25, 1977
(〃)	2	20.0	10.0	0	0	70.0	22.2	74.3±10.5 (7)	63.0±8.3 (3)	50	〃
(1973)	3	52.3	22.7	2.3	0	22.7	70.6	328.5±145.1 (16)	222.1±154.6 (7)	44	May 9. 1975
(〃)	4	51.0	33.3	0	0	15.6	76.7	225.0±138.6 (18)	272.8±152.6 (4)	45	〃
(1975)	5	8.0	50.0	0	0	42.0	16.0	36.0±1.0 (2)	36 (1)	50	Jun. 1, 1976
(1976)	6	28.0	0	8.0	0	64.0	36.0	57.6±13.1 (10)	43.0±0.0 (3)	50	Jun. 25, 1977
11 (1976)	1	31.3	29.2	0	0	43.8	41.7	70.7±4.5 (7)	64.8±1.5 (8)	48	Jun. 25, 1977
(〃)	2	54.0	8.0	0	0	38.0	58.7	63.4±14.0 (16)	65.0±18.6 (10)	50	〃
(1973)	3	70.2	14.9	2.1	0	12.8	85.0	224.4±172.5 (30)	150.7±116.7 (3)	47	May 9. 1975
(〃)	4	85.4	6.3	4.2	2.1	2.1	97.7	90.0±99.6 (27)	92.9±99.5 (14)	48	〃
(1976)	6	84.0	4.0	2.0	0	10.0	89.6	53.6±6.9 (32)	53.7±8.0 (9)	50	Jun. 25, 1977
14 (1974)	1	36.0	14.0	2.0	0	48.0	44.2	70.5±6.9 (8)	58.8±5.3 (10)	50	May 7, 1975
(〃)	2	77.1	8.3	2.1	6.3	6.3	92.7	56.2±9.0 (18)	51.4±7.2 (19)	48	〃
(〃)	3	91.8	4.1	0	4.1	0	100	50.4±4.7 (29)	50.9±11.1 (16)	49	〃
(〃)	4	92.0	4.0	4.0	0	0	100	45.0±3.0 (29)	41.4±2.1 (17)	50	〃
(1975)	5	92.0	8.0	0	0	0	100	40.0±3.6 (21)	35.5±2.8 (25)	50	Jun. 1, 1976
(1976)	6	92.0	4.0	4.0	0	0	95.8	42.8±3.6 (32)	37.8±3.3 (12)	50	Jun. 25, 1977
18 (1976)	1	8.0	18.0	0	0	74.0	9.8	64 (1)	154.5±90.5 (2)	50	Jun. 25, 1977
(1974)	2	70.0	14.0	4.0	2.0	10.0	88.0	55.6±3.4 (17)	47.7±6.4 (18)	50	May 7, 1975
(〃)	3	82.0	8.0	2.0	4.0	4.0	95.5	47.7±3.0 (19)	40.7±3.4 (22)	50	〃
(〃)	4	94.1	2.0	0	2.0	2.0	98.0	42.3±2.9 (32)	40.8±5.7 (16)	51	〃
(〃)	5	91.8	8.2	0	0	0	100	39.0±3.0 (30)	34.6±1.8 (14)	49	〃
20 (1976)	1	2.0	10.0	0	0	88.0	2.2	68 (1)	– (0)	50	Jun. 25, 1977
(〃)	2	4.0	4.0	2.0	0	90.0	6.3	50 (1)	186 (1)	50	〃
(〃)	3	30.0	10.0	0	0	60.0	33.3	45.1±5.7 (10)	34.8±6.4 (5)	50	〃
(〃)	4	28.0	0	0	0	72.0	28.0	28.3±7.0 (6)	22.3±4.4 (8)	50	〃
(〃)	6	40.0	0	0	0	60.0	40.0	16.7±7.7 (6)	11.6±8.9 (14)	50	〃

[1)] Presumably, deceased by fungus. [2)] Obtained by A + B/A + B + C. [3)] Cocoons were dissected to check contents.

Table 6. Percentage of prepupae of *Megachile esakii* of which diapause was broken subjecting to six different low temperatures.

Low temperature (℃)(Year examined)	Duration (months) of subjection	% of adults eclosed in cocoons^{A}	% of dead[1)]		% of living prepupae^{C}	% of diapause broken[2)]	Days from incubation to eclosion of adults				*N*	Date of final cheking[3)]
			prepupa	pupa^{B}			Female	(*N*)	Male	(*N*)		
9 (2004)	1	60.0	40.0	0	0	100	48.2±1.9	(10)	46.5±2.7	(4)	23	Apr. 30, 2005
(〃)	2	52.0	12.0	36.0	0	100	43.7±2.5	(3)	37.1±4.0	(10)	25	〃
(〃)	3	52.0	0	48.0	0	100	38.3±0.9	(3)	31.3±1.4	(10)	25	〃
12 (〃)	1	20.0	16.0	64.0	0	100	–	(0)	43.8±1.0	(5)	25	Apr. 30, 2005
(〃)	2	52.0	48.0	0	0	100	–	(0)	35	(1)	25	〃
(〃)	3	56.0	12.0	32.0	0	100	37.0±1.8	(7)	31.1±1.9	(7)	25	〃
15 (2004)	1	92.0	4.0	4.0	0	100	47.7±1.1	(15)	43.2±1.0	(9)	25	Apr. 30, 2005
(〃)	2	16.0	0	84.0	0	100	43	(1)	32.7±3.3	(3)	25	〃
(〃)	3	80.0	12.0	8.0	0	100	32.3±2.1	(12)	28.9±1.5	(8)	25	〃
(2008)	1	100	0	0	0	100	41.0±0.8	(10)	37.9±1.8	(13)	23	Jan. 19, 2009
(〃)	2	95.5	4.5	0	0	100	36.4±1.8	(8)	30.5±1.3	(13)	22	〃
18 (2004)	1	84.0	16.0	0	0	100	47.8±1.3	(12)	43.6±1.7	(9)	25	Apr. 30, 2005
(〃)	2	76.0	12.0	12.0	0	100	37.2±1.0	(9)	34.2±1.1	(10)	25	〃
(〃)	3	76.0	24.0	0	0	100	29.7±2.6	(10)	24.2±1.6	(9)	25	〃
(〃)	4	64.0	28.0	8.0	0	100	20.1±2.0	(6)	14.1±1.5	(10)	25	〃
(2008)	1	100	0	0	0	100	45.8±0.7	(8)	40.9±2.7	(13)	23	Jan. 19, 2009
(〃)	2	95.7	4.3	0	0	100	34.5±1.4	(10)	31.4±1.7	(13)	23	〃
21 (2004)	1	76.0	24.0	0	0	100	48.6±0.5	(11)	45.6±2.1	(8)	25	Apr. 30, 2005
(〃)	2	4.0	48.0	48.0	0	100	–	(0)	23	(1)	25	〃
(〃)	3	24.0	20.0	56.0	0	100	12	(1)	16.0±2.4	(5)	25	〃
(2008)	1	91.3	0	0	8.7	91.3	59.2±4.0	(12)	52.4±4.9	(9)	23	Jan. 19, 2009
(〃)	2	100	0	0	0	100	30.7±3.7	(9)	26.6±2.2	(14)	23	〃
24 (2004)	1	28.0	32.0	40.0	0	100	61.0±0.0	(4)	48.7±0.5	(3)	25	Apr. 30, 2005
(〃)[4)]	2	68.0	12.0	20.0	0	100	25.6±2.4	(9)	20.4±3.8	(8)*	25	〃
(〃)[4)]	3	92.0	4.0	4.0	0	100	4.3±12.3	(11)*	0.3±6.3	(12)*	25	〃

1) Infested by *Melittobia sosui* and presumably deceased by fungus. 2) Obtained by A + B/A + B + C. 3) Cocoons were dissected to check contents.

4) Some adults have emerged during subjecting to 24℃ (indicated with an asterisk in the table). Duration (days) of these adults from incubation was calculated by the date of emergence minus that of incubation.

males, and 16.2℃ in females and 16.3℃ in males. The developmental threshold temperatures of both stages were a little lower in *M. spissula* than in *M. esakii*, 0.9℃ in females and 2.2℃ in males of prepupae, and 1.9℃ in females and 3.1℃ in males of pupae. These thermal differences may show that climatically adaptive traits of the both species, occurring in different temperate zones.

Duration of prepupal stage of *M. spissula* and *M. esakii* is accurately incomparable, because of that the state of free-dormant prepupae differed between 2 species. However, a comparison of the pupal duration was possible between both species. The values of duration (days) of pupal stage in *M. spissula/M.esakii* at 3 different temperatures obtained from the regression lines (Fig. 1) did not significantly differ at 26℃ and 30℃ in both sexes ($p > 0.01$, significant test was made, basing on

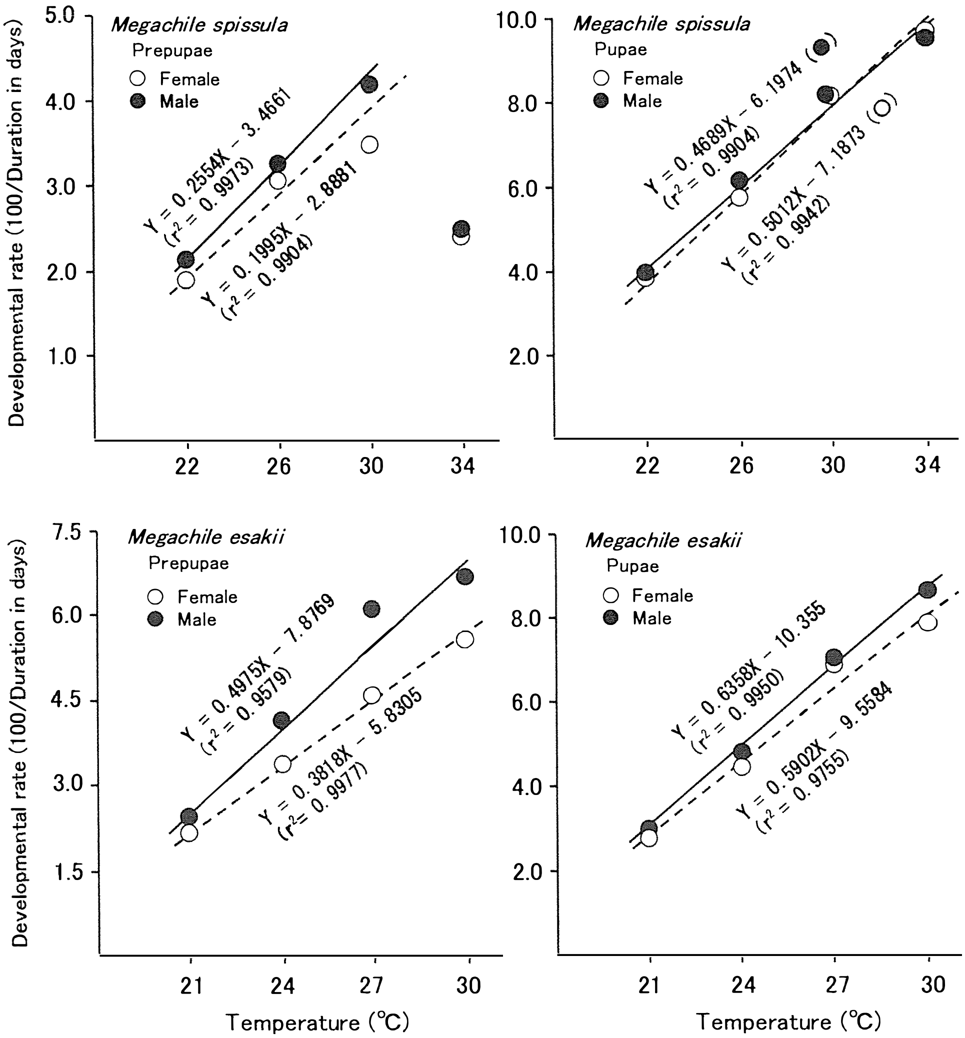

Fig. 1. Relationship between four different temperatures and developmental rates of non-dormant prepupae and subsequent pupae in *Megachile spissula* and *M. esakii*. Incubation was made on March 2, 1973 in *M. spissula* and on November 19, 2008 in *M. esakii*. Regression line is drawn except 34℃ at which developmental inhibition was occurred in *M. spissula*.

the regression analysis of 95 % confidence interval of the secondary regression equation calculated from a relationship between temperatures and developmental rate), except for 22℃ in both sexes ($p < 0.01$, ditto), as described below.

	22℃	26℃	30℃
Females	24.3/29.2	16.7/17.3	12.7/12.3
Males	26.1/27.6	17.1/16.2	12.8/11.5

The distribution of sexes in nest tubes of *M. spissula* and *M. esakii* is a typical ♀♂ type (female cells are placed at the inner- and males at the outer-part of nest tubes, as in most megachilid bees (KROMBEIN, 1967; MAETA, 1978; SUGIURA & MAETA, 1989). Protoandry is maintainable, because the developmental duration was always shorter in males than females in both prepupal and pupal stages under any temperature (Table 7).

5. Comparison of the effective low temperatures needed to break diapause in various taxa of temperate bees

As mentioned above, duration needed to break prepupal diapause by subjecting low temperatures was very shorter in *M. esakii* (one month or less) than *M. spissula* (3 months), although similar low temperatures in both species (14℃–18℃) and also a little higher temperature (21℃) in *M. esakii* functioned effectively. Moreover, the developmental threshold temperatures in both non-dormant prepupae and pupae were a little lower in *M. spissula* than *M. esakii*, as mentioned above. These

Table 7. Developmental duration for prepupal and pupal stages in *Megachile spissula* and *M. esakii* incubated at four different temperatures.

Species	Temperature (℃)	Sex	Duration in days from prepupa to pupa[1)]		Duration in days from pupa to adult		*N*
			Range	Mean ± SD	Range	Mean ± SD	
spissula	22	♀	45–65	53.3±4.9	19–32	26.1±2.2	26
		♂	33–62	47.1±5.9	23–29	25.4±1.4	31
	26	♀	24–38	32.8±3.5	16–22	17.5±1.3	26
		♂	21–38	30.9±3.6	11–24	16.3±1.9	28
	30	♀	20–38	28.8±4.8	11–13	12.3±0.7	22
		♂	19–28	24.0±2.4	11–14	12.3±0.9	24
	34	♀	24–60	41.8±10.7	10–12	10.3±0.6	13
		♂	24–63	40.3±10.5	9–14	10.5±1.3	20
esakii	21	♀	44–52	46.7±2.7	35–37	35.6±0.9	7
		♂	38–46	42.5±2.7	32–35	33.0±0.8	14
	24	♀	25–34	29.8±3.4	21–24	22.8±1.0	9
		♂	22–29	24.3±2.3	20–22	21.1±0.5	13
	27	♀	20–25	21.9±1.7	14–15	14.5±0.5	8
		♂	15–18	16.4±1.3	13–15	14.2±0.7	13
	30	♀	14–24	18.0±3.7	12–14	12.7±0.7	9
		♂	12–19	15.0±2.1	10–12	11.6±0.6	14

[1)] Duration implies that for the later developmental phase of free-diapaused prepupae, *i.e.*, from the day on which incubation started to the day on which pupation occurred.

Table 8. Comparison of the effective low temperatures to break diapause of various taxa of bees in temperate zone.

Species	Flying period	Overwintering stage	Effective (optimum) temperatures (℃)[1]		Months required[2]	References
Andrenidae						
Andrena prostomias	Early summer	Prepupa	10−16	(13)	4	MAETA (2000)
Megachilidae						
Osmia cornifrons	Spring	Adult	2−8	(5)	3	MAETA (1978); MAETA & KITAMURA (2007)
O. taurus	〃	〃	5−14	(11)	4	
O. cornuta	〃	〃	?−?	(7)	3	BOSCH & KEMP (2004)
O. lignaria	〃	〃	3−5	(?)	?	BOSCH & KEMP (2001)
O. caerulescens	Spring − Summer[3]	〃	5−14	(11)	4	MAETA (unpubl.)
Megachile sculpturalis	Summer	Prepupa	11−14	(14)	3	SASAKI & MAETA (1994)
M. spissula	〃	〃	14−18	(14−18)	3	Present study
M. pseudomonticola	〃	〃	11−20	(17)	3	GÔUKON *et al.* (1996)
Apidae						
Ceratina flavipes	Spring − Autumn	Adult	2−11	(5−11)	3	MAETA *et al.* (1993)

[1] Range of low temperatures which are capable to break diapause almost completely, and the shortest duration (days) required from the commencement of incubation to emergence of adults from their cocoons regarded as the optimum temperature. However, only termination rate of diapause was used for *C. flavipes*.

[2] Duration (months) was required to break diapause at optimum low temperature.

[3] Partial bivoltine. Other species are all univoltine.

differences of thermal response between 2 species may closely relate to their distributional ranges. *Megachile esakii* seems to adapt inhabiting in the subtropical zone, where low temperatures are for a short period time.

In Table 8, 10 species of temperate bees, including *M. spissula*, of which effective low temperatures to break diapause are summarized. The difference of the effective low temperatures among bee species seems to be mainly determined by the flying periods of bees. In vernal species, the effective low temperatures are apparently lower than those of summer species, irrespective of overwintering stages. Presumably, vernal species need lower temperatures, so as to emerge timely in spring season.

Monthly mean temperature (℃) in Iriomote Island from January to December is as follows: 15.2, 15.6, 17.4, 20.0, 22.9, 24.9, 25.8, 25.3, 24.4, 22.4, 19.9 and 17.1 (Iriomote Observatory, based on the climatological data for 25 years, 1955–1980). So far as concerning to the temperature sequence in this island throughout the year, the effective low temperatures to break the prepupal diapause of *M. spissula* (14–18℃) are continued for 4 months from December to March. It suggests that possibility to inhabit for the species in subtropical region, but does not occur there. Interestingly, *M. spissula* is also occurring from Southern Asia, Taiwan and South China (MAETA, unpubl.; WU, 2006). The prepupal diapause pattern of this species in these areas seems to be similar to that of *M. esakii*, not likely to that of *M. spissula*, occurring in temperate zone.

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