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Masanori Hirata · Adam L. Cronin · Midori Kidokoro
Noriko Azuma

Spatio-temporal variation of colony structure and eusociality level of the Japanese sweat bee *Lasioglossum* (*Evylaeus*) *duplex* (Hymenoptera: Halictidae)

Abstract Colony structure and eusociality level of the sweat bee *Lasioglossum* (*Evylaeus*) *duplex* were studied in 2001 in Sapporo and Assabu, Hokkaido, northern Japan. Sakagami and his colleagues had also studied this species in Sapporo in 1957–1968. Brood size, sex ratio and queen–worker size dimorphism were geographically and temporally variable, indicating spatio-temporal variation at the eusociality level. Inseminated workers constituted only 7.9% of the populations in 1957–1968 Sapporo but about 60% in 2001 in Sapporo and Assabu. A few of the inseminated workers were believed to leave natal nests for independent colony founding. Thus, partial bivoltinism is likely in this sweat bee species. The presence of workers with developed ovaries and/or corpora lutea suggests the occurrence of worker oviposition.

Keywords Colony structure · Eusociality · Halictidae · *Lasioglossum* · Worker fecundity

Introduction

Halictine bees show a wide variety of social behaviors and have served as model organisms in studies of social evolution (Wcislo and Danforth 1997). In general, sweat bees of high eusociality levels are expected to exhibit more female-biased sex ratios, lower rates of worker reproductivity, a greater degree of queen–worker size dimorphism, and relatively large brood size (Michener 1974; Breed 1976; Packer and Knerer 1985). Phyloge-

netic analyses by Packer (1997) and Soucy and Danforth (2002) suggest that evolutionary reversions to solitary behavior or social polymorphism are common in this bee group.

Some studies have also indicated that sociality is flexible even within species (Wcislo and Danforth 1997; Richards 2001; Cronin and Hirata 2003). For instance, Wyman and Richards (2003) showed that *Lasioglossum malachurum* exhibits clinal variation in social behavior across Europe. This species produces only a single worker brood and then a gyne brood in northern areas such as Isle of Wight, England, but as many as three worker broods and a gyne brood in southern areas such as Agios Nikolaos Monemvasias, Greece. In the northern populations, more males are produced in the early brood and workers are more likely to mate but have lower rates of ovarian development. In the southern populations, few or no males are produced in the early broods, and workers are less likely to mate but have higher rates of ovarian development.

Lasioglossum (*Evylaeus*) *duplex* is a common species in northern Japan, and previous studies by Sakagami and colleagues have indicated that colonies of this species exhibit primitively eusocial behavior (Sakagami 1977; Sakagami and Hayashida 1958, 1960, 1961, 1968; Goukon et al. 1986). Here, we present data from a recent study on two populations of this species which reveal geographical variation, and we compare our data with those of Sakagami and his colleagues to examine temporal variation in the same population.

Materials and methods

During the bee-active season of 2001, a total of 207 and 121 *Lasioglossum* (*Evylaeus*) *duplex* colonies were excavated at Sapporo (Hokkaido University, 141°22′E, 43°05′N) and Assabu (140°15′E, 41°55′N), respectively, Hokkaido, northern Japan. The nest excavation was performed in early morning or on cloudy days when nests were closed and all colony members stayed inside.

M. Hirata (✉) · A. L. Cronin · M. Kidokoro · N. Azuma
Graduate School of Environmental Earth Science,
Hokkaido University, Sapporo 060-0810, Japan
E-mail: tombo@ees.hokudai.ac.jp
Tel.: +81-11-7062251
Fax: +81-11-7064867

Present address: A. L. Cronin
Department of Biology, University College London,
Wolfson House, 4 Stephenson Way, London, NW1 2HE, UK

After all adults and immatures were collected, we measured depth from ground surface to the middle of cells and to the bottom of the deepest shafts (nest depth). The number of cells per colony was almost always one in spring colonies and one to four in summer colonies, and the cell depth was represented by the depth of the shallowest cell.

In the laboratory, we sexed adults and pupae, and reared larvae and prepupae for sexing. However, the rearing and sexing were unsuccessful for many larvae. Especially in Assabu, the number of colonies where the sexing was successful for all colony members was only 10 in spring and 5 in summer. Therefore, only the overall sex ratio (male ratio in brood) was given for Assabu, while for Sapporo the mean sex ratio per colony and the overall sex ratio were given.

Adult females were preserved in a $-20°C$ freezer until dissection of their abdomens. The dissection was performed to determine the reproductive condition of females: insemination status was inferred from the opacity of spermathecae; previous oviposition was inferred from the presence of corpora lutea; classifications of ovarian development and mandibular wear were performed according to Sakagami and Hayashida (1968); and wing wear was measured by the number of nicks on both wings. Head width was represented by outerorbital distance, and the degree of queen–worker size dimorphism was given by $100 \times Hq–Hw/Hq$ where Hq and Hw were head width of queen and worker, respectively. The wing length was the distance from the base of the costa to the end of the stigma. Female bees that were inseminated and had corpora lutea, developed oocytes, larger body size and well-worn mandibles and wings were classified as queens. If a female did not fulfill all of these conditions, she was regarded as a worker. In addition to the comparison of colony structures between Sapporo and Assabu, our data were also compared with data of Sakagami and Hayashida (1968) and Goukon et al. (1986) who studied colony structures of *Lasioglossum duplex* from 1957–1968 in Sapporo (Botanic Garden of Hokkaido University). Although we closely followed the sampling and dissection methods of Sakagami and his colleagues, in that paper the cell depth was represented as the distance from ground surface to the bottom of cells. Since the height of a cell is about 4 cm, we corrected their cell depth by subtracting 2 cm from their data.

Results

Phenology in Sapporo 2001

The bee-active season was divided into two phases with an intermediate inactive period of about 2 weeks (Fig. 1). In the first phase, each foundress started reuse of an old nest or construction of a new nest in early spring and reared first brood up to pupae by mid-June. Although multiple foundresses coexisted and cooperated in 2.2% of all colonies collected in the first phase, all the
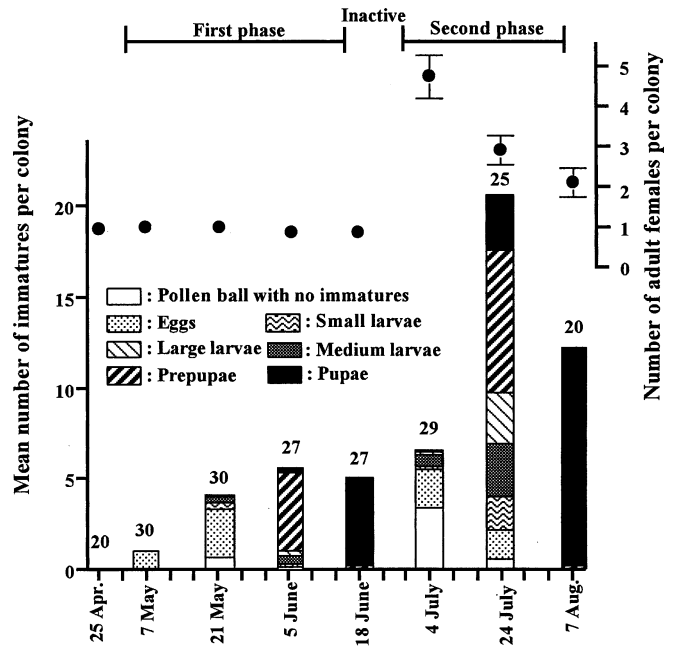

Fig. 1 Seasonal trend of colony makeup in Sapporo 2001. Sample size (number of nests excavated) is indicated *above each bar*

colonies appeared to become monogynous by the end of the first phase. The second phase started in early July when callows eclosed. Males eclosed earlier and flew away from natal nests, while many of females stayed with the foundress to become workers in natal nests. Since the mean number of adult females per colony was 4.8 ± 2.19 on 4 July but only 2.7 ± 1.31 on 24 July, some new females probably left their natal nests to found independent colonies. Workers reared a second brood which eclosed by mid-August. The foundress died by the end of July and most, if not all, workers died by mid-August. Callows stayed in natal nests until they carried out mating in early September. Thereafter, males died but females overwintered below ground to become colony foundresses in the following bee-active season.

Seasonal comparison of colony structures between first and second phases in Sapporo 2001

As mentioned above, this bee species was solitary in the first phase but eusocial in the second phase. Colony structures in Sapporo 2001 are shown in Table 1. Mean brood size was significantly larger in the second phase (20.6 ± 12.02) than in the first phase (5.7 ± 2.61) $(t_{76} = -8.66, P < 0.001)$. Nests and cells were significantly deeper in the second phase $(19.7 \pm 3.41$ and 12.4 ± 3.43 cm, respectively) than in first phase $(11.2 \pm 2.34$ and 6.3 ± 1.38 cm) $(t_{74} = -8.59, P < 0.001$ for nest depth; $t_{74} = -11.0, P < 0.001$ for cell depth). Sex ratio per colony deviated significantly from 0.5 in the first phase $(0.3 \pm 0.36$; one sample *t*-test: $t_{22} = -2.99, P = 0.007)$ but not in the second phase $(0.5 \pm 0.17; t_{15} = -0.79, P = 0.44)$.

Table 1 A comparison of colony structure and eusociality level in *Lasioglossum duplex* among Assabu (2001), Sapporo (2001) and Sapporo (1957–1968). *N* Number of nests, *n* number of individuals, *NA* data not available

	Assabu (2001)	Spatial comparison	Sapporo (2001)	Temporal comparison	Sapporo (1957–1968)[a]
First phase (solitary)					
Brood size	3.7 ± 1.83 $N = 24$	$t_{75} = -3.71$ $P < 0.001$	5.7 ± 2.61 $N = 53$	NS	5.1 ± 1.82 $N = 113$
Sex ratio per colony	NA		0.3 ± 0.36 $N = 23$		NA
Overall sex ratio	0.05 $n = 39$		0.26 $n = 131$		0.03 $n = 803$
Nest depth (cm)	8.1 ± 1.56 $N = 24$	$t_{75} = -5.89$ $P < 0.001$	11.2 ± 2.34 $N = 53$	$t_{141} = 5.51$ $P < 0.001$	9.4 ± 2.55 $N = 90$
Cell depth (cm)	4.6 ± 1.03 $N = 24$	$t_{75} = -5.50$ $P < 0.001$	6.3 ± 1.38 $N = 53$	NS	6.4 ± 2.54 $N = 76$
Number of multifoundress colonies	5 (11.6%) $N = 43$		3 (2.2%) $N = 134$		4 (0.01%) $N = 500$
Second phase (eusocial)					
Brood size	9.5 ± 6.62 $N = 28$	$t_{51} = -4.23$ $P < 0.001$	20.6 ± 12.02 $N = 25$	$t_{130} = 3.76$ $P < 0.001$	13.5 ± 7.70 $N = 107$
Sex ratio per colony	NA		0.5 ± 0.17 $N = 16$		NA
Overall sex ratio	0.6 $n = 15$		0.46 $n = 216$		0.56 $n = 368$
Nest depth (cm)	13.8 ± 3.08 $N = 28$	$t_{49} = -6.48$ $P < 0.001$	19.7 ± 3.41 $N = 23$	$t_{169} = 3.12$ $P < 0.001$	16.2 ± 5.23 $N = 148$
Cell depth (cm)	7.6 ± 1.64 $N = 28$	$t_{49} = -6.59$ $P < 0.001$	12.4 ± 3.43 $N = 23$	NS	12.7 ± 4.26 $N = 119$
Mean number of workers	1.3 ± 1.24 $N = 24$	$t_{51} = -5.99$ $P < 0.001$	4.1 ± 2.00 $N = 29$	NS	3.6 ± 2.03 $N = 41$
Workers inseminated(%)	59.5 $n = 74$		58.8 $n = 194$		7.9 $n = 277$
Workers with developed ovaries (%)	17.6 $n = 74$		33.0 $n = 194$		20.2 $n = 277$
Workers with corpora lutea (%)	28.4 $n = 74$		16.0 $n = 194$		NA
Queen/worker size difference (%)	8.0 ± 5.47 $N = 6$		4.5 ± 5.26 $N = 16$		6.12 $N = 70$

[a]Data from Sakagami and Hayashida (1968) and Goukon et al. (1986)

Geographical comparison of colony structure and eusociality level between Sapporo and Assabu in 2001

In both the first and second phases, colonies of Sapporo were significantly larger than those of Assabu in brood size ($t_{75} = -3.71$, $P < 0.001$; $t_{51} = -4.23$, $P < 0.001$), nest depth ($t_{75} = -5.89$, $P < 0.001$; $t_{49} = -6.48$, $P < 0.001$) and cell depth ($t_{75} = -5.50$, $P < 0.001$; $t_{49} = -6.59$, $P < 0.001$). In eusocial colonies, mean number of workers was significantly larger in Sapporo (4.1 ± 2.00) than in Assabu (1.3 ± 1.24) ($t_{51} = -5.99$, $P < 0.001$). Intriguingly, about 60% of workers were inseminated, and 33% (Sapporo) or 17.6% (Assabu) had developed ovaries. Workers with corpora lutea constituted 16% of the colony in Sapporo and 28.4% in Assabu. Each queen was larger in head width than her daughters by $4.5 \pm 5.26\%$ in Sapporo and $8.0 \pm 5.47\%$ in Assabu, suggesting greater caste differentiation in Assabu.

Temporal comparison of colony structures and eusociality between 2001 and 1957–1968 in Sapporo

After comparison with data of Sakagami and his colleagues, brood size in the second phase was significantly larger in 2001 (20.6 ± 12.02) than in 1957–1968 (13.5 ± 7.70) ($t_{130} = 3.76$, $P < 0.001$), whereas there was no significant difference in brood size in the first phase. Nests were deeper in 2001 than in 1957–1968 not only in the second phase (19.7 ± 3.41 and 16.2 ± 5.23 cm, respectively; $t_{169} = 3.12$, $P < 0.001$) but also in the first phase (11.2 ± 2.34 and 9.4 ± 2.55 cm; $t_{141} = 5.51$, $P < 0.001$). There was no significant difference in cell depth. Sakagami and Hayashida (1968) did not show mean sex ratio per colony but provided overall sex ratio in population. While the overall sex ratio in the second phase was close to 0.5 in both 2001 (0.46) and 1957–1968 (0.56), that in the first phase was much higher in 2001 (0.26) than in 1957–1968 (0.03).

Although temporal variation was not detected in the mean number of workers, the percentage of inseminated workers was much higher in 2001 (58.8%) than in 1957–1968 (7.9%). According to the data of Sakagami and his colleagues, 20.2% of workers had developed ovaries, suggesting that most of the workers with developed ovaries were virgin, unlike in 2001 when most of the workers with developed ovaries were inseminated.

Discussion

Nest depth and cell depth are spatio-temporally variable in *Lasioglossum duplex* as has already been reported in some sweat bee species (Packer et al. 1989). In *Augochlorella striata*, for instance, mean depth of deepest cells within a nest ranges from 5–20 cm, with deeper nests in drier locations and during different seasons of a year (Ordway 1966). As a general rule in sweat bees, deeper nests are associated with comparatively dry soils (Sakagami and Michener 1962), probably because relatively higher humidity is required for the survival and growth of larvae. Monthly precipitation in May was 17 mm in Sapporo in 2001, which was much lower than in Assabu in 2001 (60 mm) and lower even than the mean 61.5 ± 26.97 mm in Sapporo in 1957–1968. In fact, the soil was considerably drier in Sapporo than in Assabu in 2001. The present study further suggests that the nest depth is affected by social structure in addition to soil moisture, since the nest is significantly deeper in eusocial phase when workers can expand the space of their nests.

A higher female-biased sex ratio in first brood, lower rates of worker reproductivity, greater degree of queen–worker size dimorphism and large brood size have been suggested as indicators of high-level eusociality (Michener 1974; Breed 1976; Packer and Knerer 1985). In the present study, brood size, sex ratio and queen–worker size dimorphism all showed geographical and/or temporal variations, many of which were statistically significant. Thus, the eusociality level is variable even in *Lasioglossum duplex*, which has been considered to be eusocial in all populations (Sakagami and Hayashida 1958; Sakagami 1977; Goukon et al. 1986). Although worker reproductivity was not clear in the present study, the presence of workers with developed ovaries and/or corpora lutea suggests that a few workers lay eggs.

In the subgenus *Evylaeus*, the ratio of inseminated workers has been considered as low as 0–18% (Packer and Knerer 1985), demonstrating the strong control of worker insemination by foundresses (Kukuk and May 1991). However, Richards (2000) found that the ratio of inseminated workers occasionally exceeds 50% in a population of *Lasioglossum malachurum* in Greece. Cronin and Hirata (2003) also reported that the ratio of inseminated workers is 57% in *L. baleicum*. The present study revealed that the populations of *L. duplex* are variable in the ratio of inseminated workers, reaching up to about 60%.

Here one question arises: why are so many workers inseminated in eusocial sweat bees? Another genus, *Halictus* of the Halictinae family, often shows a ratio of inseminated workers as high as 0.63, in *H. sexcinctus*, and 0.52, in *H. ligatus* (Richards 2001; Richards and Packer 1995), probably because the mortality of foundresses is 50 and 55%, respectively, and the inseminated workers can succeed to the position of queen whenever foundresses are dead (Richards 2001). In *Lasioglossum duplex*, however, the mortality of foundresses is about 25% (Sakagami and Hayashida 1968) and the "succession of queens" hypothesis is unlikely. Instead, Sakagami (1977) found a few *L. duplex* nests constructed by solitary females in summer when most colonies were eusocial. In the present study, the number of adult females sharply decreased soon after the eclosion of new adults, suggesting that some females leave their natal nests without becoming workers. Therefore, we conclude that there is probably partial bivoltinism in this sweat bee species as in *Halictus ligatus* (Richards and Packer 1995).

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