

Both cooperation and nest position improve larval survival in the sweat bee, *Lasioglossum (Evylaeus) baleicum*

Norihiro Yagi · Eisuke Hasegawa

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Abstract Nesting by multiple females in sweat bees raises the question of the benefits associated with grouping. Adult numbers of the sweat bee, *Lasioglossum (Evylaeus) baleicum*, exhibited variation within a nest population. The effects of adult number and nest position on larval survival were analyzed in a nest aggregation. Both variables independently affected larval survival, with the presence of multiple adults in a nest markedly improving survival rate. Nests located near the periphery of the aggregation of nests suffered high larval mortalities. Increased frequency of cell-inspection in multiple-female nests seems to be associated with a concomitant increase in larval cell protection from external predation. Such predation pressure was assumed, given the existence of an underground ant colony that employs a recruitment system while foraging in the study area.

Keywords Nest aggregation · Positioning effect · Cooperative nesting · Synergy · Sociality · Selfish herd

Introduction

The sweat bee, *Lasioglossum (Evylaeus) baleicum*, exhibits a form of social polymorphism in which the number of adults varies between nests (Hirata 2005). Multiple-female nesting is considered to improve reproductive success through increasing per capita productivity and larval

survival. In several sweat bee species, females open cells to inspect the developing larvae within, possibly as a defense against predators that penetrate cells directly (Packer 1991; Plateaux-Quenu 2008). The advantage of cooperative brooding for larval survival is thus that females in cooperative nests are able to protect their developing larvae while solitary females must leave their larvae unprotected while foraging. While few studies have examined the contribution of grouping to larval survival (Mappes et al. 1995), the contribution of the synergistic effect associated with this behavior has recently received increased attention (West et al. 2007; Wilson 2008).

Sweat bees have been reported to aggregate their nests within a small area (Sakagami 1977; Sakagami and Fukuda 1989; Cronin and Hirata 2003; Hirata 2005; Richards et al. 2005; Hirata and Higashi 2008; Ulrich et al. 2009), raising the question as to whether the location of a nest within such an aggregation affects larval survival. Since predator attacks originate from outside these nesting areas, peripheral nests are likely to be the most susceptible to predation. Indeed, this “positioning effect” has been observed in groupings of birds and fishes (reviewed in Krause and Ruxton 2002). Similarly, the stinkbug, *Elasmucha signoreti*, lays smaller eggs near the periphery of its egg-clumps in order to maximize the fitness of the parents (Kudo 2006).

In group-nesting bees, the number of adults at a nest and the position of the nest within the nesting area are both likely to affect larval survival. The nest aggregations of *L. (E.) baleicum*, which consist of both solitary and eusocial nests in the second reproductive season of a year (Hirata 2005), are typically found in sunny areas on bare ground (Cronin and Hirata 2003). Since such areas are also commonly favored by a variety of ant and ground wasp species (i.e., potential predators) (Sakagami and Fukuda 1989), *L. (E.) baleicum* is well suited for testing the

N. Yagi (✉) · E. Hasegawa
Laboratory of Animal Ecology,
Department of Ecology and Systematics,
Graduate School of Agriculture,
Hokkaido University, Kita-ku,
Sapporo 8589, Japan
e-mail: vespa@res.agr.hokudai.ac.jp

combined effect of grouping and nest position on larval survival. We examined the contribution of both grouping and positioning effect on larval survival in an aggregation of *L. (E.) baleicum* nests and present our findings here.

Materials and methods

The aggregation of *L. baleicum* nests described in this study were observed from late May to late August 2009 in a research forest belonging to the Forestry and Forest Products Research Institute located south of Sapporo, Japan. In *L. baleicum*, there are two reproductive seasons within a year in Sapporo (Cronin and Hirata 2003). In the first season (late May to late June), females that have overwintered (F1 generation) found nests and rear a brood (F2 generation); all the nests are thus founded by solitary sweat bees in the first reproductive season. After a short period of inactivity (ca. 3 weeks), the second reproductive season starts and the second brood is produced (F3 generation). In this second season, some of the nests contain multiple adult females belonging to the F1 and F2 generations (Hirata 2005). The F3 females hibernate after mating and become the new F1 generation the following spring.

We checked each nest over a 5-day period in late July for the existence of adult bees. We confirmed whether bees were foraging by observing the departure of adult bees from the nest. This period is the start of the second season, and nests were considered as having “successfully started” when at least one such departure was confirmed. Only successfully started nests were used in subsequent investigations. To estimate the number of adults in a nest, we marked the bees that left the nest at two points on the thorax using a marker pen (PaintMarker®; Mitsubishi, Japan). A combination of seven colors and two marking positions were used to mark individual bees and to discriminate between nests.

We excavated all the nests in late August and determined the number of cells and the existence of pupae. Digging took place at the start of autumn when it gets cold quickly in Sapporo. With the exception of several empty cells, all the cells contained pupae. Taken together, the absence of pupae so late in the season suggests that the empty cells were not awaiting pollen provisioning. Dissections and morphological degeneration indicated the maturity of all the adults, suggesting that an empty cell is not simply the result of the eclosion of its owner. Consequently, since empty cells were considered to have been attacked by predators, the survival rate of larvae in each nest could be calculated as a function of the total number of cells and the number of empty cells.

In Sapporo, several *Lasioglossum* species may coexist in an aggregation of nests, and morphological discrimination between species is difficult. We therefore confirmed the

identity of species by comparing the mtDNA sequence of an adult from each nest with the known sequence (Access no. AB299367; Hirata and Higashi 2008).

At the time of digging up the nests, any unmarked adults were considered to be members of the nest. In a few cases, collected females had markings that differed from those of that nest ($n = 2$). In such cases, the bees were considered to belong to the nest where they were finally collected.

After placing a 1.5-m steel tape measure on the ground for scale, the aggregation of nests was photographed from above using a digital camera ($\mu 750$; Olympus, Japan). The digital image was then imported into an image analysis software package (ImageJ ver.1.42, <http://www.nih.gov/>), and the abscissa and ordinates of each nest entrance were recorded as pixel numbers. The average of three measurements was then taken as the coordinates of a nest, and the center of the aggregated nests was calculated as the average of the coordinates of all the nests. The distance of each nest from the center of the aggregated nests was then measured, and pixel numbers were transformed into actual distances (mm) using the relationship between pixel numbers and the tape measure.

The effects of adult number and distance from the center of the nest aggregation on larval survival were then analyzed by using a generalized linear model (GLM). We used a logit-link function with a binomial error distribution to perform a multivariate logistic-regression with the GLM. The model having the lowest Akaike’s information criterion (AIC) was selected as the best model among possible sets of variables. Regression coefficients were estimated using maximum likelihood estimations, and statistical significance of the coefficients was tested by the Wald test. All calculations were conducted using the statistical software R (ver. 2.9.2; R Development Core Team).

Results

The nest aggregation consisted of 20 *L. (E.) baleicum* nests. The number of adult females in each nest ranged from 1 to 5, and each category of adult number contained 4, 3, 3, 4, and 6 nests, respectively.

One nest contained a single adult female but no cells, and this nest was omitted from the analysis because the survival rate of the larvae could not be determined. Since workers of the omnivorous ant, *Tetramorium tsushimae*, were found in the galleries of two nests that contained only empty cells, the ant was assumed to have attacked these nests. The other 17 nests reared at least one pupa, and larval survival was observed to increase markedly as the number of adults in a nest increased (Fig. 1).

The best model selected using AIC criterion included both variables but excluded the interaction term (Table 1),

indicating that the two variables affected larval survival independently. In the best model, the regression coefficients of both variables were highly significant (Table 1). In addition, the number of adults was found to have a positive effect on larval survival (Fig. 1), and there is a marked difference in larval survival between solitary and multiple-female nests. Similarly, the distance from the center of the aggregated nests was negatively correlated with larval survival (Fig. 2).

Discussion

Having multiple adults within a nest and being located near the center of the nest aggregation improved the rate of larval survival in *L. (E.) baleicum*; the former shows a

synergistic effect associated with multiple-female nesting, while the latter demonstrates the advantages of the relative position of a nest within an nest aggregation.

The finding that workers of the ant, *T. tsushimae*, were observed in nests with empty cells suggests that this ant species is a predator of sweat bee larvae. Workers of *T. tsushimae* are very small (ca. 2.5 mm) and employ a recruitment system while foraging. A scout searches for food in shallow underground crevices (Sanada-Morimura, personal communication). When large food items are located by individual scouts searching for food, additional workers are recruited using pheromone trails (Sanada-Morimura, personal communication). Adult *L. (E.) baleicum* guard the nest entrance and larval cells are located several centimeters under the ground surface (Hirata 2005). Since *T. tsushimae* scouts would thus be able to locate

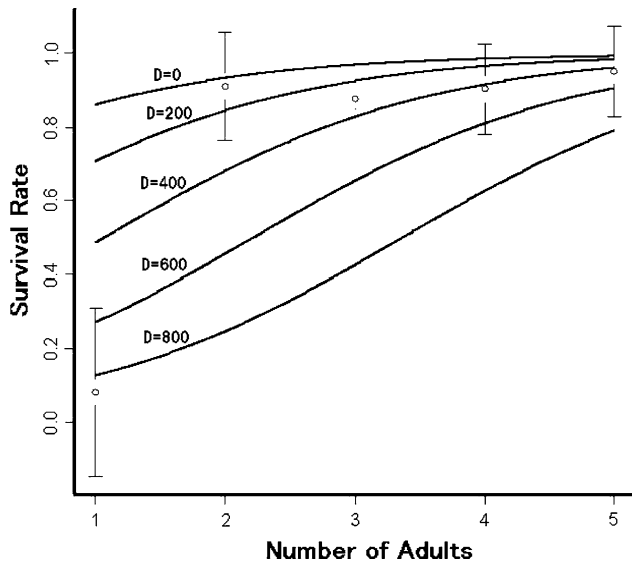


Fig. 1 Relationship between the number of adult sweat bees, *Lasioglossum (Evyllaesus) baleicum*, in a nest and larval survival rate. Lines are multivariate logistic regressions with values representing the distance (mm) of the nest from the center of the aggregated nests (*D*)

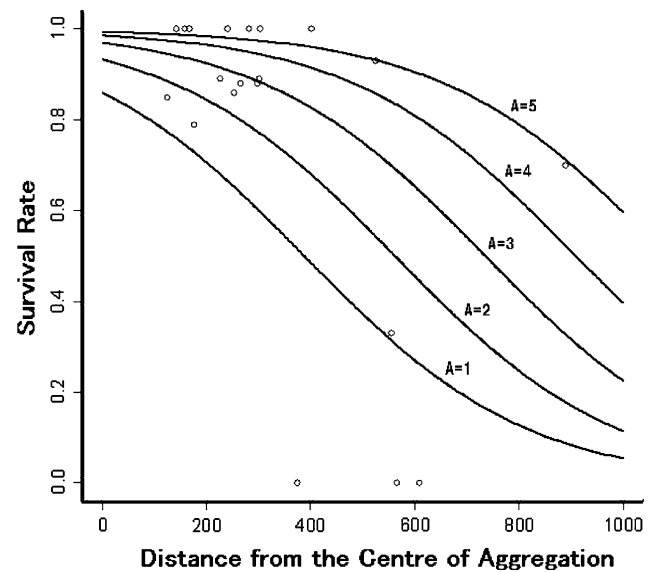


Fig. 2 Relationship between the distance from the center of the aggregated nests and larval survival rate. Lines are multivariate logistic regressions for different numbers of adults (*A*)

Table 1 Results of the GLM analysis using *A* the number of adult sweat bees, *Lasioglossum (Evyllaesus) baleicum*, in a nest and *D* the distance from the center of the aggregated nests as variables

Model	Deviance	Residual deviance	AIC		Regression coefficient	SE	<i>z</i> value	<i>p</i>
A + D + I	40.33	19.34	48.71					
A + D	42.33	20.96	48.33	Intercept	1.0003	0.6469	1.546	0.1220
				A	0.8121	0.2097	3.872	0.0001
				D	-0.0467	0.0120	-3.863	0.0001
A	57.25	35.88	61.25					
D	61.02	39.65	65.02					

The interaction term of both the variables is represented by *I*

L. (E.) baleicum larval cells with relative ease, adult sweat bees must kill the scouts before they return to the ant colony and recruit additional workers.

In several bees belonging to the same subgenus *Evyllaesus*, adult females have been reported to inspect cells by opening them (Packer 1991; Plateaux-Quenu 2008). Unlike the females in cooperative nests, which are able to conduct such inspections frequently if necessary, solitary bees must leave their brood untended while foraging. Since inspection frequency would increase with the number of adult bees in a nest, frequent inspections would increase the likelihood that potential predators are detected. In addition, the gap in the inspection frequency between solitary and multiple-female nests is likely to be large, and this may explain the observed disparity between larval survival and the number of adults in a nest.

Nests located near the center of an aggregation of nests had a relatively higher larval survival rate (Fig. 2). This is because any attacks by predators would likely come from beyond the periphery of the area of aggregated nests, which was not located within an ant-nesting area. There is a report for *L. (E.) duplex* that a nest was taken into the expanded nesting area of an ant, *Formica japonica* (Sakagami and Fukuda 1989). Thus, bee nests located at the periphery of a nest aggregation have higher associated predation risks than nests located near the center of such aggregations. This type of positioning effect has been observed in several organisms that form groups (reviewed in Krause and Ruxton 2002). Indeed, it seems likely that the variable predation risks within such nesting areas explains the observed relationship between nest position and larval survival in *L. (E.) baleicum* in this study.

Since the establishment of a new nest at another location (e.g., at a site closer to the center of the aggregation of nests) would be costly compared to remaining in the natal nest, this choice may affect the decision-making of an F2 female selecting between independent and cooperative nesting. An F2 female with a peripheral nest could easily compensate for the disadvantages associated with nest location by remaining in the natal nest and employing cooperative nesting. Certainly, future research should examine the distribution of multiple-female nests within nest aggregations.

Importantly, the benefits associated with multiple-female nests appear to be independent of kinship between nest members. In this study, several females were collected from nests other than their original nests, suggesting the occurrence of intrusion of unrelated females into the nests of other females. Such intrusion has been reported previously in several bee species (Paxton 2002; Lopez-Vaamonde 2004; Richards et al. 2005; Ulrich et al. 2009). Intruders were previously considered to be social parasites that were harmful to recipients (Lopez-Vaamonde 2004).

However, in solitary *L. (E.) baleicum* bees with nests near the periphery of the area of a nest aggregation, acceptance of unrelated adults may in fact benefit the recipient and increase larval survival. Intruders may also gain direct fitness by producing their own offspring as occurs in a bumblebee species (Lopez-Vaamonde 2004). It is therefore important that future studies examine the genetic relationships among nest members.

Darwin (1859) was the first to introduce the paradox of sociality, i.e., why sterile workers transmit their sterility to the next generation without reproducing. Since Hamilton (1964) proposed the kin selection theory to explain this problem, the evolution of cooperation has been a central theme in evolutionary ecology, with extensive debate about the roles of kin and synergistic effects in social evolution (West et al. 2007; Wilson 2008). In this study, we demonstrated the existence of social polymorphism within a population as well as the synergistic effect of cooperative nesting. In addition, we also demonstrated the potential advantages of using *L. (E.) baleicum* for studying evolution of cooperation. Other than the effects on larval survival, changes in per capita productivity relative to the number of females is considered to be a critical factor affecting social decision-making among females (see Smith et al. 2007). Further studies including productivity data with information on kinship within individual nests will improve our understanding of social evolution in organisms.

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