

EVOLUTIONARY ECOLOGY

The benefits of grouping as a main driver of social evolution in a halictine bee

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Over the past decade, the cause of sociality has been much debated. Inclusive fitness [br in Hamilton's rule ($br - c > 0$)] has been criticized but is still useful in the organization of a framework by elucidating mechanisms through which br (benefit \times relatedness) becomes larger than c (cost). The bee *Lasioglossum baleicum* is suitable for investigation of this issue because of the sympatric occurrence of both social and solitary nesting in its populations. We show that a large part (approximately 92%) of the inclusive fitness of a eusocial worker can be attributed to the benefits of grouping. A 1.5-fold relatedness asymmetry benefit in singly mated haplo-diploids explains a small part (approximately 8.5%) of the observed inclusive fitness. Sociality enables this species to conduct foraging and nest defense simultaneously, which is not the case in solitary nests. Our results indicate that this benefit of grouping is the main source of the increased inclusive fitness of eusocial workers.

INTRODUCTION

A recent prominent and much-debated topic in evolutionary biology involves identification of the major drivers of social evolution (1–9). Relatedness asymmetry (RA) between full sisters (0.75) and their offspring (0.5), as found in singly mated haplo-diploids, confers a 1.5-fold genetic benefit [RA benefit (RAB)] to daughters by altering their reproductive efforts toward the rearing of full sisters rather than to raising their own offspring (6). One focus of debate is the role of RAB versus the benefit of grouping as primary drivers of sociality (4, 5). The question seeks to understand how the fitness of a cooperative individual increases in comparison with that of a solitary individual. On this issue, there is no current obvious, robust empirical answer.

In an evolutionary theory of sociality, Hamilton's rule posits that kin selection in social species causes genes to increase in frequency when the benefit to the recipient (b) multiplied by the genetic relatedness of a recipient to an actor (r) is greater than the reproductive cost to the actor (c), that is, $b \times r - c > 0$, where b is the increase in the number of offspring of the recipient [usually the colony mother (queen) through the altruistic behavior of a donor], c is the decrease in the number of offspring of the donor as a result of altruism, and r is the relatedness of the donor to the recipient. This indicates that if b is sufficiently large, sociality can evolve even if r is small (5). Because $r \leq 1$, the condition that fulfills this rule is $|b| \gg |c|$ in organisms without RA (for example, $2|b| > |c|$ for diplo-diploid organisms) but $(0.5 \text{ to } 0.75) \times |b| > |c|$ in haplo-diploid organisms with RA (7). Thus, Hamilton argued that sociality would evolve more frequently in haplo-diploid organisms than in the other organisms (6) because of this low evolutionary hurdle for altruistic behaviors. In the original Hamilton's rule, b and c are defined as real numbers representing the number of focal allele copies passed to the next generation from the recipient and the donor [Hamilton's rule special (HRS)]. HRS has been shown to fail in predicting the direction

of selection (5, 10–13). There are several strong constraints in efforts to determine this directionality of an altruistic allele following this rule (5, 10–14). In addition, there are other constraints [for example, weak selection and additivity of direct and indirect fitness terms in the inclusive fitness (14)] that must be satisfied to determine the direction of selection of an altruistic allele (13, 14). To relax these constraints, another version of Hamilton's rule has been proposed in which b and c are estimated as the slopes of regression of fitness on the focal trait value [Hamilton's rule general (HRG)] (1, 15). However, theoretical analysis has shown that HRG does not generate any prediction of the direction of selection of the focal trait because the trait itself is used to estimate b and c , which determine the direction of selection, a logical circulation (16). Therefore, as HRG is limited by its assumptions, a selective force toward sociality cannot be examined empirically by using HRG (16).

There are claims from several viewpoints (see above) for the concept and use of inclusive fitness and HRG based on it. However, a recent study has shown that HRG is still useful to clarify the organization framework by knowing how br becomes larger than c (17). Thus, knowing the degree of nonlinear trade-off between $|b|$ and $|c|$ caused by cooperation is important for clear understanding of the driving force leading toward sociality in nature (4, 5, 18–20). If the number of reared brood per female (brood productivity) in a cooperative nest is relatively large compared to that of a solitary nest, then we could assume that grouping effects were the most important factors acting to select for relevant altruistic alleles (18). However, recent debates have been based only on theoretical studies because there are few species in which the sympatric occurrence of both social and solitary nests can be investigated.

Therefore, qualitative data on relative brood productivity in the compared social and solitary nests of a sympatric, socially polymorphic species are crucially important. A previous study examined fitness differences between solitary and social females in a socially polymorphic bee species (*Xylocopa sulcatipes*), but the result was ambiguous because solitary females attained greater fitness in one study year, while cooperative females exhibited greater fitness in another (21). An alternative subject, the sweat bee, *Lasioglossum baleicum*, is highly appropriate for the study of this issue because its populations show sympatric co-occurrence of both solitary and social

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nesses (22–25), comparison of which could help to clarify the benefits of sociality (23, 25). A previous study demonstrated that in two different populations in different years, eusocial workers were able to rear more brood per capita than solitary females (23). This finding suggests a larger degree of increase of fitness of eusocial workers, evidenced as inclusive fitness. This factor has been estimated from brood number reared and kin structure within eusocial nests (23).

In addition, solitary nesting in *L. baleicum* is likely to represent a strategy serving to make the best of the existing situation [the so-called the best of bad jobs strategy (23)]; for example, solitary females have lost association with their mothers and are forced to maintain a nest by themselves irrespective of the disadvantages of solitary nesting. Thus, the observed increase in brood productivity and a higher inclusive fitness in social bees compared to that in solitary individuals (23) could not be explained as being due to the maintenance of a genetic polymorphism with the equalized fitness of multiple genotypes. It has proved possible to quantify the advantage of grouping in this species to know how inclusive fitness of a eusocial worker increase (23). Here, we focus on the question “how do eusocial *L. baleicum* workers increase brood productivity and resulting inclusive fitness?” We investigate how *L. baleicum* in cooperative nests is able to realize a much higher (ca. ninefold) brood productivity than in social nests (23) by measuring ecological parameters in both nest types that affect brood productivity. More specifically, we estimate the relative contributions of RAB and the benefits of grouping to the observed inclusive fitness under the assumption of $|b| = |c|$, considering the question “how is inclusive fitness increased by sociality” to establish an organizing framework for social evolution (17).

Female sweat bees defend their broods by plugging their nest entrance from the inside using their heads. Presumably, this “head plugging” habit protects the nests from predation by ants, which we have observed to be the main predators of *L. baleicum* (23). If there are multiple females in a nest, then the colony can conduct foraging simultaneously with nest guarding, which a solitary bee cannot. Thus, sociality might be more advantageous than solitary nesting in this regard. Many of the nests of this species are eusocial (23), and therefore, we can compare the degree of RAB benefit and grouping under an assumption of $|b| = |c|$.

Here, we tested the hypothesis that coping with foraging and nest defense in social nests increases brood production in the nests (as a benefit of grouping) and estimated the relative contributions of RAB and of grouping as contributors to the inclusive fitness of a eusocial worker. We observed five nest aggregations in various regions in Hokkaido and measured foraging parameters (number and duration of foraging trips) and nest defense efficiency for both solitary and social nests. In addition, we conducted a simulation under the assumption of random foraging by members of social nests, by which we compared the estimated time during which no female occupied the nest (empty time) with the observed empty time. When the observed empty time is significantly shorter than the expected time, cooperation among nest members is considered to have occurred. These data will provide an answer to the much-debated question we are considering.

RESULTS

We usually found that solitary bees undertake only a few foraging trips, all of which were conducted in the afternoon. Bees in social nests

foraged more frequently during the whole day (Fig. 1, A to E). Because this pattern was similar across the subject populations, we combined all data for statistical analyses (Fig. 1F).

The average number of trips per worker was significantly greater for bees in social nests (Wilcoxon rank sum test, $W = 2878.5$, $P < 0.0001$; Fig. 2A). This trend is the same when data were analyzed using a generalized linear model (GLM). The total number of foraging trips per female was regressed on three independent variables {ant activity, nest type [solitary (0) or social (1)], and the site of the investigated aggregation}. A GLM with a negative binomial distribution with a log link function showed a highly significant positive effect of nest type on the number of trips per female [slope = 2.902, $t = 7.078$, $P < 0.0001$, $dp = 1.119$; dp is the dispersion parameter of the model, and when this is 1, the dispersion of the data from the model is within the predicted variance from the model (meaning the model fitted well to the observed data)]. A less than 1.5 dp suggests that there is not so much deviations of the observed data from the model.

The average foraging time per worker was significantly longer for individual bees from social nests (Wilcoxon rank sum test, $W = 464$, $P = 3.63 \times 10^{-6}$; Fig. 2B). The GLM analysis showed that the nest type

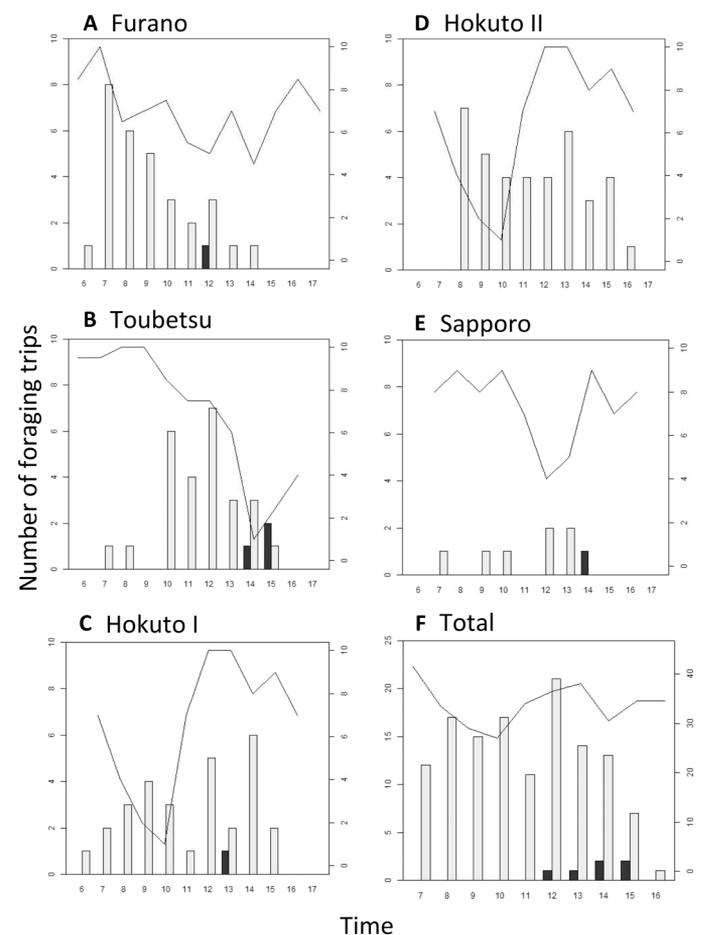


Fig. 1. Foraging activities of predatory ants (line) and of solitary sweat bees (*L. baleicum*) (black bars) and bees from social nests (gray bars). (A to E) Results of the five nest aggregations. (F) Combined data. In all aggregations, solitary bees conducted only a few trips during the afternoon, whereas bees from social nests conducted frequent trips throughout the day.

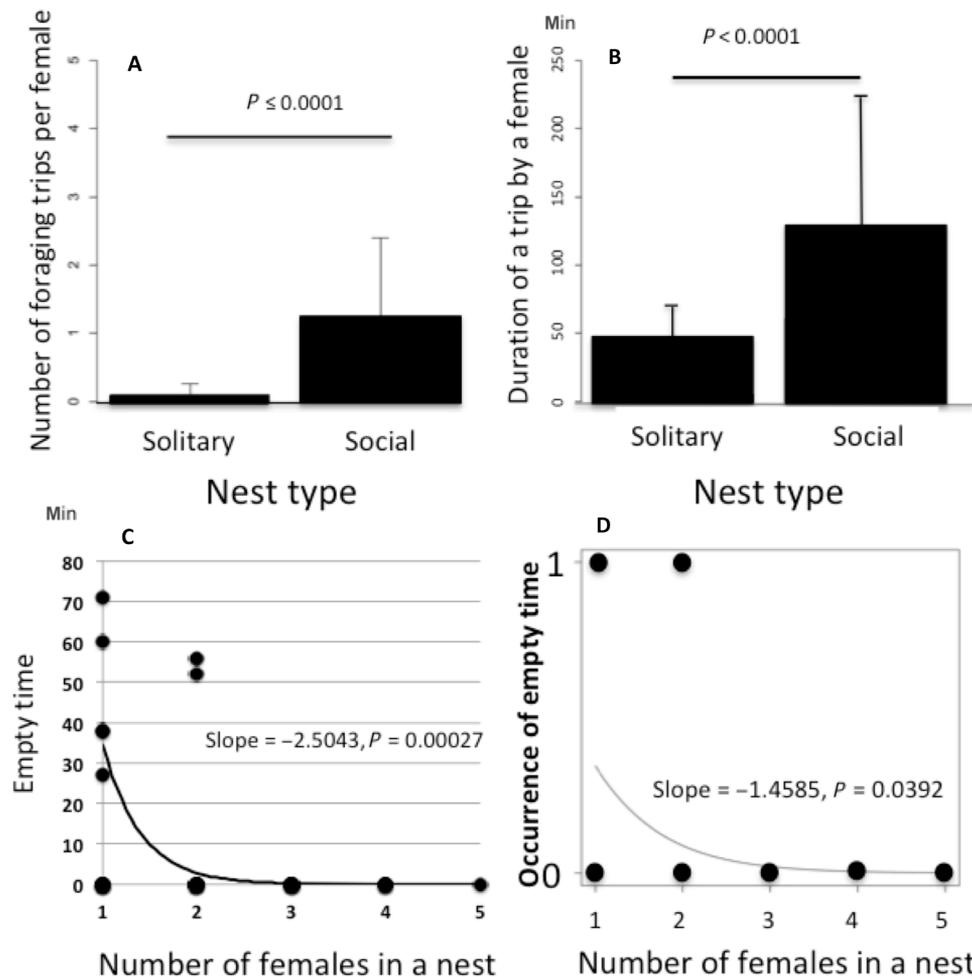


Fig. 2. Comparisons of foraging activities and the time nests were empty between solitary *L. baleicum* and bees in social nests. (A) The number of foraging trips per worker was significantly greater for bees in social nests than for solitary bees. (B) The total duration of foraging trips per day per female was also significantly longer for social bees. The average + 1 SD was shown for each nest type. (C) GLM regression of the observed empty time on number of females in nests. Black circles are the observed values, and the curve is the exponential regression line using the estimated parameters. For (A) and (B), we presented the results of analyses using GLM in the text (see Results), but the results showed the same tendency with (A) and (B).

positively affected the total duration of trips per female (slope = 8.20954, $t = 5.340$, $P < 0.0001$, $dp = 1.1040$). Predation risk (ant activity) was negatively correlated to foraging duration in the solitary nests (slope = -0.03576 , $t = -2.903$, $P = 0.0037$, $dp = 1.4302$) but was not correlated to foraging duration in the social nest (slope = 0.000723, $t = 0.342$, $P = 0.7322$, $dp = 1.4317$). The ratio of foraging duration between social and solitary bees was approximately 15:1 at the nest level and 4.9:1 at the level of individual foragers.

From the records of the foraging activity from 5:00 to 17:00 for each nest, we calculated the duration of periods during which the nest was not occupied by adults (empty time) for each colony. When the nest is empty, the nest is not defended from predations during this time. The observed empty time was regressed on the number of females in the nest using GLM with a negative binomial distribution with a log link function. We selected this distribution because, when GLM was conducted using a Poisson distribution with a log link function, there was a large degree of overdispersion ($dp = 49.24$). The dp of the model was improved to 1.17 when we used the negative binomial distribution. In the later GLM, the empty time shows

a significant negative correlation with the number of females in the nest (slope = -2.5043 , $t = -3.899$, $P = 0.00027$; Fig. 2C). Occurrences of empty time also negatively correlated with number of females in a nest (slope = -1.4585 , $t = -2.062$, $P = 0.0392$, $dp = 0.7593$; Fig. 2D). In addition, the solitary nests were empty more frequently (five times in 19 nests for solitary nests versus two times in 38 nests for social nests; Fisher's exact probability test, $P = 0.035$).

In the case of social nests, we conducted a simulation to estimate the empty time with the experimental assumption of coexistence (each individual executes a foraging trip irrespective of behaviors of the other nest members) rather than effective cooperation (for details, see Materials and Methods) and compared the expected and observed empty times to test for the existence of cooperation. The occurrence score for empty time is significantly different from that observed and simulated data (2 of 33 and 14 of 33 in the observed and expected, respectively; Fisher's exact probability test, $P = 0.0011$). In addition, the observed empty time was significantly shorter than that expected (Wilcoxon signed rank test, $V = 0$, $n = 15$, $P = 0.0313$; Fig. 3). Thus, cooperation among adult females serving to minimize

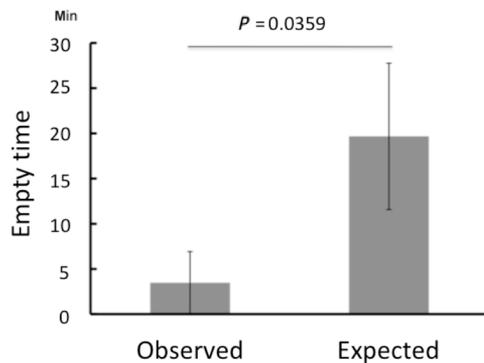


Fig. 3. The observed and the expected durations in which a nest is empty for social *L. baleicum*. The expected durations that nests were empty were calculated on the basis of the assumption that each individual forages independently, that is, the individuals within a nest coexist but do not cooperate. The observed duration that nests were empty was significantly shorter than the expected duration under coexistence, thus indicating that the nest members cooperate to minimize the time that a nest is empty.

predation risks by plugging nest entrances exists in social colonies. The estimations of contributions of both RAB and benefits of grouping showed that the estimated contributions of RAB and the benefits of grouping are ca. 8.5 and ca. 92.0%, respectively (for detailed calculation, see section S1, tables S1 to S6, and Table 1).

DISCUSSION

Our investigations demonstrated the following facts: (i) Multiple female nests conduct many more foraging trips across most of the day (Figs. 1, A to F, and 2A). (ii) The duration of any single trip by a forager is longer in the multiple female nests than that in the solitary nests (Fig. 2B). (iii) Empty time is negatively correlated with the number of females in the colony (Fig. 2C), and solitary nests were empty more frequently than multiple female nests. Therefore, cooperative nests were more efficiently defended. Thus, these benefits of grouping should increase relative levels of brood production in social nests. This prediction matches the observed difference in offspring production between the two nest types in the relevant previous field study (23). These results demonstrate that foraging efficiency is much improved for bees in social nests, which would result in relatively high brood productivity compared to the relatively inefficient solitary nests in addition to the improvements in defense efficiencies.

The advantages of cooperative nesting in *L. baleicum* are demonstrated by the much longer duration of foraging trips per day (approximately 4.9-fold at individual level; Fig. 2B) and greater efficiency of defense in those nests (see Results). These results matched with the results of a previous study that has shown ca. nine times greater brood numbers for a cooperative female compared to a solitary female (23). These data indicate that the benefits attained by grouping (that is, nonlinear trade-off between **b** and **c**) are the main cause in the increase of fitness in social nests in this species. In addition, this group advantage helps to explain why cooperative nests comprised only nonkin individuals in this species (23).

Grouping brought two major merits for social females. The first is avoidance of predation risks (26–28). This appears to be the primary benefit for *L. baleicum* in social nests because solitary bees shorten empty time in the nests by reducing the time spent foraging.

Head plugging has been shown to be necessary for nest survival in these species (29). The empty times were longer for solitary bees. Thus, the efficient nest defense afforded by grouping should be important in the evolution of sociality in cavity-dwelling organisms. The second is advantage in foraging efficiency (Fig. 2, A and B). The low productivity of a solitary female (23) is explained by her reduced foraging effort. The foraging efficiencies of social nests increased 15-fold at nest level and ca. fivefold at individual level. This advantage of grouping appears to be the most important evolutionary driver of sociality in this bee species (18).

A cooperative female can increase her fitness when $r = 0$ and if grouping sufficiently increases the direct fitness of each individual in that group (that is, although $b \times r = 0$, $-c$ becomes positive) (4, 5). In *L. baleicum*, unrelated females that obtain a higher direct fitness than that of a solitary female maintain a portion of the social nests (9.1%) (23). Furthermore, 36.4% of eusocial colonies contain at least one unrelated adult female (23). Kin selection cannot explain the evolution of this cooperation by unrelated females, but the benefits of grouping can. The 1.5-fold of RAB is arisen only when workers are full sibling (that is, they are daughters of a singly mated mother). Although a part of F0 females are likely to mate with two males (see fig. S2), daughters of both types of F0 females cooperate with their mothers, suggesting a low importance of RAB in this bee. However, grouping merits work similarly on a group consisting of a mother and her daughter. *L. baleicum* becomes social at the second reproductive period in a colony year at which predatory ants are active (30). Thus, in the early stage of social evolution, both the mother and her daughters would gain a fitness benefit by mere coexistence (without cooperation).

In haplo-diploids, the 1.5-fold RAB is considered to promote eusociality (1–3, 6, 8). However, our calculation under the assumption of $|b| = |c|$ as in the Hamilton's original argument (for details, see tables S1 to S6) (6) showed that only ca. 8.5% was attributable to RAB, whereas ca. 92% was explained by the benefits of grouping (Table 1). This result indicates that a large degree of benefit from grouping ($|b| \gg |c|$) seems to be the main driver of social evolution in *L. baleicum* (4, 5, 16). However, note that this does not mean that RAB is unimportant in eusocial evolution. Eusocial workers showing no reproduction have the only way to transmit their genes to the next generation through their mother (queen). In other words, the evolution of "eusociality" could not achieve without kin selection. Eusociality has evolved many times in haplo-diploids with RAB but only occasionally in diplo-diploids without RAB (6). Certainly, the small contribution of RAB would be important in multiple evolutionary origins of sociality in haplo-diploids.

Another important consideration involves the cost of grouping in *L. baleicum*. This species digs a short (several centimeters) vertical burrow with several brood chambers (23). Thus, the number of available chambers per female decreases with increases in the number of females sharing the nest (23). This cost may answer the question of why daughters become sterile after sharing a nest with their mothers because they would thus not have to compete with one another for limited reproductive space.

The benefits of grouping described here could exist for all organisms that form cavity nests. A RAB can apply only to haplo-diploid organisms (1, 2, 6). However, diplo-diploid eusocial organisms [for example, termites (31), ambrosia beetles (32), shrimp (33), and naked mole rats (34)] or clonal eusocial organisms [for example, aphids (35)] lack this genetic advantage toward eusociality (36), but all of them

Table 1. The inclusive fitness values of a eusocial *L. baleicum* worker and the proportions attributable to each RAB and the benefits of grouping. The RAB explained 8.5% of the observed inclusive fitness of a eusocial worker, whereas the benefits of grouping explained 92.0%. The demographic data used for calculation are that in (23), and the calculation procedures are presented in tables S1 to S7.

	Attributable to				Total
	RAB	Benefits of grouping		Unexplained	
		For foraging	For defense		
Estimated fitness	0.155	1.058	0.621	-0.008	1.826
Explained (%)	8.49	57.94	34.01	-0.44	100
Total	8.49	91.95		-0.44	100

are cavity dwellers. Our findings can explain the establishment of group living in these organisms. Group living seems to be a prerequisite for the evolution of sociality. Many solitary wasps (with RA) have a similar lifestyle with halictid bees (making a nest with several brood chambers to which collected preys are stuffed with an egg). However, most of them leave the nest before the emergence of adult offspring although they are multivoltine (37, 38). This habit deprives a daughter of the chance to choose to coreproduce with her mother. Thus, the coexistence of multiple individuals in a nest could be another crucial factor underlying the evolution of sociality in cavity dwellers.

Finally, the cost of grouping (for example, a spatial limitation for brood chambers) may be another key to the evolution of sterile workers in organisms without RA because the increasing individuals in a nest will increase the degree of competition, and this large cost makes easy the evolution of sterility when the recipient is kin. Because the kin selection model is theoretically compatible with the group selection model (39–41), additional empirical studies to elucidate the relationships between r and the degree of $|b|$ relative to $|c|$ are crucial to understanding how and why sociality has evolved in nature (18).

MATERIALS AND METHODS

Biology of *L. baleicum*

L. baleicum is a small sweat bee found in Japan. It constructs nest aggregations (several to 20 to 30 nests per site) at suitable sites (bare and sunny areas at forest edges) and produces two broods per year. Many F0 females mated with a single male, but some females were likely to mate with two males (see fig. S2). Social nests are formed only during the second reproductive season (24, 30). This species shows sympatric social polymorphism in which three types of nests exist: (i) Many include a mother and a few of her sterile daughters (eusocial), and this type of nest occasionally contains several unrelated females; (ii) some nests consist of multiple assembled unrelated females (parasocial); and (iii) a small proportion of nests are maintained by single females (solitary) (23). Because the fitness of a solitary individual is much lower than that of a social individual (23), the solitary state appears to be an enforced, unfavorable condition (that is, solitary individuals have failed to find partners). As we did not measure structure among members within social nests in this study, we treated the sum of eusocial and parasocial nests (when the latter were present) as “social nests” since the aim of this study is to elucidate differences in ecological parameters between the two nest types. There is no difference between the two (solitary and social)

nest types in terms of the per capita number of brood cells (23), but a difference in fitness arises from marked increases in the number of larvae reared in social nests (that is, most of the brood chambers in solitary nests were empty) (23, 25). The causes of this lower brood productivity could not be elucidated by the previous data, and the present results in this study would clarify this point. The main predators of this bee species appear to be the ants (23) (*Myrmica kotokui*, *Pheidole fervida*, and *Tetremorium tsushimae*) and a parasitic wasp (*Nomada* sp.). Although there were social nests that consisted of only nonkin females (23), we summed the data of both the eusocial and nonkin nests in this study because the aim of this study is to elucidate improved ecological parameters by sociality. The difference between kin and nonkin nests will be published elsewhere. The permission of owners was obtained for all study sites.

Behavioral observations

Five nest aggregations of *L. baleicum* in Hokkaido, Japan were observed. The day before observations began, all adults in the nests were marked with paint dots at two points (head and tip of the abdomen) using different colors (Paint Marker, Mitsubishi) on 20 July 2013 in Furano, 5 July 2014 in Toubetsu, 10 July 2015 in Hokuto I and II, and 22 July 2015 in Sapporo. After marking, the bees were returned to their nests. The numbers of solitary and social nests observed were Furano (3, 7), Toubetsu (9, 10), Hokuto I (3, 5), Hokuto II (1, 7), and Sapporo (1, 7). We studied these nests on 21 July 2013 in Furano, 6 July 2014 in Toubetsu, 11 July 2015 in Hokuto I and II, and 23 July 2015 in Sapporo. Each nest in each aggregation was observed from 5:00 to 17:00, and departure and returning times for each forager were recorded using two digital video recorders (DMX-CA100, SANYO; DMC-H700M, Panasonic). From the recorded movies, we determined the ecological parameters, number, and duration of foraging trips per female and empty time for each colony. In some cases, we could not identify the time of departure or return, and the number of foraging duration records became smaller than the number of foraging trips. The empty time represents a vulnerability to predation of each nest because during which the bees cannot defend their brood from predatory ants. A previous study confirmed predations of brood by ants intruding into bee nests (23). Twenty traps constructed from aluminum foil (5 cm × 5 cm) and baited with meat were randomly placed within a circle (approximately 5-m radius) around each nest aggregation. The number of traps where ants were observed (*P. fervida* or *M. kotokui*) was counted every hour and used as an index of predatory activity of the ants. Each aggregation was observed only once to avoid pseudoreplications.

GLM model analyses for the measured parameters

To examine the effects of measured parameters on foraging activities, we used GLM analyses. For the number of foraging trips per female, we constructed a GLM model “number of trips = site of aggregation + type of nest (solitary or social) + predation risk (ant activity) + the interaction term (type of nest \times predation risk).” When the interaction term was not significant, we removed it and reanalyzed with the remaining independent variables. We intended to evaluate the effect of nest type on the number of foraging trips by this model. Thus, when variables other than nest type became significant, we used the effect of nest type from that model.

For the duration of foraging trips per female, we made a GLM model separately for nest type. The model is “total foraging duration per nest or female = number of workers in their nest (total females – 1, for social nests only) + predation risk + the interaction term.” When the interaction was not significant, we reanalyzed a model without it. For empty time, we regressed the observed empty time of a nest on number of workers (total females, –1) using a GLM model (empty time = number of workers).

In all the GLMs used in this study, we checked distributions of dependent variables before analyses. When the distribution significantly deviated from normality (examined by the Shapiro-Wilk test), we assigned another more adequate distribution (see fig. S1, A to E). For most cases, we used a negative binomial distribution in GLMs using the `glm.nb()` functions in the MASS package for R, which automatically estimated the required parameters for the negative binomial distribution. In the case of GLM between empty time and number of females in the nest, we sought parameters of a negative binomial distribution by which the dp of the model becomes less than 1.5. In all the analyses, overdispersion using a Poisson regression was relaxed by using a negative binomial distribution to be dp that becomes less than 1.5. This criterion ($dp < 1.5$) does not have a theoretical basis, but this treatment much improved the degrees of overdispersions (see Results).

Simulation estimating the time nests spent empty under the assumption of independent foraging

From the observed data, we estimated empty time under the assumption that each female forages independently (mere coexistence with no cooperation). For each social nest, the observed foraging pattern of an individual was randomized along a time axis (from the first to the last leaving time). For example, when an individual foraged three times with different durations, these three foraging times were placed at random on the time axis. This treatment was performed for all individuals in each nest, and the empty time of each nest was calculated from the randomized data. When multiple foraging trips by an individual overlapped, the results were discarded and resimulated because these situations are not possible in the real world. The expected empty time was calculated under the assumption that each individual foraged independently irrespective of behavior of other females. The averages between the observed and estimated empty times were then compared.

Statistics

All the statistic analyses were conducted by using R (version 3.2.1).

SUPPLEMENTARY MATERIALS

Supplementary material for this article is available at <http://advances.sciencemag.org/cgi/content/full/4/10/e1700741/DC1>
Section S1. Additional methods
Fig. S1. Frequency distributions of dependent variables of GLMs used in the analyses.

Fig S2. Distribution of relatedness between females belonging F1 and F2 generations.
Table S1. Colony demographic data and estimated fitness of workers for both solitary and social nests of *L. baleicum* during 2009–2010 at Sapporo.
Table S2. The recorded foraging durations in social and solitary nests.
Table S3. The obtained parameters to estimate expected inclusive fitness of a eusocial worker.
Table S4. Observed and estimated (inclusive) fitness of a worker (or female) in each nest.
Table S5. Estimated inclusive fitness from the defense benefit.
Table S6. Estimated inclusive fitness from RAB for a solitary female by becoming sterile worker to rear sisters ($|b| = |c|$ and the maximum RAB is assumed).

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