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Extended Parental Care in Communal Social Groups

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Abstract

Recent developments in social insect research have challenged the need for close kinship as a prerequisite for the evolution of stable group living. In a model communal bee species, *Lasioglossum (Chilalictus) hemichalceum,* previous allozyme work indicated that groups of cooperating adult females are not relatives. Yet at any given time, not all group members perform the risky task of foraging. We previously hypothesized that tolerance for non-foragers was a component of extended parental care, previously known only for kin based social systems. DNA microsatellites were used to study colony genetic structure in order to test this hypothesis. Microsatellite polymorphism was substantial (*He* = 0.775). Overall intracolony relatedness, mainly of immatures, was low but significant in nine, late season nests ($r = 0.136 \pm 0.023$), indicating that broods contain five to six unrelated sib ships. Detailed analyses of kinship between pairs of individuals revealed that most pairs were unrelated and most related pairs were siblings. Mothers are absent for 89-91% of the developing immature females, and 97% of developing males. Alternatively, 46% of adult females had neither sibs nor offspring in their nests. These findings indicate that the extended parental care model applies broadly to both kin based and nonkin based social systems in the Hymenoptera.

Keywords: Halictidae, *Lasioglossum*, relatedness, kinship, extended parental care

Introduction

Communal associations in which group members occupy a single nest, all group members are reproductively active, and brood care is in some way shared are common in the Hymenoptera (Eickwort, 1981; Kukuk and Sage, 1994; Wcislo and Engel, 1996). Recent empirical work indicates that, in the Apoidea, members of communal groups are not relatives (Danforth et al., 1996; Kukuk and Sage, 1994; Paxton et al., 1997). Communal bees thus afford the opportunity to examine the ecological constraints acting on individuals to produce the evolutionary transition from solitary to group life in the absence of indirect fitness effects. Moreover, while a large body of evidence supports the notion that the evolution of eusociality can be explained by kin selection (Hamilton, 1963, 1964ab), explaining complex cooperation among nonkin remains problematic. If an individual can benefit from cooperation with nonrelatives, each one might do better by taking advantage of the cooperative behaviors of others (Axelrod and Hamilton, 1981). In communal bees, the limitations on cheating are not yet fully understood but evidence from natural nests of *Lasioglossum (Chilalictus) hemichalceum* suggests that egg guarding may prevent cheating (Ward and Kukuk, 1998).

L. hemichalceum is a communal, mass provisioning, halictine species in which groups of unrelated adult females occupy a single nest (Kukuk, 1997; Kukuk and Sage, 1994). Females are remarkably cooperative and exchange food by oral trophallaxis with both familiar and unfamiliar individuals (Kukuk, 1992, Kukuk and Crozier, 1990). At any given time only a subset of the females occupying a single nest are foragers (Ward and Kukuk, 1998).

Field studies of the communal halictine bee *L. hemichalceum*, a model species for the study of communal sociality, suggest that extended parental care may be the most important factor leading to cooperation with nonrelatives (Kukuk et al., 1998). The theory of extended parental care (also termed Assured Fitness Returns by Gadagkar, 1990) suggests that group life is advantageous because if an individual dies her offspring will then be reared by other members of the group. This theory applies to kin-based systems with progressive provisioning and depends on indirect fitness returns through continued feeding of related immatures after the death of their mother (Clark and Dukas, 1994; Field et al., 1998, 2000; Gadagkar, 1990, 1994, 1996; Queller, 1994, 1996; Strassmann and Queller, 1989). Cooperating adults in communal species are not relatives (Kukuk and Sage, 1994) and halictine bees are mass provisioners so that developing immatures do not depend on the presence of adults for food (Michener, 1974). Confirmation of that extended parental care if found in a communal, mass provisioning species would extend the generality of this mechanism to a broader array of social systems than previously thought.

Field data suggest the hypothesis that extended parental care occurs in this species and is driven by the following constraints: (1) the egg to adult development time is long (about six weeks); (2) foraging is very risky so that the life expectancy of a forager is less than half of the egg to adult development time; and (3) orphaned brood is vulnerable to catastrophic predation by ants (Kukuk et al. 1998). The presence of adults appears to be required, not to provide food after a forager's death, but to protect her offspring from predation.

The occurrence of group living *per se* and the tolerance of nonforagers in nests by foragers at a specific time can both be explained if the *L. hemichalceum* social system involves extended parental care (Kukuk et al., 1998). This would produce a colony cycle as depicted in Figure 1. The continued care of brood results if some spring females delay foraging. This delay ripples through the season so that adult females are always present in nests containing brood but are not the mothers of that brood. While the field evidence led to the formulation of this hypothetical colony cycle, additional supporting evidence is required. If the colony cycle suggested by field data is correct, then immatures in nests that were protected from ant predation by adults will not be the offspring of those adults.

Using microsatellite genetic markers, we examined the genetic structure of *L. hemichalceum* colonies in detail to test this prediction. Colonies that served as controls for a field experiment in which removal of adults resulted in greatly increased ant predation on orphaned brood were examined genetically because adults in these nests did in fact protect immatures. Even though active colony defense was not observed the presence of these adults in these nine colonies prevented ant predation on brood (Kukuk et al., 1998).

Materials and Methods

The entire contents of nine control nests of *L. hemichalceum* were obtained from the 5-Way nest aggregation located in Cabboboonee State Forest in South Eastern Victoria, Australia at the intersection of Fish Holes and Cut out Dam Roads. These nests were of equal age as all were naturally established in January of the previous year. They were excavated late in the reproductive season and contained developing brood and adults (see vertical dotted line in Figure 1). The nests were used as controls for an experiment in which females were removed from natural nests and excavated at a time when active provisioning was decreasing substantially as fall approached (Feb. 16-22, 1995, see Kukuk at al., 1998). Nest excavation techniques followed those of Abrams and Eickwort (1980). All excavations were carried out in the evening so that all colony members were present. All individuals were placed in 95% ethanol for later genetic analysis. Individuals were assigned to one of 11 age classes from worn adult (class 11) to larvae (class 1) based on wing wear and cuticular hardness (for adults and tenerals, stages 11-9), pigmentation (for pupae, stages 8-3), prepupae (stage 2) and larvae (stage 1).

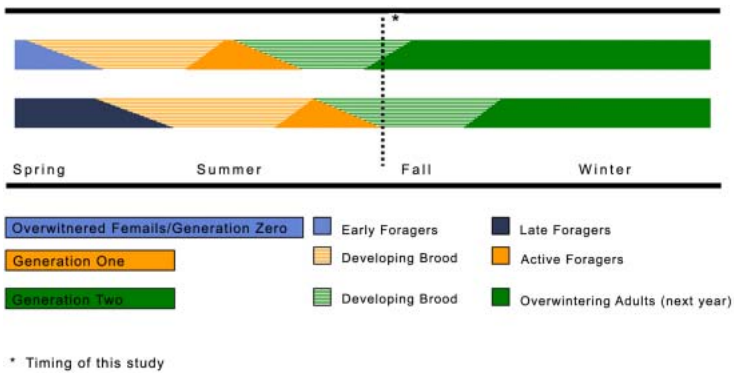

Figure 1. The annual colony cycle of *Lasioglossum (Chilalictus) hemichalceum.* In spring, colonies consist of mated, overwintered females (blue) some females forage early (light blue) and others do not forage until later (dark blue). Foragers die before their brood matures but unrelated adults are present in colonies because not all adults forage at the same time. The hatched vertical line indicates timing of this study.

Tissue extractions used the entire head or half the thorax from adults and pupae, and approximately one third of the body from prepupae and larvae. The tissue was crushed, suspended in 200 µl H_20 containing 5% w/v Chelex resin (Bio101, Inc.), vortexed, autoclaved for 5 min., cooled, and centrifuged in a high-speed microfuge to pellet the cell debris and resin beads. DNA microsatellite loci were amplified using the polymerase chain reaction (PCR). Reactions contained 2ul of the Chelex preparation supernatant in a total volume of 10ul, using Perkin-Elmer *Amplitaq* DNA polymerase under the manufacturer's recommended conditions. One primer for each locus was fluorescently labeled with HEX or FAM dye and the PCR products were visualized in denaturing polyacrylamide sequencing gels using an FMBIO-101 gel scanner (Hitachi Inc.). Ten microsatellite primers were used in this study but one of these loci proved to have a null allele and so was excluded from all analyses (see Kukuk et al., in press for detailed methods).

Basic genetic parameters were calculated using *BIOSYS-1* (Swofford and Selander, 1989). Genetic relatedness (*r*) among nest mates was estimated by the method of Queller and Goodnight (1989) using the program *Relatedness 5.0*. For measures between females we used the "symmetrical relatedness" capability of version 5.0. However, *r* between the sexes in haplodiploids is not expected to be symmetrical, so both F-M and M-F values are reported. Estimation of pair-wise individual relatedness, performance of exclusion and likelihood ratio tests of kinship, and kinship simulations are based on the observed allele frequencies using *Kinship 1.3* (Goodnight and Queller, 1999). All *Relatedness* and *Kinship* calculations used allele frequency bias correction by colony.

Pair-wise Kinship tests were used assessed colony structure using a three-step process. First, *Kinship* was used to detect all pairs of females sharing an allele at each locus. For females in a haplodiploid system, both mother-daughter pairs and full sib pairs must meet this criterion so that their relationship is excludable by a single incompatible locus. With nine codominant microsatellite loci each having high allelic variation, the type II error rate is effectively zero even at $\alpha = 0.001$. Because they have the same criteria, these exclusion tests for mother-daughter and full sister relationships reveal exactly the same sets of related females so two additional steps were needed to distinguish between mother-daughters and full sisters. Second, all adult-adult pairs and immature-immature pairs were eliminated. Third, because full sister and mother-daughter pairs, while both share an allele at each locus, have expected *r* values of 0.75 and 0.50 respectively, we graphed the pair wise relatedness values by age for the remaining adult-immature pairs to provide additional criteria for distinguishing between the two relationships.

We repeated similar steps for female-male pairs using tests appropriate for diploid-haploid relationships, to assess whether these are most likely to be mother-son or brother-sister pairs.

Results

Colony Composition

The sample consisted of 231 bees (180 females, 51 males) from nine natural nests. Because nests were excavated in the evening after all activity had ceased, all nest occupants were assumed to have been obtained. All colonies contained adults but most individuals were immatures (n = 41 adults, n = 190 young). Most immatures were pupae or tenerals (n = 175) and few were larvae (n = 15). The nine microsatellite loci had from eight to 19 alleles in the entire sample, and the number of alleles per l locus within colonies ranged from 4.4 to 9.0 (Table 1). Within colonies, binomial expected heterozygosity (H_e) ranged from 0.691 to 0.834 (mean = 0.775); these values were significantly heterogeneous among colonies. Of the 36 pair wise comparisons of H_e (Table 1) among colonies, eight were significant at the P<0.05 level (paired *t*-tests comparing H_e at individual loci; Nei, 1987).

Overall relatedness (*r*) within colonies was low compared to most eusocial insects: 0.136±0.023 for all bees and 0.153±0.028 for females only. These values are significantly lower than the maternal half-sib level (*r* approaches 0.25 with extensive multiple mating) and slightly but significantly higher than unrelated (*r* = zero). Figure 2 compares the frequency distributions of relatedness values between all intracolony pairs of females (*N* = 2220) to a simulation of the same number of unrelated pairs sampled from the same allele frequencies. The peak in pair-wise relatedness values for the sample is at zero, as are the simulated values. In addition the distribution shows a long "tail" to the right of higher than expected values.

Female offspring with mothers present

Out of 2220 total female pairs within colonies, only 233 pairs shared an allele at each locus indicating full sib OR mother-daughter relationships. Of these there were seven pairs of adults (likely to be full sisters) and 183 pairs of immatures that could only be full sisters. This left only 43 adult-immature pairs that could possibly be mothers and their daughters. Table 2 shows the counts of the females in these pairs listed by age of immatures from teneral adults (class 9) to larvae (class 1). There were 14 different adults with one or more immature relatives and 32 different immatures with one or more adult relatives. These counts suggest a maximum of 21 percent of immatures (32/153) have a possible mother present. The distribution of ages in Table 2 shows that most immature relatives of adults are older brood. Figure 3 charts pair-wise *r* values for the 50 first-order female pairs with an adult discussed above (seven adult-adult, 43 adult-immature pairs). As expected if close age-mates are sisters and age-distant pairs are mother-daughters, mean relatedness values are near 0.75 for adults and older brood (forms 11- 7) and near 0.50 for adults and younger brood (forms 6-1) with a transition at age class 6. In addition, 47% of adult females in nests have neither sisters nor daughters present. Nearly half of the adults are preventing predation on brood that is not related to them.

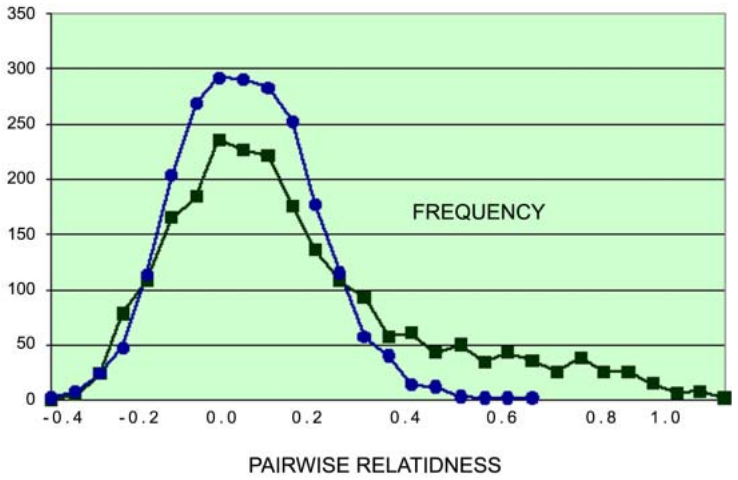

Figure 2. Observed relatedness (*r*) for pairs of females within colonies (green line and squares) and simulated values for the same number of pairs and *r* = 0 using the observed allele frequencies at nine loci (blue line and circles).

Table 1. Genetic variation and relatedness using nine microsatellite loci in nine colonies. For colony *r*, the standard error (±*SE*) was jackknifed over loci. For mean *r*, colonies were weighted equally and *SE* was jackknifed over groups. *A* = alleles per locus per colony, H_e = binomial expected heterozygosity, *r* = relatedness.

			r (SE)	
Colony	*A*	H_e	All	Females
1	7.0	0.747	0.165±0.055	0.200±0.068
2	5.6	0.777	0.091±0.023	0.095±0.026
3	9.0	0.834	0.031±0.016	0.037±0.022
4	8.6	0.791	0.120±0.017	0.131±0.017
5	5.2	0.741	0.192±0.055	0.240±0.074
6	5.2	0.754	0.159±0.032	0.172±0.036
7	8.3	0.794	0.115±0.032	0.118±0.031
8	4.4	0.691	0.307±0.051	0.348±0.058
9	5.9	0.819	0.073±0.033	0.074±0.043
Mean	6.6	0.775	0.136±0.023	0.153±0.028

Male offspring with mothers present

Results were similar for female-male pairs of relatives. Like the mother-daughter test, the mother-son test is powerful because this relationship is excludable; mother-sons share one allele per locus. Only 11 female-male pairs met this criterion and appeared genotypically to be mother-sons. But only four pairs (11 percent of all immature males) included an adult female and an immature male. Even these four apparent mother-son pairs based on genotypes and age could in fact be siblings because a small proportion of sister-brothers can meet the one-allele-per-locus criterion by chance.

Using the observed allele frequencies, we employed *Kinship* to simulate 10,000 sister-brother pairs, and 203 pairs (2.0 percent) shared one allele per locus, thus testing positively as mother-sons. In light of the large number of sister-brother pairs in the simulation, the presence of up to four genotypically possible mother-sons in the sample (out of more than 289 apparent sister-brothers) may be

Table 2. Counts by age of female bees with relatives that qualify as possible mothers or daughters both genotypically and by age category. Shown for each colony are the total number of adult females, the number of these having immature first-order relatives, the number of immatures with adult first-order relatives (listed by age form), the total number of immatures with first-order adult relatives, and the total number of immature females. Adults are age forms 11 and 10; forms 9-1 are successively younger brood.

Total Adult Females	Adults with Immature Relatives	Immature Females With Adult Relatives – Age Classes										Total Immature Females
		9	8	7	6	5	4	3	2	1	Total	
3	1	1	—	—	—	1	—	—	—	—	2	17
2	0	—	—	—	—	—	—	—	—	—	0	8
6	0	—	—	—	—	—	—	—	—	—	0	23
6	4	5	1	—	2	2	2	1	1	—	14	35
0	0	—	—	—	—	—	—	—	—	—	0	10
3	3	1	2	1	1	1	1	—	—	1	8	15
3	2	—	—	1	—	—	—	—	—	—	1	27
3	3	2	1	—	2	—	—	—	—	—	5	12
1	1	—	—	—	1	1	—	—	—	—	2	6
26	14	9	4	2	6	5	3	1	1	1	32	153

explained entirely by this phenomenon. In addition, three of these four males are from the oldest brood age forms (two 9s and one 8), making them unlikely to be sons of resident females. This leaves only a single form 11 male (3 percent of all immature males) that is likely to have his mother present. This age distribution is similar (but with much smaller numbers) to that for first-order female pairs described above, further supporting a lack of mothers present for late-season brood.

Discussion

Colony composition

It is important to recall that the nests used in this analysis are also used as controls in a field experiment. Treatment nests had females removed. Brood in treatment nests was preyed upon by ants, while brood in the control nests, examined genetically here, escaped ant predation (Kukuk et al. 1998). Thus, the brood in the colonies studied here was protected from ant predation by the adult females present in natural nests in the field.

As expected for any undisturbed nest late in the active season, nest contents include some adults but predominantly immatures. The stage of the young indicated that most were between 15 and 40 days old (see Kukuk et al, 1998). These nest contents and the date of nest excavation are the basis for the placement of the hatched vertical line in Figure 1. There was little evidence of active, pollen gathering forgers (presence of eggs or small larvae) suggesting that most adults were inactive at the time these nests were excavated. Low intracolony relatedness values for brood are compatible with the hypothesis that within a nest, individuals constitute a set of unrelated sib ships. If so, each individual, then, occupies a nest with many nonrelatives and a few close relatives.

Previous work indicates that on average there are an average of 5.5 unrelated, actively foraging adult females in summer nests (Kukuk and Sage, 1994). If five to six unrelated females are each singly mated to unrelated males and all reproduce equally the expected relatedness among their collective brood would be between 0.125 and 0.150. This is congruent with the values of intracolony relatedness found in this study suggesting that the colonies consisted of a set of unrelated sib ships where each sib ship is the brood of a once-mated, single female. The two-fold range in the number of adults and significant differences in H_e among colonies also suggest that the number of sib ships is variable, and colonies consist of the

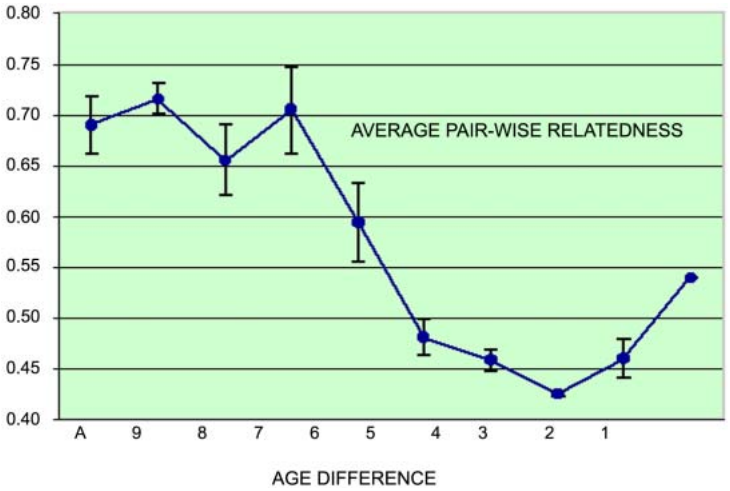

Figure 3. Average, and standard errors, for relatedness of female pairs within colonies that (1) include an adult and (2) share one allele per locus (i.e. are either full-sister or mother-daughter pairs). A = adult (forms 11-10), immatures include tenerals (9), pupae (8-2) and larvae (1).

offspring from different numbers of reproductive adults.

Additional evidence for the existence of sets of sib ships in colonies is found in Figure 2 where the distribution of pair-wise relatedness values within colonies is graphed. This figure shows that most pairs are nonrelatives but a few pairs are close relatives. The pattern of this distribution indicates that colonies are not single families because the peak of the distribution is at zero, showing that most pairs of individuals in a nest are not relatives, i.e. they belong to different sib ships. The long "tail" on the right of pairs that are close relatives represents pairs of individuals in the same sib ship. This pattern of relatedness values is similar to that reported for other communal bees (Danforth et al., 1996, Paxton et al., 1997) suggesting that communal bee broods, in general, are of sets of unrelated sib ships. Within nests an individual is associated with nonrelatives or close relatives. The distribution in Figure 2 does not support the notion that individuals are part of a large extended family, i.e. all individuals are slightly or moderately related to one another.

Offspring with mothers present

Microsatellite data were used to examine pair-wise relatedness values between all adults present and all immatures. For immature females, both mother-daughter and full sisters share at least one allele at each locus and all such pairs were identified. The relationship between average pair-wise relatedness values and the age of the immature in each pair (Figure 3) indicate that only pairs where the immature is age class 6 or lower are mother-daughter pairs. Thus 11% of immature females have their mothers present. An overwhelming majority of immature females do not have their mothers present. In addition only 21% of immatures (32 of 153) have any close adult relative present. This combined with the absence of moderately close relatives in colonies (Figure 2) suggests that over 3/4 of immature females lack not only mothers but also lack even moderately or slightly related adult relatives. The situation for males is very similar but even fewer immature males have their mothers present. These genetic data supports the hypothesis that extended parental care occurs in *L. hemichalceum*. Immatures are protected from ant predation by the presence of adults who are not their mothers and who, in most cases, are not their relatives at all.

This care for nonrelatives could be a byproduct of the care given to relatives, either sibs or offspring. Because colony members are a set of sib ships, each adult is expected to have a few close relatives and also many nonrelatives present in her nest. If this were the case, we would expect that all females would have at least one close relative in the nest. However we find that nearly half, 46%, of all females have no close relatives in their nests. Moreover, because cooperation in this species is indiscriminate, it appears that individuals do not or can not discriminate between kin and nonkin (Kukuk et al., 1992b; Kukuk & Crozier, 1996).

Social cooperation in order to extend parental care beyond the lifespan of the actual parents is thought to be an important evolutionary force in social Hymenoptera in kin-based societies that progressively provision their brood (see Field et al., 2000). This is due to a general phenomenon of short adult life spans relative to brood development times. The genetic data on *L. hemichalceum* confirm that the phenomenon of extended parental also applies to social Hymenoptera in societies that are not kin based and in which brood is mass provisioned. As argued above, communal species are common and found in most bee families so this represents an important generalization. It indicates that extended parental care is very general throughout the Hymenoptera and can occur in the absence of kin selection. It appears to occur in communal species as "byproduct mutualism" where the activities of individuals that provide increased individual fitness also benefit unrelated colony members.

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