

Adam R. Smith · William T. Wcislo · Sean O'Donnell

Assured fitness returns favor sociality in a mass-provisioning sweat bee, *Megalopta genalis* (Hymenoptera: Halictidae)

Received: 8 April 2002 / Revised: 23 January 2003 / Accepted: 24 January 2003 / Published online: 19 March 2003
© Springer-Verlag 2003

Abstract Assured fitness returns models for the evolution of sociality emphasize the selective value of ensuring that offspring receive adequate parental care to reach maturity. If a member of a social group dies, it can accrue returns on investment in offspring through the efforts of surviving social partners. We provide evidence that in the mass-provisioning, facultatively social sweat bee *Megalopta genalis*, adult presence in the nest throughout brood development provides protection from ant predation. Nests with adults present were well protected, and brood in nests with adults removed suffered higher predation. Females in observation nests showed effective defensive behavior against experimentally introduced ants, and bees in natural nests repulsed naturally occurring ant raids. *Megalopta* nest architecture and behavior are such that the brood of several cooperating females can be defended with little additional cost relative to solitary nesting. The benefits of cooperative defense may favor group living in mass provisioning bees. Our observations and experiments suggest that parental care throughout brood development can be adaptive in mass provisioning species, supporting the predictions of assured fitness returns models.

Keywords Assured fitness returns · Social evolution · Ant predation · Brood defense

Introduction

Although much attention has been focused on the role of high relatedness in promoting the evolution of eusociality via kin selection (Hamilton 1964, reviewed in Queller and Strassmann 1998), recent studies of sweat bees (Halictidae) highlight the importance of extrinsic factors in favoring social over solitary behavior (Wcislo 1997a, 2000). While some species of halictid bees are obligately and permanently eusocial, members of some populations express both solitary reproduction and eusociality (reviewed in Wcislo 1997a, 2000). Because of their ability to switch between modes of social organization, individuals may exhibit solitary behavior when it is advantageous over social behavior, and vice versa. Although current social flexibility may not represent an intermediate step toward an evolutionary endpoint of obligate eusociality, socially flexible species can be used to test hypotheses for the costs and benefits of group living (Danforth and Eickwort 1997; Wcislo and Danforth 1997; Danforth 2002; Soucy and Danforth 2002).

A key question that must be addressed in any explanation for the expression of social behavior is: Why do some adults forgo direct reproduction and act as helpers rather than leave to found their own nests? Assured fitness returns (AFR) models argue that patterns of adult mortality combine with offspring dependence on parental care to select for group living (Queller 1989, 1994, 1996; Strassmann and Queller 1989; Gadagkar 1990, 1991; Bull and Schwarz 2001). AFR models posit that group living is selectively favored because it increases the likelihood of fitness payoffs to parental and kin investment. In a social group, if an adult dies before the brood reach maturity, other adults remain to provide parental care. Studies of progressive provisioning species (those species that must feed offspring repeatedly throughout larval development) such as paper wasps and hover wasps (Vespidae), and allodapine bees (Apidae) support AFR models (Queller 1989; Gadagkar 1990; Bull and Schwarz 1996, 1997; Schwarz et al. 1997, 1998; Field et al. 2000; Hogendoorn et al. 2001). In these cases the benefits are clear, because

Communicated by R.F.A. Moritz

A. R. Smith (✉) · S. O'Donnell
Animal Behavior Area, Department of Psychology,
University of Washington,
Seattle, WA 98195, USA
e-mail: arsmith@u.washington.edu
Tel.: +1-206-5250403
Fax: +1-206-6853157

W. T. Wcislo
Smithsonian Tropical Research Institute,
Unit 0948, APO AA 34002, Panama

progressively provisioned offspring would starve without continual feeding by adults.

Some social insect species, including halictid bees, are mass provisioners, meaning that females provide all necessary food for offspring development before laying an egg (Wilson 1971; Michener 1974). Because offspring could potentially reach maturity even if the parent died immediately after oviposition, AFR models were not initially applied to mass provisioners (Queller 1989; Gadagkar 1990). However, mass-provisioned offspring may be dependent on other forms of parental care, such as defense against nest predation and parasitism (Queller 1994; Eickwort et al. 1996; Kukuk et al. 1998). For example, Eickwort et al. (1996) showed that the brood of naturally orphaned solitary nests of a mass-provisioning, facultatively social bee, *Halictus rubicundus*, suffered greater fly parasitism than those with adults present at the time of nest collection. Similarly, Kukuk et al. (1998) showed that in the communal bee *Lasioglossum hemichalceum*, unattended brood suffered greater ant predation than brood protected by an adult. These studies were correlational. Experimental demonstration of selective disadvantages if brood are orphaned would argue for the importance of parental care throughout brood development, and confirm the applicability of AFR models to mass provisioning species.

We used the facultatively social, Neotropical sweat bee *Megalopta genalis* (Halictidae: Augochlorini) to experimentally test whether defense against predators may select for group living in a mass provisioning species. Ant predation is a major threat to many social insects (Jeanne 1975; Hölldobler and Wilson 1990; Schwarz et al. 1998), and is especially strong in the tropics (Jeanne 1979; Kojima 1993). Thus, we focused our studies on *M. genalis* defense against ant predation. We investigated what contribution, if any, adult *Megalopta* made to nest defense against ants. If the brood rely on adult protection, *Megalopta* could realize improved fitness by living in groups and thereby avoid brood orphanage.

We conducted three field studies. First, we observed naturally occurring *M. genalis* defense against ant predation. We next experimentally removed adults from some *M. genalis* colonies, and predicted that nests with adult females would suffer less ant predation than orphaned nests. Finally, we observed bees in artificial nests to document their responses to invading ants, and we tested for the application of ant repellent chemicals at the nest entrance. We discuss the implications of our results for the adaptive value of group living in *Megalopta*, and in other mass provisioning species.

Methods

Study site

All studies were conducted in the Barro Colorado Nature Monument (BCNM), principally on Barro Colorado Island (BCI; 9°09'N,

79°51'W), Panamá Province, Republic of Panamá. BCNM is a lowland tropical semi-deciduous moist forest with a pronounced dry season, usually from mid-December through mid-April (Rau 1933; Leigh 1999).

Overview of natural history

M. genalis are collected at light traps the year round on BCI (Wolda and Roubik 1986; Roubik and Wolda 2001), but they provision nests and reproduce primarily in the dry season and first half of the wet season (for details of their nesting and social biology, see Arneson and Wcislo 2003; W.T. Wcislo et al., unpublished data.). Nests occur in relatively dry, broken sticks that are usually suspended in tangles of vegetation (Fig. 1; Sakagami 1964; Janzen 1968). The nest entrance has a constricted collar made of chewed wood, with a diameter slightly wider than the resident(s)' head (Fig. 1c). The entrance opens into a single tunnel that has cells adjacent to it; cell entrances are flush with the tunnel wall (Fig. 1b).

At the start of the dry season, most nests (>75%) contain a solitary female. Females forage during the approximately 1.5 h after sunset and before dawn. Development from egg to adult takes approximately 35 days. Newly eclosed females are fed by older adult(s) for 1–14 days, and may then disperse or join the natal nest. Females younger than 2 weeks have undeveloped ovaries.

Later in the dry season ~25–50% of nests have multiple females, presumably because some daughters joined their natal nests. Multi-female nests average 2.5 ± 0.09 (SE) females, but group size varies seasonally. Multi-female nests have up to ten females, although nests with more than four females are uncommon. Throughout the year, some bees establish new nests, as inferred from the continual occurrence of singleton nests with one or a few brood cells.

Within multi-female observation nests, a dominant bee is generally larger, older, mated and has fully developed ovaries; she rarely forages and frequently receives nectar from a subordinate via trophallaxis. In contrast, the subordinate(s) tend to be younger, smaller, mated or unmated, and have slender or developed ovaries; they are the primary foragers and frequently give nectar to dominants via trophallaxis. The first brood has a female-biased sex ratio and the proportion of males increases throughout the season. Brood rearing is not synchronized and adults continue to forage as brood develop. The distribution of reproduction within natural multi-female nests is not known, but based on dissections

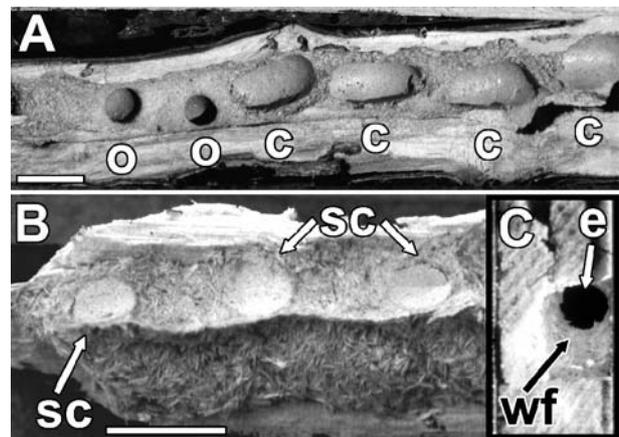


Fig. 1a–c *Megalopta genalis* nest architecture. Both scale bars=1 cm. **a** Cells inside the nest, constructed of chewed wood fiber: *c* exposed cells, *o* cells' natural opening. **b** Sealed, provisioned brood cells. Arrows indicate closed and sealed cell openings (*sc*). Note that cell openings (light-colored circles) are flush with the tunnel wall. Brood cells angle down and right. **c** Constricted nest entrance (5 mm diameter), *wf* chewed wood fiber used to construct the entrance collar

all females had developed ovaries in some nests, while in others only a single female had developed ovaries. Furthermore, in observation nests subordinate females can supercede a dominant and take over the nest, if the nesting substrate remains suitable. Female longevity in natural nests is unknown, but it can be up to 8 months in observation nests. Nests are built in dead sticks that are rarely suitable for use the following season, so bequeathing a long-lasting nest as a valuable resource is unlikely. Females were largely inactive in the latter half of the wet season (September–November), which is when relatively few floral resources are available for bees (Wright and Calderon 1995).

Observations of nest defense in the field

To observe ant attacks and *Megalopta* nest defense, we used a Sony MiniDV or Digital8 camcorder with an infrared light source, or a Canon Hi8 camcorder with red light illumination, to videotape the nest entrance ($n=12$ nests) when bees were provisioning and guarding the nests. These nests were moved from their natural location to sites near the laboratory, where they were suspended from vegetation, for ease of observation. Video-taping periods ranged from 45–90 min. Because the field of view was limited to the nest entrance and its immediate vicinity, we could not usually distinguish between several ant foragers encountering a nest over the course of a taping period, or the same forager wandering in and out of view. Thus, we recorded whether or not the *Megalopta* nest was encountered by an ant during the first 45 min of a session (in order to compare sessions of different lengths), but not how often the nest was encountered.

Experiment 1: ant exclusions

To test the ability of *M. genalis* adults to resist ant attack we performed two experiments. First, we compared the survival of adult-occupied control nests with the survival of adult-occupied nests protected with Tanglefoot pest barrier in the field over 42 days (Tanglefoot is a sticky, resinous substance that prevents passage by walking insects). We determined a *Megalopta* nest to be occupied if we saw an adult inside, or if the entrance was rebuilt after we blocked it with crushed wood fiber. We randomly assigned occupied *Megalopta* nests to either ant-exclusion treatment ($n=23$) or control ($n=25$) groups. To exclude ants from treatment nests, we applied an unbroken band of Tanglefoot to the nest stick approximately 10 cm behind the entrance, and trimmed, or applied Tanglefoot to, any other vegetation that provided access to the entrance. We checked nests for the presence of adult bees weekly from 12 April to 23 May 2001. We determined a nest to have lost all adults if we saw no bees inside and if the entrance remained blocked by added wood fiber for two consecutive weeks. We placed mesh traps over these nests to monitor for any brood emergence for the remainder of the study. All nests were dissected and checked for brood at the end of the study.

Experiment 2: removing adult bees

We removed *Megalopta* adults from nests in the field to compare the survival of brood in nests with and without adults. We removed adults from the treatment nests by placing a mesh trap over the end of the stick for 4 days. Previous experience showed that this was ample time for all bees to leave the nest and be captured in the trap (A.R. Smith, unpublished data; all subsequent references to adult removals refer to this technique). Between 30 January and 17 February 2000, we randomly assigned nests with adults present to treatment ($n=24$) or control ($n=22$) groups. We removed all adults from the treatment nests and left both treatment and control nests in their natural locations. We collected and dissected all nests after 40 days. Nests with intact brood cells and/or live adults were scored as surviving. Nests with emptied brood cells were scored as predated.

Experiment 3: ant baiting and adult removal

We baited ants to *Megalopta* nest entrances to further test the ability of adult bees to resist ant attack in the field. We collected nests from their natural locations and moved them to a common site. We used 19 nests 18–20 March 2000, and 17 nests 14–16 March 2001. We alternately placed nests with and without adult bees present at a height of approximately 1.5 m in vegetation every 10 m along a transect through the forest. We baited all nests with either a *Megalopta* (2000) or *Polistes* wasp (2001) pupa or late-instar larva. We wrapped the bait in mesh, so that it could not be carried away whole, and attached it by wire to the nest stick 0.5–1.0 cm from the entrance. We collected and dissected all nests after 48 h. Nests with emptied brood cells were scored as predated, those with brood cells still sealed were scored as surviving, even if ants were present. Nests that did not yet contain provisioned brood cells were excluded from the analysis.

Observations of defensive behavior within nests

Between 5 April and 8 May 2001, we introduced ants into observation nests to determine whether and how *Megalopta* adults would defend against intruders. We constructed *Megalopta* observation nests by putting balsa wood between two panes of glass that were covered except during behavioral observations. For all introductions we used nests in which bees were actively provisioning brood cells. We opened the cover and left the nests undisturbed for at least 5 min prior to each trial. The resident bees were then filmed for 3 min before a single forager of either *Crematogaster* sp. or *Camponotus* sp. ants (both common *Megalopta* brood predators; A.R. Smith and W.T. Wcislo, personal observation) was introduced into the nest entrance with forceps or flexible tubing. Ants were not re-used, and nests were left undisturbed for at least 2.5 h before re-use. Filming continued throughout the resulting interaction with the ants, until 3 min after the ant had been either killed or expelled from the nest. We scored five defensive behaviors exhibited by *Megalopta* against intruding ants, typical of defensive behavior in other halictines (Michener 1974; Wcislo 1997b): (1) bite: the bee closes its mandibles on ant, or attempts to, but the ant moves away; (2) C-posture: the bee curls its abdomen ventrally to form a C-shaped posture, and both the sting and mandibles face the ant; (3) sting: the bee adopts a C-posture and repeatedly stings the ant while vigorously biting it with the mandibles; (4) backout: the bee walks backwards, pushing the ant out of the nest with her abdomen; (5) kill: the bee kills the ant before it can flee the nest.

We performed 30 trials on nests with multiple bees (*Camponotus*: one two-bee nest, two trials; *Crematogaster*: three two-bee nests, 20 trials; one four-bee nest, eight trials), and 17 trials on single bee nests (*Camponotus*: ten nests; *Crematogaster*: seven nests). We recorded all behaviors of all bees during the trials. We pooled observations from multiple trials on the same nest to avoid pseudoreplication in the statistical analyses. Thus, each nest was counted as a single trial for each ant species. The unpooled data showed similar patterns to those which we present below for the pooled observations.

Test for chemical repellency

Some bees produce aromatic ant-repellent mandibular gland secretions which they apply to the nest entrance (Cane and Michener 1983; Cane 1986). We placed wood fibers scraped from the constructed nest collar of active nests ($n=10$; Fig. 1c) in the center of a clear plastic petri dish within 4 h of removing the fibers from the nest. Ten *Crematogaster* sp. ants were placed in the dish for 5 min. We videotaped the dish from above with a camcorder centered on the disk, and measured the average of the distance from the center to each ant at 30 s intervals. The same experiment was repeated using wood fiber from the same *Megalopta* nest stick, but not part of the constructed nest collar or nest, as a control. The same ants were used in both treatment and control trials for each nest, but

not for multiple nests. We alternated the order of presentation of the control or treatment between nests.

Megalopta interactions with army ants

Many insects are capable of defending their brood against all types of ants except army ants (Ecitonini) (Chadab 1979; Gotwald 1995). To test whether *Megalopta* nests were susceptible to army ant predation, we placed active nests upright in front of a foraging column of the army ant *Eciton hamatum* by attaching the nest to a tent stake which was inserted perpendicular to the ground, between 16 March and 6 June 2001. *E. hamatum* feed almost exclusively on social insects. *Eciton* foragers enter sticks to raid wood-nesting ants, and readily climb vertical surfaces, often extending their foraging columns into the tree canopy (Rettenmeyer et al. 1983). For all trials, we placed a nest in the path of an advancing column that was actively foraging. We never used the same column front twice, but we did use multiple columns from the same colony, which presumably contained different ants (we used six *E. hamatum* colonies in total). To induce attack, we rubbed a pupa or late-instar larva of the paper wasp *Polistes canadensis* from the base of the *Megalopta* nest stick to the nest entrance. *P. canadensis* is a common prey item of *E. hamatum* (Chadab 1979; Pickering 1980). A nest was scored as being under attack when an *E. hamatum* forager reached the *Megalopta* nest entrance. The attack was scored as ended when all ants had left the nest stick and remained off for 5 min (these 5 min were not counted in the attack time).

We provided *Megalopta* and *Polistes* larvae to an *E. hamatum* colony to test whether the ants accepted *Megalopta* larvae as readily as the known prey item *Polistes*. One *Megalopta* and two *Polistes* larvae were provided to a small column front, and another six *Polistes* and five *Megalopta* larvae were provided to the ants guarding a prey cache between the column front and bivouac. We conducted all of these presentations on the afternoon of 6 June 2001, on a single column front and single prey cache of the same *E. hamatum* colony.

Statistical analyses and voucher specimens

In the Tanglefoot study, we performed a Cox one-factor regression survival analysis using SPSS 10.0 (SPSS 1999), with experimental treatment entered as the factor, to test for differences between the treatment and control groups' survival rates. We used SYSTAT 10 (SPSS 2000) to perform Fisher's exact probability tests to analyze all 2x2 contingency tables. We used a χ^2 test for differences in *Megalopta* behavioral responses to introduced *Crematogaster* and *Camponotus* ants. We used the total frequency for each behavioral act, weighted by the percentage of total trials for each ant species, to generate expected values for the null hypothesis that the probability of expression of each *Megalopta* behavior was independent of the ant species used in the trial. We compared army ant attack time toward occupied and vacant nests using a Mann-Whitney *U*-test because the data violated the parametric assumption of homogeneity of variance between groups. Voucher specimens of the bee are deposited in the Dry Reference Collection of the Smithsonian Tropical Research Institute.

Results

Observations of nest defense in the field

Ants frequently encountered *Megalopta* nest entrances. In 86% ($n=59$) of the video taped foraging periods, at least one ant encountered the nest entrance within 45 min. We observed sustained raids that lasted up to 2 h by *Azteca* sp. and *Camponotus* sp. In response, a resident bee blocked

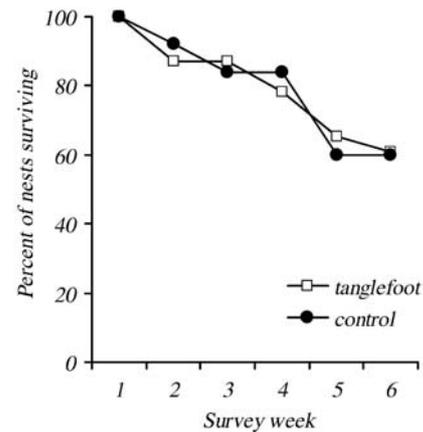


Fig. 2 Natural *Megalopta genalis* nests with and without a protective ring of Tanglefoot showed similar patterns of survival over 6 weeks. The y-axis shows the percent of nests still active at each week of our survey. The overall survival rate was 60% ($n=48$), and there was no significant difference between the two treatments

the constricted nest entrance with her metasomal tergites (abdomen), or the ant entered but then quickly exited the nest. In both sustained raids, one or two marked bees left the nest and returned, but did not enter the nest until after the raids ended.

We video-taped two instances of single foragers of the ponerine ant *Ectatomma tuberculatum* poised for ambush attack at *Megalopta* nest entrances, similar to the posture of *Ectatomma ruidum* when attacking other halictid bees (Schatz and Wcislo 1999). In both cases, the ant immediately retreated when the bee, which was larger than the ant, emerged from the nest.

On the afternoon of March 8 2001, we observed a large ponerine ant forager attack the entrance of a nest that we had blocked with crushed wood fiber to monitor bee activity. After removing the fibers (~5 min), the ant proceeded directly into the nest, and then ran out 1–2 s later. We saw a *Megalopta* female about 2 cm inside the tunnel after the ant left.

Experimental exclusion of ants and bee removals

Unmanipulated nests with adult bees present were as well protected from ants as those treated with Tanglefoot. There was no difference in survival between control (60% survival, $n=25$) and Tanglefoot treated (61% survival, $n=23$) nests over a 6 week survey (Fig. 2; Cox one-factor regression survival analysis $\chi^2_1=0.003$, $P=0.96$). These survival rates are similar to the 50% survival rate at ~5 weeks found by W.T. Wcislo et al. (unpublished data) in 1999. Only one bee emerged from a nest after we determined that it contained no adults. None of the nests that had lost adults contained living brood upon collection. To directly measure the ability of *Megalopta* adults to defend against ants, we baited ants to the nest entrance and checked nests for survival after 48 h. Pooled across 2 years, all 20 nests with adults present remained intact,

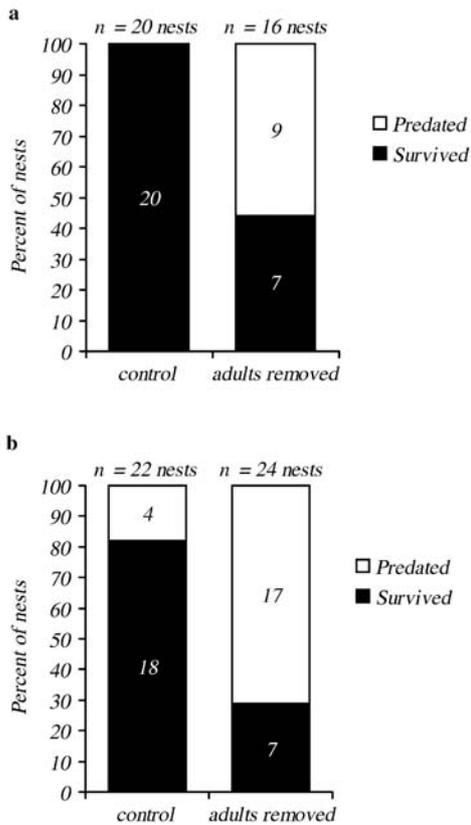


Fig. 3a, b *Megalopta genalis* nests with adults experimentally removed were more likely to be predated. Black bars represent the nests that survived, and white bars those nests that did not. Numerals in each bar show the number of nests in each group. The percent of nests is on the y-axis, and the x-axis shows treatment. **a** Result of 48 h of baiting ants to the nest entrance. **b** Results after being left in the field for 40 days (without bait). The difference between control and adult removal groups is significant (Fisher's exact test $P < 0.001$ for each experiment)

while nine of the 16 nests with adults removed suffered brood predation (Fig. 3a; Fisher's exact test $P < 0.001$; the difference between treatments was statistically significant in both years; 2000: Fisher's exact test $P = 0.033$; 2001: Fisher's exact test $P = 0.003$). All baits were either completely removed or were being eaten by ants at the end of 48 h, when the nests were collected, so the differences were not a result of some nests remaining undetected by ants. In each year, one adult-removal nest was found with ants inside, but the brood unharmed (these nests were counted as surviving). Nine of the 20 (45%) nests with adults present contained only one female. Thus, a single female can effectively defend her nest against ants for up to 48 h.

In natural nesting locations, *Megalopta* nests with adults removed were much more likely to be raided by ants than were control nests (Fig. 3b; Fisher's exact test $P < 0.001$). Ant predators found consuming *Megalopta* brood in the field included *Camponotus* sp., *Crematogaster* sp., and *Azteca* sp. One nest in each treatment group was attacked by an unidentified predator that ripped

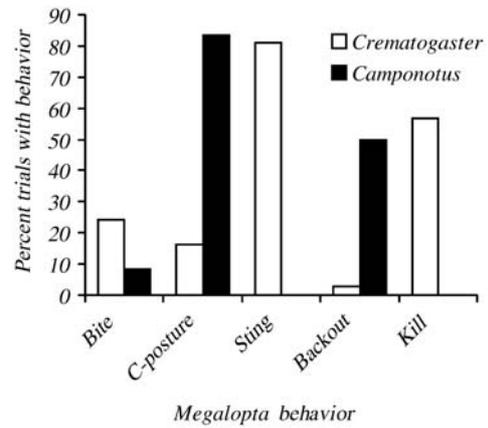


Fig. 4 *Megalopta genalis* behavioral responses to *Crematogaster* and *Camponotus* ants in the nest. Bar heights represent the percentage of the trials conducted with each ant species that elicited the given behavior at any point during the trial. Black bars represent behaviors in response to introduced *Crematogaster* foragers, and white bars represent responses to introduced *Camponotus* foragers. The distributions of *Megalopta* responses differed between ant species ($\chi^2_3 = 16.62$, $P < 0.001$). *Megalopta* were also significantly more likely to kill *Crematogaster* than *Camponotus* foragers (Fisher's exact test $P < 0.001$)

a slit down the side of the stick, from which it extracted the bee brood.

Observations of *Megalopta* defensive behavior within nests

Differences in *Megalopta* behavioral responses to the two ant species were highly significant (Fig. 4; $\chi^2_3 = 16.62$, $P < 0.001$). Resident *Megalopta* females ($n = 11$ nests) reacted to the larger-bodied (> 1 cm length) *Camponotus* ants by sometimes biting, then assuming a C-posture and approaching the ant with both her mandibles and sting facing the intruder. If the *Camponotus* did not turn and leave upon contact with the bee, the *Megalopta* female walked backwards toward the ant, pushing it out of the nest with her abdomen (Fig. 4). *Megalopta* females ($n = 11$ nests) reacted differently to the much smaller (< 5 mm length) *Crematogaster* ants. *Megalopta* females curled their bodies ventrally to simultaneously bite and sting *Crematogaster*, and often killed them (51% of trials; $n = 13$). No *Camponotus* ($n = 11$) were killed. This difference in the probability of killing the ants was significant (Fisher's exact test $P < 0.001$).

We never observed female bees cooperating in nest defense ($n = 32$ trials on four nests with multiple females; 2–4 adult bees were present). The narrow nest tunnel precludes bees from standing abreast to face an intruder. The first bee to contact the ant was the one that defended against it in almost all cases. Twice a *Crematogaster* ant passed the bee nearest the entrance without contact, and was attacked by the second bee upon contact. Once a *Crematogaster* ant contacted a male *Megalopta*. The male bee immediately fled the nest and never returned.

Tests for chemical ant repellence

Crematogaster ants showed no aversion to wood fibers taken from the constricted nest entrance collar relative to control wood fibers taken from 5–10 mm away on the same stick. The mean ant distance from control fiber \pm SD was 6.23 \pm 1.69 cm, versus 6.12 \pm 1.19 cm for nest entrance fiber (paired *t*-test: *t*=0.25, *df*=9, *P*=0.8). Ants observed in the field showed no obvious aversive reactions when entering *Megalopta* nests.

Interactions with army ants

No *E. hamatum* army ant foragers entered any of the 15 *Megalopta* nests, even though all sticks were encountered by foraging ants. In all cases ants climbed other vertical surfaces in the vicinity, so the ants did not avoid the *Megalopta* nest sticks due to a reluctance to climb. Ants attacked all 20 nests baited with *Polistes* brood. In seven of ten nests with *Megalopta* adults, and nine of the ten nests without adults, at least one *Eciton* forager completely entered the nest. In seven of the ten occupied nests a resident bee visibly blocked the nest entrance with her tergites (any blocking further down the tunnel would not have been visible). *E. hamatum* attack time was significantly longer for occupied than vacant nests (occupied: mean=18 min 45 s \pm 5:40; vacant: mean=6:07 \pm 1:13; two-sample, unequal variance *t*-test: *t*=2.18, *df*=9.8, *P*=0.05). However, *E. hamatum* foragers did not prey upon brood or adults of either group. When presented with *Megalopta* and *Polistes* larvae or pupae, *E. hamatum* foragers carried *Polistes* back to their bivouac, but ignored *Megalopta* larvae (Fisher's exact test *P*=0.026). Of the three larvae/pupae presented to the column front, one *Polistes* was taken, and one each of *Polistes* and *Megalopta* were declined. Of the remaining larvae/pupae (six *Polistes*, five *Megalopta*), which we placed next to the prey cache, all *Polistes* and only one *Megalopta* were brought into the cache. In one case, a *Megalopta* pupae was lifted from the vine being used as the ants' substrate and dropped over the side, typical of the ants' response to obstacles on their trail (A.R. Smith, personal observation). In another trial, we presented a *Megalopta* larva that had a larva of the parasitoid beetle *Macrosiagon gracilis* (Falín et al. 2000) attached to it. The army ants took the parasitoid larva, but not the larger *Megalopta*.

Rejection of *Megalopta* larvae was not simply a result of satiation, as we alternated *Megalopta* and *Polistes* presentations. Also, all prey experimentally removed from the *Eciton* food cache (epiponine wasp brood) were brought back in by the ants.

Discussion

Protection from brood predation can be a strong selective factor that favors extended parental care, even in mass provisioning species. *Megalopta* nests with adults were

well protected from ants. Observations of nest defense against sustained attacks by *Camponotus* and *Azteca* spp. showed that the bees were able to protect their brood under natural conditions. In their natural sites, over the approximate egg-to-adult development time, *Megalopta* nests showed no additional survival benefits when protected with Tanglefoot. Both over the 2-day period of the ant-bait study, and the 42 days of the Tanglefoot study, adult presence was an effective defense against ants. The ant bait experiments showed that females defended their nests against direct attack, and that ants raided *Megalopta* brood when they had access to it. The only two instances in which we observed female-occupied nests being predated by ants presented unusual circumstances: one *Megalopta* colony tunneled into an existing *Camponotus* nest, and another fell to the ground. In contrast, nests that we experimentally orphaned by removing adult bees were not likely to survive the egg-to-adult development time. These results are consistent with Eickwort et al.'s (1996) finding that orphaned sweat bee (*H. rubicundus*) nests were more susceptible to brood parasitism than nests with an adult present.

Ants are more abundant and ant predation is more intense in the tropics than in temperate habitats (Jeanne 1979; Hölldobler and Wilson 1990; Kojima 1993; Kaspari et al. 2000). Ant predation has strongly influenced the evolution of tropical bee and paper wasp social behavior (Jeanne 1975; Roubik 1989; Kojima 1993; Smith et al. 2000). The frequent ant encounters recorded in our videotapes of nest entrances suggests that there is nearly constant probing of *Megalopta* defenses by ant foragers.

Our observations of in-nest defensive behavior showed that adult *Megalopta* actively expelled or killed intruding ants. *Megalopta* interactions with army ants are more difficult to explain than those with other ants. *Megalopta* females plugged the nest entrance with their abdominal tergites to block *E. hamatum* foragers from entering. However, the army ants never consumed *M. genalis* brood, even when they freely entered undefended nests. In fact, army ant foragers spent more time attacking the adult-occupied nests, suggesting that the adult bees and brood were more attractive than brood alone. Rettenmeyer et al. (1983) noted that *Eciton* army ants often specialize on a subset of available prey. Perhaps, with their individually sealed cells in wood, *Megalopta* brood are not profitable prey items. During one of the trials we also saw *E. hamatum* enter and leave unharmed the excavated wood nest of a carpenter bee (*Xylocopa* sp.), supporting the possibility that individually prey in sealed cells in wood were not valuable. The food choice trials with *Polistes* larvae suggest that *E. hamatum* foragers avoid *Megalopta* brood as prey even in the absence of any protection conferred by nest architecture, although these trials require replication with more *E. hamatum* colonies.

Adult bee presence appears to be critical in *Megalopta* brood defense. Unlike some other bees (Cane 1986) and paper wasps (reviewed in Smith et al. 2000), preliminary evidence suggests that *Megalopta* bees do not apply chemical ant repellent to their constricted nest entrances.

Active *M. genalis* nests are rarely left unguarded, as the bees remain in their nests for most of the day and night. Even the brood of an active, solitary-nesting female is usually left unattended for brief periods only at dusk and dawn (W.T. Wcislo and A. Kelber, unpublished data). These foraging patterns and defensive behaviors against ants are similar to those found by Burgett and Sukumalanand (2000) in the Palearctic, wood-nesting, nocturnal bee *Xylocopa tranquebarica*.

A *Megalopta* female is capable of defending a nest alone because its architecture allows her to block the constricted entrance. Nest architecture largely precludes cooperative defense because the tunnel is generally too narrow to permit two or more bees standing abreast. As expected, the ant bait experiment showed that protection did not depend on the number of bees present, and the ant introductions to observation nests showed that even when multiple females were present, only one bee defended the nest at a time. We hypothesize, therefore, that ant predation selects for group living not because the brood is safer when defended by more than one bee, but because having two or more bees cohabiting increases the likelihood that at least one bee will remain to defend the nest. Queller (1994, 1996) argued that it should be easier to evolve helping behavior through AFRs if the helper can take over the investments made by deceased colony mate(s) without giving up investments of her own. *Megalopta* appears to be just such a case because nest defense often takes place near the entrance, and thus protects all brood inside. Unlike progressive provisioners, which must assume feeding responsibilities for any brood left by others (Field et al. 2000; Hogendoorn et al. 2001), a *Megalopta* female can defend all brood in her nest, including her own (if any), by simply remaining in the nest.

We have shown that AFR models for the evolutionary origins of sociality can be successfully applied to at least one mass-provisioning species, and that ant predation is an extrinsic factor that may select for social behavior to the extent that sociality prevents brood orphanage. The AFR benefits of helping depend in part on *Megalopta* female demography. Future studies are needed to measure *Megalopta* adult mortality rates and success in founding new nests. The success rate of newly founded nests is unknown, but 40–50% of existing nests fail before completing the approximate egg-to-adult development time. This suggests that assuring the survival of partially reared young in the natal nest before attempting to found a new nest may be selectively advantageous. Gadagkar (1991) notes that even if a female is going to disperse, she may accrue indirect fitness by helping at her natal nest until she establishes her own nest. Newly eclosed *Megalopta* females can temporarily defend the nest with presumably little cost to future reproductive success because they remain in the nest for up to 2 weeks (Wcislo and Gonzalez 2003), and evade the physiological costs and risk of death associated with foraging (O'Donnell and Jeanne 1995). Consequently, selection for this type of helping may be strong.

Food exchange among adults (trophallaxis) is extremely rare in Halictidae, and in the tribe Augochlorini it is known only in *Megalopta* (Kukuk 1994; Wcislo 2000; Wcislo and Gonzalez 2003). This fact, coupled with the observations that newly eclosed bees and young adults are sometimes fed by older, dominant bees in multi-female nests (W.T. Wcislo and V.H. Gonzalez, unpublished data), further suggest that it is advantageous for the dominant female to induce a bee to remain in the natal nest. In turn, the distribution of foraging effort within social groups suggests that young bees avoid foraging when possible (Wcislo and Gonzalez 2003). In multi-female nests, the dominant female never makes more than 10% of the foraging trips, and in two-bee, three-bee and four-bee nests, the youngest bee makes 90%, 48% and 15% of the total trips, respectively. The dominant female is proportionally older relative to the youngest bee as the number of females per nest increases, suggesting there is a greater likelihood of superceding as the dominant becomes senescent or dies, and thus the younger bees have greater incentive to avoid risky tasks. Taken together, these data suggest that selective pressure favors group living as a way to maintain parental protection throughout brood development.

Acknowledgements A.R.S. was supported by a Smithsonian Tropical Research Institute (STRI) short-term fellowship and by general research funds from STRI to W.T.W. S.O'D. was supported by NSF grant IBN-9904885. Additional general research funds from STRI to W.T.W. are also acknowledged, including the Baird Restricted Endowment. Victor Gonzalez and Kari Roche helped with fieldwork through the STRI Behind the Scenes Volunteer Program; Edgardo Garrido provided additional field assistance. Sabine Spehn, Alexander Lang, and Ingeborg Tuppener loaned equipment. Scott Powell provided army ant advice, Almut Kelber gave constructive criticism, and the staff of STRI provided logistical support. Two anonymous reviewers provided helpful comments. Research was conducted under INRENARE scientific permit no. 75-99 in accordance with the laws of the Republic of Panamá.

References

- Arneson L, Wcislo WT (2003) Dominant-subordinate relationships in a facultatively social, nocturnal bee, *Megalopta genalis* (Hymenoptera: Halictidae). *J Kans Entomol Soc* (in press)
- Bull NJ, Schwarz MP (1996) The habitat saturation hypothesis and sociality in an allodapine bee: cooperative nesting is not "making the best of a bad situation". *Behav Ecol Sociobiol* 39:267–274
- Bull NJ, Schwarz MP (1997) Rearing of non-descendent offspring in an allodapine bee, *Exoneura bicolor* Smith (Hymenoptera: Apidae: Xylocopinae). *Aust J Entomol* 36:391–394
- Bull NJ, Schwarz MP (2001) Brood insurance via protogyny: a source of female-biased sex allocation. *Proc R Soc Lond B* 268:1869–1874
- Burgett DM, Sukumalanand P (2000) Flight activity of *Xylocopa (Nyctomeliitta) tranquebarica*: a night flying carpenter bee (Hymenoptera: Apidae). *J Apic Res* 39:75–83
- Cane JH (1986) Predator deterrence by mandibular gland secretions of bees (Hymenoptera: Apoidea). *J Chem Ecol* 12:1295–1309
- Cane JH, Michener CD (1983) Chemistry and function of mandibular gland products of bees of the genus *Exoneura* (Hymenoptera, Anthophoridae). *J Chem Ecol* 9:1525–1531

- Chadab R (1979) Army-ant predation on social wasps. PhD dissertation, University of Connecticut, Storrs, Conn.
- Danforth BN (2002) Evolution of sociality in a primitively eusocial lineage of bees. *Proc Natl Acad Sci USA* 99:286–290.
- Danforth BN, Eickwort GC (1997) The evolution of social behavior in the augochlorine sweat bees (Hymenoptera: Halictidae) based on a phylogenetic analysis of the genera. In: Choe JC, Crespi BJ (eds) *Social behavior in insects and arachnids*. Cambridge University Press, Cambridge, pp 270–292
- Eickwort GC, Eickwort KR, Eickwort JM, Gordon JM, Eickwort A (1996) Solitary behavior in a high-altitude population of the social sweat bee *Halictus rubicundus*. *Behav Ecol Sociobiol* 38:227–233
- Falin ZH, Arneson LC, Wcislo WT (2000) Night-flying sweat bees *Megalopta genalis* and *Me. ecuadoria* (Hymenoptera: Halictidae) as hosts of the parasitoid beetle *Macrosiagon gracilis* (Coleoptera: Rhipiphoridae). *J Kans Entomol Soc* 73:183–185
- Field J, Shreeves G, Sumner S, Casiraghi M (2000) Insurance-based advantage to helpers in a tropical hover wasp. *Nature* 404:869–870
- Gadagkar R (1990) Evolution of eusociality: the advantage of assured fitness returns. *Philos Trans R Soc Lond B* 329:17–25
- Gadagkar R (1991) Demographic predisposition to the evolution of eusociality: a hierarchy of models. *Proc Natl Acad Sci USA* 88:10993–10997
- Gotwald WH Jr (1995) *Army ants: the biology of social predation*. Cornell University Press, Ithaca
- Hamilton WD (1964) The genetical evolution of social behaviour, I and II. *J Theor Biol* 7:1–52
- Hogendoorn K, Watiniasih NL, Schwarz MP (2001) Extended alloparental care in the almost solitary bee *Exoneurella eremophila* (Hymenoptera: Apidae). *Behav Ecol Sociobiol* 50:275–282
- Hölldobler B, Wilson EO (1990) *The ants*. Harvard University Press, Cambridge, Mass.
- Janzen DH (1968) Notes on nesting and foraging behavior of *Megalopta* (Hymenoptera: Halictidae) in Costa Rica. *J Kans Entomol Soc* 41:342–350
- Jeanne RL (1975) The adaptiveness of social wasp nest architecture. *Q Rev Biol* 50:267–287
- Jeanne RL (1979) A latitudinal gradient in rates of ant predation. *Ecology* 60:1211–1224
- Kaspari M, O'Donnell S, Kercher JR (2000) Energy, density, and constraints to species richness: ant assemblages along a productivity gradient. *Am Nat* 155:280–293
- Kojima J (1993) A latitudinal gradient in intensity of applying ant-repellent substance to the nest petiole in paper wasps (Hymenoptera: Vespidae). *Insectes Soc* 40:403–421
- Kukuk PF (1994) Nutrient transfer and reproduction in primitively social bees. In: Hunt JH, Nalepa CA (eds) *Nourishment and evolution in insect societies*. Westview Press, Boulder, Colo., pp 329–344
- Kukuk PF, Ward SA, Jozwiak A (1998) Mutualistic benefits generate an unequal distribution of risky activities among unrelated group members. *Naturwissenschaften* 85:445–449
- Leigh EG Jr (1999) *Tropical forest ecology: A view from Barro Colorado Island*. Oxford University Press, Oxford
- Michener CD (1974) *Social behavior of the bees*. Harvard University Press, Cambridge, Mass.
- O'Donnell S, Jeanne RL (1995) Implications of senescence patterns for the evolution of age polyethism in eusocial insects. *Behav Ecol* 6:269–273
- Pickering J (1980) Sex ratio, social behavior and ecology in *Polistes* (Hymenoptera, Vespidae), *Pachysomoides* (Hymenoptera, Ichneumonidae) and *Plasmodium* (Protozoa, Haemosporida). PhD dissertation, Harvard University, Cambridge, Mass.
- Queller DC (1989) The evolution of eusociality: reproductive head starts of workers. *Proc Natl Acad Sci USA* 86:3224–3226
- Queller DC (1994) Extended parental care and the origin of eusociality. *Proc R Soc Lond B* 256:105–111
- Queller DC (1996) The origin and maintenance of eusociality: the advantage of extended parental care. In: Turillazzi S, West-Eberhard MJ (eds) *Natural history and evolution of paper wasps*. Oxford University Press, Oxford, pp 218–234
- Queller DC, Strassmann JE (1998) Kin selection and social insects. *BioScience* 48:165–175
- Rau P (1933) *Jungle bees and wasps of Barro Colorado Island*. Von Hoffmann Press, St. Louis, Mo.
- Rettenmeyer CW, Chadab-Crepet R, Naumann MG, Morales L (1983) Comparative foraging by Neotropical army ants. In: Jaisson P (ed) *Social insects in the tropics*, vol 2. Université de Paris-Nord, Paris, pp 59–73
- Roubik DW (1989) *Ecology and natural history of tropical bees*. Cambridge University Press, Cambridge
- Roubik DW, Wolda H (2001) Do competing honey bees matter? Dynamics and abundance of native bees before and after honey bee invasion. *Popul Ecol* 43:53–62
- Sakagami SF (1964) Wiederendeckung des Nestes einer Nachtfurchenbiene, *Megalopta* sp am Amazonas (Hymenoptera, Halictidae). *Kontyû* 32:457–463
- Schatz B, Wcislo WT (1999) Ambush predation by the ponerine ant *Ectatomma ruidum* Roger (Formicidae) on a sweat bee, *Lasioglossum umbripenne* (Halictidae), in Panama. *J Insect Behav* 12:641–663
- Schwarz MP, Silberbauer LX, Hurst PS (1997) Intrinsic and extrinsic factors associated with social evolution in allodapine bees. In: Choe JC, Crespi BJ (eds) *Social behavior in insects and arachnids*. Cambridge University Press, Cambridge, pp 333–346
- Schwarz MP, Bull NJ, Hogendoorn K (1998) Evolution of sociality in the allodapine bees: a review of sex allocation, ecology, and evolution. *Insectes Soc* 45:349–368
- Smith AR, O'Donnell S, Jeanne RL (2000) Correlated evolution of colony defense and social structure: a comparative analysis in eusocial wasps (Hymenoptera: Vespidae). *Evol Ecol Res* 3:331–344
- Soucy SL, Danforth BN (2002) Phylogeography of the socially polymorphic sweat bee *Halictus rubicundus* (Hymenoptera: Halictidae). *Evolution* 56:330–341
- Strassmann JE, Queller DC (1989) Ecological determinants of social evolution. In: Breed MD, Page RE Jr (eds) *The genetics of social evolution*. Westview Press, Boulder, Colo.
- Wcislo WT (1997a) Behavioral environments of sweat bees (Halictinae) in relation to variability in social organization. In: Choe JC, Crespi BJ (eds) *Social behavior in insects and arachnids*. Cambridge University Press, Cambridge, pp 316–332
- Wcislo WT (1997b) Invasion of nests of *Lasioglossum imitatum* by a social parasite, *Paralictus asteris* (Hymenoptera: Halictidae). *Ethology* 103:1–11
- Wcislo WT (2000) Environmental hierarchy, behavioral contexts, and social evolution in insects. In: Martins RP, Lewinsohn TM, Barbeito MS (eds) *Ecologia e comportamento de insetos*. Oecol Bras [Suppl] 8:49–84
- Wcislo WT, Danforth BN (1997) Secondarily solitary: the evolutionary loss of social behavior. *Trends Ecol Evol* 12:468–473
- Wcislo WT, Gonzalez VH (2003) Social and ecological contexts of trophallaxis in facultatively social, nocturnal sweat bees, *Megalopta genalis* and *M. ecuadoria* (Hymenoptera: Halictidae). *Ethology* (in press)
- Wilson EO (1971) *The insect societies*. Harvard University Press, Cambridge, Mass.
- Wolda H, Roubik DW (1986) Nocturnal bee abundance and seasonal bee activity in a Panamanian forest. *Ecology* 67:426–433
- Wright SJ, Calderon O (1995) Phylogenetic patterns among tropical flowering phenologies. *J Ecol* 83:937–948