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The evolution of nocturnal behaviour in sweat bees, *Megalopta genalis* and *M. ecuadoria* (Hymenoptera: Halictidae): an escape from competitors and enemies?

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Evolutionary transitions to dim-light foraging (predawn matinal, crepuscular, nocturnal) have occurred repeatedly in bees, and may be associated with an escape from enemies or competitors. To date, however, little information has been available to test these hypotheses. Here we provide the first detailed information on the nesting behaviour of two species of Neotropical, nocturnal sweat bees, *Megalopta genalis* and *M. ecuadoria* (Hymenoptera: Halictidae). Females are facultatively social or solitary, and construct nests in dead wood. Nocturnal foraging behaviour is bimodal. Bees began foraging after sunset (~18:30 h) and ceased foraging approximately 1 h later even though nocturnal flowers with pollen were still abundant; a second foraging bout occurred in the predawn morning, which began at ~04:45 h and ended around sunrise (~06:15 h) when diurnal-blooming flowers were abundant. Bees are capable of controlled flight in full light. They utilized pollen from both canopy and understory plant species, which have diurnal or nocturnal pollen anthesis. *Megalopta* nests are attacked by generalist predators such as ants, as well as the endoparasitic fly *Melaloncha* sp. nov. (Phoridae), the beetle *Macrosaigon gracilis* (Rhipophoridae), the parasitic wasp *Lophostigma cincta* (Mutillidae), and the brood parasite *Megalopta byroni* (Halictidae). Overall nest survivorship rates were comparable to those for diurnal relatives, but rates of cell parasitism for *Megalopta* (< < 5%) were substantially lower than they are for day-flying relatives, offering some support for the hypothesis that the evolution of nocturnal behaviour enables escape from natural enemies. © 2004 The Linnean Society of London, *Biological Journal of the Linnean Society*, 2004, **83**, 377–387.

ADDITIONAL KEYWORDS: flexibility – nocturnal foraging – parasitism – social pollination.

INTRODUCTION

Bee biologists tend to work 'in warm sunny places, during pleasant . . . times of day' (Michener, 2000: 1), when most bees are foraging (Roubik, 1989; Wcislo & Cane, 1996). Except for diurnal species such as stingless bees (Meliponini) or honey bees (*Apis*) that occasionally fly in the early dawn or under a full moon (e.g. Dyer, 1985; Roubik, 1989; Warrant, Porombka & Kirchner, 1996), relatively little is known about bees that regularly forage in dim-light environments: at dawn, dusk or night (e.g. Jörgensen, 1912; Rau, 1933; Linsley, 1958; Chandler, 1961; Sakagami, 1964; Sakagami & Moure, 1967; Janzen, 1968; Linsley & Cazier, 1970; Roberts, 1971; Rozen & Rozen, 1986; Burgett & Sukumalanand, 2000; Arneson & Wcislo, 2003; Smith, Wcislo & O'Donnel, 2003).

Evolutionary transitions to dim-light foraging have occurred repeatedly in bees. This foraging specialization is known, or inferred from anatomical traits, in four of the seven currently recognized bee families (Colletidae, Andrenidae, Halictidae, Apidae) (e.g. Smith, 1862; Bingham, 1897; Graenicher, 1911;

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Stevens, 1920; Cockerell, 1923; Friese, 1926; Linsley, 1958; Michener, 1966; Kerfoot, 1967a; Eickwort, 1969). Dim-light species are typically characterized externally by their enlarged ocelli (Kerfoot, 1967a), large-diameter ommatidia (compound eye facets) (Jander & Jander, 2002), and pale body coloration (Friese, 1926; Hunt *et al*., 1995). In different lineages dim-light foragers are matinal, crepuscular, facultatively nocturnal or obligately nocturnal (e.g. Bingham, 1897; Kerfoot, 1967b; Roberts, 1971; Burgett & Sukumalanand, 2000). The evolution of nocturnal foraging on night-blooming flowers apparently opened a new niche for bees, but usually these evolutionary transitions have not generated subsequent radiations. In the Halictidae, for example, dim-light foraging evolved at least four times, but in most cases this has given rise to relatively few species (Moure & Hurd, 1987). *Lasioglossum (Sphecodogastra)* (five species) are crepuscular and forage on *Oenethera*, although they extend foraging flights later in the evening when there is a full moon (Kerfoot, 1967a, b); *Megommation* (five species) are presumably nocturnal because of their large ocelli, and they have been observed visiting flowers before sunrise and found within their nests during the day (Jörgensen, 1912; de Bertoni, 1911; Michener & Lange, 1958; Sakagami & Moure, 1967; Janzen, 1968); *Megaloptidia* (three species) are also presumed to be nocturnal because of the enlarged ocelli, and an individual of one species had been collected from a flower (*Dichorisandra ulei*) with nocturnal anthesis (Engel & Brooks, 1998), though pollen usage was not documented. *Megalopta* (~28 species), in contrast, has undergone adaptive radiation (*sensu* Wilson, 1992), which includes parasitic species that attack congeners.

What factors favour an evolutionary transition from diurnal to nocturnal foraging among pollinators? Bats (Chiroptera) are one of the better-known taxa that evolved associations with night-blooming trees and shrubs, acting as pollinators or seed dispersers for the nocturnal flora (e.g. Park, 1940; Baker & Harris, 1957; Baker, 1961; Faegri & van der Pijl, 1979; Marshall, 1983; Luckow & Hopkins, 1995). Two ecological hypotheses have been proposed to account for the evolution of nocturnal behaviour in bats (Park, 1940; Baker, 1961; Jones & Rydell, 1994; Rydell & Speakman, 1995). These hypotheses invoke benefits associated with escape from competitors for food, and with reduced mortality following escape from natural enemies. Assessing the generality of these hypotheses by applying them to the evolution of nocturnality in other groups such as bees has been hindered by the limited data available.

This study provides the first detailed account of the biology of nocturnal sweat bees, *Megalopta genalis* and *M. ecuadoria*, with the aim of ascertaining how they utilize floral resources and their susceptibility to predators and other natural enemies. We evaluate hypotheses that nocturnal foraging is associated with enemy-free and competitor-free space.

MATERIAL AND METHODS

Study species

Megalopta is a Neotropical genus of sweat bees (Hymenoptera: Halictidae) that contains ~28 named species (Moure & Hurd, 1987; Engel, Brooks & Yanega, 1997). All known species nest in dead wood, and most species are believed to be nocturnal or crepuscular because of anatomical features shared with other dim-light foraging aculeate Hymenoptera (see Introduction). *M. genalis* is found in Panama and northern Colombia, while *M. ecuadoria* is found in Panama, Colombia, Ecuador and Brazil (Moure & Hurd, 1987).

Field sites

All field sites were in the Republic of Panama. Most nests were collected in the Barro Colorado Nature Monument (BCNM) (9°9′N, 79°51′W, Colon Province), principally on Barro Colorado Island (for details of the site, see Rau, 1933; Leigh, 1999), but some were collected along Pipeline Road in the adjacent Parque Nacional Soberanía, Parque Natural Metropolitano near Panama City (Panama Province), and adjacent to Castillo San Lorenzo at the Sherman Canopy Crane Site near Colon (Colon Province), between 1998 and 2001. BCNM supports a semideciduous tropical forest with a mature canopy height of ~35 m. Mean monthly temperatures are 25–26 °C, annual rainfall averages ~2500 mm, and an approximately 4-month-long dry season begins in mid-December or early January (see Windsor, 1990). The site south of BCNM (Parque Metropolitano) has a lower annual rainfall and a longer dry season, while the site to the north (Sherman Crane site) has a higher rainfall and a shorter dry season.

Nest collections and analyses

We searched for nests by walking through the forest in areas where dead or broken branches were abundant, and the understory was not too dense. At two sites (Parque Metropolitano and Sherman) we searched for nests in the forest canopy using canopy-access cranes. Nests were collected during the day to ensure that resident bees were captured. Nest entrances were plugged with cotton, and nests were transported to the laboratory where they were either opened immediately, or placed in a freezer and opened later to score

parasitism rates. Nests were opened with a sharp knife by cutting away the wall opposite the cells. Pollen samples were collected from individual nest cells, and from individual foraging bees, and prepared for examination and identification using standard palynological techniques, and a pollen reference collection at the Smithsonian Tropical Research Institute (STRI) (Roubik & Moreno, 1991). Cells were opened to examine their contents, which were preserved or transferred to plastic tissue culture trays for rearing parasites. A sample of adults was dissected and examined for internal parasites. We described nest architectural features following Sakagami & Michener (1962) and Wcislo & Engel (1996), and measurements were made with Mitutoyo digital calipers or Fowler dial calipers.

Nest survivorship

To ascertain survivorship among nests for comparison with diurnal species, active nests ($N = 113$) were identified in the field and tagged, and were examined weekly or biweekly for activity. Nests were considered 'active' when the presence of resident bees was confirmed visually using an opthalmascope (Titan Tool MicroViewing System). In some cases the curvature of the nest tunnel precluded viewing the entire nest, so a fine gauge wire was inserted into the tunnel to perturb the bees; vibrations are easily detected through the substrate when a bee is present. For survivorship studies we pooled nests of the two species because it is not possible to distinguish them reliably based on nest architecture.

Population density

To provide an estimate of population density, a series of randomly selected transects was searched systematically on the Barro Colorado Island Forest Dynamics Plot, a 50 ha section divided into 5-m^2 grids. This plot supports mature forest and has escaped human disturbance for >500 years (see Condit, 1998). We searched 60 transects of 100 m (20×5-m^2 grids per transect), and in each grid we examined every stem with diameter ≥1 cm to locate nests.

Statistical analyses and voucher specimens

Data were analysed using SYSTAT v.10.0 on a personal computer, and Oriana v.1.0 for circular statistics. Unless otherwise stated, mean values are presented with standard errors. Voucher specimens of bees and nests were deposited in the Dry Reference Collection of STRI and the Natural History Museum, University of Kansas (Lawrence, USA).

RESULTS

Nest sites

Bees used dead wood – branches, vines and lianas – as nesting substrata. Nearly all nests were found tangled in vegetation in the understory of primary and secondary forests, where dead sticks are abundant. No nests were found in ~15 h of searching in the forest canopy at each of two sites using canopy cranes, even though nests were found in the understories at these sites.

Nests were in wood that varied from firm to soft and nearly crumbling (Fig. 1). Occupied sticks had a minimum diameter of 1 cm and 1.5 cm for *M. ecuadoria* and *M. genalis*, respectively, and were rarely found in sticks with a diameter greater than 10 cm. On average *M. genalis* nested in sticks having a larger diameter [$x = 21.6 \pm 0.47$ ($N = 108$)] compared with *M. ecuadoria* [$x = 16.3 \pm 1.01$ ($N = 67$)] (Mann–Whitney U-test = 5010, $P < 0.0001$, ×2 approximation = 43.9, d.f. = 1). Stick diameter may be an important factor in shaping *Megalopta* social behaviour, because there was a significant correlation between the number of females per nest and stick diameter in *M. genalis* (Spearman's rho = 0.41, $N = 108$) ($P < 0.05$) but not in *M. ecuadoria* (Spearman's rho = –0.138; $N = 67$).

Nest architecture

Nests were easily recognized by examining the ends of sticks. Bees construct a textured collar around the circular entrance, which usually had a different colour from the surrounding wood (when the wood was dry), producing a concentric series of rings surrounding a black centre. This collar was constructed of very fine

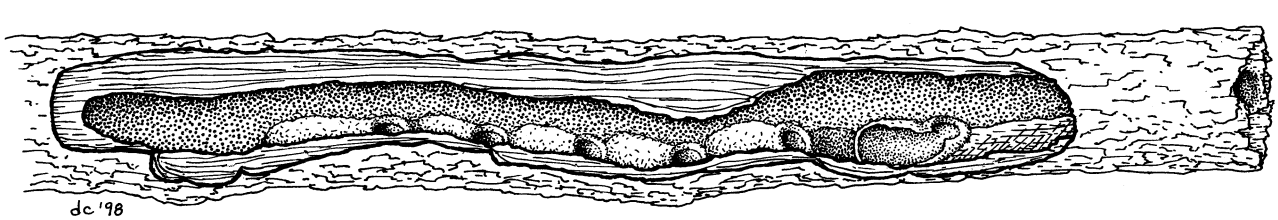

Figure 1. Cut-away view of a nest of *Megalopta genalis,* showing the nest structure and cells; the nest entrance is to the far right.

comminuted wood (possibly with secretions added) to form a matrix similar to pressed particle-board (Fig. 1). The entrance diameter approximately matched the female head size [entrance diameter = 5.8 ± 0.13 mm ($N = 62$), and 4.1 ± 0.07 mm ($N = 55$) for *M. genalis* and *M. ecuadoria*, respectively] (Mann–Whitney U-test = 2655, $P < 0.0001$, using ×2 approximation = 64.64, d.f. = 1). Beyond the nest entrance, the tunnel diameter widened to 9.1 ± 0.6 mm ($N = 62$) and 6.9 ± 0.3 ($N = 55$) for *M. genalis* and *M. ecuadoria*, respectively. The bees constructed each cell within a cavity using wood fibres. The first cell was usually >4 cm from the nest entrance, and subsequent cells were contiguous or separated by varying distances (Fig. 1). Cell shape differed from those of other halictine bees in that the cell often had a recurved neck such that the cell entrance was nearly parallel to the long axis of the cell (Figs 1, 2). This long axis ran roughly parallel to the tunnel, or at an oblique angle. Consequently, cell walls also served as the walls of the tunnel system. In sticks with a diameter wider than ~4 cm, cells were less recurved and some were nearly perpendicular to the tunnel. Cell entrances had internal diameters of 4.7 ± 0.06 mm ($N = 54$) and 4.7 ± 0.05 mm ($N = 42$) for *M. genalis* females and males, respectively, and 4.2 ± 0.08 mm ($N = 26$) and 3.9 ± 0.06 mm ($N = 29$) for *M. ecuadoria* females and males, respectively. The mean internal cell length (sample sizes as above) was: *M. genalis*, 1.8 ± 0.02 cm (females) and 1.7 ± 0.02 cm (males); *M. ecuadoria*, 1.5 ± 0.02 cm (females) and 1.5 ± 0.03 cm (males). The mean internal cell width was: *M. genalis*, 0.9 ± 0.02 cm (females) and 0.8 ± 0.02 cm (males); *M. ecuadoria*, 0.65 ± 0.01 cm (females) and 0.67 ± 0.03 cm (males). Cells were only slightly flattened on the surface that receives the pollen loaf, relative to other halictine cells (Fig. 2). The inner walls of the cells were coated with a shiny, hydrophobic substance, presumably secretions of Dufour's glands. Cells were sealed with a wood-fibre plug that was not coated on the inside. Within a cell pollen was placed on the surface away from the tunnel. Each pollen loaf was formed into an ovoid-rectangular shape (Fig. 2), and an egg was deposited on the top of the pollen loaf. Following larval development, faeces were smeared in bands on the rear wall of the cell as for other halictines (Sakagami & Michener, 1962; Wcislo & Engel, 1996).

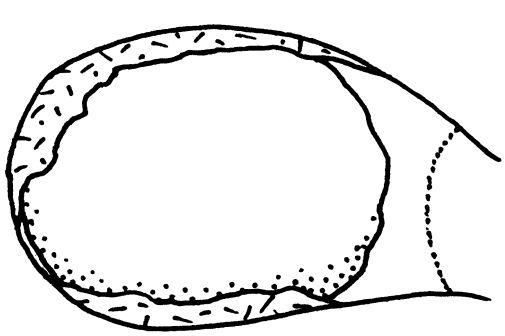

Figure 2. Cut-away view of a nest cell of *Megalopta genalis*, showing the pollen loaf and the recurved cell entrance.

Seasonal cycle

From 1 to 11 females shared a nest (Fig. 3). Singleton and multifemale nests occurred throughout the year (Fig. 4). Females were largely inactive in the latter half of the wet season (September–November), when few floral resources were available (Wright & Calderon, 1995). At this time females were little-worn, inseminated, but with slender ovaries (W. T. Wcislo, unpubl. data). Bees were quiescent but not in diapause and flew away if the nest was opened. Some females presumably passed the inactive period in natal nests because those nests contained one or more old, used cells; other females presumably had dispersed because they were found in nests having a tunnel but no cells (44% of 27 *M. genalis* nests collected Oct–Nov were simple tunnels, while the remaining had at least one cell).

Females began provisioning nests at the start of the dry season in late December or early January, depending on when the rains ended. The majority of nests were solitary early in the dry season and the percentage of nests that developed into multifemale nests increased as the dry season progressed (Fig. 4), possibly when daughter females emerged and joined their

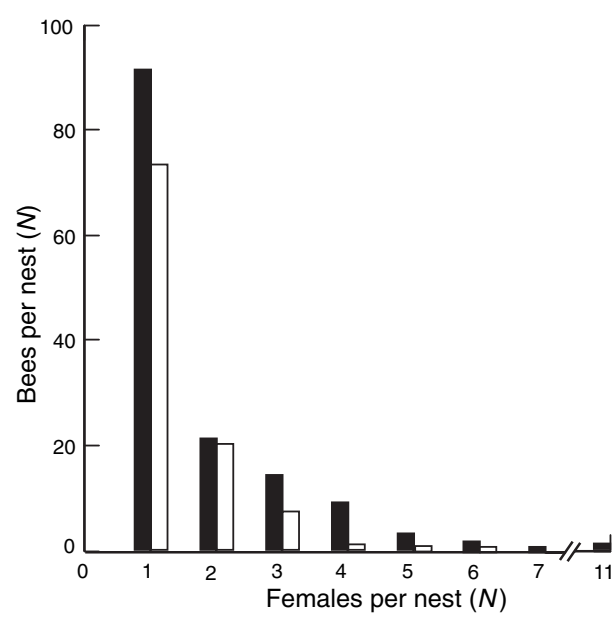

Figure 3. Numbers of bees per nest for *Megalopta genalis* (■) and *M. ecuadoria* (□).

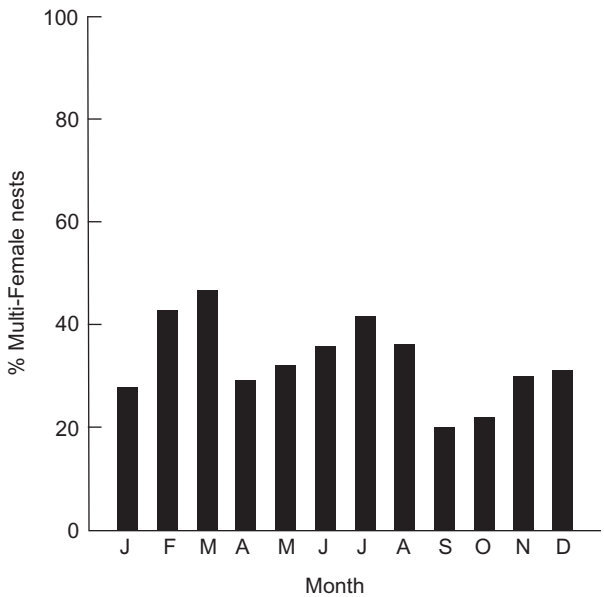

Figure 4. Proportion of multifemale nests of *Megalopta genalis* throughout the year.

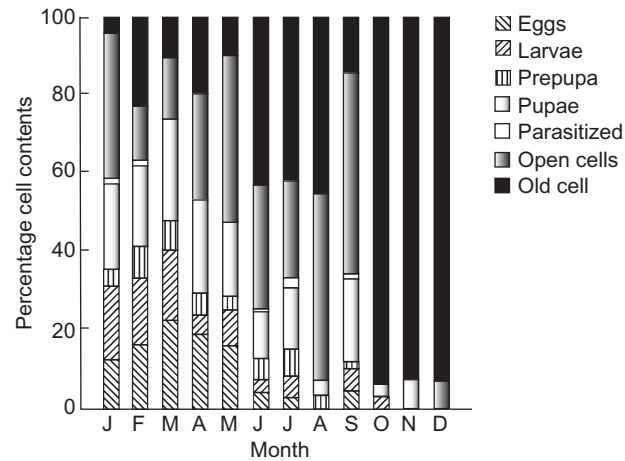

Figure 5. Brood composition of nests of *Megalopta genalis* throughout the year.

natal nests. Bees that emerged in the dry season either left the natal nest and survivors presumably established their own nests as suggested by the occurrence of singleton nests throughout the dry season, or remained in the nest to form part of a social group; we do not know if some of these early brood females immediately entered diapause after emergence (see Yanega, 1997).

Brood composition

Nests that contained eggs were most frequent during the dry season and the first months of the wet season, and no eggs were found in nests at the end of the wet season (Figs 5, 6), suggesting that the bees were not reproductively active year-round. Brood composition was relatively synchronized early in the dry season, but less so subsequently, presumably because some nests developed into multifemale colonies, and other females established new nests. Based on the relative timing of the first appearance of eggs and callow adults within nests, we estimate the egg-to-adult development time to be approximately 35 days (Figs 5, 6).

Ecological abundance

Based on transects within a 50-ha plot on BCI, the mean density of active nests was $5.3 \times 10^{-3} \pm 0.001$ nests m^{-2} (both species pooled, excluding 37 inactive nests; $N = 60$ transects, 1200 5-m^2 grids).

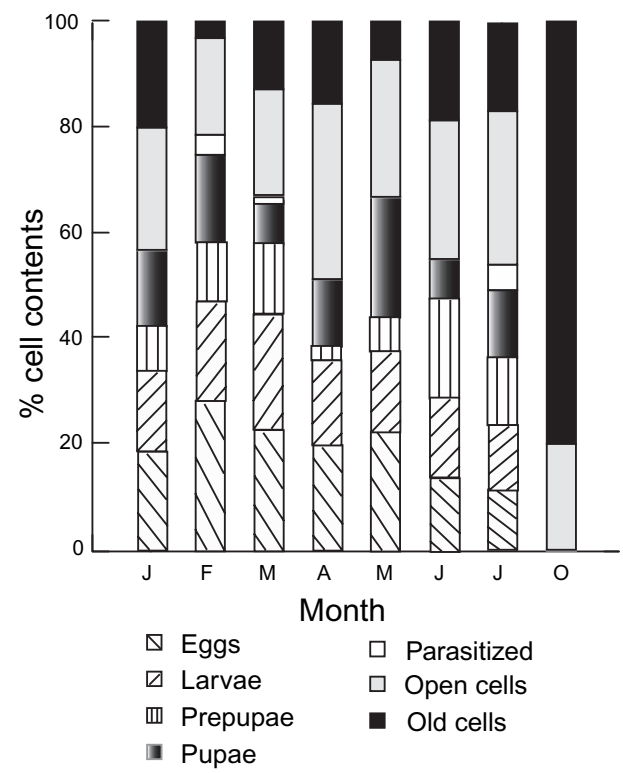

Figure 6. Brood composition of nests of *Megalopta ecuadoria* in January to July, and October.

Foraging behaviour and pollen utilization

On occasion individual females were still afield in the early morning after sunrise, and very rarely females left nests and returned in the afternoon (W. T. Wcislo & A. R. Smith, unpubl. data), indicating they are capable of controlled flight under light conditions experienced by day-flying bees. Nevertheless, most foraging

flights occurred in the dark. In the dry season the mean time of first departure in the evening was 18:45 h ($N = 104$; Rayleigh's test of uniformity, $P < 0.001$), roughly 15–30 min after sunset, and evening foraging trips ceased by 19:30 h. In the pre-dawn morning, bees began foraging again before 05:00 h, and the circular mean for last entry in the morning was 06:14 h ($N = 147$; Rayleigh's test of uniformity, $P < 0.001$), roughly at sunrise. From dusk to dawn, departure times were strongly bimodal, though on occasion flights occurred sporadically during the night, especially when artificial light was used (see also Roulston, 1998). Flights away from the nest ranged from <10 s to more than 60 min in length, but following very short trips bees did not enter with pollen. Foraging flights were longer in the early morning [$x = 19.1 \pm 2.9$ min, $N = 63$] than in the early evening [$x = 12.9 \pm 1.3$ min, $N = 35$], but differences were not significant (Mann–Whitney U-test = 1128, $P > 0.1$, ×2 approximation = 0.036). Detailed behavioural and neural analyses of temporal patterns of foraging, and mechanisms of nest orientation in relation to light levels, will be reported elsewhere (Warrant *et al.,* 2004).

Individual females captured at an ultraviolet light in the evenings from 4 to 14 January 2000 carried pure pollen loads of *Ceiba pentandra* ($N = 6$). Other females collected in the mornings from 14 to 26 January 2000 carried pure loads of either *Bombacopsis quinata*, *Vismia baccifera* or *Pseudobombax septenatum* ($N = 5$). The most frequent pollens in nest cells were from the family Bombacaceae, particularly *Pseudobombax septenatum,* which was present in >80% of nest cells, and *Ceiba, Bombacopsis* and *Ochroma*. Other plants frequently used as pollen sources included *Spondias* (Anacardiaceae), *Vismia* (Guttiferae), two species of *Cecropia* (Cecropiadaceae), *Psidium, Acacia, Aegiphila*, the palm *Chamaedorea*, and the shrub *Miconia* (Melastomataceae). These records, along with published observations (see Discussion), indicate that bees foraged both in the canopy and understory, and used plants that have both nocturnal and diurnal anthesis. Pollen from > 40 plant species was used by the bees in both dry and wet seasons; a detailed analysis of its utilization will be presented elsewhere (W. T. Wcislo & D. W. Roubik, unpubl. data).

Nest survivorship and natural enemies

Approximately 50% of nests survived less than 2 months, although some nests persisted up to 7 months (Fig. 7). In most cases we do not know the cause of morbidity. Bees can successfully defend nests against predatory ants, including army ants (Ecitonini) (Smith *et al.*, 2003), which presumably selects for group-living. Failed nests frequently (10–85% of dead sticks) had slits in the wall of the stick adjacent to cells and the cells had been ripped open; the identity of the 'slit-maker' is unconfirmed, but based on forensic marks it may have been the silky anteater (*Cyclopes didactylus*). Little is known about the predators and parasites of adult foragers. Females sometimes harbour in their metasomal glands large numbers of a new genus of nematodes (R. Giblin-Davis & W. T. Wcislo, unpubl. data; see also Lello, 1971); nothing is known of the biological relationships between these nematodes and bees. Female *M. genalis* infrequently (2% of $N = 120$ dissected females) contained larvae of the endoparasitic fly *Melaloncha* sp. nov. (Diptera: Phoridae) (W. T. Wcislo, V. Gonzalez & B. Brown, unpubl. data). Oviposition by these flies has not been observed. *Megalopta* appear to be slow fliers, at least in the understory near nests, and whether they are hunted by bats in nature is unknown. Preliminary trials suggest insectivorous bats catch but release females (A. R. Smith & W. T. Wcislo, unpubl. data). Foragers transport triangulin larvae of the beetle *Macrosaigon gracilis* (Rhipohporidae) (Falin, Arneson & Wcislo, 2000), and inside the nest these larvae then crawl to a cell and feed on the stored provisions. Rates of cell parasitism by *Macrosaigon* were low (<1%) for both species of *Megalopta*. Brood cells are also attacked infrequently by females of the parasitic wasp, *Lophostigma cincta* (Mutillidae) (Cambra, Gonzalez & Wcislo, in press). Approximately 2.5% of 177 cells from 66 *M. ecuadoria* nests were parasitized by *Lophostigma,* and similar rates of parasitism occurred in *M. genalis* (~2.1% of 388 cells from 119 different nests). The brood parasite *M. byroni* was also

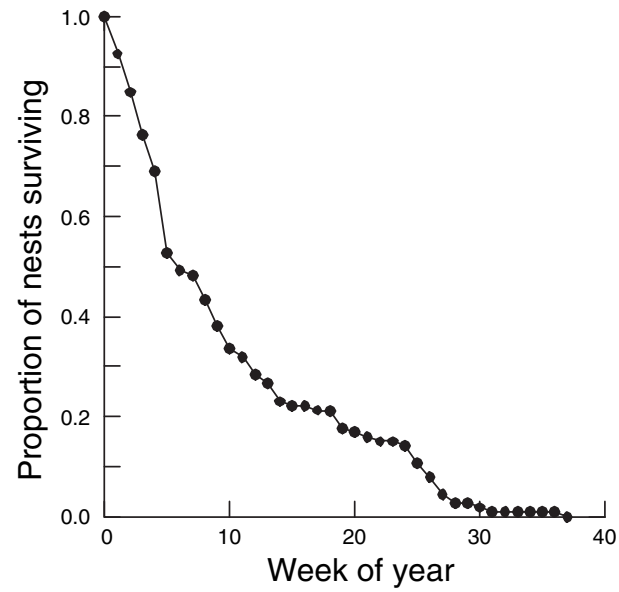

Figure 7. Relative survivorship of nests of *Megalopta genalis* ($N = 113$ nests).

relatively rare. Three of > 300 nests contained both an adult *M. genalis* and one female *M. byroni* (< < 1%), which is the first confirmed host association for this parasite. Although nesting in rotting wood in the tropics would seem to invite fungal attack, only 2% of >1000 cells contained fungi. It was not possible to determine that fungi were the cause of mortality. Preliminary studies showed that whole-gland extracts of Dufour's gland secretions had no antifungal activity against fungi cultured from bees' nests (W. T. Wcislo & K. Roesch, unpubl. data).

DISCUSSION

The dark-loving Halictidae are noteworthy in that the behaviour has a biased phyletic and geographical distribution: most are Neotropical Augochlorini, except the largely solitary Nearctic *Lasioglossum (Sphecodagastra)* in the tribe Halictini. As discussed below, major sources of pollen for nocturnal bees are flowers usually associated with bat pollination. Such plants are most common in the Neotropics, which may help explain the geographical distribution of neotropical nocturnal bees.

Ecological drift aside (Hubbell, 2001), two adaptive hypotheses have been discussed to account for the evolution of dim-light foraging specializations in bees, which parallel arguments to account for the evolution of nocturnal foraging in bats (Rydell & Speakman, 1995). The first draws a temporal analogy with the concept of 'enemy-free space' invoked to explain host shifts in phytophagous insects (Bernays & Chapman, 1994). There are legions of generalist predators and parasites that attack diurnal bees (e.g. Roubik, 1989; Danforth & Eickwort, 1997; Wcislo, 1997, 2000; Schmid-Hempel, 1998), and a shift to nocturnal or crepuscular activity may offer an evolutionary escape route, as suggested by Kerfoot (1967b). With the exception of *Macrosaigon* (Coleoptera: Rhipophoridae) (Falin *et al*., 2000) and predatory ants, none of the other observed enemies of *Megalopta* also attack diurnal hosts, apparently providing modest support to this hypothesis. However, the natural histories of diurnal relatives of *Megalopta* and their enemies are not well known, so the absence of host records may not be informative. Overall nest survivorship is somewhat comparable to that of diurnal, temperate sweat bees. Batra (1966), for example, showed that 50% survivorship of *Lasioglossum zephyrum* nests varies from ~3 weeks to 2 months, roughly comparable to *Megalopta* (see also Michener & Wille, 1961). These rates are somewhat higher than reported for a eusocial sweat bee, *Lasioglossum duplex* (Sakagami & Fukuda, 1989), although there was considerable yearly variation in survival rates (Sakagami, 1977). A measure more relevant to the concept of enemy-free space is rate of cell parasitism, rather than overall survival. Rates of cell parasitism in *Megalopta (*< < 5%) were nearly six-fold lower compared with a random sample of 25 twig-nesting temperate bees, for which mean rates of cell parasitism are ~29% (Wcislo, 1996), and were approximately four-fold lower compared with rates of some tropical, ground-nesting sweat bees (Wcislo *et al*., 1993).

The second hypothesis to account for nocturnality is associated with competitor-free space. In addition to the floral resources used by bees in this study (Results), *Megalopta* females also gather nectar or pollen from *Solanum, Asplundia, Bactris, Desmoncus, Mimosa, Ipomoea* and *Parkia* (Janzen, 1968; Bullock *et al*., 1987; Mori & Boeke, 1987; Gottsberger, 1991; Listabarth, 1996; Roulston, 1998; Hopkins, Hopkins & Sothers, 2000). Thus, *Megalopta* females exploit both the nocturnal and diurnal flora. Foraging behaviour among bees is thought to be intensely competitive, and typically resources are available on a 'first come-first served' basis (Linsley, 1958; Roubik, 1989; Shelly *et al*., 1993; Wcislo & Cane, 1996). Release of pollen (anthesis) in many species of diurnal flowers occurs early in the morning (e.g. Endress, 1994; Minckley *et al*., 1994; Gribel, Gibbs & Queiróz, 1999), and bees that forage earlier and earlier to beat the competition enter a progressively dimmer environment. In the Sonoran desert, for example, a crepuscular species, *Ptiloglossa arizonensis* (Colletidae), begins foraging on *Solanum* flowers 1–2 h prior to the diurnal *Bombus sonorus* (Apidae). On average each flower is visited by 12 *Ptiloglossa* bees before the first diurnal *Bombus* arrives (Shelly *et al*., 1993), and there is decreasing pollen availability for late-comers (J. Cane, cited in Roulston, 1998). A number of plant lineages have evolved nocturnal anthesis in apparent association with pollination by bats and large moths (e.g. Park, 1940; Baker & Harris, 1957; Baker, 1961; Faegri & van der Pijl, 1979; Marshall, 1983), and some flowers open in the late afternoon and early evening (e.g. Pettersson & Knudsen, 2001; Miyake & Yahara, 1999). In Mexico, for example, flowers of *Ipomoea wolcottiana* open at night and were visited by *Megalopta* sp. prior to sunrise, after which 20 diurnal bee species visited them to collect resources (Bullock *et al*., 1987). Many tropical social stingless bees apparently obtain substantial nutritional resources by foraging early in the morning and opportunistically exploiting nocturnal floral resources by collecting the dregs of pollen left behind by bats (Roubik, 1989). In these environments the survival of the fittest may be equivalent to the 'survival of the first' (see Hopf, 1988), favouring a shift to nocturnal foraging to exploit predawn anthesis. In addition to potentially escaping competition, these nocturnal resources may be especially valuable to bees because of their copious nectar production and pro-

tein-rich pollen. In general, pollen consists of ~3–61% protein by dry weight (Buchmann, 1986), and pollen of putatively bat-pollinated species tends to be at the upper end of this scale, with a higher protein content compared with pollen of bee-pollinated plants (Howell, 1974; Roulston, 1998). In our study the pollen and possibly nectar used most frequently by *Megalopa* generally seemed to come from large flowers that opened at night. Nevertheless, these bees did not specialize on Bombacaceae, and in fact diurnal flowers characterized most of the plant taxa that they utilized to a lesser extent (also W. T. Wcislo & D. W. Roubik, unpubl. data).

A common assumption in discussions of the evolution of nocturnal behaviour in tropical bees is that they evolved to exploit a niche opened by the evolution of bat–plant associations (e.g. Wolda & Roubik, 1986; Roulston, 1998). Some phylogenetic data are inconsistent with this hypothesis. Cladistic analysis of the amphitropical *Parkia* (Leguminosae) shows that the entomophilous species are basal, while a derived clade consists of bat-associated species (Luckow & Hopkins, 1995; see also Baker & Harris, 1957). A *Megalopta* sp. is a frequent visitor to a basal, entomophilous *Parkia* sp. with late afternoon pollen anthesis, and Hopkins *et al*. (2000) suggest that nocturnal bee visitation may have played a role in shaping the floral environment in which bats evolved. Unfortunately, the fossil record for both bees and bats is not extensive (e.g. Hand *et al*., 1994; Engel, 2001). Early close relatives of extant flower-visiting bat species date from the Miocene or Oligocene (Hand *et al*., 1994). Nothing is known of the fossil history of *Megalopta*, nor of other nocturnal halictids; one fossil of a derived augochlorine (*Oligochlora*) related to *Megalopta* (three steps in a cladogram of genera) is probably from the Miocene (Engel, 2000, 2001). These considerations raise the hypothesis that the role of bees as nocturnal visitors may antedate pollinators such as flower-visiting bats in opening a new niche.

Based on long-term data from light traps on BCI, Wolda & Roubik (1986; see also Roubik & Wolda, 2000) concluded that the only consistent seasonal trend in *Megalopta* abundance was a decline in capture rate during the dry season, although they note there was extensive variation among years. Furthermore, these authors note their data 'clearly establish the year-round activity of *Megalopta*.' Our data contradict their findings so far as nest provisioning is concerned. Bees provisioned nests during the dry season and early wet season [based on proportion of nest cells that contained eggs (Figs 5, 6), or observations of nesting behaviour (W. T. Wcislo & V. Gonzalez, unpubl. data)], and virtually no nests contained cells with eggs at the end of the wet season, indicating that bees were *not* active in nest provisioning the year round. That bees go to artificial light year-round (Wolda & Roubik, 1986) suggests that they may forage for nectar even at times when they are not provisioning nests.

Foraging in dim light highlights the point that adaptations arise not in response to an autonomous external world, but rather evolve as solutions to problems that animals make for themselves, such that novel behaviour generates novel selective environments (see Wcislo, 1989; Lewontin, 2000; Odling-Smee, Laland & Feldman, 2003). Among diurnal bees there is considerable variation in foraging times, and some individuals facultatively behave as crepuscular or even nocturnal foragers (e.g. Warrant *et al*., 1996). Such behaviour generates a dim-light foraging environment, which will select for traits that enhance dim-light foraging. Unlike facultative dim-light foragers, crepuscular and nocturnal species that regularly forage in low light levels usually have enlarged simple and compound eyes (Kerfoot, 1967a; Jander & Jander, 2002), as is true for other dark-active animals (e.g. Fernald, 1997; Thomas *et al*., 2001; Garamszegi, Møller & Erritzøe, 2002). These changes, along with changes in receptor sensitivity and size, enhance the ability to see in dimmer light environments (Warrant *et al.*, 2004). However, inferences about behaviour drawn from morphology can be misleading (Russell, 1916), and clearly more studies are needed on additional species to shed light on the ecological significance of the evolution of nocturnal behaviour in bees.

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