

Phenological, but not social, variation associated with climate differences in a eusocial sweat bee, *Halictus ligatus*, nesting in southern Ontario

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Academic editor: Jack Neff | Received 15 October 2014 | Accepted 10 March 2015 | Published 27 March 2015

<http://zoobank.org/E0C14ABA-65CE-48A9-9544-6FE9529A95C7>

Citation: Richards MH, Onuferko TO, Rehan SM (2015) Phenological, but not social, variation associated with climate differences in a eusocial sweat bee, *Halictus ligatus*, nesting in southern Ontario. Journal of Hymenoptera Research 43: 19–44. doi: 10.3897/JHR.43.8756

Abstract

Studies of annual and geographic variation in eusocial bee populations suggest that more stringent environmental conditions result in stronger reproductive skew favouring queens, while moderate conditions favour increasing worker reproduction. To test these predictions, we compared the phenology and colony development of *H. ligatus* nesting in St. Catharines, Ontario, Canada to a previously studied aggregation 90 km north of St. Catharines, in Victoria, Ontario. Despite the close proximity of these two locations, St. Catharines has markedly shorter winters and longer summers. Comparisons between St. Catharines in 2006 and Victoria in the 1980s and 1990s incorporate both geographic differences in climate and temporal differences due to climate change. We predicted that St. Catharines foundress queens should emerge from hibernation and initiate nests earlier in spring, giving them time to produce more workers. Since earlier studies indicated that queens have difficulty suppressing worker reproduction in larger colonies, we also predicted higher rates of worker ovarian development in St. Catharines. In spring and summer 2006, we excavated 65 *H. ligatus* nests, comparing their contents to 713 specimens collected in pan traps. As predicted, nests were initiated about a month earlier in St. Catharines than in Victoria, but contrary to prediction, fewer workers were produced in St. Catharines. St. Catharines workers were just as likely to have developed ovaries as Victoria workers. About 40% of St. Catharines workers were classified as reproductive, and larger reproductive workers tended to have higher ovarian scores. Early queen mortality in the longer nest cycle of St. Catharines bees may have enhanced opportunities for worker reproduction despite their smaller numbers. Novel features of *H. ligatus* sociobiology in St. Catharines included

evidence that queens can initiate new nests following the loss of their first brood, overlap between worker and gyne production within some nests, and high rates of independent nest founding by worker-sized females, suggesting that many worker-brood females overwinter. Overall, the distinctly warmer climate of St. Catharines compared to Victoria led to earlier nest initiation and lengthening of the flight season, but not to the predicted differences in colony social organisation or queen-worker reproductive skew. A second objective of our study was to assess how well pan trap collections capture important information about demographic and social parameters important in assessing social variability in sweat bees. Nest excavations and pan traps produced similar results, suggesting that pan traps are a good alternative when nest excavations are impossible.

Keywords

Halictinae, social evolution, geographic variation, pan trap, worker reproduction

Introduction

Eusociality is the most frequent, caste-based form of colony social organisation in sweat bees (Halictidae) (Michener 2007). In sweat bees, eusociality is characterized by the behavioural totipotency of all adult females, such that any newly eclosed female is potentially capable of taking on either the queen or worker role (Yanega 1989, 1990). The flexibility inherent in the behaviour of individual females is also observed at the level of entire colonies or populations, as there is considerable evidence for both intra- and inter-population variation in colony social organisation, especially in response to local climatic conditions (Yanega 1993, Miyanaga et al. 1999, Hirata et al. 2005, Kocher and Paxton 2014). Ultimately, variation in colony social organisation seems to reflect colony or population-typical outcomes of conflict between queens and workers over control of reproduction, the conflict being moderated by the environmental conditions experienced by individual bees and colonies.

Halictus ligatus Say is one of the most widespread eusocial bees in North America, and together with its closely related and morphologically cryptic sister species, *H. poeyi* Lepeletier, represents a spectrum of queen-worker reproductive skew from high skew at high latitudes to low skew at lower latitudes (Michener and Bennett 1977, Packer and Knerer 1986, 1987, Dunn et al. 1998). Studies of annual variation within populations and geographic variation among populations suggest that more stringent environmental conditions result in stronger reproductive skew favouring queens, whereas moderate conditions favour increasing worker reproduction (Richards and Packer 1995, 1996, Richards et al. 1995, Richards 2004). At a nesting aggregation in Victoria, Ontario (northwest of Toronto), variation in colony social behaviour was ultimately due to extreme annual variation in local rainfall and temperature patterns, that led to dynamic changes in queen-worker behavioural interactions. In 1990, poor weather conditions (higher than normal rainfall) limited queen foraging time, leading to the production of workers with relatively small body sizes. The wet conditions also led to high levels of juvenile mortality, so worker brood survival was low. As a result, queens had to contend with relatively few, relatively small workers in 1990, which

provisioned relatively small gynes. In contrast, the summer of 1991 was unusually warm, with normal rainfall levels. That year, the small queens born in 1990, experienced excellent conditions during the worker brood provisioning phase and raised relatively large numbers of large-bodied workers. The weather variation that directly led to differences in worker body size and numbers indirectly led to significant social variation. In 1990, large queens had to contend with relatively few, small workers, and were more successful in preventing worker oviposition, whereas in 1991, small queens had large numbers of large workers, and workers increased their contribution to oviposition of Brood 2.

The social variation observed within a single population of *H. ligatus* in response to temporal variation in local environmental conditions, supports the view that much or most social variation is due to phenotypic plasticity. Temperature and breeding season length are among the most important factors influencing colony social variation, because almost all eusocial halictines must produce at least two broods per year, whereas solitary halictines need produce only one brood per year (Kocher et al. 2014). Studies in several species of facultatively social *Halictus* and *Lasioglossum* indicate that colonies develop socially when foundresses can initiate nests early, but develop solitarily when nest initiation is delayed (Packer 1990, Yanega 1993, Hirata and Higashi 2008, Field et al. 2012). Warmer soil temperatures can also speed up colony development, which can lead to production of more workers or extra worker broods (Weissel et al. 2006, Hirata and Higashi 2008). Direct environmental effects such as these can be amplified by indirect effects of longer breeding seasons. For instance, production of extra workers can lead to increased production of reproductive brood (gynes and workers) or may result in declines in reproductive skew, as increased colony size often is associated with increased rates of worker ovarian development (Packer et al. 1989, Richards et al. 1995, Strohm and Bordon-Hauser 2003).

In the current study, we examined the phenology and colony development of *H. ligatus* at a nesting aggregation in St. Catharines, in the Niagara region of southern Ontario in 2006. St. Catharines is about 90 km south of Victoria, Ontario, but has markedly shorter and milder winters and longer summers (Table 1). Moreover, examination of temperature records for southern Ontario (based on data from the “St. Catharines A” and “Orangeville” stations, available at climate.weather.gc.ca) demonstrates that from 2000–2006, average monthly temperatures were higher than they had been in the 1980s (summarized in Table 1). This suggests that comparisons of bees nesting in Victoria in the 1980s and 1990s and St. Catharines in 2006 incorporate both geographic variation in climate and temporal differences due to climate change. Accordingly, we predicted that the longer spring and summer seasons in Niagara in 2006, compared to Victoria 15–22 years earlier, should be associated with differences in colony phenology that translate into differences in colony social organisation. In Niagara, foundress queens should emerge from hibernation and initiate nests earlier in spring than in Victoria, and start provisioning the worker brood earlier. This would give them time to produce more workers and thus larger colonies by mid-summer. Larger colony sizes, in turn, would lead to relatively high rates of worker egg-laying, as queens would be less able to

Table 1. Geographic variation in temperature and precipitation patterns in St. Catharines and Victoria, as indicated by climate normals for the period 1981–2010. Climate data were obtained from for the St. Catharines A and Orangeville stations (http://climate.weather.gc.ca/climate_normals). Bees nesting in St. Catharines experience considerably shorter winters and longer flight seasons and higher temperatures, especially during the spring. Precipitation patterns are very similar at the two sites. Degree-days represent the number of days per year in which the temperature exceeds the given average temperature. Note that *H. ligatus* foragers cannot fly at temperatures below 14 °C (M.H. Richards, pers. obs.).

Event, 1981–2010	St. Catharines	Victoria (Orangeville)
Average date of last spring frost	24 April	20 May
Average date of first autumn frost	21 October	30 September
Average length of frost-free period (days)	179	132
Days with minimum temperature above 0 °C	238.1	194.6
Degree-days above 15 °C	656.2	403.5
Degree-days above 18 °C	334.9	169.2
Rainfall (mm)	754.2	750.1
Snowfall (cm)	137.1	151.5
Total precipitation (mm)	880.1	901.5
Days with precipitation >0.2mm, April–September	71.6	66.7

suppress worker oviposition (Richards et al. 1995). Longer breeding seasons might also be associated with an increased likelihood that queens die prior to completion of Brood 2, promoting worker oviposition, especially by the youngest workers which might often emerge into nests without large foundress queens. The early initiation of spring might not be the only factor influencing bee behaviour; the shorter, milder winters in Niagara compared to Victoria might also have an influence. Most females that found nests are gynes (large, fat females produced in Brood 2 of the previous year). However, workers (small, skinny females produced in Brood 1 of the current year) also attempt to nest independently or to overwinter and become foundresses the following spring (Rehan et al. 2013; Richards and Packer 1994). Worker-sized females might be more likely to survive hibernation in areas with milder winters. This would increase the number of small foundresses in spring, possibly influencing rates of pleometrotic (multi-foundress) nest-founding if worker-sized females are more likely to become subordinate females rather than founding their own nests (Packer 1986a).

Collecting detailed field observations of colonies is very important in studies of demographic and social variation among sweat bee nesting aggregations, but nesting aggregations can be difficult to locate, a serious impediment to extensive comparisons among populations. An alternative source of demographic data, season-long collections of bees in pan traps, is widespread, but mostly aimed at assessing variation in abundance and diversity of entire bee communities (e.g. Richards et al. 2011). To date, only one study has evaluated the social status of a halictine species based solely on pan trap data. Packer et al. (2007) used dissection and measurement data of pan trapped specimens to show that *H. tripartitus* is eusocial in California. A second objective of our study was to assess how well pan trap collections capture important information about demographic and social parameters important in assessing social variability in sweat bees.

Methods

Nest excavations

Nest excavation techniques for *H. ligatus* nesting in St. Catharines, were as previously described (Rehan et al. 2013). Briefly, 65 *Halictus ligatus* nests, including four previously described nests founded by workers in mid-summer (Rehan et al. 2013) were excavated over the period from late May to late August 2006 from a temporary pile of dirt created by landscaping activities on the Brock University campus, St. Catharines, Ontario, Canada (N 42°07'11", W 79°14'57"). We aimed to excavate five nests per week, weather permitting. Nests were excavated early in the morning or late in the day to ensure that all occupants were inside. Talcum powder was blown down nest entrances to enhance visibility prior to nest excavation. Adults, young larvae and injured specimens were collected directly into 95% ethanol, while older larvae and pupae were collected in parafin-lined petri dishes to be raised to adulthood in the lab. In classifying the life stages of brood, prepupae (post-defecatory larvae) were classified as larvae, while newly eclosed bees still in their brood cells (sometimes referred to as imagoes) were classified as adults.

Pan trapping

Pan trapping techniques and locations were as previously described (Richards et al. 2011). In brief, pan trap lines were set out weekly at six sites (406, BrNW, BrS, Esc, Pon, and StD) on the Brock University campus and the nearby Glenridge Quarry Naturalization Site, within 1–2 km of the nesting aggregation described below. Pan trapping was carried out from the last week of April (week 1) until late September (week 23). A total of 713 *H. ligatus* specimens were pinned and identified, and used to assess flight patterns of adult males and females, thus revealing breeding season phenology (Richards et al. 2010). A subset of pan-trapped females, selected from the peak collecting periods, was measured and dissected as described below.

Status of adult females

All adult females (N = 171) collected from nests were measured and dissected to determine head width, wing length, mandibular wear, wing wear, ovarian development, and whether they had mated. In addition, 133 females collected from nests as larvae or pupae and reared to pupation or adulthood, were measured. We also measured head width and wing wear and dissected 153 of the 463 females collected in pans. Most of these dissected specimens were queens collected in mid-May (week 4), workers in mid-July to mid-August (weeks 13–16), and a mix of workers and early gynes in late August (week 19). The procedures for dissections and measurements followed those used in previous studies (e.g. Richards et al. 2010). Briefly, mandibular wear (nest bees

only) and wing wear (both nest and pan bees) were each scored from 0 (unworn) to 5 (extremely worn) and then added together to give a total wear score (TW). Ovarian development (OD) was assessed by assigning to each developing oocyte a fraction indicating its size relative to a fully developed oocyte (1, $\frac{3}{4}$, $\frac{1}{2}$, or $\frac{1}{4}$) and summing. OD provides a cardinal measure of each female's total volume of developing oocytes. An OD value of 0.1 was assigned to females with thickened ovaries but no developing oocytes, and 0 was assigned to females with thread-like ovaries.

In eusocial sweat bees, caste is associated with differences in behaviour, body size, wear, and ovarian development (Schwarz et al. 2007). In general, queens fly and provision brood in spring, remain in the nest in summer, are larger than their own workers, become quite worn by mid-summer, are mated, and have high rates of ovarian development. Workers emerge and provision brood in summer, are almost always smaller than queens, become progressively more worn by late summer, are often mated, and may or may not have developed ovaries (Packer and Knerer 1985, Packer 1986a, Richards 2001, Richards et al. 2010). Therefore, we used the following criteria for categorizing females collected in nest excavations. Adult females collected before week 10, when Brood 1 had not yet begun to emerge as adults, were categorized as queens, regardless of size. From week 10 onward, we used the median head width for all females (median HW = 2.82 mm) to categorize females as large (above the median) or small (below the median). From weeks 10–16, small (HW < 2.82) adult females were designated as workers. Two worker-sized females collected in nests excavated in weeks 16 and 17, were categorized as foundresses rather than workers, because their unusually high wear scores (TW = 10) suggested that they had been foraging for much longer than the workers in their own nests. After week 16, large, very worn (TW > 5) females were designated as queens, small, worn females (TW > 1) were designated as workers, and large, unworn females (TW ≤ 1) were designated as gynes. Females that did not fit these criteria were not assigned to caste. Note that the term 'gyne' is used here to describe females that overwinter prior to reproduction, whereas 'reproductive workers' are Brood 1 females that attempt to breed shortly after eclosion.

Assignment of caste to females caught in pan traps was also based on the above criteria, as well as comparisons to the colony development phenology inferred from nest excavations. Adult females collected before week 10 were categorized as queens. From weeks 10–16 onwards, all females caught in pan traps were designated as workers because gynes had not yet eclosed in excavated nests. It is possible that some of the small, worn females captured at this time were not workers, but very late-foraging small queens or subordinate foundresses that continued foraging after worker emergence (Packer 1986b). From week 17 onwards, females with head width above the median (2.82 mm), low wing wear (WW ≤ 1), and no ovarian development were designated as gynes, while those with head width below the median and worn wings (WW > 1) were designated as workers. Females that did not fit these criteria were designated as caste unknown.

Statistical analyses

We checked our initial caste assignments in two ways. We used Principal Components Analysis (PCA) to examine differentiation among queens, workers, and gynes. Bees collected in nests and pans were analysed separately. For nest bees, the PCA was based on head width, total wear, and ovarian development, whereas for pan-trapped bees, the PCA was based on head width, wing wear, and ovarian development. The PCA analyses were carried out using the `princomp` function on the rescaled variables in R version 2.15.0. Visual inspection of the principal components plots indicated general separation of the castes. We also used Discriminant Functions Analysis (DFA) to examine the caste classifications of individual females using the `lda` and `predict` functions (R, library MASS). For 171 queens, workers, and gynes collected in nest excavations, a DFA based on head width, wing length, and wear (TW) (but not ovarian development) produced a list of 22 females that were re-classified to a different caste. Of these, 12 reclassifications were wholly implausible given the time of collection (queens collected before worker emergence cannot reasonably be reclassified as workers or gynes), but 10 reclassifications of females collected from week 16 onward (workers that might have been gynes, and vice versa) were incorporated into the data set. We then combined the nest and pan trap bees for a second DFA to classify females collected from week 16 onward (based on HW, WW, and TOD), as these were the most difficult to assign. Females whose caste was initially assigned as ‘unknown’ and which were still unclassifiable after the DFA, were excluded from statistical analyses in which caste was a factor.

In eusocial sweat bees, size differentiation between castes is often measured by proportional differences between queens and workers. We calculated queen-worker differences as $[(\text{queen value} - \text{worker value}) / \text{queen value}] \times 100$. Proportional differences were compared for queens and daughters from their own nests or using the average queen and worker trait values within the nest and pan-trapped individuals separately.

Where parametric statistics are presented, these were based on model statements which generated error terms with normal distributions. The response variables in general linear models were cardinal variables (e.g. head width, ovarian development). Where it was not possible to achieve normally distributed error terms using standard data transformations and where ordinal variables (e.g. wing wear) were analysed, we used non-parametric statistics. All analyses were carried in R-Studio, using R, version 2.15.1. Except where otherwise noted, degrees of freedom (df) = 1.

Data resources

The data underpinning the analyses reported in this paper are deposited in the Dryad Data Repository at doi: [10.5061/dryad.vm11c](https://doi.org/10.5061/dryad.vm11c).

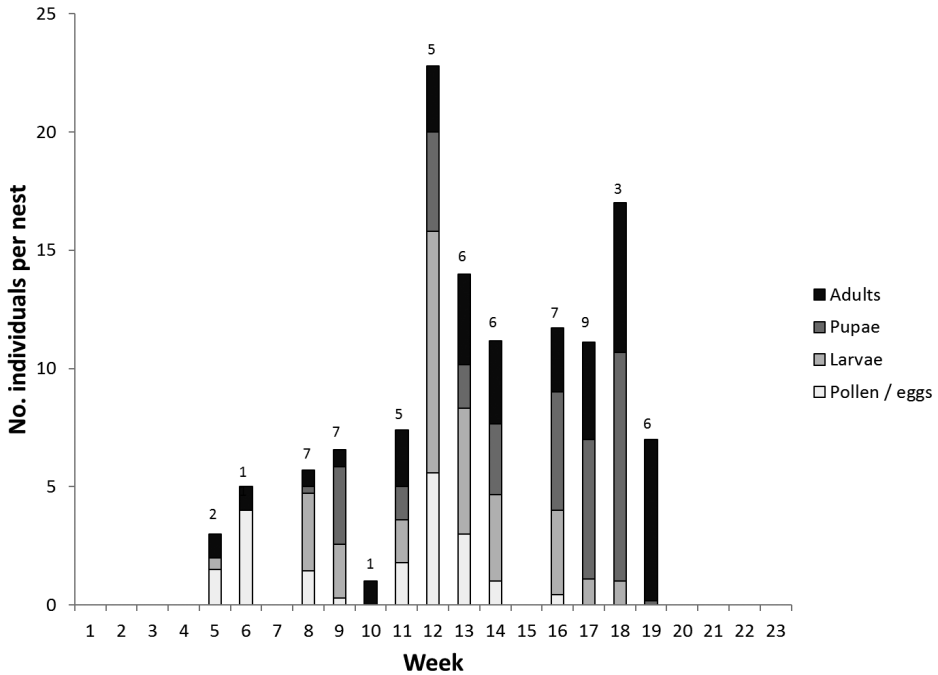


Figure 1. Timing of brood production and development in *Halictus ligatus* nests excavated at Brock University in St. Catharines, Ontario in 2006. Week 1 was the last week of April, and week 11 was the first week of July. Numbers above each bar are the numbers of nests excavated each week.

Results

Brood and colony development based on nest excavations

We excavated 67 nests excavated in St. Catharines from May to August 2006 (weeks 5 to 19). The contents of excavated nests were used to infer the timing of brood production and development (Figure 1).

Nests excavated in weeks 5–6 contained pollen masses, eggs, and small larvae. Pupae were first detected in week 8, and by week 9 very few Brood 1 provision masses were being constructed, so worker brood provisioning was mostly complete. In weeks 8 and 9, the apparent peak of Brood 1 production, nests contained an average of 5.5 ± 3.2 (SD) brood (range = 1 to 10, $n = 14$ nests). Based on 21 sexable pupae collected during this 2-week period, 9.5% of brood were males ($n = 2$).

Nests excavated from week 11 onward contained juveniles representing both broods. Pollen masses attributed to Brood 2 were collected from weeks 11–16. Gyne provision masses, which are identifiable by their distinctive saddle shape (Boomsma and Eickwort 1993), were collected from weeks 11–14, suggesting that Brood 2 was protogynous. The first gyne pupae were collected in week 14 and newly eclosed gynes (still in the brood cell) were collected in week 17. During weeks 16–18 (mid to late

Table 2. Evidence for simultaneous production of workers and gynes in three nests of *Halictus ligatus* based on ages of pupae, which indicate that some worker pupae were younger than gyne pupae in the same nest. Caste was assigned based on head width; the sizes of adult workers from the same nests are shown for comparison. Individuals born “out of order” are indicated in boldface.

Week	Date	Nest	Developmental stage when collected (oldest to youngest)	Head width (mm)	Caste
14	25-Jul-06	45	Adult	2.75	W
			Adult	2.45	W
			Black-eyed pupa	2.35	W
			Brown-eyed pupa	3.20	G
			Red-eyed pupa	2.68	W
			Pink-eyed pupa	2.73	W
			White-eyed pupa	3.20	G
			Prepupa	3.01	G
			Prepupa	3.15	G
17	14-Aug-06	271	Adult	2.59	W
			Adult	2.54	W
			Adult	2.85	W
			Adult	2.85	W
			Adult	2.87	W
			$\frac{3}{4}$ -pigmented pupa	3.29	G
			Black-eyed pupa	3.15	G
			Brown-eyed pupa	2.82	W
			White-eyed pupa	2.96	G
17	15-Aug-06	278	Adult	2.73	W
			Adult	3.15	G
			Black-eyed pupa	3.01	G
			Brown-eyed pupa	2.45	W

August), 56/96 (58%) female larvae and pupae that could be measured, were gyne-sized. In most nests, juvenile females identified as workers were clearly older than those identified as gynes. However, in three nests, the ages of pupae of distinctly different sizes suggested that workers and gynes had been produced at about the same time (Table 2). For instance, in nest 45 (Table 2), a gyne-sized, brown-eyed pupa was evidently older than the next two female offspring, which were distinctly smaller and worker-sized.

The fact that many nests excavated from week 11 onward contained juveniles representing both broods, as well as the extended period of brood development, complicates evaluation of the number of offspring in Brood 2. That most juveniles found in nests during weeks 16–18 were probably members of Brood 2 is supported by the observation that provision masses were not found after week 16 and the earliest (oldest) individuals of Brood 2 began to eclose in week 17. During weeks 16–18, the average number of brood per nest was 9.9 ± 6.0 (range = 1–23, $n = 19$ nests), and 36 of 117 sexable pupae (31.0%) were male. The number of brood per nest began to decline around week 19 (Figure 1), suggesting that reproductive brood were completing their development and dispersing.

Ten of 14 nests excavated in weeks 8 and 9 contained a queen, while 4 of 5 nests contained a queen in week 11. This suggests that up to 80% of queens survived to worker emergence. Only five of 16 nests excavated in weeks 16–18 contained queens, suggesting that only 31% survived to the end of Brood 2 egg-laying. Both these survival rates are likely over-estimates as we do not include nests that failed early in the season and therefore were not marked. During weeks 11 and 12, the first two weeks of the worker foraging period, excavated nests contained an average of 1.8 ± 1.6 adult workers (range 0–6, $n = 10$ nests). Later, during weeks 16–18, the average was 1.5 ± 1.6 (range 0–6, $n = 19$ nests), which was not significantly different (Kruskal-Wallis $X^2 = 0.615$, $df = 1$, n.s.).

Rates of pleometrosis were inferred from demographic data. Only one nest contained two foundress queens; however, this nest was excavated on 5 July after *Astata* wasps had begun excavating burrows among the bee nests (Rehan et al. 2013), and the resulting soil disruption may have caused two separate nests to be joined. A nest excavated on 14 July (week 12) contained only a single adult with HW = 2.44 mm and TW = 6. The oldest brood in this nest were pigmented pupae and the youngest were larvae, so this female could have been a small subordinate that outlived the dominant foundress in a pleometrotic association. A nest excavated on 8 August (week 16) contained a highly worn, worker-sized female (HW = 2.59 mm and TW = 10) and six workers, all of them larger and none of them with TW > 4. Similarly, a nest excavated on 15 August (week 17), contained a highly worn, worker-sized female (HW = 2.54 mm and TW = 10) and a larger worker with little wear (TW = 2). In a fifth nest, excavated on 22 June (week 9), the only indication of pleometrosis was that the worker pupae were all slightly larger than the nest queen. In all these nests, the highly worn, small females were either tiny haplometrotic queens or subordinate foundresses that had outlived larger dominants. Since preliminary analyses based on microsatellite DNA genotyping suggested high rates of pleometrosis (A. Giroux and M.H. Richards, unpub. data), we estimate the rate of pleometrosis was as high as 5/65 excavated nests (7.1%).

Two nests suggest the possibility that queens occasionally start new nests after losing the first one. A nest excavated in week 10 (29 June) contained only a large, worn female (HW = 2.92 mm, WW = 0, MW = 5) but no brood cells, so the nest may have been newly founded. A nest excavated in week 11 contained a worn adult female of intermediate size (HW = 2.82, WW = 2, MW = 5), as well as an unfinished provision mass, a provision mass with an egg, and two larvae, but no workers or empty brood cells.

Flight activity and phenology based on pan trapping

The numbers of bees caught in weekly pan trap collections were used to infer the timing of important events in the colony cycle of *Halictus ligatus* in 2006 (Figure 2). Spring foundresses (queens whose workers have not yet emerged) were trapped from 3 May (week 2) to mid-June (week 8), suggesting a queen foraging period about 6 weeks in duration. However, the peak foraging period was in mid-May (weeks 4 and 5).

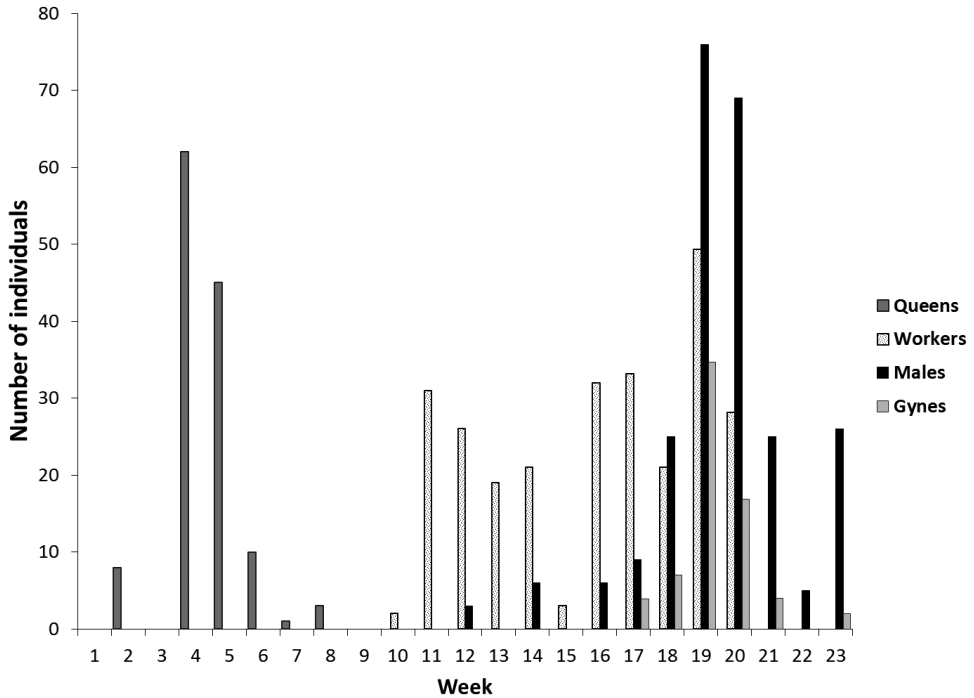


Figure 2. Seasonal phenology of *Halictus ligatus* inferred from weekly pan trap samples at six sites at Brock University and the adjacent Glenridge Quarry Naturalization Site in 2006. Week 1 was the last week of April, and week 11 was the first week of July.

Unworn workers were trapped from late June to late July, suggesting that in most nests, Brood 1 completed development as adults during weeks 10–14, about 7 weeks after provisioning by queens. Worn workers were collected until the last week of trapping in early September (week 20), except for an apparent hiatus during week 15, suggesting that population-wide, the worker foraging period was at least 10 weeks in duration. Males trapped during weeks 10–14 were likely produced in Brood 1; based on the proportional representation of males and workers in pan traps during weeks 10–14, about 8.3% of Brood 1 were males and 91.7% were workers. The first gynes were trapped in mid-August (week 17), signalling the beginning of Brood 2 emergence 6–7 weeks after initiation of worker provisioning. The largest numbers of gynes and males were caught in late August and early September (weeks 19–20), suggesting that most of Brood 2 eclosed as adults around this time. Males and gynes were caught as late as the end of September (week 23), when pan-trapping ceased. During weeks 16–18, the period for which the Brood 2 sex ratio was estimated from nest data based on pupae, the majority (40 / 44) of females trapped were workers; since most gynes were still pupae, this period was too early to estimate the Brood 2 sex ratio from pan trap collections. During weeks 19–21, gynes represented 42.8% (24/56) of dissected females, indicating that they were emerging in large numbers. Applying this propor-

tion to the total 404 females caught in weeks 19–21, we collected about 173 gynes and 324 males, suggesting that the Brood 2 sex ratio was about 65%.

Characteristics of adult females

PCA outcomes for females collected in nests and pans are presented in Figure 3. In both data sets, the PCA suggests three clusters of females congruent with our caste assignments. In nest bees, there was better separation of queens, workers and gynes, likely because more information was available for nest bees (mandibular wear scores and demographics of whole colonies) than for pan trap bees.

More detailed comparisons of body size, wing wear, and ovarian development of queens, workers, and gynes collected from nests and pan traps are presented in Figure 4. Queens collected by the two methods were similar in size and ovarian development (Kruskal-Wallis tests: HW $X^2 = 0.12$, n.s.; WW: $X^2 = 2.87$, n.s.; TOD: $X^2 = 0.21$, n.s.). Workers from nests were significantly smaller ($X^2 = 11.03$, $p < 0.001$), less worn ($X^2 = 26.15$, $p < 0.0001$), and had more ovarian development ($X^2 = 25.10$, $p < 0.0001$) than those from pan traps. Gynes from nests were significantly larger ($X^2 = 5.87$, $p < 0.05$) than those from pan traps. One nest contained a queen that was about 2.8% smaller than her worker pupae (the possible pleometrotic queen described above). In the remaining nests, queens were from 1.5 to 29.7% larger than their own workers (mean based on one randomly chosen worker per nest = 16.5%). Using the aggregate average sizes of all nest queens and workers, the size difference was 11.8% (Kruskal-Wallis $X^2 = 9.56$, $p < 0.01$), while the difference was 8.4% in the pan-trapped bees (Kruskal-Wallis $X^2 = 27.90$, $p < 0.0001$). Despite these differences in average body size, there was still considerable overlap in the size distributions of queens and workers; in particular, many queens that were active before worker emergence, were actually worker-sized.

Figures 3 and 4 demonstrate that many workers had developing ovaries. We further classified workers as non-reproductives (OD = 0 or 0.1) or as reproductives (OD ≥ 0.25). Among 80 nest workers dissected, 47 (59%) were reproductive and 33 (41%) were non-reproductive. Among 97 pan trap workers dissected, 27 (28%) were reproductive and 70 (72%) were non-reproductive. These proportions were significantly different ($X^2 = 17.223$, $df = 1$, $p < 0.0001$ with Yates correction), indicating a higher proportion of reproductive workers among nest bees. Among workers in which the spermatheca could be clearly seen upon dissection (nest and pan trapped specimens pooled), a significantly greater proportion of reproductive workers were mated (26/51 = 51%) compared to non-reproductive workers (9/38 = 24%; Yates $X^2 = 5.704$, $df = 1$, $p = 0.017$).

We then compared both head width and wing wear for nest and pan trapped bees combined, as well as mandibular wear for nest bees (Figure 5). Overall, non-reproductive and reproductive workers did not differ in size (Kruskal-Wallis $X^2 = 0.56$, ns.). However, there was a body size effect on ovarian development among reproductive

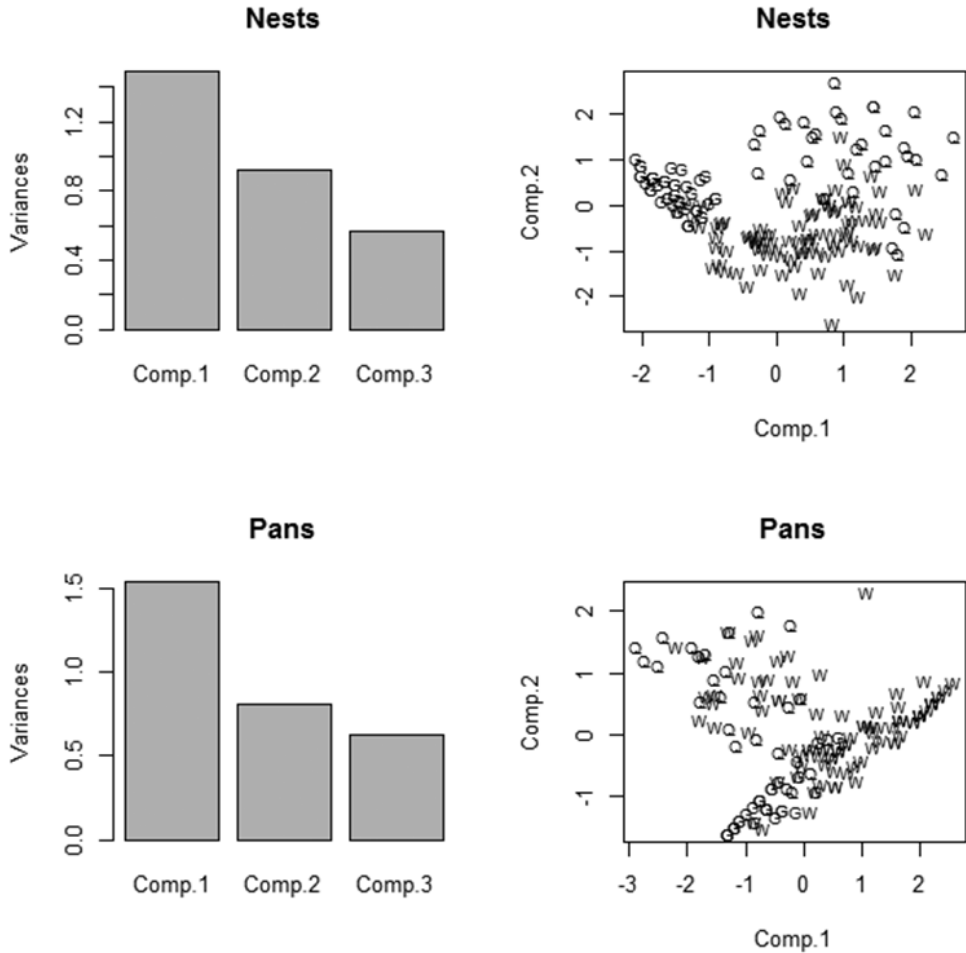


Figure 3. Principal components analyses (PCA) indicating caste differentiation and supporting caste classifications of female *Halictus ligatus* collected in nests and pan traps. The histograms are scree plots indicating the significance of the first three principal components for each analysis. The scatterplots indicate the relationship between the first two principal components for each analysis. PCA for nest bees was based on head width, total wear, and ovarian development, while that for pan trap bees was based on head width, wing wear, and ovarian development. Note the greater separation among queens (Q), workers (W), and gynes (G) in the nest sample.

workers, with larger females having significantly higher ovarian scores than smaller females ($F = 18.32$, $df = 1,72$, $p < 0.0001$).

Non-reproductive workers exhibited significantly higher wing wear scores, indicating that they flew more than reproductive workers (Kruskal-Wallis $X^2 = 42.11$, $p < 0.0001$). However, they did not have significantly different mandibular wear scores (Kruskal-Wallis: $X^2 = 0.77$, n.s.), suggesting that both non-reproductive and reproductive workers excavated brood cells and nest tunnels.

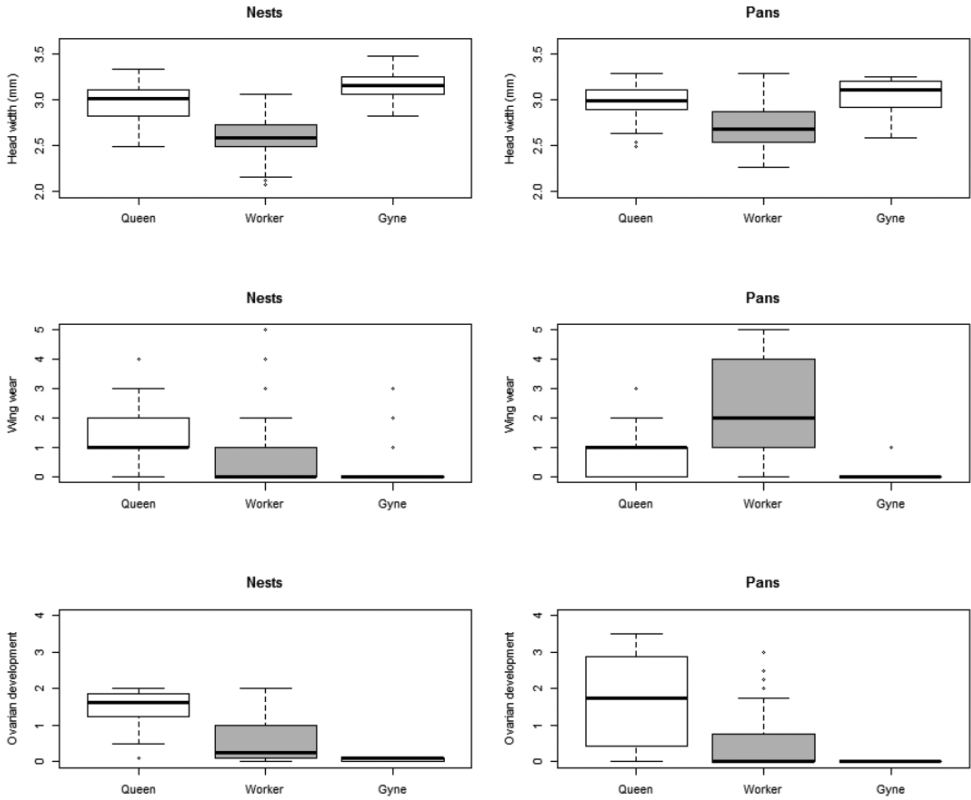


Figure 4. Variation in head width, wing wear, and ovarian development among *H. ligatus* queens, workers, and gynes collected in nest excavations and pan traps in 2006. Box plots represent means and quartiles, with unfilled circles indicating outliers. Gynes were classified by their lack of wear or ovarian development (as well as by time of emergence), and are included here to emphasize the phenotypic differences among the three groups of females.

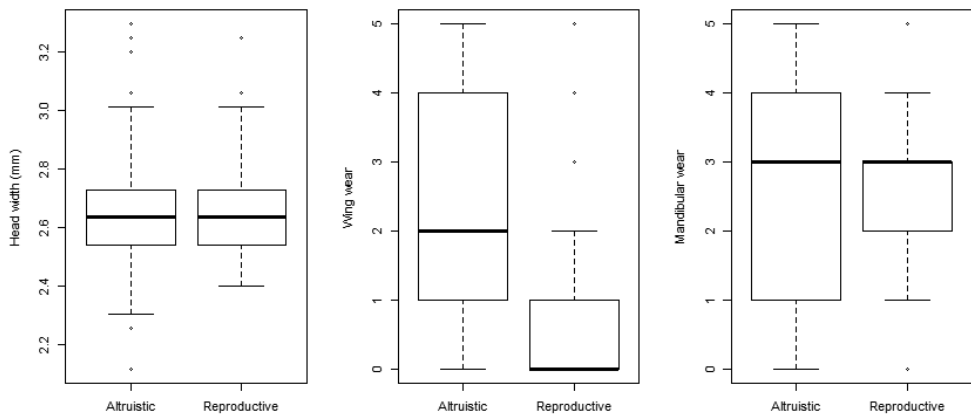


Figure 5. Size and wear distributions compared between altruistic, non-reproductive (OD = 0 or 0.1) and reproductive workers (OD ≥ 0.25).

Discussion

Phenological and social variation in *Halictus ligatus*

The colony cycle of *Halictus ligatus* was typical for this species and most temperate, eusocial halictids (Litte 1977, Packer 1986a, Richards and Packer 1995). Foundress queens established nests in spring, provisioned Brood 1, then remained inside their nests for the remainder of the breeding season. Across the population, Brood 1 comprised 90% workers and 10% males. After emerging as adults, workers provisioned a brood of gynes and more males, which mated prior to the onset of gyne hibernation in late summer and early autumn.

Table 3 summarizes phenological and colony traits of the St. Catharines and Victoria populations. The most obvious differences between the populations are phenological. As predicted, foundress queens in the St. Catharines population initiated reproductive activities, including nest construction and brood provisioning, considerably earlier than in Victoria. In St. Catharines, the first foraging queens were pan trapped on 1 May in 2006, and this is not unusual at our study sites- in 2012, we collected a *Halictus ligatus* queen on 22 March, during an unusually early warm spell when many bees left their hibernacula (R. Kutby and M. Richards, unpub. data).

We predicted that earlier initiation of brood production in St. Catharines would result in a longer Brood 1 provisioning period, allowing queens time to produce more workers, eventually resulting in larger colony sizes. Spring nest initiation and brood provisioning by foundress queens, as well as worker emergence, were indeed considerably earlier in St. Catharines than in Victoria. In 2006, St. Catharines temperatures were warmer than average from January to July, which likely encouraged foundresses to initiate even earlier than usual. However, the nest excavation data indicated that contrary to prediction, Brood 1 was somewhat smaller in St. Catharines (Table 3). Since St. Catharines queens started and finished provisioning the worker brood earlier than in Victoria, but ended up with fewer brood, the length of spring may not be the proximate factor influencing brood size. More likely, spring resource (pollen and nectar) availability, mediated by local weather conditions, dictates annual brood size. Another possibility is that the average number of workers is more or less set within a population, such that queens produce enough workers to ensure eventual production of a reproductive brood. This has been suggested for another obligately eusocial sweat bee, *Lasioglossum malachurum*, in which average size of the first worker brood is remarkably similar in much of Europe (Knerer 1992). Moreover, differences in temperature induce intra-population variation in *L. malachurum* nesting phenology (warmer nests are earlier), but not intra-population variation in colony size (i.e. number of workers in Brood 1; Strohm and Bordon-Hauser 2003). Perhaps variation in the numbers of workers produced has more to do with the local availability of pollen and nectar resources, than with the timing of nest initiation.

A possible consequence of the longer breeding season in St. Catharines was lower survival of queens past the peak of reproductive brood (Brood 2) production in August

Table 3. Colony social parameters compared between *Halictus ligatus* aggregations in St. Catharines, Ontario (current study) and Victoria, Ontario (Packer 1986; Richards et al. 1995; Richards and Packer 1995, 1998). All values are based on specimens collected in nest excavations.

Phenological or social trait	St. Catharines 2006	Victoria 1984, 1990–91
Earliest spring foragers (queens)	1 May	21 May
Earliest summer foragers (workers)	14 June	8 July
Queen survival to peak production of Brood 2	31% (maximum)	45–65%
Proportion of pleometrotic nests	7.1%	10%
Size of Brood 1 (n)	5.1	5.8–9.0
Sex ratio of Brood 1 (% males)	9.5%	5–15%
Number of Brood 1 females (n)	4.7	5.2–8.5
Average no. of adult workers in summer	3.9	≤4.5
Size of Brood 2 (n)	7.9	12–15
Sex ratio of Brood 2 (% males)	31%	45%
Queen–worker size difference ¹	16.5%	11.4–15.8%
Proportion of nest workers mated	39%	42–52%
Proportion of nest workers with developing ovaries ²	59%	60%

¹ Comparing queens to the workers in their own nests.

² Worker OD ≥0.25.

than observed in Victoria (Table 3). Lower survival of queens may explain why the proportions of mated nest workers with developing ovaries were so similar in the two populations. Although the queen-worker size difference was larger in St. Catharines, the relatively early deaths of many queens would have led to increased opportunities for workers to lay eggs that would have produced males and gynes in Brood 2. Thus the prediction that milder environmental conditions in St. Catharines would be associated with increased worker reproduction is supported, but not for the reasons predicted, namely increased colony sizes and a concomitant decline in queen control of worker behaviour. Rather, early queen mortality makes it possible for relatively large numbers of workers to become the primary reproductives in colonies, especially late in summer.

The frequency of pleometrotic nest-founding varies intra-specifically in *Halictus ligatus*. In the current study, the rate inferred from inspection of nest contents in St. Catharines was about 7%, while in Victoria, the average was similar and varied considerably from year to year (Richards and Packer 1998). We found only one nest that contained two live foundresses, but strong circumstantial evidence for pleometrosis in others. In *H. ligatus*, pleometrosis is probably an accidental by-product of overwintering behaviour; gynes hibernate beneath their natal nests, often in the same tunnel, and likely encounter each other in spring (Richards and Packer 1998). When associations form between gynes that are quite different in size, the large one becomes dominant and behaves like a queen, while the smaller ones become subordinates that behave like workers (Packer 1986b). The frequency of pleometrosis may be influenced by environmental factors that influence whether worker-sized females successfully overwinter. In St. Catharines, the relatively early mortality of queens may afford females produced

in Brood 1, especially late-emerging ones, more frequent opportunities to enter hibernation. First brood females with fewer fat stores than second brood females may be less likely to survive hibernation (Richards and Packer 1994), but their overwintering survival might be higher in places like St. Catharines with milder winters.

Production of gynes in the first brood is well documented in the facultatively eusocial sweat bee, *Halictus rubicundus* (Yanega 1989), but in obligately eusocial bees production of large gynes before small workers within a single nest appears to be rare. This phenomenon has not previously been reported for *H. ligatus*, but has been observed in another obligately eusocial species, *Lasioglossum malachurum* (Wyman and Richards 2003). Although it is always possible that undetected errors in nest excavation underlie such observations, there are plausible behavioural explanations. First, egg-layers may occasionally make mistakes about the sex of the offspring for which a provision mass has been provided. Although *H. ligatus* provision masses for gynes and males differ in both size and shape (Boomsma and Eickwort 1993), females may sometimes deposit diploid eggs on male provision masses, and the resulting female would be worker-sized. Such errors might be relatively frequent in colonies where conflict over oviposition between queens and reproductive workers may result in one female substituting her own egg for another, increasing the likelihood that a female egg is mistakenly laid on a male provision mass. Another possibility is that production of late workers is adaptive in areas where foraging seasons are long, particularly where they are longer than the average lifespan of queens. Queen production of late workers could be an insurance strategy that extends both brood care and brood production as long as provisions can be gathered.

Are there two kinds of workers in *Halictus ligatus*?

In eusocial halictids, the potential for worker reproduction is well known, and in almost all known eusocial species, dissections of workers or genetic studies of relatedness suggest the potential for worker reproduction (Packer and Knerer 1985, Packer and Owen 1994, Paxton et al. 2002, Yagi and Hasegawa 2012). Several of these studies indicate that relatively large numbers of workers have developing ovaries but that queens are often highly successful at preventing worker maternity, probably because they remove most worker-laid eggs by eating them (Michener and Brothers 1974). In both the Victoria and St. Catharines populations of *H. ligatus*, almost two-thirds of workers had developing ovaries and some workers had as much ovarian development as queens. Moreover, in the current study, reproductive workers were more frequent among nest bees, which include both foragers and non-foragers. Workers with no ovarian development, some of which must have been altruistic workers provisioning brood for queens, were proportionately more frequent among pan trapped bees, which are foragers. Body size did not predict which workers would have developed ovaries, but among workers with ovarian development, larger individuals had more and larger oocytes, a pattern also observed in *H. tripartitus* (Packer et al. 2007a). Also, workers with

ovarian development were less worn and more likely to be mated than those with no ovarian development. What determines which *H. ligatus* workers will become altruistic or reproductive? Body size is not a good predictor (Richards and Packer 1996). Queen viability is also not a good predictor, as we find reproductive workers in nests with viable queens, as well as in queenless nests. We propose that production of both altruistic and reproductive workers is actually a strategy by queens to maximize colony brood production.

Worker reproduction in eusocial sweat bees and other social insects is often conceptualized in terms of queen-worker reproductive conflict, with worker reproduction resulting from the failure of queens to completely suppress worker oviposition (Schwarz et al. 2007). However, worker egg-laying might actually be favoured by queens when queens cannot produce eggs fast enough to utilize all the brood provisions brought to nests by workers. Most halictid queens probably cannot lay more than two eggs per day; we have dissected many hundreds of individual females of several species (*Halictus ligatus*, *Halictus confusus*, *Halictus sexcinctus*, and *Lasioglossum malachurum*) and have never observed one with more than two fully developed oocytes (M. Richards, pers. obs.). Egg limitation may mean that queens are unable to utilize all available brood provisions, at times when these are being brought to the nest at a rate allowing more than two eggs per day to be provisioned. Under these circumstances, queen fitness is actually enhanced by worker reproduction. On the other hand, it is certainly in a queen's interest that at least some of her daughters behave as altruistic workers, as queens can produce far more reproductive offspring with worker help than without it (Strohm and Bordon-Hauser 2003, Richards et al. 2005). How a *H. ligatus* queen could bias the developmental programs of her worker-brood daughters is unclear, but in the facultatively eusocial sweat bee, *Megalopta genalis*, there seem to be inherent differences among workers that will become altruistic helpers versus those that have the potential to become egg-layers (Kapheim et al. 2012). Possibly, *H. ligatus* queens nutritionally bias the developmental programs of worker-brood females such that some larvae are more likely to develop into “reproductively suppressable”, altruistic workers that will help raise the queen's reproductive brood, while others are more likely to develop their ovaries when circumstances favour worker egg-laying (Richards and Packer 1994, Kapheim et al. 2011). Under such a scenario, higher rates of worker reproduction would be expected when queens cannot or do not suppress worker reproductivity.

Halictus poeyi is the morphologically cryptic sister species of *Halictus ligatus*. It has a much more southerly distribution, including subtropical and tropical regions (Dunn et al. 1998). Mature colonies of *H. poeyi* reach sizes much larger than those of *H. ligatus* colonies in southern Ontario (Michener and Bennett 1977, Packer and Knerer 1986). Workers are also larger, so queen-worker size dimorphism is relatively low. Predictably, rates of worker reproductivity in *H. poeyi* are high (Michener and Bennett 1977). Thus both *H. ligatus* and *H. poeyi* share the behavioural tendency for some workers to become egg-layers when colonies outstrip the ability of queens to monopolize oviposition.

Nest excavations versus pan traps

The second major objective of this paper was to use pan trap collections as a source of information about demographic and social parameters important in assessing intra-specific social variation. Clearly, the best option for sociobiological data collection is to observe nest occupants in order to quantify behavioural interactions among nestmates and the nature of colony social organization. Unfortunately, nesting aggregations of eusocial sweat bees are difficult to find even where the bees are very common, so nest-based study is often impossible. The alternative is to collect bees outside their nests. For instance, Dunn et al. (1998) compared several sociobiologically important traits of *Halictus ligatus* and *H. poeyi* in their zone of sympatry, using bees netted on a biweekly schedule throughout the flight season. This allowed Dunn et al. to infer the phenology of colony development (e.g. when females fly, when males emerge, etc.) and to collect females for dissection and body size measurements. Pan trapping presents the possibility for a further refinement of this approach, because it is less time-consuming and more or less eliminates collector bias. Pan trapping data have been used to supplement nest excavation data in several behavioural studies of *Halictus confusus* (Richards et al. 2010) and *Ceratina* (Rehan and Richards 2010, Vickruck et al. 2011), and were the sole source of data in a study on *H. tripartitus* (Packer et al. 2007a).

To what extent can we rely on pan trap data when nest data are completely unavailable? Some sociobiologically important information, namely data on colony sizes, numbers of workers, colony-specific sex ratios, and nestmate relatedness, simply cannot be obtained from specimens collected with pan traps; observations and collections from colonies are required. In our study, there was very close agreement between pan traps and nest collections in the timing of major events in colony development, such as Brood 1 provisioning by queens, Brood 2 provisioning by workers, and emergence of male and female brood. Our previous studies also showed good phenological agreement based on nest and pan data (Richards et al. 2010, Rehan and Richards 2010, Vickruck et al. 2011). The advantage of the pan traps was that sample sizes were larger and collection effort was much less. However, in the current study, nest excavations suggested that brood provisioning was over by about week 19, whereas we collected foraging workers until week 23. This discrepancy could indicate that many bees continue foraging for themselves even after brood provisioning has ceased, but is more likely a sample size effect: we excavated relatively few nests per week (e.g. 6 in week 19) and from a single aggregation, whereas the pan traps were likely sampling bees from a wider area. Foraging workers from very late nests might be relatively rare, but given the large sampling area covered (trapping sites were up to a km apart) might be quite likely to be collected in pan traps. This suggests that the traps provided more precise information about timing of bee flight activity. Some subtle effects, such as the correlation between body size and ovarian status in reproductive workers detected in this study and in *H. tripartitus* (Packer et al. 2007b) may only be detectable with the very large sample sizes often collected in pan traps.

Other sociobiologically important parameters critical for comparing colony social organisation among populations or species, such as the proportion of mated workers, the proportion of workers with ovarian development, caste size dimorphism, and the Brood 1 sex ratio can also be obtained from pan trapped specimens. A second sociobiologically important parameter that is rarely estimated is Brood 2 sex ratio, which is difficult to measure because nest excavations rarely obtain complete second broods. We obtained good agreement between the Brood 1 sex ratio derived from nests (proportion of males, 9.5%) and that derived from pan traps (8.3%). However, the Brood 2 sex ratios derived from nests (31% males) and pan traps (65% males) were quite disparate, and it is difficult to determine which sex ratio is more accurate. The nest estimate may be biased by broods that were not complete at the time of excavation, either because the youngest brood had not yet been produced or because the oldest had already emerged and dispersed. However, both pan trapping and sweep netting probably underestimate the numbers of gynes in the population, because gynes enter hibernation shortly after emergence, whereas males spend the rest of their lives searching for flowers and females and thus are more likely to be trapped. In the current study, the Brood 2 sex ratio of 65% males based on pan traps was likely an over-estimate, because individual males had more chances to be caught.

For some kinds of demographic information, the pan trap data were arguably superior to the nest excavation data, because the use of a standardized pan trap protocol makes it possible to infer relative abundances of different groups of individuals across years or species. For instance, we can compare the pan trapping patterns and phenology of different species caught in the same pan traps, as this may reveal sociobiologically relevant, interspecific variation for species living in close proximity. To illustrate, we compare *Halictus ligatus* to *Halictus confusus* (Figure 6), another eusocial sweat bee that we studied at the same sites in 2006 (Richards et al. 2010). Comparison of pan trap collections reveals contrasting patterns in the two species. In *H. ligatus*, many queens were caught in spring, relatively few workers were caught in summer, and then many gynes were caught in late summer. In *H. confusus*, relatively few queens were caught in spring, many workers were caught in summer, and few gynes were caught in late summer. Although seasonal differences in floral abundance might influence the relative trappability of queens and workers within species (see below), it is unlikely that the overall differences in the patterns illustrated in Figure 6 are due simply to trappability bias caused by caste and species-specific responses to pan traps. More likely, the differences in pan trap “syndromes” reflect real differences in demography and colony social organization. For *H. ligatus*, the large numbers of queens followed by small numbers of workers suggest high rates of queen mortality and nest failure. For *H. confusus*, the large peak in female abundance at the time of worker provisioning of Brood 2, followed by a low abundance of gynes later in the summer, suggests the possibility of differential diapause by Brood 1 females, which instead of helping to raise Brood 2, begin hibernation in mid-summer, preparatory to founding nests the following spring (Yanega 1988, 1989).

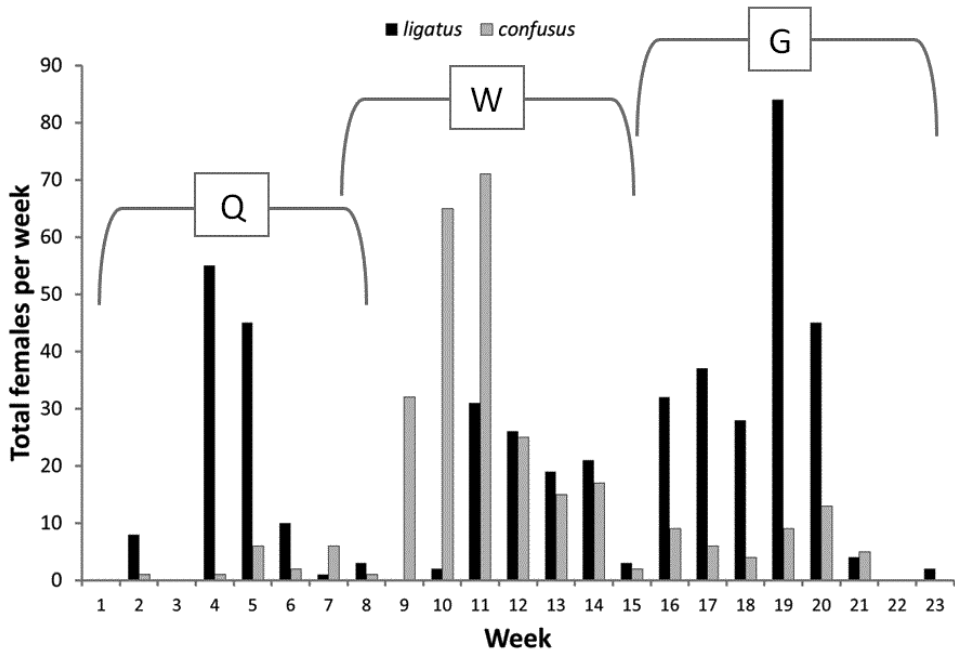


Figure 6. Comparison of pan trapping phenologies of *Halictus ligatus* and *H. confusus* collected at the same sites in 2006. The three main flight periods are indicated, for queens (**Q**), workers (**W**), and the late summer mix of workers and gynes (**G**). Note the differences between species. In *H. ligatus*, more females were caught towards the end of the flight season (the gyne flight period), whereas in *H. confusus*, more females were caught in the middle of the flight season, during the worker foraging period. Week 1 was the last week of April, and week 11 was the first week of July.

A disadvantage of pan traps is that their attractiveness to bees seems to be inversely proportional to blossom availability (Cane et al. 2000, Baum and Wallen 2011). In our study sites, flowers were much less abundant before week 6 than after, so early-foraging queens may have been more attracted to pan traps than late queens or workers. It is also possible that early and late workers were differentially trappable. However, biases caused by differential trappability over time would mainly bias temporal analyses of abundance, which were not a part of this study. In fact, differences in trappability among different sets of individuals may sometimes be informative, as in the contrasting proportions of non-reproductive workers among nest and pan trapped samples, which supported the contention that non-reproductives are altruistic workers that were more likely to forage (and be trapped) than reproductive workers.

Conclusions

Based on previous studies of geographical and annual variation in colony social organisation of *Halictus ligatus*, and evidence that this was due to local climatic variation,

we predicted that in St. Catharines, the longer flight season would translate into earlier nest initiation, larger colony sizes and lower reproductive skew. Although earlier springs in St. Catharines clearly resulted in early nest initiation, the result seems to have been to shift the entire first phase of colony development forward, with no more workers being produced than if nests had been initiated a month later, as they were in Victoria. One reason for this may be that earlier onset of spring in St. Catharines is not associated with an increase in floral resources; ultimately the number of brood that a foundress queen produces may have more to do with pollen and nectar availability than with temperatures. Within sites, warmer temperatures may be associated with increased floral resources, as seems to have been true in Victoria (Richards 2004), but this relationship is not necessarily true for comparisons between sites.

Earlier colony initiation in St. Catharines does not seem to have resulted in early completion of flight and nesting activity, so overall, the bee activity season was considerably longer than in Victoria. The longer colony season helps to explain why measures of queen-worker interactions and reproduction, such as the proportions of reproductive workers, were similar in the two sites and time periods, despite the phenological differences. This does not mean there were no behavioural differences between the sites. In St. Catharines, queens had fewer workers to contend with but died relatively sooner in the colony cycle, and the net result was rates of worker reproductivity as high as in Victoria. Another possible behavioural difference may be indicated by the observation that many St. Catharines queens were worker-sized. It is possible that extended flight seasons coupled with milder winters allow a greater proportion of worker-sized females to overwinter and found nests the following spring. Interestingly, the observed rate of pleometrosis was no higher in St. Catharines than in Victoria so higher overwintering survival for small females did not translate into higher rates of subordinacy in spring multifoundress assemblages. Global climate change will likely mean that nesting seasons for temperate bee populations become even more extended, affording late workers even more opportunities for reproduction.

We found that pan trapping bees throughout the breeding season was a useful complement to collections based on nest excavations. There was good phenological agreement between nest data and trap data, while pan trap data provided much larger sample sizes for assessing caste-related variation in size, wear and ovarian development. Nest-based studies (e.g. Yanega 1989, 1990) provide a “gold standard” for revealing many crucial aspects of social behaviour in insects, because they allow us to infer colony social organisation and the consequences of interactions among nestmates. But when nests are difficult to find in large enough numbers to justify excavating and destroying them, sociobiological studies based on pan trapped specimens generate inferences about colony social organization and female reproductive behaviour that are a viable alternative. The study of *Halictus tripartitus* by Packer et al. (2007), which was based solely on pan-trapped specimens, is an excellent example of how our understanding of bee social behaviour can be advanced even when colonies cannot be found. We hope that the increasing use of pan trapping studies, including those aimed primarily at assessments of bee community abundance and diversity, will generate additional

specimens that can be used for studies of intra- and inter-specific social variation in what is arguably the most critical taxon for understanding the origins and extinctions of sociality in insects.

Acknowledgements

We greatly appreciate the assistance of Mark Frampton for diligent pan trapping and specimen curation in 2006. We thank Andrew Giroux for lab work and Jess Vickruck, David Awde and the reviewers for helpful comments on the manuscript. This project was funded by an NSERC Discovery grant to MHR, an NSERC USRA to SR, and Brock University.

References

- Baum KA, Wallen KE (2011) Potential bias in pan trapping as a function of floral abundance. *Journal of the Kansas Entomological Society* 84: 155–159. doi: 10.2317/JKES100629.1
- Boomsma JJ, Eickwort GC (1993) Colony structure, provisioning and sex allocations in the sweat bee *Halictus ligatus* (Hymenoptera: Halictidae). *Biological Journal of the Linnean Society* 48: 355–377. doi: 10.1111/j.1095-8312.1993.tb02097.x
- Cane JH, Minckley RL, Kervin LJ (2000) Sampling bees (Hymenoptera : Apiformes) for pollinator community studies: Pitfalls of pan-trapping. *Journal of the Kansas Entomological Society* 73: 225–231.
- Dunn M, Mitchell PL, Packer L (1998) Phenology and social biology of two sibling species of *Halictus* in an area of sympatry. *Canadian Journal of Zoology* 76: 2207–2213. doi: 10.1139/cjz-76-12-2207
- Field J, Paxton R, Soro A, Craze P, Bridge C (2012) Body size, demography and foraging in a socially plastic sweat bee: a common garden experiment. *Behavioral Ecology and Sociobiology* 66: 743–756. doi: 10.1007/s00265-012-1322-7
- Hirata M, Cronin AL, Kidokoro M, Azuma N (2005) Spatio-temporal variation of colony structure and eusociality level of the Japanese sweat bee *Lasioglossum (Evyllaesus) duplex* (Hymenoptera: Halictidae). *Ecological Research* 20: 75–79. doi: 10.1007/s11284-004-0005-x
- Hirata M, Higashi S (2008) Degree-day accumulation controlling allopatric and sympatric variations in the sociality of sweat bees, *Lasioglossum (Evyllaesus) baleicum* (Hymenoptera: Halictidae). *Behavioral Ecology and Sociobiology* 62: 1239–1247. doi: 10.1007/s00265-008-0552-1
- Kapheim KM, Bernal SP, Smith AR, Nonacs P, Wcislo WT (2011) Support for maternal manipulation of developmental nutrition in a facultatively eusocial bee, *Megalopta genalis* (Halictidae). *Behavioral Ecology and Sociobiology* 65: 1179–1190. doi: 10.1007/s00265-010-1131-9
- Kapheim KM, Smith AR, Ihle KE, Amdam GV, Nonacs P, Wcislo W (2012) Physiological variation as a mechanism for developmental caste-biasing in a facultatively eusocial

- sweat bee. *Proceedings of the Royal Society B-Biological Sciences* 279: 1437–1446. doi: 10.1098/rspb.2011.1652
- Knerer G (1992) The biology and social behaviour of *Evylaeus malachurus* (K.) (Hymenoptera; Halictidae) in different climatic regions of Europe. *Zoologische Jahrbuch Systematik* 119: 261–290.
- Kocher SD, Paxton RJ (2014) Comparative methods offer powerful insights into social evolution in bees. *Apidologie* 45: 289–305. doi: 10.1007/s13592-014-0268-3
- Kocher SD, Pellissier L, Veller C, Purcell J, Nowak MA, Chapuisat M, Pierce NE (2014) Transitions in social complexity along elevational gradients reveal a combined impact of season length and development time on social evolution. *Proceeding of the Royal Society London Series B* 281: 20140627. doi: 10.1098/rspb.2014.0627
- Litte M (1977) Aspects of the social biology of the bee *Halictus ligatus* in New York State. *Insectes Sociaux* 24: 9–36. doi: 10.1007/BF02223277
- Michener CD (2007) *The Bees of the World*, 2nd ed. Johns Hopkins University Press, Baltimore.
- Michener CD, Bennett FD (1977) Geographical variation in nesting biology and social organization of *Halictus ligatus*. *University of Kansas Science Bulletin* 51: 233–260. doi: 10.1073/pnas.71.3.671
- Michener CD, Brothers DJ (1974) Were workers of eusocial Hymenoptera initially altruistic or oppressed? *Proceedings of the National Academy of Sciences, USA* 71: 671–674.
- Miyanaga R, Maeta Y, Sakagami SF (1999) Geographical variation of sociality and size-linked color patterns in *Lasioglossum (Evylaeus) apristum* (Vachal) in Japan (Hymenoptera, Halictidae). *Insectes Sociaux* 46: 224–232. doi: 10.1007/s000400050138
- Packer L (1986a) The social organisation of *Halictus ligatus* (Hymenoptera; Halictidae) in southern Ontario. *Canadian Journal of Zoology* 64: 2317–2324. doi: 10.1139/z86-345
- Packer L (1986b) Multiple-foundress associations in a temperate population of *Halictus ligatus* (Hymenoptera; Halictidae). *Canadian Journal of Zoology-Revue Canadienne De Zoologie* 64: 2325–2332. doi: 10.1139/z86-346
- Packer L (1990) Solitary and eusocial nests in a population of *Augochlorella striata* (Provancher) (Hymenoptera: Halictidae) at the northern edge of its range. *Behavioral Ecology and Sociobiology* 27: 339–344. doi: 10.1007/BF00164004
- Packer L, Gravel AD, Lebuhn G (2007a) Phenology and Social Organization of *Halictus (Seladonia) tripartitus* (Hymenoptera: Halictidae). *Journal of Hymenoptera Research* 16: 281–292. <http://biostor.org/reference/389>
- Packer L, Gravel AID, LeBuhn G (2007b) Phenology and social organization of *Halictus (Seladonia) tripartitus* (Hymenoptera: Halictidae). *Journal of Hymenoptera Research* 16: 281–292.
- Packer L, Jessome V, Lockerbie C, Sampson B (1989) The phenology and social biology of four sweat bees in a marginal environment: Cape Breton Island. *Canadian Journal of Zoology* 67: 2871–2877. doi: 10.1139/z89-407
- Packer L, Knerer G (1985) Social evolution and its correlates in bees of the subgenus *Evylaeus* (Hymenoptera: Halictidae). *Behavioral Ecology and Sociobiology* 17: 143–149.
- Packer L, Knerer G (1986) The biology of a subtropical population of *Halictus ligatus* Say (Hymenoptera, Halictidae). 1. Phenology and social organization. *Behavioral Ecology and Sociobiology* 18: 363–375. doi: 10.1007/BF00299667

- Packer L, Knerer G (1987) The biology of a sub-tropical population of *Halictus ligatus* Say (Hymenoptera, Halictidae) 3. The transition between annual and continuously brooded colony cycles. *Journal of the Kansas Entomological Society* 60: 510–516.
- Packer L, Owen RE (1994) Relatedness and sex ratio in a primitively eusocial halictine bee. *Behavioral Ecology and Sociobiology* 34: 1–10. doi: 10.1007/BF00175452
- Paxton RJ, Ayasse M, Field J, Soro A (2002) Complex sociogenetic organization and reproductive skew in a primitively eusocial sweat bee, *Lasioglossum malachurum*, as revealed by microsatellites. *Molecular Ecology* 11: 2405–2416. doi: 10.1046/j.1365-294X.2002.01620.x
- Rehan SM, Richards MH (2010) Nesting biology and subsociality in *Ceratina calcarata* (Hymenoptera: Apidae). *Canadian Entomologist* 142: 65–74. doi: 10.4039/n09-056
- Rehan SM, Rotella A, Onuferko TM, Richards MH (2013) Colony disturbance and solitary nest initiation by workers in the obligately eusocial sweat bee, *Halictus ligatus*. *Insectes Sociaux* 60: 389–392. doi: 10.1007/s00040-013-0304-8
- Richards MH (2001) Nesting biology and social organization of *Halictus sexcinctus* (Fabricius) in southern Greece. *Canadian Journal of Zoology* 79: 2210–2220. doi: 10.1139/cjz-79-12-2210
- Richards MH (2004) Annual and social variation in foraging effort of the obligately eusocial sweat bee, *Halictus ligatus* (Hymenoptera: Halictidae). *Journal of the Kansas Entomological Society* 77: 484–502. doi: 10.2317/E37.1
- Richards MH, French D, Paxton RJ (2005) It's good to be queen: classically eusocial colony structure and low worker fitness in an obligately social sweat bee. *Molecular Ecology* 14: 4123–4133. doi: 10.1111/j.1365-294X.2005.02724.x
- Richards MH, Packer L (1994) Trophic aspects of caste determination in *Halictus ligatus*, a primitively eusocial sweat bee. *Behavioral Ecology and Sociobiology* 34: 385–391. doi: 10.1007/BF00167329
- Richards MH, Packer L (1995) Annual variation in survival and reproduction of the primitively eusocial sweat bee *Halictus ligatus* (Hymenoptera: Halictidae). *Canadian Journal of Zoology* 73: 933–941. doi: 10.1139/z95-109
- Richards MH, Packer L (1996) The socioecology of body size variation in the primitively eusocial sweat bee, *Halictus ligatus* (Hymenoptera: Halictidae). *Oikos* 77: 68–76. doi: 10.2307/3545586
- Richards MH, Packer L (1998) Demography and relatedness in multiple-foundress nests of the social sweat bee, *Halictus ligatus*. *Insectes Sociaux* 45: 97–109. doi: 10.1007/s000400050072
- Richards MH, Packer L, Seger J (1995) Unexpected patterns of parentage and relatedness in a primitively eusocial bee. *Nature* 373: 239–241. doi: 10.1038/373239a0
- Richards MH, Rutgers-Kelly A, Gibbs J, Vickruck JL, Rehan SM, Sheffield CS (2011) Bee diversity in naturalizing patches of Carolinian grasslands in southern Ontario, Canada. *The Canadian Entomologist* 143: 279–299. doi: 10.4039/n11-010
- Richards MH, Vickruck JL, Rehan SM (2010) Colony social organisation of *Halictus confusus* in southern Ontario, with comments on sociality in the subgenus *H. (Seladonia)*. *Journal of Hymenoptera Research* 19: 144–158.

- Schwarz MP, Richards MH, Danforth BN (2007) Changing paradigms in insect social evolution: Insights from halictine and allodapine bees. *Annual Review of Entomology* 52: 127–150. doi: 10.1146/annurev.ento.51.110104.150950
- Strohm E, Bordon-Hauser A (2003) Advantages and disadvantages of large colony size in a halictid bee: The queen's perspective. *Behavioral Ecology* 14: 546–553. doi: 10.1093/beheco/arg039
- Vickruck JLL, Rehan SMM, Sheffield CSS, Richards MHH (2011) Nesting biology and DNA barcode analysis of *Ceratina dupla* and *C. mikmaqi*, and comparisons with *C. calcarata* (Hymenoptera: Apidae: Xylocopinae). *Canadian Entomologist* 143: 254–262. doi: 10.4039/n11-006
- Weissel N, Mitesser O, Liebig L, Poethke HJJ, Strohm E, Liebig J (2006) The influence of soil temperature on the nesting cycle of the halictid bee *Lasioglossum malachurum*. *Insectes Sociaux* 53: 390–398. doi: 10.1007/s00040-005-0884-7
- Wyman LM, Richards MH (2003) Colony social organization of *Lasioglossum malachurum* Kirby (Hymenoptera, Halictidae) in southern Greece. *Insectes Sociaux* 50: 201–211. doi: 10.1007/s00040-003-0647-7
- Yagi N, Hasegawa E (2012) A halictid bee with sympatric solitary and eusocial nests offers evidence for Hamilton's rule. *Nature Communications* 3: 939. doi: 10.1038/ncomms1939
- Yanega D (1988) Social plasticity and early-diapausing females in a primitively social bee. *Proceedings of the National Academy of Sciences, USA* 85: 4374–4377. doi: 10.1073/pnas.85.12.4374
- Yanega D (1989) Caste determination and differential diapause within the first brood of *Halictus rubicundus* in New York (Hymenoptera: Halictidae). *Behavioral Ecology and Sociobiology* 24: 97–107. doi: 10.1007/BF00299641
- Yanega D (1990) Philopatry and nest founding in a primitively social bee, *Halictus rubicundus*. *Behavioral Ecology and Sociobiology* 27: 37–42. doi: 10.1007/BF00183311
- Yanega D (1993) Environmental influences on male production and social structure in *Halictus rubicundus* (Hymenoptera: Halictidae). *Insectes Sociaux* 40: 169–180. doi: 10.1007/BF01240705