Colony Social Organisation of *Halictus confusus* in Southern Ontario, with Comments on Sociality in the Subgenus *H. (Seladonia)*

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Abstract.—*Halictus (Seladonia) confusus* Smith is one of the most common bees in North America. Classified as eusocial, its colony social organization is known only from qualitative descriptions of a population in Indiana. We studied the phenology and social behaviour of this bee in the Niagara Region of southern Ontario, using nest excavations, dissections and measurements of adult females, and pan trap samples of foraging bees to elucidate key elements of colony social organisation. The colony cycle in Niagara is typical of temperate-zone halictines, with overwintered foundresses producing a first brood of worker-sized females and a few males, followed by production of Brood 2, consisting of gynes and more males. Many Brood 1 females become reproductive: about one-quarter of Brood 1 females dissected exhibited levels of ovarian development rivalling queens. In contrast, only about one-quarter of Brood 1 females become classically altruistic, sterile workers. High rates of worker reproductivity may result from early queen mortality and supersedure or from the inability of viable queens to control worker behaviour – the average queen-worker size difference was only 5.6%, and queens were not always larger than the workers in their own nests. Comparisons with the Indiana population suggest a geographic component to variation in colony social organisation. Comparisons with other members of the subgenus for which detailed information is available, suggest that in *Seladonia*, as in other eusocial halictines, queen control of worker behaviour depends on the ability of queens to dominate small numbers of small-bodied workers.

Key words.—Halictidae, eusociality, pan traps, sweat bee

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In halictine bees, evolutionary transitions from solitary to eusocial behaviour involve two components, a demographic change from univoltine to multivoltine colony phenology, and a behavioural change from maternal care by a lone foundress, to associations between mothers and daughters that raise brood cooperatively (Schwarz et al. 2007). Likewise, evolutionary transitions from eusociality to solitary behaviour, involve the reverse changes in demography and behaviour. Therefore, to understand evolutionary transitions between solitary and social behaviour, it may be particularly fruitful to examine species that exhibit intraspecific variability in either or both of these traits. If the adaptive significance of intraspecific demographic and social variability can be understood, this in turn may help to illuminate patterns observed at higher taxonomic levels, such as differences among subgenera or genera. For instance, socially polymorphic sweat bees such as *Lasioglossum calceatum* and *Halictus rubicundus*, exhibit solitary, univoltine colony cycles in regions with short breeding seasons, and eusocial, bivoltine (actually, double-brooded) colony cycles in regions with long breeding seasons (Sakagami and Munakata 1972; Eickwort et al. 1996). There are also obligately eusocial species, such as *H. ligatus* and *L. malachurum* that exhibit considerable demographic variation, with colonies growing to larger sizes in areas with longer breeding seasons (Michener and Bennett 1977; Knerer 1992). These intraspecific patterns suggest that one

cause of the phylogenetic lability of social behaviour observed in several halictine genera, might be geographic or temporal variability in the harshness of local environmental conditions. Indeed, this prediction is borne out by recent evidence that halictine sociality may have first evolved during a period of global climate warming (Brady et al. 2006), when it would have been possible for univoltine halictine lineages to adopt bivoltine or multivoltine nesting phenologies.

One of the most common eusocial halictines in North America is *Halictus (Seladonia) confusus* Smith, but detailed information on its nesting and social biology are distinctly lacking. Dolphin (1966) studied the nesting biology and social behaviour of this bee in Indiana, USA, from 1963–1965. Although many crucial details were never published, Dolphin suggested that *H. confusus* was demographically and socially polymorphic. His study population contained nests that produced one, two, or three broods, comprising both solitary and eusocial colonies. Eickwort et al. (1996) commented that *H. confusus*, presumed by Knerer and Atwood (1962) to be solitary in boreal Ontario, is social in New York. These tantalizing descriptions suggest that *H. confusus* may exhibit considerable demographic and social variability within and between populations. Understanding the ecological factors associated with such variation is key to investigating hypotheses about the origins and extinctions of sociality in bees.

In this paper, we describe the colony phenology and social organisation of *H. confusus* in southern Ontario. We studied a mixed nesting aggregation of halictine bees, including a small number of nests of *H. confusus*. We also used pan traps to collect adult females and males throughout the breeding season, in order to supplement the information from colony excavations. We show that while *H. confusus* is predominantly eusocial in southern Ontario, there is evidence that large numbers of Brood 1 females become reproductives, rather than sterile workers, suggesting that the population contains a mix of solitary and social strategies, as well as univoltine and bivoltine phenologies. We also compare *H. confusus* to other well studied members of the subgenus *Seladonia*, in order to assess the level of social variation in the subgenus as a whole.

METHODS

Study sites.—All study sites were on or within walking distance of the Brock University Campus in St. Catharines, Ontario (W 79 14′ 57″ N 43 07′11″). We excavated nests from a small nesting aggregation on the north shore of Lake Moodie that contained nests of *Halictus confusus* and *H. ligatus*, and hibernacula of *H. rubicundus*. The nests were on a gentle, south-facing slope. Nests were excavated using a standard technique in which baby powder was blown in at the nest entrances to coat the sides of the burrows, which were then carefully exposed using a kitchen knife. Nests were excavated in the morning before the entrances were open in or in the late afternoon after they were closed. All adult occupants were preserved in 95% ethanol, while brood were placed in wax-lined petri dishes indented with small chambers and brought back to the lab to be raised to adulthood. When these died or emerged as adults, they also were preserved in ethanol.

In addition to nest excavations, we used pan traps to capture flying bees at six sites on the Brock University campus and at the contiguous Glenridge Quarry Naturalization Site; pan trap sites were within 2 km of the nesting aggregation. At each site, 30 pan traps were laid out in an X or other space-filling pattern, alternating yellow, white, and blue pans at 10m intervals, according to standard protocols (Lebuhn et al. 2003). Pans were set out weekly from 1 May to 30 September 2006 at six locations. Bees caught in pan traps were used to determine the timing of important pheno-

logical events, including nest founding, the first and second brood-provisioning phases, and brood emergence from the nests. Since trapping effort was constant over the course of the summer, the numbers of bees caught per week should provide a consistent estimate of bee density and flight activity. Weeks were numbered starting with 1 May 2006 as the beginning of week 1.

Dissections.—Adult bees were measured, assessed for wear, and females were dissected. Body size was measured in terms of head width (HW, the distance across the widest part of the head, including the compound eyes) and length of the forewing costal vein (CVL, from the stigma to the end of the marginal cell); the head widths of pupae were also measured. Queen-worker size difference was calculated as (queen HW – worker HW) / (queen HW) * 100. Mandibular wear (MW) was assessed on a scale of 0–5, with 0 representing completely unworn mandibles with sharp teeth and 5 representing mandibles so worn as to be completely blunted. Wing wear (WW) was also assessed on a scale of 0–5, 0 representing wings with no damage to the margin and 5 representing wings with the margin completely obliterated by nicks and tears. A total wear (TW) score was obtained by summing mandibular and wing wear scores for each female. As wings can be nicked during handling and because unworn mandibles sometimes appear somewhat blunt, bees were categorized as worn if TW≥2.

Females were dissected to determine mating status (whether the spermatheca was opaque, indicating that it was filled with sperm, or transparent, indicating that it was empty) and ovarian development. For the latter, all developing oocytes were assigned fractional scores of ¼, ½, ¾, or 1, indicating their size relative to a fully developed oocyte. These scores were then summed to make a total ovarian development (OD) score. Females with undeveloped or only thickened ovaries but no visible oocytes, were assigned OD scores of 0 or 0.1, respectively.

Caste assignments for females were based primarily on seasonal activity patterns and secondarily on body size, based on the assumption that in Niagara, *Halictus confusus* would exhibit the bivoltine phenology typical of primitively eusocial halictines in the temperate zone (Schwarz et al. 2007). The term 'foundress' is used for overwintered females that excavate burrows and forage in spring. The term 'worker' is used for Brood 1 females. After workers emerge, a foundress may be referred to as a 'queen'. A gyne is a Brood 2 female that will overwinter and found a nest the following spring. A 'replacement queen' is a Brood 1 worker that takes over the role of queen from a dead or moribund foundress.

Caste designations were assigned to females caught in pan traps and nests based on the following criteria. When newly emerged from hibernation, foundresses are unworn, becoming progressively more worn as they excavate nests and provision brood cells. Thus in midsummer, we can use wear scores to distinguish worn, late-foraging foundresses from unworn, early workers. In late summer, we used wear scores to distinguish worn workers from unworn gynes. Ovarian development was not used to assign caste designations, thus avoiding teleological complications in comparisons of the reproductive status of queens and workers. All adult females caught in nest excavations, as well as the majority of pan-trapped females (all foundresses, all gynes, and 100 workers) were measured and dissected.

Interspecific comparisons.—To examine interspecific variation in the subgenus *Seladonia*, the best approach would be to map these data onto a phylogeny and then investigate evolutionary correlations among the various traits (Felsenstein 1988). However, in the absence of a phylogeny, several authors have used

principal components analysis (PCA) to quantify social variation among halictine bees and to construct hypotheses about how social traits co-evolve (Michener 1974; Breed 1976; Packer and Knerer 1985). Hypotheses constructed without a phylogenetic framework, can then be tested when an appropriate phylogeny becomes available. For comparisons among *Seladonia* populations, we used five variables commonly assessed in studies of halictine sociality: the proportion of males in Brood 1, the number of workers per nest (or the number of females produced in Brood 1), the proportion of workers with developing ovaries, the proportion of mated workers, and the queen-worker size difference based on head width. Values for each variable were either taken directly from the literature, recalculated from figures in the literature, or recalculated as midpoints of ranges. The initial PCA was based on all five variables, retaining factors with eigenvalues $\geq$ 1.0. However, since Kaiser's Measure of Sampling Adequacy (MSA) with all five variables had a value of only 0.55, the variable with the lowest communality measure (proportion of workers mated) was dropped from the PCA. With the remaining four variables, MSA=0.77, which exceeds the 0.6 criterion. We present both factor loading scores (the degree to which each variable influences the inferred factors) and communality estimates (a reliability score which estimates the proportion of variance in each variable that is jointly explained by all three factors). Note that the interspecific comparisons based on the PCA are presented in the last section of the Discussion, rather than in the Results.

RESULTS

Colony cycle.—In southern Ontario, *H. confusus* exhibits a foraging and nesting cycle typical of temperate zone, eusocial halictines (Fig. 1). The beginning of the foundress foraging period was marked by the capture on 1 May 2006 of an over-wintered foundress. Since only two foundresses were captured in the first 3 weeks, they likely emerged from overwintering diapause in late April and early May, but mostly did not venture out of their nests until mid-May when the weather became more suitable. Foundresses continued to be caught in pan traps for about eight weeks, with the last foraging foundress caught on 28 June (week 9). Most foundress foraging and provisioning of Brood 1 probably occurred from weeks 4–8.

There was a sharp increase in the number of females caught beginning in week 8, many of them small and unworn. Large numbers of brood continued to be caught until week 11 after which pan trap catches declined. Weeks 8–11 thus represented the peak emergence period of Brood 1 and the peak worker foraging period. In the population as a whole, there was no quiescent period between the foundress and worker foraging periods, as the first Brood 1 females (which were small and unworn) were caught on 21 June (week 8) when clearly identifiable foundresses (large, worn females) were still flying. The first Brood 1 males were caught in week 9, so emergence of Brood 1 was slightly protogynous. Based on pan trap samples from weeks 8–11, the proportion of males in Brood 1 was about 1.9%.

The emergence of Brood 2 was marked by a small increase in trap numbers of both males and females beginning around week 15 (7–13 August), with the majority of Brood 2 emerging between weeks 18–20 (Fig. 1). Week 15 was marked not only by the appearance of large, unworn females from Brood 2, but also by the last capture of small, unworn females deemed to be from Brood 1, suggesting that the last of Brood 1 had emerged as adults by week 15. The worker foraging period was mostly finished by week 17, although one small, worn forager was captured in week 19. Based on pan trap samples from weeks 12–20, the proportion of males produced in Brood 2 was about 22%.
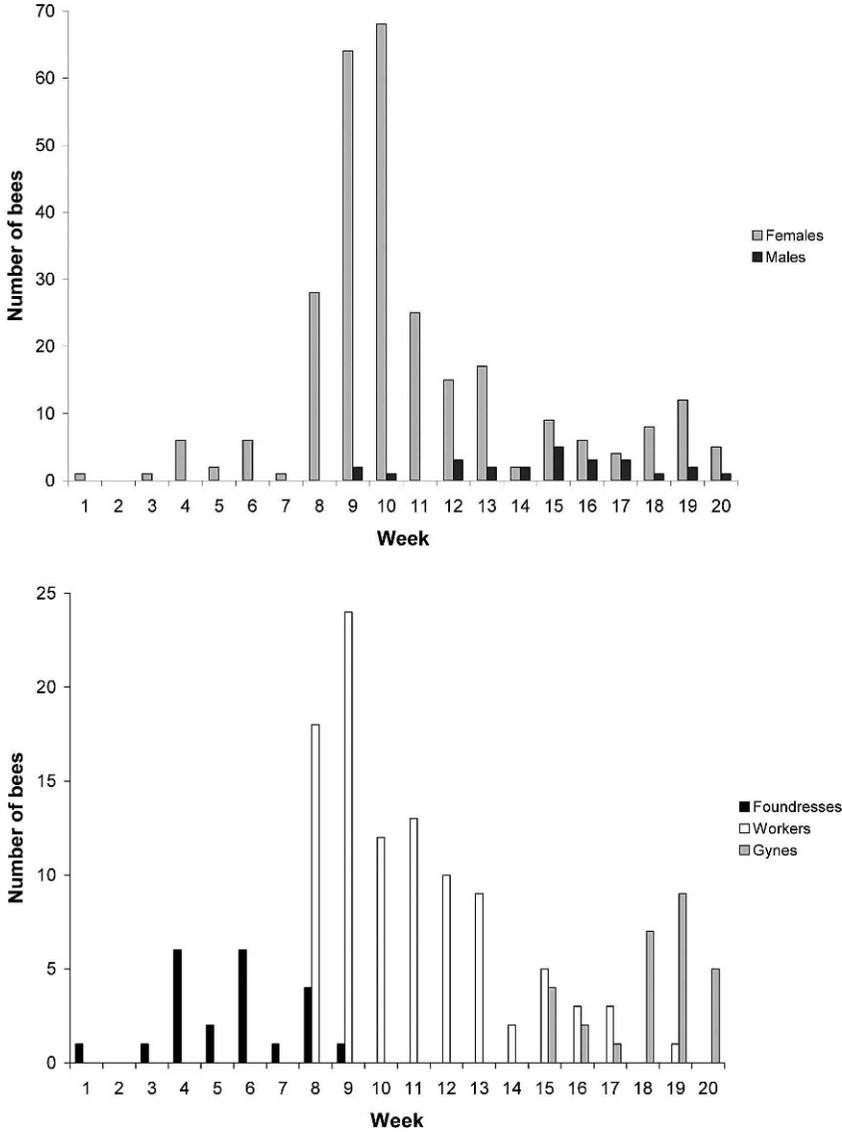
Fig. 1.   Flight phenology of *H. confusus* based on 2006 pan trap samples. Top: Phenology of all adult bees collected in pan traps. The sharp rise in numbers of females caught in mid-summer (beginning with week 8) corresponds with the first appearance of males in week 9, suggesting that this mid-summer peak marks the emergence of Brood 1. Bottom: Timing of female caste emergence based on size and wear patterns. Foundresses emerge in early May and continue to forage until mid-summer, slightly overlapping with females of Brood 1 (workers). Gynes first begin to appear in week 15. Sample size differences between top and bottom graphs are because only 100 of the workers caught in pan traps was dissected.

Nest contents.—Fourteen nests were excavated in total, four prior to worker foraging and ten later in the summer. A single nest excavated during week 5 contained a foundress and three brood cells, comprising one provision mass with an egg, one medium larva, and one early stage pupa (damaged during excavation). In week 8, three nests were excavated. The first nest contained a queen and 3 worker pupae; the second nest contained a queen, one worker with worn mandibles, three worker pupae, and an unfinished provision mass; and the third nest contained a
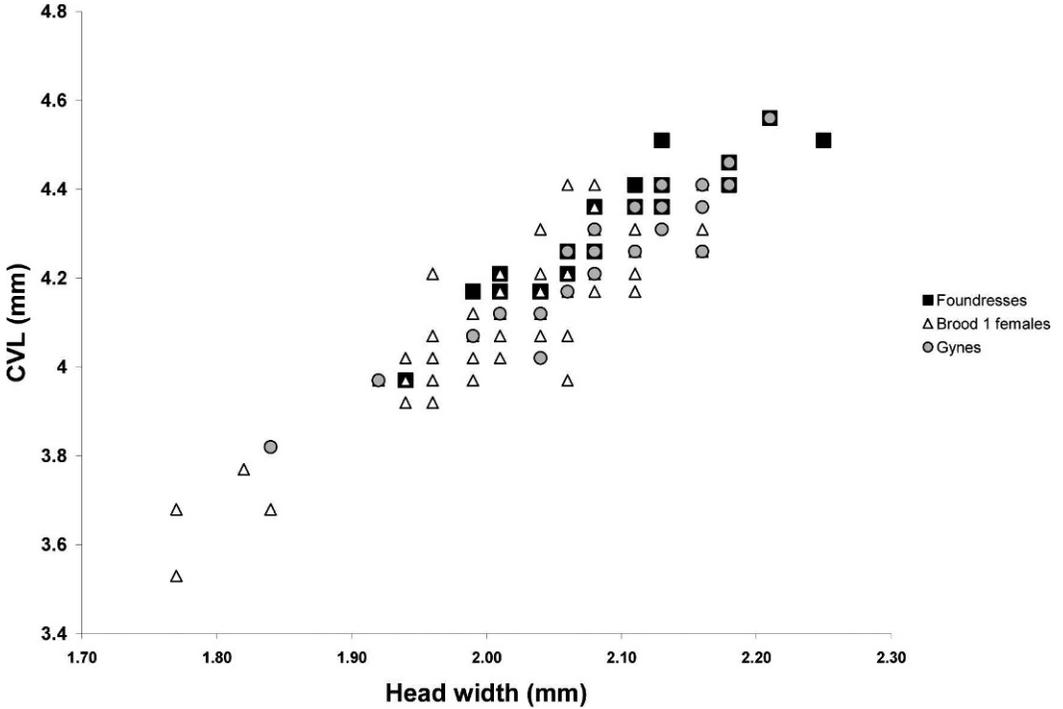
Fig. 2. Body size distributions of pan trapped *H. confusus* foundresses, Brood 1 females, and gynes based on head width and wing length (measured as costal vein length, CVL).

queen, two workers with worn mandibles, five female pupae, four male pupae, one larva that had completely consumed its provisions, one provision mass with an egg, and one unfinished provision mass. The average brood size of these three nests was 6.3, and 22% of the sexable brood were males. The latter figure is considerably higher than the estimate of 2% males based on pan traps and implies that males are under-represented in pan trap samples. The presence of workers in these nests, together with evident age gaps between younger (eggs and larvae) and older brood (pupae), indicates that the younger brood were from Brood 2 and that within individual nests there is a hiatus or quiescent period between Broods 1 and 2.

Ten nests were excavated during weeks 17 and 18. None contained a live foundress (queen). Four nests contained a total of six worn adult workers. Dissections showed that three of these had undeveloped ovaries but were mated (the other three were poorly preserved and could not be scored). The youngest brood were pigmented pupae, so it is likely that older adult brood had already dispersed from their natal nests. The average number of brood per nest had fallen to 3.4, and only 3% of sexable brood were males (as compared with 22% in pan trap samples). Of the 23 gynes in these nests, 21 had mated and 20 had noticeable fat deposits in their abdomens. There was no evidence that gynes had begun digging hibernacula below their natal nests.

Female body size.—Foundresses and gynes were very similar in size, and both were larger than Brood 1 females; there was no indication of a body shape difference between the gyne and worker castes (ANOVA, $F=8.56$, df$=2,147$, $p=0.0003$; Fig. 2 and Table 1). Queen and worker size measurements were available for two nests (both excavated in week 8). In Nest 166, the queen was larger than all four of her workers (adults and pupae), and the

Table 1.   Caste characteristics of females caught in pan traps. Smaller sample sizes for ovarian and mating success reflect technical difficulties with dissections. Females were considered as worn if MW≥2 or WW ≥2, and ready to lay if they contained at least one, ¾-developed oocyte. Statistical comparisons of foundresses versus Brood 1 females were based on ANOVA (F statistics), Kruskal-Wallis tests (H statistics), and chi square tests.

Trait	Foundresses (n=22)	Brood 1 females (n=100)	Gynes (n=28)	Statistical comparison (foundresses vs. Brood 1 females)
HW (mm ± 1 sd)	2.09 ± 0.07	2.03 ± 0.08	2.09 ± 0.09	$F=11.22$, df=1, p=0.0011
CVL (mm ± 1 sd)	4.33 ± 0.16	4.15 ± 0.16	4.25 ± 0.17	$F=21.91$, df=1, p=0.0001
Proportion with worn mandibles	13/22 (60%)	32/100 (32%)	0/28 (0%)	$\chi^2=5.68$, df=1, p<0.0171
Proportion with worn wings	1/22 (4%)	8/100 (8%)	0/28 (0%)	$\chi^2=0.32$, df=1, n.s.
OD score (mean and range)	1.82 (0.5–2.75)	0.58 (0–2.25)	0.03 (0–0.1)	$F=21.64$, df=1, p=0.0001
Proportion ready to lay	16/22 (73%)	23/100 (23%)	0/28 (0%)	$\chi^2=20.50$, df=1, p<0.0001
Proportion mated	18/18 (100%)	39/80 (49%)	17/28 (61%)	$\chi^2=15.86$, df=1, p<0.0001

queen-worker size difference was 7.2% based on head width and 9.9% based on wing length. In Nest 168 the situation was very different. The small, worn queen was the same size as one worker, but smaller than four others (two worker pupae were not measured), resulting in a queen-worker size difference of negative 4.0% based on head width and negative 1.2% based on wing length. Since the above calculations were based on females from only two nests, we also calculated the average size differences for pan trapped foundresses and workers: these were 2.9% based on head width and 4.2% based on wing length.

Wear and reproductive status.—Based on females caught in pan traps, foundresses sustained higher levels of mandibular wear than Brood 1 females (Table 1). Few females had worn wings, but one notable exception was the queen of Nest 168 (excavated in week 8), with a total wear score of 10; this female was so much more worn than other bees examined that she might have been nesting for the second time, having overwintered twice.

Potential for reproduction by foundresses and Brood 1 females is compared in Table 1. All foundresses dissected (18 from pan traps and 4 from nest excavations) had sperm in their spermathecae, whereas only about half of the Brood 1 females examined

had sperm in their spermathecae. Foundresses also had significantly higher OD scores than Brood 1 females, and were more likely to have at least one oocyte ready or almost ready to lay.

Four types of 'workers' could be distinguished based on wear and ovarian development, each category comprising about 25% of the total among Brood 1 females caught in pan traps (Table 2). The first group comprised unworn (TW ≤ 1) females with undeveloped ovaries (OD ≤ 0.1); these were evidently newly eclosed workers. The second group were worn (TW≥2) but exhibited no ovarian development, suggesting that they were engaged in nest maintenance or foraging activities, but were not laying eggs; these bees were categorized as sterile altruists. The third group were queen-like, at least in terms of their readiness to lay eggs: most of these (18.3% of all Brood 1 females) contained at least one fully developed oocyte ready to lay, while the remainder contained at least one ¾-developed oocyte. The remaining group of Brood 1 females can be categorized as potentially reproductive workers, exhibiting a distinct degree of wear and some ovarian development, but not sufficient to be ready to lay eggs. These workers likely provision both queen-laid and sometimes their own eggs, and could also be referred to as 'partial altruists'.

Table 2.   Comparison of ovarian development and wear in Brood 1 females collected in pan traps. Unworn females had total wear ($TW = MW + WW$) scores of 0 or 1, whereas worn females had $TW \geq 2$. Percentages represent proportions of the total (n=93). Four categories of workers can be distinguished: 'newly eclosed' females that have not yet accumulated either wear or ovarian development; 'altruists', worn, working females with no ovarian development, 'queen-like' females with very high rates of ovarian development, and the remainder, with intermediate levels of wear and ovarian development, that can be referred to simply as 'workers'.

Size of largest oocyte	Unworn	Worn	Total
None	24 (25.8%)	21 (22.6%)	45 (48.4%)
	Newly eclosed	Altruists	
¼	6 (6.5%)	10 (10.8%)	16 (17.2%)
	Potentially reproductive workers	Potentially reproductive workers	
½	2 (2.2%)	7 (7.5%)	9 (9.7%)
	Potentially reproductive workers	Potentially reproductive workers	
¾	2 (2.2%)	4 (4.3%)	6 (6.5%)
	Queen-like	Queen-like	
Full	6 (6.5%)	11 (11.8%)	17 (18.3%)
	Queen-like	Queen-like	
Total	**40 (43.0%)**	**53 (57.0%)**	**93 (100%)**

Roughly half of Brood 1 females caught in pan traps were mated (this value underestimates the rate of worker mating as it includes newly eclosed individuals that might not yet have met males). Those with developed ovaries (OD scores ≥ 0.25) were more likely to have mated than females with no ovarian development (Likelihood ratio chi-square, G=14.46, df=1, p<0.0001; Table 3), and this was significant even when newly eclosed females are excluded from consideration (Likelihood ratio chi-square, G=4.978, df=1, p=<0.0257). Degree of ovarian development was not correlated with body size (head width: $\rho=-0.01$, n=95, n.s.; costal vein length:

Table 3.   Association between mating status and ovarian development in 80 *H. confusus* Brood 1 females collected from pan traps. The minimum OD score for a female with at least one visibly developed oocyte is 0.25. Statistical analysis is given in the text.

Ovarian score	Mating status		Total
	Unmated	Mated	
OD ≤ 0.1	29 (36%)	11 (14%)	40 (50%)
OD ≥ 0.25	12 (15%)	28 (35%)	40 (50%)
Total	41 (51%)	39 (49%)	80 (100%)

$\rho=0.02$, n=95, n.s.), even when females assumed to be newly eclosed were excluded.

Comparisons of queens to the workers in their own nests suggest that queens dominated but did not completely monopolize oviposition. Nest 166 was excavated on 22 June 2006 (week 8), and contained a queen, one adult worker, three worker pupae, and an unfinished provision mass. The queen (TW=6) had an OD score of 2.75, including three ¾-developed oocytes but no fully developed oocytes. The adult worker was slightly worn (TW=3) and had probably collected the pollen provisions. She was mated and her OD score was 0.75, comprising a single ¾-developed oocyte. Evidently, the queen or the worker could have had a mature oocyte to lay by the time the provision mass was completed. In nest 168, excavated on the same day, the queen, which was the most worn bee we found (TW=10) had an OD score of 1.75, comprising one fully developed and one ¾ developed oocyte. Of the two adult workers in the nest, the one smaller than the queen was worn (TW=3), was unmated and had only slightly thickened ovaries,

while the one larger than the queen was a bit less worn (TW=2), was mated and had an OD score of 1.0, including a ¾-developed oocyte. Since both workers were worn, they were probably both foragers, but only the former would be categorized as a 'sterile altruist'.

Dissections of pan-trapped bees revealed that 4/22 foundresses, 14/100 workers, and 0/28 gynes had been parasitized by conopid larvae, many of them large enough to fill their host's abdominal cavity. Perhaps noteworthy is the fact that two foundresses caught in pan traps in late May contained conopid parasites so large as to prevent any ovarian development. Two gynes from nests excavated in late August were also parasitized by conopids.

DISCUSSION

Phenology and colony social organization in southern Ontario.—In southern Ontario, *Halictus confusus* exhibits a colony cycle which in broad terms, is typical of eusocial, temperate zone halictines (Schwarz et al. 2007). In spring, large females excavate new burrows, then provision a first brood that is composed mainly of workers and a few males. Foundresses cease provisioning shortly after the summer solstice, and then are replaced as small Brood 1 females emerge from their nests, and begin to provision Brood 2. Most individuals of Brood 2 are provisioned by the end of July, emerging as adults until mid-September. Since many queens evidently survive until mid-summer when workers emerge and begin foraging, this suggests that many surviving colonies become eusocial. As in other halictine bees (Packer 1992; Richards et al. 1995; Paxton et al. 2003; Richards et al. 2005), foundress queens likely produce the majority of Brood 2 gynes and males. Dissections indicate that queens have higher reproductive potential on average, and that workers can have high rates of ovarian development even in queen-right nests. The relatively large numbers of unworn workers with highly developed ovaries caught in midsummer, suggest that when foundress queens die or become moribund, they are replaced by one of their Brood 1 daughters, and colonies become parasocial.

Halictus confusus nests are probably founded haplometrotically (singly), as the few (n=4) nests that we excavated in spring each contained a single foundress. Haplometrotic nest founding is more likely when gynes overwinter away from the summer nesting sites, while pleometrotic co-founding is more likely when gynes overwinter together near the nesting site (Packer 1993; Richards and Packer 1998). Atwood (1933) and Dolphin (1966) suggested that *H. confusus* gynes overwinter away from their natal nests, and nests that we excavated near the end of August contained newly eclosed gynes but no evidence that these were preparing hibernacula. Nevertheless, pleometrosis cannot be ruled out entirely, as we did excavate a nest in which the queen was smaller than most of her workers. In eusocial halictines, queens control worker body size by manipulating the size of larval provision masses (Richards and Packer 1994), making workers that are almost always smaller than themselves (Richards and Packer 1996), so the finding of a very worn queen smaller than some of her own workers suggests that she may have been a small subordinate co-foundress that outlived a larger dominant (Packer 1986; Richards and Packer 1996).

In *H. confusus*, it appears that females produced in Brood 1 may adopt one of three or four different reproductive options. Some Brood 1 females become classical, sterile, altruistic workers that provide provisions for eggs laid by the queen but produce no offspring of their own. Some Brood 1 females become reproductive workers, a category that comprises workers that collect provisions upon which a queen will lay eggs, but whose developing ovaries suggest that they also will lay eggs given the chance. For many,

perhaps most, of these 'reproductive workers', egg-laying opportunities may never present themselves, so observations that many workers have ovarian development do not necessarily translate into high rates of worker oviposition in queen-right nests (Packer 1992; Packer and Owen 1994). Nevertheless, worker maternity in queen-right nests does occur even in strongly eusocial halictines (Richards et al. 2005) so in *H. confusus*, it is likely that at least some reproductive workers, successfully produce brood, even in queen-right nests. The workers with queen-like ovaries would almost certainly be egg-layers, and most likely were replacement queens. We found no new *H. confusus* nests in midsummer after the first emergence of workers, so it is unlikely that workers with queen-like ovaries were Brood 1 females that leave their natal nests to found new nests in summer, either solitarily or communally (Sakagami and Hayashida 1968; Richards et al. 2003).

A curious feature of the flight phenology of *H. confusus* in Niagara was the small number of females captured in late summer, following emergence of Brood 2, compared to the far greater numbers captured in midsummer following emergence of Brood 1. Several explanations present themselves. First, gynes might have been under-represented in pan traps relative to workers, due to changes in flower and forage availability. Pan traps are known to capture relatively fewer foragers when flower availability increases (Roulston et al. 2007). Pan traps may therefore be less attractive to gynes (and males) because they are not active provisioners, and because flower availability may be higher after midsummer than before. It is also possible that the pattern of lower gyne than worker densities is real. If so, then one explanation would be high rates of colony failure prior to worker emergence (Richards and Packer 1995a). Another possibility is that some Brood 1 females leave their natal nests to enter diapause preparatory to becoming foundresses the following spring, a phenomenon known as differential diapause and well documented in *Halictus rubicundus* (Yanega 1988). It would be interesting to compare pan trap phenologies with detailed nesting data for several species with different colony cycles, in order to assess concordance in the patterns inferred using the two types of information.

Geographic variation in colony social organisation.—Demographic differences between Indiana (Dolphin 1966) and Ontario likely stem from differences in the timing of important colony events. In Indiana, foundresses emerge from hibernation as early as March or April and complete foraging by late May or early June, with first brood workers emerging from mid-May to early June, second brood workers emerging in mid to late July, and gynes emerging from mid-July to early September. In Ontario, foundresses emerge from hibernation in late April and forage until about the third week of June, with workers emerging from about June until the end of July, and gynes from mid-August to mid-September. This suggests that Dolphin's population experienced a breeding season about three weeks longer than we observed in Niagara in 2006. In Indiana, many colonies produced two worker broods. This seems unlikely for our Ontario population, as pan traps suggested that the majority of Brood 1 workers emerged between weeks 8 and 13, a six week period that is only slightly shorter than the period encompassing most foundress foraging activity between weeks 3 and 9. However, the intriguing, small peak in captures of females around weeks 14 and 15, might have signaled the emergence of a secondary worker brood. We did capture some small, unworn females at this time, which we categorized as gynes, but which were possibly workers. The ability to interpolate a second worker brood in areas with long enough breeding is well known in *Lasioglossum malachurum*, which produces a

single worker brood at the northerly edge of its range, but two or three worker broods in warmer environs and at the southerly extent of its range (Knerer 1992; Wyman and Richards 2003; Weissel et al. 2006). *Augochlorella striata* apparently has sufficient behavioural flexibility that it can respond to annual weather conditions by producing workers when conditions will create a long breeding season or omitting workers and producing gynes directly when conditions will create a short breeding season (Packer 1990).

Another phenological difference between Ontario and Indiana *H. confusus* was the absence in Ontario of a distinct quiescent period between the foundress and worker foraging periods. Not only was there no quiescent period, but there was at least a week of overlap in the foraging periods of foundresses and workers. This was somewhat unexpected as a quiescent period between the two flight periods is typical of many primitively eusocial halictines, even when multiple worker broods are produced. The overlap suggests an extended rather than a synchronized period of nest establishment in spring, with the result that some foundresses continued to provision brood as long as 2–3 weeks after the earliest foundresses had completed their first broods. However, lack of synchronicity may not be typical of Niagara *H. confusus*, if weather conditions in the spring of 2006 led to early nesting activity by some foundresses. In *H. ligatus* nesting near Victoria, Ontario, unusually warm conditions in spring 1991 led to an extended nest founding period that obliterated the usually predictable hiatus between the foundress and worker foraging periods (Richards and Packer 1995b).

Sociality in the subgenus Seladonia.—Table 4 compares *H. confusus* to other species of the subgenus *Seladonia* for which sufficiently detailed sociobiological data are available. All members of the subgenus are thought to be primarily social (Packer et al 2007), including *H. tumulorum* which

originally was thought to be solitary (Sakagami 1974). For three species, *H. confusus*, *H. hesperus*, and *H. lucidipennis*, social data are available for intraspecific comparisons between populations. Variation between populations suggests considerable geographic variability in rates of worker mating and ovarian development. In *H. confusus*, there seems to be a link between breeding season length and colony size, as additional worker broods were interpolated before the gyne brood in Indiana. A similar pattern seems to occur in *H. lucidipennis*, which produces more workers in southern than in northern India (Batra 1966).

In the absence of a phylogeny for behaviourally known members of the subgenus *Halictus (Seladonia)*, we used principal components analysis (PCA) to quantitatively explore correlations among five sociobiologically important variables (Table 4). Only six populations (*H. confusus* from Ontario, *H. aerarius*, *H. hesperus* from Mexico, *H. lucidipennis* from northern and southern India, *H. tumulorum* from France, and *H. vicinus* from southern India) could be included as basic information was missing for the remainder. We did not attempt a hierarchical analysis to distinguish between inter and intraspecific variation, although it is possible that intra- and interspecific patterns might differ (this could eventually be tested phylogenetically). As noted in the methods, proportion of workers mated explained little of the variation among populations and was dropped from the analysis. Its lack of explanatory power could stem either from difficulties in data collection (Table 4 suggests considerable variability depending on when workers are captured for dissection) or could reflect a genuine lack of relevance to explaining behavioural variation among populations. The remaining four variables together explained 76% of variability among populations, ranked along a single eigenvector (principal component) with eigenvalue = 3.051. The

Table 4. Sociobiological comparison of *H. confusus* and other *Halictus* (*Seladonia*) species. Figures in boldface were used for the principal components analysis presented in Table 5. Where the figure '1+' is given for the number of worker broods, it is likely that worker brood production is extended such that early workers provision subsequent worker brood. Figures in parenthesis were inferred as noted.

Species	Location (reference)	No. worker broods	Males in Brood 1 (%)	No. workers (Brood 1)	Workers with developed ovaries (%)	Workers mated (%)	Queen-worker size difference based on head width (%)
confusus	Ontario (this study)	1	**22** (based on excavated nests)	**4.9**	**44.2** (18.3 with mature oocyte)	**51.3**	Nest bees: **5.6** / Pans: 4.0
confusus	Indiana (Dolphin 1966)	1–2	13.8	2–3 foraging per day	-	-	Brood 1: 4.8 / Brood 2: 1.9
aerarius	Japan (Sakagami and Fukushima 1961)	1	(0)[1]	>10	0	0	**17.1** (recalculated based on Fig. 2)
hesperus	Mexico (Packer 1985)	-	**0**	**6.5**	**9.3**	**5.6**	**23.4**
hesperus	Panama (Brooks and Roubik 1983)	1+	0	97–149 (probably not first brood)	4.7	10.7	19.7
hesperus	Costa Rica (Wille and Michener 1971)	1+	0	37.8	25	0.0	not available (10.6% based on wing length)
lucidipennis	southern India (Batra 1966)	1+	**61.7**	**21**	**51.1**	**12.8**	>**2.8**
lucidipennis	northern India (Batra 1966)	1+	**76.6**	**14.7**	**39.1**	**26.1**	**(2.8)**[2]
lutescens	Costa Rica (Wille and Michener 1971)	1+	>0	187.5	26	31	-
lutescens	Guatemala (Sakagami and Okazawa 1985)	1+	0	>600	18	-	-
tripartitus	California, USA (Packer et al. 2007)	1+	-	-	34–55	13.7–34.2	5.1 (early workers)
tumulorum	France (Plateaux-Quénu and Plateaux 1994)	1	**30**	**3** (1 nest)	**9** (5% with a mature oocyte)	**90**	8.8 (foragers) / 15.0 (1 nest) / (**11.9**)
tumulorum	Japan (Sakagami and Fukushima 1961, Sakagami 1974)	0–1	-	10–20 females per nest	-	-	-
vicinus	southern India (Batra 1966)	1+	**29.4**	**18**	**24.1**	**10.3**	**7.8**

[1] Since no workers were mated, it was inferred that no males were available.
[2] Used same value as for northern Indian population.

Table 5. Results of principal components analysis for 7 populations of *Seladonia* (*H. confusus* from Ontario, *H. aerarius*, *H. hesperus* from Mexico, *H. lucidipennis* from northern and southern India, *H. tumulorum* from France, and *H. vicinus*) compared in Table 4. Only the first principal component (eigenvalue = 3.051) was retained based on the criterion that eigenvalue ≥ 1.000.

Variable	Factor 1 loading score	Communality estimate
Proportion of males in worker brood	0.917	0.846
Number of workers per nest	0.723	0.994
Proportion of workers with developed ovaries	0.897	0.866
Queen-worker size difference	−0.940	0.932

results showed that about 76% of the variation among these populations and species was explained by this principal component (Table 5). The factor loading scores indicate strong negative correlations between queen size relative to workers versus number of workers produced in Brood 1 (which are usually provisioned by the queen herself), the proportion of males in this brood, and rates of worker ovarian development. Taken together, this suggests that in the subgenus *Seladonia*, that sociality, especially the degree of queen-worker reproductive skew, is related to the ability of queens to dominate workers. This is a well-known pattern in eusocial halictines, in which greater skew occurs when queens must contend with fewer and/or smaller workers (Schwarz et al. 2007).

Two species not included in the PCA, *H. hesperus* and *H. lutescens* (Wille and Michener 1971; Brooks and Roubik 1983; Sakagami and Okazawa 1985), can develop extraordinarily large colony sizes numbering in the many hundreds. It seems unlikely that such large colonies result from egg production by only one female (but see Plateaux-Quénu (1962) for an important exception), and more likely these colonies contain multiple egg-layers. This suggests that queens might dominate oviposition early in the colony cycle, but eventually are superseded or lose control of worker reproduction as colonies grow. This switch to 'worker' reproduction would allow colonies to grow even larger and would also blur the distinction between queens and reproductive workers. Interestingly, queen supersedure was suggested by Dolphin (1966) for *H. confusus* in areas with colony cycles longer than average queen lifespan. Another *Seladonia* species not included in our comparative analysis due to a lack of nesting data, is *H. lanei*. In this species, the degree of queen-worker size dimorphism is extraordinary, with queens being as much as eight times larger than their workers (Janjic and Packer 2001). This exceptional degree of size differentiation may allow queens to dominate oviposition and effectively control worker behaviour even with large colony sizes. Clearly, more behavioural data on more species in this interesting subgenus are required for us to better understand how queen control and reproductive skew co-evolve.

CONCLUSION

Across *Halictus* (*Seladonia*), all species thus far studied exhibit sociality (Packer et al. 2007). However, the social behaviour of *H. confusus* suggests a high degree of intraspecific social variability. Moreover, specimens have been collected as far north as Alaska and Finland, where breeding seasons may be too short to allow foundresses to produce workers, so there is a high probability that at least some *Seladonia* populations or species are monomorphically solitary. Social polymorphism, co-occurrence of solitary and social nests within populations, might represent an ecological intermediate between solitary populations in areas with very short breeding seasons and social populations in areas with longer seasons (Packer 1990). Behaviourally, variation in the strength of dominance hierarchies and in queen-worker reproductive skew may also be

taken as evidence of intermediate stages in social evolution. The behavioural variability that we have observed in *H. confusus* and which others have observed in other members of the subgenus, make it likely that further study of *H. (Seladonia)* populations will shed considerable light on the ecological and evolutionary factors that promote social transitions in halictine bees. Species like *H. confusus*, with wide geographic ranges encompassing very short to relatively long breeding seasons, would be ideal candidates for studies of reproductive skew using microsatellite markers to specifically investigate the circumstances under which queens lose control of worker reproduction.

ACKNOWLEDGMENTS

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