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Author(s): J.L. Vickruck, S.M. Rehan, C.S. Sheffield, M.H. Richards
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Nesting biology and DNA barcode analysis of *Ceratina dupla* and *C. mikmaqi*, and comparisons with *C. calcarata* (Hymenoptera: Apidae: Xylocopinae)

J.L. Vickruck, S.M. Rehan, C.S. Sheffield, M.H. Richards

Abstract—Using DNA barcode analysis, nest collections, and pan-trapping we compared molecular differences, nesting behaviour, and phenology of three of the four species of *Ceratina* Latreille present in the Niagara Region of southern Ontario, Canada: *C. dupla* Say, *C. calcarata* Robertson, and *C. mikmaqi* Rehan and Sheffield. *Ceratina dupla* and *C. mikmaqi* were separated by five fixed nucleotide differences and an average sequence divergence of 1.86%. In our population, *C. mikmaqi* and *C. calcarata* were common and *C. dupla* was rare. *Ceratina dupla* nested earlier than *C. mikmaqi* and *C. calcarata,* and sometimes produced a second brood in late July – early August. Each species constructed linear nests in the pith of dead twigs, *C. mikmaqi* and *C. dupla* usually in Fuller's teasel (*Dipsacus fullonum* L.; Dipsacaceae) and *C. calcarata* usually in raspberry (*Rubus* L.; Rosaceae). Genetically distinct, each species occupies a slightly different niche in the Niagara bee assemblage.

Résumé—À l'aide d'une analyse des codes de barre d'ADN, de récoltes de nids et de piégeage avec des plateaux, nous comparons les différences moléculaires, le comportement de nidification et la phénologie de trois des quatre espèces de *Ceratina* Latreille présentes dans la région de Niagara du sud de l'Ontario, Canada, soit *C. dupla* Say, *C. calcarata* Robertson et *C. mikmaqi* Rehan et Sheffield. *Ceratina dupla* et *C. mikmaqi* se distinguent par des différences fixes dans cinq nucléotides et la divergence moyenne de leurs séquences est de 1,86 %. Dans notre peuplement, *C. mikmaqi* et *C. calcarata* sont communs, alors que *C. dupla* est rare. *Ceratina dupla* niche plus tôt que *C. mikmaqi* et *C. calcarata* et produit quelquefois une seconde portée à la fin de juillet − début d'août. Chaque espèce construit un nid linéaire dans la moelle de brindilles mortes, *C. mikmaqi* et *C. dupla* généralement de cardère des foulons (*Dipsacus fullonum* L.; Dipsacaceae) et *C. calcarata* de framboisiers (*Rubus* L.; Rosaceae). Chacune des espèces, génétiquement distincte, occupe une niche légèrement différente au sein du peuplement d'abeilles de la région de Niagara.

[Traduit par la Rédaction]

Introduction

Four species of dwarf carpenter bees, *Ceratina* Latreille (Hymenoptera: Apidae: Xylocopinae), occur in the Niagara Region of southern Ontario. *Ceratina calcarata* Robertson and members of the *C. dupla* Say species group are very common, whereas *C. strenua* Smith is extremely rare (Richards *et al.* 2011). *Ceratina dupla* represent a species complex (Sheffield *et al.* 2009), represented in Niagara by *C. dupla* and *C. mikmaqi* Rehan and Sheffield (Rehan and Sheffield 2011). *Ceratina calcarata* and the *C. dupla* species group are phylogenetically closely related (Rehan *et al.* 2010), are morphologically very similar (Rehan and Richards 2008; Rehan and Sheffield 2011), and have nearly identical geographical ranges (Daly 1973). They

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J.L. Vickruck,[1] S.M. Rehan, M.H. Richards, Department of Biological Sciences, Brock University, 500 Glenridge Avenue, St. Catharines, Ontario, Canada L2S 3A1
C.S. Sheffield, Department of Biology, York University, 4700 Keele Street, Toronto, Ontario, Canada M3J 1P3

[1]Corresponding author (e-mail: jess.vickruck@brocku.ca).
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also are polylectic (Rutgers-Kelly 2003) and nest in the exposed pith of twigs and stems (Comstock 1911; Kislow 1976). This ecological similarity suggests that *C. calcarata* and the *C. dupla* species group may be an interesting group for studies of competition for important resources (*e.g.*, nest sites and pollen) and of subtle niche differences between closely related species.

Little is known of the nesting biology of *C. dupla* and *C. mikmaqi* (but see Comstock 1911; Grothaus 1962), and only *C. calcarata* has been previously studied (Rau 1928; Grothaus 1962; Kislow 1976; Johnson 1988, 1990; Rehan and Richards 2010). *Ceratina calcarata* is univoltine, is mass-provisioning, and commonly nests in exposed pithy stems of sumac (*Rhus* L., Anacardiaceae), raspberry (*Rubus* L., Rosaceae), and cultivated rose (*Rosa* L., Rosaceae). Mothers are devoted, long-lived, and remain with their broods at least until adult emergence. Brood sex ratios are often male-biased, and the innermost brood cell usually produces a small daughter (Kislow 1976; Johnson 1988; Rehan and Richards 2010). As in other species of *Ceratina* (Sakagami and Maeta 1977; Michener 1985), this daughter may aid her mother in feeding younger brood as adults. Though females are usually solitary in nature, there is one account of coerced multi-female nesting in captivity (Chandler 1975).

This study had two objectives. The major objective was to describe the nesting biology of *C. dupla* and *C. mikmaqi* in the Niagara Region and to compare it with that of *C. calcarata,* with the goal of identifying interspecific differences that might represent evidence of niche differentiation. Because *C. mikmaqi* has only recently been identified and described (Rehan and Sheffield 2011), a secondary objective was to use DNA barcode analysis to confirm and delineate the presence of *C. dupla* and *C. mikmaqi* in southern Ontario. To address these objectives, specimens of *C. calcarata*, *C. dupla*, and *C. mikmaqi* were collected with their nests or by using pan traps over the course of the 2008 nesting season. Investigating these three species in the same season allowed us to compare their nesting biology without confounding environmentally based variation that might occur between breeding seasons.

Methods

Study sites

Ceratina nests were collected at three locations in St. Catharines, Ontario: around the Brock University campus (43°119′N, 79°249′W), at the Glenridge Quarry Naturalization Site (GQNS) (43°122′N, 79°237′W), and in an old field on Glendale Avenue (43°147′N, 79°180′W). Pan-trap collections took place on the Brock University campus and at GQNS.

Collection of foraging *Ceratina*

Foraging *Ceratina* bees were collected in pan traps at five sites on the Brock University campus and at GQNS to help determine flight phenology of each species (Packer *et al.* 2007; Richards *et al.* 2010). The pan-trapping protocol was based on the Bee Inventory Plot (LeBuhn *et al.* 2003). At each site, two 50 m long transects were established at 90° to one another, forming an X pattern. Fifteen pans (plastic bowls, SOLO PS6-0099) filled with soapy water were equally spaced along each transect, for a total of 30 pans (10 white, 10 yellow, and 10 blue) per site. Each site was sampled weekly in random order from 14 April to 28 September 2008; a few samples were missed because of inclement weather. Pans were set out at each site by 0900 and brought in after 1500. Insects were strained from the pans using a small sieve, after which they were rinsed with water and stored in 70% ethanol.

Nest collections

At least 15 *Ceratina* nests were collected weekly in early morning, prior to the initiation of foraging, from 14 April to 16 September 2008 at each of the three field sites. Nests were chilled in a refrigerator to cold-anesthetise the occupants, then carefully split open longitudinally to expose nest contents, leaving brood-cell partitions intact. Nest contents were recorded, including the number and sex of adults, the developmental stage and number of immatures, and the presence of parasites.

Based on their contents, nests were classified into one of five categories modified from Daly (1966). ''Hibernacula'' contained adults and varying levels of debris but no brood cells, pollen balls, or larval faeces; occasionally lines

left from old cell partitions were visible. ''Founding nests'' had clean walls (no old cell lines visible) and no pollen balls, eggs, or cell partitions. ''Active brood nests'' contained at least one pollen ball and egg. In ''full-brood nests'', either the brood cells filled the nest, leaving only enough space for the mother to guard the entrance, or the brood cell nearest to the entrance contained a juvenile that had reached the small larva stage or older (Daly 1966), indicating that at least 5 days had elapsed since an egg had been laid. ''Mature brood'' nests contained newly eclosed adults, immatures, and usually a foundress (very worn female). Clutch size was calculated as the total number of provisioned brood cells per nest.

All immatures (eggs, larvae, and pupae) were reared to adulthood or death in the laboratory at room temperature (approximately 21 °C). Each immature was observed daily to assess developmental stage and day of adult emergence. Immatures were classified into 1 of 18 developmental stages following Daly (1966). Developmental rates were calculated by dividing the number of stages completed by the number of days taken to complete those stages. Individuals collected during the egg and prepupal stages were not included in development-time analysis because these stages are considerably longer than the others (Rehan and Richards 2010) and we could not be certain how far through the stage each newly collected individual had progressed when first collected. Some juveniles died in the laboratory as a result of handling; others died before adulthood, through natural causes, including parasitism and apparent developmental failures. Numbers of brood that died as a result of handling and those that reached adulthood were combined to provide the total number of surviving brood per nest.

Descriptions of adult bees

To compare average body sizes among the three species, the head widths of adult females collected in nests were measured across the widest portion of the face, including the compound eyes, at 40 × magnification using a dissecting microscope fitted with an eyepiece micrometer. Wing wear was examined to assess flight activity (Cartar 1992) and scored from 0 to 6, where 0 indicated undamaged wings containing no nicks or tears along the wing margin, 5 indicated obliterated wing margins, and 6 indicated no visible wing margin and damage extending to wing veins and cells.

Specimen identification and DNA sequencing

Specimens were identified morphologically as *C. calcarata*, *C. dupla*, and *C. mikmaqi* using Rehan and Sheffield (2011). To confirm and test identifications we also sequenced 654 base pairs of the mitochondrial gene cytochrome oxidase 1 (COI, the DNA barcode region) from 84 and 9 individuals morphologically identified as *C. mikmaqi* and *C. dupla*, respectively. Sequencing was performed at the Canadian Centre for DNA Barcoding at Guelph University (Guelph, Ontario). DNA was extracted from a single leg of each specimen using automated extraction protocols for 96-well plates (Ivanova *et al.* 2006). One set of primer pairs (LepF1 and LepR1) was used to amplify the DNA barcode region Hebert *et al.* 2004). Polymerase chain reaction (PCR) and sequencing reactions followed standard protocols (Hajibabaei *et al.* 2005). Sequences were edited using BIOEDIT (Hall 1999) and aligned using CLUSTAL (Thompson *et al.* 1994) using default settings with the exception of gap open penalties increased to 50. Analysis of molecular variance (AMOVA), as implemented in Arlequin 3.11 (Excoffier *et al.* 2005), was used to compare levels of genetic variation within and between *C. dupla* and *C. mikmaqi,* based on the hypothesis that these groups are reproductively isolated. COI sequences for all 93 specimens are available at GenBank (accession numbers GU707555−GU707648).

Data analysis

Statistical analyses were carried out using SAS (SAS Institute Inc. 2004). Almost all variables were normally distributed and had equal variances and were analyzed with parametric statistical functions with post hoc Tukey's tests where appropriate. The variable wing wear was not normally distributed and changes over the course of the season were investigated using Spearman's rank correlations. Data are presented as means ± standard deviations.

Table 1. Comparison of important results comparing demographic and life-history traits of *Ceratina mikmaqi, C. dupla*, and *C. calcarata* in the Niagara Region of Ontario in 2008.

Type of trait	Trait	*C. mikmaqi*	*C. dupla*	*C. calcarata*
Resource use	Common nest substrates	80% *Dipsacus*; 20% *Rubus*	89% *Dipsacus*; 11% *Rubus*	46% *Rubus*; 36% *Dipsacus*; 18% *Rhus*
	Nest length (cm)	15.5 ± 5.3	11.9 ± 7.9	15.6 ± 4.6
	Nest diameter (mm)	3.5 ± 0.4	3.6 ± 0.3	3.6 ± 0.4
	Brood-cell length (mm)	7.41 ± 1.4*a*	6.11 ± 0.65*b*	7.01 ± 1.2*a*
Demographic	Female head width (mm)	1.90 ± 0.18*a*	1.74 ± 0.18*b*	1.96 ± 0.16*c*
	Clutch size	11.5 ± 4.07*a*	Brood 1: 9.25 ± 1.53*a* Brood 2: approx. 2.0	7.56 ± 4.08*b*
	Surviving brood per nest	7.5 ± 1.4*a*	3.0 ± 1.9*b*	4.0 ± 3.2*b*
	Brood cell parasitism rate	101/437 (23)	24/40 (60)	109/295 (37)
Temporal	Life cycle	Univoltine	Bivoltine?	Univoltine
	First full brood nest	30 June	10 June	7 July

Note: Values are expressed as the mean ± SD. Numbers in parentheses are percentages. Values followed by a different letter are significantly different.

Results

Ceratina diversity in Niagara

We collected 336 *Ceratina* females and 201 males in pan traps in 2008. Based on females, the *Ceratina* community was 49% *C. mikmaqi* ($n = 164$), 49% *C. calcarata* ($n = 165$), and 2% *C. dupla* ($n = 7$). Based on males, the community was 79% *C. mikmaqi* ($n = 158$), 16% *C. calcarata* ($n = 33$), and 5% *C. dupla* ($n = 10$). No *C. strenua* were collected in 2008.

Patterns of COI sequence divergence confirmed that *C. dupla* and *C. mikmaqi* are genetically distinct. Sequences fell cleanly into two groups, distinguished by five fixed nucleotide differences between the two species. AMOVA indicated significantly greater sequence divergence between than within species (AMOVA: $F_{ST} = 0.8812$, df = 1,92; $P < 0.00001$). The mean pairwise difference between *C. mikmaqi* and *C. dupla* was 1.86%. Within *C. mikmaqi* average divergence was only 0.42% for the six haplotypes among the 84 specimens. All 9 specimens of *C. dupla* shared a single haplotype.

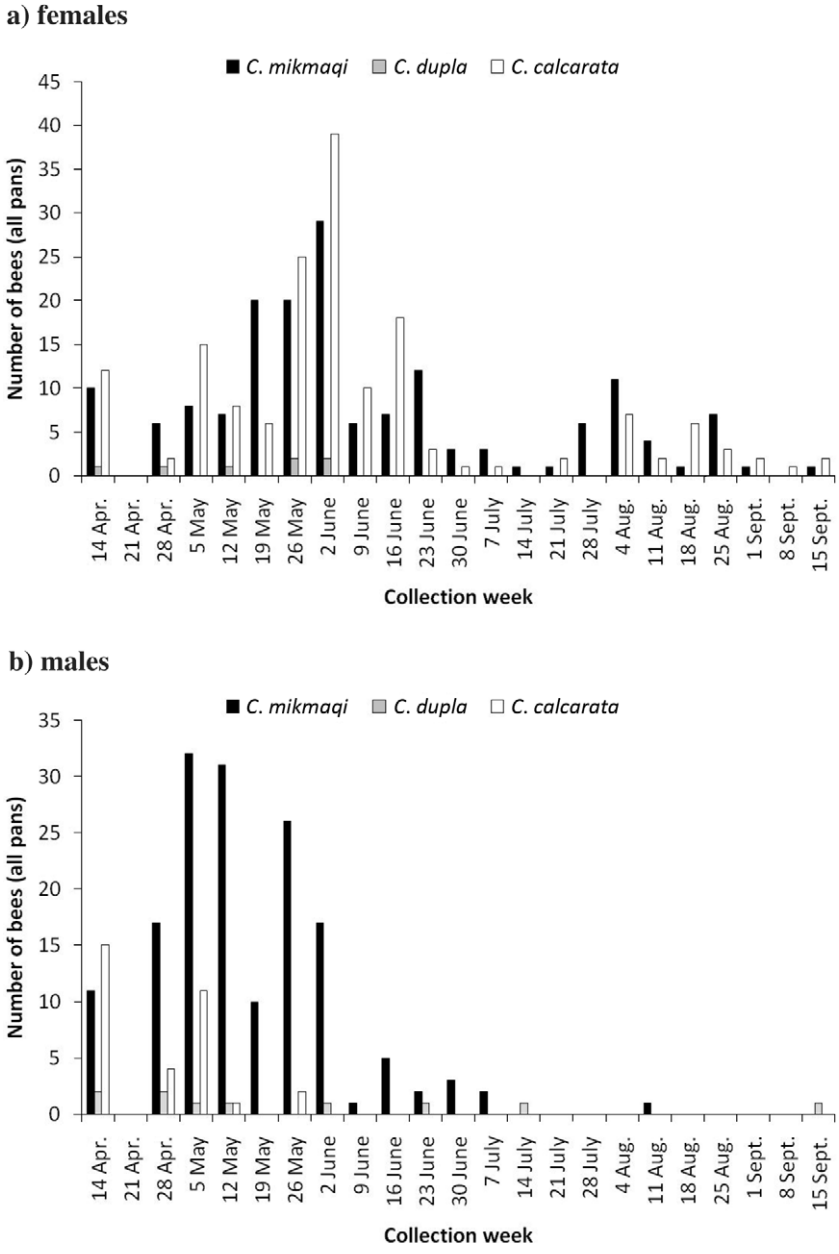
Adult female body size was distinct for each species ($F_{2,337} = 11.05$, $P < 0.0001$; Table 1). *Ceratina calcarata* females were the largest, *C. mikmaqi* females were intermediate, and *C. dupla* females were the smallest.

Phenology based on pan-trap samples

Flight and foraging phenology were inferred from bee abundance in weekly pan-trap samples. *Ceratina mikmaqi* females began to emerge from hibernation during the week of 14 April 2008 (Fig. 1*a*). The number of females collected peaked during the week of 2 June, then declined, and was followed by a period of few catches in mid-July. Females again became more abundant from late July to early August. Female wing wear increased over the course of the season (Spearman's $\rho = 0.46$, $n = 165$, $P < 0.001$). Seven females with wing-wear scores of 0 or 1 were collected in pan traps in July and August and were likely newly emerged adults of that year. In spring, most *C. mikmaqi* males emerged earlier than females, with captures peaking during the week of 5 May and declining steadily thereafter (Fig. 1*b*). Males collected during the week of 14 April had low wing-wear scores (mean 0.91 ± 1.04), but wing wear increased significantly over the course of the season (Spearman's $\rho = 0.52$, $n = 158$, $P < 0.0001$). One unworn male was captured during the week of 11 August and was probably newly emerged.

Only seven *C. dupla* females were caught in pan traps, providing limited data on their flight phenology. All seven were caught by the week of 2 June (Fig. 1*a*). Their wing-wear scores did not change significantly over this period (Spearman's $\rho = -0.49$, $n = 7$, ns). *Ceratina dupla* males were caught at low frequencies until mid-July, after which none were collected until the week of 15 September. As in females, wing wear did not increase significantly over

Fig. 1. Flight phenology of *Ceratina mikmaqi*, *C. dupla*, and *C. calcarata* in the Niagara Region of Ontario, based on weekly pan-trap collections.



the course of the season (Spearman's $\rho = 0.46$, $n = 10$, ns).

Ceratina calcarata females showed pan-trap abundance and wing-wear patterns similar to those of *C. mikmaqi*. Females were first caught the during week of 14 April and had peak abundance the week of 2 June. Unworn (newly emerged) *C. calcarata* females were collected in pan traps in August and September; however, wing wear did increase over the course of the season (Spearman's $\rho = 0.39$, $n = 165$, $P < 0.0001$; Fig. 1*a*). *Ceratina calcarata* males had the highest abundance in pan traps during the week of 14 April. Wing wear was low but

did increase over the season (Spearman's $\rho = 0.37$, $n = 33$, $P = 0.03$). Unlike *C. mikmaqi* and *C. dupla*, newly emerged males were not caught in August or September.

Nesting biology

We collected 401 *Ceratina* nests (including hibernacula), comprising 178 (44%) *C. mikmaqi*, 9 (2%) *C. dupla*, 207 (52%) *C. calcarata*, and 7 (2%) hibernacula that contained at least one *C. calcarata* together with either *C. mikmaqi* or *C. dupla.* Nesting females of *C. mikmaqi* and *C. dupla* were collected most frequently in Fuller's teasel (*Dipsacus fullonum* L., Dipsacaceae; hereinafter teasel) twigs (*C. mikmaqi* (80%), *C. dupla* (89%)), less often in raspberry (20% and 11%, respectively), and never from sumac. Nesting *C. calcarata* were commonly collected from raspberry (46% of nests) or teasel (36%), and were relatively common in sumac (18%).

Nesting phenology was similar in *C. mikmaqi* and *C. calcarata* (Fig. 2). Both species exhibited a univoltine colony cycle, progressing sequentially from spring hibernacula, through new nests, active broods, full broods, mature broods, and lastly fall hibernacula. *Ceratina dupla* exhibited a different colony cycle. The first active brood nest was collected on 2 June and the first full brood nest 8 days later, on 10 June. Two active brood nests were subsequently collected on 25 July and 1 August, each containing a foundress with a wing-wear score of 6. This indicates that *C. dupla* is bivoltine, producing a spring brood earlier than either *C. mikmaqi* or *C. calcarata*, followed by a second brood in late summer.

Nest architecture was similar among the species (Table 1). *Ceratina dupla* nests had significantly shorter brood cells (*i.e.*, the distance between cell partitions) than those of the other species (ANOVA: $F_{2,87} = 5.37$, $P = 0.006$), reflecting the smaller body size of their occupants. Tunnel length and diameter did not differ among the species (ANOVA: $F_{2,70} = 1.16$, ns, and $F_{2,75} = 0.37$, ns, respectively; Table 1). Hibernacula were never reused as brood-rearing nests by any species. Rather, all nests that contained brood were newly constructed by females digging linear tunnels in the exposed pith of twigs.

Ceratina calcarata clutch sizes were significantly smaller than those of *C. mikmaqi* and the first brood of *C. dupla* (ANOVA: $F_{2,77} = 9.21$, $P < 0.001$; Table 1). Two *C. dupla* nests collected late in the season were inferred to contain second broods; however, neither was complete. If *C. dupla* females are iteroparous, then average annual brood size for this species in the Niagara Region would be about 11.25.

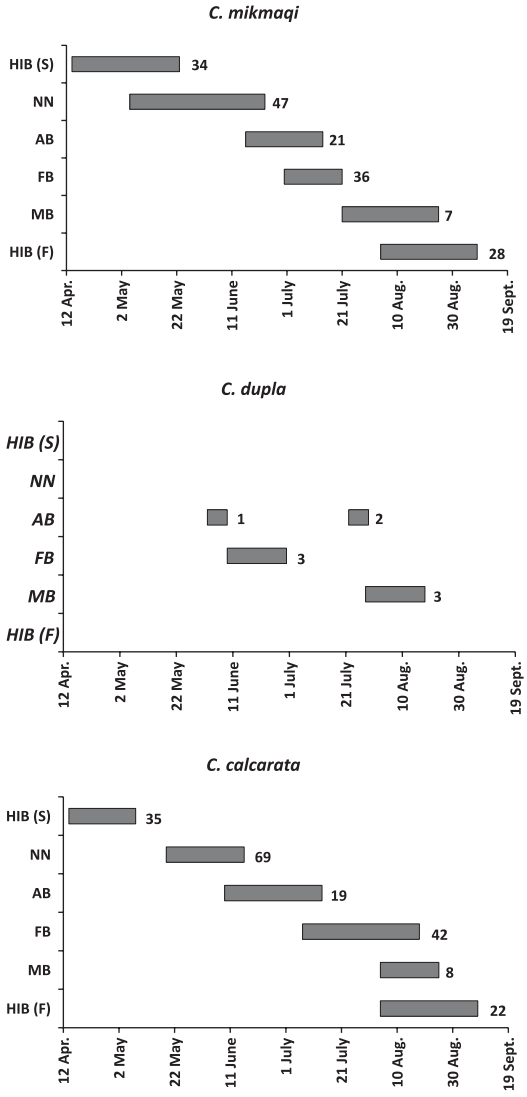
Brood parasitism occurred at different rates among *Ceratina* species, with *C. dupla* being the most heavily parasitized ($\chi^2 = 32.23$, df = 2, $P < 0.0001$; Table 1). *Ceratina calcarata* and *C. mikmaqi* were parasitized by several species of Hymenoptera, including *Axima zabriskiei* Howard (Eurytomidae), *Baryscapus americanus* (Ashmead) (Eulophidae), *Eurytoma* sp. near *apiculae* Bugbee (Eurytomidae), a species of *Coelopencyrtus* Timberlake (Encyrtidae), *Hoplocryptus zoesmairi* (Dalla Torre) (Ichneumonidae), *Eupelmus vesicularis* (Retzius) (Eupelmidae), and a species of *Pyemotes* Amerling (Acari: Pyemotidae). In our samples, *C. dupla* was only parasitized by an unidentified species of *Baryscapus* Förster. The average number of surviving brood differed significantly among all three *Ceratina* species (ANOVA: $F_{(2,77)} = 9.40$, $P < 0.0002$; Table 1).

Discussion

DNA barcodes and biological data both indicate that there are three *Ceratina* species in the Niagara Region. Pan-trap abundances and nest frequencies suggest that the rarest species is *C. dupla* (2%–5% of the total); *C. calcarata* and *C. mikmaqi* comprise the remainder of the community. The relative abundance of the latter two is unclear because abundances of pan-trapped females and nests suggest that the species are about equally frequent, whereas abundances of pan-trapped males suggest that *C. mikmaqi* is much more common.

In the Niagara Region the three species occur sympatrically and each exhibits unique aspects in its nesting biology, suggesting that each occupies a slightly different niche in the local bee community. *Ceratina mikmaqi* and *C. calcarata* are univoltine, whereas *C. dupla*

Fig. 2. Nesting phenology by nest type for *Ceratina mikmaqi*, *C. dupla*, and *C. calcarata* from 2008 nest collections in the Niagara Region of Ontario. Bars indicate the period during which nests of that type were collected (HIB (S), spring hibernacula; NN, new nests; AB, active brood; FB, – full brood; MB, mature brood; HIB (F, fall hibernacula). Note that active brood nests of *C. dupla* are divided into two sections. Summer nests housing only males and hibernacula containing more than one species have been excluded. The sample size for each nest type is included.



is probably bivoltine. Comstock (1911) and Grothaus (1962) also reported that *C. dupla* females sometimes provisioned two nests per season, which implies a mix of univoltine and bivoltine nesting strategies within populations. Although intraspecific variation in life-history pattern cannot be ruled out, Comstock (1911) and Grothaus (1962) may have been observing interspecific variation between *C. dupla* and *C. mikmaqi*. The low level of DNA sequence divergence between *C. mikmaqi* and *C. dupla* suggests that they may have diverged relatively recently. Recently diverged species that live sympatrically often may be more strongly temporally isolated from one another than older, more distantly diverged species, and temporal isolation is likely crucial to the speciation process (Rice 1987; Quinn *et al.* 2000; Friesen *et al.* 2007). The divergent life-history strategies of *C. mikmaqi* and *C. dupla* suggest that temporal isolation may be involved in niche differentiation between these two morphologically similar species, based on the timing of important events such as nest founding and brood provisioning.

In addition to phenological differences, there were marked differences in nesting-substrate preferences among species. *Ceratina mikmaqi* and *C. dupla* were collected most often from nests in teasel and sometimes in raspberry, and Grothaus (1962) noted that *C. dupla* nested in sumac, rose, and bramble (*Rubus* spp.). *Ceratina calcarata* was collected most commonly in raspberry, was fairly common in teasel, and was the only species found nesting in sumac. Kislow (1976) and Rehan and Richards (2010) reported collecting *C. calcarata* in raspberry and sumac; Johnson (1988) reported collecting *C. calcarata* from cultivated roses. Nesting in teasel has not previously been reported for any *Ceratina*. Although teasel nesting was frequent in our study, it may be rare overall in North America because teasel is a relatively recent introduction to the Nearctic Region (Rector *et al.* 2006).

Ceratina mikmaqi and *C. dupla* have similar clutch sizes that are significantly larger than those of *C. calcarata.* Average clutch size for *C. calcarata* observed by us in 2008 was 7.6, similar to the size (6.9) reported in the Niagara Region in 2006 (Rehan and Richards 2010). Clutch size may be influenced by nest location, because proximity to larval food resources is

positively correlated with the number of brood cells bees can produce (Peterson and Roitberg 2006*a*, 2006*b*; Zurbuchen *et al.* 2010). Teasel grows in open fields, often in close proximity to wildflower pollen sources, whereas raspberry tends to grow at shady, forest edges, where pollen sources may not be as plentiful. The observed differences in clutch size may be a result of the nesting-substrate preferences of the three species.

Although the three species are morphologically similar and nest in the exposed pith of twigs and stems, there are subtle but significant genetic and behavioural differences between them. DNA barcode analysis indicates that *C. mikmaqi* and *C. dupla* are genetically distinct, and our field observations indicate interspecific differences in phenology and nesting behaviour. These differences suggest that, at least in the Niagara Region, *C. mikmaqi*, *C. calcarata*, and *C. dupla* occupy slightly different niches and that competition among them is lowered by temporal isolation (differences in phenology) and resource partitioning (differences in nesting-substrate usage). Further studies on resource use, especially nest sites, would be useful to understand how species of *Ceratina* interact within the bee community in the Niagara Region.

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References

Cartar, R.V. 1992. Morphological senescence and longevity: an experiment relating wing wear and life span foraging wild bumble bees. Journal of Animal Ecology, **61**: 225–231. doi:10.2307/5525.

Chandler, L. 1975. Eusociality in *Ceratina calcarata* Robertson (Hymenoptera: Anthophoridae). Proceedings of the Indiana Academy of Science, **84**: 283–284.

Comstock, A.B. 1911. Handbook of nature study. Comstock Publishing Associates, Ithaca, New York.

Daly, H.V. 1966. Biological studies on *Ceratina dallatoreana*, an alien bee in California which reproduces by parthenogenesis (Hymenoptera: Apoidea). Annals of the Entomological Society of America, **59**: 1138–1154.

Daly, H.V. 1973. Bees of the genus *Ceratina* in America north of Mexico. University of California Press, Berkeley, California.

Excoffier, L., Laval, G., and Schneider, S. 2005. Arlequin version 3.0: an integrated software package for population genetics data analysis. Evolutionary Bioinformatics Online, **1**: 47–50.

Friesen, V.L., Smith, A.L., Gomez-Diaz, E., Bolton, M., Furness, R.W., Gonzalez-Solis, J., and Monteiro, L.R. 2007. Sympatric speciation by allochrony in a seabird. Proceedings of the National Academy of Sciences of the United States of America, **104**: 18589–18594. doi:10.1073/pnas.0700446104.

Grothaus, H.G. 1962. The biology of the species of *Ceratina* (Hymenoptera, Xylocopidae) in Indiana. M.S. thesis, Purdue University, West Lafayette, Indiana.

Hall, T.A. 1999. Bioedit: a user friendly biological sequence alignment editor and alignment program for Windows 95/98 NT. Nucleic Acid Symposium Series, **41**: 95–98.

Hajibabaei, M., deWaard, J.R., Ivanova, N.V., Ratnasingham, S., Dooh, R.T., Kirk, S.L., *et al.* 2005. Critical factors for assembling a high volume of DNA barcodes. Philosophical Transactions of the Royal Society of London B Biological Sciences, **360**: 1959–1967. doi: 10.1098/rstb.2005.1727.

Hebert, P.D.N., Stoeckle, M.Y., Zemlak, T.S., and Francis, C.M. 2004. Identification of birds through DNA barcodes. PLoS Biology, 2:e312. doi: 10.i.S7i;journal.pbio.0020312.

Ivanova, N., deWaard, J., and Hebert, P. 2006. An inexpensive, automation-friendly protocol for recovering high-quality DNA. Molecular Ecology Notes, **6**: 998–1002. doi: 10.1111/j.1471-8286.2006.01428.x.

Johnson, M.D. 1988. The relationship of provision weight to adult weight and sex ratio in the solitary bee, *Ceratina calcarata*. Ecological Entomology, **13**: 165–170. doi:10.1111/j.1365-2311.1988.tb00344.x.

Johnson, M.D. 1990. Female size and fecundity in the small carpenter bee, *Ceratina calcarata* (Robertson) (Hymenoptera: Anthophoridae).

Journal of the Kansas Entomological Society, **63**: 414–419.

Kislow, C.J. 1976. The comparative biology of two species of small carpenter bee, *Ceratina strenua* F. Smith and *C. calcarata* Robertson (Hymenoptera: Xylocopinae). Ph.D. thesis, University of Georgia, Athens, Georgia.

LeBuhn, G., Droege, S., Griswold, T.L., Minckley, R.L., Roulston, T., Cane, J.H., *et al.* 2003. Standardized method for monitoring bee populations — the bee inventory (BI) plot [online]. Available from http://online.sfsu.edu/beeplot/pdfs/Bee%20Plot%202003.pdf [accessed September 2008].

Michener, C.D. 1985. From solitary to eusocial – need there be a series of intervening species? Fortschritte der Zoologie, **31**: 293–305.

Packer, L., Gravel, A.-I.D., and Lebuhn, G. 2007. Phenology and social organization of *Halictus* (*Seladonia*) *tripartitus* (Hymenoptera: Halictidae). Journal of Hymenoptera Research, **16**: 281–292.

Peterson, J.H., and Roitberg, B.D. 2006*a*. Impacts on flight distance on sex ratio and resource allocation to offspring in the leafcutter bee, *Megachile rotundata*. Behavioural Ecology and Sociobiology, **59**: 589–596. doi: 10.1007/s00265-005-0085-9.

Peterson, J.H., and Roitberg, B.D. 2006*b*. Impact of resource levels on sex ratio and resource allocation in the solitary bee, *Megachile rotundata*. Environmental Entomology, **35**: 1404–14. doi: 10.1603/0046-225X(2006)35[1404:IORLOS]2.0.CO;2.

Quinn, T.P., Unwin, M.J., and Kinnison, M.T. 2000. Evolution of temporal isolation in the wild: genetic divergence in timing of migration and breeding by introduced chinook salmon populations. Evolution, **54**: 1372–1385. doi: 10.1554/0014-3820(2000)054[1372:EOTIIT]2.0.CO;2.

Rau, P. 1928. The nesting habits of the little carpenter bee, *Ceratina calcarata*. Annals of the Entomological Society of America, **21**: 380–396.

Rector, B.G., Harizanova, V., Sforza, R., Widmer, T., and Wiedenmann, R.N. 2006. Prospects for biological control of teasels, *Dipsacus* spp., a new target in the United States. Biological Control, **36**: 1–14. doi: 10.1016/j.biocontrol.2005.09.010.

Rehan, S.M., and Richards, M.H. 2008. Morphological and DNA sequence delineation of two problematic species of *Ceratina* (Hymenoptera: Apidae) from Eastern Canada. Journal of the Entomological Society of Ontario, **139**: 59–67.

Rehan, S.M., and Richards, M.H. 2010. Nesting biology and subsociality in the small carpenter bee, *Ceratina calcarata* (Hymenoptera: Apidae). The Canadian Entomologist, **142**: 65–74. doi: 10.4039/n09-056.

Rehan S.M., and Sheffield C.S, 2011. Morphological and molecular delineation of a new species and new combination in the *Ceratina dupla* species-group (Hymenoptera: Apidae) of eastern North America. Zootaxa. In press.

Rehan, S.M., Chapman, T.W., Craigie, A.I., Richards, M.H., Cooper, S.J.B., and Schwarz, M.P. 2010. Molecular phylogeny of the small carpenter bees (Hymenoptera: Apidae: Ceratinini) indicates early and rapid global dispersal. Molecular Phylogenetics and Evolution, **55**: 1042–1054. doi: 10.1016/j.ympev.2010.01.011.

Rice, W.R. 1987. Speciation via habitat specialization: the evolution of reproductive isolation as a correlated character. Evolutionary Ecology, **1**: 301–314. doi: 10.1007/BF02071555.

Richards, M.H., Vickruck, J.L., and Rehan, S.M. 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.

Richards, M.H., Rutgers-Kelly, A., Gibbs, J., Vickruck, J.L., Rehan, S.M., and Sheffield, C. 2011. Bee diversity in naturalizing patches of Carolinian grasslands in southern Ontario. The Canadian Entomologist, **143**: 280–300.

Rutgers-Kelly, A. 2003. The bees of Niagara: a test of the intermediate disturbance hypothesis. M.Sc. thesis, Brock University, St. Catharines, Ontario.

Sakagami, S.F., and Maeta, Y. 1977. Some presumably presocial habits of Japanese *Ceratina* bees, with notes on various social types in Hymenoptera. Insectes Sociaux, **24**: 319–343. doi: 10.1007/BF02223784.

SAS Institute Inc. 2004. SAS version 9.1 [computer program]. SAS Institute Inc., Cary, North Carolina.

Sheffield, C.S., Hebert, P.D.N., Kevan, P.G., and Packer, L. 2009. DNA barcoding a regional bee (Hymenoptera: Apoidea) fauna and its potential for ecological studies. Molecular Ecology Resources, **1**: 196–207. doi: 10.1111/j.1755-0998.2009.02645.x.

Thompson, J.D., Higgins, D.G., and Gibson, T.J. 1994. CLUSTAL W: improving the sensitivity of progressive multiple sequence alignment through sequence weighting, position-specific gap penalties and weight matrix choice. Nucleic Acids Research, **22**: 4673–4680. doi:10.1093/nar/22.22.4673.

Zurbuchen, A., Cheesman, S., Klaiber, J., Mueller, A., Hein, S., and Dorn, S. 2010. Long foraging distances impose high costs on offspring production in solitary bees. Journal of Animal Ecology, **79**: 674–681. doi: 10.1111/j.1365-2656.2010.01675.x.