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# Phenological synchrony between the hoary squash bee (*Eucera pruinos*) and cultivated acorn squash (*Cucurbita pepo*) flowering is imperfect at a northern site

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## ABSTRACT

The phenology of crop flowering and pollinator reproduction can become asynchronous at the edge of their respective ranges. At a northern site in Peterborough County, Ontario, we evaluated offspring emergence of *Cucurbita* pollen specialist hoary squash bees (*Eucera pruinos*) from nests in enclosures to determine their phenological synchrony with a squash crop (*Cucurbita pepo*). For the crop, we evaluated the percentage of bees that emerged in time to provide pollination services during the crop pollination window. For the bees, we compared the period when both male and females were present and could mate to the whole crop flowering period. We found that fewer than half the bees had emerged by the time the crop pollination window closed and only 34.1% of the flowering period of the crop could support the reproductive activities of the bees, suggesting that phenological synchrony was imperfect from the perspective of both the crop and the pollinator at this northern site.

## Introduction

Among the important elements in a crop-pollinator relationship is the overlap between the goals of the crop (i.e., pollination leading to reproduction) and the goals of the pollinator (i.e., resource acquisition supporting reproduction). Phenological synchrony is an important temporal element that affects the crop-pollinator overlap. Whether synchrony exists, or the overlap supports both crop and pollinator equally well is often overlooked.

Ground-nesting bees comprise about 70% of solitary bee species, many of which are important to crop pollination (Packer et al., 2007; Pindar et al., 2017; USDA, 2017). As a group, their relationship to agricultural crops is understudied because collectively so little is known about them. Among this important group of bees, the solitary hoary squash bee (*Eucera (Peponapis) pruinos* (Say, 1837)) is one of the most well understood ground-nesting species, though many knowledge gaps about the species' biology remain.

Here we approach the subject of phenological synchrony between a *Cucurbita* crop (represented by acorn squash, *Cucurbita pepo ovifera*) and the solitary, ground-nesting hoary squash bee (*E. pruinos*). Following the domestication of *C. pepo* from the wild buffalo gourd (*C. foetidissima*), the hoary squash bee, a Meso-American *Cucurbita* pollen specialist, expanded its range to include much of temperate North America, with its present northern limits in southern Ontario and Quebec, Canada (Hurd et al., 1971; Smith, 2006; López-Uribe et al., 2016).

Across much of its present range, including Ontario, the hoary squash bee is now entirely dependent upon cultivated *Cucurbita* (pumpkins, squash, and gourds) for its source of pollen because its wild host, *Cucurbita foetidissima*, is not present (López-Uribe et al., 2016).

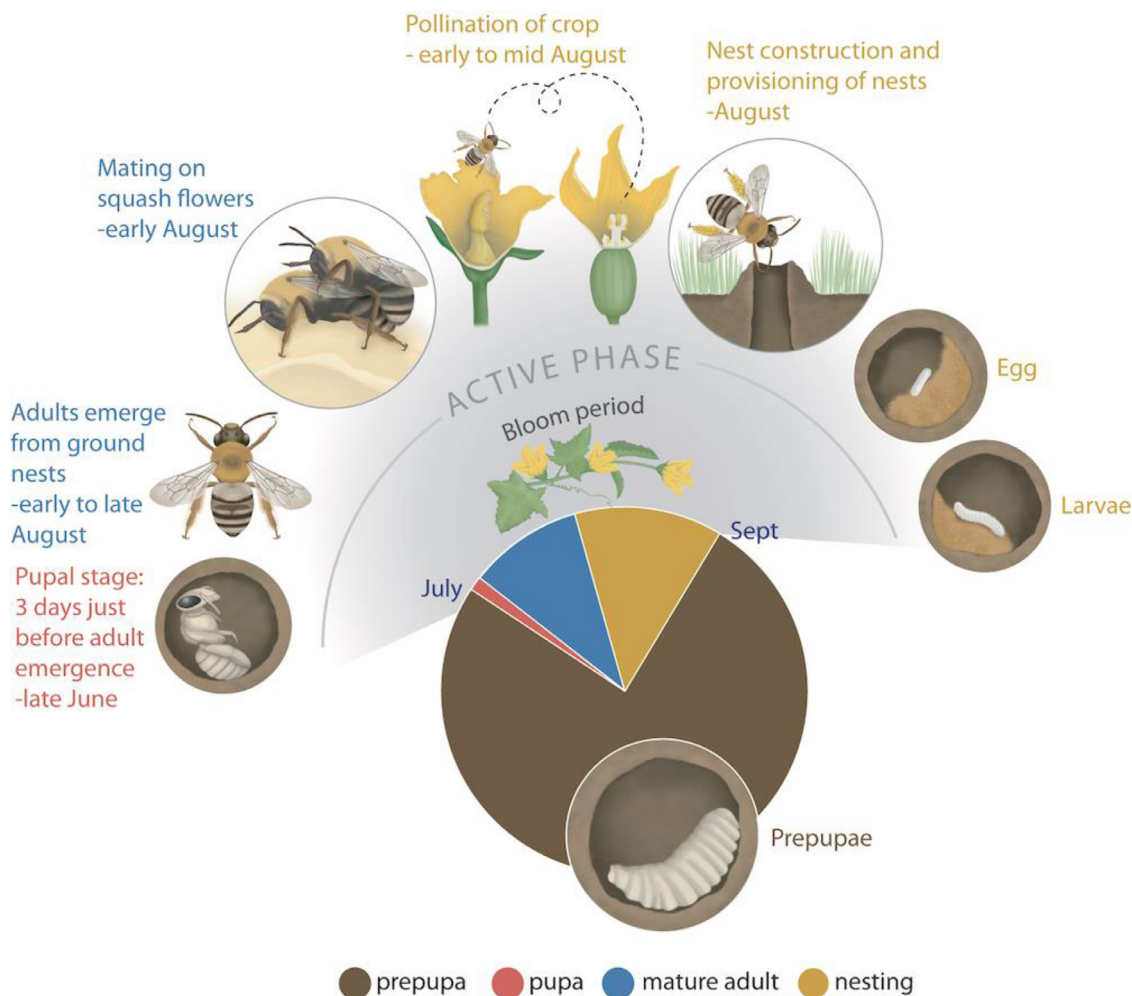
In Ontario, the land area devoted to *Cucurbita* crop production is increasing in response to a large demand for the crops, both for food and cultural purposes (Mailvaganam, 2018). *Cucurbita* crops grown in Ontario are more constrained by the length of the growing season and the average temperatures than are crops grown in more southerly locations.

The timing of planting of *Cucurbita* crops in Ontario is constrained in the spring by soil temperature and risk of frost, generally occurring in late May or early June. Although bloom begins in July, pistillate (fruit-bearing) flowers do not appear until early August, marking the beginning of the crop pollination window (Willis Chan & Raine, 2021a). The timing of harvest is constrained by the time it takes squash or pumpkins to mature after pollination (~60 days) and the timing of the biggest markets for the fruit, which are Thanksgiving (early October in Canada) and Halloween (October 31st) (Westerfield, 2014). Working backwards 60 days from an October 15<sup>th</sup> harvest date, *Cucurbita* flowers would have to be pollinated by August 15<sup>th</sup> in order to set marketable fruit in Ontario, marking the end of the crop pollination window, although flowering continues after this (Willis Chan & Raine, 2021a).

Hoary squash bees nest in the ground, often in large aggregations, in association with *Cucurbita* crops (Hurd et al., 1974; Kevan et al., 1988; Willis Chan, 2020). Each female builds her own nest and produces a

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**Figure 1.** The lifecycle of a hoary squash bee (*Eucera pruinosus*) showing development from egg to prepupa in one season followed by pupation, emergence as the adult phase, mating, foraging, nesting, and reproduction the next season. Hoary squash bees produce one generation of offspring per year, and emergence is linked to flowering of *Cucurbita* crops across much of its range. Figure designed by DSWC and drawn by Ann Sanderson.

single generation of offspring per year that emerges during the summer the following year (Mathewson, 1968; Figure 1).

As a pollen specialist, the foraging activity of hoary squash bees corresponds seasonally to bloom of wild and/or cultivated *Cucurbita*, though their activity period is variable across their range. For example, in California, Utah, and Mississippi, hoary squash bees are active on *Cucurbita* crops in May and remain active over a two-month period, but in Ontario at the northern edge of the species' range, hoary squash bees are active between mid July and the end of August (Figure 1) (Hurd et al., 1974; Willis & Kevan, 1995; Cane et al., 2011; Willis Chan & Raine, 2021a). Squash bees are matinal foragers, avoiding competition by acquiring squash or pumpkin floral resources before other bee species begin to forage on the crop (Willis Chan & Raine, 2021a).

Despite their dependence upon *Cucurbita* for pollen, hoary squash bees may visit other flowers for nectar, allowing them to sustain themselves (though not reproduce) if they emerge before pollen from *Cucurbita* flowers is available (Hurd & Linsley, 1964; Nunes-Silva et al., 2020).

Hoary squash bees emerge as adults from ground nests over an extended period within a season and reportedly undergo a 2- to 3-week period of sexual maturation, during which they consume pollen and nectar and mature their oocytes (Mathewson, 1968; Hurd et al., 1974; Cane, 2016). However, in Ontario we tracked the time between hoary squash bee emergence and nest initiation within semi-field enclosures in 2018 and found a lag of about 4 days, providing some preliminary

evidence that sexual maturation may occur within days, not weeks, of emergence (Willis Chan, 2020). If hoary squash bee emergence coincides with flowering in *Cucurbita* crops (i.e., July or August in Ontario), a 2-week lag before nest initiation could seriously curtail the reproductive output of these northern populations and could partly define the northern boundaries for this species.

Female hoary squash bees are likely monandrous (accepting a single mate), whereas males are likely polygynous, attempting to mate with many females (Hurd et al., 1974; Alcock et al., 1978). In such a mating system, there is likely to be a selective pressure towards protandry (males emerging before females) because only newly sexually mature females would be receptive to mating and early emerging males are more likely to mate with more females in their lifetime than those that emerge later (Bulmer, 1983).

Male hoary squash bees search for mates on flowers where both receptive and unreceptive (previously mated) females forage (Linsley, 1958; Willis Chan, 2020). Mating occurs in the base of the *Cucurbita* flower corolla and can best be described as scramble competition in which many males cluster around a female, competing to mate with her (Linsley, 1958; Thornhill & Alcock, 1983).

While there are many accounts of hoary squash bee activity on *Cucurbita* flowers or at nests (Mathewson, 1968; Hurd et al., 1974; Willis, 1991; Cane et al., 2011), empirical data about hoary squash bee emergence are lacking.



**Figure 2.** The inside of an enclosure showing the 28 squash plants and the net covering. Eight mated hoary squash bee females were introduced into each of nine such enclosures in 2017. We followed reproductive output of the second generation and emergence patterns of the third generation of bees from the initial foundress population. Photo credit: Beatrice Chan, used with permission.

Although important itself, the hoary squash bee has a wider significance. Because of its commonness, ubiquity across a large range, and ease of maintenance in captivity under field-realistic conditions, it may be the best model species available to represent other more elusive ground-nesting bees in agricultural pesticide risk assessments that are presently honey bee centric (Willis Chan et al., 2019; Franklin & Raine 2019; Willis Chan & Raine, 2021a,b). However, compared to the honey bee, we have very little information about the hoary squash bee.

Here we present a study of the species' basic ecology and behaviour. We provide some of that basic information, including baseline data on reproductive output in a confinement scenario, emergence patterns, and sex ratios, to improve the value of the hoary squash bee as a model species and to elucidate bee-crop phenological synchrony for the hoary squash bee and acorn squash in southern Ontario at the northern edge of this bee's range. Our objectives are to (1) measure the reproductive output of female hoary squash bees; (2) describe emergence patterns of hoary squash bees over the season; (3) quantify the intra-sexual competition among males for receptive females over the season using sex ratios; and (4) evaluate the synchrony between squash crops (*Cucurbita pepo*; Acorn squash) and hoary squash bees at the northern edge of the squash bee range.

## Materials and Methods

### Study Site

Our work was undertaken in nine large mesh-covered enclosures (Width 4.80m x Length 6.10m x Height 3.05m) that were established at a site in Peterborough County, Ontario, Canada (44.421466°N, -78.346122°W) in 2017.

Each enclosure had 28 acorn squash (*Cucurbita pepo*, Cucurbitaceae) plants grown in it each year from 2017-2019 (Figure 2). The acorn squash were planted from seed in the last week of May or the first week of June, depending on the season. All planting methods and timing followed those used by commercial *Cucurbita* crop growers in the region. The plants began to bloom in the enclosures in late July, in synchrony with bloom in commercial fields, and female flowers were produced in early August.

In 2017, we collected mated female bees from a large nesting aggregation on a farm near Guelph, Ontario and introduced eight of them into each enclosure after the squash plants had begun to bloom in early August for a total of 72 female foundress bees.

We managed the enclosures to closely mimic field conditions throughout the lifecycle of the bees. Although relative humidity was not measured, temperatures were measured 24 hours per day during

the whole bee-active season and were not different within and outside of enclosures.

During the bees' adult active period, the enclosures were covered with netting, allowing in light and precipitation but preventing the bees from escaping. After adult activity ceased (September), we removed the nets and reapplied them in early June the following year before adults emerged from the ground.

Because hoary squash bees specialize on pumpkin and squash crops, avoid competition with other foragers by foraging matinally, nest within the cropping area, and can be established easily in confinement, we believe that reproductive behaviour of populations confined in our large enclosures planted with 28 acorn squash plants closely mimics the behaviour of the species in the wild in the Peterborough area of Ontario.

We studied the second (emerging in 2018) and third generation (emerging in 2019) of bees from the population of 72 females we introduced to enclosures in 2017. To provide a detailed picture of emergence for this population, we quantified the number of reproductive females, the number of nests they produced, and the number of flowers in all enclosures in 2018 (parent generation); and the number of bees emerging from those nests in 2019 (offspring generation). Because the bees in each enclosure were maintained in isolation from each other, we treated each enclosure as a separate sample of hoary squash bees that have all experienced the same conditions. Despite the soil classification of the location as Otonabee loam, there was some localized variability in soil texture among the enclosures, with three enclosures having a coarser sandy soil, three having soil with an intermediate texture and three having a finer textured soil.

The nine enclosures used in this study were part of another study undertaken between 2017-2019 that used 12 enclosures to compare the potential effects of exposure to systemic insecticides on hoary squash bee reproduction (see Willis Chan & Raine, 2021b). In that study insecticides were applied to the crop in 2017, then bees were introduced and the nests they established were counted. In 2018, the same insecticides were applied before the offspring from 2017 emerged. That generation was allowed to forage in the enclosures. The number of mated females and the number of nests they established were again counted. In 2019, no insecticides were applied and offspring from 2018 were counted, sexed, and removed for this study.

In the insecticide trial we found a strong effect of one insecticide (imidacloprid-#3, applied in three enclosures) and no effects of the others (thiamethoxam-#1, and chlorantraniliprole- #2, and an untreated control, represented by nine enclosures) on nest establishment, offspring production or sex ratio (see Willis Chan & Raine, 2021b). Here we have used data collected from the nine enclosures which showed no significant effect of the insecticide treatments to examine phenological synchrony with the crop.

### Parent Generation (2018)

In 2018, we counted flowers and observed nesting activity within each enclosure on eight days (August 7,9,10,13,14,15,20,23) across the bee-active season. We marked each nest with a unique identifier. At the end of the season all the tags were collected and counted to determine the number of nests created. On August 23-24, 2018, we removed all bees that were found resting in wilted squash flowers in the afternoon from the enclosures. This effectively removed all males and all unmated females from the enclosures because both males and unmated females rest in wilted *Cucurbita* flowers (Hurd & Linsley, 1964). On August 25, 2018, we captured, counted, and removed all mated female bees foraging on the flowers in enclosures. We rechecked the enclosures on August 26 to make sure we had not missed any bees. From flower counts, we calculated a seasonal mean flower count for each enclosure.

### Counting Emerging Bees (2019)

The following summer (July 25 to September 4, 2019), we recorded the number and sex of hoary squash bee offspring emerging from the nests established in 2018 in each enclosure by checking all open flowers on 25 observation days (every 1-3 days). We repeated the inspection the next observation day until there were no more bees emerging over a 3-day period. On each observation day, we released the bees outside the enclosure after counting them. Upon emergence, bees had access to acorn squash flowers in which to forage and rest. Bee emergence counts were then totaled by sex for each week of the crop flowering period (July 25-September 4). A seasonal running total emergence for males was calculated by adding each week's male emergence count to the count of the previous week for each enclosure.

### Reproductive Output

As measures of reproductive output, we calculated the mean number of nests per female parent (Equation 1), offspring per nest (Equation 2), and flowers available per offspring (Equation 3) for each enclosure.

$$\text{Nests per female parent} = \frac{\text{Number of nests constructed in 2018}}{\text{Number of mated females in 2018}} \quad (1)$$

$$\text{Offspring per nest} = \frac{\text{Number of offspring emerged in 2019}}{\text{Number of nests constructed in 2018}} \quad (2)$$

$$\text{Flowers per offspring} = \frac{\text{Mean number of flowers in 2018}}{\text{Number of offspring emerged in 2019}} \quad (3)$$

The percent of males and females in the emergent population was also calculated for each enclosure.

### Offspring Emergence Patterns (2019)

The number of days to first emergence, 25% emergence, 50% emergence, 75% emergence and 100% emergence were calculated in each enclosure by multiplying the seasonal total number of emerging bees by category (total bees, males, and females) in that enclosure by the percentage (25, 50, 75, 100%) and relating this back to the day of emergence that most closely reflected that value. The time lag (in days) between male and female first emergence across all enclosures was calculated by taking the mean difference between first male and female emergence dates for each enclosure population.

### Sex Ratios

Sex ratios are expressed as a single number referring to the number of males per female (sex ratio = number of males/ number of females; West et al., 2002). A seasonal sex ratio was calculated for each enclosure

and across all nine enclosures (seasonal sex ratio = total number of males emerged in 2019 / total number of females emerged in 2019).

The change in sex ratio over time was tracked by calculating the sex ratio in each enclosure by week. Although emergence data were collected more frequently, sex ratios were calculated by week because sex ratios were incalculable on some days when females did not emerge on that day.

We calculated a 'functional sex ratio' to quantify the intra-sexual competition among polygynous males for receptive monandrous females (functional sex ratio = running total of males in an enclosure/ number of newly emerged females in a week in that enclosure). The functional sex ratio assumed that only newly emerged females would be receptive to mating. A sexual maturation period was not included in the calculations because the duration of that period is not conclusively known. Functional sex ratios could only be calculated by week.

We defined the hoary squash bee mating window for each enclosure as beginning when the functional sex ratio > 0 (i.e., there were both males and females in an enclosure) and ending when the squash crop ceased to bloom because mating takes place on the flowers. The mating window for the site was the mean of the enclosure mating windows.

### Phenological Synchrony

Phenological synchrony between hoary squash bee emergence and the flowering period of the *Cucurbita* crop was evaluated from the perspective of the squash crop and the bee. From the perspective of the crop, ideal synchrony would exist if all the bees emerged and were foraging on the crop flowers during the crop pollination window, beginning when both staminate (male) and pistillate (female) flowers were present and ending by August 15, allowing for a 60-day fruit growth and maturation period before harvest in mid-October (Westerfield, 2014; Willis Chan & Raine, 2021a). We measured synchrony from the perspective of the crop by calculating the percent of the bee population that had emerged within the crop pollination window.

We calculated percent synchrony from the perspective of the bee by counting the number of days from the beginning of the bee mating window (i.e., functional sex ratio > 0) to the end of the crop flowering period (September 4, 2019) across all nine enclosures and compared that to the total number of flowering days of the crop in 2019 (July 25-September 4, 2019; 41 days). Ideal synchrony would exist if all emerging bees had sufficient time within the crop flowering period to produce offspring.

### Statistical Analysis

SAS University Edition (version 3.8) was used to generate general linear mixed models to test the significance of effects on a dependent variable. Dependent variables included nests per female, total offspring per nest, male offspring per nest, female offspring per nest, flowers per offspring, number of male, female, and total hoary squash bees emerging, days to 1<sup>st</sup>, 25%, 50%, 75%, and 100% emergence from natal nests, sex ratio and functional sex ratio. Model effects varied with the dependent variables being measured (see Table S1). Model information (Table S1) and pairwise comparisons for each significant effect in each model are presented in Table S2 in supplemental information. F-tests were used to determine the significance of effects in all models and t-tests were used in *post hoc* pairwise comparisons between levels of significant effects based on a significance level of 0.05. Where multiple pairwise comparisons were undertaken, p-values were Tukey adjusted. To ensure that all dependent variables measured in this research were not affected by the pesticide treatments applied in the enclosures in our previous study, we included an insecticide treatment effect in all models tested. For the dependent variables time to 1<sup>st</sup>, 25%, 50%, 75%, and 100% emergence, the effect of soil texture was also included in the model in case soil texture was impacting larval development or survival. The experimental design was balanced with equal number of enclosures for each soil

**Table 1**

Reproductive output of female hoary squash bee parents in nine net-covered enclosures at a site in Peterborough County, Ontario, Canada. Female parents constructed nests, provisioned nest cells by foraging on acorn squash flowers and laid a single egg in each nest cell. Eggs hatched and larvae developed within nest cells and emerged as adults in 2019 when they were counted. Flower counts are means of flower counts within the enclosures on 10 days over the 2018 season.

Enclosure	2018			2019			Reproductive Output Measures						
	Count			Offspring Count			Offspring/Nest			Percent of Total Offspring (%)			
	Mean Flowers	Female Parent	Nest	Male	Female	Total	Nests/Female Parent	Flowers/Offspring	Male	Female	Total	Male	Female
1	45.1	9	8	44	14	58	0.9	0.8	5.5	1.8	7.3	76	24
2	57.6	2	6	35	27	62	3.0	0.9	5.8	4.5	10.3	56	44
3	43.6	27	19	19	12	31	0.7	1.4	1.0	0.6	1.6	61	39
4	46.3	12	11	45	4	49	0.9	0.9	4.1	0.4	4.5	92	8
5	41.1	7	10	30	4	34	1.4	1.2	3.0	0.4	3.4	88	12
6	32.4	12	14	7	10	17	1.2	1.9	0.5	0.7	1.2	41	59
7	34.6	21	13	9	6	15	0.6	2.3	0.7	0.5	1.2	60	40
8	44.9	6	10	8	3	11	1.7	4.1	0.8	0.3	1.1	73	27
9	41.6	10	18	25	12	37	1.8	1.1	1.4	0.7	2.1	68	32
Mean	43.0	11.8	12.1	24.7	10.2	34.9	1.4	1.2	2.5	1.1	3.6	68.3	31.7
SE	2.3	2.4	1.4	4.7	2.4	5.9	0.2	0.4	0.7	0.4	1.0	5.0	5.0
Sum		106	109	222	92	314							

texture and insecticide treatment. All insecticide treatments were randomly assigned with respect to enclosures (Willis Chan & Raine, 2021b). All means are reported with their associated standard error.

## Results

### Reproductive Output

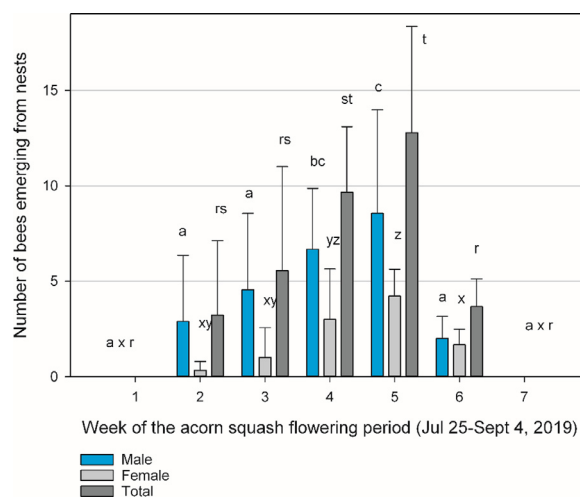
Overall, a total of 106 mated female bees (mean  $\pm$  SE:  $11.8 \pm 2.4$  per enclosure; max = 27, min = 2; n = 9 enclosures) constructed 109 nests (mean  $\pm$  SE:  $12.1 \pm 1.4$  per enclosure; max = 21, min = 6; n = 9 enclosures) across all enclosures in 2018 (Table 1). The mean number of nests produced per female parent was  $1.4 \pm 0.2$  (max = 3.0; min = 0.6; n = 9 enclosures) (Table 1).

In 2019, a total of 314 bees emerged from the nests constructed in 2018, of which just over two thirds were male (male:  $68.3 \pm 5.0\%$ ; female:  $31.7 \pm 5.0\%$ ; n = 9 enclosures; Table 1). The mean number of offspring produced per nest was  $3.6 \pm 1.0$  (max = 10.3; min = 1.1; n = 9), with more males per nest ( $2.5 \pm 0.5$ ; max = 5.8; min = 0.7; n = 9 enclosures) than females ( $1.1 \pm 0.4$ ; max = 4.5; min = 0.3; n = 9 enclosures) (Table 1).

On average, there were  $43.0 \pm 2.3$  flowers per enclosure (max: 57.6; min 32.4; n = 9 enclosures) with  $1.2 \pm 0.4$  (max = 4.1; min = 0.8) flowers available per female parent per day to provision each offspring produced (Table 1). There was no effect of treatment on any measure of reproductive output (Table S1). We tested if the reproductive output values calculated for 2018-2019 held up for the previous 2017-2018 generation in the same enclosures. In 2017, 72 females were introduced into the nine enclosures under study (8 mated females/enclosure). Assuming no mortality, if each of those females produced 1.4 nests with 3.6 offspring/nest with an overall seasonal sex ratio of 2.4 (222 males/92 females; Table 1), then by 2018, we would have expected 363 bees in the enclosures, with 107 females. This is surprisingly close to the number of females counted in the enclosures in 2018 (Female count = 106; Table 1).

### Offspring Emergence Patterns

Emergence in the enclosures occurred over five weeks (31 days), from July 30 to August 29, 2019, with a significant increase in emergence of both male ( $F_{6,48} = 4.66$ ,  $p = 0.0008$ ) and female ( $F_{6,48} = 7.23$ ,  $p < 0.0001$ ) bees from the start of this period until peak emergence at



**Figure 3.** Emergence of hoary squash bees by week of the acorn squash flowering period (July 25-September 4, 2019). Total = male + female. Bars within a category (male, female, or total) marked with the same letter are not significantly different.: letters a-c are used for males, x-z are used for females, and r-t are used for total bees.

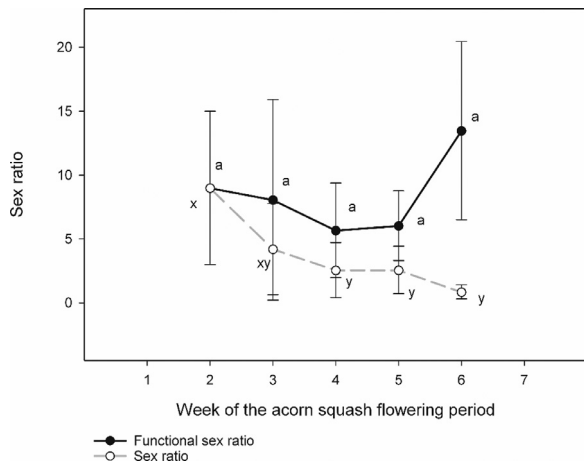
weeks 4 and 5, with a decline in emergence thereafter (Figure 3; Table S1).

There was a significant effect of bee sex on the day of first emergence ( $F_{1,8} = 24.19$ ;  $p = 0.0012$ ; Table S1). Across all enclosures, the mean first day of emergence for males and females was day  $11.5 \pm 1.8$  and day  $21.8 \pm 2.9$  respectively, a lag of ~10 days between mean male and female first emergence ( $t_{16} = 6.02$ ,  $p < 0.0001$ ; Table 2; Table S2). This lag was not found at either the 25% emergence ( $F_{1,8} = 3.31$ ;  $p = 0.1065$ ) or 50% emergence points ( $F_{1,8} = 1.57$ ;  $p = 0.2459$ ; Figure 3; Table S1). However, a significant effect of bee sex reappeared by 75% emergence ( $F_{1,8} = 7.58$ ;  $p < 0.0249$ ; Table S1), with females reaching this threshold about two days later than males (Figure 3; Table S2). There was no detectable difference in the time to 100% emergence for either sex ( $F_{1,8} = 2.53$ ;  $p = 0.1305$ ), with males and females reaching this threshold at virtually the same time (at  $36.2 \pm 0.3$  and  $36.2 \pm 0.2$  days after the onset of flowering respectively; Table 2; Table S1; Table S2). Neither soil texture, nor insecticide treatment had a significant effect on the number of days to 1<sup>st</sup>, 25%, 50%, 75% or 100% emergence (Table S1).

**Table 2**

The number of days to first, 25%, 50%, 75%, and 100% emergence for male and female hoary squash bee populations in each of nine net-covered enclosures at a site in Peterborough County, Ontario, Canada.

Sex	Emergence Variable	Enclosure Number									Mean ± SE
		1	2	3	4	5	6	7	8	9	
Female	Days to first emergence	9	10	21	28	29	18	23	28	21	21.8 ± 2.9
	Days to 25% emergence	15	13	23	28	29	18	23	28	26	24.0 ± 2.1
	Days to 50% emergence	25	21	25	28	30	18	28	28	28	27.3 ± 0.8
	Days to 75% emergence	28	22	28	29	35	30	30	31	31	30.5 ± 1.1
	Days to 100% emergence	37	27	35	30	36	35	36	36	37	36.2 ± 0.3
Male	Days to first emergence	6	6	13	18	14	18	9	18	9	11.5 ± 1.8
	Days to 25% emergence	13	10	18	23	21	28	14	25	21	18.7 ± 1.9
	Days to 50% emergence	15	13	22	26	28	28	25	28	25	23.8 ± 2.0
	Days to 75% emergence	23	21	28	29	29	28	30	29	29	28.0 ± 1.0
	Days to 100% emergence	37	36	36	36	36	35	36	36	36	36.2 ± 0.2



**Figure 4.** The change in the weekly sex ratio and functional sex ratio of hoary squash bees in nine net-covered enclosures at a site in Peterborough County, Ontario, Canada. Sex ratio = weekly total of emerging males / weekly total of emerging females. Functional sex ratio = seasonal running total of emerging males / weekly total of emerging females. Functional sex ratio is an indication of intra-sexual competition among males for unmated females. Points marked with the same letter are not significantly different. No statistical comparison between the sex ratio and the functional sex ratio is intended.

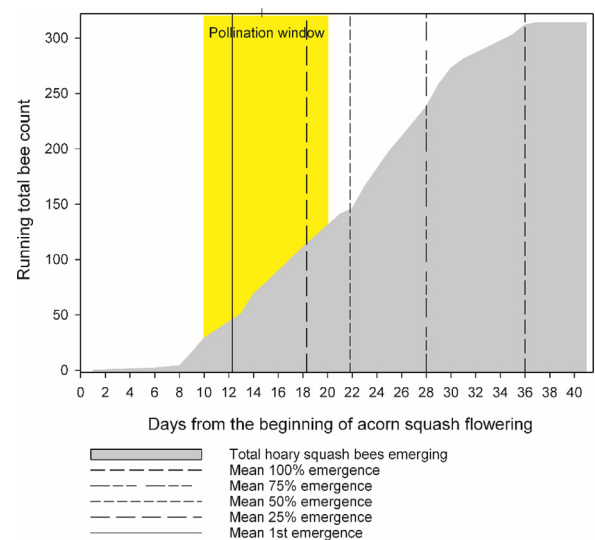
**Sex Ratios**

The overall seasonal sex ratio, calculated from the total number of males and females found in all enclosures by the end of the season, was male biased with 2.4 males per female (222 males/ 91 females; Table 1). Sex ratio was strongly influenced by week ( $F_{2,4} = 7.20, p = 0.0023$ ; Table S1). Sex ratio was most strongly male biased in week 2, during the lag between male and female emergence, followed by a significant decline to week 4, after which sex ratio stabilised over the rest of the emergence period (weeks 4-6: Figure 4; Table S2).

Functional sex ratio did not vary significantly by week or treatment (Figure 4; Table S1). The mean functional sex ratio across all enclosures was  $8.5 \pm 1.4$  males per receptive female, 3.5 times greater than the overall seasonal sex ratio (Figure 4; Table 3).

**Phenological Synchrony**

Pollination services began to be provided by hoary squash bees  $11.5 \pm 1.8$  days after the onset of flowering in the acorn squash crop (Figure 5). At the end of the acorn squash crop pollination window (August 15), between 25 and 50% of the total population of hoary squash bees had emerged and were available to provide pollination services (Figure 5; Table 2).



**Figure 5.** Synchrony of *Cucurbita* crop flowering and hoary squash bee emergence from the crop perspective at a site in Peterborough County, Ontario, Canada. The yellow rectangle represents the pollination window in which both staminate and pistillate flowers are available and pollination provided by bees can result in a mature marketable acorn squash fruit by October 15. The grey area is the running (cumulative) total count of all hoary squash bees in all enclosures. Vertical lines indicate the mean days to first, 25%, 50%, 75%, and 100% emergence of hoary squash bees from nests established in 2018. Emergence occurred over the squash flowering season (July 25-September 4, 2019) in nine net-covered enclosures.

The start of the bee mating window (i.e., the period during which bees in the enclosures could find a mate; functional sex ratio > 0) across all enclosures was  $3.9 \pm 0.7$  weeks (27.2 days) after the onset of squash flowering (Table 3), leaving only about 14 days in which the population of bees could carry out all the activities related to reproduction (mating, building and provisioning a nest, and laying eggs) before crop flowering ceased on day 41. The mean duration of the mating window was  $3.0 \pm 0.4$  weeks (Max: 6 weeks, Min: 1 week, n = 9 enclosures; Table 3). There was a 34.1% overlap between the total number of days in which this population of hoary squash bees could reproduce (14 days) and the total days in which the squash crop was in bloom (41 days).

**Discussion and Conclusion**

The only available information we have on the number of offspring per hoary squash bee nest comes from Mathewson (1968) in Rhode Island, who counted 4-5 cells per excavated nest. This could be considered the potential reproductive output for the species. In this study, on average,  $3.6 \pm 1.0$  offspring emerged per nest, about 72% fewer than Math-

**Table 3**

Functional sex ratio broken down by week and enclosure. Functional sex ratio = Running total count of males/ Total weekly count of newly emerged females in an enclosure. Functional sex ratio quantifies the intra-sexual competition among males for females in a polygynous-monandrous mating system. Mating window includes all weeks where the functional sex ratio >0. Weekly means marked with the same letter are not significantly different (see Table S1 for model). \*Overall seasonal functional sex ratio across weeks and enclosures. †Overall mean mating window duration across enclosures. Overall means are in bold font.

Enclosure	Functional Sex Ratio						Enclosure Mean $\pm$ SE	Mating Window Begin-End (# weeks)
	Week 2	Week 3	Week 4	Week 5	Week 6			
1	12.0	12.0	12.3	10.3	11.0	11.5 $\pm$ 0.4	2-6 (5)	
2	6.0	4.1	2.6	6.8	35.0	10.9 $\pm$ 6.1	2-6 (5)	
3	-	-	3.5	2.4	19.0	8.3 $\pm$ 5.4	4-6 (3)	
4	-	-	-	10.0	-	10.0 $\pm$ n/a	5 (1)	
5	-	-	-	13.0	15.0	14.0 $\pm$ 1.0	5-6 (2)	
6	-	-	0.7	2.3	7.0	3.3 $\pm$ 1.9	4-6 (3)	
7	-	-	5.0	2.7	4.5	4.1 $\pm$ 0.7	4-6 (3)	
8	-	-	-	4.0	8.0	6.0 $\pm$ 2.0	5-6 (2)	
9	-	-	10.0	2.9	8.3	7.1 $\pm$ 2.1	4-6 (3)	
<b>Mean <math>\pm</math> SE</b>	9.0 $\pm$ 3.0 <sup>a</sup>	8.1 $\pm$ 3.9 <sup>a</sup>	5.7 $\pm$ 1.9 <sup>a</sup>	6.0 $\pm$ 1.4 <sup>a</sup>	13.5 $\pm$ 3.5 <sup>a</sup>	<b>8.5 <math>\pm</math> 1.3*</b>	<b>3.0 <math>\pm</math> 0.4†</b>	
<b>n</b>	2	2	6	9	8	27		

ewson's maximum potential output of 5 offspring per nest. Differences in reproductive output may be attributable to the differing climatic conditions over time (50 years) or space (Rhode Island vs Ontario) or may be related to the different methods used in the studies. Mathewson excavated nests and counted nest cells to determine the number of offspring per nest whereas we counted nests and the number of offspring emerging from those nests. Mathewson's values reflect potential offspring per nest whereas our values are actual offspring per nest including winter mortality. Differences also may be related to the confinement of our study population within an enclosure, but because the reproductive output in the enclosures was consistent for the 2017-2018 and the 2018-2019 generations despite expanding populations, this seems unlikely. Lastly, it is also possible that differences are caused by the phenological asynchrony between the crop and the bees at the northern limit of the species range which limits the fecundity of hoary squash bees.

Hoary squash bee emergence (in 2019) was continuous and prolonged, with an abrupt end at day 39, likely related to the abrupt end of flowering the season before (2018). Extended emergence suggests that there is a strong relationship between the time an egg was laid and the time the adult emerged. However, environmental factors such as temperature will likely influence rates of development and emergence times in ground-nesting bees, though they are less critical for survival in summer emerging bees than for spring emerging bees (Bartomeus et al. 2011; Sgolastra et al. 2012; Vinchesi et al., 2013).

On average, males emerged about 10 days before females, providing clear evidence of protandry in the population as would be expected in a monandrous-polygynous mating system. However, both male and female emergence peaked at a similar time (in weeks 4 and 5) providing good overlap and opportunities for mating between sexes in this population. Although sex ratios were most male-biased (highest) at the beginning of emergence and declined thereafter, males effectively experienced similar levels of competition for receptive females throughout the season (functional sex ratio = 8.5). Therefore, early-emerging males would not benefit from reduced competition for receptive females but would have more potential mating opportunities over the season than later-emerging males. Early emerging females could likely provision more nest cells than late females because they had more days in which to forage. Although not undertaken here, it would be interesting to determine if male size changes over the season at this northern site. In the hoary squash bee polygynous sexual system where males engage in scramble competition for receptive females, larger males would have a mating advantage over smaller ones (Fraberger & Ayasse, 2007). If those larger males emerge earlier in the season, they can be expected to leave more offspring by mating with more monandrous females, especially when there is no change in the functional sex ratio over the

season. Despite having a mating advantage, if larger males emerge later in the season, they may leave no offspring because the females they mate with may not have enough time to undertake all the activities needed to reproduce before the flowering season of *Cucurbita* crops ends.

Synchrony between the squash crop flowering and hoary squash bee emergence at this site appeared to be low. From the perspective of the grower, the crop pollination window was from August 5-15 before which pistillate (fruit bearing) flowers were not present and after which there was not enough time to fully mature seeds and fruit before harvest or frost in Ontario. On average, hoary squash bee emergence coincided with the beginning of that pollination window (i.e., males emerged at day 11 or August 6), but between 50% and 75% of bees emerged after the pollination window had closed, meaning that the crop did not benefit from the full pollinating force of the bees at this northern site. Whether this apparent asynchrony might impact crop yields remains unknown.

Synchrony from the perspective of the bees was based upon the ability of the crop to provide flowers both as mating platforms and to support nest provisioning and reproduction through collection of pollen and nectar. At this northern site, there was only a 34% overlap between the number of days when hoary squash bees could find a mate (functional sex ratio > 0) and the number of days when crop flowers were available to provide floral resources and mating platforms, meaning that synchrony was low between crop flowering and bee reproduction. It would be interesting to compare synchrony between bee emergence and crop flowering across sites throughout the range of *E. pruinosa* and *C. pepo*. The poor synchrony at this site may be partly responsible for the reduced reproductive output of hoary squash bees here in comparison to the potential of 5 offspring per nest noted in Rhode Island (Mathewson, 1968), possibly indicating that the species is near the northern edge of its range.

Clearly, evaluating synchrony between crop flowering and bee reproduction requires two different lenses that cannot be substituted one for the other. Whether phenological synchrony between hoary squash bees and *Cucurbita* crops will improve at the northern edge of their respective ranges as the climate warms is a matter of conjecture. More information about all aspects of the biology of the hoary squash bee is needed to understand the full extent of the relationship between this important ground-nesting bee and the *Cucurbita* crops it pollinates across North America. Furthermore, as mentioned earlier, studies like ours contribute to knowledge about hoary squash bee biology that can expand their utility as a model species to represent other ground-nesting bees in pesticide risk assessments where honey bee models are inadequate.

#### Data Availability

Data are available in Supplementary Information.

## Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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## Author Contributions

D.S.W.C. conceived and designed the project, carried out the experimental work and did the subsequent data analyses. D.S.W.C. and N.E.R. wrote the paper.

## Additional Information

D. Susan Willis Chan and Nigel E. Raine each declare no competing interests. Supplementary Information is available.

## Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.cris.2021.100022.

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