



# Linear dominance hierarchies and conditional reproductive strategies in a facultatively social carpenter bee

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Received: 13 April 2018 / Revised: 30 July 2018 / Accepted: 13 August 2018 / Published online: 21 August 2018  
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## Abstract

In social groups, dominance rank may have important fitness consequences, as higher ranking individuals tend to have higher overall fitness. In social nests of the eastern carpenter bee, *Xylocopa virginica*, females in social nests demonstrate a complete division of labour where one female is the dominant egg layer and forager while other females in the nest are non-reproductive. We investigated the nature of reproductive queues in this species by performing removal experiments across 3 years to observe how females respond to new reproductive opportunities in the nest. When a primary female was removed, a secondary female always assumed her position as replacement primary and reproductive queues formed in a linear fashion. A third type of female in the nest, the tertiary female, did not become reproductive, even if she was the only female remaining. In delaying reproduction, tertiary females were able to overwinter a second time and were often successful at becoming reproductive in their second summer. Tertiary females were smaller than primary or secondary females, had higher fat stores and lower ovarian development. When all other females in the nest were removed, tertiary females were observed ejecting the offspring of previous dominant females in the nest. Tertiary females appear to represent a novel reproductive strategy among the Hymenoptera who can drastically alter their physiology and behaviour, essentially doubling their life span to maximize reproductive potential.

**Keywords** Behavioural plasticity · Delayed reproduction · Reproductive queue · *Xylocopa virginica*

## Introduction

The formation of dominance hierarchies has been hypothesized as one of the key pre-adaptations to the evolution of eusociality, increasing the efficiency and ecological success of the group (Wilson 2008). In many kinds of hierarchical animal social groups, one or few dominant individuals monopolize opportunities for mating and offspring

production. As a result, subordinate individuals may be forced to queue for the chance to breed in their current group. Another possibility is that subordinates might adopt alternative behavioural strategy that increases their chances of breeding (Michener 1974; Bridge and Field 2007; Smith et al. 2009; Lucas et al. 2011). Linear queues are often observed in female social insects with small group sizes, including carpenter bees, hover wasps, and polistine wasps (Cronin and Field 2007; Bridge and Field 2007; Ishikawa et al. 2010). In a linear queue, subordinates await the chance to assume the dominant, breeding position, and a dead or weak dominant is predictably replaced by the second-ranked individual, whose position is then taken by the third-ranked individual, and so on (Bridge and Field 2007). Several factors affect an individual's position within a queue, including size, age, residency status, and chemical signals (Hogendoorn and Velthuis 1999; Cant et al. 2006; Bridge and Field 2007; Zanette and Field 2009; Lucas et al. 2011). In some species, including the sweat bees and carpenter bees, larger individuals are often better able to compete for reproductive opportunities, especially when aggressive interactions

**Electronic supplementary material** The online version of this article (<https://doi.org/10.1007/s00040-018-0653-4>) contains supplementary material, which is available to authorized users.

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determine dominance status among group members (Hogendoorn and Velthuis 1999; Pabalan et al. 2000), but in others, body size does not influence dominance rank (Zanette and Field 2009; Smith et al. 2009; Leadbeater et al. 2011). The age of group members is often negatively correlated with dominance rank, with older individuals more likely to be at the front of the queue. Chemical control of hierarchies is well documented in many highly eusocial species such as honey bees and ants, where pheromones excreted by the queen provide her with control of the subordinate individuals in the colony (Fletcher and Blum 1981; Keller and Nonacs 1993). Indeed some species, such as the wasp *Ropalidia marginata*, form strong linear hierarchies with very few clues to the underlying mechanism (Bang and Gadagkar 2012).

When reproductive queues are long and replacement of dominant females occurs infrequently, subordinate individuals may have no real chance of moving up to the dominant breeding position while reproduction is still possible; this problem would be especially acute for short or climatically defined breeding seasons. Under these circumstances, subordinate or low-quality individuals might achieve greater reproductive success via an alternative reproductive strategy that might help to avoid the breeding delays associated with remaining in a reproductive queue. In the eastern carpenter bees, *Xylocopa virginica*, reproductive queues may be comprised of six or more individuals, but only subordinates in positions 2 and 3 are likely to succeed to the position of dominant egg layer before the end of the relatively short egg-laying season (Richards and Course 2015). For species such as hover wasps living in tropical climates, nests can persist for much longer periods of time, giving subordinates further down the queue a better chance at becoming the dominant egg layer (Field et al. 1998; Bridge and Field 2007).

In eastern carpenter bees, subordinate females (also referred to as “secondaries”, Richards 2011) exhibit two sets of tactics that likely increase their lifetime chances of reproduction. First, they can disperse from their natal nests and either join another group of females or nest solitarily, however, solitary nests are typically the minority in the aggregations we have observed (Richards 2011; Peso and Richards 2011). Females that disperse to new nests frequently can become dominant females (also referred to as “primaries”, Richards 2011) or high-ranking subordinates in their new colonies (Richards and Course 2015), suggesting that joining a new colony is a strategy at least as successful as remaining in the natal colony. A second, rarer strategy, is exhibited by females referred to as “tertiaries” (Richards 2011). Tertiary females remain in their natal nests and have pristine, unworn wings and mandibles even at the end of the foraging season, because they do no work either outside or inside the nest (Richards 2011). After spending their first breeding season quietly inside their natal nests, they

overwinter a second time, prior to breeding in their second year (Gerling and Hermann 1978). The relatively small body size of tertiary females suggests that overwintering twice is a conditional reproductive strategy in which subordinate individuals delay reproduction for a year (Richards and Course 2015). The success of this strategy is exhibited by multiple observations of small primary females that in their second spring begin foraging earlier than most other primary females (Richards and Course 2015). Worn primary and secondary females very rarely overwinter twice and forage in their second spring (observed in 1 of 500 bees studied), but approximately 10% of spring foragers may be former tertiary females (Richards and Course 2015; J. Vickruck, unpub. data). This suggests that tertiary females make physiological, as well as behavioural adjustments, to successfully overwinter twice, prior to becoming breeders in their second year.

In the current study, we further examine the links between dominance hierarchies and reproductive strategies employed by *X. virginica* females. Previous observations suggest that secondary females have the behavioural flexibility to move into new nests and improve their queue position, whereas tertiary females appear to be non-participants in reproductive queues until their second breeding season. To test the behavioural flexibility of secondary and tertiary females, we carried out serial removal experiments of dominant females across 3 years, creating opportunities for subordinates to become the primary reproductive in their colony. Our second goal was to compare the physical characteristics of primary, secondary and tertiary females, to identify traits associated with the reproductive behaviours employed by each female. Third, we aimed to track tertiary females across both years of their lives to quantify overwintering success and reproductive potential in their second year. Quantifying the behaviour of tertiary females across their lifespan will help explain why this strategy has evolved and how it has persisted in *X. virginica* social groups.

## Methods

### Life history of *Xylocopa virginica*

In social carpenter bees, group size is small, reproductive division of labour is complete, and dominant females both forage and lay eggs (van der Blom and Velthuis 1988; Gerling et al. 1989; Stark 1992; Richards 2011). The eastern carpenter bee, *X. virginica*, is facultatively social, nesting in groups that are slightly larger than those of either *X. pubescens* or *X. sulcatipes* (2–8 females for *X. virginica*, 2–4 for *X. pubescens* and *X. sulcatipes*, Richards and Course 2015). Bees overwinter as adults, and the majority of dispersal takes place in spring (Peso and Richards 2011). In southern Ontario, there are two distinct foraging phases: the nestmate

provisioning phase, when pollen is collected to feed to adult nestmates, and the brood-provisioning phase, when pollen is gathered to feed to developing offspring (Richards and Course 2015; Vickruck and Richards 2017). The nestmate provisioning phase lasts 2–3 weeks and is followed by the brood-provisioning phase. Dispersal takes place during the nestmate provisioning phase, when females can stay in their natal nest, leave the population altogether, excavate a new nest of their own, or join an established nest. During the brood-provisioning phase, dominant females provision offspring, although other adults in the nest presumably could also consume these provisions. *Xylocopa virginica* females are mass provisioners, collecting pollen and laying eggs in a serial manner. The brood-provisioning phase lasts approximately for 6–7 weeks. Previous work demonstrates that in social nests, there is usually only one primary forager, rarely two, although secondary females occasionally bring tiny pollen loads as well (Richards and Course 2015).

### Field site and activity periods

*Xylocopa virginica* nests were studied at the Glenridge Quarry Naturalization Site (GQNS), in St. Catharines, Ontario, Canada (43.122, – 79.236 decimal degrees). Within the park were five wooden bridges constructed over dry ditches; each bridge housed a separate nesting aggregation of 10–22 nests (bridges named A, B, C, D and F). Eastern carpenter bees reuse nests for many years and these bridges became available to the bees in 2004, 7 years prior the start of the study. All aggregations were within 500 m of one another.

In 2011, bees were first observed foraging on 12 May, and the last day of foraging was 7 July. That year, marking and observation of females took place after the brood-provisioning phase had already commenced. In 2012, the first foraging day (also the first day of the nestmate provisioning phase) was 15 May. The brood-provisioning phase began on 28 May and lasted until 29 June (the last foraging day). In 2013, the nestmate provisioning phase began on 20 May, the brood-provisioning phase began 29 May and the last day of foraging was 1 July. We determined the onset of the brood-provisioning phase using pollen trip phenology as described by Richards and Course (2015). Detailed dates for the brood-provisioning phases across years can be found in Supplementary figure S1.

### Bee capture and marking

Bees were caught at the nest by placing ‘cup traps’ over nest entrances which comprised a plastic cup with a small hole in the bottom and a sealed lid was placed over each

nest entrance. Bees leaving the nest became trapped in the cup, at the time when they were chilled for approximately 10 min before marking and measuring.

Each bee was marked on its thorax with a unique two-paint combination with Testor’s © enamel model paint. Head width was measured with digital calipers across the widest portion of the head including the compound eyes. Wing wear was scored from 0 to 5, where 0 indicated perfect wing margins with no nicks or tears and 5 indicated wings with completely obliterated margins. One person (JLV) did all measurements to eliminate measuring bias. After marking, each bee was placed just outside its nest entrance to resume normal activities.

### Foraging observations and determination of nest status

Nest entrances were observed during the brood-provisioning phase of 2011, and during both the nestmate and brood-provisioning phases of 2012 and 2013, to track individual female activity across breeding seasons and between years. Once females began to fly in spring, nests were observed daily from 8:00 to 16:00 h, weather permitting. Observations ceased each year when an entire observation day passed (8 h) without seeing a single pollen trip by a female bee, indicating that the brood-provisioning phase was complete.

Cup traps were placed over all nest entrances in the morning to trap any females leaving the nest. Time, nest of departure, and bee colour code were recorded for each bee in the trap, and then the bees were released and the trap was replaced over the nest entrance. When a bee returned to the nest, the trap was removed to allow her entry. The time of her return, the nest which she returned to, as well as whether she was carrying pollen was recorded. At the end of the day, all cup traps were removed.

To determine if other bees remained inside a nest after a female had departed, a small plastic transfer pipette was inserted into the nest entrance. Females remaining inside would buzz or bite the end of the plastic transfer pipette, or block the entrance with their abdomen, preventing the transfer pipette from entering the nest. The presence or absence of a guarding female was recorded and used to determine whether the nest was solitary or social. Nests were classified as solitary if during the brood-provisioning phase only one female was ever seen bringing pollen to the nest and a second bee was never observed guarding the nest entrance. Nests were classified as social if more than one female were recorded in the nest during the brood-provisioning phase. As we were interested in the structure of queues in social nests, solitary nests were not included in these analyses.

## Assigning reproductive strategies to female bees

Assigning reproductive strategies to *X. virginica* females can be challenging, as it is difficult to know which females are inside a nest at any given time without destroying it. Richards (2011) categorized females at the end of the breeding season based on wing and mandibular wear patterns. In this study, we categorized females based on flight and foraging activity, as it was necessary to categorize primary females during the reproductive period to conduct removals. Females were categorized as primaries if they made at least three pollen trips over an 8-h observation period, based on the average daily foraging rate reported (pollen trips/day) by Richards and Course (2015). Rarely, two females from the same nest made three or more pollen trips on the same day. Under these circumstances, both females were designated as primary foragers. Following the removal of a primary female, another bee was categorized as the replacement primary if she made at least three pollen trips over an 8-h observation period. Bees were categorized as secondary females if they were seen flying outside the nest more than once during the brood-provisioning phase, but never did more than two pollen trips in a single observation day. To avoid confusion, secondary females that assumed the dominant position after a primary were removed and are sometimes referred to as “replacement primaries”, and in statistical comparisons, are referred to as “secondaries”.

Bees were categorized as tertiaries if they were never observed flying outside their nests prior to removal of all their primary and secondary nestmates. Tertiaries that eventually left their nests after removals were checked for wing wear to confirm that they had not previously engaged in flight activity. All tertiaries had wing wear scores of 0 or 1 (occasionally, bees slightly damage their wing margins while inside their tunnels) when first captured.

## Forager removal experiments

Social nests (those containing at least two females) at each of the five bridges were assigned to one of two groups: control or removal (Supplemental Figure S2). No nests identified as solitary at the beginning of the brood-provisioning phase became social by the end of the season. Females in both control and removal nests were marked and measured in the same manner. Since control nests represented unmanipulated reproductive queues, these were used to observe foraging activity and the frequency of changes in reproductive queues. Removal nests from which all females were eventually removed or marked were used to precisely assess group size and queue length during the brood-provisioning phase. In removal nests, foragers were caught in cup traps when leaving the nest and immediately stored in 70 or 100% ethanol on ice in the field and transferred to a  $-20^{\circ}\text{C}$  freezer at

the end of each day. Adult females removed from nests were dissected to assess ovarian development, matedness, and abdominal fat content. Ovarian development was assessed by scoring oocytes as fractional scores as compared to a fully developed egg (1,  $\frac{3}{4}$ ,  $\frac{1}{2}$ ,  $\frac{1}{4}$ ). After scoring, oocytes were summed to give total ovarian development. Females were classified as mated if there was sperm present in their spermatheca. Females were categorized as fat if they had visible fat deposits in their abdomens and skinny if there were no visible fat deposits.

The procedures used for removal experiments varied from year to year. In 2011, removals were done at a single nesting aggregation containing 16 social nests (Bridge F). Primary females were removed from 8 nests on 13 June 2011. There were no control nests that year, as the remaining nests were used for a different experiment. After the primary was removed, each female that made three or more pollen trips on a single day was deemed to be the replacement primary and was removed via cup trap the next time she left the nest. The time it took between the removal of the primary female and a replacement taking over was recorded. Tertiary females that eventually left their nests were marked and measured, then returned to their nests so we could assess survival and dominance status in the following year. Nests were observed daily from 13 June to 7 July, when foraging had ceased in all nests.

In 2012, observations and experiments were carried out at 60 social nests across all five nesting aggregations (A, B, C, D and F), with 22 nests designated as controls and 38 designated for removals. To test whether the timing of removal would affect the likelihood that a subordinate would become a replacement primary, we implemented two removal periods, early (30 May–6 June 2012) and late (13–15 June 2012). In total, 56 females were removed from the 38 removal nests. The last female to emerge from a nest was marked and measured when possible and was then returned to the nest to overwinter.

In 2013, only 17 nests (aggregations A, B, C, D, and F) were selected for removals, due to a population decline and increased solitary nesting. Removals began on 9 June. As this was the last year of the experiment, all replacement primary and tertiary females were removed from experimental nests when possible so that ovarian development, fat content and matedness could be assessed.

## Data analysis

All data analyses were conducted in R version 3.1.2, running under RStudio version 0.98.1056 (RStudio, Boston, Massachusetts, USA). Although nests are reused from year to year, colonies were treated as statistically independent units. Several variables including wing wear, ovarian development, rate of wing wear accumulation, and the number of bees per



nest were not normally distributed and were analyzed using Kruskal–Wallis tests. Rate of wing wear accumulation calculated as wing wear at time of removal minus wing wear at time of marking divided by the number of days between the two dates. Fisher's exact tests were used to examine whether the last bee in the queue was significantly more likely to be a secondary or a tertiary female and whether the number of nests from which immatures were ejected differed across years. Chi square analysis was used to ask whether the proportion of females with fat in their abdomens changed across reproductive strategy. Lastly, to understand the effects of body size on reproductive strategy, we used a linear model with head width (HW) as the explanatory variable. In this model, reproductive strategy was nested within removal nest to account for variation within group members.

## Results

### Group size and queue length

Group size and composition were examined in removal nests in which every female was individually identified; note that all of these colonies occupied nests constructed in previous years. From 2011 to 2013, removal nests contained 2–4 females (Table 1). In five two-bee (P+T) nests from 2012, in which the primary female was removed early in the brood-provisioning period, the female initially identified as a tertiary eventually began to forage, despite never previously leaving the nest. In some analyses below, we refer to these five females as “late replacement primaries”, since they initially presented as tertiaries but eventually made pollen trips, technically making them replacement primaries.

All social nests with three females contained one primary, one secondary and one tertiary, and all social nests with four females contained one primary, two secondaries, and a tertiary female. The number of bees per social nest varied significantly among years (Kruskal–Wallis  $\chi^2 = 10.13$ ,  $df = 2$ ,  $P < 0.006$ ).

### Foraging behaviour in control colonies

Control colonies (2012 and 2013 only) were used to assess the foraging behaviour of primary females and to quantify how often they were replaced during the brood-provisioning phase. Most nests (21/22, 95%) in 2012 and all 18 nests in 2013 contained a primary forager. The one nest in 2012 in which no female was designated as the primary contained females that made fewer than three pollen trips per observation day. It is possible that this nest did contain a primary female, as she could have made additional pollen trips outside the 8-h observation window. One colony in 2012 and

**Table 1** Composition of social colonies, based on the numbers of primary, secondary, and tertiary females identified in removal nests

Type of colony	Year			Total
	2011	2012	2013	
Social (all nests)	16	60	35	111
Nests designated as controls	0	22	18	40
Nests designated for removals	8	38 <sup>a</sup>	17	63
<i>Composition of removal nests</i>				
P+T	7	12	13	32
P+S	0	0	0	0
P+S+T	0	22	3	25
P+2S+T	1 <sup>2</sup>	4	1	6
Total females removed <sup>b</sup>	9	56	28	93
Females per nest	2.2 ± 0.7	2.7 ± 0.6	2.3 ± 0.6	2.55 ± 0.7

<sup>a</sup>Twenty-five early removal nests, thirteen late removal nests. In five early removal nests, the tertiary female eventually began to forage

<sup>b</sup>In each year, some secondary females did not return from a pollen trip or disappeared between days. In addition, not all tertiaries were successfully removed from their nests (2011: 1 female not captured; 2012: 12 females not captured; 2013 12 females not captured)

one in 2013 contained two primary females that foraged simultaneously.

In 23% (5/22) of nests in 2012 and 11% (2/18) of nests in 2013, the primary female was succeeded by a second forager mid-season. In 2012, the last observed foraging day was 29 June, but in three nests, foraging finished on or before 13 June, more than 2 weeks before the end of the brood-provisioning phase. A female was present inside all three nests, but never foraged. These three nests may represent instances where the primary female did not survive to the end of the brood-provisioning phase, but the final female in the nest did not become a replacement primary.

### The structure of dominance hierarchies within social nests

The vast majority of social nests (109/112, experimental and control nests combined) contained a single primary forager at the start of the brood-provisioning phase. Only four nests across all three sample years contained two primary females that foraged simultaneously. Two of these were control nests (one nest in 2012 and one nest in 2013) and two were removal nests (both in 2012). With a single exception, all removals ( $n = 93$ ) produced one of two outcomes: either a single female began to forage and became the replacement primary in the nest, or the nest became inactive, with a tertiary female remaining inside, leaving the nest very occasionally for nectar feeding but never making a pollen trip.

In a single nest in 2012, the removal of the primary female resulted in two secondary females simultaneously becoming replacement primaries in the nest. In no removal nest did a female retain secondary status (making less than two pollen trips a day) once the primary female had been removed.

The final bee in a reproductive queue was significantly more likely to be a tertiary female than a secondary (Fisher's exact  $P < 0.00001$ ). In cases where the removal of the primary left two or more females in the nest (31 occasions), a secondary female always began to forage and became the replacement primary (31/31 removals). After 32 removals, only one bee remained in the nest. In 27 of these 32 occurrences, the final bee was a tertiary female that never made a pollen trip, even though she was now the sole occupant of the nest. However, in five cases, the final bee, which had been classified as a tertiary, eventually began to forage and thus became a late replacement primary. These five females all belonged to two-bee, early removal nests (Table 1).

Following the removal of the primary bee, secondary females that became replacement primaries made their first pollen trips  $5.0 \pm 3.2$  (range 1–13) days after removal, while late replacement primaries took  $8.5 \pm 3.8$  (range 4–13) days to begin foraging. With the exception of the late replacement primaries, tertiary females did not make pollen trips, but this was not because there was no time remaining in the brood-provisioning phase. On average,  $16.0 \pm 4.9$  (range 7–31) days remained until the end of the brood-provisioning phase (Kruskal–Wallis  $\chi^2 = 33.19$ ,  $df = 2$ ,  $P < 0.0001$ ).

### Physical characteristics of primary, secondary and tertiary females

Head widths of females in social nests varied not only across female type but also within nest. Reproductive strategy was a significant predictor of body size, late replacement primaries were smaller than primary, secondary or tertiary females ( $F = 7.27$ ,  $df = 3$ ,  $P = 0.01$ , Fig. 1a). The interaction between reproductive strategy and nest was also significant, indicating that head width differences within nests were important (reproductive strategy nested within nest  $F = 2.99$ ,  $df = 164$ ,  $P = 0.048$ , overall model  $F_{(8,166)} = 3.07$ ,  $P = 0.04$ ). Primary females were on average  $0.14 \pm 0.39$  mm (1.8%) larger than tertiaries and  $0.05 \pm 0.34$  mm (0.6%) larger than secondaries in their own nests. Secondary females were on average  $0.19 \pm 0.36$  mm (2.6%) larger than tertiaries with which they were nesting.

Patterns of wing wear differed among primary, secondary and tertiary females (Fig. 1b, c). At the time of removal, primary females had the most worn wings, followed by secondary females and then tertiary females (Kruskal–Wallis  $\chi^2 = 35.80$ ,  $df = 2$ ,  $P < 0.00001$ ; Fig. 1b). However, primary and secondary females accumulated wing wear at similar rates (Kruskal–Wallis  $\chi^2 = 0.85$ ,  $df = 1$ ,  $P = 0.36$ ; Fig. 1d).

Tertiary females were removed at first sight and were not given the opportunity to accumulate wing wear.

Dissections of females removed from social nests showed differences in ovarian development and fat stores among different types of females. Ovarian development was significantly higher in primary and secondary females than in tertiary bees (Kruskal–Wallis  $\chi^2 = 8.47$ ,  $df = 2$ ,  $P = 0.01$ ; Fig. 1d). Females of different reproductive strategies also showed differences in whether or not fat was present in their abdomens. The majority of primary females contained no fat in their abdomens, while all tertiary females dissected had abdomens that contained fat stores ( $\chi^2 = 38.33$ ,  $df = 2$ ,  $P < 0.00001$ ; Fig. 2). Of the 93 females dissected, all were mated with the exception of one tertiary female from 2012.

### Longevity and success of tertiary females

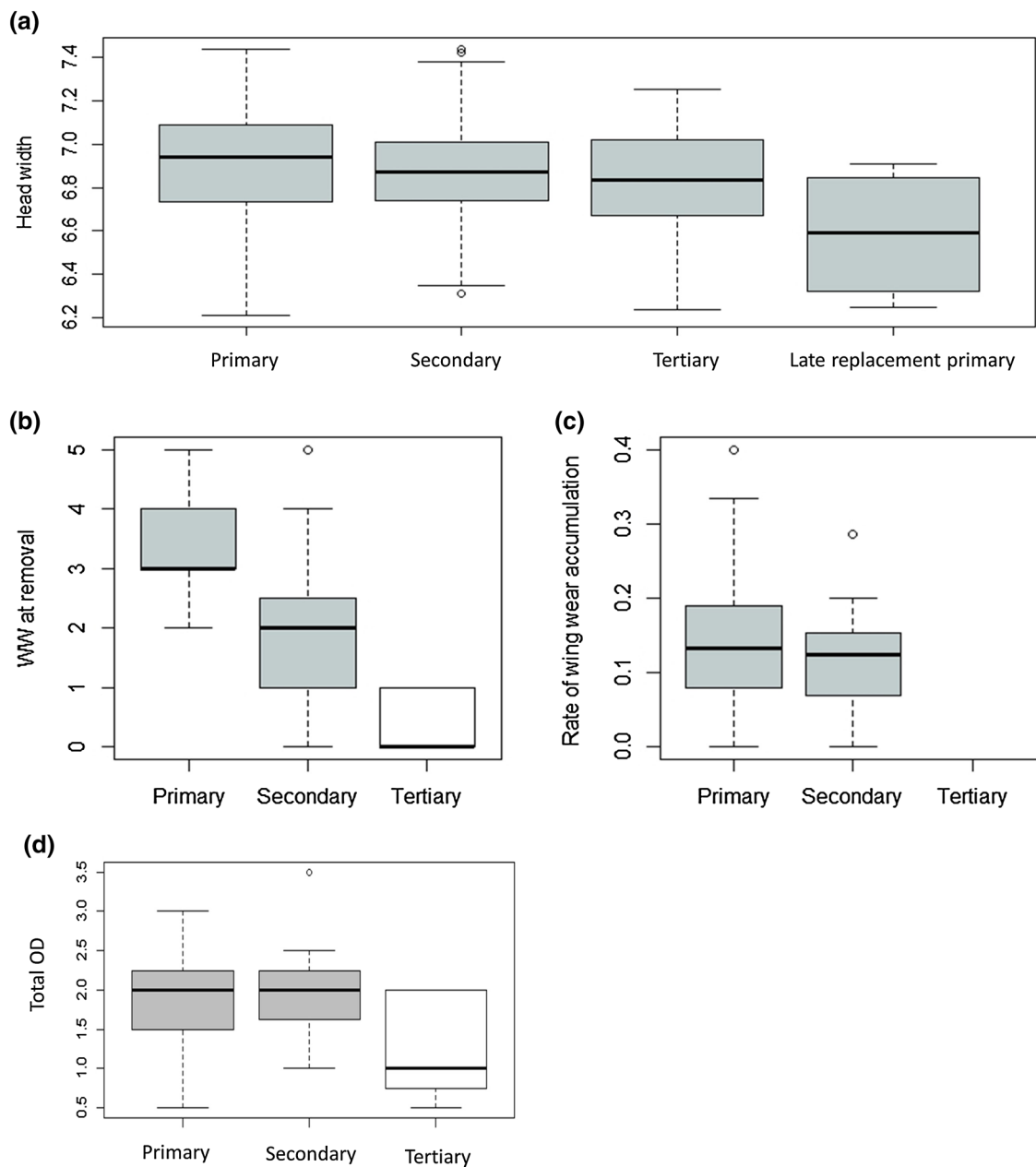
Over the 3 years of the study, we were able to track the fate of tertiary females that had been marked from the previous year. Ten tertiary females were marked at a single aggregation during the 2011 field season. Seven (70%) of them successfully overwintered twice and were seen again in 2012. Two of these seven bees (29%) became primary females in their second summer, two (29%) became secondary females and three (42%) were never seen again. In 2012, fourteen tertiaries were marked across all aggregations, six (43%) of which were seen again in 2013. Three of these six (50%) became solitary females in 2013 and three were not seen again.

Tertiary females were also seen ejecting immature offspring of primary females and replacement primary females out of experimental nests. This behaviour only occurred in experimental nests after all primary and secondary females had been removed. Offspring ejection was observed in 2/8 (25%) of removal nests in 2011, 8/40 (20%) removal nests in 2012 and 4/17 (24%) removal nests in 2013. The proportion of removal nests from which immatures were ejected did not differ across years (Fisher's exact,  $P = 0.87$ ). In contrast, offspring were never ejected from control nests or when secondaries or primaries were present in the nest.

## Discussion

### Characteristics of primary and secondary females

In this study, we built on previous observations in which three female behavioural strategies were inferred from late summer patterns of wing wear and ovarian development (Richards 2011), and observational studies that suggested turnover of principal foragers (Peso and Richards 2011; Richards and Course 2015). Here, we used an experimental approach to demonstrate that eastern carpenter bee



**Fig. 1** Physical characteristics of females in social nests. **a** Head widths (mm) among different types of females in social nests. **b** Wing wear at time of removal; **c** Rate of wing wear accumulation. **d** Total ovarian development for females removed from experimental nests in

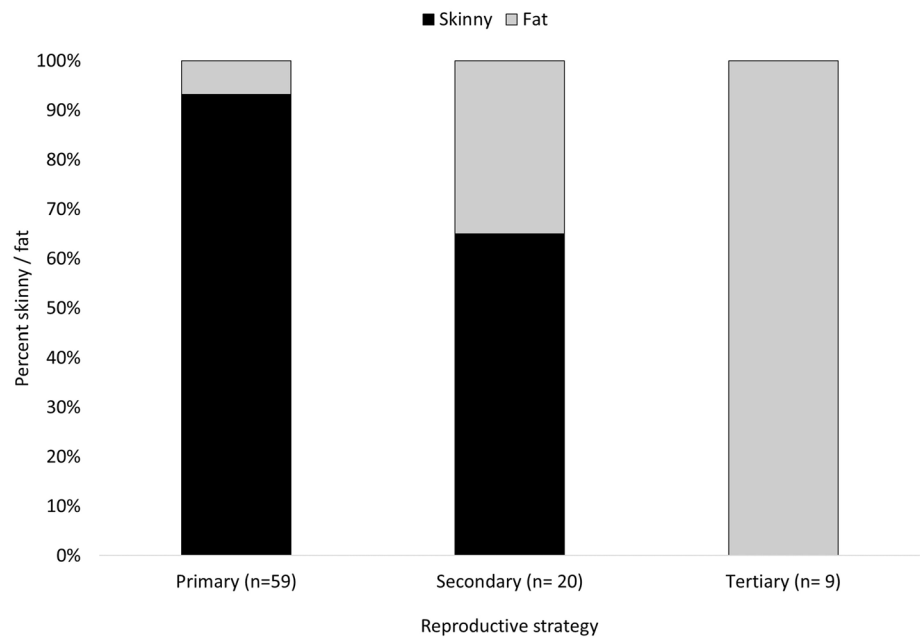
2012 and 2013. All secondary females were replacement primaries at the time of removal. Grey boxes represent groups of reproductive females, white indicates non-reproductive females

societies are indeed based on reproductive queues in which dominant, primary females monopolize egg laying, while subordinates wait for their chance to move up the queue into the dominant position. We also confirmed that there are two subordinate strategies: secondary females wait for the chance to reproduce in the current breeding season, while tertiaries wait for the chance to reproduce in the next breeding season, a year later. Below, we describe physical

characteristics of each type of female, focussing on tertiary females for which we know the least.

In *X. virginica* colonies, primary females are active reproductives. In our study population, they were on average larger than tertiary, but not secondary, females, and had high-ovarian development scores, worn wings and minimal fat stores in their abdomens. The vast majority of nests contained a single primary forager, indicating that there is

**Fig. 2** The number of females from different reproductive strategies that either possessed fat in their abdomen (fat) or did not (skinny) across all years. Primary females were the dominant foragers in the nest, secondary females were replacement foragers at the time of removal. Tertiary females were the last females to be removed from each nest



typically only one position at the top of the reproductive queue. Primary females were also never observed ejecting brood from the nest. Because they occupy queue position one, primary females immediately gain direct fitness by producing their own offspring. They may also derive the benefits of assured fitness returns if they die before the end of the brood-provisioning season, because their broods are protected by the presence of other adult females in the nest (Gadagkar 1990; Smith et al. 2003).

In this study, primary survival to the end of the brood-provisioning phase was high (79% in 2012 and 86% in 2013), whereas in previous studies, only 40–65% of primaries survived this long (Richards and Course 2015). This suggests that in some years, secondary females have limited reproductive opportunities, whereas in other years they have a high chance of becoming the dominant egg layer. When experimentally given the opportunity to become the dominant reproductive, secondary females quickly initiate brood provisioning. Secondary females were larger than tertiaries (but not primaries) and had ovarian development scores comparable to those of primary females. Secondary females had intermediate levels of wing wear; many likely accumulate significant wear when they leave their natal nests and try to join a new social group (Richards and Course 2015). Some secondaries had fat stores in their abdomens while others did not, suggesting that queue position might correlate with physical condition, but whether fat stores influence potential fecundity is an open question. The subfertility hypothesis suggests that less fecund females become subordinate helpers because they gain more inclusive fitness by helping more fecund relatives than by breeding themselves (Craig 1983) and has been supported in the eusocial sweat

bee *Halictus sexcinctus* (Richards 2003) and the paper wasp *Ropalidia marginata* (Gadagkar 2016), but not in the hover wasp, *Liostenogaster flavolineata*, or the sweat bee, *Megalopta genalis* (Field and Foster 1999; Smith et al. 2009). Neither primary nor secondary females in our study were ever seen to make a pollen trip in a second season, indicating that joining the dominance hierarchy limits reproductive lifespan to one season.

### Characteristics of tertiary females: a novel behavioural strategy in social bees?

The tertiary strategy of postponing reproduction to a later breeding season appears to be unique among social insects, and it requires not only a change in behaviour but also doubling of female lifespan. However, a similar phenomenon is known in desert-specialist bees that can delay emergence until enough rain has fallen to induce flowering of their host plant (Rozen Jr. 1990; Houston 1991; Danforth 1999). For instance, *Amegilla dawsoni* (Family: Apidae), can delay emergence for as long as 10 years until conditions are suitable (Houston 1991). However, these desert specialists extend diapause. While tertiary females of eastern carpenter bees do not forage or lay eggs, they hibernate twice, and in their first summer are capable of flight and nest defence when necessary.

The tertiary females of *Xylocopa virginica* are both physically and behaviourally distinct from primary or secondary females. In our studies, tertiaries are significantly smaller than primary or secondary bees. They accumulate almost no wing wear, because they do not leave their natal nest until their second year. When dissected, all tertiary



females had large fat deposits in their abdomens, suggesting that by mid to late summer they are prepared for a second hibernation. Tertiary females had significantly more fat in their abdomens than primary or secondary females. Fat stores are an important source of energy during diapause, and if tertiary females are attempting to overwinter twice, having a sufficient fat store would likely increase overwintering success. Tertiary females likely maintain fat stores by remaining in the nest and eating pollen. Interestingly, Gerling and Hermann (1978) mentioned they could discern 1-year-old and 2-year-old *X. virginica* females in Georgia by the amount of fat in their abdomens, but did not provide details of how abdominal fat stores related to behaviour in the nest. It seems likely that the tertiary strategy exists in southern carpenter bee populations but was not noticed in earlier studies.

When given the opportunity to become the new dominant female in the nest, tertiary females almost all remained as tertiaries rather than becoming breeders in the current year. Given that almost all tertiary females delayed reproduction until the following year even when other females were removed, it is surprising that they displayed some ovarian development (although significantly less than primaries and secondaries). A related strategy has been noted in several species of eusocial bees and wasps, in which some first brood females (which usually become workers) disperse and begin diapause early, presumably to become queens in the subsequent year (Yanega 1988; Reeve et al. 1998; Mueller 2018). One possibility is that the presence of primary and secondary females suppresses ovarian development in tertiary females via aggressive interactions or chemical inhibition. Aggressive behaviour by dominant queens was shown to suppress ovarian development in workers of the sweat bee, *Lasioglossum zephyrum* (Michener and Brothers 1974) and have already been observed within *X. virginica* nests (Vickruck and Richards 2017), so it is possible that the removal of all other females in the nest allowed tertiary females to reactivate their ovaries. Currently, there is no evidence that dominant females influence others by pheromonal control.

All but one tertiary female had mated by the time of her removal. This indicates that the vast majority of tertiaries would have been able to lay either diploid or haploid eggs. Because tertiaries were never observed leaving their nests, this also indicates that mating may have taken place inside. Males are often allowed to enter nests at the end of the day (Peso and Richards 2011), potentially permitting mating between unrelated pairs inside. Twig-nesting carpenter bees (genus *Ceratina*) from the same subfamily (Xylocopinae) have been observed mating inside nests (J. Vickruck, pers. obs.). If tertiaries mate in their first year, they may have a slight advantage in the spring, as they would not have to mate prior to provisioning female offspring.

Tertiary females in experimental nests were observed occasionally ejecting the offspring of removed females. The killing of immature offspring produced by the previous dominant in the group has been observed in mammals (Packer and Pusey 2008), birds (Schmaltz et al. 2008; Quinn et al. 2010) and insects (van der Blom and Velthuis 1988; Hogendoorn 1996). Interestingly, in *X. virginica*, immature offspring were only ejected by tertiary females only after all other bees in the nest had been removed. This suggests that tertiary females are prevented from killing larvae and pupae while primary and secondary females remain in the nest. One explanation for this behaviour is that tertiaries are removing their competition for reproductive opportunities in the subsequent spring. By ejecting larvae and pupae that will compete with her for reproductive opportunities, tertiary females increase their chances of becoming the primary or perhaps even solitary female in the nest. Gerling and Hermann (1978) noted that one of the *X. virginica* nests they were using for X-ray analysis appeared to lose larvae at one point in the season. Destruction of immature brood cells is common in the carpenter bee *X. pubescens*, when usurping females often destroy much of the brood laid by the previous dominant female (Hogendoorn and Leys 1993).

Why do females become tertiaries? The only clue we have so far is their small size. We do know that there is considerable activity inside nests in spring (Vickruck and Richards 2017). Before females ever leave their nests to forage, they eject debris (dead bees, other insects, sawdust) from the nest entrances. Sometimes they also eject injured females with intact wings, suggesting that there are serious aggressive interactions prior to the onset of foraging. This is when we think reproductive queues and dominance hierarchies are formed. We suspect that small females rapidly lose in such interactions and can leave the queue and become tertiaries prior to the onset of any foraging activity. Once the nestmate provisioning period begins, tertiaries will be fed by primary and secondary foragers. In their second year, tertiaries must somehow overcome the disadvantages of small size to become the primary foragers in their nests. Over the years, we have accumulated multiple observations of small, second-year females that become primaries. In one case, a second-year female began nestmate provisioning before any other female in the population (Richards and Course 2015), suggesting that she reinforced her position as dominant by feeding her nestmates (Vickruck and Richards 2017). In fact, this particular tertiary female was never replaced as the dominant forager and survived to the end of the brood-provisioning period.

### Dominance hierarchies and conditional reproductive strategies in social nests

Behavioural observations and removal experiments suggest that eastern carpenter bee social groups comprise

linear dominance hierarchies in which subordinates await opportunities to move into the dominant primary position. They also provide further evidence that females have alternative options to joining reproductive queues. When a primary female was removed from the nest and a secondary female remained, she always assumed the role of replacement primary. This indicates that primary and secondary females form linear dominance hierarchies, in which primaries are dominant to secondary females. Both types of female are ready to capitalize on reproductive opportunities that may be presented to them, and along with solitary females, have assumed the conditional strategy of breeding in the current reproductive year. Turnover of primary females in social colonies (control nests in this study, Richards and Course 2015), and the ability of secondary females to become replacement primaries allows them to capitalize on direct fitness opportunities as they become available. Simple two-bee, dominant-subordinate relationships have also been documented in *Xylocopa pubescens* (Hogendoorn and Leys 1993) and linear dominance hierarchies are also seen in primitively eusocial wasps in the genera *Liostenogaster* and *Polistes* (Bridge and Field 2007; Zanette and Field 2009; Ishikawa et al. 2010).

In contrast to the primaries and secondaries that clearly form a reproductive queue, the tertiary females of eastern carpenter bees appear to have removed themselves from the reproductive queue in an attempt to delay reproduction until the following year. Postponement of reproduction requires females to hibernate twice and seems to be dependent on remaining more or less inactive in their natal nest throughout the entirety of their first breeding season. The small size of tertiaries suggests this is a conditional reproductive strategy that females choose in early spring, shortly after emerging from hibernation. Removal experiments demonstrated that this decision must be made during the nestmate provisioning phase, since no tertiaries left their nests until all other females had been removed. Even then, only 5 of 33 eventually began to forage during the brood-provisioning phase of the colony cycle, and only after more than 8 days. This suggests that after the decision to delay reproduction has been made, it is largely inflexible. These five females were among the smallest females in the dataset, all had the last position in their queues, and took a long time to begin foraging, supporting their classification as tertiary females, and suggesting that tertiaries occasionally can alter their reproductive strategy mid-season if the opportunity arises early enough to raise brood. Alternatively, these females might have been small secondaries in poor condition. This group, termed here as 'late replacement primaries' adds additional complexity to the system, and further demonstrates the remarkable behavioural flexibility of this social species.

## Conclusions

Eastern carpenter bee females in social nests display two different conditional reproductive strategies, either attempting to breed in the current year, or delaying reproduction until the following year. Primary and secondary females who are hoping to reproduce in the current year form linear dominance hierarchies, while tertiary females who are delaying reproduction rarely forage even when given the opportunity. Tertiary females are able to maintain fat stores, minimize wear and likely alter some key physiological components to double their life span. Given that reproductive hierarchy decisions are not sorted until after spring emergence, behavioural flexibility must be maintained across all individuals, as conditional reproductive strategy decisions are likely made based on interactions within the nest in spring.

**Acknowledgements** We would like to thank Jessi deHaan, Andrew Giroux and Konrad Karolak for fieldwork assistance as well as the Niagara Region Waste Management Division for supporting research at the Glenridge Quarry Naturalization Site. This research was supported by a National Science and Engineering Research Council (NSERC) postgraduate scholarship and an Ontario Graduate Scholarship to JLV and an NSERC Discovery grant to MHR.

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