Ecological Entomology

Ecological Entomology (2012), **37**, 283–292 DOI: 10.1111/j.1365-2311.2012.01363.x

An ecological analysis of mating biology of *Xylocopa virginica* in southern Ontario

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Abstract. 1. *Xylocopa virginica virginica* Linnaeus is a wide-ranging species with plastic nesting behaviour that appears to represent an intermediary between solitary and social nesting species. Over 3 years, a natural population was studied with the objective of quantifying the relationship among population dynamics, climate, female nest provisioning behaviour, and male mating strategy.

2. Males in the population congregated around female-occupied nesting sites before the beginning of nest provisioning by females; both resident and satellite male mating strategies were observed. Overall, the present results are consistent with female defence polygyny.

3. Male mating strategies were consistent across the three breeding seasons of our study, in spite of annual variation in population size, sex ratio, and weather. Male mating behaviour was also consistent with that seen in other populations with longer breeding seasons.

4. Adult non-breeding females that never leave nests are observed in nests throughout the breeding season and we hypothesise that males continue to defend territories after breeding females have mated because of a small probability they can mate with one of these non-breeding females.

5. These results are important to our understanding of the relationship between mating systems and the evolution of sociality, contributing data on the role of ecological factors to male mating behaviour. Collection of such data for a variety of species that differ in sociality is necessary for the comparative analysis that is required to fully elucidate coevolution of mating systems and sociality.

Key words. Annual variation, carpenter bee, mating behaviour, population dynamics, satellite, territorial.

Introduction

Seminal work by Emlen and Oring (1977), later expanded on by Shuster and Wade (2003), postulated that animal mating systems, and in particular male (female) mating strategies, depend on the spatial and temporal distribution of females (males). This theoretical work created a foundation on which our understanding of the evolution of mating behaviour now rests. In particular, for taxa with diverse mating systems, theory predicts ecological differences that can be tested. Paxton (2005) explicitly extended these predictions to bees. Mating strategies in bees have been well studied, partly because of the impressive range of strategies they display (reviewed in Alcock *et al.*, 1978; Eickwort & Ginsberg, 1980; Gerling *et al.*, 1989).

One group particularly well suited to the study of mating systems and the role of ecological factors is large carpenter bees, *Xylocopa* (Gerling *et al.*, 1989). These large bees are easily observed in nature and species within the taxon exhibit a wide range of mating systems. The group displays a range of social nesting patterns, from solitary to semi-social (non-breeding females act as guards) and foraging habits (Eickwort & Ginsberg, 1980; Gerling *et al.*, 1989), both relevant to theoretically predicted mating strategies (Paxton, 2005). Species within the group display all possible male mating strategies: some species have a lek-like mating behaviour in which males defend locations that have no apparent resources for females

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and males rely heavily on pheromones to attract females (Vinson & Frankie, 1990; Alcock, 1993, 1996; Leys, 2000). In other species, males may intercept females at foraging sites or nesting sites (Velthuis & Gerling, 1980; Alcock, 1991). Finally, in many *Xylocopa* species intra-specific variation in mating strategies exists. For example, Barrows (1983) suggests that *Xylocopa virginica virginica* Linnaeus males may show as many as five different male mating strategies.

Xylocopa virginica is particularly suited to inclusion in comparative studies to test hypotheses regarding the relationship between ecology and mating behaviour because of its variable social behaviour (Gerling *et al.*, 1989). In this species, a single female builds and provisions her own nest and lays all the eggs in this nest (Rau, 1933; Gerling & Hermann, 1978). Yet, other adult females are also often present in the nest and tolerated by the breeding female, suggesting the group represents a transition either to or from social nesting behaviour (Gerling & Hermann, 1978; Prager, 2008). Furthermore, the species is wide-ranging, with populations from the southern U.S. into southern Ontario (Hurd, 1978). Thus, intra-specific comparisons among populations provide an additional layer of variance in ecological factors that may influence both social and mating behaviour. Such comparative analyses must be preceded by a thorough description of male and female mating behaviour in each population. Several descriptive studies of male behaviour in this species exist (Gerling & Hermann, 1978; Barrows, 1983; Barthell & Baird, 2004; Barthell *et al.*, 2006), providing a good background on observed male mating behaviour in populations in the central and southern parts of the extensive latitudinal range of *X. virginica*. In the present study, we take advantage of a contained natural population that allows all individuals to be identified and readily observed, to achieve several goals: (i) we use combined demographic data and behavioural data to assess the role of ecological factors on mating strategies. (ii) We studied the same population over three consecutive breeding seasons to obtain general insight into mating behaviour that is independent of annual variation in the environment, especially weather. (iii) We collected female behavioural data in conjunction with male data, allowing us to detect shifts in male mating behaviour that occur in response to female behaviour. (iv) We related the phenology and mating behaviour of this northern-most population of the species to studied phenological data for the same species at lower latitudes. Overall, it is our goal to determine how ecological factors influence mating behaviour in a plastic system with multiple strategies.

Methods

Study site

Research was conducted from 2003 to 2005 on the campus of Brock University, St. Catharines, Ontario, Canada (43°07′21′N, 79°14′37′W). The study site was situated between academic buildings and residences, had limited shade and consisted mostly of a concrete walkway through a grass lawn. *Xylocopa virginica virginica* nested in seven cedar benches located along the walkway. In 2003, the site contained a total of 71 nest entrances, this number increased to 90 in 2004 and to 101 in 2005. Each nest entrance was uniquely identified and labelled. The study site was defined as all the area contained within approximately 10 m around each bench, for a total area of approximately 900 m^2. Detailed descriptions of the study site and benches can be found in Prager (2008). Throughout the study, benches remained in the same relative positions with the exception of a few small moves (< 1 m), and an approximately 10 m move of bench 7 during the winter of 2004–2005.

In our study site, bees nested exclusively in seven cedar benches; nests and females were thus clearly in a clumped distribution within the site. Each bench (approximately 2.5×1.5 m) was considered a territory based on male behaviour described below; this size is within the range of territory sizes previously observed in male *X. virginica* (Barrows, 1983; Barthell & Baird, 2004).

We identified all active individuals in this study site during the breeding season for the years 2003–2005. All flying individuals were caught in hand nets, marked using Testors® (Testor Corporation, Rockford, Illinois) enamel paint applied to the thorax and abdomen, and measured for size (Barthell & Baird, 2004). Marking bees made it possible to identify individuals in observational studies. Marking began with the first sighting of a bee (male or female) and continued daily throughout the season. While it is possible that some bees immediately and permanently left the site at the time of emergence from the nest, because every nest was observed on multiple occasions, all individuals that remained in the population were eventually marked. Thus, the number of marked individuals approximates the maximum active adult population size.

Male territorial behaviour

Each year of the study, we began behavioural observations of males when the first male of the season was observed hovering near a bench and continued until males were no longer observed at the study site. Patterns of residency throughout the day and season were determined from 10-min censuses at each bench. After a 1-min acclimation (males often react initially to human presence), the position and identity of all males and females within 1 m of the bench was recorded for 10 min. Horizontal position was recorded by dividing the three-dimensional space surrounding a bench into perimeters of 0.5 and 1 m from the bench; these distances were easily identified as the walkway was constructed of 0.5-m cement squares. Five censuses of each bench were carried out each day; we conducted censuses in the periods between observations quantifying male behaviour; approximately every 15 min.

We quantified male behaviour in a series of 15 min observation periods (Barrows, 1983) carried out throughout the day at each bench in turn. The order of observations was randomised each day with respect to bench to avoid bias that might be associated with daily variability in male activity patterns. In instances where no activity was observed for 5 min, observations at the bench were suspended until later in the day. If no activity was observed in two attempts, the bench was declared inactive for that day. *Xylocopa virginica virginica* is not active when it rains so observations were not made on days with precipitation. Complete sets of observations (i.e. those uninterrupted by rain) were conducted on 18 days in 2003, 7 days in 2004, and 9 days in 2005, for a total of 122.5 h of observations.

In 2005, we moved benches to determine whether males were particular to a specific bench, or location. In the first experiment, we moved benches small distances (1 or 2 m) and observed the response of both males and foraging females. In the second experiment, we moved bench 7, which was initially the most remote (nearly 25 m from the next nearest) to a more central position, less than 10 m from three other benches. The benches were moved during winter before any bees were active.

In association with another study, we collected data on the foraging rates of females at nests throughout the study site. During these observations, the identities of all individuals arriving, entering, and departing from a nest were recorded, as well as the time of day and whether pollen was visible on the legs. A period of behavioural observation began with the first indication of daily activity and lasted 6 h. However, if during the initial 30 min of the day's observation no activity occurred at any bench, then conditions were presumed inappropriate for bee activity and observations were cancelled for that day. Thus, observations should closely approximate daily activity. In 2003 and 2004, the order of observation was randomised with respect to bench. In 2005, observations could not be randomised with respect to order of observation owing to a concurrent study. These data were collected mid-June until late August of each year; each nest was observed at least once per week. Also in conjunction with another study, we examined various signs of nest use and re-use including debris below entrances, other signs of nest use, and video observation of behaviour within nests. Video observation was conducted with a video-boroscope (Everest VIT, GE Measurement and Control); a fibre-optic camera small enough to be placed and manipulated within a burrow. We incorporated this information on female foraging and nest maintenance behaviour with all other methods to create a comprehensive phenology for male and female bees in this population.

Data analysis

Weather data. Climate data were obtained from Environment Canada meteorological stations in the proximity of Brock University and retrieved from the Environment Canada online database of Canadian climate normals (http://www.climate.weatheroffice.ec.gc.ca/climate_normals/index_e.html). Data for 2003, 2004, and part of 2005 were obtained from the weather station at Port Weller, Ontario (WMO ID-71432, 43°15′N, 79°13′, approximately 15 km from the study site. Data from this station were not available from October 2005 through to September 2006, so data from the Niagara Falls station (43°1.800′N, 79°4.800′W, 13 km from study site) were used for this period. Based on these data, we calculated the number of 'good bee days'; days where the temperature was greater than 14 °C and there was no precipitation. *Xylocopa virginica virginica* does not fly when there is precipitation or when temperatures are below 14 °C (S. Prager, unpubl. data).

Territoriality. The difference in male residency patterns among benches was assessed in an ANOVA that modelled the average number of males at a bench against the independent categorical variables bench and year; year was included to account for annual variation in population size and in number of nests. We considered several possible independent factors that might explain patterns in male residency: the number of nests in a territory (whether active or not), the number of females observed within the territory, and the number of foraging trips by females within the territory. These factors were tested in separate regressions; all results obtained were robust to the most conservative Bonferroni correction for repeated tests (uncorrected P-values are reported in results). In each regression, the independent variable was nested within years to control for yearly variation.

We examined the spatial relationships between censused males, and both nests and females using the SADIE (spatial analysis by distance indices) method for testing spatial association, implemented via the SADIEShell graphical interface. Details of the SADIE method are available in Perry *et al.* (1999) and Perry and Dixon (2002). In simple terms, SADIE analysis uses spatially referenced counts to generate a clustering index that measures the proportions of data that fit into patched clusters or gap clusters. The SADIE association index is a two-tailed test that compares overlap between these clusters; generally a $P < 0.025$ is considered associated, a P between 0.025 and 0.975 indicates no association and a $P > 0.975$ indicates disassociation. We report all our results as a Dutilleul (1993) adjusted P. All analyses used the maximum of 9999 randomisations.

Behavioural data. To quantify male behaviour, we measured a series of male behaviours: loop (L), chase (C), chase and touch (CT), and chase and fight (CF). These behaviours are consistent with those previously observed in *X. v. virginica* territorial males (Eickwort & Ginsberg, 1980; Barrows, 1983; Vinson & Frankie, 1990; Barthell & Baird, 2004). A L was defined as a previously hovering male briefly leaving a territory and flying in a circular path not directed at an intruder or object before returning to his original position. Looping behaviour was conspicuous and appeared to be an advertisement or a male checking for the presence of other males (Barrows, 1983). Chase was defined as a hovering male flying towards an object (other than a female and typically another male), but not making contact with it. The CT was defined as a chase that was followed by light contact between the male and the object of pursuit. A CF was defined as a chase followed by prolonged contact, grappling, biting or the individuals falling to the ground; this is roughly equivalent to the 'pouncing' described by Barrows (1983).

Two behaviours related to male–female interactions were also quantified. Chase female (CFEM) was defined as a hovering male flying towards a female but not contacting her. A mating attempt (MA) was defined as a male chasing and grabbing a female with the result that the pair landed on a surface or flew out of the territory together. This is distinguished from CFEM where no contact was observed. Mating attempt events follow CFEM events; however, not all CFEM events result in mating attempts. It was not possible to observe copulation in most instances but MA behaviours are consistent with the precopulatory behaviour described by Barrows (1983). Behaviours were treated as single events, regardless of duration, and were recorded relative to a focal individual (male of interest), defined as the male that initiated the behaviour. All behaviours performed by all males within 1 m of the bench were recorded. As in previous studies on male bee territorial behaviour, we define territory based on the space in which a male hovers; in our study site a single male controlled the airspace immediately surrounding a bench ('nest site' in Barrows, 1983). Thus, males censused above a bench in the plurality (most instances but not necessarily half) of the census periods conducted on that day were categorised as residents. All other males within 1 m surrounding the bench ('nest periphery' in Barrows, 1983) were categorised as satellites ('interlopers' in Barrows 1983).

As behaviours are potentially associated, and to reduce the number of variables, we performed principal components analysis (PCA) on all the behaviours recorded during 15-min observations. We then compared the significant principal components (PC) between satellite and resident males (as defined above). Since analyses are unique to a day, a male may be a resident on one day and a satellite on another day. We compared each PC individually using General Linear Models conducted in SAS 9.1.3 (SAS Institute Inc., Carey, NC) (SAS Institute, 2004). Each model used PC score as the dependent variable and the following predictors: male identity (nested within year), status (resident or satellite), and days (days since the first territory was established in that year). The male identity term was included to account for repeat observations of a male on multiple days within a season and was nested within years to account for repeated use of male identification marks in different years. There was no effect of days in any model and this variable was subsequently excluded from the analyses.

We considered two factors that might affect male behavioural activity: territory quality and amount of male competition. We regressed total number of chasing behaviours (C+CT+CF) on the number of marked females in the territory and the number of female foraging trips from nests in that territory, hypothesizing that these were two possible predictors of territory quality. In both analyses, year was included to account for annual variation in behaviour. The role of male density on behaviour was examined with a similar model that substituted number of males censused near a bench as a predictor instead of females.

To see if resident males had greater access to females than satellite males, we compared the rates of both CFEM and MA events between resident and satellite males. As none of the principal components was significant in explaining CFEM or MA behaviours (Table S2), we performed analyses directly on the frequency of these behaviours. CFEM events were compared via ANCOVA using the number of events per male per 15-min observation period as the dependent variable and the fixed term status (satellite or resident); male identity (nested within year), days (the number of days since males established territories in a given year), and bench were included as random effects terms. Bench was included to control for potential differences in encounter rate associated with territory quality. We also performed these analyses substituting the number of nests in a bench or the number of females marked in proximity of the bench for bench, with no increase in the variation explained (r^2) and thus report only analysis using bench as a predictor. After removing non-significant terms from the model, the final model included only male status and days as predictors. Mating attempts were compared between resident and satellite males using χ^2 goodness-of-fit tests because too few attempts were observed to consider mating attempts as a continuous variable.

Results

Mating phenology of Xylocopa virginica

The phenology of *X.v. virginica* as it relates to mating was similar throughout the three seasons of the present study (Table S1). A few females first appear in late April, at a point which is approximately concurrent with temperatures rising above 14 °C. A few weeks later, females begin constructing and renovating nests and males begin guarding territories (Table S1). Critically, territories were always formed by males before females began flying to and from the nests (i.e. foraging) and thus males were in a position to mate with females almost immediately, as evidenced by the timing of male–female interactions (Table S1). Likewise, most males entered the population before females each year, and females remained active after males were no longer in territories (see last six rows of Table S1).

Territoriality

In each year of this study, overwintered males and females were first observed at about the same time, although males tended to become active before females (Fig. 1, Table S1). At this time, males were aggregating around benches. The total number of males in the population increased for a period of about 6 weeks after which it reached an asymptote; during this time, there were more active males than females at the study site (Fig. 1).

Variation the in number of males aggregating around benches each year suggests benches varied in quality (Fig. 2). The average number of males censused daily differed among benches (ANOVA: $F_{6,12} = 9.50$, $P < 0.001$) and among years (ANOVA: $F_{2,12} = 22.05$, $P < 0.001$) (Fig. 2a), but the relative preferences are consistent. Male density was highest in bench 5 in 2years and contained the most nests in all years, while bench 4 had the most males in the third year (Fig. 2a,b). Benches 3 and 1 appeared to be visited the least among the 3years. Bench 3 also had the fewest nests every year. This suggests that benches 4 and 5 were high quality whereas 3 and 1 were low quality.

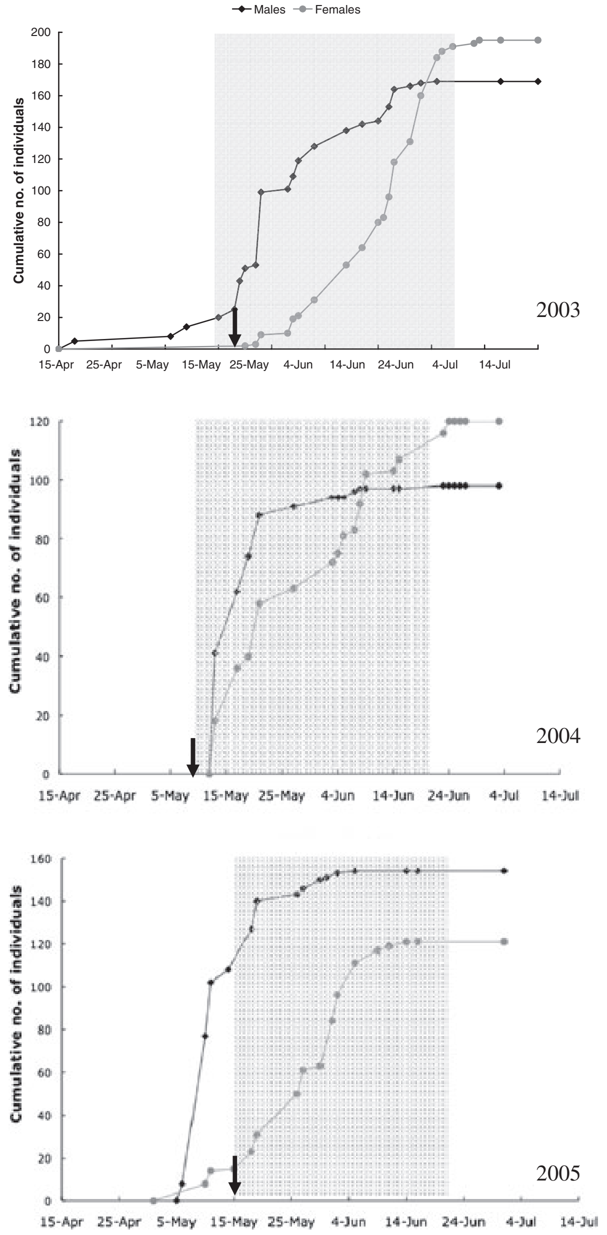

Fig. 1. The cumulative number of marked males (black diamonds) and marked females (grey circles) at the Courtyard site for each year of the study. Grey areas indicate the time from the first observation of a male defending a territory to the last male observed defending a territory. Arrows indicate first observed mating attempt.

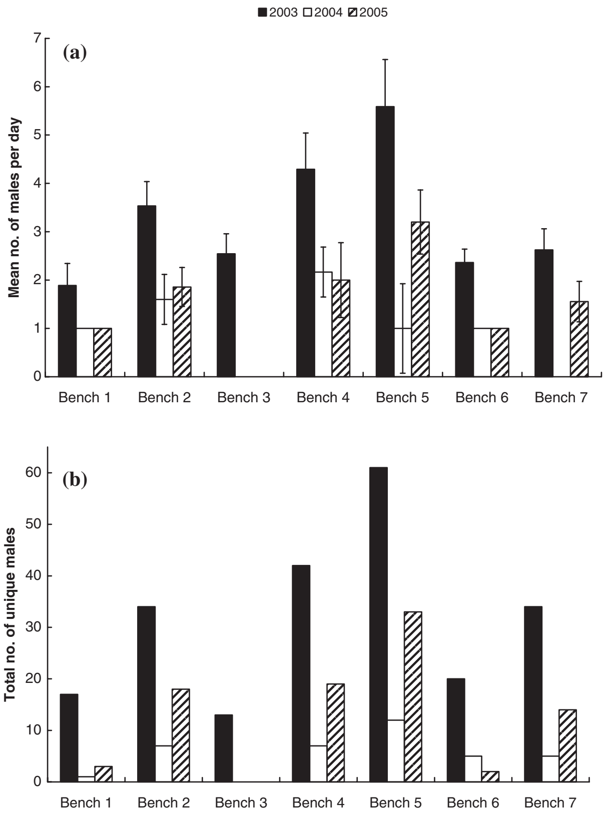

Fig. 2. (a) The number of unique males (mean±SE) censused per day by bench for 2003 (solid bars) ($n = 18$ days), 2004 (white bars; $n = 7$), and 2005 (hatched bars; $n = 9$). In 2004 and 2005, no males were censused near Bench 3. In 2004 and 2005, bench 6 was active on a single day and only one male was censused on that day. No SE indicates a sample size of one for that year. (b) The number of males marked near each bench for 2003–2005.

We interpret variation in the number of males within 1m of a bench to indicate variation in the perceived quality of a territory (the area around a bench). We considered whether males might be judging territory quality based on the number of nests (as expected in resource defence polygyny), or based on the number of females or female activity (as expected for female defence polygyny). The number of males at a bench was significantly associated with female presence (ANCOVA: $F_{1,15} = 12.09$, $P < 0.004$; Fig. 3a) but not with the number of nests (ANCOVA: $F_{1,15} = 1.78$, $P = 0.2$; Fig. 3b), although the non-significant result appears to be as a result of one outlier (Bench 1 in 2003). When this point was removed from the analysis, there was a significant relationship among the number of nests and males (ANCOVA: $F_{1,14} = 26.03$, $P < 0.01$). Furthermore, the number of males marked near a bench was associated with both the mean (Regression: $F_{3,17} = 9.01$, $P = 0.0009$, $r^2 = 0.62$) and the total number of foraging trips observed at that bench ($F_{3,17} = 30.82$, $P = 0.0001$, $r^2 = 0.84$; Fig. 3c). Analyses using SADIE showed that both nests ($\chi^2_{100} = 0.9397$, $P = 0.0001$, $n_{\mathrm{adj}} = 75.3$) and females ($\chi^2_{100} = 0.9319$, $P = 0.0001$, $n_{\mathrm{adj}} = 75.3$) are spatially correlated with the number of males.

The effects of moving bench 7 during the winter of 2004–2005 were complex. In 2005, we marked 27 males near bench 7 versus 9 in 2004. We also observed more males in 2005 (48 versus 22). This may correspond to an increase in active nests; three new nests were constructed in bench 7 in 2005. Conversely, this may reflect the greater number of female trips in 2005; we observed only 2 trips in 2004 and 12 in 2005. There was no difference in unique females observed in 2004 and 2005. Finally, moving the bench did not result in a change

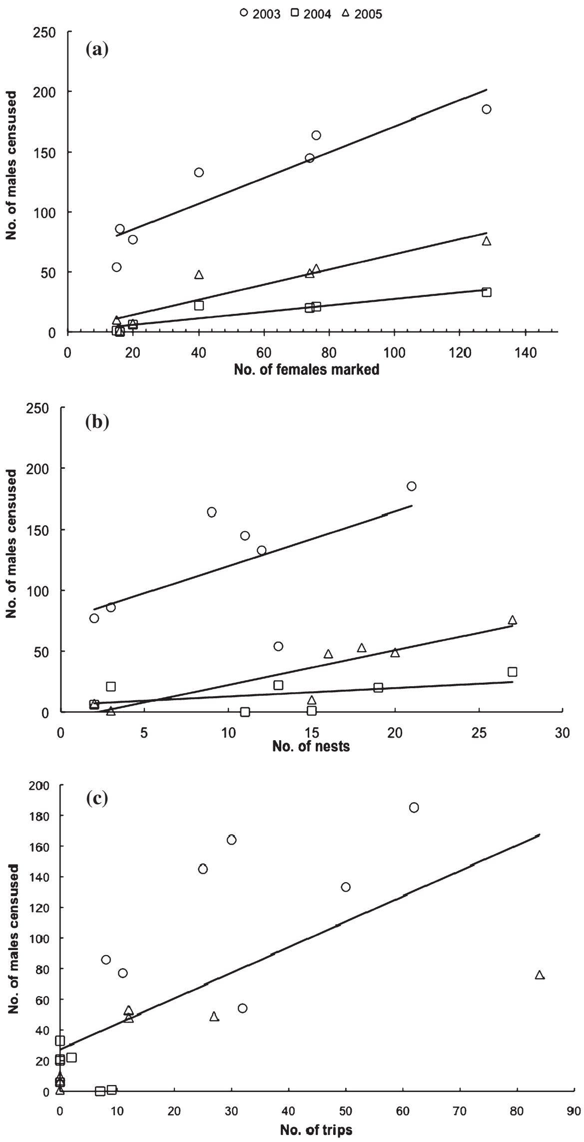

Fig. 3. The total number of males observed in censuses relative to (a) number of nests in the focal bench of a territory, (b) female density (females marked in that territory), and (c) female foraging. Sample sizes are 21 for all panels. The years 2003 (open circles), 2004 (open squares), and 2005 (open triangles) are shown separately. Data for each plot were analysed in a single ANOVA that included both bench and year as factors.

in the mean number of males per census at bench 7 versus the other six benches.

Censuses of male positions around benches clearly revealed the presence of a distinct resident (positioned above the bench) and satellite males (positioned around the periphery of the bench). These were observed both simultaneously at the same bench, and separately (e.g. only satellites or only residents at a bench). In each year, most censused males were observed only as residents above a bench whereas some individuals were only seen on the periphery (satellite). In both 2003 and 2004, some males were observed being both resident and satellite males on different occasions (Table 1). Typically, male status did not change at a given bench, but a resident would occasionally be dominant when they moved to another bench. All possible combinations of resident and satellite males were observed during the census study: only resident males present, only satellite males present, and both present. We also observed that when resident males were removed for marking, one of the satellite males typically assumed a territorial position above the bench. Removed males typically re-established their position when released and the temporary occupant of the territory returned to the periphery. In addition, when benches were moved short distances during the summer, we found that females became disoriented. Males, though, attempted to mate with these females and then re-established their original positions within the territories. Interestingly, males did not appear to change roles; those that had been territory holders moved to correspond with the same territorial bench. Satellite males would establish a position on the periphery of that, or one of the nearby, benches. Male ability to establish a position above the bench was not related to size. The head width of resident males did not differ significantly from that of all marked males in the population or from satellite males (Table 2; Wilcoxon's signed ranked test, $Z = 0.71$, $n = 998$, NS).

Behaviour

The PCA of the six male behaviours quantified returned four principal components (PC) with eigenvalues > 1 (Table S2). PC1 explained nearly 60% of the variation in behaviour and represents an increase in all male behaviours except MA. PC2, accounting for 20% of the variation in behaviour, primarily explains variation in male behaviours that involve contact (CT and CF) relative to those behaviours that do not (L and CFEM). PC3 reflects variation in mating attempts and accounts for 14% of the variation in behaviour. PC4 was most closely tied to CFEM behaviour and explained only 5% of behavioural variation. Resident and satellite males differed significantly only with respect to PC1 scores ($F_{1,362} = 37.89$, $P < 0.0001$, Table S2), with territorial males showing more chasing behaviours (C, CT, and CF) than satellite males; no other PC's showed significant differences.

We define C, CT, and CF as 'chasing' behaviours as they all relate to chasing another male away from a territory. We tested

Table 1. The number of censuses in which males were located above a bench, on the periphery, and in both positions.

	Only above bench	Only periphery	Above and periphery
2003	240 (67%)	32 (9%)	87 (24%)
2004	87 (91%)	9 (9%)	0 (0%)
2005	57 (45%)	16 (13%)	53 (42%)
Mean	128 (66%)	19 (10%)	47 (24%)

Data are presented for each year of the study; in each year seven benches were censused.

whether males perform more chasing behaviours in the defence of 'higher quality' territories using both a direct estimate of territory quality (number of males present) and an indirect measure of territory quality (number of female foraging trips). The number of chasing behaviours was positively associated with the number of males censused at a bench (Regression: $F_{3,17} = 25.07$, $P < 0.0001$, $r^2 = 0.94$; see Table S2). While this is an imperfect test because if all males are equally likely to chase any male coming within a certain distance, then this relationship will be positive even if male behaviour does not change with territory quality, this result was corroborated by a positive association between the number of chasing behaviours and the number of female foraging trips (Regression: $F_{3,16} = 16.91$, $P < 0.0001$, $r^2 = 0.76$; see Table S2).

Behavioural interactions with females varied between resident and satellite males. The model of CFEM behaviours was significant (ANOVA: $F_{145,218} = 1.45$, $P < 0.008$, $r^2 = 0.69$; status term: $F_{1,362} = 251.32$, $P < 0.001$), and resident males chased females more frequently than satellite males (frequency of CFEM ± SE per 15-min observation period: residents: 3.7 ± 3.6, satellites: 0.8 ± 1.7). Males were occasionally observed 'grabbing' and mounting a female, followed by the individuals flying together, often out of view. In other instances, the pair crashed to the ground. As these events usually took the pair out of the territory being observed, it was difficult to estimate the length of the events and impossible to know if the flight resulted in copulation. However, males often returned during the same 15-min observation period. Females frequently and visibly rejected males attempting to 'grab' them near nests.

We observed few mating attempts in any year. In 2003, we observed 7 attempts by resident males and 11 by satellites, when examined against each of 3 sets of expected values: equal, 9 to 9 ($\chi_1^2 = 0.5$, $P = 0.48$); and skewed as 2 to 16 ($\chi_1^2 = 2.37$, $P = 0.21$) and 3 to 15 ($\chi_1^2 = 1.246$, $P = 0.26$) there were no significant differences. The sole MA observed in 2004 was performed by a resident. In 2005, all five observed MA were by resident males. Summed across all years, residents did not perform significantly more mating attempts than satellites (13 resident and 11 satellite) (χ^2 goodness of fit: $\chi_1^2 = 0.04$, $P = 0.8$).

Seasonal and geographical variation

We did not find a direct influence of weather (good bee days) on mating behaviour (Table 2). Sex ratio (measured as marked males and females) differed when pooled across years ($\chi_3^2 = 10.75$, $P = 0.01$; Table 2). However, in no individual year, did the ratio of males: female differ from an expectation of even (2003: $\chi_1^2 = 0.93$, $P = 0.37$; 2004: $\chi_1^2 = 1.2$, $P = 0.23$; 2005: $\chi_1^2 = 1.76$, $P = 0.16$; 2006: $\chi_1^2 = 0.95$, $P = 0.33$). Similarly, while we detected variation, we did not find any distinguishable patterns relating male strategy to weather. There was variation among years in the number of active nests and the total population size, as well as in the number of males that permanently left the site. Furthermore, in all 3 years for which we have data, we observed satellite males, territory holders, and males that permanently left the study site. While there was variation in both the proportion of nest sites that were active and in the proportion of males that left the study site, no trend linking the two was discernible from our data (Table 2).

Discussion

Males of *X. v. virginica* in southern Ontario are clearly territorial and have a mating strategy that resembles female defence polygyny, but which may actually be defence of a resource (nests). We identified the presence of an alternative mating strategy, satellite males, in the population; this substantiates earlier work from more southern populations of *X. v. virginica.* The consistency of males establishing territories at female nesting sites through three seasons that varied in population size, relative number of males and females, and timing of emergences and activity, is of particular interest given the extensive variability observed generally in male mating behaviour within *Xylocopa* (Alcock *et al.*, 1978; Eickwort & Ginsberg, 1980; Gerling *et al.*, 1989). Males establish territories before females are actively provisioning brood cells, and generally before females are active. Moreover, most males have been marked at a point in time when females are still entering the population. This suggests that females will encounter a male while still a virgin. It is not clear that all these encounters will result in copulation; however, it does suggest that males will continue to encounter females for an extended period, and that not only early territorial males will encounter virgins.

We found that density of males near a territory (territorial plus satellite males) is associated with the number females marked in a territory, the number of foraging trips conducted by females in the territory, but not the number of nests in a given territory; although, this last result may be the effect of a single data point (see the Results). Males almost entirely ignored potential territories with no active nests (S. Prager, unpubl. data), but these often have few females as well. We found that the number of both nests and females are spatially associated with censused males, and with each other. In addition, when a nesting substrate was moved males retained their preferences regardless of the proximity to other benches or territories. *Xylocopa* (*Lestis*) *aerates* males do not defend territories with no receptive females, but they do inspect the entrances of empty nests (Leys, 2000), something we saw no evidence of in *X. virginica*. Other *X. virginica* populations are purported to show female defence polygyny (Barthell *et al.*, 2006) and our results are consistent with males defending females directly but also defending a resource (nest substrate). Distinguishing between resource and female defense polygyny can be difficult and may even be impossible in some systems (Barthell & Baird, 2004), and this is likely for *X. virginica* also. A key and missing requirement to assessing this further in *X. virginica* is knowledge about the receptivity of females. Most bees are assumed to mate soon after emergence and then be unreceptive to further mating attempts (Eickwort & Ginsberg, 1980). There is a remarkable lack of data on female receptivity in *Xylocopa*, but some

Table 2. Male demographical and behavioural data relative to weather.

	2002	2003	2004	2005	2006
No. of good bee days w/males	–	20	12	15	28
Days males in censuses	–	18	6	9	–
No. of active nests	43	69	90	87	65
Total no. of nests	54	71	90	100	102
Total marked individuals	–	364	221	275	358
Number of females marked	–	195 (53%)	123 (56%)	121 (44%)	165 (46%)
Number of males marked	–	169 (47%)	98 (44%)	154 (56%)	193 (54%)
Number of males disappeared	–	64 (38%)	69 (70%)	90 (58%)	–
No. of marked males observed	–	105 (62%)	29 (30%)	64 (42%)	–
No. satellites	–	45	5	33	–
No. territorial	–	6	5	1	–
No. of both	–	22	0	19	–
Mean aggressive events/male/obs	–	3.9 ± 4.9	3.1 ± 4.3	2.4 ± 3.9	–
Satellites only	–	2.5 ± 3.7	1.8 ± 3.5	2.3 ± 4.0	–
Residents only	–	7.7 ± 5.8	5.1 ± 4.3	3.2 ± 3.0	–
No. mating attempts	–	12	1	1	–
Satellites only	–	8	0	1	–
Residents only	–	4	1	0	–
Mean chase-female events	–	3.5	4.3	2.7	–
Satellites only	–	4.5	5.6	2.6	–
Residents only	–	2.5	2.6	2.7	–
Mean HCW females	6.8 ± 0.25	6.8 ± 0.3	6.7 ± 0.29	6.7 ± 0.26	6.9 ± 0.34
Mean HCW males	6.3 ± 0.22	6.1 ± 0.39	6.2 ± 0.23	6.2 ± 0.27	6.2 ± 0.28
Satellites	–	6.5 ± 0.5	6.0 ± 0.26	6.2 ± 0.3	–
Residents	–	6.4 ± 0.4	6.3 ± 0.1	5.8	–

Number of males disappeared = number of males marked at emergence and not observed again on study site subsequently. HCW, head capsule width (mm). Good bee days are defined as those with a temperature above 14 °C and no rain. Only one male was exclusively a resident in 2005.

communal bees (Adrenidae) continue to mate throughout the flight season (Paxton *et al.*, 1999). We propose that male status (resident or satellite) is determined by a male's ability to chase competitors and through an advantage to initially holding a territory. Resident males performed more aggressive actions than satellite males even although aggressive behaviours involving contact have been shown to damage participants in other species of bee (Hurd, 1978). Numerous studies of male territoriality in insects have demonstrated that resident males have an advantage in male–male contests (Alcock & Bailey, 1997; Kemp & Wiklund, 2001, 2004), and numerous studies of *Xylocopa* indicate that males that are taken from, or leave, a territory, always re-gain resident status on return (Gerling & Hermann, 1978; Barrows, 1983; Alcock, 1991). Theory on resident advantage predicts that residents will be more aggressive in chasing away competitors. In tarantula hawk wasps contest durations between replacement residents and returning residents (experimentally removed from the territory) were positively correlated with duration of time the replacement resident had been on the territory, but uncorrelated with relative size of the two males (Alcock & Bailey, 1997). While we did not explicitly test resident male advantage, we do know that *X. virginica* males show no size difference between satellite and resident males (the present study; Barthell & Baird, 2004; Barthell *et al.*, 2006), whereas residents males were more aggressive (did more chasing of other individuals) and mostly retained their positions even when temporarily removed from the territory. Males also appear to become more aggressive over time; however, the available data do not allow this result to be empirically tested (S. Prager, unpubl. data).

Resident males pursued females with a greater frequency than did satellite males. This is possibly because of the increased potential for interactions associated with holding a territory, but it may also reflect less cost to residents if they chase unreceptive females. *Xylocopa virginica virginica* flights often take the pair out of a territory (Gerling & Hermann, 1978) and resident males that can regain their territories may be more likely to pursue females. In spite of pursuing females more often, resident males did not engage in significantly more mating attempts, although the number of observed mating attempts might have simply been too low to detect any differences. While we recorded over 700 male–female chases in three seasons, we observed only 24 mating attempts. Previous work in related taxa has found similarly low levels of mating (Alcock & Smith, 1987; Alcock & Johnson, 1990; Vinson & Frankie, 1990; Alcock, 1991; Leys, 2000). It is the rarity with which copulations are observed that has generated the presumption that females mate only once (Gerling & Hermann, 1978; Barrows, 1983; Barthell & Baird, 2004). While *Xylocopa* females appear able to refuse male mating attempts (Prager, 2008), there are circumstances in which females might benefit from mating multiple times (Alcock *et al.*, 1978; Arnqvist & Nilsson, 2000).

If females mate only once at first emergence in spring, the persistence of males defending nests through to the end of June might appear maladaptive. One possibility is that males that continue to defend territories are repelling parasites and predators from the nest and thereby increasing their offspring fitness (Barrows, 1983). This is unlikely; woodpeckers (or other nest predators) were never observed on the study site and resident bees were not observed chasing away either birds or parasitic flies. What does seem likely is that an extended female emergence period may mean that males remaining at the nesting site until nest provisioning has begun in all nests will acquire more mates than males that leave too early.

One might equally ask why males leave the nesting sites when they do. In some nests, non-breeding adult females are present throughout the season; the average number of females per nest in this population is greater than two (Prager, 2008). These adult females apparently rarely, if at all, leave the nest and may be unmated. We hypothesise that these females remain in their natal nest for their first breeding season and subsequently breed in their second year, in keeping with inferences about the life history of *X. v. virginica* in Georgia (Gerling & Hermann, 1978). If a small percentage of these females without brood are receptive, and the male has any non-zero probability of gaining access to and mating with such a female, then we would expect selection on males to remain territorial and actively chase away other males. These males are also expected to chase any female that emerges because of the small but non-zero possibility of one of these receptive females emerging, however briefly, from the nest. In addition, half of unworn females collected in the fall and winter are mated (S. Prager, unpubl. data; Barrows, 1983), indicating that some females must mate in late summer or fall. It is not clear how these females become inseminated, although males do patrol landmarks or plants in the summer and fall (S. Prager, pers. obs.). Males return to nests at night, but we have no evidence that males mate while in nests and it seems unlikely given the nature of mating flights and the spatial constraints imposed by nests. Note also that non-breeding females get food from the provisioning female, and thus do not need to leave the nest to forage (Prager, 2008). In this population, both females and nests were spatially clumped relative to foraging sites and desirable plant species, which were widely dispersed. Consequently, it should be far easier for males to locate females at nesting sites than at foraging patches. As males are emerging from the same nests that females are provisioning, there is a further reduction in the time necessary to search for mates. Paxton (2005) and others have predicted that in such a scenario males should exhibit some territorial behaviour at nests. Our data confirms this prediction across multiple years, and in varying densities of females and nests. We cannot rule out the existence of a third male mating strategy in this population: some males may be patrolling for females at foraging sites. Extensive acreage of suitable flowering plants near the study site, however, mean that this resource is widely scattered and thus female encounter rates are likely to be low.

Gerling and Hermann (1978) reported females first flying in Georgia in March, with provisioning from May to July and mating occurring from April to May. Barrows (1983) reports for Washington, DC that females make their first vernal flights in April; males form territories in April and May but only mated in the fall. Barthell and Baird (2004) reported that interactions among males and females in Oklahoma occurred between 11 and 31 May, and as late as June. In our study in Ontario, males emerged and formed territories in late April and May, the first important female activity began in May, and male–female interactions occurred primarily in June. This suggests a slight shift with mating events occurring later in Ontario, but it also demonstrates that events occur in the same phenological order.

We propose that *X v. virginica* has multiple mating strategies that may represent an evolutionary stable strategy, as proposed by Barthell and Baird (2004). Small males that cannot compete leave to guard at flowers, whereas larger males are territorial. Those males that emerge first take on resident roles, while later emerging males become satellites. Although satellite males appear to mate with similar frequency to residents based on our data, there is presumably some slight benefit to resident males (molecular data may be required to adequately assess this) so that with the physical costs of defending a territory, overall fitness is similar among the male strategies. Thus, all three strategies are maintained. Data on female receptivity through the flight season are required to test the veracity of this proposal.

In the present study, we found that *X. virginica* have a variety of mating strategies that are independent of ecological factors. This suggests that alternate mating strategies and tactics can be stable, and may not be in response to current ecological factors. Alternative mating strategies that are stable have the potential to promote evolution in a population in a way that plastic environmentally-induced traits do not. Mating systems are well known to be influenced by distribution characteristics of males and females, yet we found multiple male mating strategies can persist across years that vary substantially in population dynamics and weather variables. That male mating behaviour and rudimentary social behaviour (females tolerated non-nesting adult females in their nest) were consistent in an ecological time frame, further supports the potential inter-connectedness of mating systems and social systems. This study provides the basis for future work linking mating systems and levels of sociality in a variety of species with the ultimate aim of elucidating co-evolution between mating systems and social behaviour generally.

Acknowledgements

We thank Andrew Ironside, Candice Kerling, Elvis Law, Margaret Macanowicz-Hall, and Sandra Rehan for help in the field. John Barthell, Fiona Hunter, Xavier Martini, and an anonymous reviewer provided advice on various versions of this manuscript. S.M.P. was funded by a National Science Engineering Research Council discovery grant to M.H.R. and by funding from the Brock University Faculty of Mathematics and Science.

Supporting Information

Additional Supporting Information may be found in the online version of this article under the DOI reference:
10.1111/j.1365-2311.2012.01363.x

Table S1. Important phenological dates related to mating in *Xylocopa virginica* in St. Catharines, Ontario for the years of this study. All dates are based on behavioral observations, presence of nest debris below nest entrances, and video observation (except 2003). 2003 also includes data from nest dissections.

Table S2. Factor loading values for the first four principal components (PC) of variables related to male behaviour, and results of statistical comparisons between resident and satellite males.

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Accepted 10 April 2012