Drifting bumble bee (Hymenoptera: Apidae) workers in commercial greenhouses may be social parasites

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Abstract: Commercial greenhouses require high densities of managed bumble bee (*Bombus occidentalis* Greene, 1858 and *Bombus impatiens* Cresson, 1863) colonies to pollinate crops such as tomatoes (*Lycopersicon esculentum* Miller). We examined drifting, a behavioural consequence of introducing closely aggregated colonies into greenhouse habitats, to determine possible explanations for observed drifting frequencies. Bee drift is normally associated with increased individual mortality and disease transfer between colonies. In this study, individual bees frequently drifted into and remained within foreign colonies. More drifting bees were found in colonies with higher worker and brood populations and greater pollen stores. Increased intracolony aggressive interactions were not associated with a higher number of drifting bees. Drifting bees had a significantly greater number of mature eggs in their ovaries than did resident worker bees residing in colonies hosting drifters, suggesting that drifting could potentially increase the fitness of individual worker bees and may not be solely a function of disorientation and (or) nectar robbing. Taken together, our results suggest that drifting of workers into foreign colonies within greenhouses may demonstrate a predisposition to social parasitism. This selfish worker reproduction challenges our previous understanding of social insect societies as being cooperative societies.

Résumé : Dans les serres commerciales, il faut de fortes densités de colonies aménagées de bourdons (*Bombus occidentalis* Greene, 1858 et *Bombus impatiens* Cresson, 1863) pour assurer la pollinisation de plantes telles que les tomates (*Lycopersicon esculentum* Miller). Nous avons étudié la dérive, une conséquence comportementale de l'introduction de colonies fortement regroupées dans des habitats de serre, afin de connaître les facteurs possibles qui pourraient expliquer les fréquences de dérive observés. La dérive des bourdons est généralement associée à une augmentation de la mortalité individuelle et de la propagation de maladies d'une colonie à l'autre. Dans notre étude, les bourdons individuels dérivent fréquemment vers des colonies étrangères et y demeurent. Il y a un plus grand nombre de bourdons en dérive dans les colonies qui contiennent de plus fortes densités d'ouvrières et de larves et qui ont de plus grandes réserves de pollen. Aucune augmentation des interactions agressives à l'intérieur des colonies n'est observée en réaction à l'accroissement du nombre de bourdons en dérive. Les bourdons en dérive ont un nombre significativement plus élevé d'oeufs matures dans leurs ovaires que les ouvrières résidantes dans les colonies qui accueillent ces bourdons en dérive; il semble donc que la dérive puisse potentiellement accroître la fitness des ouvrières individuelles et ne pas être reliée seulement à la désorientation et (ou) au vol de nectar. Dans leur ensemble, nos résultats indiquent que la dérive des ouvrières vers les colonies étrangères dans les serres peut démontrer une tendance au parasitisme social. Une telle reproduction égoïste individuelle des ouvrières fait planer un doute sur notre interprétation des colonies d'insectes sociaux comme des sociétés de coopération.

[Traduit par la Rédaction]

Introduction

Bumble bees (*Bombus occidentalis* Greene, 1858 and *Bombus impatiens* Cresson, 1863) are the primary managed pollinator of commercial greenhouse tomatoes (*Lycopersicon esculentum* Miller) (Banda and Paxton 1991; Kevan et al. 1991; Dogterom et al. 1998). Techniques to establish and maintain colonies have been successful, but substantial adult mortality can occur in greenhouse environments (Morandin et al. 2001*a*, 2001*b*, 2001*c*; Whittington 2003), resulting in colony population declines and decreased pollination efficiency that are costly for growers. Management research has focused on pollination effectiveness, optimal colony density, the effects of nutrition provided by tomato pollen, interspecific interactions among bees, and the benefits of glass versus plastic greenhouse coverings (Dogterom et al. 1998; Pressman et al. 1999; Morandin et al. 2001*a*; Whittington 2003). However, conspecific behavioural interactions have not been well-studied in this environment.

Bee drift, when a bee leaves one colony to join another, generally has been associated with orientation errors (Jay 1966; Pfeiffer and Crailsheim 1998; Paar et al. 2002) and could be a factor contributing to bumble bee loss. Drift is a

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well-documented phenomenon for honey bees housed in similar-looking hives kept in close proximity to one another (Free 1958; Jay 1965, 1966; Pfeiffer and Crailsheim 1998) and for alfalfa leaf cutter bees (Goerzen et al. 1995). Beekeepers attempt to minimize drift because of the potential for disease transfer (Goodwin et al. 1994; Fries and Camazine 2001), nectar robbing, and adult mortality (Free and Butler 1959; Jay 1966, 1968). Drifting out of colonies may increase individual worker fitness by increasing their chance at reproduction, but likely decreases natal colony fitness (Jay 1968; Goodwin et al. 1994), and nest-mate recognition is important in this context to minimize the extent of drifting (Downs and Ratnieks 2000). Aggressive interactions from foreign bees entering a hive could lead to less time spent foraging and the early development of reproductives (Bloch et al. 2000*a*; Duchateau and Velthius 1989), resulting in a smaller foraging work force and a decrease in the amount of pollination achieved by each colony.

Orientation cues such as nest entrance patterns and spatially nonuniform apiary layouts are usually employed to decrease the amount of bee drift (Jay 1966; Goerzen et al. 1995). Bumble bees generally nest in the ground in preexisting burrows, commonly disguise their hive entrances (Free and Butler 1959), and normally do not aggregate their nests (Gamboa et al. 1987; Kearns and Thomson 2000), whereas in greenhouses colonies are highly visible and are located in one central area. Commercial producers provide nest boxes with identical outward appearances, and hive entrances are not cryptic. A typical commercial greenhouse contains a homogenous distribution of tomato plants repeated in regularly spaced rows, providing worker bees with clear visual access to a large number of similar-looking hives. The uniformity of a greenhouse environment may further promote bee disorientation and loss, leading to increased bee drift, subsequent pollination decline, and colony expenditure increase. However, when bumble bees were provided with nest entrance patterns or large landmarks scattered throughout the greenhouse, the amount of bee loss and drift was not reduced (Birmingham and Winston 2004), suggesting that alternative biologically important factors may affect the propensity for worker bees to drift.

Bumble bee queens exert reproductive dominance over workers through pheromones and aggression, resulting in reduced or absent worker ovary development (van der Blom 1986; Duchateau and Velthius 1989; Cnaani et al. 2000). Social interactions also influence ovary activity (Duchateau and Velthius 1989), but some dominant workers develop ovaries and lay eggs (Bloch et al. 2000*a*). This occurs mostly in the final phase of a colony's life cycle when worker populations are high (Duchateau and Velthius 1989; Cnaani et al. 2000). Workers perceive the onset of larval queen development, signifying the end of a colony's cycle, and often begin to lay unfertilized male eggs (Cnaani et al. 2000). Since greenhouses contain many colonies at varying stages in their life cycles, worker bees might have unusual opportunities to drift into foreign colonies to achieve a higher dominance ranking via agonistic acts (Duchateau and Velthius 1989) and (or) lay eggs.

Social parasitism, when a parasite benefits from brood care or resources of a host, is common in *Bombus* species (Schmid-Hempel 1998). *Bombus occidentalis*, the primary pollinator of greenhouses in British Columbia, recognizes and attacks foreign bees and other intruders, as its nests are commonly usurped by the closely related social parasite *Psithyrus* Lepeletier, 1833 in nature (Free and Butler 1959; Kupper and Schwammberger 1995). *Bombus occidentalis* should therefore have strong kin and colony recognition mechanisms (Gamboa et al. 1987; Plowright and Fuller 1988) to minimize such social parasitism. Conspecific reproduction of foreign bees entering a colony may represent an incipient stage of such social parasitism as observed in *Bombus terrestris* (L., 1758), where worker bee drifters in foreign colonies successfully laid and reared male eggs to adulthood (Lopez-Vaamonde et al. 2004).

In this study, we report characteristics of colonies and of drifting bees that suggest commercial greenhouse environments encourage a predisposition for parasitic behaviour through drifting, rather than drift being only a consequence of disorientation or other errors. We predicted that (*i*) drifting workers would drift into more populous late-stage colonies with good pollen stores because they would provide more likely opportunities for worker reproduction, (*ii*) drifting bees would have more developed ovaries than resident bees in colonies hosting drifters, and (*iii*) aggressive behaviour would be higher in colonies hosting more drifters because drifters would need to defend themselves and assert their dominance.

Methods

Data were gathered from three different greenhouses: Gipaanda Greenhouses Ltd., Century Pacific Greenhouses Ltd., and Windset Greenhouse, in Delta, British Columbia. Data were collected from Gipaanda between May and June 2001 and May and August 2002, from Century Pacific between June and August 2001, and from Windset between April and August 2002.

Greenhouse descriptions

Gipaanda Greenhouses Ltd.

The 6-ha greenhouse produced tomatoes (*Lycopersicon esculentum* 'Rhapsody') planted in north–south directional rows from a central aisle. Colonies were situated along the centre aisle in vertical stacks of three hives 3.5–4.5 m above the leaf canopy and were placed on support beams with a 20-cm separation from the top of one colony to the bottom of the next. Each stack of colonies was separated by five rows of tomato plants, with each stack alternated north and south along the centre aisle and with hive entrances facing either east or west.

Colonies of *B. occidentalis* were supplied by Biobest Biological Systems Ltd. and shipped from a rearing facility in Leamington, Ontario, Canada. Bees were contained in 35 cm × 25 cm × 20 cm plastic boxes inside a Styrofoam-insulated cardboard box with a bottom feeder containing a sugar and preservative solution. Colonies arrived at the greenhouse once per week and each contained 30–100 worker bees. Wax cells already built by the workers had been insulated with cotton wool, which was removed prior to bee marking and not returned to the colonies.

Century Pacific Greenhouses Ltd.

The 9-ha greenhouse produced tomatoes planted in north–south directional rows from a centre aisle. Colonies were stacked in horizontal groups of four, set 5 m deep from the centre aisle at a height of 1.5 m above the ground (within the leaf canopy). There were five rows of plants between stacks. Colonies were placed on a shelf with 1–5 cm of space between each colony, with hive entrances facing either east or west.

Colonies of *B. impatiens* were supplied by Koppert Industries and shipped from a rearing facility in Romulus, Michigan, USA. Bees were contained in 35 cm × 30 cm × 20 cm plastic boxes inside a Styrofoam-insulated cardboard box with a bottom feeder containing a sugar and preservative solution. Colonies arrived at the greenhouse once per week and contained 30–50 worker bees. Wax cells built by the workers prior to shipping had been insulated with cotton wool, which was removed prior to bee marking and not returned to the colonies.

Windset Greenhouse Ltd.

Windset Greenhouses produced 5.8 ha of tomatoes on the vine, of which the experimental arena (phase III) was 1.93 ha. Tomatoes were planted in north–south directional rows from a centre aisle. Hives were positioned in groups of three and stacked two high, with a third hive beside the bottom hive. Groups of colonies were placed on a shelf 3.5 m above the leaf canopy, with hive entrances facing either east or west. There were 10 rows of plants between stacks. Each set of stacks was directly across the central aisle from another set.

Biobest Biological Systems Ltd. supplied the *B. occidentalis* colonies as described above.

Marking procedure

All bees in a study colony from Gipaanda and Century Pacific in 2001 were marked on their thorax or abdomen, or thorax + abdomen, with a colony-specific colour or combination of colours using Liquid Paper™. Bees from Gipaanda and Windset studied in 2002 were marked with coloured numbered tags supplied by Opalithplattchen (Christian Graze KG, Weinstadt-Endersbach, Germany) that were glued to their thoraces. During marking, all bees were removed from a colony at one time and kept on ice until they were sluggish enough to be handled, marked in succession, and then placed back into their hive. Hive boxes were marked according to the colour of the bee tags and week of introduction to the greenhouse. Placement of hives into the greenhouse was random with respect to possible hive locations that were available.

Colony assessments

The colour and (or) number of all marked bees were noted for every hive surveyed. Data were collected on the number of marked workers, unmarked workers, queens, males, brood (the number of pupae, open pupae, larval masses, and egg masses), honey pots, and pollen pots. Drifters were defined as marked bees that were found in a colony from which they did not originate.

Gipaanda (2001)

Every bee from 18 *B. occidentalis* colonies were marked over 7 weeks starting in May. For 3 weeks following greenhouse introduction, hives with marked bees, all hives in a stack containing an experimental hive, and the hive in the stack positionally adjacent to a marked hive were assessed. From July onward, an additional 12 colonies were marked over 6 weeks.

Century Pacific (2001)

A total of 12 *B. impatiens* hives were introduced over 3 weeks, one each to the end of a group of hives. Marked colonies were observed and assessed for 3 weeks after greenhouse introduction, but colonies surrounding the study hive were not surveyed.

Gipaanda and Windset (2002)

A total of 32 *B. occidentalis* colonies were marked over 16 weeks at each greenhouse. Hives were assessed similarly as described above for Gipaanda (2001) (with surrounding colonies surveyed) for 2 weeks following greenhouse introduction.

Aggression survey

Behavioural surveys of colonies of *B. occidentalis* were conducted at Gipaanda and Windset greenhouses in 2002. Bees were observed for 60 s prior to colony assessments, and again 5 min later. Colonies were assigned an aggression index value from 1 to 5 when they were first opened, with 5 being the highest aggression value based on the number of aggressive acts witnessed. Specific behaviours recorded included the number of bees seen buzzing, running, flying, and posturing (raising one or more legs in a defensive manner). To score a value of 5, all the specific behaviours would had to have been seen and (or) a stinging event would had to have occurred.

Bee collection and dissection procedure

A total of 125 marked *B. occidentalis* were collected from Gipaanda and Windset greenhouses between May and August 2002 for dissection, consisting of 5 marked drifters and 5 marked original resident bees selected randomly from on top of the brood wax cells of the same colony. Bees were removed from hives 2 weeks after their introduction to the greenhouse. Drifter and original resident bees were placed in separate plastic petri dishes and stored frozen prior to dissection, which occurred within 3 months.

Bombus occidentalis were removed from the freezer and allowed to thaw for a minimum of 5 min. Bee tag colour and number were noted and then removed, and the distance between the eyes of each bee was also measured to indicate size (Wilson 1971). An index from 1 to 3 of wing wear was used to approximate the age of the bee, with 3 indicating the greatest amount of wear because wing wear is considered a reasonable measure in comparing bee ages (Cartar 1992). All measurements were taken using an ocular micrometer.

Bees were dissected in 70% ethanol under a dissecting microscope. The basal oocyte length of the terminal oocyte and ovary length to the last nutritive cell excluding the membrane was measured from the left and right ovaries. The distinction between mature eggs and oocytes was determined using a descriptive index (Duchateau and Velthius 1989).

The number of mature eggs and the total number of oocytes were counted. The left and right ovaries then were weighed separately then discarded.

Data analysis

Square-root transformations ($\sqrt{x + 3/8}$) were performed when the data were non-normally distributed because the small counts formed a Poisson distribution (Zar 1984). Regressions were examined in JMP IN version 4.0.3 (SAS Institute Inc. 2001) and ANOVA comparisons were analysed with SAS version 6.12 (SAS Institute Inc. 1997). Site-specific differences and interactions between greenhouses were accounted for in the statistical models. Results are reported as means with 95% confidence intervals (±95% CI).

When the number of drifters was positively correlated with increased worker populations, worker relationships to the amount of queens, males, brood, honey pots, and pollen pots were determined. Where the worker population relationship to other colony factors was significantly positive, a drifter to worker ratio was used to remove the covarying effect that all colony variables increase as the worker population increases.

Results

Colony assessments

For *B. impatiens* in 2001, a positive relationship was found between the mean number of drifters and the size of the adult worker populations ($r^2 = 0.36$, $p = 0.04$; Fig. 1) and male populations ($r^2 = 0.38$, $p = 0.03$; Fig. 2). There was no relationship between the mean number of drifters and of queens ($r^2 = 0.0020$, $p = 0.89$; Fig. 2), brood ($r^2 = 0.089$, $p = 0.35$; Fig. 1), honey pots ($r^2 = 0.20$, $p = 0.14$; Fig. 3), or pollen pots ($r^2 = 0.073$, $p = 0.39$; Fig. 3).

For *B. occidentalis* in 2001, a positive relationship also was found between the number of drifters and of adult workers ($r^2 = 0.070$, $p = 0.005$; Fig. 1). Worker populations (48.0 ± 5.9) increased significantly with the number of males (2.58 ± 1.2; $F_{[1,16]} = 16.7$, $p = 0.0003$), brood (84.8 ± 12; $F_{[1,16]} = 31.7$, $p < 0.0001$), and pollen pots (0.67 ± 0.30; $F_{[1,16]} = 4.67$, $p = 0.04$), but there was no relationship between the number of drifters and the number of males ($r^2 = 0.039$, $p = 0.68$; Fig. 2), the amount of brood ($r^2 = 0.059$, $p = 0.42$; Fig. 1), or pollen pots ($r^2 = 0.13$, $p = 0.30$; Fig. 3), when the number of workers was added as a covariate to the statistical model. The amount of honey pots ($r^2 = 0.23$, $p = 0.10$; Fig. 3) and the number of queens also did not have an effect on the number of drifters ($r^2 = 0.018$, $p = 0.53$; Fig. 2).

In 2002, *B. occidentalis* worker populations (44.8 ± 2.8) increased significantly with brood populations (60.1 ± 3.6; $F_{[1,30]} = 35.5$, $p < 0.0001$) and pollen pots (33.0 ± 2.2; $F_{[1,30]} = 17.1$, $p < 0.0001$), and was therefore added as a covariate to the statistical model. The number of drifters also increased positively with the amount of broods ($r^2 = 0.23$, $p < 0.0001$; Fig. 1), but there was no relationship with the amount of honey pots ($r^2 = 0.093$, $p = 0.10$; Fig. 3). The number of *B. occidentalis* drifters increased positively with increasing numbers of workers ($r^2 = 0.13$, $p < 0.0001$; Fig. 1), males ($r^2 = 0.034$, $p = 0.006$; Fig. 2), and pollen pots ($r^2 = 0.17$, $p < 0.0001$; Fig. 3), but not with the numbers of queens ($r^2 = 0.0026$, $p = 0.45$; Fig. 2).

In 2002, more *B. occidentalis* drifters were observed in hives that had been in the greenhouse for longer than 5 weeks, but younger than 11 weeks ($n = 12$; drifters: $\bar{y} = 1.55 + 0.124x$; $r^2 = 0.53$, df = 1, $p = 0.008$; Fig. 4). Greenhouse growers did not normally keep hives in the greenhouse longer than 13 weeks. Satellite colonies (small auxiliary colonies of mostly pupae and egg masses separate from the main colony area) and egg cannibalism by workers were observed during assessments of both bee species in both years.

Ovary dissections

Dissected drifting bees (*B. occidentalis*) had a greater number of mature ($F_{[1,125]} = 5.64$, $p = 0.02$) and immature ($F_{[1,125]} = 4.41$, $p = 0.04$) eggs in their ovaries than did resident marked bees of the colony they drifted into (41% of drifting bees versus 22% of resident bees had mature eggs; Fig. 5). Head width and wing-wear measurements of drifters (6.2 ± 0.16 mm and 0.82 ± 0.24, respectively) and residents (6.1 ± 0.18 mm and 0.78 ± 0.24, respectively) were not significantly different (head width: $F_{[1,125]} = 0.00$, $p = 0.97$; wing wear: $F_{[1,125]} = 0.09$, $p = 0.77$), suggesting that they were of similar size and age.

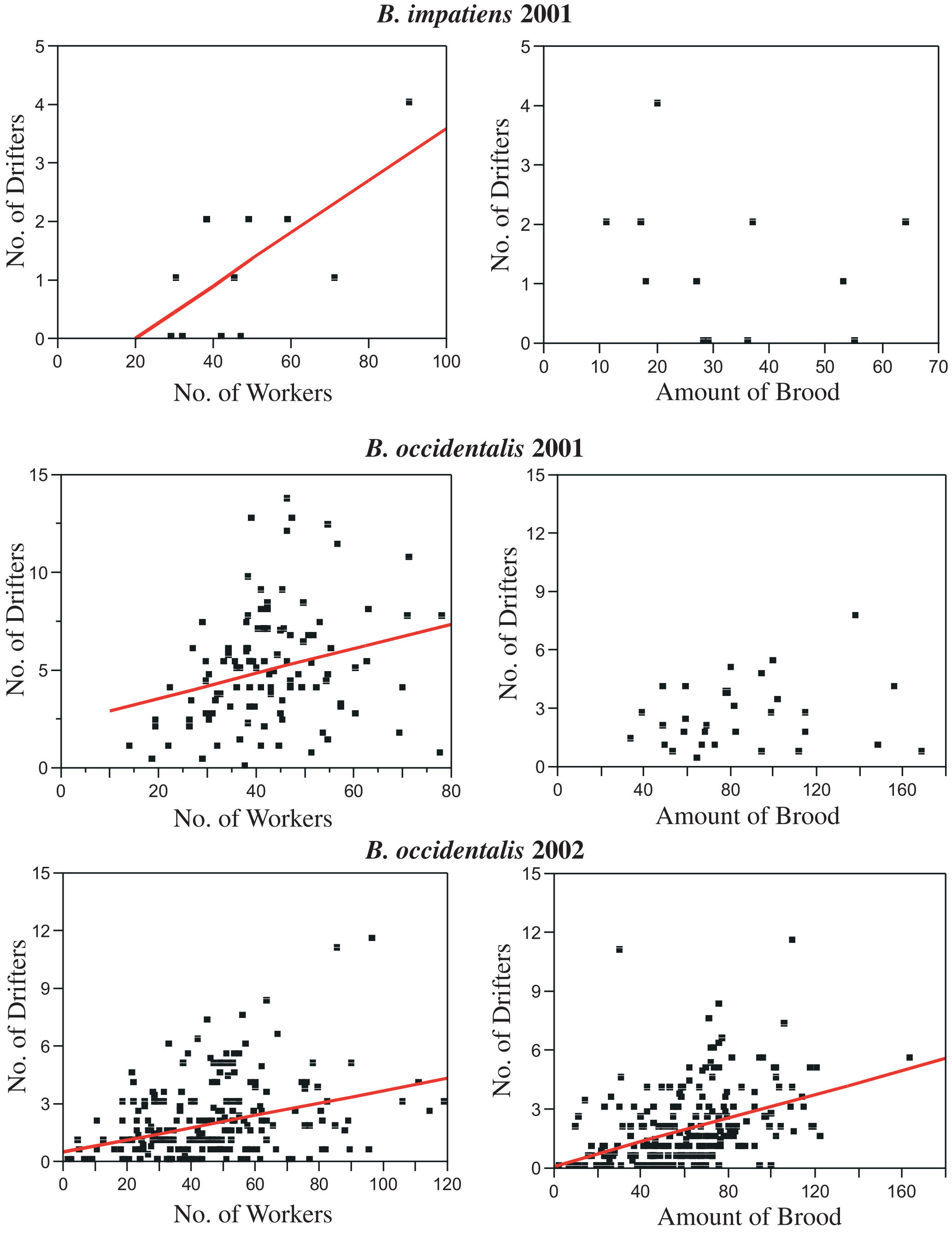
Colony assessment data of 16 *B. occidentalis* drifters with mature eggs in their ovaries revealed that they left hives with smaller worker populations and entered hives with larger worker populations ($F_{[1,14]} = 7.46$, $p = 0.02$), greater numbers of queens ($F_{[1,14]} = 19.9$, $p = 0.0006$), larger amounts of brood ($F_{[1,14]} = 18.4$, $p = 0.0009$), and less pollen pots ($F_{[1,14]} = 4.58$, $p = 0.05$; Fig. 6). The number of drifters ($F_{[1,14]} = 3.70$, $p = 0.08$), remaining marked bees ($F_{[1,14]} = 2.14$, $p = 0.17$), males ($F_{[1,14]} = 1.58$, $p = 0.23$), and honey pots ($F_{[1,14]} = 3.38$, $p = 0.09$) had no effect on colony choice for drifters with egg-laying potential (Fig. 6). Drifters without mature eggs in their ovaries were found in hives where colony characteristics were not significantly different from those in the colonies they left (drifters: $F_{[1,14]} = 0.0012$, $p = 0.97$; workers: $F_{[1,14]} = 0.00220$, $p = 0.963$; queens: $F_{[1,14]} = 0.348$, $p = 0.56$; males: $F_{[1,14]} = 0.407$, $p = 0.53$; brood: $F_{[1,14]} = 0.0347$, $p = 0.85$; pollen pots: $F_{[1,14]} = 2.33$, $p = 0.14$; honey pots: $F_{[1,14]} = 0.786$, $p = 0.39$; Fig. 6).

Drifting bees that were observed more than once comprised 31% of the total drifting population (leaving 69% of all marked drifting bees that were not seen in colonies after the first week they were described as drifters). The drifters seen on more than one occasion displayed three distinct patterns of drift: transient (found in 3.2 ± 1.6 foreign hives over a 4-week period), immigrant (drifted once and then remained in the new colony), and voyeur (drifted into a foreign hive but later returned to their original hive).

Aggression

Indices of aggression for *B. occidentalis* were not positively correlated with the number of drifters when the number of workers was used as a covariate in the statistical model ($n = 51$; index: 1.79 ± 0.22, $F_{[2,50]} = 2.47$, $p = 0.12$; buzzing: 2.79 ± 0.65, $F_{[2,50]} = 0.08$, $p = 0.78$; running: 1.71 ± 0.71, $F_{[2,50]} = 2.99$, $p = 0.09$; fliers: 0.91 ± 0.39, $F_{[2,50]} = 0.325$, $p = 0.57$; posturing: 0.90 ± 0.37, $F_{[2,50]} = 0.373$, $p = 0.54$), indicating that the number of foreign bees was not a predictor of the levels of aggression in a colony.

Fig. 1. The number of drifters in a colony of *Bombus impatiens* and *Bombus occidentalis* increased significantly with increasing colony population. The number of *B. occidentalis* drifters increased significantly with the amount of brood in both 2001 and 2002. As worker and brood populations increased between April and August 2002, the number of *B. occidentalis* drifters increased (worker: $r^2 = 0.13$, $p < 0.0001$; brood: $r^2 = 0.23$, $p < 0.0001$; $n = 221$).



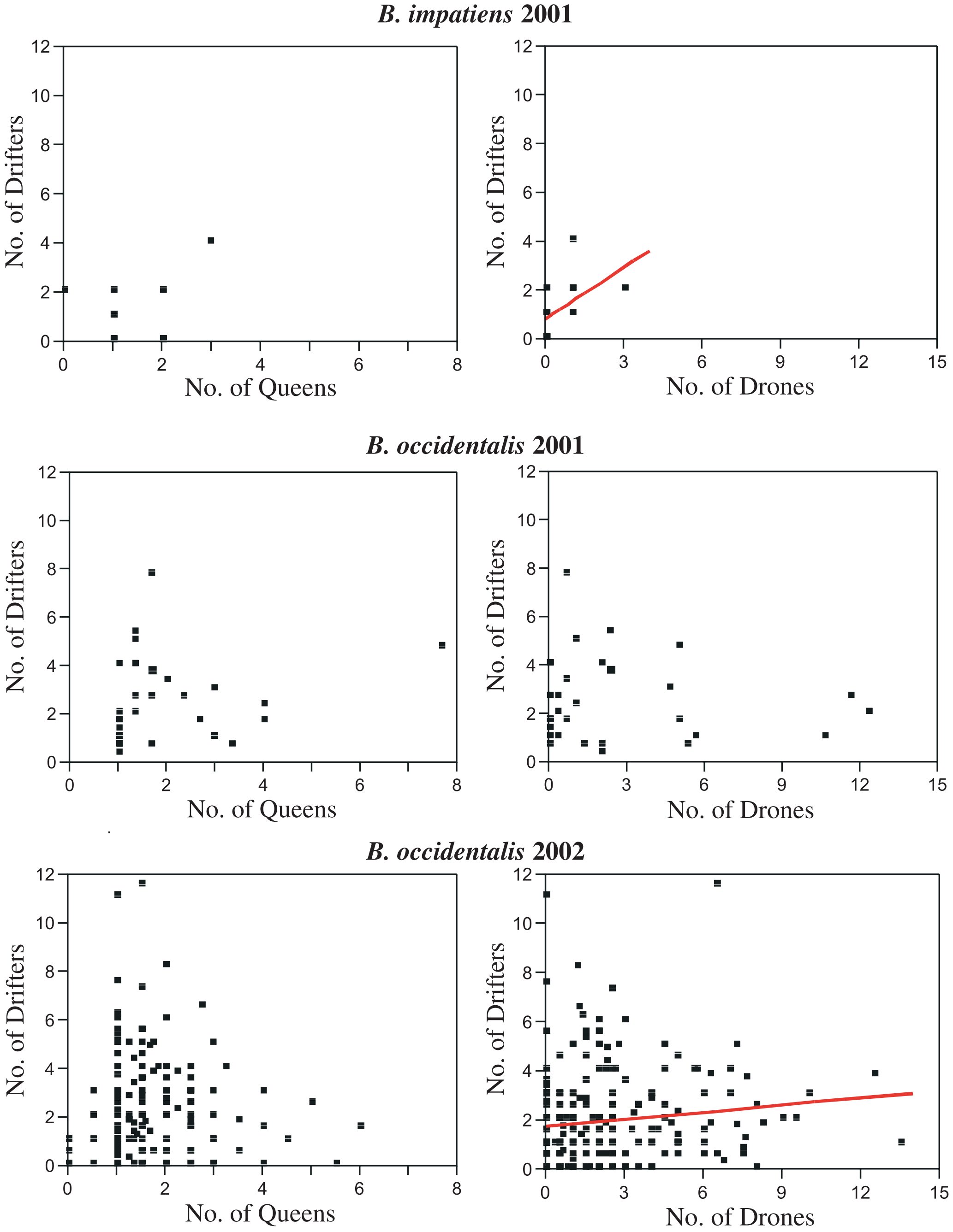
Indices of aggression increased significantly with increasing worker population ($n = 51$; index: $F_{[2,50]} = 3.95$, $p = 0.05$; buzzing: $F_{[2,50]} = 6.50$, $p = 0.01$; running: $F_{[2,50]} = 5.03$, $p = 0.03$; fliers: $F_{[2,50]} = 4.87$, $p = 0.03$; posturing: $F_{[2,50]} = 7.51$, $p = 0.008$), indicating that larger colonies were more aggressive.

Discussion

Bumble bee drifters (*B. occidentalis*) often were found in older hives with greater numbers of adult workers, brood, pollen pots, and males. Furthermore, *B. occidentalis* drifters had more developed eggs in their ovaries than resident nondrifting marked bees. Results for *B. impatiens* drifters were not as extensive, but we found significant positive relationships between increasing numbers of drifters and adult worker and male populations. Together, these results suggest that drifting may be a biologically meaningful strategy by workers to increase their individual fitness.

Aspects of these data provide evidence that bumble bee drifters act as opportunistic social parasites when in the greenhouse environment. Drifters entered hives with greater

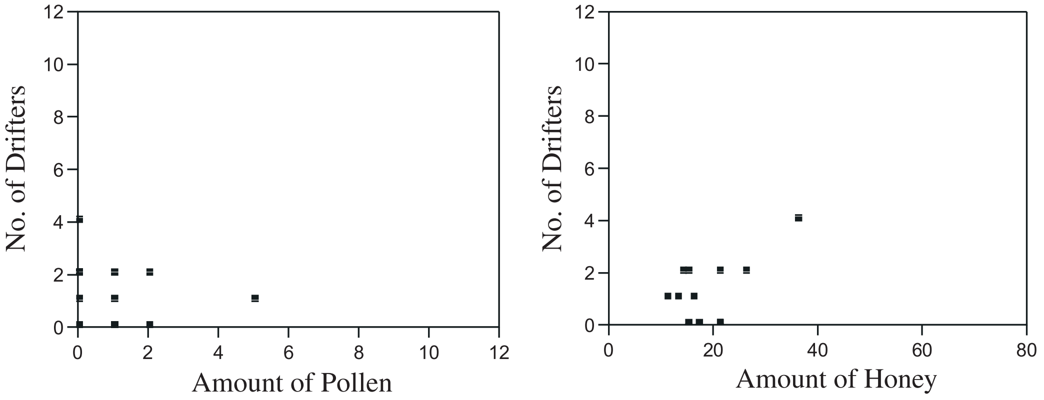
Fig. 2. The number of *B. impatiens* drifters increased significantly with the number of males (drone: $r^2 = 0.38$, $p = 0.03$), but was not significantly affected by the number of queens ($r^2 = 0.0020$, $p = 0.89$) ($n = 12$). The number of *B. occidentalis* drifters increased with the number of males only in 2002 ($r^2 = 0.034$, $p = 0.006$), but did not increase with an increase in the number of queens ($r^2 = 0.0026$, $p = 0.45$) ($n = 221$).



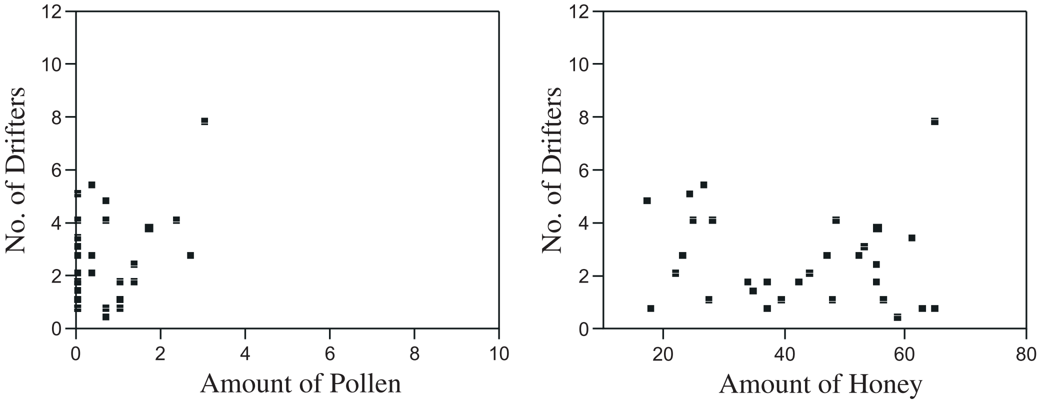
numbers of workers, greater pollen resources, and potentially less queen control because of their higher population, age, and onset of male production, which are colony attributes that would enhance the likelihood of successful egg production. The extent of ovary development was not dependent on the age or size of dissected drifters versus resident bees, suggesting that the increased ovary development seen for drifters was not an artefact of nutrition. Drifters with egg-laying potential left significantly smaller colonies to enter older, larger colonies with a greater amount of brood and where more reproductives had been produced, a point in colony cycles when other workers are less likely to eat worker-laid eggs (van der Blom 1986; Ratnieks 1988). Drifters without egg-laying potential did not demonstrate the same pattern of dispersal. Queen reproductive control over workers is reduced in colonies where reproductives are being produced, normally near the end of a colony's cycle (Duchateau and Velthius 1989; Cnaani et al. 2000). These factors taken together suggest that drifters with reproductive potential did not randomly choose which hives to enter but

Fig. 3. The number of *B. occidentalis* drifters increased in colonies with more pollen pots in 2002 ($r^2 = 0.17$, $p < 0.0001$, $n = 221$). There was no observed relationship between the amount of honey and the number of drifters.

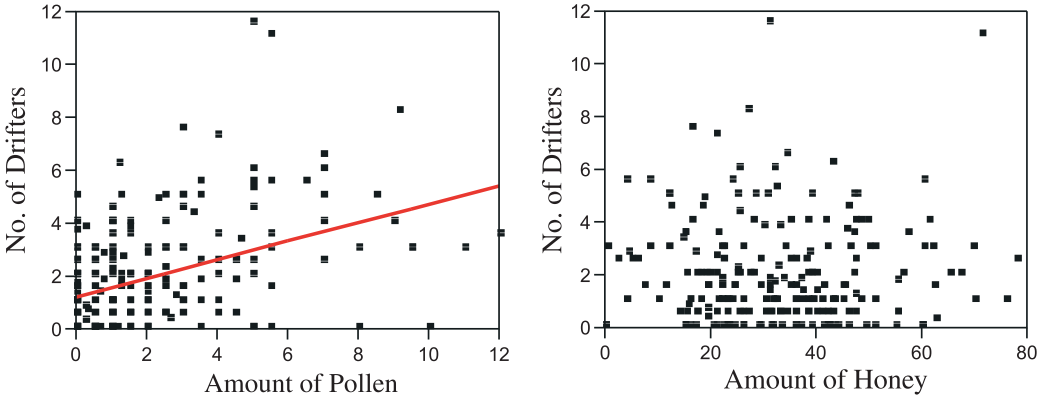
***B. impatiens* 2001**



***B. occidentalis* 2001**



***B. occidentalis* 2002**



selected colonies where their individual fitness would be enhanced.

Colony aggression increased with worker population, but higher numbers of drifting bees were not found in more aggressive colonies. However, we only observed colonies with smaller than average greenhouse worker populations, and increased aggression is normally associated with large colonies that are producing reproductives (Plowright and Fuller 1988; Duchateau and Velthius 1989; Bloch et al. 2000*b*). Dissected drifters were not larger than dissected resident bees, suggesting that drifters with reproductive capabilities were not always aggressive or dominant within the hives they entered. Potentially, drifters with mature eggs in their ovaries are entering colonies that are less aggressive and more accepting of foreign bees, thereby evading possible conflict. An individual bee may trade-off the risk of attack and potential death when entering a colony with mature eggs in her ovaries for individual fitness gain.

Drifting behaviour may involve disorientation, but our data suggest that drifting can represent a possible early stage in the evolution of a parasitic species, somewhere between the social bumble bee parasite *Psithyrus* species and a colony's own reproducing workers. *Psithyrus* species are obligatory social parasites of *Bombus* species that have lost the

Fig. 4. A greater number of *B. occidentalis* drifters entered older hives ($r^2 = 0.56$, $p = 0.008$, $n = 12$), but their numbers declined once the colony was past the age of 11 weeks. The numbers of hives at each age are in parentheses.

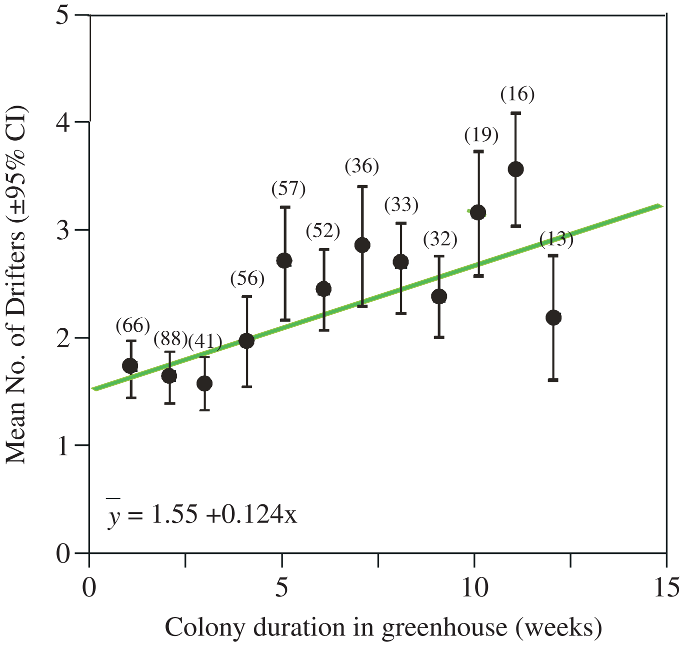
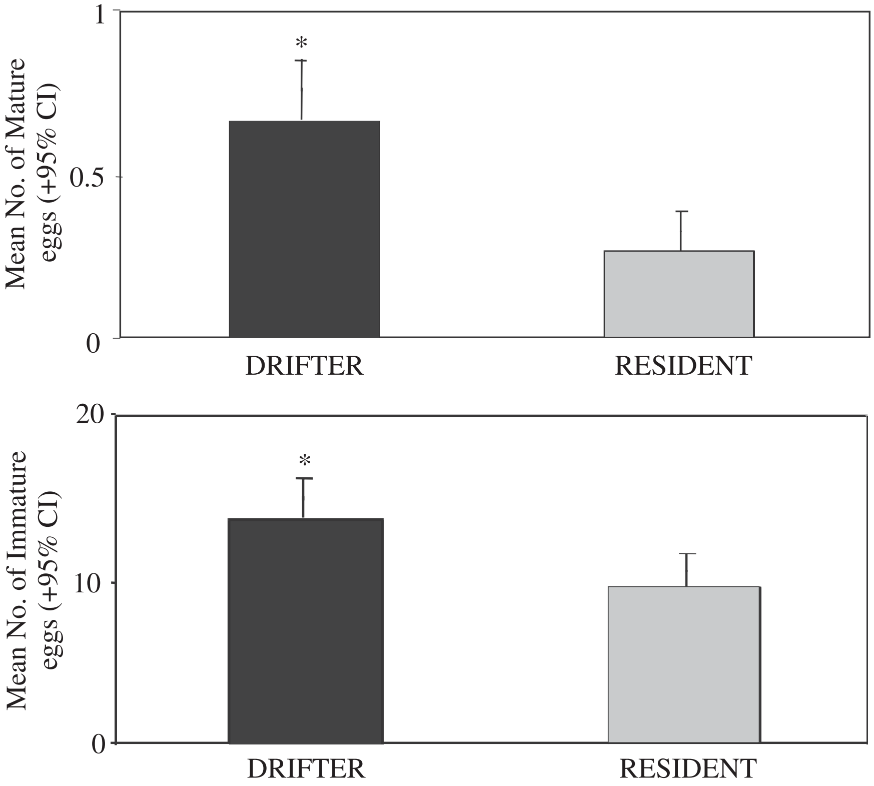
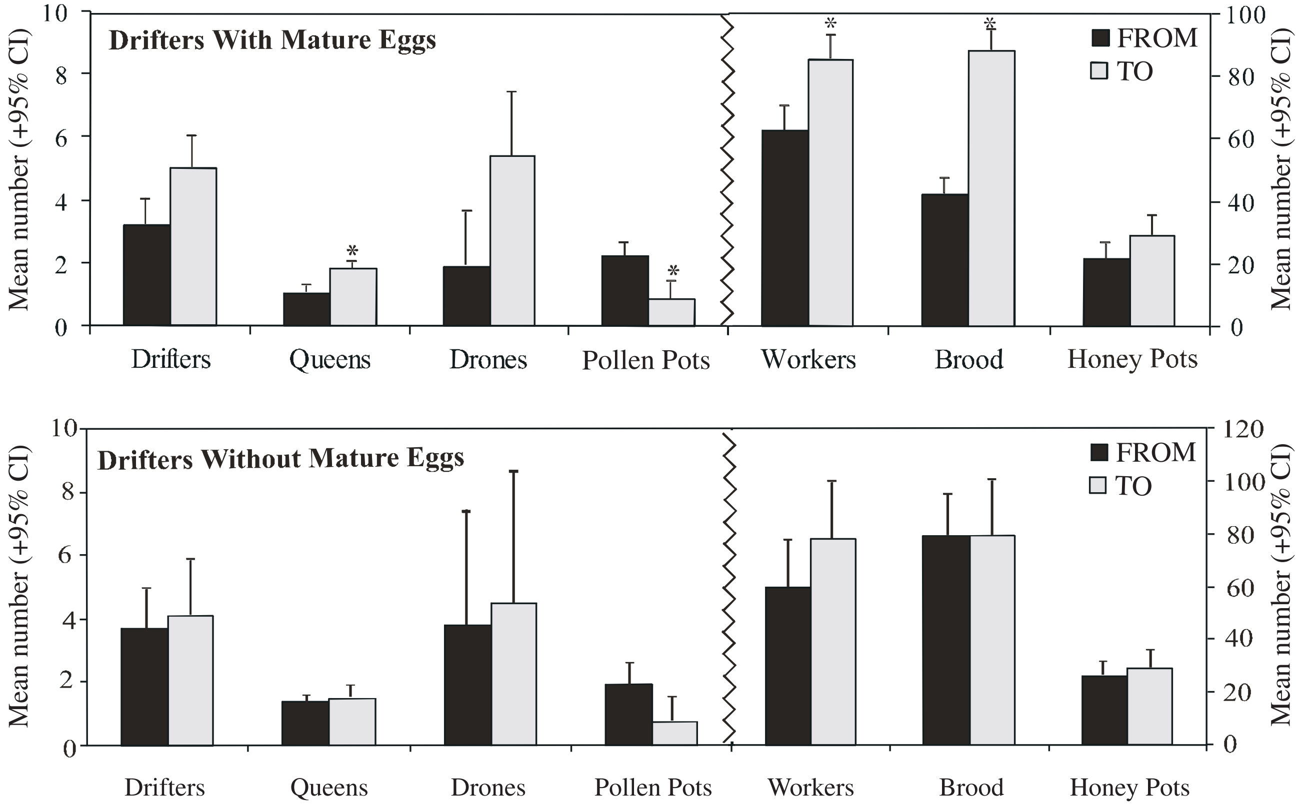


Fig. 5. *Bombus occidentalis* drifters had more mature ($F_{[1,125]} = 5.64$, $p = 0.02$) and immature eggs ($F_{[1,125]} = 4.41$, $p = 0.04$) in their ovaries than marked resident bees of the same colony. Asterisks indicate significantly different results.



worker caste (Schmid-Hempel 1998). Their queens establish nests by either killing or evading the queen, and behaviourally and chemically blend into the hive to allow workers to become accustomed to their presence (Kearns and Thomson 2000). *Psithyrus* queens will then reproduce instead of or alongside the host *Bombus* queen as the *Bombus* workers continue bringing nectar and pollen back to the hive (Kupper and Schwammberger 1995). Worker bumble bees also lay eggs in the presence of the queen at the end of the colony life cycle (Roseler 1974; Owen and Plowright 1982;

Fig. 6. (*a*) Colony composition of the home (from) and the destination colonies (to) of 16 *B. occidentalis* drifters with mature eggs in their ovaries. Destination colonies had significantly more queens ($F_{[1,14]} = 19.9$, $p = 0.0006$), workers ($F_{[1,14]} = 7.46$, $p = 0.02$), and brood ($F_{[1,14]} = 18.4$, $p = 0.0009$), but fewer pollen pots ($F_{[1,14]} = 4.58$, $p = 0.05$). The number of drifters ($F_{[1,14]} = 3.70$, $p = 0.08$), males ($F_{[1,14]} = 1.58$, $p = 0.23$), and honey pots ($F_{[1,14]} = 3.38$, $p = 0.09$) did not differ. Asterisks indicate that the means are significantly different (Tukey–Kramer HSD). (*b*) Colony composition of the home (from) and the destination colonies (to) of 22 *B. occidentalis* drifters without mature eggs in their ovaries. Destination colonies did not differ in their composition from home colonies.



Duchateau and Velthius 1989; Bloch et al. 2000*a*; Lopez-Vaamonde et al. 2004), and drifting greenhouse bees may take advantage of the unrelated workers in older colonies, using the food brought back to the hive to rear their brood. Furthermore, *B. terrestris* workers act as intraspecific social parasites, drifting into foreign colonies, laying (male) eggs, and rearing them to adulthood (Lopez-Vaamonde et al. 2004). Social parasites often are derived from species that share an immediate ancestry with their hosts (Emory's rule) and molecular evidence suggests a close relationship between *Psithyrus* species and *Bombus* species (Koulianos et al. 1999). Commercially reared colonies have similar nesting types, which also should facilitate social parasitism (Schmid-Hempel 1998).

Not all drifting bees had reproductive development, and some may have drifted for nonreproductive reasons, such as disorientation or nectar robbing. The transient, resident, and voyeur drifting behaviours observed here may reflect three different reasons why bumble bees might drift. Foraging bees might drift into foreign colonies where there is an abundant energy supply to fuel their foraging trips, and are accepted because of the pollen loads they carry (Free and Butler 1959; Downs and Ratnieks 2000). It is possible that drifters did not recognize they were in a foreign colony and (or) were not recognized as foreign by guard bees because of the similar environmental conditions that they may have been raised in (Foster and Gamboa 1989), and the constant supply of similar pollen close to the nest. Foraging bees with large pollen loads were observed entering and then leaving foreign colonies with pollen loads intact after a brief (4 s) stay. Further, behavioural observations might determine how much drift could be attributed to disorientation, nectar robbing, or social parasitism.

Results for *B. impatiens* were less extensive than for *B. occidentalis*, since fewer *B. impatiens* colonies were studied, surrounding hives were not surveyed, bees were not individually marked, and smaller proportions of bees were seen a subsequent time than for *B. occidentalis*. *Bombus impatiens* were observed to be and normally are more aggressive than *B. occidentalis*, and therefore may have been more successful at excluding drifting bees, but at this point it would be premature to speculate whether differences between *B. occidentalis* and *B. impatiens* were due to biological differences or to a less intensive experimental effort for *B. impatiens*.

The novel greenhouse environment increases the potential for worker reproduction because bees can drift into foreign, highly visible hives or search for a hive where egg-laying conditions may be optimal. The higher brood populations associated with drifting could have been due to worker reproduction, but this hypothesis cannot be explored further by the present study, as we did not take samples to determine maternity. However, commercially reared *B. occidentalis* have the same low level of isozyme variability as wild populations (Mullen and Rust 1994), suggesting that any lack of nest-mate recognition was not due to inbreeding at commercial rearing facilities. Commercially reared populations genetically mimic wild populations, suggesting that increased drifting behaviour is mostly a consequence of the greenhouse environment.

Drifting bumble bees in a novel environment exhibit important preadaptations for social parasitism. Their drift behaviour could be an active dispersal to establish themselves in nests where egg laying was more likely than in natal nests, and to survive adult worker aggression and evade egg removal, as found in *B. terrestris* (Lopez-Vaamonde et al. 2004) and the parasitic Cape honey bee *Apis mellifera capensis* Escholtz, 1821 (Neumann and Hepburn 2002). By drifting into foreign colonies, workers may have their male offspring reared by unrelated workers, simultaneously avoiding competition with siblings also trying to rear male offspring, and increasing the total male production by members of their natal colony. These drifters might accumulate both indirect fitness, by helping at the nest, and direct fitness, by laying reproductives, much like wasps of the *Polistes* genus (O'Donnell 1996). Our study did not determine whether bees with developed ovaries were successful in laying and (or) rearing male eggs; however, a recent study by Lopez-Vaamonde et al. (2004) demonstrated the absolute ability of worker bumble bees to drift into another hive, lay eggs, and rear them to a full adult.

Bloch et al. (2000*a*) and Duchateau and Velthius (1989) also found that adult *B. terrestris* workers with developed ovaries could successfully lay eggs, provided they have adequate amounts of protein because bees without access to pollen will not develop ovaries (Duchateau and Velthius 1989). Resources are not limited in a greenhouse, where colonies are provided with sugar solution and pollen is readily available, and thus is not expected to be a limiting factor. Greenhouse colonies may also be less aggressive and more accepting of foreign bees because resources are abundant (Reeve 1989; Downs and Ratnieks 2000).

Reproductively developed drifting bumble bees were found in more populous, late-stage colonies with greater pollen stores, indicating that bumble bee drift in this novel environment may provide opportunities to increase individual fitness, challenging the theory of eusocial cooperation (Hamilton 1964; Lopez-Vaamonde et al. 2004). Bumble bees adapt well to the greenhouse environment, demonstrating a high degree of behavioural plasticity (Guirfa et al. 2001) that was expected from a genus whose natural habitat ranges from the Arctic to the equator. As the greenhouse environment may encourage a natural drifting behaviour, we suggest future studies might focus on three ideas: (1) the frequency and motives for bumble bee drift; (2) whether this phenomenon is common in wild habitats; and (3) the degree of success in egg laying by drifting workers.

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