Bumble bee workers drift to conspecific nests at field scales

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Abstract. 1. Workers in several bee species travel to conspecific nests (‘drifting’), enter them, and produce male offspring inside them, so acting as intra-specific social parasites. This adds a new dimension to bees’ reproductive behaviour and spatial ecology, but the extent to which drifting occurs over field scales, i.e. at natural nest densities in field conditions, has been unclear.

2. Using the bumble bee Bombus terrestris (Linnaeus) as a model system, we sought to determine rates of worker drifting at field scales and the frequency of potential drifter workers in wild nests.

3. A field experiment with 27 colonies showed that workers travelled to, and became accepted in, conspecific nests that were up to 60 m away, although the number of accepted drifter workers within nests fell significantly with distance. The rate at which nests were entered by drifters was relatively high and significantly exceeded the rate at which drifters became accepted.

4. Microsatellite genotyping of eight field-collected nests from Greater London, U.K., showed that a low frequency (3%) of workers were not full sisters of nestmate workers and hence were likely to have been drifter workers.

5. It is therefore concluded that workers can drift to conspecific nests over field scales and confirmed that successful drifting occurs in natural populations. Drifting appears to be a natural but low-frequency behaviour permitting B. terrestris workers to gain direct fitness.

Key words. Bombus terrestris, drifting, social insect, social parasitism, worker reproduction.

Introduction

Recent studies have shown that worker bees, as well as foraging in the external environment, travel to conspecific nests (so-called ‘drifting’), are able to enter them, and may then produce their own offspring inside them (Beekman & Oldroyd, 2008). Such intra-specific social parasitism by workers is possible because, in common with workers in many other haplodiploid Hymenoptera, worker bees are capable of producing male offspring asexually (Bourke, 1988). Drifting combined with intra-specific social parasitism by workers has been described in several species of honey bee (Nanork et al., 2005, 2007; Chapman et al., 2010a) and bumble bee (Birmingham et al., 2004; Lopez-Vaamonde et al., 2004; Lefebvre & Pierre, 2007; Takahashi et al., 2010; Blacher et al., 2013; O’Connor et al., 2013). Workers in these eusocial Hymenoptera can increase their inclusive fitness by rearing related sexual nestmates (indirect fitness) or by producing their own male offspring (direct fitness). Reproduction by drifter workers is an important phenomenon because it represents an additional means, on top of producing male offspring within the natal nest (e.g. Zanette et al., 2012), by which workers may gain direct fitness (Beekman & Oldroyd, 2008). In addition, if workers enter other nests frequently, as part of an evolved reproductive tactic, then how pathogens are transported across landscapes by insect vectors may not be solely a function of workers’ foraging behaviour and range (O’Connor et al., 2013). Consequently, the effects of worker...
drifting in bees may need to be taken into account by models of pathogen transmission (Schmid-Hempel, 1998) for these major insect pollinators. Several previous studies of bees and wasps have investigated worker drifting in wild nests or experimentally under field conditions (Nanork et al., 2007; Sumner et al., 2007; Ulrich et al., 2009; Chapman et al., 2010a,b; Takahashi et al., 2010; O’Connor et al., 2013). In addition, two studies have recently shown that wild bumble bee colonies contain workers and male brood that do not originate from nestmate queens or workers, consistent with successful reproduction by drifter workers in nature, i.e. in Bombus deuteronymus Schulz (Takahashi et al., 2010) and B. terrestris (Linnaeus)(O’Connor et al., 2013). However, the spatial scale over which drifting can or cannot occur remains unclear. Hence the possibility remains that drifting over field scales (i.e. at natural nest densities) is unusual and, specifically, that drifting is sometimes an artefact of the relatively high densities at which experimental nests are often kept. In this study we therefore had two goals. The first was to determine experimentally, rates of worker drifting in a bumble bee at field scales by measuring the rates at which nests were entered by drifter workers over varying distances and the rates at which such workers successfully became accepted. We selected B. terrestris as the study species because a previous study detected widespread worker drifting and intra-specific social parasitism in semi-natural B. terrestris nests (Lopez-Vaamonde et al., 2004). Our second goal was to conduct a genetic analysis of field-collected B. terrestris nests in order to measure the frequency of foreign conspecific workers and so to add to the sparse evidence base for worker drifting in bumble bees in nature.

Materials and methods

Experiment to determine rates of worker drifting at field scales

Field methods. Bombus terrestris colonies (n = 27) were reared from 91 queens caught in the field on or near the University of East Anglia (UEA) campus, near Norwich, U.K., in March–April 2008 (with the remaining 64 queens failing to raise sufficiently large colonies). Each colony was housed in a wooden nest-box (internal dimensions 17 × 27.5 × 16 cm high) with a Perspex lid on top of which was placed a second, larger lid of opaque plastic. Workers could not exit from the nest-boxes, so there was no mixing of workers during colony-rearing. The colonies were divided into three replicate sets of nine colonies, with the replicates being used in the experiment sequentially (as their colonies reached a mean size threshold of approximately 50 workers). Before being placed in the field, all workers were marked with an individually numbered disk glued to the thorax (Queen marking kit; Thorne Ltd, Market Rasen, U.K.) and (within replicates) with a colony-specific enamel paint mark. The mean size of colonies (± SD) when placed in the field was 52 ± 23 workers.

The first replicate set of nine colonies was placed in the field on 6 July 2008 and randomly allocated, in sets of three, to each of three distance treatments (2, 20, and 60 m; Fig. 1). In each treatment, three nest-boxes were placed on the ground at one of three sites (A, B, and C) on or near the UEA campus, with each nest-box being positioned at an apex of an imaginary equilateral triangle the sides of which equalled the treatment distance in length. The sites consisted of two large gardens and a rough pasture and were at least 1 km apart (Fig. 1). Recent genetic studies have estimated the natural densities of bumble bee nests to vary with species and population from 8 to 193 nests km⁻² (Chapman et al., 2003; Darvill et al., 2004; Knight et al., 2005, 2009; Herrmann et al., 2007; Kraus et al., 2009; Charnam et al., 2010). At the midpoint of this range (100 nests km⁻²), the mean nearest-neighbour distance of randomly-distributed nests (Clark & Evans, 1954) would be 50 m. [We use the midpoint of the Bombus species range as a whole because individual species densities vary widely, even within sites across years (Charman et al., 2010).] Bumble bee nests are probably clumped rather than randomly distributed (Harder, 1986; Osborne et al., 2008), which would result in lower nearest-neighbour distances. The 60 m distance treatment was therefore selected as lying within a realistic range of natural nearest-neighbour distances for wild bumble bee nests. The 2 m distance treatment was selected as a control (Lopez-Vaamonde et al., 2004) and the 20 m distance treatment as an intermediate treatment.

Nest-boxes were left in the field for 12 days. Over the first 3 days, their entrances were progressively opened as described below. In the remaining 9 days, nests were inspected daily for the presence of workers from other nests in the experiment, i.e. drifter workers, which were recognisable from their different disk numbers and/or colony-specific paint-marks. After 12 days, the nest-boxes of the first replicate set were removed after 21.00 hours and the next replicate set of nine colonies was then treated in exactly the same way, followed by the third set, with each distance treatment being replicated within each site, i.e. so that distance treatment was not confounded by site (Fig. 1). While in the field, workers of eight colonies built a wax canopy over the brood area of their nests. In addition, nine of the colonies lost their queen, a mean of 3.3 days (range, 1–8 days) into their 12-day field period. The extent of wax canopy and degree of queenlessness were included in the statistical analyses as factors potentially affecting the number of drifter workers observed.

While nest-boxes were in the field, a block of concrete was kept on top of each to stabilise it and the resulting ensemble (nest-box plus block) was draped in camouflage netting. Bees could enter or leave the nest via an entrance tunnel (short plastic tube) protruding through the netting at the base of one side of the nest-box (Fig. 1). For the first 3 days after nest-boxes were placed in the field, the opening of their entrances was staggered within distance treatments. Each day, only one nest-box was allowed to have an open entrance, and the identity of this nest-box was rotated over successive days. This arrangement allowed workers in each nest-box the opportunity to learn their new surroundings without the possibility of drifting to another nest-box within their treatment. In addition, because drifting at the start of each 9-day observation period could have been more likely to stem from orientation errors, only data from the last 7 days of this period were included in analyses. All nest-boxes were inspected twice daily (at 08.00–10.00 and 16.00–17.00 hours). The netting and concrete block were removed and the interior of the nest-box was viewed through
Fig. 1. Design of experiment to determine rates of worker drifting at field scales in Bombus terrestris. Central figure: diagram (not to scale) representing a replicate of the experiment showing the three distance treatments; grey squares represent bumble bee nest-boxes and horizontal bar represents minimum separation of sites. Lower chart: schedule of experiment, with each replicate (with nine nests) being performed sequentially and distance treatments being rotated within sites. Upper right image: an experimental nest-box in the field, with camouflage netting in place; black arrow points to nest entrance (sealed with bung for photography).

the Perspex lid for up to 5 min. Any drifter worker present in the interior of the nest was recorded. Such workers were termed ‘accepted’ drifter workers (without implying that ‘acceptance’ necessarily involved a behavioural reaction by host colony members). Note that workers entering the experimental nests from wild B. terrestris colonies (Lopez-Vaamonde et al., 2004) would not have been detectable in this experiment because any unmarked workers could also have been (a) natal workers that had lost their labels or (b) natal workers that had eclosed in the nests since the start of each replicate.

In the second and third replicates (but not in the first, because of poor weather), the entrance of each nest-box was digitally filmed to estimate the rate at which nests were entered by drifter workers, as not all drifters entering nests necessarily became accepted drifters. Digital filming was carried out using a camcorder (Sony DCR-SR32) mounted c. 50 cm outside the nest-box entrance. Each entrance (n = 18) was filmed for a total of 3.5 h in two bouts of 105 min each, except in the case of four nests in the third replicate in which there was no filming over the second bout because of poor weather. The bouts took place between 10.00 and 12.00 hours on two randomly-selected days (during the final 7 days of each replicate). Each bout was split into a period of 15 min ending immediately before the interior of the nest was inspected for drifter workers and a period of 90 min starting immediately after the inspection was completed. During the viewing of the films, only cases in which a worker went inside the foreign nest’s entrance tunnel were scored as drifting attempts (the worker being recognized as not native to the entered nest via its disk number and/or colony-specific paint mark). Of the 23 drifter workers recorded this way, three were accepted drifters, so these were excluded from the analysis of drifting attempts.

The data from filming were also used to test whether disturbance brought about by the inspection caused workers to exit the nest, so making them more likely to drift. From the film data, the frequency with which natal workers (i.e. workers produced in a focal colony) left the nest-boxes during the 15 min before a given inspection was compared with the frequency with which they did so during the first 15 min after that inspection.

Statistical analysis. A generalised linear model (GLM) was used to compare the number of drifter workers observed in each distance treatment (Faraway, 2004; Crawley, 2005).
number of accepted drifter workers recorded inside each nest was the response variable and distance between nests, extent of wax canopy, degree of queenlessness (proportion of days in the field when the queen was present), colony size (number of workers present on first day in the field), site (A, B or C), and replicate (first, second or third) were tested as explanatory variables. A negative binomial error distribution was assumed because of the large number of zeros in the response variable (Crawley, 2005). A GLM was also used to compare the rates at which nests were entered by drifer workers at each distance treatment, as measured by filming the nest-entrances. The rate per observation hour at which nests were entered by different drifer workers (i.e. workers attempting to drift, excluding accepted drifer workers) was the response variable and the explanatory variables were the same as those used in the previous GLM, except for site which was replaced by the interaction of distance between nests and site to account for the fact that only two distances were tested in each site, i.e. the first replicate was missing. A quasi-Poisson error distribution was assumed because of overdispersion and lack of normality in the response variable (Crawley, 2005). In both GLMs, the minimally adequate model was found by removal of non-significant terms. A paired t-test was used to compare rates at which natal workers exited nests in the 15 min before and after each nest inspection. All analyses were performed in R (versions 2.9, 2.11.1 and 3.02.2, R Core Team, 2013). All means are quoted ± 1 SD unless otherwise stated.

Genetic analysis of field-collected nests

As a result of advertisements placed in the local press and contacts with pest control officials, eight *B. terrestris* nests were collected from the field within Greater London, U.K., in June–July 2005 (Table 1). In U.K. populations, isolated workers of *B. terrestris* may be hard to distinguish from workers of the closely related *B. lucorum*, but *B. terrestris* queens and males and workers en masse are reliably distinguishable from their *B. lucorum* counterparts by coat colour (Benton, 2006), rendering our specific identification of the sampled nests robust. Adult workers were sampled as completely as feasible from the nests, but we did not obtain sufficient brood for brood-genotyping (e.g. because several nests were in relatively inaccessible positions within private property). Nests were in mature condition, with seven of eight nests producing either males or queens. Incomplete collection meant that, although the colony queen was found in two nests (nos. 1 and 2), in the others it is unknown whether they were queenright (contained the colony queen) or queenless at the time of collection. Adult workers were individually frozen, with each nest being handled separately from the others to prevent the accidental transfer of workers between nests.

DNA was extracted from an antenna or leg using the HotSHOT protocol (Truett et al., 2000) and samples were genotyped at six unlinked polymorphic microsatellite loci: B10, B11, B96, B118, B121, and B124 (Estoup et al., 1995, 1996; Lopez-Vaamonde et al., 2003). Multiplex polymerase chain reactions (PCR) were carried out in a 10-μl mixture containing 5 μl of QIAGEN multiplex PCR mix, 1 μl of primer mix (with concentrations of 1.32 μM for B10 and B121, 2 μM for B11, B118 and B124, and 3.6 μM for B96), 3 μl of water and 1 μl of DNA extraction (∼50 ng). Reactions were performed in a Biometra TProfessional Standard Thermocycler as follows: an initial denaturation step at 95°C for 15 min, 30 cycles of 94°C for 30 s, 57°C for 90 s, and 72°C for 60 s, and a final incubation at 60°C for 30 min. PCR products were run with a 500 LIZ™ (Applied Biosystems, Foster City, California) size standard on an ABI Prism 3730 automated DNA sequencer. Allele sizes were scored with GENEMAPPER (version 3.7 or 4.0; Applied Biosystems). A total of 196 workers from 8 nests were successfully genotyped (Table 1), i.e. 24.5 ± 14.8 workers per nest, which represents 30% of the mean worker number within U.K. *B. terrestris* colonies (Lopez-Vaamonde et al., 2009). All workers with genotypes not grouping with nestmates were repeat-genotyped from the PCR stage onwards and confirmed as correct. In addition, a random set of samples (35% of individuals) was repeat-genotyped at all six loci and comparisons of the allele sizes in the two runs were used to calculate genotyping error rates.

Formal tests for Hardy–Weinberg equilibrium were not performed on the genotypic data because of the low number of independent genotypes within the sample, but a previous study using four of the same six microsatellites showed that *B. terrestris* populations within London are panmictic (Chapman

<table>
<thead>
<tr>
<th>Nest</th>
<th>Date collected (dd/mm/yy)</th>
<th>Latitude, longitude</th>
<th>Total n adult workers collected</th>
<th>n adult workers genotyped</th>
<th>n anomalous workers detected</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>24/06/05</td>
<td>51°34’51'”N, 00°12’24’”E</td>
<td>50</td>
<td>32</td>
<td>1</td>
</tr>
<tr>
<td>2</td>
<td>26/06/05</td>
<td>51°29’12’”N, 00°16’18’”W</td>
<td>75</td>
<td>57</td>
<td>0</td>
</tr>
<tr>
<td>5</td>
<td>05/07/05</td>
<td>51°32’34’”N, 00°10’08’”E</td>
<td>23</td>
<td>21</td>
<td>1</td>
</tr>
<tr>
<td>7</td>
<td>14/07/05</td>
<td>51°29’07’”N, 00°23’40’”W</td>
<td>19</td>
<td>19</td>
<td>0</td>
</tr>
<tr>
<td>8</td>
<td>19/07/05</td>
<td>51°33’50’”N, 00°26’60’”W</td>
<td>11</td>
<td>10</td>
<td>0</td>
</tr>
<tr>
<td>9</td>
<td>19/07/05</td>
<td>51°24’23’”N, 00°15’17’”W</td>
<td>15†</td>
<td>15</td>
<td>0</td>
</tr>
<tr>
<td>11</td>
<td>14/07/05</td>
<td>51°34’46’”N, 00°24’43’”W</td>
<td>26†</td>
<td>26</td>
<td>3</td>
</tr>
<tr>
<td>12</td>
<td>08/07/05</td>
<td>51°22’54’”N, 00°16’35’”W</td>
<td>16†</td>
<td>16</td>
<td>1</td>
</tr>
<tr>
<td>Totals</td>
<td></td>
<td></td>
<td>235</td>
<td>196</td>
<td>6</td>
</tr>
</tbody>
</table>

*Anomalous workers* † were those that were found not to be full sisters of nestmate workers.
† Minimum totals collected, as actual total was not recorded in these three nests.
et al., 2003). Colony 2.0 was used to group worker genotypes within a nest into full sisterhoods (applying error rates estimated from the repeat-genotyping), which also allowed the parental mating type to be reconstructed (Wang, 2004). Results were inspected to identify any workers not grouping as full sisters with nestmate workers. Such workers would not be offspring of the colony queen because *Bombus terrestris* queens are obligately monandrous (Estoup et al., 1995; Schmid-Hempel & Schmid-Hempel, 2000; Lopez-Vaamonde et al., 2004).

**Results**

**Experiment to determine rates of worker drifting at field scales**

Analysis of the digital films showed that there was no significant difference between the rates at which natal workers left the nest-boxes before and after inspection (means of $4.4 \pm 3.3$ vs. $4.3 \pm 3.9$ departing workers per 15 min, respectively; paired t-test, $t = 0.06$, d.f. = 16, $P = 0.94$). Therefore, inspecting the nests to check for drifter workers had no detected effect on the rate of drifting.

Across all replicates, the numbers of marked, drifter workers found in the experimental nests (accepted drifter workers) were 26, 4, and 5 at the distance treatments of 2, 20, and 60 m, respectively. Therefore, as 1313 workers were marked in total, 2.7% of workers (35/1313) became accepted drifter workers.

The number of nests successfully exporting these workers was 11 and the number receiving them was 12, of which 7 also exported drifers. All drifting occurred within sites, i.e. to one of the other two nest-boxes in each set of three nest-boxes within a distance treatment and replicate. Hence *B. terrestris* workers were able to successfully enter conspecific nests up to 60 m away under field conditions. The number of accepted drifter workers within nests decreased significantly as distance between nests increased (GLM: Table 2). Extent of wax canopy, degree of queenlessness, colony size, site, and replicate had no significant effect on the number of accepted drifter workers, although the effect of colony size approached significance (GLM: Table 2). Nests that were successful in exporting accepted drifter workers ($n = 11$) did not differ significantly from those that did not export them ($n = 16$), either in degree of queenlessness or in colony size (Wilcoxon’s rank sum test: $W = 97.5, P = 0.607$ and $W = 95, P = 0.748$, respectively).

**Table 2.** Results of analysis of number of drifter workers found during inspections of *Bombus terrestris* nests experimentally placed in field.

<table>
<thead>
<tr>
<th>Explanatory variables tested</th>
<th>d.f.</th>
<th>Log-likelihood ratio</th>
<th>$P$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Distance between nests</td>
<td>24</td>
<td>7.613</td>
<td>0.023</td>
</tr>
<tr>
<td>Extent of wax canopy</td>
<td>18</td>
<td>1.116</td>
<td>0.291</td>
</tr>
<tr>
<td>Degree of queenlessness</td>
<td>17</td>
<td>1.479</td>
<td>0.223</td>
</tr>
<tr>
<td>Colony size</td>
<td>23</td>
<td>3.751</td>
<td>0.052</td>
</tr>
<tr>
<td>Site</td>
<td>19</td>
<td>0.445</td>
<td>0.801</td>
</tr>
<tr>
<td>Replicate</td>
<td>21</td>
<td>1.323</td>
<td>0.516</td>
</tr>
</tbody>
</table>

Analysis was by generalised linear model. $P$-values are from a likelihood ratio test used to compare models after removal of explanatory variables. Variables are as defined in Statistical analysis.


**Fig. 2.** Results of experiment to determine rates of worker drifting at field scales in *Bombus terrestris*. Open squares: mean rates (per nest per week) at which nests were found to contain drifter workers as revealed by daily inspections. Filled circles: mean rates (per nest per week) at which nests were entered by drifter workers as revealed by filming. All data are from replicates 2 and 3 only. Rates were calculated by estimating, given the data, the number of events (number of drifter workers successfully becoming accepted or recorded entering nests, respectively) per nest in a period of 7 days. For the film data, this calculation assumed 10 h of worker activity per day. Error bars represent ±1 SD. Observed rates of drifting attempts (by different workers) for each colony per hour of film were: 0, 1.28, 0.31, 0, 0.46, 0.72 (2 m treatment); 0.25, 0.27, 0.32, 0, 0, 0.26 (20 m treatment) and; 0.8, 0, 0, 0, 0.52, 0 (60 m treatment).

Once present, drifter workers did not always remain in their host nests for the entire duration of the period over which the host nest was in the field. The mean duration of the stay of drifter workers in host nests was $1.9 \pm 1.6$ days (range, 1–7, $n = 35$ drifter workers). In spite of this incomplete tenure, no accepted drifter worker was observed to have returned to its nest of origin or to have transferred to a second foreign nest during the experiment.

The data from the filming of the nest-box entrances showed that nests were entered by marked, drifter workers at a relatively high rate, i.e. $0.29 \pm 0.35$ times per host nest per observation hour, averaged across all distance treatments. There was a tendency for nests to be entered by drifter workers at lower rates as distance between nests increased (Fig. 2), although this difference was not significant (GLM: deviance = 4.21, scaled deviance = 0.885, $P = 0.195$). Neither the extent of wax canopy, degree of queenlessness, colony size nor the interaction between treatment and site had a significant effect on the rate at which
nests were entered by drifter workers (GLM: all \( P > 0.111 \)). However, for unknown reasons, nests were entered by drifter workers at a significantly lower rate in the third replicate compared with the second replicate (GLM: deviance = 5.086, scaled deviance = 2.681, \( P = 0.001 \)). The rate at which nests were entered by different drifter workers (expressed as a weekly rate) was significantly greater than the weekly rate at which accepted drifter workers were observed within foreign nests during the inspections (Wilcoxon’s signed rank test: \( V = 3, \) d.f. = 18, \( P = 0.005 \); Fig. 2). This meant that many entries by drifter workers into nests did not lead to the acceptance of those workers, i.e. were unsuccessful. Consistent with this, the films showed that, in several cases (34%), drifter workers exited nests shortly after entering them (mean time in the nest = 81 ± 52 s, \( n = 8 \)).

**Genetic analysis of field-collected nests**

In our sample of field-collected *B. terrestris* nests, the expected heterozygosity of loci and the number of alleles per locus (calculated from worker genotypes only) were 0.85 (12 alleles) for B10, 0.39 (three alleles) for B11, 0.36 (three alleles) for B96, 0.33 (nine alleles) for B118, 0.58 (three alleles) for B21, and 0.85 (11 alleles) for B24. The mean rate of genotyping error per locus was 2.6%.

Analysis with COLONY 2.0 revealed that 6 out of 196 workers genotyped (3.1%) did not group as full sisters with their respective nestmates (Table 1). These ‘anomalous’ workers came from four nests and differed from nestmate workers at a mean of 2.7 ± 1.0 loci. The detection of anomalous workers was not attributable to genotyping error because (i) we ran COLONY 2.0 incorporating our observed level of genotyping error and (ii) anomalous workers returned consistent genotypes on repeat-genotyping. None of them grouped as full sisters with the other nests (as they would have done if they were contaminants from within the sample) or with any of the other five such workers. The latter result meant that, in the case in which three anomalous workers were found in one nest (no. 11, Table 1), they did not arise from a single, usurping queen. We therefore conclude that these workers were putative drifter workers.

**Discussion**

Our first goal was to determine experimentally the rates of worker drifting (attempted and successful) in bumble bees at field scales. We found that *B. terrestris* workers visit, and successfully drift to, conspecific nests up to 60 m away, but that the number of accepted drifter workers fell significantly with distance between nests. The decline in drifting success with distance between nests matched a similar finding of Lopez-Vaamonde *et al.* (2004) using an array of nests (with access to the external environment) placed at a higher density in a building. A surprising and unexpected result of the field experiment was that nests were entered by different drifter workers at a high rate. The observed mean rate at which this occurred (0.29 times per host nest per observation hour) would amount to over 20 visits from foreign workers per nest per week (if we assume that drifting attempts occur uniformly over the day and, conservatively, that there are 10 h of flight activity per day). Moreover, the estimated rate at which nests were entered by drifter workers was much higher than the rate at which these workers successfully became accepted (by a factor of 11–23; Fig. 2). For example, in the 60-m distance treatment, foreign nests were entered by drifter workers at an estimated rate of 15.4 entries per nest per week, whereas the corresponding rate at which workers successfully became accepted in such nests was less than one (Fig. 2).

Our second goal was to measure the frequency of foreign conspecific workers within field-collected *B. terrestris* nests. Using microsatellite genotyping of workers, we found that the frequency of workers that were not daughters of the queen was above-zero but low (3%), a value congruent with the observed frequency of accepted drifter workers in our experiment (2.7%). In the field-collected nests, workers that were not daughters of the queen could have been drifter workers or offspring of usurping conspecific queens, as intra-specific social parasitism by *B. terrestris* queens has been reported (Alford, 1975; Goulson, 2010; O’Connor *et al.*, 2013). The latter origin is unlikely, because the anomalous workers in the single nest with more than one such worker were not full sisters of one another and hence not the daughters of the same mother, as they would have been if they had been produced by a usurping queen (Paxton *et al.*, 2001; O’Connor *et al.*, 2013). Therefore, workers found within nests that were not daughters of the colony queen were almost certainly naturally-occurring drifter workers.

These findings have two main implications. The first concerns strategies of gaining direct fitness on the part of workers. Two previous studies have provided evidence of worker drifting and intra-specific social parasitism in natural Bombus populations. Takahashi *et al.* (2010) found field-collected *B. deuteronymus* nests to contain frequencies of drifter workers of 2% and of their male offspring of 7% (i.e. percentage of all males). O’Connor *et al.* (2013) found field-collected *B. terrestris* nests to contain frequencies of drifter workers of 1.2% and of their male offspring of 0.6%. Our finding of an estimated 3% of drifter workers in a second *B. terrestris* population matches these results. The studies of Takahashi *et al.* (2010) and O’Connor *et al.* (2013), taken together with our results, therefore suggest that intra-specific social parasitism by drifter workers is a real phenomenon in natural populations of bumble bees, albeit one occurring at low frequency. As a corollary, these studies, in combination with the results of our field experiment showing drifting at field scales, suggest that, although the proximity of nests increases the rate of drifting, the occurrence of drifting in previous studies using denser arrays of nests was not an artefact (Birmingham *et al.*, 2004; Lopez-Vaamonde *et al.*, 2004; Lefebvre & Pierre, 2007). Lopez-Vaamonde *et al.* (2004) presented evidence that, through elevated rates of aggression, egg-laying and reproductive success, as well as through reproducing earlier in the colony cycle, *B. terrestris* drifter workers exhibit a syndrome of reproductive behaviours that differs from that shown by reproductive natal workers. Recent work showing that ovary-activated *B. terrestris* workers introduced to a foreign colony retain their ovarian activation, whereas those
reintroduced to their natal colony do not, supports this view (Yagound et al., 2012; Blacher et al., 2013). Overall, therefore, we conclude that intra-specific social parasitism is an evolved but low-frequency behaviour for gaining direct fitness in worker bumble bees. Similar behaviours are likely to exist in workers in other eusocial insects (Abbot et al., 2001; Beekman & Oldroyd, 2008; Dobata et al., 2009).

The second main implication concerns the movement of pathogens across landscapes by worker bumble bees. Previous research has suggested that workers from different bumble bee nests exchange pathogens at flowers (Durrer & Schmid-Hempel, 1994). If workers can deliver pathogens directly to foreign conspecific nests, and if direct nest-to-nest transmission occurs at relatively high rates, then, as argued by O’Connor et al. (2013), current views of how bee pathogens are transported across landscapes require revision. This argument is strengthened by our current results showing that the rate of contact within nests between workers from different nests can be far greater than the realized frequency of drifter workers within nests suggests. Hence the possibility of substantial nest-to-nest transmission of pathogens via drifter workers should inform our understanding of disease dynamics in populations of wild bees (Schmid-Hempel, 1998; O’Connor et al., 2013).

Why worker bumble bees initially visit and enter foreign, conspecific nests remains unclear. Establishment of drifter workers within nests could begin with workers (a) ‘mistakenly’ entering foreign nests, e.g. if returning foragers made orientation errors, or (b) ‘deliberately’ entering foreign nests either (i) to steal honey, as has been recorded (Free & Butler, 1959), or (ii) to produce sons (Beekman & Oldroyd, 2008). In cases (a) and (b) (i), reproductive behaviour might, following acceptance, be initiated opportunistically. Our findings do not discriminate between these possibilities, although recent studies suggest that prior ovary activation increases the probability of workers drifting (Yagound et al., 2012; Blacher et al., 2013). The large difference in our field experiment between the rate at which foreign nests were entered by drifter workers and the rate at which these workers were accepted suggests that nestmate recognition, which has been experimentally demonstrated in Bombus (Free, 1958; Dronnet et al., 2005), may be used to reject a high proportion of drifter workers. This is consistent with our observations of drifter workers exiting foreign nests shortly after entering them (although this could also stem from workers recognizing that they have entered a foreign nest ‘mistakenly’).

Hence, in bumble bees in nature, it appears that nestmate recognition serves to exclude many drifter workers but is not so effective that successful drifting is impossible. Much remains to be discovered regarding the proximate and ultimate factors underlying worker drifting and intra-specific social parasitism in wild bees and their effects on workers’ behaviour and spatial ecology.

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References


Lopez-Vaamonde, C., Raine, N.E., Koning, J.W., Brown, R.M., Pere-


