Artificial Domicile Use by Bumble Bees (*Bombus*; Hymenoptera: Apidae) in Ontario, Canada

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Abstract

Bumble bees are an important group of pollinating insects that are of increasing conservation concern due to relatively recent and dramatic species-specific declines. Nesting ecology can vary significantly between species, and nest site selection may be affected by many factors, including heredity, individual experience, and habitat availability. Data on bumble bee nesting ecology are inherently difficult to collect in the wild as nests are often cryptic. Artificial domiciles (nest boxes) can be a useful tool for gathering information on species-specific nesting behavior to inform conservation management of native pollinator populations. The aim of this study was to examine the use of three different domicile designs for monitoring bumble bees: aboveground, underground, and false underground, while collecting information on occupying species identity and richness to compare with sampling with traditional netting survey methods. Across Ontario, the majority of sites had at least one domicile occupied, with the aboveground installation method proving most successful whereas no false-underground domiciles were occupied. Occupied domiciles appeared to preferentially sample certain species compared to netting surveys, and rarefied species richness of both methods was similar. Given that some bumble bees did occupy artificial domiciles, and species richness relative to sample size was high, with further refinement, this method may be useful for bumble bee research and monitoring: filling in nesting ecology knowledge gaps and potentially as a conservation management tool.

Key words: domicile, conservation, methodology, diversity, colony

Bumble bees [*Bombus* (Latreille)] are ecologically and economically important due to their key role pollinating native and agricultural plants (Losey and Vaughan 2006, James and Pitts-Singer 2008, Potts et al. 2010). Growing evidence suggests multiple species are declining (Colla and Packer 2008, Goulson et al. 2008, Grixti et al. 2009, Cameron et al. 2011), and their unique colony cycle and habitat requirements may be contributing factors (Bartomeus et al. 2013). However, bumble bee nesting ecology is poorly understood; nests are often inconspicuous, making large surveys difficult (Kells and Goulson 2003, Lye et al. 2011). Developing reliable methods for studying nesting ecology is critical for conservation management.

Artificial domiciles have potential as tools to investigate bumble bee life history. With domiciles, colonies can be monitored to examine nesting behavior, foraging, sociality, habitat requirements, and response to environmental stressors. Domiciles may also provide a method of augmenting populations, requiring research to understand their capacity to support declining species.

Bumble bee domestication and rearing for crop pollination and research has a long history, including the development of in situ (domiciles) and ex situ (lab rearing of spring-caught queens) methodologies (Sladen 1912, Velthuis and Van Doorn 2006). Domicile use began in early-20th-century England (Sladen 1912) and later expanded through the United Kingdom (Lye et al. 2011) and into New Zealand (e.g., Donovan and Wier 1978) and North America (e.g., Frison 1926). Historically, occupancy ranged from <10% (Macfarlane 1974) to between 20 and 50% (Sladen 1912, Frison 1926, Fye and Medler 1954, Donovan and Wier 1978, Richards 1978). Recent use of domiciles has been less successful, with occupancy typically between 0 and 10% (Barron et al. 2000, Lye et al. 2011). Research design is variable, but occupation by a total of 7 European bumble bees (5 common species and 2 rare species), all 4 New Zealand species (introduced), and 21 North American species (including the declining *Bombus terricola* (Kirby), *Bombus occidentalis* (Greene), and *Bombus pensylvanicus* (De Geer)) has been observed.

Assessing whether domiciles are useful for conservation management requires determining whether the local bumble bee community uses them unbiasedly. To date, efficacy investigations have not addressed this, and recent studies in North America are lacking. We

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explore the effectiveness of three domicile installation methods and contrast occupying species to netting surveys in Ontario, Canada. Quantifying use will help determine the future value of domiciles for bumble bee conservation.

Materials and Methods

Domiciles were installed at 15 sites throughout south-central Ontario between 1 and 15 April 2017 (Fig. 1). Fourteen sites were 'type 1', containing underground and aboveground domiciles, and one site was 'type 2', containing two sizes of false-underground (covered with vegetation at ground level) domiciles across five subsites.

Netting surveys were conducted at 33 sites throughout the same regions to quantify the surrounding community (Fig. 1). Bumble bees were not always netted at the same locations as domiciles, but minimum one survey was conducted within 20 km from each site.

Domiciles were constructed using untreated ³/₄" spruce plywood (West Fraser wood products) and lined with upholsterers' cotton, based on previously successful designs (Hobbs et al. 1960). Canopy cover (densiometer) and entrance aspect were quantified for every domicile.

At type 1 sites, seven underground and seven aboveground domiciles (measuring $18 \times 18 \times 19$ cm; Fig. 2a and b) were deployed (=196). Underground domiciles were installed on slopes and fitted with 20-cm PVC pipe entrances (20 mm external diameter). Aboveground domiciles were mounted to trees (70–150 cm in height). Lids were covered with waterproofing plastic sheeting (Munn 1998). Each domicile was 3- to 10-m distance from its nearest neighbor.

At the type 2 site, 150 false-underground domiciles were deployed on the ground, obscured by vegetation: 75 small ($15 \times 15 \times$ 15 cm) and 75 large ($30 \times 15 \times 15$ cm; Fig. 2c), all with 30-cm pipe entrances. Five subsites were chosen based on queen observations and habitat type. Thirty domiciles were installed per subsite, minimum 2 m apart. Large and small domiciles were paired.

All sites were visited before collection to assess colony progression, and domiciles were removed after senescence. Domiciles with signs of bumble bee presence (e.g., dead bumble bees, wax structures like brood cells, nectar pots) were scored as occupied. For established colonies, counted brood cells were a proxy for colony success.

Bumble bee netting data from 33 sites between 24 April 2017 and 8 June 2017 (n = 1,221 individuals) were used to measure local community composition. Sites were surveyed 0.5–4 h, depending on bumble bee abundance, and 1–146 bees were sampled per visit (mean 37 bees per site). Only queens were included to ensure relevant comparison to nest-founding individuals. All individuals were temporarily collected, identified, and released. Only confidently identified individuals were analyzed.

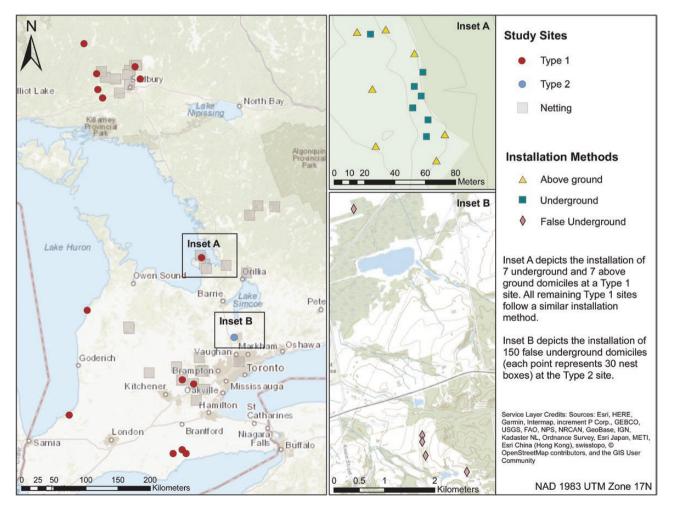


Fig. 1. Map of 15 domicile and 33 netting study sites located throughout south-central Ontario, Canada. On the left, large-scale site locations and types are displayed, and on the right, smaller insets show domicile arrangement for each site type.



Fig. 2. Photographs of each domicile installation method: (a) underground domicile before complete burial, (b) aboveground strapped to tree, and (c) falseunderground hidden in vegetation.

To test whether species in domiciles differed from the netted community, we generated a null distribution for domicile species occupancy (using R version 3.4.3 (R Core Team 2018)). This distribution was constructed by repeatedly filling occupied domiciles with bumble bees sampled probabilistically from the netting dataset. We employed three spatial models: 1) all observations in the netted dataset sampled with equal probability to fill each domicile, 2) bees only sampled within a specified distance, and 3) a netted bee's sample probability was inversely proportional to that observation's distance. These models yielded the same conclusions, so we only present the most conservative (inverse distance). We ranked bee species according to their probability of occupying each domicile (domicile-specific ranks), such that the species most likely to occupy a domicile was assigned rank 1, the species second most likely assigned rank 2, and so on. We then calculated the mean rank of the species collection for each of our randomly sampled sets of occupants to produce the null distribution of mean rank of occupants. The proportion of mean ranks that are more extreme than the mean rank of the species that actually occupied domiciles provides an empirical P-value.

To relate species richness of domicile and netting samples, netting sample size was rarefied to the number of occupied domiciles using the 'rarefy' function in package 'vegan' (Oksanen et al. 2018).

Results

Of the 346 domiciles installed, 17 (4.9%) were occupied by bumble bees; type 1 sites had an occupation rate of 8.6%, and the type 2 site was unsuccessful. Occupied domiciles were distributed unequally across 60% of all sites (Fig. 3). Four of 98 (4.1%) underground and 13 of 98 (13.3%) aboveground domiciles were occupied. The highest site-level occupation rate was 36% (Table 1). A summary of site-level mean habitat variables per domicile is included in Table 1, though low sample size precluded comparison of unoccupied and occupied domiciles. The mean entrance aspect for occupied domiciles was 55° (NE) and canopy cover was 72%.

Of the 17 occupied domiciles, only 13 contained remains that were identifiable to species. Nine showed signs of parasitism by *Achroia grisella* (Lepidoptera: Pyralidae) Fabricius, where larvae completely destroyed wax remains preventing the counting of brood cells in eight of those nine. Of the preserved nests, colonies ranged dramatically in size from 4 to 782 brood cells. Six different species were observed, including *Bombus bimaculatus* Cresson

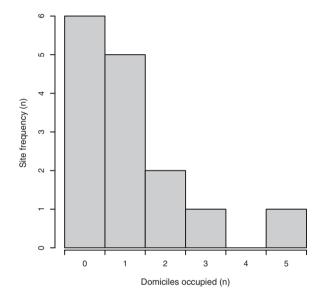


Fig. 3. Number of domiciles occupied per site displayed as a site-level frequency histogram, indicating how often an individual site contained from zero to five occupied domiciles.

(n = 2), Bombus griseocollis De Geer (n = 2), Bombus impatiens Cresson (n = 1), Bombus perplexus Cresson (n = 5), Bombus rufocinctus Cresson (n = 2), and Bombus ternarius Say (n = 1). Most species inhabited exclusively aboveground (B. griseocollis, B. perplexus, and B. rufocinctus) or underground (B. impatiens and B. ternarius) domiciles with one exception—B. bimaculatus occupied one of each.

Species that occupied domiciles were on average significantly locally rarer than expected by distance-weighted netted observations (P < 0.001, Fig. 4). This difference appears to be driven by the overrepresentation of *B. perplexus*, *B. griseocollis*, and *B. rufocinctus* and underrepresentation of *B. bimaculatus*, *B. impatiens*, and *B. ternarius* in domiciles compared with their likelihood of selection based on distance-weighted abundance (Fig. 4). When rarefied to a sample size equal to the number of occupied domiciles (n = 17), species richness for queen netting surveys in domicile regions was adjusted from 9 to 4.71 species ± 1.03 (SE), indicating that domiciles captured a community at least as rich (n = 6 species) as netting, despite low overall occupancy.

Table 1. Bumble bee domicile occupation summary by site, including per-domicile habitat variable averages.

Sites	Latitude	Longitude	Occupied domiciles	Canopy cover (mean)	Aspect (mean)
1 (Awenda Provincial Park)	44.824741	-79.987468	2 A, 3 U	68%	230° (SW)
2	46.38699	-81.37376	3 A	25%	150° (SSE)
3	43.55766	-80.09742	2 A	97%	0° (N)
4 (Inverhuron Provincial Park)	44.29961	-81.58835	2 A	93%w	150° (SSE)
5 (Guelph Lake Conservation Area)	43.60075	-80.25816	1 A	73%	260° (WSW)
6	42.88733	-80.26023	1 A	61%	15° (NNE)
7	42.84967	-80.2035	1 A	50%	125° (ESE)
8 (Fairbank Provincial Park)	46.46868	-81.43967	1 A	90%	40° (NE)
9 (Windy Lake Provincial Park)	46.620813	-81.456546	1 U	48%	240° (WSW)
10	42.84971	-80.38653	0 A, U	76%	40° (NE)
11 (Pinery Provincial Park)	43.24315	-81.84042	0 A, U	90%	15° (NNE)
12 (Halfway Lake Provincial Park)	46.90849	-81.63226	0 A, U	57%	275° (W)
13	46.68774	-80.91743	0 A, U	50%	105° (ESE)
14	46.57001	-80.84492	0 A, U	34%	185° (SSW)
15 (Koffler Scientific Reserve)	44.029596	-79.53159	0 F	13%	150° (SSE)

Occupied domiciles by installation method: A (aboveground), U (underground), and F (false underground).

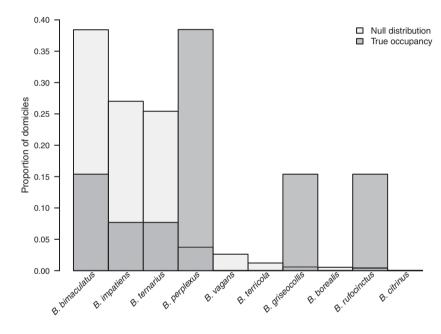


Fig. 4. Comparison of bumble bee species observed in occupied boxes versus the null distribution as sampled from netting surveys. Proportion is calculated from n = 13 for true occupancy and n = 130,000 (13 occupied domiciles generated over 10,000 runs) for the null distribution.

Discussion

Domicile occupancy rate was low for all sites, but success per type and installation method varied significantly. Given the range by installation method from 0% (false underground) to 13% (aboveground) and from 0 to 36% between sites, many factors are probably influencing the establishment of colonies. Aboveground domiciles were our most successful, though installation method does not reliably predict occupation success: aboveground and underground domiciles have been both successful (20-100%, Sladen 1912, Fye and Medler 1954, Richards 1978) and comparatively unsuccessful (0-14%, Hobbs et al. 1960, Barron et al. 2000, Lye et al. 2011). In this study, both underground and false-underground domiciles were often subject to improper drainage, and entrances were frequently blocked by soil/vegetation. It is difficult to disentangle site-level effects from installation method effects for type 2 subsites containing all 150 empty false-underground domiciles-false-underground domiciles too have been both successful and unsuccessful in past

work (0–43%, Hobbs et al. 1960, Macfarlane 1974, Richards 1978). Domicile design has also been historically variable and does not appear associated with success rate—metal coffee tins (Sladen 1912, Frison 1926), stock lumber, clay (Fye and Medler 1954, Hobbs et al. 1960, Macfarlane 1974), and plywood (Richards 1978, Barron et al. 2000, Lye et al. 2011) constructions, most often cubic and similar dimensions, have all been both successful and unsuccessful at attracting queens.

No previous domicile studies have attempted to make diversity comparisons to alternate sampling methods. We found that community-level local species abundance from netting surveys was not strongly predictive of species found in domiciles. Several occupying species were observed frequently in domiciles but rarely in nets, suggesting the potential influence of species-specific nesting preferences or a species-level likelihood for acceptance of artificial nesting structures. *Bombus perplexus*, our most common occupant, is known to accept manmade structures and has been previously observed using domiciles (Farmer 2014; S. Johnson, unpublished data). *Bombus hypnorum* (Linnaeus), a European species closely related to *B. perplexus* (Hines 2008), occasionally inhabits bird boxes (Lye et al. 2011), indicating a potential subgenus-level component to this overrepresentation. If domiciles are species biased, application for nesting structures in life-history research in the occupying species will still be invaluable.

Previous North American studies have domicile occupation by 21 of 46 (45.6%) species (Frison 1926, Fye and Medler 1954, Hobbs et al. 1960, Hobbs 1967, MacFarlane 1974, Richards 1978). Each of our occupants has previously accepted domiciles, but three never before in Ontario (*B. bimaculatus*, *B. griseocollis*, and *B. ternarius*; Macfarlane 1974). Species richness in domiciles was at least as diverse as rarefied netting richness, suggesting that domiciles could be valuable for collecting data for a wide range of bumble bees, pending increased occupation.

It is difficult to compare success between studies due to substantial temporal (Sladen 1912 to current), spatial (Europe, New Zealand, and North America), and methodological (design and installation) variation. Factors such as landscape disturbance levels, interactions between local bumble bee, floral, and natural nest site abundance, nesting preferences, and domicile design (Barron et al. 2000, Lye et al. 2011) are probably all important. Given our observed levels of between-site variation, between-method variation, and the tendency of overrepresentation of certain species compared with netting, site-level characteristics and species preferences may strongly influence occupation rates in Ontario. To disentangle these effects, manipulative experiments are probably necessary.

Our findings suggest domiciles have utility as a tool for ecological study if occupation rates can be increased. Given the overrepresentation of certain species that accept domiciles in Ontario, care should be taken when considering application to at-risk species conservation management, or for examining bumble bee communities independent of other survey methods. Recent North American domicile research is lacking, and in light of species declines, more work is needed to assess whether reduced modern occupancy (e.g., Barron et al. 2000, Lye et al. 2011) is associated with conservation status. Given the potential value of using domiciles to fill knowledge gaps in bumble bee nesting behavior and colony development, additional evaluation of different installation methods will be valuable for clarifying influences on per-species occupation.

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