THE CONSERVATION MANAGEMENT AND ECOLOGY OF NORTHEASTERN NORTH AMERICAN BUMBLE BEES

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Abstract

Bumble bees (*Bombus* spp.; Apidae) are among the pollinators most in decline globally with a main cause being habitat loss. Habitat requirements for bumble bees are poorly understood presenting a research gap.

The purpose of my dissertation is to characterize the habitat of bumble bees at different spatial scales using: a systematic literature review of bumble bee nesting and overwintering habitat globally (Chapter 1); surveys of local and landcover variables for two at-risk bumble bee species (*Bombus terricola*, and *B. pensylvanicus*) in southern Ontario (Chapter 2); identification of conservation priority areas for bumble bee species in Canada (Chapter 3); and an analysis of the methodology for locating bumble bee nests using detection dogs (Chapter 4).

The main findings were current literature on bumble bee nesting and overwintering habitat is limited and biased towards the United Kingdom and agricultural habitats (Ch.1). Bumble bees overwinter underground, often on shaded banks or near trees. Nests were mostly underground and found in many landscapes (Ch.1). *B. terricola* and *B. pensylvanicus* have distinct habitat characteristics (Ch.2). Landscape predictors explained more variation in the species data than local or floral resources (Ch.2). Among local variables, floral resources were consistently important throughout the season (Ch.2). Most bumble bee conservation priority areas are in western Canada, southern Ontario, southern Quebec and across the Maritimes and are most often located within woody savannas (Ch.3). Climate change is predicted to shift priority areas to more northerly latitudes and to higher elevations (Ch.3). These priority areas do not overlap highly with current protected areas (Ch.3). Using detection dogs to locate the scent of bumble bee nests was more nuanced than expected (Ch.4). The detection distance for nests was short, so dogs needed to conduct detailed searches for nests (Ch.4). Nests may also have multiple entrances which complicates confirming detections made by dogs (Ch.4). The challenges with deploying detection dogs to locate bumble bee nests could be mitigated with careful study design should be carefully considered in future research.

This research addresses many gaps in our understanding of bumble bee habitats and will be valuable in informing conservation policy.

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Chapter 1: A systematic review of the nesting and overwintering habitat of bumble bees globally

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Author's Contributions:

ARL, SRC, conceived of the ideas and literature review search terms. ARL conducted the literature review, processed all articles, extracted relevant data from articles retained for the review, and summarized and analyzed the results. ARL led the writing of the manuscript. Both authors contributed to editing and gave final approval for publication to the journal Insect Conservation and submission for this dissertation.

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Summary

Some bumble bee species are in decline globally. Declines have been attributed to many factors including habitat loss. Habitat is an integral component of any species' survival and should be a central focus of conservation efforts to protect at risk species. However, the habitat of bumble bee species is not fully understood. We conducted a systematic review of the peer-reviewed literature using Web of Science to summarize articles that have described the habitat of bumble bee species. In total, 55 nesting and 10 overwintering habitat studies are described in this review. We described common patterns associated with bumble bee studies including overwintering habitat, landscape type, and ground position. We found that bumble bee nests are more frequently found underground and that studies were biased towards United Kingdom and agricultural habitats. There are some preferences in nesting and overwintering habitat, but further research is needed to draw any substantial conclusions. Detection of nesting and overwintering site studies may be improved using citizen science initiatives and possibly through employing detection dogs or radio-telemetry. Increasing the detection of nesting and overwintering sites is an important priority to improve our understanding of bumble bee habitat. It is critical that we identify all aspects of bumble bee habitat to ensure the protection, restoration and creation of important resources to ensure their conservation.

Introduction

Some bumble bee species (34.7%) are in decline globally (Arbetman et al., 2017)**.** Suggested threats that are contributing to bumble bee declines include climate change, pathogen spillover, pesticides, invasive species competition and habitat loss (Brown and Paxton, 2009; Colla et al., 2006; Goulson et al., 2015; Grixti et al., 2009; Thomson, 2016). Habitat loss especially has being cited as the main threat to bumble bees in Europe (Goulson et al., 2008; Williams and Osborne, 2009). Bumble bee declines are problematic both to crop and wild plant systems, especially in temperate regions. This is because bumble bees are efficient pollinators in conditions where other pollinators are not effective such as during cool and wet weather (Bishop and Armbruster, 1999; Willmer et al., 1994). Bumble bees are also able to buzz pollinate plant species that require this method of pollination (Goulson, 2003). To address these observed declines there has been a focus on protecting and improving pollinator habitat (Carvell, 2002; Lye et al., 2009; Roulston and Goodell, 2011). However, these efforts have mainly focused on increasing available forage (Carvell et al., 2007; Dicks et al., 2015; Moquet et al., 2017) and this is only one component of bumble bee habitat. Bumble bees require three main resources to complete a colony cycle: nesting, forage, and overwintering sites (Kearns and Thomson, 2001). Overall, we lack understanding about what specifically characterizes bumble bee habitat besides describing very general ecosystem types where bumble bees are found and the types of forage species bumble bees utilize (Colla, 2016). Nesting and overwintering requirements for bumble bees are extremely understudied likely due to the difficulty in locating these sites (Darvill et al., 2004; O’Connor et al., 2017; Waters et al., 2011). Compiling available literature, exploring patterns in previous research as well as outlining knowledge gaps are next critical steps for the conservation of bumble bees as we need to ensure we are protecting all aspects of bumble bee habitat.

Additionally, this information is often needed when defining a species' critical habitat for legal protections. The nesting and overwintering requirements for bumble bees need to be better described to effectively address habitat protection for those in decline.

Methods

Literature review

A systematic literature review was conducted on February 5, 2018 using Web of Science. This search was part of a larger literature review for all of bumble bee habitat. The following search terms were used: (nest*) OR (forag* OR floral OR flower*) OR (overwinter* OR hiberna*) AND (*bombus* OR bumble bee OR bumblebee) AND (habitat OR resource*). A total of 955 articles resulted from this search. A preliminary refinement excluded 430 articles that were reviews, did not study bumble bees, or were not focused on bumble bee habitat. Of the remaining 525 articles, 53 appeared to be related to nesting or overwintering habitat (51 for nesting and 2 for overwintering) and were refined further for this review. These 53 studies were identified as potentially relevant to the review by reading the paper's title and abstract. These 53 studies were read in their entirety and we determined that 25 of these studies were not relevant (i.e. were focused on crop yields, did not actually involve finding nesting/overwintering sites or describing nesting/overwintering habitat), or we could not access (3 studies) due to no digital copy available. The literature cited section of the remaining 28 articles (26 nesting, 2 overwintering) was searched for additional relevant articles. This resulted in an additional 29 nesting and 8 overwintering studies that were included in this review. The search terms were then input into Scopus to locate any additional missing studies. The resulting papers from the Scopus search did not add any missing papers from our original Web of Science and literature cited searches (i.e. all papers in the Scopus search results were already included in our literature review). The final total number of studies included in this review were 55 nesting and 10 for overwintering. Some of the studies included in the review looked at both nesting and overwintering habitat and were coded as separate studies even though they were from the same article. This was done to allow more efficient summaries of nesting and overwintering habitat. The 55 and 10 nesting and overwintering studies respectively, resulted from 59 separate articles (i.e. 6 articles looked at both nesting and overwintering bumble bee habitat) (Table 1).

Data extracted

The data extracted from each study was the study location (latitude and longitude), study species information (species, subgenus), and descriptions of the study site location (landscape and patch types, soil). If GPS coordinates were not provided in the study, we used description of the study site to approximate where the study took place and estimated the GPS coordinates using Google Earth. Ground cover (i.e. bare ground, litter, moss, etc.) was also described for overwintering studies. Additional information was extracted (when available) for nesting studies including the number of nests per species in each study, ground position of nests (aboveground, surface, underground), detection method used to find nests (detection dogs, molecular methods, nest searching queens, observational, and statistical modelling), nesting materials (i.e. what the nest was made from including artificial domiciles), and nest density. Observational methods are those that visually located nests (e.g. by walking transects at a study site). Molecular methods and some of the statistical models used to detect nests did not locate or describe the actual nest.

Data analysis

The data were summarized by subgenus, landscape type or ground position of nests to determine if there is a relationship between i) where nests are located by landscape type and subgenus, ii) ground-position of nests and landscape type and, iii) subgenus and ground-position of nests. To examine these potential relationships the proportion of nests by subgenus, landscape type or ground position was compared. Landscape types were classified as: agricultural, alpine, forest, forest edge, grassland, tropical forest, urban or other. Agriculture includes all types of agriculture including intensive, conventional, and stewardship practices. It also includes non-arable areas such as hedgerows, pastures, woodlots, and grasslands. Grasslands include meadows, heathlands, and machair. The 'other' category includes landscape types that were not well represented across the studies: wetlands, dunes, shrublands, and human-associated (but not developed enough to be considered urban). If a range of nests were included in a study, the lowest number of nests in the range was assigned as the number of nests to keep a conservative estimate. If a study included more than one category (i.e. more than one ground position for the same species) the study was double coded. To determine if there is a relationship between subgenus, landscape type or ground position, three chi-square tests were performed comparing: i) subgenus and landscape type, ii) subgenus and ground-position, iii) ground-position and landscape type.

Results and Discussion

A total of 59 unique articles studies were retained for analysis. Ten studies examined overwintering habitat and 55 examined nesting habitat (some studied described both nesting and overwintering habitat) (Table 1). The lack of studies investigating nesting or overwintering is apparent when comparing the number of primary articles related to bumble bee habitat in general (525 articles identified from our preliminary refinement). Since most studies were focused on nesting habitat, most analyses in this review were focused on nesting habitat. These studies spanned five continents with most studies based in the United Kingdom (Fig. 1). Three overwintering studies took place in Europe while the others were in North America (Fig. 1). The small sample of articles examining bumble bee nesting habitats makes deriving substantive conclusions about the requirements for nesting and overwintering habitat challenging. To overcome this limitation somewhat, we summarized our findings at the subgeneric level. By summarizing our data this way, however, we may be confounding potential regional preferences in nesting or overwintering habitats and this should be considered. Additionally, the presence of a bumble bee nest or overwintering queens does not necessarily mean that the surrounding areas are high quality or preferred habitat. However, the available content provides an important summary of our current knowledge on bumble bee nesting habitat and areas of focus for future studies.

Overwintering habitat

The 10 overwintering studies occurred in North America and Europe with publication dates spanning from 1912 to 1973. These studies include 23 different bumble bee species and nine subgenera (Table 1). These studies described different aspects of bumble bee overwintering habitat including the landscape and patch characteristics, ground cover, soil type as well as the depth, density, depth, distance from the nest, that overwintering queens were found. Finally, a single study examined the temperature during overwintering and spring emergence of *Bombus impatiens* queens at the University of Guelph, Ontario, Canada (Szabo and Pengelly, 1973).

The overwintering studies took place in mountainous areas, grasslands, forests as well as a university campus (surrounded by urban development and agricultural areas). All overwintering queens were found underground. Overwintering queens were most often observed in shaded areas usually near trees and in banks without dense vegetation (Alford, 1969; Bols., 1937; Hobbs, 1967; Plath, 1927; Sladen, 1912). There were a few observations by Alford (1969) and Sladen (1912) where queens were found in the open (*B. lapidarius* (Sladen, 1912)) or away from trees (*B. mesomelas*, *B. lucorum* – found both away and under trees, (Alford, 1969)). Overwintering queens were most often found in north-facing slopes (Alford, 1969; Bols., 1937; Hobbs, 1967; Sladen, 1912), but they were also found in slopes facing east, west and south by Hobbs (1967), and a few overwintering sites were flat, namely for *Bombus impatiens* (Plath, 1927), *B. pratorum* and *B. mesomelas* (Alford, 1969). Alford (1969) noted that bumble bee queens may preferentially overwinter in north-facing slopes to prevent emerging too early on a warm or sunny day in the winter. The soil type was often described as sandy, well-drained, or loose where queens were found overwintering (Alford, 1969; Bols., 1937; Hobbs, 1967; Plath, 1927), however, *B. huntii* was found only in moist soil by Hobbs (1967). Whether this pattern of queens preferentially selecting shaded north facing banks is consistent across species would be an important future study question.

The ground cover where bumble bee queens overwintered was similar across the studies. Overwintering queens avoid areas with dense vegetation and will overwinter in bare-earth, moss, under tree litter, or in bare-patches within short grass (Alford, 1969; Bols., 1937; Hobbs, 1965; Hobbs, 1965; Hobbs, 1967, 1966a; Plath, 1927; Sladen, 1912). Whether there are preferences for a certain ground cover at the species or subgenus level cannot be determined with the current level of overwintering studies, however, this would be an interesting avenue of future research.

Overwintering queens hibernate at varying depths within the soil, at large densities, and they may or may not hibernate close to their nest. Queens within the overwintering studies were observed overwintering at depths between 2 -15cm. The depth queens hibernate at may vary by ecosystem type and region in order to best regulate temperature to optimize emergence time (Alford, 1969; Hobbs, 1967, 1966a, 1966b; Szabo and Pengelly, 1973). The average overwintering depths of queens reported are: *B. nevadensis* 11.43 cm in loose soil, 7.62 cm in compact soil (Hobbs, 1965); *B. rufocinctus* 4.32 cm (Hobbs, 1965); *B. flavifrons* and *B. bifarius* 10 cm, *B. frigidus* and *B. sylvicola* 2.54 cm (Hobbs, 1967); *B. appositus* 6.35 cm (Hobbs, 1966a); *B. fervidus* 2-4 cm (Hobbs, 1966b). The overwintering studies reported finding large densities of queens hibernating in the same location and sometimes multiple queens were found in the same hibernation chamber (G. A. Hobbs, 1965). Alford (1969) also reported that the same overwintering site may be used repeatedly. There were conflicting reports as to whether the overwintering sites were located near nests. Plath (1927) found 103 hibernating queens of *B. impatiens* that chose overwintering sites very close to their nests (~ 60 cm), however, they could not find any hibernating queens of *B. affinis* or *B. fervidus* close to their nests. Alford (1969) stated that queens do not preferentially overwinter close to their nests. A more recent study by

Carvell *et al* (2017) reported varying dispersal distances of spring queens from natal nests with a positive correlation observed between spring queen dispersal and habitat quality at various spatial scales. It may be possible that there are species-specific preferences in relation to nest distance, but it may also depend on whether there is suitable overwintering habitat near the nest location.

The soil temperature measured in the *B. impatiens* overwintering sites at the University of Guelph (Ontario, Canada) varied between -2.5 and 1.1°C depending on the air temperature and snow pack (Szabo and Pengelly, 1973). Soil temperature was found to have a strong influence on queen emergence in the spring with emergence occurring at temperatures between 13 and 17°C. It is unclear whether the temperature range for overwintering and emergence as determined by Szabo and Pengelly (1973) would be similar across other bumble bee species and this should be the focus of future physiological studies of bumble bee overwintering.

Nesting habitats

Bumble bees exhibit a generalist nesting pattern across landscape types with no clear preferences in landscape types for nesting within subgenera (Fig. 2). Wild nests, artificial domiciles and nests in human structures were collectively considered. Bumble bee nests were found in eight landscape types: agricultural, alpine, dune, forest, forest edge, grassland, tropical forest and urban. Wild nests were found in more landscape types (agriculture, alpine, dune, forest, shrubland, tropical forest, urban and wetland) than nests found in human structures or artificial domiciles (agriculture, alpine, forest, forest-edge, grassland, urban). Although the Chi-square test showed that there was a significant relationship between bumble bee subgenera and locations of nests within the different landscape types (Chi-squared = 148.91, df=70, p = <0.001), there were large differences in the number of studies that reported each subgenus that likely contributed to this result. The most common landscapes for bumble bee nests were grasslands, agriculture and forests. Wild nests were found most commonly in forests followed by agriculture, grasslands, urban areas, tropical forests, alpine, dunes, shrublands, wetlands. Occupied artificial domiciles and nests in human structures were also found most commonly in forested areas followed by urban areas, grasslands, forest-edge, alpine, and agriculture. There is a need to expand studies across more landscape types and geographical locations especially in alpine, dune, and urban habitats. Studies in semi-arid areas, dunes, tundra, and alpine are especially needed because we found no or few studies on bumble bee nesting in these habitats but bumble bees are found in these areas (Eidesen et al., 2017; Koch et al., 2012; Shelly et al., 1991). This should allow a clearer understanding of the nesting preferences of bumble bees across all habitats.

Most studies on the nesting habitat of bumble bees had at least part of their observations occur in agricultural landscapes (81.8%), but few nests were found within arable land. Most bumble bee nests (79.5%) within agricultural landscapes were found in field margins/hedgerows, in surrounding patch types (woodlands, meadows, gardens) or pastures. Five of the eleven studies that found nests within arable lands used molecular techniques to determine nesting density (Darvill et al., 2004; Geib et al., 2015; Goulson et al., 2010; Herrmann et al., 2007; Knight et al., 2009, 2005). Since these studies did not locate the nests, it may be that nests were not actually within arable lands, but rather the surrounding landscape. The remaining six studies that found bumble bee nests in arable lands used observational methods to detect the nests (Barron et al., 2000; Chavarria, 1996; de Oliveira et al., 2015; Janzen, 1971; Rao and Skyrm, 2013; Rau, 1941).

Nests were found within arable lands in these studies because planting and harvesting of crops was not highly disruptive, e.g. no tilling (Rao and Skyrm, 2013) such as banana or coffee plantations (Chavarria, 1996; de Oliveira et al., 2015; Janzen, 1971), or through supplementation of nesting sites using artificial domiciles (Barron et al., 2000). Previous studies have indicated that arable lands are not suitable habitat for bumble bees and expansion of intensive arable lands without creating or protecting natural habitat patches or margins may be contributing to the decline in some bumble bee species (Carvell et al., 2007; Kells and Goulson, 2003; Lye et al., 2009; Svensson et al., 2000). However, some crop types may increase the availability of nest sites for bumble bees. Rao and Skyrm (2013) found that rye fields attract voles who make their burrows within the field. The increase in burrow availability may benefit bumble bees by providing additional nesting sites (Carreck et al., 2009). Field margins and other habitat patches within an agricultural area can provide suitable nesting habitats because they diversify the landscape, are less disturbed than arable land, can provide cover needed for some species, and are more complex in vegetation pattern and resource availability (Kremen et al., 2015; Lye et al., 2009; Svensson et al., 2000). Improving field margins may increase the available nesting habitat for bumble bees within agricultural environments.

The availability of forage resources influences nest density and colony survival. Nest-searching queens may choose nesting sites that are near areas with spring forage available to optimize their energy intake during foraging trips (O’Connor et al., 2017; Suzuki et al., 2009, 2007). Increasing floral resources in a landscape have been shown to increase bumble bee nest densities (Goulson et al., 2010; Knight et al., 2009; Osborne et al., 2008). Forests often have a high density of early-flowering plant species (Kaemper et al., 2016; Wray et al., 2014) and may attract early-emerging bumble bee species. Conversely, later in the season, forests often have lower floral resources compared to other landscapes such as grasslands or urban areas (Wray and Elle, 2015). Although high floral availability may attract bumble bee queens, nests are usually not found in areas of high floral cover (O'Connor et al., 2017), but this pattern may reflect the difficulty in finding nests in highly vegetated areas and not nesting preferences. Suzuki *et al* (2007) attempted to develop a statistical model to predict bumble bee nest locations using floral resource availability. Their model overall was not successful in predicting nest locations except for May forage. This low predictability may be because floral resource availability can change over the season. Seasonal fluctuations in floral resources can have negative effects on the survival of bumble bee colonies if sufficient food sources are not available (Bowers, 1985; Moquet et al., 2017). Given that nesting, overwintering and foraging resources are all essential to bumble bee survival, efforts at predicting bumble bee nest locations and their survival throughout the season should consider temporal fluctuations in floral resources and investigate the potential influence of non-forage variables (i.e. forest cover, plant debris, animal burrows, ground cover, soil type, microclimates etc.) on nesting and overwintering habitat.

Ground position and materials of bumble bee nests

Bumble bees were found to nest underground, on the ground surface and aboveground. All three ground nesting positions were observed for most subgenera (Fig. 3) and in most habitats (Fig. 4). Underground nests were the most common ground position for wild nests, artificial domiciles and nests in human structures, followed by surface nests and aboveground nests. However, the proportion of nests found within each ground position differed between wild nests and artificial domiciles/nests in human structures. For wild nests: 52.1 % were underground, 33.3% were on the surface, and 14.6% were aboveground. For artificial domiciles/nests in human structures: 39.1% were underground, 38.5% were on the surface and 22.3% were aboveground. Artificial domiciles/nests in human structures may be increasing the representation of aboveground nests. There is no significant relationship between ground position and subgenus (Chi-square = 28.7, df= 20, p =0.09), or ground position and landscape-type (Chi-square = 16.9, df= 14, p = 0.27). Underground nests were the most commonly observed ground position for all subgenera and landscapes except *Thoracobombus, Megabombus, Cullumanobombus* and *Sibiricobombus* (although there was only one observation for this subgenus). The most common ground nesting position for *Thoracobombus, Megabombus* and *Cullumanobombus* were surface nests, while the one *Sibiricobombus* nest was found aboveground. Surface nests were more common in tropical forests and were as common as below-ground nests in agricultural landscapes (Fig. 4). Most bumble bee species were found nesting at one ground position (58.5%) and fewer species were found nesting at two (24.4%) or all three (17.1%) ground positions.

The materials used to construct bumble bee nests varied with the nest's ground position. Underground nests were often abandoned mammal burrows or nests (De Meulemeester et al., 2011; Hoffmann et al., 2004; Rao and Skyrm, 2013; for example, Svensson B G and Lundberg, 1977), or other cavities or holes in the ground (Fussell and Corbet, 1992; Lye et al., 2012). These holes or cavities could include human-constructed materials including buried PVC pipe or underneath buildings (Fussell and Corbet, 1992; Inoue et al., 2010; Lye et al., 2012). Some studies reported entrances to underground burrows would sometimes be covered with vegetation (either dried or living) and this is thought to help prevent the nest from being detected (De Meulemeester et al., 2011; de Oliveira et al., 2015; Hoffmann et al., 2004). Underground nests may also be particularly susceptible to flooding. For example, Harder (1986) reported that common ground nesting species were found infrequently in the late season possibly due to an unseasonably wet spring that may have resulted in colony failures. This may explain why underground nests are less common in tropical forests (Fig. 4).

Surface nests were either constructed of plant material or associated with certain vegetation. The surface nests found in tropical forests were constructed from dried grass, cut leaves, and twigs (Hines et al., 2007; Sakagami et al., 1967; Taylor and Cameron, 2003). These nests were constructed by bumble bees in a dome-like shape to provide shelter for the colony. Additionally, the nests were often associated with other vegetation including shrubs or trees to act as structural support (Chavarria, 1996; Hoffmann et al., 2004; Taylor and Cameron, 2003). Non-tropical surface nesting bumble bees would also construct their nests under the vegetation cover, especially tussock grasses, leaf litter, or log piles to protect their nests (Fussell and Corbet, 1992; Kells and Goulson, 2003; Lye et al., 2012; Sakagami and Katayama, 1977; Svensson et al., 2000). Surface nests in more human-developed areas included within buildings or walls (Fussell and Corbet, 1992). Surface nesting bumble bees would be vulnerable to ground disturbances such as development and mowing, and in agricultural landscapes to tilling or grazing livestock (Harmon-Threatt and Chin, 2016). Although surface nests are still detected in agricultural landscapes (Fig. 4), they are not likely to be found in arable lands. Indeed, the only study that found bumble bee nests within arable lands found them underground (Rao and Skyrm, 2013). Setting aside undisturbed areas in agricultural areas (such as field margins) or in other developed regions (such as roadsides) may be a strategy to reduce impacts to surface nesting bumble bees, and underground nesting bees if tilling/soil disturbances occurs. Surface nesting species may also be susceptible to flooding or wet conditions.

Aboveground bumble bee nests were observed the least often and this could be because of the inherent difficulties in observing them. Most of the aboveground bumble bee nests included in this review were occupied artificial nest boxes (47% of papers). Most other observations of aboveground nests (85%) occurred in gardens with bumble bees found in bird houses, within buildings/walls, in trees, tree cavities, and in cavities between rock piles/walls (for example, Janzen, 1971; Lye et al., 2012; Osborne et al., 2008; Richards, 1978). The remaining aboveground nests were in trees or within dense piles of grass and leaves. This might explain why most aboveground nests were in forest edge and urban sites as there would be naturally occurring or human-made cavities in trees, logs, exposed rock, and in buildings/walls. These aboveground nests may be going undetected as observational studies and the use of detection dogs focus on searching the ground for nests or search for low-flying nest-searching queens (O’Connor et al., 2017, 2012; Waters et al., 2011). Future studies should also attempt to search for nests aboveground particularly in forested habitat to help determine the true extent of aboveground nesting bumble bees.

Detecting bumble bee nests

Bumble bee nests are difficult to detect, and this has likely contributed to the lack of studies on nesting habitat. Some methods that have been employed to detect bumble bee nests include locating nests by observation - “observational”, using observations of nest-searching queens as a proxy for nest locations - “nest-searching queens”, using genetic analysis to determine number of colonies in an area – “molecular”, using statistical modeling to predict nest locations – “modeling” and using trained detection dogs to locate nests – “detection dogs” (Table 1).

Observational studies vary from standardized (i.e. searching predetermined transects or areas (e.g. O’Connor et al., 2017)) to opportunistic searches for nests (e.g. De Meulemeester et al., 2011). Observational studies have the advantage of identifying the exact location of bumble bee nests for further analysis of habitat preferences. However, the major disadvantage of observational studies is the amount of time and effort needed to find often difficult to detect nests. The most successful application of observational methods to detect nests was by Lye *et al.* (2012) who used citizen scientists to monitor their gardens for bumble bee nests across the United Kingdom. This study located an impressive 1022 bumble bee nests from ten species (including species classified as “other”, or “unknown”) between 2007-2009 versus an average of 21 nests for all other observational studies. The efficiency of using citizen science data to gather large amounts of data also comes at the cost of biased data towards areas readily surveyed by participants (i.e. gardens) and this should be considered when employing this method. Other observational studies monitored artificial nest-boxes or domiciles. This method can be useful for gathering information on the nesting habitat for bumble bees and be easier than looking for natural bumble bee nests. Occupancy of artificial domiciles is likely inversely related to the number of natural nest sites such as rodent burrows (Richards, 1978). Finding natural nests would still be preferred for modelling nesting habitat to determine what natural materials are required for bumble bee nest sites. (O’Connor et al., 2012; Waters et al., 2011). Detection dogs have the potential to significantly reduce human search effort and rapidly and accurately identify bumble bee nests, and they were successfully used in two studies thus far (O’Connor et al., 2012;

Waters et al., 2011). In these studies, dogs were trained to locate bumble bee nests by scent. Once a nest is located, the dogs signal to their handlers the location of the nest in a non-destructive manner. Nests identified by dogs are also visually confirmed by researchers. However, the O'Connor *et al.* (2012) study which compared the nest detection ability between humans and dogs concluded that dogs were no more efficient than trained humans, and given the cost involved with training dogs, they would recommend using humans over dogs. This is the only study that has compared the efficiency of using detection dogs as a method for identifying nests and more research into the potential use of this method is needed.

Another potentially useful method for locating bumble bee nesting and overwintering sites would be the use of radio telemetry. Advances in radio-tracking technology has made it possible to track some insect species including bumble bees. Radio-telemetry and radio-frequency identification (RFID) has previously been used to study foraging and flight distances in bumble bees (Hagen et al., 2011; Minahan and Brunet, 2018), but this method has not (to our knowledge) been applied to bumble bee nesting or overwintering studies. Spring queens or workers could be fitted with trackers so that researchers can follow individual bees back to their nest. Additionally, young queens can be tracked later in the season to find overwintering habitats. There are some challenges to radio-tracking that need to be considered when deploying this method. These are reviewed in Kissling *et al* (2014) and include: short battery life, limited range of tracking, weight of the trackers, and the potential for the trackers to alter the behaviour of the individual.

Use of proxy measures to identify bumble bee nests can be an effective alternative, but with some limitations. Observing areas with a high density of nest-searching queens allows a greater sample size than looking for nests due to the easier detectability of nest-searching queens (O’Connor et al., 2017; Svensson et al., 2000). These methods can be inaccurate as a survey technique of bumble bee habitat because it does not actually find the physical location of nests. Molecular methods are likely the most rapid method of identifying many colonies within a given area (Geib et al., 2015; Goulson et al., 2010). Similarly, actual nests are not identified that can limit its applicability to more specifically determining nesting habitat especially at finer scale than landscape-level descriptions of habitat. Using a statistical modeling approach to predict nest locations have been applied to few studies thus far and have had limited success (Carvell et al., 2017; Suzuki et al., 2009, 2007). These studies have attempted to use forage availability to predict nesting or overwintering sites, with mixed results. If these models can be improved this could be an extremely powerful method to decrease survey times for nesting. However, these models likely are weakened by an incomplete understanding of bumble bee habitat. Future bumble bee nesting habitat studies may need to consider the trade-off between obtaining a good sample size with accurately predicting bumble bee nesting habitat.

Conclusions

Bumble bees appear to be generalists within and across subgenera in terms of nesting habitat preferences. Bumble bees may preferentially overwinter in shaded banks either with moss or in bare-ground, but more research is needed on overwintering habitat. Future studies should attempt to increase the knowledge available on the nesting habitat of bumble bees by investigating understudied habitat areas such as dunes, and alpine areas and by recording detailed descriptions of the habitat surrounding nests. For increased specificity in bumble bee habitat requirements it is suggested that observational methods especially citizen science projects be employed to increase the likelihood of finding a suitable number of nests and overwintering sites and look further into the efficiency of detection dogs. Increasing knowledge on the specific nesting and overwintering requirements for bumble bees is important to ensure their protection and aid in conservation efforts for declining species. Current management efforts focusing on bumble bee habitat are mainly interested in expanding the available forage for bumble bees. If nesting or overwintering habitat is limiting, focusing solely on forage will do little to protect or increase populations.

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Tables and Figures

Table 1: Bumble bee species information for all studies included in the literature review. Species are divided into subgenera. Numbers in brackets indicate the number of studies reported, square brackets are the number of overwintering studies. The IUCN red list status (IUCN, 2017) for each species is given. Bumble bee nest or overwintering detection methods are shown under method and include detection dogs (dogs trained to find nests) molecular (i.e. genetic analysis), observational (i.e. transect visual surveys), Nest-searching queen surveys (as a proxy for bumble bee nest density), and modeling (i.e. predicting nest locations based on floral resources).

	Species	Continent	IUCN status[2]	Method
Alpigeno bombus (1)	*Bombus wurflenii* (1)	Europe	Not Assessed	Observational[1]
Alpinobombus (8)	*Bombus alpinus* (1)	Europe	Vulnerable	Observational[2] Nest-searching queens[2]
	Bombus balteatus (3)	Europe[2] North America[3,4]	Not Assessed	Nest-searching queens[2] Molecular[3] Observational[3,4]
	Bombus hyperboreus (2)	Europe[2] North America[31]	Not Assessed	Observational[31] Nest-searching queens[2]
	Bombus polaris (2)	Europe[2] North America[31]	Data Deficient	Observational[31] Nest-searching queens[2]
Bombias (3)[1]	*Bombus auricomus* (1)	North America	Least Concern	Observational[5]
	Bombus nevadensis (2)[1][5]	North America	Least Concern	Observational[5,6]
Bombus sensu stricto (33)[4]	*Bombus affinis* (2)	North America	Critically Endangered	Observational[7,8]
	Bombus hypocrita (1)	Asia	Not Assessed	Observational[9]
	Bombus ignitus (1)	Asia	Not Assessed	Observational[9]
	Bombus lucorum (10)[2][55,56]	Europe	Not Assessed	Detection Dogs[10] Nest-searching queens[2, 11-14] Observational[1,2,14-16]
	Bombus occidentalis (4)	North America	Vulnerable	Nest-searching queens[17] Observational[7, 18, 19]
	Bombus terrestris (14)[2][55,56]	Europe[10-14, 20, 23] Asia[26] Australia[27, 28]	Not Assessed	Detection dogs[10] Molecular[20, 23] Nest-searching queens[11-14] Observational[14-16, 25-28]
	Bombus terricola (2)	North America	Vulnerable	Observational[7,8]
Cullumanobom bus (46)[1]	*Bombus griseocollis* (1)	North America	Least Concern	Observational[29]
	Bombus melaleucus (1)	South America	Data Deficient	Observational[59]
	Bombus rufocinctus (4)[1][30]	North America	Least Concern	Nest-searching queens[17] Observational[18,29,30]
Megabom bus (13)[3]	*Bombus diversus* (1)	Asia	Not Assessed	Observational[9]
	Bombus hortorum (9)[2][55,56]	Europe	Not Assessed	Detection dogs[10] Nest-searching queens[11-14] Observational[14,15,25,27]

	Bombus ruderatus (3)[1][56]	Europe Australia	Not Assessed	Observational[25,27,28]
Melanobombus (11)[2]	*Bombus lapidarius* (11)[2][55,56]	Europe	Not Assessed	Detection dogs[32] Molecular[22,23] Nest-searching queens[11-14] Observational[1,14-16,25]
Mendacibombus (1)	*Bombus handlirschianus* (1)	Asia	Not Assessed	Observational[33]
Pyrobombus (46) [10]	*Bombus ardens* (4)	Asia	Not Assessed	Observational[9,34-36] Statistical model[34-36]
	Bombus beaticola (1)	Asia	Not Assessed	Observational[9]
	Bombus bifarius (4)[1][37]	North America	Least Concern	Molecular[3] Nest-searching queens[17] Observational[18,37]
	Bombus bimaculatus (1)[1][8]	North America	Least Concern	Observational[7]
	Bombus centralis (1)	North America	Least Concern	Observational[37]
	Bombus flavifrons (4)[1][37]	North America	Least Concern	Molecular[3] Nest-searching queens[17] Observational[18, 37]
	Bombus frigidus (2)[1][37]	North America	Least Concern	Observational[18,37]
	Bombus huntii (2)[1][37]	North America	Least Concern	Nest-searching queens[17] Observational[37]
	Bombus hypnorum (2)	Europe	Not Assessed	Nest-searching queens[2] Observational[16]
	Bombus impatiens (2)[2][8,58]	North America	Least Concern	Observational[7,8]
	Bombus jonellus (2)	Europe	Data Deficient	Detection dogs[32] Nest-searching queens[2]
	Bombus lapponicus (1)	Europe	Least Concern	Nest-searching queens[2]
	Bombus melanopygus (1)	North America	Least Concern	Observational[37]
	Bombus mixtus (2)	North America	Least Concern	Observational[18,37]
	Bombus monticola (1)	Europe	Not Assessed	Nest-searching queens[2]
	Bombus pratorum (9)[2][55,56]	Europe	Not Assessed	Molecular[23] Nest-searching queens [2,11-14] Observational[2,14,16,25]
	Bombus sylvicola (2)[1][37]	North America	Least Concern	Molecular[3] Observational[37]

	Bombus ternarius (2)	North America	Least Concern	Observational[18,37]
	Bombus vagans (3)	North America	Least Concern	Observational[7,29,37]
Sibiricobombus (1)	*Bombus niveatus* (1)	Asia	Not Assessed	Observational[38]
Subterraneobombus (7)[2]	*Bombus appositus* (2)[1][39]	North America	Least Concern	Observational[18,39]
	Bombus borealis (2)	North America	Least Concern	Nest-searching queens[7] Observational[39]
	Bombus distinguendus (2)	Europe	Data Deficient	Detection dogs[32] Molecular[40]
	Bombus subterraneus (1)[1][56]	Europe	Not Assessed	Nest-searching queens[12]
Thoracobombus (41) [2]	*Bombus brevivillus* (1)	South America	Data Deficient	Observational[43]
	Bombus deuteronymus (1)	Asia	Data Deficient	Observational[9]
	Bombus fervidus (5)[1][44]	North America	Vulnerable	Observational[7,8,18, 29, 44]
	Bombus medius (2)	North America	Vulnerable	Observational[45,46]
	Bombus mesomelas [1]	Europe	Not assessed	Observational[55]
	Bombus mucidus (2)	South America	Least Concern	Observational[41,42]
	Bombus muscorum (1)	Europe	Not Assessed	Detection dogs[32]
	Bombus pascuorum (15)	Europe	Not Assessed	Detection dogs[10] Molecular[20-24] Nest-searching queens[11-14,] Observational[1,14, 16,]
	Bombus pensylvanicus (1)	North America	Vulnerable	Observational[29]
	Bombus pseudobaicalensis (1)	Asia	Not Assessed	Observational[9]
	Bombus pullatus (3)	South America	Data Deficient	Observational[47-49]
	Bombus ruderarius (2)	Europe	Not Assessed	Nest-seeking queens[13] Observational[25]
	Bombus schrencki (2)	Asia	Not Assessed	Observational[49,50]
	Bombus transversalis (5)	South America	Least Concern	Observational[51-54]
Psithyrus [5]	*Bombus barbutellus* [1][55]	Europe	Not Assessed	Observational
	Bombus campestris [1][55]	Europe	Not Assessed	Observational
	Bombus sylvestris [1][55]	Europe	Not Assessed	Observational
	Bombus vestalis [2][55,57]	Europe	Not Assessed	Observational

1. (Dramstad, 1996) 2. (Svensson B G and Lundberg, 1977) 3. (Geib et al., 2015) 4. (Hobbs, 1964) 5. (G. A. Hobbs, 1965) 6. (Rao and Skyrm, 2013) 7. (Plath, 1922) 8. (Plath, 1927) 9. (Sakagami and Katayama, 1977) 10. (O'Connor et al., 2012) 11. (Lye et al., 2009) 12. (Svensson et al., 2000) 13. (Kells and Goulson, 2003) 14. (O'Connor et al., 2017) 15. (Osborne et al., 2008) 16. (Lye et al., 2012) 17. (Bowers, 1985) 18. (Richards, 1978) 19. (Gamboa et al., 1987) 20. (Darvill et al., 2004) 21. (Knight et al., 2009) 22. (Goulson et al., 2010) 23. (Knight et al., 2005) 24. (Herrmann et al., 2007) 25. (Fussell and Corbet, 1992) 26. (Inoue et al., 2010) 27. (Barron et al., 2000) 28. (Palmer, 1968) 29. (Harder, 1986) 30. (G A Hobbs, 1965) 31. (Milliron and Oliver, 1966) 32. (Waters et al., 2011) 33. (De Meulemeester et al., 2011) 34. (Suzuki et al., 2007) 35. (Nakamura and Toquenaga, 2002) 36. (Suzuki et al., 2009) 37. (Hobbs, 1967) 38. (Rasmont et al., 2008) 39. (Hobbs, 1966a) 40. (Charman et al., 2010) 41.(Sakagami et al., 1967) 42. (Gonzalez et al., 2004) 43. (de Oliveira et al., 2015) 44. (Hobbs, 1966b) 45. (Michener and Laberge, 1954) 46. (Rau, 1941) 47. (Chavarria, 1996) 48. (Hines et al., 2007) 49. (Janzen, 1971) 50. (Sakagami and Nishijima, 1973) 51. (Taylor and Cameron, 2003) 52. (Olesen, 1989) 53. (Ramirez and Cameron, 2003) 54. (Cameron et al., 1999) 55. (Alford, 1969) 56. (Sladen, 1912) 57. (Bols., 1937) 58. (Szabo and Pengelly, 1973) 59. (Hoffmann et al., 2004)

Fig. 1 Map of study locations. Blue circles = overwintering study locations, green = nesting study locations, turquoise = studies that described both overwintering and nesting habitat. The numbers correspond to the study number found in Table 1). 1. (Dramstad, 1996) 2. (Svensson B G and Lundberg, 1977) 3. (Geib et al., 2015) 4. (Hobbs, 1964) 5. (G. A. Hobbs, 1965) 6. (Rao and Skyrm, 2013) 7. (Plath, 1922) 8. (Plath, 1927) 9. (Sakagami and Katayama, 1977) 10. (O’Connor et al., 2012) 11. (Lye et al., 2009) 12. (Svensson et al., 2000) 13. (Kells and Goulson, 2003) 14. (O’Connor et al., 2017) 15. (Osborne et al., 2008) 16. (Lye et al., 2012) 17. (Bowers, 1985) 18. (Richards, 1978) 19. (Gamboa et al., 1987) 20. (Darvill et al., 2004) 21. (Knight et al., 2009) 22. (Goulson et al., 2010) 23. (Knight et al., 2005) 24. (Herrmann et al., 2007) 25. (Fussell and Corbet, 1992) 26. (Inoue et al., 2010) 27. (Barron et al., 2000) 28. (Palmer, 1968)29. (Harder, 1986) 30. (G A Hobbs, 1965) 31. (Milliron and Oliver, 1966) 32. (Waters et al., 2011) 33. (De Meulemeester et al., 2011) 34. (Suzuki et al., 2007) 35. (Nakamura and Toquenaga, 2002) 36. (Suzuki et al., 2009) 37. (Hobbs, 1967) 38. (Rasmont et al., 2008) 39. (Hobbs, 1966a) 40. (Charman et al., 2010) 41.(Sakagami et al., 1967) 42. (Gonzalez et al., 2004) 43. (de Oliveira et al., 2015) 44. (Hobbs, 1966b) 45. (Michener and Laberge, 1954) 46. (Rau, 1941) 47. (Chavarria, 1996) 48. (Hines et al., 2007) 49. (Janzen, 1971) 50. (Sakagami and Nishijima, 1973) 51. (Taylor and Cameron, 2003) 52. (Olesen, 1989) 53. (Ramirez and Cameron, 2003) 54. (Cameron et al., 1999) 55. (Alford, 1969) 56. (Sladen, 1912) 57. (Bols., 1937) 58. (Szabo and Pengelly, 1973) 59. (Hoffmann et al., 2004)

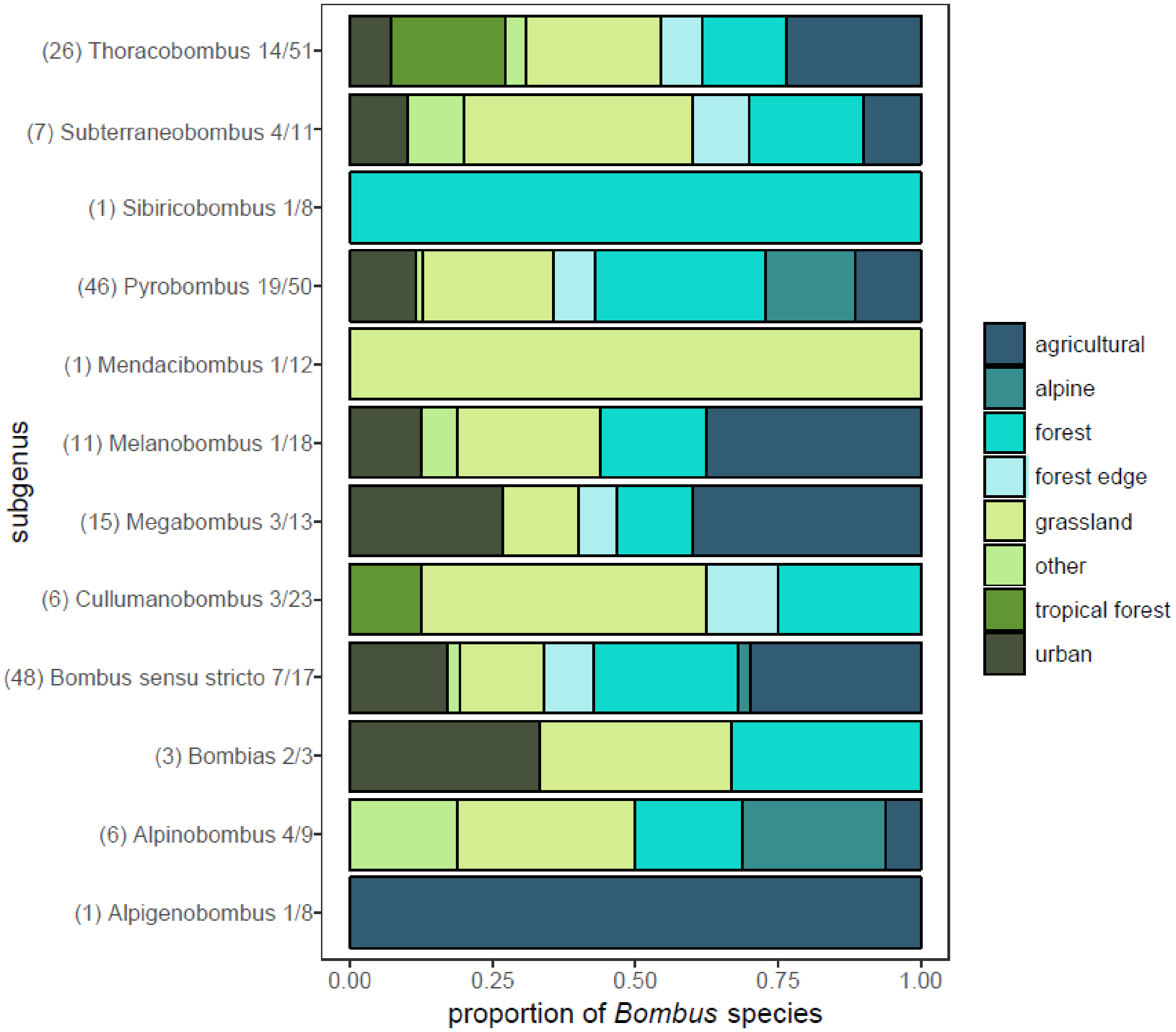

Fig 2. Landscape-level nesting habitat for the bumble bee subgenera. Values are the proportion of nests found for each subgenus by landscape type. The number of studies that included each subgenus is indicated in brackets. Fractions denote the number of species represented in the review per the total number of species within the subgenus.

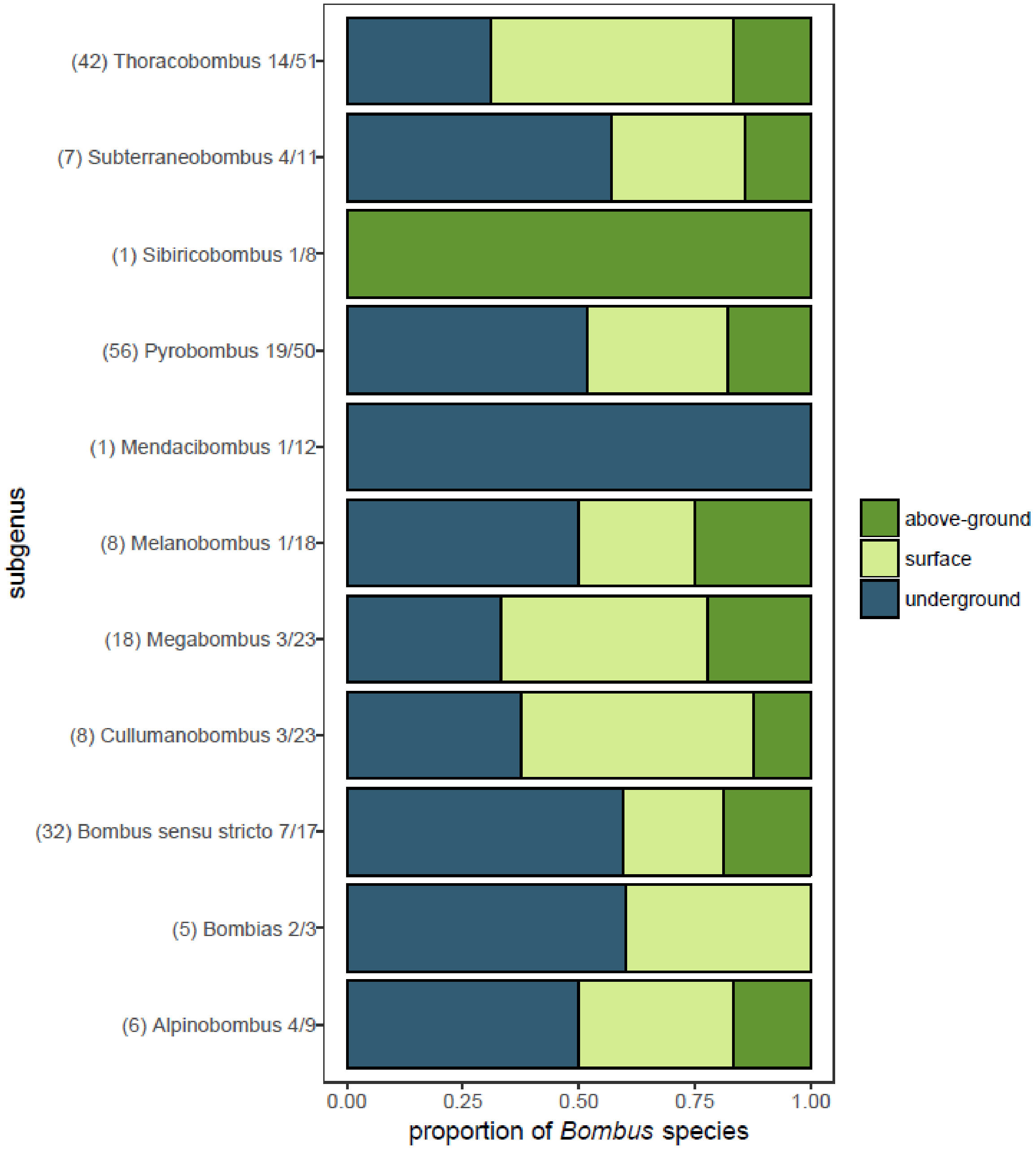

Fig. 3 The ground nesting position (underground, surface or aboveground) for the bumble bee subgenera. Values are the proportion of nests for each subgenus found per ground position. The number of instances where the ground position for each subgenus was described is shown in brackets. Fractions denote the number of species represented in the review per the total number of species within the subgenus. Not all nesting studies described the position of nests (i.e. molecular detection method studies).

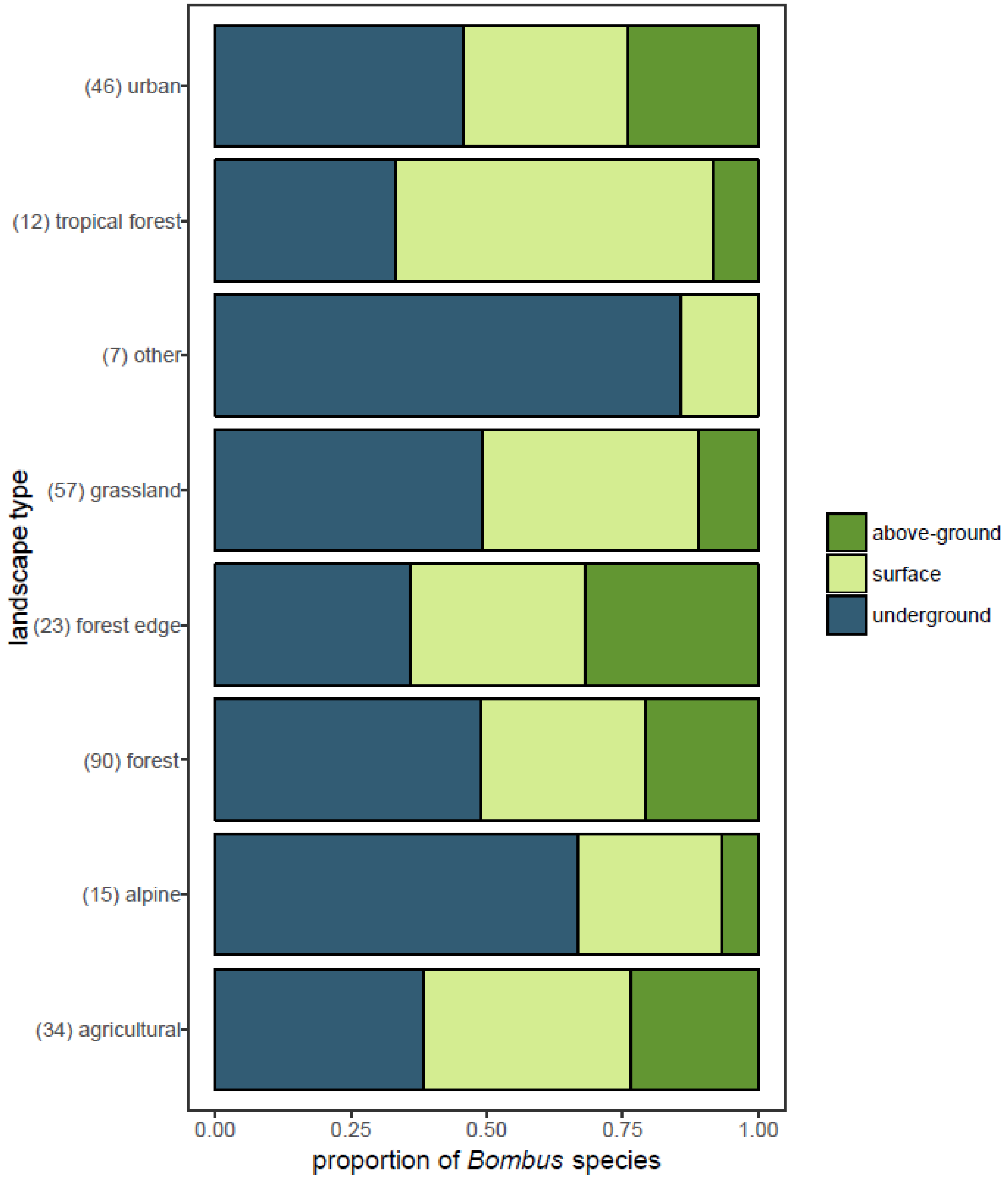

Fig. 4 Nesting position (underground, surface or aboveground) for bumble bees across eight habitat types as well as generalist species. Values the proportion of nests for each landscape-type found per ground position. The number of instances where the ground position for each landscape-type is shown in brackets. Not all nesting studies described the ground position of nests (i.e. molecular detection method studies).

Chapter 2: One size does not fit all: at-risk bumble bee habitat management requires species-specific and local and landscape considerations

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Author’s contributions:

ARL, SRC, conceived of the ideas and developed the methodology. ARL selected the final survey sites, devised the survey periods, recruited research volunteers, conducted field work and data collection, input all data, and completed all data analyses. ARL led the writing of the manuscript. Both authors contributed to editing and gave final approval for publication in Insect Conservation and Diversity and submission as a part of this dissertation.

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Summary

Declining bumble bees are threatened by habitat loss, pathogens and climate change. Despite policy and management recommendations to create pollinator habitat, habitat requirements for at-risk bumble bees remains unclear. Most studies on bumble bee habitat are descriptive, focus on floral resources, occur at one spatial scale, or do not examine at-risk species. We provide the first thorough habitat description for two North American bumblebee species (*Bombus terricola* and *Bombus pensylvanicus*) at-risk of extinction. We asked the following questions: 1) What characterizes *B. terricola* and *B. pensylvanicus* habitat? 2) Are landscape variables, local variables, or flowering plant species more important aspects of habitat? 3) do important variables change throughout the season? Surveys were conducted at 25 sites with a recent occurrence of either *B. terricola, B. pensylvanicus,* or both species across southern Ontario, Canada. Landscape variables were extracted from a 1-km buffer around each site. Local variables related to bumble bee resource requirements (floral, nesting and overwintering) and flowering species cover were measured in spring, mid-summer, and late-summer. We found that the proportion of different land cover classes at 1 km was a more important predictor of *B. terricola* and *B. pensylvanicus* presence than local transect based variables such as floral richness or the patchiness of floral cover. We did not find any evidence of important variables changing temporally, but floral resources were consistently important throughout the season. Our results highlight that management of at-risk pollinator species requires consideration of species-specific habitat requirements.

Introduction

Bumble bees are important pollinators in temperate and montane regions due to their ability to buzz pollinate and forage under cooler temperatures than other species (Goulson, 2003). Some bumble bee species (*Bombus* spp.; Apidae; Hymenoptera) are among the pollinators most in decline (Bartomeus et al., 2013). Threats contributing to bumble bee declines include: habitat loss (Goulson et al., 2008; Williams and Osborne, 2009), pathogen spillover (Colla et al., 2006; Szabo et al., 2012) climate change (Kerr et al., 2015), pesticides (Rundlöf et al., 2015; Whitehorn et al., 2012), and competition from non-native species (Herbertsson et al., 2016; Thomson, 2016). Declines in bumble bees and other pollinator species are cause for concern as losses in pollination services can negatively impact ecosystem functioning and agricultural production (Biesmeijer et al., 2006; Potts et al., 2010). Given the importance of these species both economically and ecologically, it is vital to effectively develop conservation management plans to help mitigate declines.

Although bumble bee declines are likely caused by multiple interacting factors including habitat loss, pathogen spillover, and climate change (Brown and Paxton, 2009; Cameron and Sadd, 2020; Goulson et al., 2015, 2008), focusing on habitat requirements is important to optimize species survival where remnant populations occur or may be reintroduced. Having enough good quality habitat can make species more resilient to other stressors (Hodgson et al., 2011, 2009). However, the habitat requirements for bumble bees is not well understood especially for nesting and overwintering resources (Liczner and Colla, 2019). Despite this, many jurisdictions have implemented policies to conserve declining bee species (Byrne and Fitzpatrick, 2009), many of which focus solely on forage resource availability for bumble bees without considering nesting or overwintering resources. This can be problematic as it can be difficult to determine which, if any, of these resources are currently limiting throughout the colony cycle if they cannot be accurately quantified and could then lead to misguided conservation efforts. To conserve declining bumble bee populations, their habitat requirements must urgently be determined.

Important habitat variables can change temporally as a species may have different needs throughout its phenology (i.e. foraging, searching for mates, dispersing/migrating, hibernation etc.). Temporal resource use may fluctuate with the colony cycle of bumble bees. Nesting resources may be important during colony initiation, followed by floral resources during colony development, and overwintering habitat for the remainder of the year. Additionally, forage resources may change spatially and temporally as bloom times of flowering plant species varies (Devoto et al., 2014; Hatfield and LeBuhn, 2007). Examining bumble bee habitat variables at multiple time periods can improve our ability to understand important resources for bumble bees at different colony stages and thus more effectively conserve at-risk bumblebee populations.

Habitat selection by species is a hierarchical process. Species respond to environmental cues at different levels (e.g. regional, landscape, local) that determine the species’ geographic range, home range, patch occupancy and resource selection (Johnson, 1980; Mayor et al., 2009; Morrison, 2013). For example, climate is a strong driver of bumble bee occurrences across regional scales (Kerr et al., 2015). Different bumble bee species are also associated with certain landcovers, such as grassland or forests (e.g. Goulson et al., 2006; Lanterman et al., 2019). These landcovers can also have differences in resource availability, for example, herbaceous meadows may have more floral resources than forests. Resources for overwintering and nesting may vary at finer scales than the landcover level. For example, bumble bees may preferentially nest in patches with particular vegetation including tussock grasses or wood piles and compost heaps (Fussell and Corbet, 1992), and bumble bees may preferentially overwinter underground with different ground coverage i.e. moss, bare ground, leaf litter etc. (Alford, 1969). Bumble bees may also preferentially foraging on some floral species within flower patches (Crowther et al., 2014; Gibson et al., 2019; Harmon-Threatt et al., 2017). To fully describe a species' habitat, it is important to consider variables from multiple environmental levels.

In this study, we provide the first detailed description of the habitat for two at-risk bumble bee species using ground surveys to measure local variables and flowering plant species cover**,** and landscape variables from geospatial data within the highly developed region of Southern Ontario, Canada. The two focal species in this study are *B. terricola* (Kirby, 1837) and *B. pensylvanicus* (DeGeer, 1773). Both species are listed as Vulnerable on the IUCN Red List (IUCN, 2017) and assessed as at-risk of extinction federally in Canada. We selected these two species because 1) they are still found in Southern Ontario (but at significantly reduced abundances and ranges), 2) represent two different subgenera with additional declining species presumably with similar ecological requirements in North America (*Bombus sensu stricto* and *Thoracobombus* respectively) and 3) are reported to have different habitat requirements, despite co-occurring (Colla, 2016)**.**

Here we addressed the following questions: 1) What characterizes *B. terricola* and *B. pensylvanicus* habitat, 2) are landscape variables, local variables, or flowering plant species more important determinants of habitat 3), and do important variables change throughout the season?

We addressed these questions by performing quadrat surveys of habitat variables at three time periods: spring, mid-summer, and late summer, and GIS analysis of landscape data including land cover, agricultural census data, soil texture data, and climate data. We expect that *B. terricola* and *B. pensylvanicus* have distinct habitat characteristics, preferring wooded and open habitats respectively, as has been reported previously (Colla, 2016). We also predict that local habitat variables would be the most important determinant of bumble bee habitat as bumble bees can respond to changing local resource amounts (Jha and Kremen, 2013). Finally, we expect that important habitat variables will change throughout the season with nesting resources, floral resources, and overwintering resources identified as the most important variables in spring, summer and late-summer respectively.

Materials and Methods

Study species habitat

The habitat for *B. terricola* has been described as within or near wooded areas and wetlands while *B. pensylvanicus* is found in grasslands and open farmlands (Williams et al., 2014). In southern Ontario, Canada, both species have a widespread distribution across North America. *Bombus terricola* is found in Eastern Temperate Forests, Boreal Forests, the Appalachian Mountains at higher elevations, the Great Plains, within Tundra/Taiga, and the Western Mountains (Williams et al., 2014). *Bombus pensylvanicus* is found in Eastern Temperate Forests, the Great Plains and Desert West (Williams et al., 2014). In southern Ontario, Canada, the emergence times for *B. terricola* and *B. pensylvanicus* are quite distinct with *B. terricola* queens emerging in early spring (April), workers emerge in early May and males emerge in late May, and *B. pensylvanicus* queens emerge in late-spring (May), workers emerge in June, and males in July (Colla et al., 2011; Williams et al., 2014).

Both species also have different nesting behaviours. *B. terricola* nests underground (Plath, 1927, 1922; Williams et al., 2014) while *B. pensylvanicus* mainly nests on the surface among long grass, although some underground nests have been found (Harder, 1986; Williams et al., 2014). Preferred forage plant species in southern Ontario for *B. terricola* and *B. pensylvanicus* have also been described (Colla and Dumesh, 2010) and include species within the genera *Crocus, Eupatorium, Linaria, Melilotus, Monarda, Ribes, Rosa, Rubus, Spiraea, Taraxacum, Vaccinium and Vicia* for *B. terricola* and *Astragalus, Cirsium, Cornus, Dalea, Echinacea, Helianthus, Kallstroemia, Liatris, Mentzelia, Silphium, Solanum, Trifolium, and Vicia* for *B. pensylvanicus,* among others.

Site selection

Twenty-five sites were selected from Southern Ontario**,** Canada that had a recent occurrence (between 2002-2017) of *B. terricola* and/or *B. pensylvanicus* (Fig. 1). Of the 25 sites, 16 had recent occurrences of *B. terricola*, six for *B. pensylvanicus,* and three sites had both species. The three sites where both species recently occurred were analysed separately from *B. terricola* or *B. pensylvanicus* sites and are hereafter referred to as *both* sites. We attempted to select an even number for each species, however, this was not possible given the difficulty in detecting at-risk species and selecting sites where access can be granted (i.e. not private property). The distance between study sites was 2.92 km - 375 km. The study sites were Provincial and National Parks, Conservation Areas and privately-owned land (Appendix A). Many of these sites were surrounded by urban/developed areas or agriculture.

The recent occurrence records used for site selection were obtained from combined survey records from field researchers, museum specimens, and community science submissions through iNaturalist and Bumble Bee Watch, so sites are not necessarily systematically nor randomly surveyed. (Richardson, 2019 https://www.leifrichardson.org/ bbna. html). Although we did not confirm if the species have persisted in these sites during this study, 21 of the sites included in this study have very recent occurrences between 2013 and 2017 for *B. terricola* or *B. pensylvanicus* (Appendix A). The four 2002-2013 sites are all for *B. pensylvanicus*. Given that most sites had very recent occurrences we have assumed that these sites still represent *B. terricola* or *B. pensylvanicus* habitat. We did not re-confirm the presence of either species due to time constraints on sampling all 25 sites as soon as possible to minimize variation between sites within each of the three survey periods (see below). Some of our study sites had an older (i.e. greater than 2 years) record of occurrence for the bumble bee species. However, we found no evidence that years since last occurrence affected the outcome of the analyses (Appendix B).

Bumble bee local variables and flowering plant species surveys

Local variables and flowering plant species percent cover were surveyed from May-August 2017. We intended to survey each site three times: spring (late April-May), mid-summer (late June-July), and late-summer (August). However, due to flooding or access issues (i.e. gaining permissions) not all sites were surveyed three times (Appendix A). The survey time periods were selected as they generally correspond to queen emergence and colony initiation in spring, worker production and colony growth in summer, the production of reproductive individuals, mating, and queen hibernation in late-summer.

A survey site was delineated by a 1-km buffer around the GPS location of each recent bumble bee occurrence in QGIS**.** A 1 km buffer was used as this is the approximate maximum flight distance for bumble bees (Elliott, 2009; Osborne et al., 2008; Redhead et al., 2016). In-field survey locations were randomly determined using QGIS. The location of two 250-m transects were randomly placed within each site using the QGIS random points function. The direction (i.e. degrees from north) of each transect was determined using a random number generator. Local variables and flowering plant species cover were surveyed every 50 m along the transect using a 10 x 10 m quadrat. Within each quadrat (10 per site per season) local variables related to floral, nesting, and overwintering resources for bumble bees, as well as the percent cover of each flowering plant species were measured (Table 1). The local variables animal burrow density and coarse woody debris measures (abundance, length, width, decay class) were only measured once in the spring as we did not expect these variables to change throughout the season. Forage variables were measured for each survey period (Figure 2). The list of variables outlined in table 1 are hereafter termed as the "local variables". Floral patchiness (Table 1) was calculated as follows:

Eq. 1. $$patchiness = \left(\sum \left(\frac{floralcover}{totalfloralcover}\right)^2\right) totalfloralcover$$

Where *floralcover* is the percent cover of each flowering plant species in the quadrat and *totalfloralcover* is the total percent cover for all flowering plant species in the quadrat. The patchiness value was calculated to indicate plots with a high percent cover of a single plant species compared to plots with a more even distribution of flowering species. The ratio (Table 1) of native-to-non-native flowering plant species was also calculated as follows:

Eq. 2. $$Ratio = \frac{(native - non\text{-}native)}{(native + non\text{-}native)}$$

The ratio of native to non-native flowering plant species was considered as different bumble bee species may preferentially forage on native vs. non-native plants (Gibson et al., 2019; Salisbury et al., 2015).

Landscape variable data extraction

Landscape variables were extracted from raster and vector files (the Statistics Canada Agricultural Census data were the only vector files) within the same 1-km study area defined above. All datasets were loaded into QGIS (v. 3.4.0) and this program was used to extract the percent cover of each landscape classification within the 1-km study area. The variables include land cover (Southern Ontario Land Resource Information System 2011 version. 2, 30 m resolution), agriculture census data including: pesticide usage, number of honey bee colonies, and gallons of other bees (1 gallon = ~10,000 larvae) (Statistics Canada Agricultural Census 2011, summarized by 2011 census boundaries), area of crop-type (Ontario Agricultural Resource inventory 2005, 25 m resolution), climate data (bioclimatic variables from WorldClim, 1 km resolution), and soil texture data (Soil Survey Complex of Ontario 2003, 1 km resolution). Agricultural census data are available at the census boundary level, therefore, these data are averaged over the entire census boundary. The percent cover of all landscape variables within each study site was calculated for subsequent analysis. We also created a habitat richness variable by counting the number of unique land classes within each 1-km buffer that was included in subsequent analyses. A description of each landcover class is shown in Appendix C.

Statistical analyses

Statistical analyses were performed independently for landscape, local variables and flowering plant species. The local variables and flowering plant species were also analysed separately for each survey period (spring, summer, late-summer).

Prior to running any analysis, the data were checked for collinearity and the flowering plant species data were transformed. Collinearity among explanatory variables was determined using the Variance Inflation Factor (VIF) with values greater than 10 identified as collinear (Hair et al., 2013) (*vifcor* function in *usdm* package) (Naimi et al., 2014). VIF is an iterative process that takes one predictor and regresses it against all other predictors. When variables were identified as collinear, the variable with the higher VIF was removed (Appendix D). The Hellinger transformation (*decostand* function in *vegan* package) was applied to flowering plant species as there were many zeros to remove the double-zero effect. The double-zero effect occurs when two sites are deemed similar because a species is not found at either site. This can be problematic when considering rare species or study designs with large gradients (Legendre and Gallagher, 2001).

To reduce the number of explanatory variables included in the landscape and flowering plant species analysis we used stepwise logistic regression by AIC (Akaike Information Criterion) (*stepAIC* function in package *MASS*). This method iteratively adds and removes predictors to identify a subset of predictor variables that forms the best model (the one with the lowest AIC value). The stepwise logistic regression outputs were not analysed in the results as it was solely used for the purpose of variable selection. The response variable for the logistic regression was the bumble bee species corresponding to each site (e.g. for *terricola* sites *terricola* =1, and *pensylvanicus* or *both* sites = 0). A summary of the final models with the reduced variables selected for analysis is shown in Appendix E

Ordination analyses (redundancy analysis) were used to determine which habitat variables are correlated with *B. terricola*, *B. pensylvanicus* and *both* sites. We first fit an unconstrainted ordination (PCA) and then fit a linear relationship with the bumble bee species using *envfit* (package vegan). The *envfit* function constrains the dimensional space of the bumble bee species to maximize correlation with the variables in the unconstrained ordination (Oksanen et al., 2013). This allows the bumble bee species to ordinate according to the habitat variables and not based on site identity (bumble bee species. E.g. when the site is *B. terricola* it is coded as 1, and *B. pensylvanicus* and both = 0). The ordinations were performed using a correlation matrix (since not all variables had the same units) and were scaled to species. Habitat characteristics for *B. terricola* and *B. pensylvanicus* were inferred from the results of the redundancy analyses. The RDAs for landscape and flowering plant species only contained the variables identified from the stepwise logistic regression (see above).

Variance partitioning analyses (*varpart* function in package *vegan*) were conducted for the local variables included in each RDA to determine the percent variance explained by the explanatory variables for each survey period (spring, mid-summer, late-summer). This was to determine if there is a temporal difference in the most important habitat variable groups. The response variable was bumble bee species (*B. terricola, B. pensylvanicus* or *both*) and the explanatory variables used in the variance partitioning analysis were: floral cover (includes: the percent cover of each flowering plant species), floral structure (includes: richness, patchiness, ratio of native plant species, total floral cover), nesting resources (includes: density of animal burrows, vegetation cover), and overwintering resources (made up of: coarse woody debris number, size and class; vegetation over). All statistical analyses were conducted using R (R Core Team, 2018 version 3.4.0).

Results

Landscape variables explained the most variation

To determine whether landscape, local variables, or flowering plant species are more important for *B. terricola* and *B. pensylvanicus* habitat, we compared the variance explained by the optimal number of axes (determined by the inflection point on scree plots) for each redundancy analysis. The total variance explained by landscape was slightly greater than the variance explained by the local variables and flowering plant species cover explained the least variation (Table 2).

Bumble bee species have distinct habitats even in overlapping ranges

Bombus terricola, *B. pensylvanicus*, and *both* sites are distinct for landscape, local variables, and flowering plant species cover (Figures 3 and 4). For landscape, *B. terricola* habitat is positively correlated with coniferous forest, and is negatively correlated with: agricultural variables (number of farms reporting insecticide/herbicide/fungicide, acres of farms using insecticide/herbicide/fungicide, and number of honey bee colonies); undifferentiated (consists of urban green spaces, and some agricultural features), transportation-based land uses (highways, roads, etc.); and maximum temperature of the warmest month. Conversely, *B. pensylvanicus* is positively correlated with agricultural variables; undifferentiated and transportation land cover classes; maximum temperature of the warmest month; and is negatively correlated with coniferous forests. *Both* sites show the same pattern as *B. pensylvanicus* except they are negatively correlated with mixed forests.

Even where the species ranges overlap in southern Ontario, local variables are mainly distinct for *B. terricola* and *B. pensylvanicus*. The exceptions are for spring and late-summer where *B. terricola* and *B. pensylvanicus* are similar and *B. pensylvanicus* and *both* are similar respectively (Figure 4). *Bombus terricola* and *B. pensylvanicus* habitats in spring have large coarse woody debris (length and diameter) that is decayed; have a high ratio of native-to-non-native flowering plant species; high floral cover and floral plant species richness; a low abundance of coarse woody debris, vegetation cover and burrows. Conversely, *both* spring local variables have high vegetation cover; high abundance of coarse woody debris; high burrow density; low richness; a low ratio of native-to-non-native flowering plant species; and small coarse woody debris (length and diameter) that is less decayed. *B. terricola* is correlated with spring flowering plant species associated with forested habitats (e.g. downy-yellow violet, sand violet and trillium), *B. pensylvanicus* spring flowering plant species are associated with open/wet habitats (e.g. Creeping bluet, marsh marigold, yellow rocket cress), and *both* flowering plant species are associated with disturbed and open habitats (e.g. dandelion and wild strawberry).

Summer local variables and flowering plant species cover were distinct for *B. terricola*, *B. pensylvanicus* and *both*. *B. terricola* habitat is positively correlated with large coarse woody debris (length and diameter) and is negatively correlated with coarse woody debris abundance. *Bombus pensylvanicus* habitat is positively correlated with coarse woody debris abundance and negatively correlated with burrow density. *Both* habitat in summer is positively correlated with vegetation cover, flowering plant species richness, flowering plant species patchiness, total floral cover, and has a low ratio of native-to-non-native flowering plant species. *Bombus terricola* flowering plant species in summer were primarily forest associated species with a few disturbance-associated plant species (e.g. herb Robert geranium, St. John's wort, wood avens, prickly wild rose, garlic mustard). *Bombus pensylvanicus* flowering plant species were mixed of open and forest associated plant species (e.g. wood lily, dames rocket, white clover, hispid buttercup). *Both* summer flowering plant species were disturbance associated (e.g. dandelion, rough-fruited cinquefoil, oxeye daisy, hop clover, field chickweed, low hop clover, red clover, cow vetch).

In late-summer, the two bumble bee species were correlated with distinct flowering plant species. For local variables, *B. pensylvanicus* and *both* habitats are similar, but opposite to *B. terricola* habitat (Figure 4). The local habitat for *B. terricola* is positively correlated with large coarse woody debris (length and diameter); burrow density; a high ratio of native-to-non-native flowering plant species; and is negatively correlated with coarse woody debris abundance. *B. pensylvanicus* and *both habitat* local habitat is opposite *B. terricola*. Late-summer flowering plant species that were correlated with *B. terricola* habitat were mixed with forest, forest-edge, open, and disturbed associated species (e.g. garlic mustard, orange jewelweed, self heal, ribgrass, viper's bugloss, catnip, herb Robert geranium and yellow wood sorrel). *Bombus pensylvanicus* habitat is positively correlated with flowering plant species found in open and disturbed habitats (e.g. crownvetch, spotted knapweed, Canadian tick trefoil). *Both* late-summer flowering plant species were disturbed and open associated species (e.g. wild teasel, bird's-foot trefoil, chicory, milkweed, bull thistle, white sweet clover, common soapwort).

There is no temporal variation in local variables

Floral resources consistently explained the most variation across all seasons (spring R^2_{adj} = 0.164; mid-summer R^2_{ad} = 0.244, late-summer R^2_{ad} = 0.234). This was followed by floral structure (spring $R^2_{ad} = 0.033$; mid-summer $R^2_{ad} = 0.022$; late-summer $R^2_{ad} = 0.028$), nesting resources (spring $R^2_{ad} = 0.016$, mid-summer $R^2_{ad} = 0.022$, late-summer $R^2_{ad} = 0.0085$) and overwintering resources (consistent $R^2_{ad} = 0$ for all seasons). Therefore, most of the variation in resource variables is determine by floral resources.

Discussion

Understanding the habitat requirements for species at-risk of extinction is critical for species recovery and long-term conservation. Given recent policy focus (and thus financial resource availability) on pollinator habitat creation in many jurisdictions across North America (e.g. https://www.whitehouse.gov/sites/whitehouse.gov/files/images/Blog/PPAP_2016.pdf), this study provides early insight into the care and focus required for effective at-risk pollinator management. The aim of our study was to describe the habitat characteristics for two declining bumble bee species, *B. terricola* and *B. pensylvanicus*, in southern Ontario throughout the growing season. We found these two at-risk species have distinct habitat characteristics particularly for landscape, and flowering plant species cover. Landscape variables explained the most variation, however, local variables also explained a large portion of variation. There was no temporal effect on important resource type across the season as floral resources were consistently the most important resource variable for these two bumble bee species. These results emphasize that current conservation efforts aimed at generally improving pollinator habitat, without an evidence-based approach, may be ineffective for conservation if species-specific needs for declining species are not considered, or may even be detrimental by over-supporting common species.

Landscape variables explained more of the variation than local variables for the two bumble bees under study. Landscape variables may be more important for bumble bees as they are generalists, and they are able to fly relatively far distances (Dramstad, 1996; Kreyer et al., 2004; Osborne et al., 1999). This large dispersal and foraging range capability allows bumble bees to use temporally and spatially variable resources (Devoto et al., 2014; Hopfenmüller et al., 2014; Pope and Jha, 2018) especially in resource limited environments such as intensive agricultural areas (Heard et al., 2007; Potts et al., 2009; Rundlöf et al., 2008). If the landscape has limited floral resources (i.e. highly forested areas) negative effects on bumble bees and their fitness have been reported (Bukovinszky et al., 2017; Spiesman et al., 2017). Our local variables also explained much of the variation. If local resources are high (such as total floral cover) bumble bees would not need to fly far to access forage (Hatfield and LeBuhn, 2007; Herrmann et al., 2017). Individual flowering plant species cover did not explain much variation and is likely not a large determining factor in bumble bee habitat characterizations. Additionally, the flowering plant species that were correlated with *B. terricola* and *B. pensylvanicus* were associated with landcover types that were also associated with *B. terricola* and *B. pensylvanicus* habitat (e.g. *B. terricola* is correlated with forest landcover and it was mainly correlated with forest-associated flowering plant species). This lends support to the result that landscape variables are the most important determinant of bumble bee habitat. Therefore, both species-specific landscape and local variables are important to consider for bumble bee habitat conservation and management.

As suspected from previous literature, we found species-specific differences in habitat preferences for landscape and local variables for *B. terricola* and *B. pensylvanicus.* Our quantitative characterisations of *B. terricola* and *B. pensylvanicus* as forest and grassland/open species respectively supports previous, descriptive, work (Colla and Dumesh, 2010; Williams et al., 2014), and quantitative work for *B. terricola* (Richardson et al., 2019). This characterisation is apparent from both the landscape results and the association of *B. terricola* with forest-associated flowering plant species and *B. pensylvanicus* with grassland/open-associated flowering plant species. These associations may be related to niche partitioning, phenology and nesting preferences. *Bombus terricola* is an early emerging species that nests underground in abandoned small mammal burrows or in decaying woody materials such as tree stumps (Plath, 1922; Williams et al., 2014). Forests often have a higher density of flowering resources early in the season compared to other landcover types (Kaemper et al., 2016; Wray et al., 2014) that would benefit early-emerging bumble bee species. *Bombus pensylvanicus* emerges later than *B. terricola*, when forested landscapes have fewer floral resources compared to other landcover types (Wray et al., 2014). Additionally, *B. pensylvanicus* nests on the surface (Harder, 1986; Williams et al., 2014), and surface nests are often under vegetation such as tussock grasses (Fussell and Corbet, 1992; Lye et al., 2012; Sakagami and Katayama, 1977). Early in the season there may be too little vegetation to provide adequate nest protection for surface nesting species. Other at-risk bumble bee species with similar life history characteristics to *B. terricola* (i.e. *B. affinis, B. franklini, B. occidentalis*) and *B. pensylvanicus* (i.e. *B. fervidus*) may also have similar habitat preferences but whether these results can be extended to other species (and other regions) should be investigated in the future. Increasing habitat heterogeneity to include many patch types might be effective at increasing the number of bumble bee species as well as increasing the availability of resources throughout the season. Additionally, it is important to ensure there are enough floral resources for each habitat type to sustain bumble bee species found in different habitats.

There were differences in the ratio of native-to-non-native flowering plant species at *B. terricola* and *B. pensylvanicus* sites. In spring and mid-summer, *B. terricola* was correlated with a high native-to-non-native flowering plant species ratio, and *B. pensylvanicus* was associated with a high native-to-non-native flowering plant species ratio in spring, and late-summer. This tendency for sites with a higher density of native plant species is an important consideration for conservation to ensure there are native flowering plant species blooming throughout the colony cycle. Although our results may suggest increasing native flowering plant species over non-natives will benefit at-risk species, land managers may need to consider the availability of floral resources prior to removing non-native plant species that may be benefitting at-risk plant species. Non-native plants may be used by at-risk bee species if they can provide adequate nutrition and if there are limited floral options in the landscape (Baldock et al., 2019, 2015; Harmon-Threatt and Kremen, 2015). Focusing on which floral resources at-risk bumble bees prefer (whether they are native or not) is an important conservation concern as there is growing evidence that declining bumble bee species may have narrower diet breadths (Gibson et al., 2019; Kleijn and Raemakers, 2008; Wood et al., 2019). This may be especially important to consider in highly disturbed areas where non-native plant species dominate that are occupied by at-risk bumble bees. Our results are unable to separate whether the correlation with native or non-native flowering plant species reflects preferential use of these resources, or if this is simply a reflection of the landscapes where these species exist and is an important avenue of future research.

The close association of *B. pensylvanicus* with agricultural areas indicates potential cause for concern. This is because agricultural practices have been implicated in pollinator declines including: use of agrochemicals, tilling, and pathogen spillover from managed bees (Goulson et al., 2015; McArt et al., 2017; Rao and Skyrm, 2013; Whitehorn et al., 2012). Previous studies have reported that *B. pensylvanicus* populations contain higher parasite loads than other non-declining bumble bee species (Cordes et al., 2012; Tripodi et al., 2014). Managed bees often have higher pathogen loads than wild bees (Murray et al., 2013), and bumble bees near greenhouses have been observed to have higher pathogen infection rates than bumble bees away from greenhouses (Colla et al., 2006). Our *B. pensylvanicus* sites had higher numbers of greenhouses (at the census boundary level) compared to *B. terricola* or *both* sites (86, 27, and 7 respectively). Additionally, the use of fungicides and herbicides, which are widely applied in agriculture, has been linked with increased pathogen loads and increased insecticide toxicity in bees (McArt et al., 2017; Tsvetkov et al., 2017), and our *B. pensylvanicus* sites and indeed much of this species' northern range is within dense agricultural regions. Not surprisingly, *B. pensylvanicus* sites had the highest inputs of these pesticides compared to our other study sites. For example, the acres of insecticide applied for our study sites (at the census boundary level) is 253,012; 49,949; and 18,763 acres for *B. pensylvanicus, B. terricola* and *both* sites respectively. Despite these stressors, *B. pensylvanicus* likely occupies these areas as agricultural regions due to lack of other suitable habitats and natural, grassland habitats are in significant decline (Hoekstra et al., 2005; Samson and Knopf, 1996). Importantly, there is interest in improving agricultural landscapes to support pollinators. Agricultural areas can support bumble bees when there is semi-natural habitats in the surrounding landscape or when farms incorporate agri-environmental schemes such as hedgerows, woodlots, or field margins (Carvell et al., 2011; Kovács-Hostyánszki et al., 2017; Lye et al., 2009). These habitat features not only increase and sustain wild pollinators (Carvell et al., 2011; Heard et al., 2007), but can result in increased ecosystem services such as the pollination of crops (Bukovinszky et al., 2017; Feltham et al., 2015; Venturini et al., 2017). Given the potential threat of agrochemicals, their use should be limited wherever possible.

Our species-specific habitat characterizations may be limited in two ways. First, we did not confirm that either species is still present at the site. We have assumed that it is still present at the site or in the surrounding area. This assumption could skew our results if these sites no longer represent the habitat of these species. However, given the relatively short time span that we have used to select sites, the difficulty in locating rare species, and the fact that most sites are within protected areas, we do not expect any large land cover changes to have occurred. Since we did not expect any large land cover changes to have taken place, we believe that the habitat variables measured (particularly the landcover variables) would have been similar throughout the timeframe of bumble bee occurrence records we used for site selection (2002-2017) despite the potential for the species to no longer persist at some sites or to be in continued decline. Although local variables and flowering plant species cover likely fluctuates with yearly variation, we have assumed that this is within the acceptable variation for each bumble bee species. Another limitation is our measured habitat characteristics are likely an overestimate of the habitat for each species as the quality of a species habitat cannot be determined without demographic data (Morrison, 2013). It is possible that a proportion of the sites we surveyed that had bumble bees could have been low quality or sink habitats and thus our measured attributes of these sites would not accurately resemble the habitat for these species. Future studies should investigate demographic responses of bumble bee populations to habitat characteristics to determine the quality of habitat for bumble bees to better inform conservation management.

We did not find any evidence of a temporal effect on resource importance for bumble bees. We expected that the importance of a resource would change with the season as the colony moves between life-stages with nesting, foraging, and overwintering resources being the most important in the spring, mid-summer and late-summer respectively as has been shown previously (Moquet et al., 2017). However, we found that foraging resources were consistently the most important variable throughout the entire season. This may be due to a few factors. Floral resources may be the most important resource for bumble bee habitat because queens may select nest sites based on the amount of available forage in the area rather than areas with high nesting resources. This may be to ensure enough forage is available to provision the colony (Elliott, 2009; Suzuki et al., 2009, 2007). A review by Roulston and Goodell (2011) outlines that limitations in floral resources have the most support for determining bee abundance and diversity. However, they also note that no studies have adequately determined a bee population's response to changes in nesting resources without any change in floral resources, so this is an area in need of further research. Overwintering variables might also be correlated with nesting habitat (Plath, 1922), but this may not always be the case (Alford, 1969). Whether bumble bees overwinter near nests may be related to habitat quality of the landscape (Carvell et al., 2017). There is little understood about nesting and overwintering habitat relative to what is known about bumble bee foraging due to the difficulty in locating these sites (Darvill et al., 2004; Liczner and Colla, 2019; O'Connor et al., 2017; Waters et al., 2011). Although we selected nesting and overwintering variables based on the best available information, it is possible that we did not adequately measure these resources. Additionally, the bumble bee occurrence records that were used to select sites were mostly from foraging observations, so this could have biased site selections towards forage patches. We attempted to correct for this by using a 1 km buffer which should have extended most bumble bee dispersal distances (Elliott, 2009; Osborne et al., 2008; Redhead et al., 2016). The dispersal and forage range distances for *B. terricola* and *B. pensylvanicus* are currently unknown so it is possible that they extend out of these ranges. As floral resources were the most important variable determining bumble bee habitat throughout the season, it is important for land managers to ensure that there are floral resources throughout the entire colony cycle (from early spring until fall) to sustain bumble bee colonies. This can be achieved by increasing the diversity of flowering plant species available which have varying bloom times (and thus increase temporal availability of flowers) and different corolla lengths which may help resource partitioning among bumble bees (Colla, 2016; Kearns and Thomson, 2001).

This study provides an important description of likely important landscape and local variables, and flowering plant species cover for *B. terricola* and *B. pensylvanicus* in Southern Ontario and the first thorough description of at-risk bumble bee habitat in North America. The detailed resource descriptions and the relatively small study extent (compared to either species' entire range) used in this study, the scalability of these results beyond southern Ontario, particularly to other ecozones should be done with caution. The habitat descriptions in this study could be applicable to other bumble bee species that have similar life history traits including other declining bumble bee species. These scientific results can also be used to help inform effective and efficient conservation management and policy development of at-risk bumblebees as well as future directions on wild pollinator habitat use. Additionally, these results give insights on the types of resources land managers may want to consider for additional management efforts and habitat restoration projects including increasing habitat heterogeneity and increasing floral resource availability spatially and temporally.

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Tables and Figures

Table 1: A description of the local variables surveyed within each 10 x 10 m quadrat (5 quadrats x 2 transects) at 25 sites. The resource category lists which local variables belong to forage, nesting and overwintering bumble bee resources.

Local variables	**Resource category**	**Description**
Richness	Floral	The number of different flowering plant species in a quadrat
Ratio	Floral	The ratio of native-to-non-native flowering plant species within a quadrat (Eq. 1).
Patchy	Floral	An estimate of the patchiness of floral resources within a quadrat (Eq. 2)
Floral cover	Floral	The total floral cover of all flowering plant species within each quadrat
Burrows	Nesting	Animal burrow density identified visually as holes with a minimum diameter of 3 cm within each quadrat
Coarse woody debris number (WDnum)	Nesting and Overwintering	The abundance of coarse woody debris within each quadrat. Coarse woody debris is defined as downed woody material with a diameter greater than 7.62 cm.
Coarse woody debris decay class (WDclass)	Nesting and Overwintering	The coarse woody debris decay class determined on a Likert scale given traits of the coarse woody debris such as structural integrity, texture, wood colour, invading roots, and ability to remove branches and twigs. Higher decay classes values indicate pieces of coarse woody decay that are more decomposed (Woodall and Williams, 2005). Average values were calculated if multiple pieces were present per quadrat.
Coarse woody debris diameter (WDdiam)	Nesting and Overwintering	Coarse woody debris diameter was measured at the widest part of the wood. Average values were calculated if multiple pieces were present per quadrat.
Coarse woody debris length (WDlength)	Nesting and Overwintering	Coarse woody debris length was measured for the full length of the wood, even if it extended outside of the quadrat. Average values were calculated if multiple pieces were present per quadrat.
Vegetation cover (vegcover)	Nesting and Overwintering	The total percent vegetation cover of all vegetation (flowering or not) within the quadrat.

Table 2: Total variance explained by the optimal number of axes (determined by the inflection point on scree plots) for each redundancy analysis.

Analysis	**Total variance explained**
Landcover variables	67.5%
Spring local	60.6%
Spring flowering species cover	24.1%
Summer local	64.3%
Summer flowering species cover	14.0%
Late-Summer local	61.6%
Late-Summer flowering species cover	14.3%

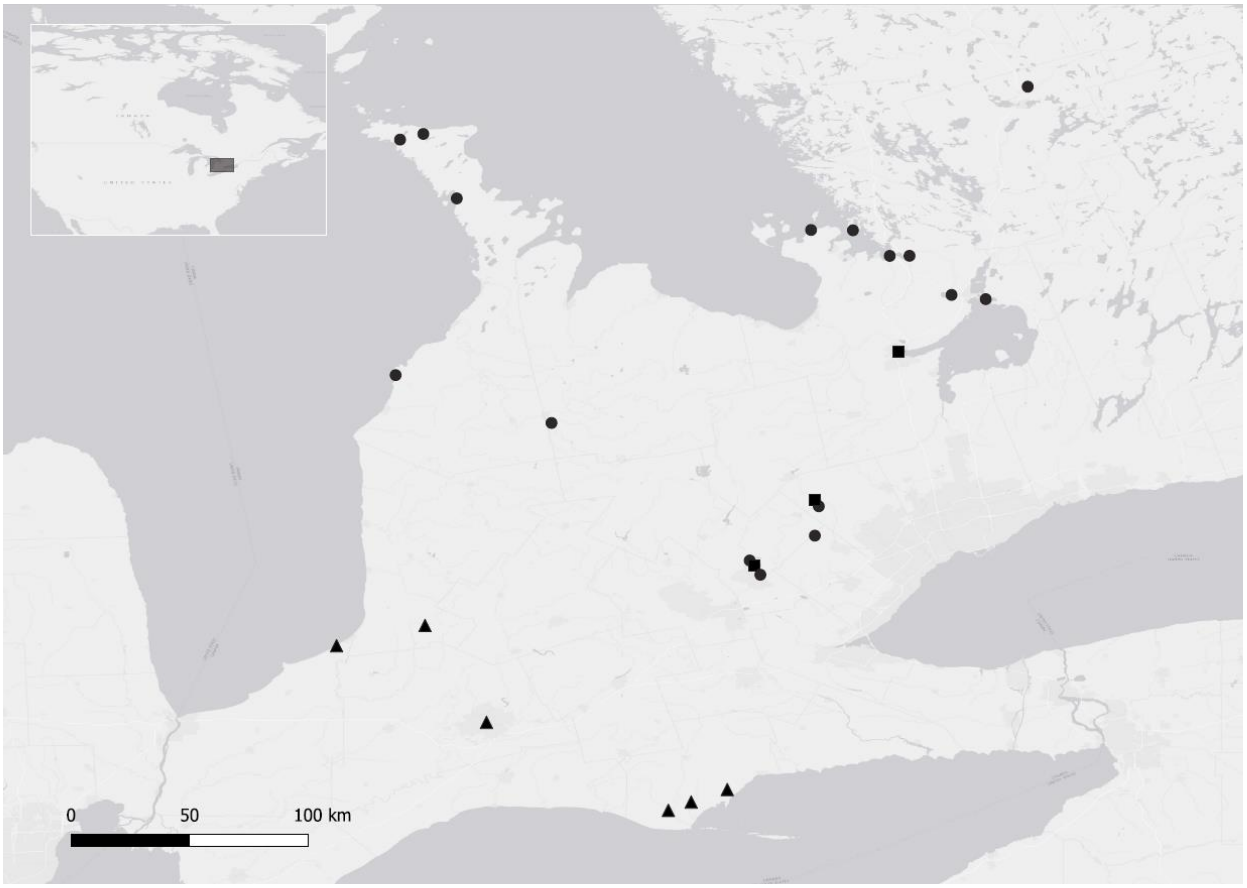

Figure 1: Map of the 25 study locations. Circles = recent *Bombus terricola* sites, triangles = recent *B. pensylvanicus* sites, squares = sites with both bumble bee species.

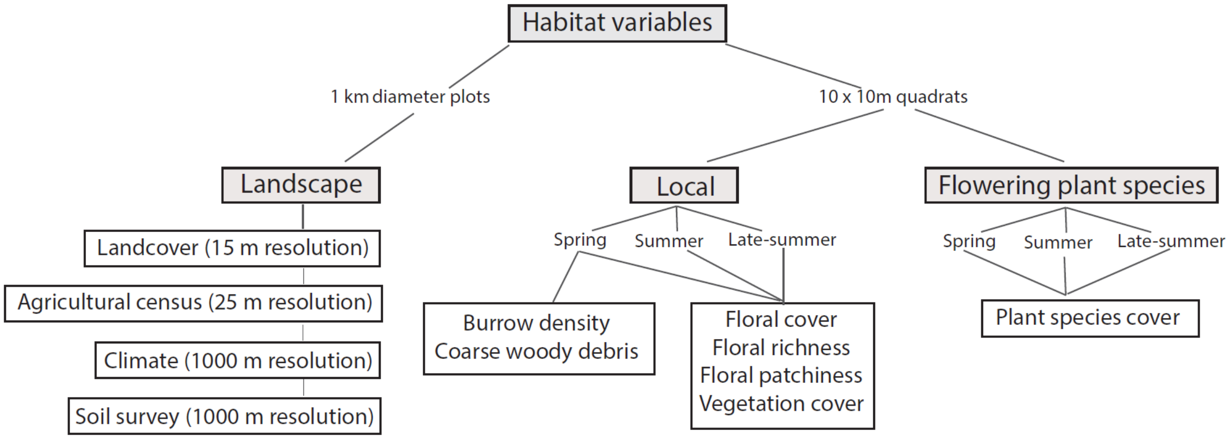

Figure 2: Methods flow chart for landscape, local variable and flowering plant species data collection. Landscape variables were extracted from a 1 km buffer around previous occurrence records of *B. terricola* or *B. pensylvanicus*. The landscape data came from raster data for landcover, climate and soil texture as well as agricultural census data summarized to the census boundary. Local variables related to forage resources (floral richness, floral cover, floral patchiness) as well as vegetation cover, were measured in all three time periods: spring, summer, late-summer. Burrow density, and coarse woody debris measures (abundance, length, width, decay class) were measured just once in spring. Flowering plant species cover was measured at all three time periods.

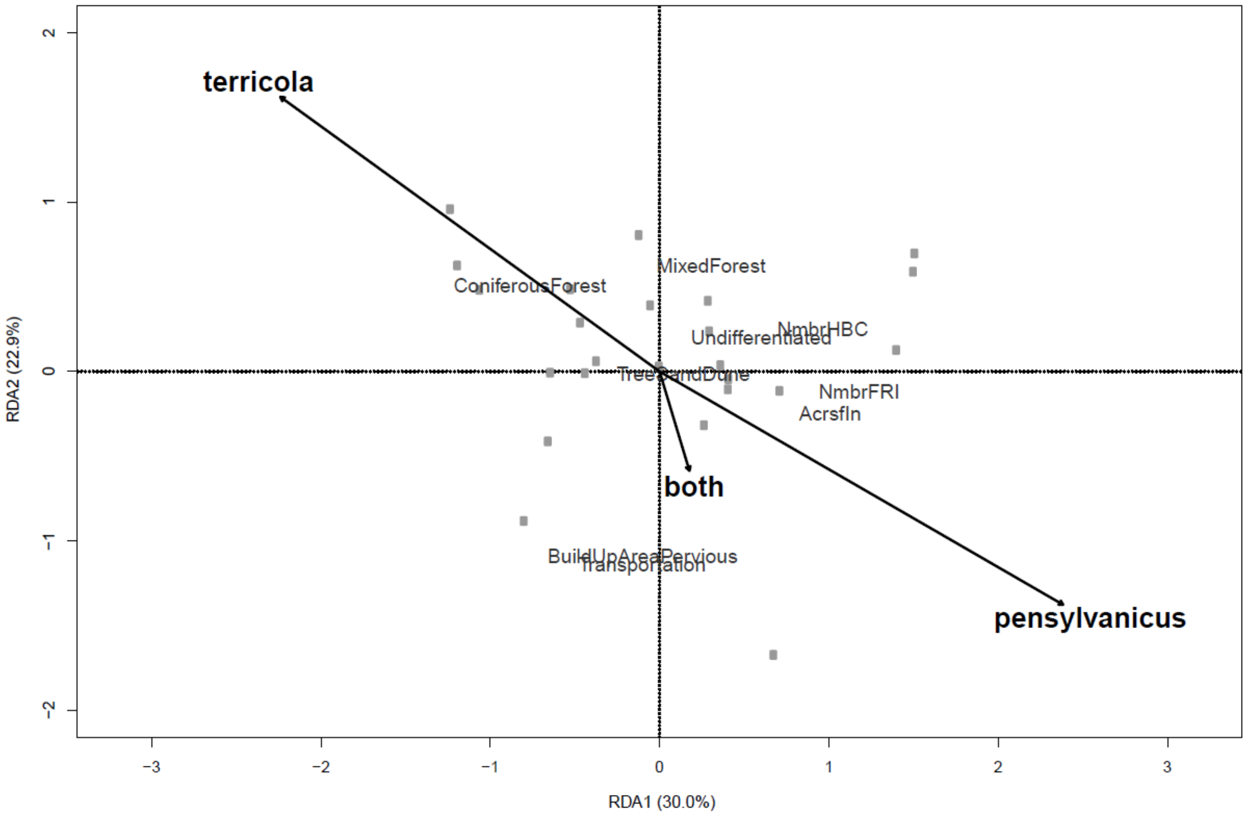

Figure 3: Redundancy analysis (RDA) for landcover habitat variables for *B. terricola, B. pensylvanicus* and *both*. The optimal number of axes for this RDA was three, but only the first two axes are shown. The total variance explained for the three optimal axes is 67.5% Undifferentiated = Includes some agricultural features not included in tilled (i.e. orchards, vineyards, perennial crops and idle land > 10 years – out of agricultural production) as well as urban brown fields, hydro and transportation right-of-way's, upland thicket and openings within forests.; NmbrHBC = The number of honey bee colonies in usage; NmbrFRI =Number of farms reporting insecticide application; AcrsfIn = Acres of insecticide applied.

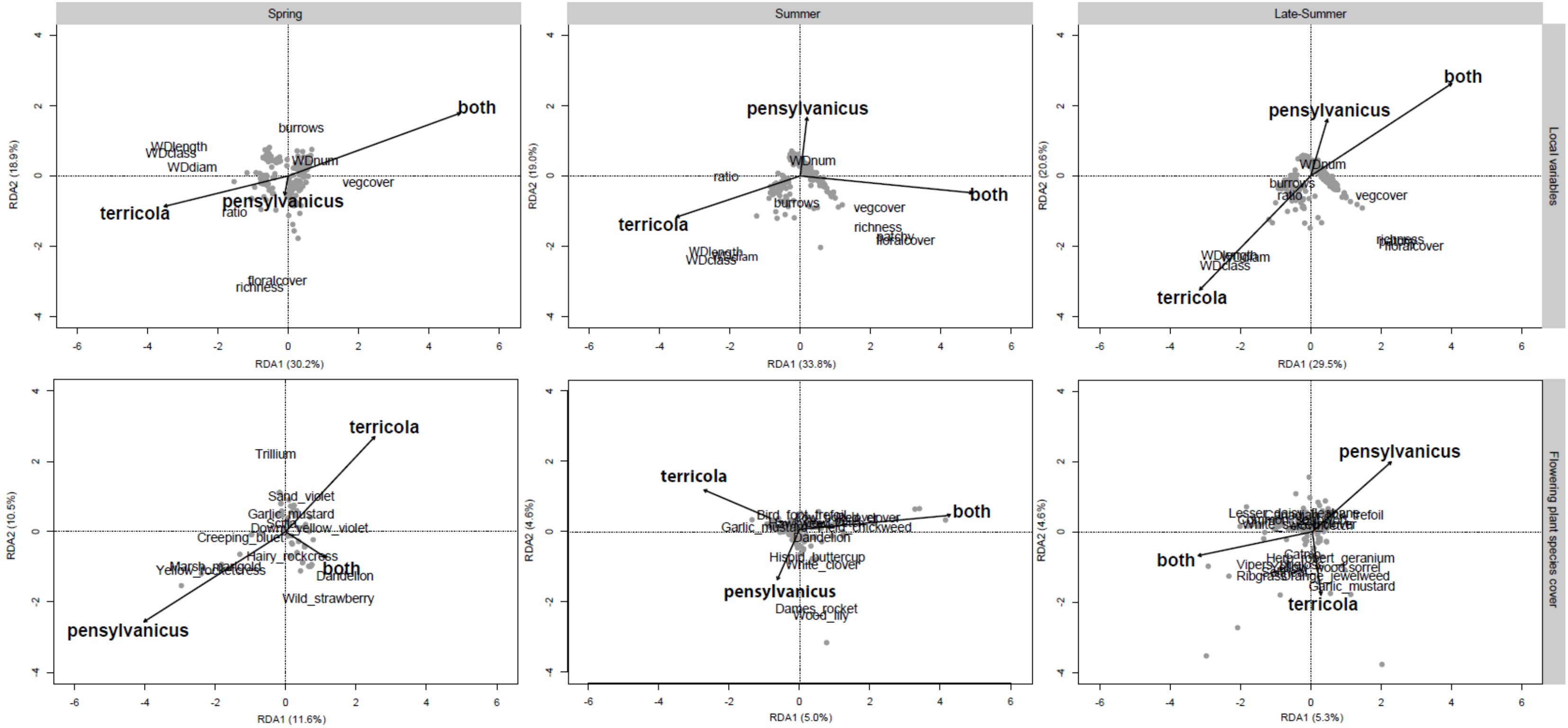

Figure 4: Redundancy analysis (RDA) for local variables (top row) and flowering plant species cover (bottom row) for *B. terricola*, *B. pensylvanicus* and *both*. Local variables and flowering plant species cover were measured for three times: spring (left column), summer (middle column), and late-summer (right column). The first two axes are shown, but the optimal number of axes for each ordination (except mid-summer) was three. The total variance explained (at three axes) is: spring local variables: 60.6%, spring flowering plant species = 24.1%, summer local variables = 64.3%, summer flowering plant species = 14%, late-summer local variables = 61.6%, late summer flowering plant species = 14.3%. Flowering plant species in summer and late summer with eigenvalues <0.1 for RDA axis 1 and 2 were removed from the plot to reduce dense clustering at the origin

Chapter 3: Conservation planning for native bumble bees under current and future climate scenarios

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Author’s contributions:

ARL, SRC, conceived of the ideas. All authors contributed to the methodology design, but it was led by ARL. LR provided the bumble bee occurrence data. ARL obtained the climate and landcover and protected areas data. ARL compiled and cleaned the data. ARL led with analysis along with RS. ARL led the writing of the manuscript. All authors contributed equally to editing and gave final approval for inclusion into the dissertation and eventual publication to a peer-reviewed journal article.

We intend to submit this manuscript for publication in the Journal of Applied Ecology

Summary

Many bumble bee species are declining globally from multiple threats including climate change. Identifying conservation priority areas under current and future climate will be important for conserving bumble bee species. We used systematic conservation planning to identify priority areas for bumble bee conservation in Canada under current and future climate scenarios (RCP 2.6 and 8.5) for the year 2050. Two different objectives were used to determine priority areas: maximize biodiversity while minimizing costs and maximize phylogenetic diversity within a budget. Bumble bee priority areas were compared to current protected areas and land cover to determine the amount of priority areas currently protected and the types of landscapes within priority areas. Bumble bee conservation priority areas are mainly within southern British Columbia, Ontario, Yukon, Quebec and the Maritimes. Conservation priority areas are expected to shift north and increase in elevation with climate change. Bumble bee conservation priority areas are not well represented by current protected areas. They are most often within woody savannas, mixed forests and evergreen needleleaf forests. Our findings identify the most important regions in Canada for conserving bumble bee species under current and future climates including areas that are consistently prioritized with climate change. Given that conservation priority areas are primarily outside of current protected areas, private lands may be an important focus for preserving bumble bee habitat.

Introduction

Reversing ongoing biodiversity loss requires recovering species at risk of extinction and protecting species from on-going threats. Efforts to protect species at risk have generally been unsuccessful with a minority of species' statuses improving while most remain unchanged (Favaro et al., 2014). This lack of effective conservation could be because current protected areas (e.g. parks and conservation reserves) do not conserve much biodiversity or species at risk (Bolliger, Raymond, Schuster, & Bennett, 2020; Deguise & Kerr, 2006). Many countries have agreed to designate at least 17% of land as protected areas by 2020 (CBD, 2010). It is important that these protected areas are designed to effectively conserve biodiversity and species at risk.

One method of identifying effective protected areas for conserving biodiversity is systematic conservation planning (SCP). SCP is a goal-based process that identifies areas of conservation priority to maximize biodiversity benefits while minimizing costs (Margules & Pressey, 2000; Schwartz et al., 2018). Conservation plans can be customized to different objectives or goals for projects such as maximizing the number of species conserved (Schuster et al., 2019), prioritizing at-risk species (Wilson et al., 2019) or maximizing phylogenetic diversity (Vereecken, 2017). Protected area planning should also consider climate change as range shifts and local extinctions have already been observed for many species (Wiens, 2016). Range shifts may cause species to move into unprotected areas if climate change is not considered (Bellard, Bertelsmeier, Leadley, Thuiller, & Courchamp, 2012; Gilbert et al., 2020). Given the uncertainty of future climate projections (Garcia, Cabeza, Rahbek, & Araujo, 2014) exploring multiple potential climate scenarios is important for SCP. To help prevent the continued loss of species, protected areas need to be established that simultaneously consider current biodiversity needs and future climate change.

Bumble bees (Apidae: *Bombus spp.*) currently experience multiple threats and are particularly sensitive to climate change. Bumble bee ranges have decreased due to warming temperatures and reduced precipitation (Biella et al., 2017; Soroye, Newbold, & Kerr, 2020) and continued range shifts are expected (Krechemer & Marchioro, 2020; Martins, Silva, De Marco Jr., & Melo, 2015). Other threats include habitat loss, pesticides, pathogen spillover and competition from non-native species (Cameron & Sadd, 2020; Szabo, Colla, Wagner, Gall, & Kerr, 2012; Thomson, 2016; Williams & Osborne, 2009). Bumble bees are important pollinators of agricultural crops and wild plants, (Williams, Thorp, Richardson, & Colla, 2014) and declines may negatively impact crop production and the reproduction of wild plants that rely on bumble bees for pollination (Biesmeijer et al., 2006; Goulson, 2003). Given the economic and ecological importance of bumble bees it is critical to identify effective conservation actions to protect declining species and bumblebee diversity. Identifying conservation priority areas for bumble bees using SCP under current and future climates could be an effective tool to help manage species at risk and support pollination services.

Our objective is to identify conservation priority areas for bumble bees with climate change. We asked the following questions: i) where are bumble bee priority areas under current and future climate scenarios? ii) do conservation priority areas change with different objectives? iii) are conservation priority areas within protected areas? and iv) what land cover types are associated with conservation priority areas? We addressed these research questions by solving conservation planning problems with integer linear programming techniques (Beyer, Dujardin, Watts, & Possingham, 2016) to identify bumble bee conservation priority areas under current and two future (year 2050) climate scenarios. These results can be important for directing bumble bee conservation efforts with future predicted climate change.

Methods

Bumble bee dataset

Bumble bee occurrence records for Canada, U.S.A and Mexico from 2008-2018 (227,735 records, 47 species) were obtained from the Bumble Bees of North America Database (Richardson, 2020) (Appendix F). Naming conventions followed the Natural History Museum's project "*Bombus"* bumble bees of the world (Williams, 2020). We removed observations at the generic level, without geospatial information or duplicate records (i.e. the same latitude and longitude for a species). Any species with fewer than ten occurrences (*Bombus cockerelli, B. distinguendus,* and *B. variabilis* with 5, 6, and 9 occurrences respectively) were removed because these species could not be reliably modelled in subsequent analyses. After refinements there were 70,164 occurrence records for 44 bumble bee species (Appendix G).

Species distribution models

All analyses were performed in R version 3.6.1 (R Core Team, 2019). The species distribution models used 19 BIOCLIM climate variables (Fick & Hijmans, 2017) at a 2.5 arcminute (~ 5 km) resolution for Canada, the U.S.A., and Mexico. A 2.5 arcminute resolution was used because we did not expect climate to vary substantially at finer resolutions and at the time of analyses finer resolutions were not available from WorldClim (www.worldclim.org). Future climate projections (year 2050) used the MRI-CGCM3 General Circulation Model and RCP (Representative Concentration Pathway) scenarios 2.6 and 8.5 to represent the lowest (significant emissions reductions) and highest ("business-as-usual") carbon emission scenarios respectively. Collinear climate variables were identified ($\theta > 10$) and removed using the Variance Inflation Factor (VIF) (*vifcor* function in *usdm* package, Naimi, Hamm, Groen, Skidmore, & Toxopeus, 2014) by comparing the climatic conditions at species occurrences to the climatic conditions at randomly assigned background points (10 x the occurrence records of each species) (Guisan, Thuiller, & Zimmermann, 2017). A list of the final eleven climatic variables used in the species distribution models is shown in Table 1.

Bumble bee occurrence records are highly concentrated near urban areas. To account for this, we generated a bias file for all bumble bee occurrence records prior to refinements (years 1805-2019, 47 species, 585425 observations) using a 2D-kernel density function (*bkde2D* function in *KernSmooth* package, Wand, 2019). This bias file down weighs heavily sampled areas in species distribution models (Fourcade, Engler, Rödder, & Secondi, 2014). Species distribution models were performed using MaxEnt across Canada, U.S.A and Mexico (*maxent* function in *dismo* package, Hijmans, Phillips, Leathwick, & Elith, 2017) for each of the 44 bumble bee species under current, future RCP 2.6 and RCP 8.5 scenarios and default model parameters. Pseudoabsences were generated by adding 10x the number of observations of each species and randomly sampling within the study area. Pseudoabsences were not spatially restricted to allow broad sampling of suitable and non-suitable climatic conditions to increase model predictability. Model evaluation was performed by withholding 20% of the occurrences and pseudoabsences to test the model. Models were compared using AUC and the correlation between testing and training models. Species distribution models with predicted occurrences outside of their known range and AUC values below 0.80 (*Bombus kluanensis, B. natvigi, B. suckleyi*) were not included in further analyses. The median AUC value for the remaining 41 bumble bee species was 0.970 (0.906 – 1.00). The change in predicted habitat suitability with climate change was assessed by determining the proportion of each species' distribution that had > 50% predicted suitability under current and future climates. A threshold of >50% was chosen so that areas of relatively high suitability were selected.

Conservation prioritization

Species distribution models were cropped to Canada and stacked by current, future RCP 2.6 and future RCP 8.5 climate scenarios. We focused on Canada because conservation legislation is often limited by political boundaries and Canada can disproportionately facilitate poleward range shifts with climate change (Coristine et al., 2019). Bumble bee species distributions were initially modelled across Canada, U.S.A and Mexico to allow species to move northward with future climate change. Two species (*Bombus fraternus* and *B. crotchii*) do not occur in Canada currently but are predicted to occur under both future climate scenarios resulting in 39 and 41 species under current and future climate respectively. No species are predicted to be lost due to climate change (under RCP 2.6 or 8.5) by the year 2050.

To reduce the influence of areas of low predicted climate suitability on conservation priority solutions, we set predicted species occurrence values from the species distribution models less than 0.20 to zero. Thresholding prevents the prioritization of areas with low predicted suitability and has been shown to increase the cost efficiency of solutions (Rodewald, Strimas-Mackey, Schuster, & Arcese, 2019). Conservation priority areas for the three climate scenarios were identified using *prioritizr* (Hanson et al., 2020) and Gurobi problem solving optimizer (Gurobi Optimization, 2019). We used two objective functions: i) *add_min_set_objective* (hereafter ‘minimize cost’) to minimize the cost of the conservation priority solution while meeting set conservation targets, and ii) *add_max_phylo_div_objective* (hereafter ‘maximize phylogenetic diversity’) to maximize the phylogenetic diversity of conservation priority areas while staying within a budget.

The objectives require different inputs and can be uniquely parameterized to fit the users needs. The minimize cost objective inputs are biodiversity data, cost data and targets. The inputs for the maximize phylogenetic diversity objective are the same with the addition of a phylogenetic tree and a budget. The biodiversity data are the stacked bumble bee species distributions. Cost data refers to the economic costs of acquiring and managing land for conservation (Ball, Possingham, & Watts, 2009). Some examples of costs include, the cost of property/land, costs associated with managing protected areas, lost potential value from alternative land uses, and land size as a proxy for actual property/land purchase costs (Naidoo et al., 2006). We used area as the cost data with each pixel assigned the same value across Canada for both objectives. This was done because it is difficult to come up with a meaningful cost for bumble bee species at a large scale. We used three targets: 17% (Aichi target) (ECCC, 2016), 30% (UN convention on biodiversity 2030 target) (CBD, 2020) and 50% (nature needs half) (Locke, 2015) for each species’ predicted distribution for both objectives. The phylogenetic trees for maximize phylogenetic diversity objective for the bumble bee species included in the analysis were accessed through the Open Tree of Life (https://tree.opentreeoflife.org/) API using the *rotl* package (Michonneau, Brown, & Winter, 2016) (Appendix H). Trees were constructed for Canada’s current bumble bee fauna and for the predicted future fauna including *B. fraternus* and *B. crotchii*. The branch lengths of the phylogenetic trees were calculated using Grafen’s method (Grafen & Hamilton, 1989) using the *compute.brlen* function in the *ape* package. Since we are designating protected areas nationally, the budget for the maximize phylogenetic diversity objective was set to the percentage of protected areas currently under Federal control (~5 % of Canada's total area) because this is the area that is managed by the Federal government. A budget based on area was selected to match the cost file (the area of each grid cell in Canada). We added weights to species listed as at risk according to the IUCN (IUCN, 2020) with species at greater risk weighted higher than species not at risk to increase their representation in the solution for the add maximum diversity objective (Juslén et al., 2016). We based weighting on IUCN status as follows: Least Concern = 1 Near Threatened = 2, Vulnerable = 4, Data Deficient = 4, Endangered = 8, Critically Endangered = 16 (Appendix G).

To determine if priority areas are shifting in latitude or elevation with climate change, we compared the mean latitude and mean elevation of priority areas under current climate relative to RCP 2.6 and RCP 8.5. As western Canada contains more elevation variation due to the Rocky Mountains, we divided the priority areas into western and eastern regions (split at -90°W). Elevation data (90 m resolution, 2018) was accessed from the SRTM digital elevation data (United States Geological Survey Earth Resources Observation and Science Center) using the *getdata* function in package *dismo*.

The proportion of distributions within conservation priority areas for at risk and least concern bumble bee species was determined for both objectives to determine if at-risk species and least concern species are adequately represented in conservation solutions. The proportion of distributions was compared for all three targets and climate scenarios.

The amount of area in each conservation prioritization solution within current protected areas and each land cover class was calculated using the *zonal* function in the *raster* package (Hijmans, 2019). The protected areas shapefile was obtained from Environment and Climate Change Canada (The Canadian Protected and Conserved Areas Database, 2019). Land cover data used was MODIS Land Cover Type (MCD12Q1) version 6 (2018) with a 500 m resolution. The land cover data was re-projected to match the resolution of the species distribution models (2.5 arcminutes) by the nearest neighbour method using *projectRaster* in the *raster* package.

Results

Bumble bee climatic distributions

Most bumble bee species were predicted to lose suitable habitat in North America under future predicted climates (n = 24; Figure 1) but some species were predicted to have an increase in suitable habitat (n = 15; Figure 1). Two species have roughly the same suitable habitat with climate change: *B. calignosus, B. crotchii* (Figure 1).

Conservation priority areas with climate change

Bumble bee conservation priority areas were mostly in the west (Yukon, British Columbia and Alberta), southern Ontario and the Maritimes under current and climate scenarios (Figures 2-3). Generally, bumble bee conservation priority areas were predicted to shift northward or into mountainous regions with climate change (Table 2). Eastern priority areas under both objectives experience a more dramatic northerly shift in priority areas compared with western priority areas and in one case there is a slight southern shift (Table 2). Priority areas increased in elevation under climate change for both objectives and both regions, but the shift in elevation is generally greater in the west.

Conservation priority areas and protected areas

Across all climate scenarios the average amount of conservation priority areas within protected areas was 10.0% (maximize phylogenetic diversity) and 13.1% (minimize cost). The amount of bumble bee conservation priority areas within protected areas was similar under both climate change scenarios with 11.7% (RCP 2.6) and 12.1% (RCP 8.5) for the minimize cost objective and 10.3% (RCP 2.6) and 10.8% (RCP 8.5) for the maximize phylogenetic diversity objective.

Species representations within priority areas

The prioritization solution for maximize phylogenetic diversity aimed to meet the objective for as many species as possible given budget/cost constraints but targets could not be met for some species (Table 3, Appendix I). Species might be poorly represented in this objective because it is attempting to increase the representation of diverse evolutionary branches (not species) and tight budget restrictions while increasing conservation targets may be a challenge.

Both at-risk and least-concern bumble bee species were well represented in all bumble bee priority solutions (Figure 4). The proportion of at-risk and least-concern bumble bee species' distributions within conservation priority areas is roughly equal for both objectives and all targets and climate scenarios.

Land cover classes of conservation priority areas

Bumble bee conservation priority areas were mainly in woody savannas across all objectives and climate scenarios. Other land cover classes frequently within priority areas include mixed forests, evergreen needleleaf forests, grasslands, croplands, savannas and open shrublands (Figure 4). With climate change, mixed forests are predicted to become more prevalent within conservation priority areas while croplands, savannas (except under maximize phylogenetic diversity) and woody savannas will become less common. Evergreen needleleaf forests, grasslands and open shrublands will remain relatively consistent for bumble bee conservation priority areas with climate change.

Discussion

Identifying priority regions for conservation action is an important step in ensuring limited conservation resources are implemented effectively and efficiently. In this study we aimed to identify spatial conservation priority areas for bumble bees across Canada, using two objectives and three climate scenarios. The areas that were identified as conservation priority areas were in western Canada, southern Ontario, southern Quebec and the Maritimes. With future projected climate change, bumble bee priority areas generally shift northward or to more mountainous regions. Consistent priority areas may be especially important to build climate resiliency for bumble bees and the recipients of the ecosystem services they provide. Bumble bee conservation priority areas did not vary much between objectives except priority areas for maximizing phylogenetic diversity remain more concentrated in the south. Bumble bee conservation priority areas under current climate scenarios are most often in woody savannas, mixed forests, and evergreen needleleaf forests. Mixed forests will become more prevalent while croplands, savannas and woody savannas become less common with climate change. A low proportion of bumble bee conservation priority areas under current and future climates were within current protected areas which has implications for how to best manage bumble bee species across Canada. These results highlight regions across Canada of focus for bumble bee conservation both now and into the future including purchasing land for new protected areas, habitat enhancement in urban and agricultural settings and mitigating threats.

A low proportion of conservation priority areas for bumble bees were within already established protected areas. This result is echoed across previous studies examining the extent of priority areas and species at risk within established protected areas (e.g. Bolliger et al., 2020; Deguise & Kerr, 2006; Krechemer & Marchioro, 2020). A challenge for protected area design in Canada is that the region with the most at-risk species (i.e. southern Canada) is also the most intensely urbanized and developed (Coristine et al., 2018; Freemark et al., 2007; Sarakinos et al., 2001). This challenge may be alleviated somewhat for bumble bees as they exist in many landscapes including those dominated by humans and habitat can be relatively easily created and improved within private lands. For example, habitat quality can be enhanced in agricultural regions by providing forage and nesting habitat in field margins by planting flowers and increasing hedgerows and incorporating areas of semi-natural habitat including forests, wetlands or meadows (Carvell, Meek, Pywell, Goulson, & Nowakowski, 2007; Carvell et al., 2011; Purvis, Meehan, & Lindo, 2020). Including bumble bee habitat within agricultural lands may be particularly important for conserving bumble bees as it was among the top land cover classes identified in priority area solutions. Other land cover classes that should be targeted for bumble bee management include needleleaf forests, savannas (western Canada), deciduous broadleaf forests (eastern Canada) and mixed forests and woody savannas in both regions. Urban areas may have fewer threats to bumble bees, such as lower pesticide and pathogen exposure than agricultural areas and urban gardens or parks can provide resources and habitat for bumble bees (Banaszak-Cibicka, Twerd, Fliszkiewicz, Giejdasz, & Langowska, 2018; Hall et al., 2017). Bumble bee conservation actions may not require the expense of purchasing large sections of land, especially in the highly developed south, if landowners can be encouraged to create bumble bee habitat on their property by planting forage, and increasing nest habitat (Huelsmann, von

Wehrden, Klein, & Leonhardt, 2015; Lye, Osborne, Park, & Goulson, 2012). However, it may be necessary to conserve specific regions to ensure connectivity between current and future priority areas for bumble bees so that species can track their climate envelopes over time.

Previous studies have also identified the southernmost regions of Canada as conservation priority areas as there is a high proportion of at-risk species in these areas (Freemark et al., 2007; Sarakinos et al., 2001). Two recent studies aimed at identifying conservation priority areas across Canada for at-risk mammal species (Cameron, & Hargreaves, 2020), and at-risk species in general (Coristine et al. 2018) identified many of the same priority areas as found in our study. There were a couple of notable differences, namely that the Prairie region was identified as priority areas in both studies, but this area was not prioritized in our study. Bumble bee priority areas shifted northward and into more mountainous regions with climate change. Bumble bee distributions have been both predicted and observed to move poleward and up in elevation under climate change (Biella et al., 2017; Martins et al., 2015; Pyke et al., 2016; Soroye et al., 2020). However, Kerr et al. (2015) found southern range contractions for bumble bees without northern range expansions. A similar study identifying bumble bee conservation priority areas in South America found priority areas will shift poleward and towards coastal areas with climate change (Krechemer & Marchioro, 2020). Bumble bees may not be tracking their shifting climate because of restrictions in dispersal ability, unequal shifts in climatic conditions (lower thermal limit has not shifted) or other non-climate related restrictions such as lack of floral resources or unsuitable habitat conditions (Biella et al., 2017; Kerr et al., 2015; Pyke et al., 2016). Determining whether bumble bees can track future climate change will be an important step to develop adaptive management plans that may support species movement such as improving connectivity by providing corridors to support expanding distributions into suitable climatic areas (Gilbert et al., 2020). Sirois-Delisle, & Kerr (2018) found that North American bumble bees will not be able to track their climate envelope with climate change when incorporating a 10 km/year dispersal rate based upon of *B. terrestris* spread in introduced regions. This dispersal distance is likely an overestimate because *B. terrestris* has been known to have some of the largest foraging distances relative to other bumble bee species (Knight et al., 2005; Walther-Hellwig & Frankl, 2000). The negative impacts of climate change on bumble bee species are likely to be more than those reported in Sirois-Delisle and Kerr (2018) because *B. terrestris* has some of the highest foraging distances relative to other bumble bees, and thus likely also dispersal distances. Determining species-specific dispersal distances, facilitating species movements (i.e. through increased connectivity), and developing adaptive management plans for climate susceptible species will be important areas of future research. (Gilbert et al., 2020). Although southern British Columbia was often selected as priority areas for conservation in our study, this region was rarely consistently selected across all climate scenarios except when greatly increasing the target. This may because southern British Columbia is expected to undergo large changes in climate (Sushama, Khaliq, & Laprise, 2010). Uncertainty around predications of climate change can make the identification of priority areas for conservation challenging.

Future research should investigate how future land cover change will impact bumble bee habitat suitability (Kremen et al., 2007) as interactions between climate and land cover change can negatively impact biodiversity (Betts, Illan, Yang, Shirley, & Thomas, 2019; Mantyka-pringle, Martin, & Rhodes, 2012). Bumble bee species are found in many different landscape types and most North American bumble bee species have large ranges (Colla & Dumesh, 2010; Liczner &

Colla, 2020; Williams et al., 2014) making modelling the influence of land cover change difficult. Using finer scales (within ecoregions) and land cover datasets with greater specificity (e.g. forest type instead of "forest") could improve models. The areas identified as consistently prioritized under current and future climates may require immediate attention as these may be important refugia if bumble bee movement is limited by other factors.

The proportion of land cover types within bumble bee protected areas are predicted to shift with climate change, including a decrease in cropland area. However, this was predicted using current land cover conditions and not future predicted landcover changes which may cause inaccuracies in our predictions. Nonetheless this may have important implications for crop pollination if the climate envelope for bumble bees shifts away from agricultural regions. Providing adequate high quality forage in agricultural landscapes throughout the bumble bee colony cycle may be an important mitigation strategy to help reduce the negative effects of heat stress for bumble bee colonies and maintain crop pollination (Vanderplanck et al., 2019).

There was considerable agreement in the conservation priority areas for bumble bees for both objectives (minimize cost and maximize phylogenetic diversity). However, previous work has shown using a different objective can produce different solutions for conservation priority areas (Wilson et al., 2019). We may not have observed any stark contrasts in our conservation priority areas as the regions with the highest species richness in Canada were also the regions with the highest density of occurrence records in our dataset (Appendix F). The complementarity across solutions is positive as conservation actions within the different priority areas will concurrently benefit the most species and species with the greatest phylogenetic diversity.

In addition to the aforementioned limitations, our study is limited in three ways. The first is the use of presence-only data for modelling species distributions. The challenge with using presence-only data to predict areas of suitable habitat is that a species' presence does not necessarily indicate high quality habitat, and in the worst-case scenario, may actually indicate a population sink (Morrison, 2013). This may lead to erroneous results in modelling habitat suitability for species. Due to the large scale and number of occurrence records in our study, we assume that most of the points used represent actual suitable habitat for species and consequently this issue would not have had a large impact on our results. However, having true absence data would increase the accuracy and by extension the conservation implications of habitat suitability models. The second limitation is the use of a non-economic cost file. Incorporating a real economic cost could have the advantage of producing more effective, efficient and realistic solutions (Rodewald et al., 2019). However, we elected to use area as a surrogate cost rather than an economic cost as true cost data are difficult to obtain at such large scales. Additionally, bumble bees might not necessarily require the purchase of land for protection because they can exist in a variety of habitat types including on private land, such as urban areas and agriculture (if appropriately managed). Future studies may consider the incorporation of a cost of the loss of bumble bee species to an area in terms of the loss in pollination services should the species become extirpated from the region (Naidoo et al., 2006). Finally, similar to previous work (e.g. Coristine et al., 2018), most bumble bee occurrence records are biased to southern Canada and bumble bee surveys are very limited in the north. This limits our ability to accurately assess suitable habitat for these species as habitat suitability models often perform poorly with low sample sizes. Without adequate occurrence records for species, it is difficult to assess their conservation status accurately. Such species are labelled as Data Deficient, which is considered equivalent to Vulnerable but limits the legal protections that may be required if the species is severely declining (IUCN, 2020). A unique challenge for norther bumble bee species is that many are within the *Alpinobombus* subgenus which is taxonomically uncertain (Williams et al. 2019). An implication of this is many observations currently used (including in this study) may be incorrect which limits our ability to effectively manage species.

Future work should include targeted conservation efforts within the priority areas and determining bumble bee species sensitivity and adaptability to changing climate conditions. Within the identified conservation priority areas, conservation actions to mitigate threats to bumble bees (i.e. habitat loss, pathogens, pesticide use) should be implemented to increase populations. This can be done effectively using priority threat management to minimize costs of managing bumble bee threats while maximizing biodiversity outcomes (Carwardine et al., 2019). Given that bumble bees may not necessarily need legally protected land for conservation, private landowners engagement in creating and protecting bumble bee habitat could be an effective conservation solution (Huelsmann et al., 2015). We did not incorporate species adaptability or sensitivity to future predicted climate change into our models which can significantly impact where species are predicted to occur in the future (Butt et al., 2016). Future studies examining species sensitivity and adaptability will be essential for effective conservation planning. This additional research will be important to prevent continued biodiversity loss now and into the future.

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Tables and Figures

Table 1: The eleven bioclimatic variables (from WorldClim) included in the species distribution models after removing collinear variables.

List of Bioclimatic variables
Mean diurnal range (mean of monthly (maximum temperature – minimum temperature))
Isothermality (Mean diurnal range/Temperature annual range) x 100
Maximum temperature of the warmest month
Temperature annual range
Mean temperature of the wettest quarter
Mean temperature of the driest quarter
Precipitation of the wettest month
Precipitation of the driest month
Precipitation seasonality (coefficient of variation)
Precipitation of the warmest quarter
Precipitation of the coldest quarter

Table 2: The shift in latitude or elevation (Δ Response) for bumble bee conservation priority areas with climate change (RCP 2.6 and RCP 8.5) for eastern (east of Manitoba) and western (west of Ontario) Canada for both objectives.

Region	**Objective**	**Δ Response**	**RCP 2.6**	**RCP 8.5**
East	Minimize cost	Latitude	4.3°N	5.1°N
East	Maximize phylogenetic diversity	Latitude	1.6°N	1.1°N
West	Minimize cost	Latitude	0.09°N	0.07°N
West	Maximize phylogenetic diversity	Latitude	0.67°N	0.12°S
East	Minimize cost	Elevation	46 m	76 m
East	Maximize phylogenetic diversity	Elevation	38 m	89 m
West	Minimize cost	Elevation	103 m	64 m
West	Maximize phylogenetic diversity	Elevation	121 m	109 m

Table 3: Percent of species that met the target/budget for each conservation prioritization objective and climate scenario.

Objective	**Targets/budget**								
	Current climate			**RCP 2.6**			**RCP 8.5**		
	17%	**30%**	**50%**	**17%**	**30%**	**50%**	**17%**	**30%**	**50%**
Minimize cost	100	100	100	100	100	100	100	100	100
Maximize phylogenetic diversity	97.4	69.2	48.7	97.6	63.4	48.8	95.1	63.4	36.6

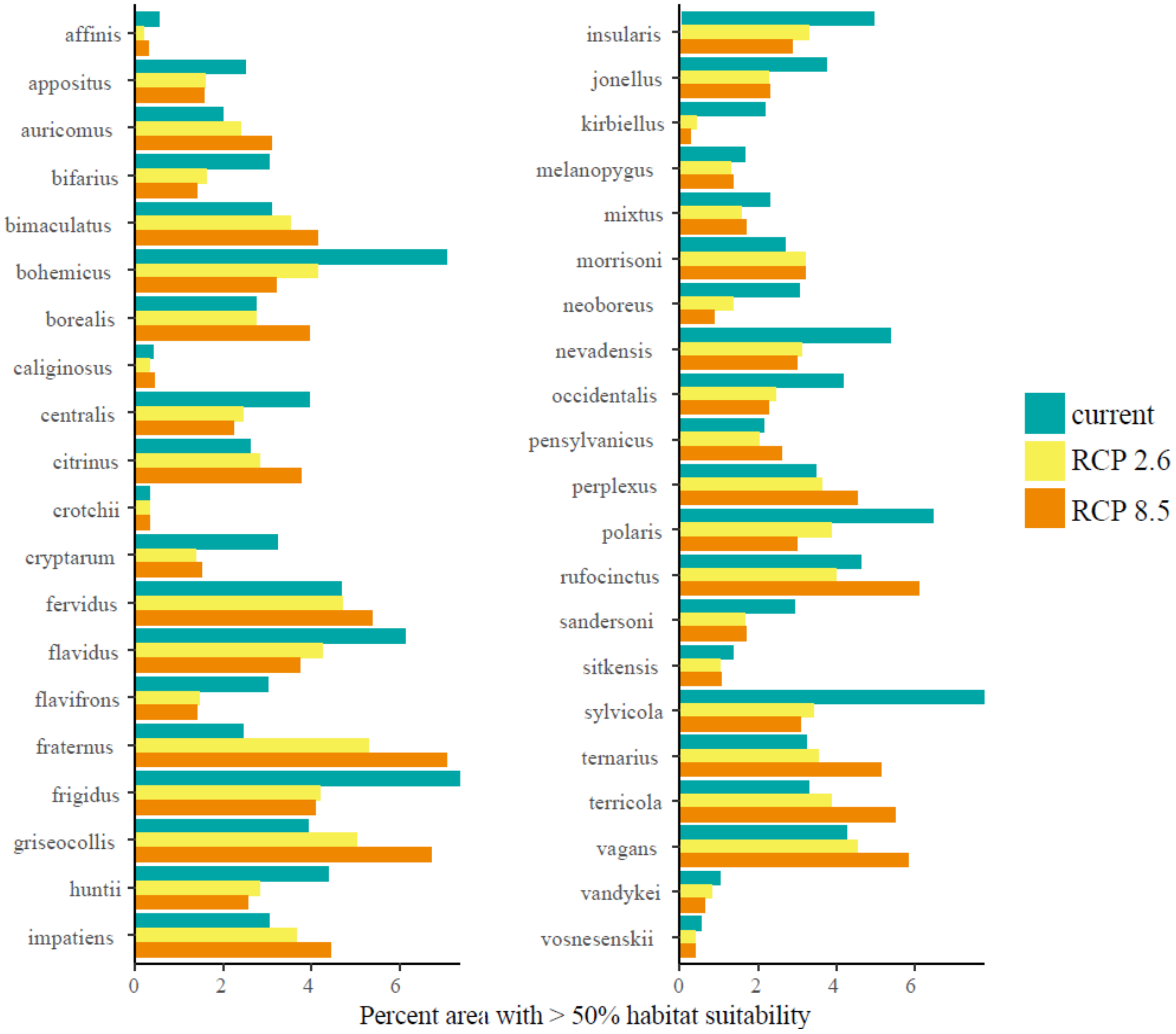

Figure 1: Percent area of North America with greater than 50% predicted habitat suitability for bumble bee species considering current and future climate conditions (RCP 2.6 and RCP 8.5 scenarios). Habitat suitability was modeled using MaxEnt.

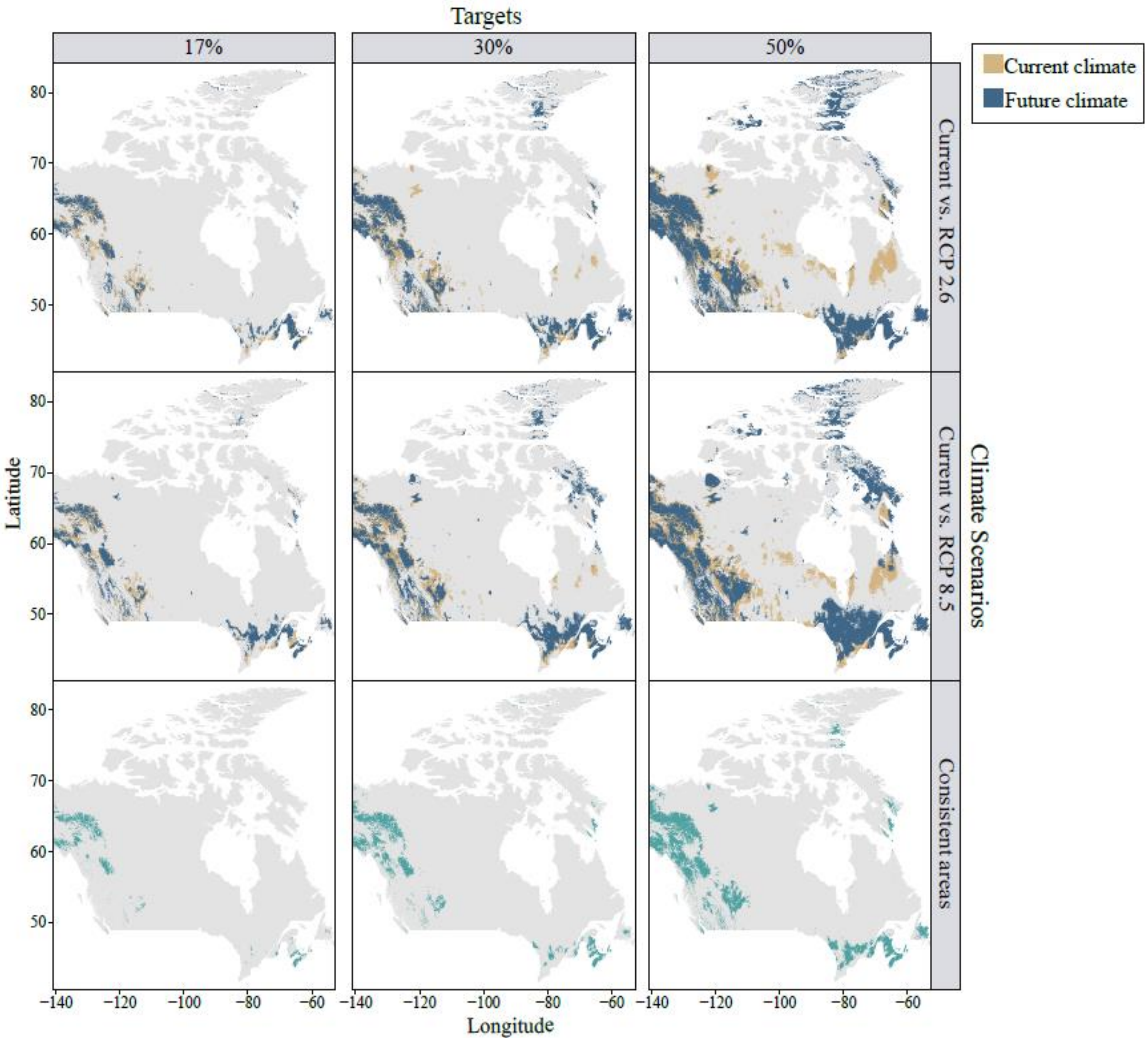

Figure 2: Bumble bee conservation priority areas using the minimize cost objective (conserves as much of each species' distribution as possible at the lowest cost) under current and future (RCP 2.6 and RCP 8.5) climate projections, and three conservation targets (the amount of area of each species' distribution to be conserved). The first two rows show the current climate and the two climate projections. The final row shows the areas that are consistently identified as conservation priority areas for all three climate scenarios.

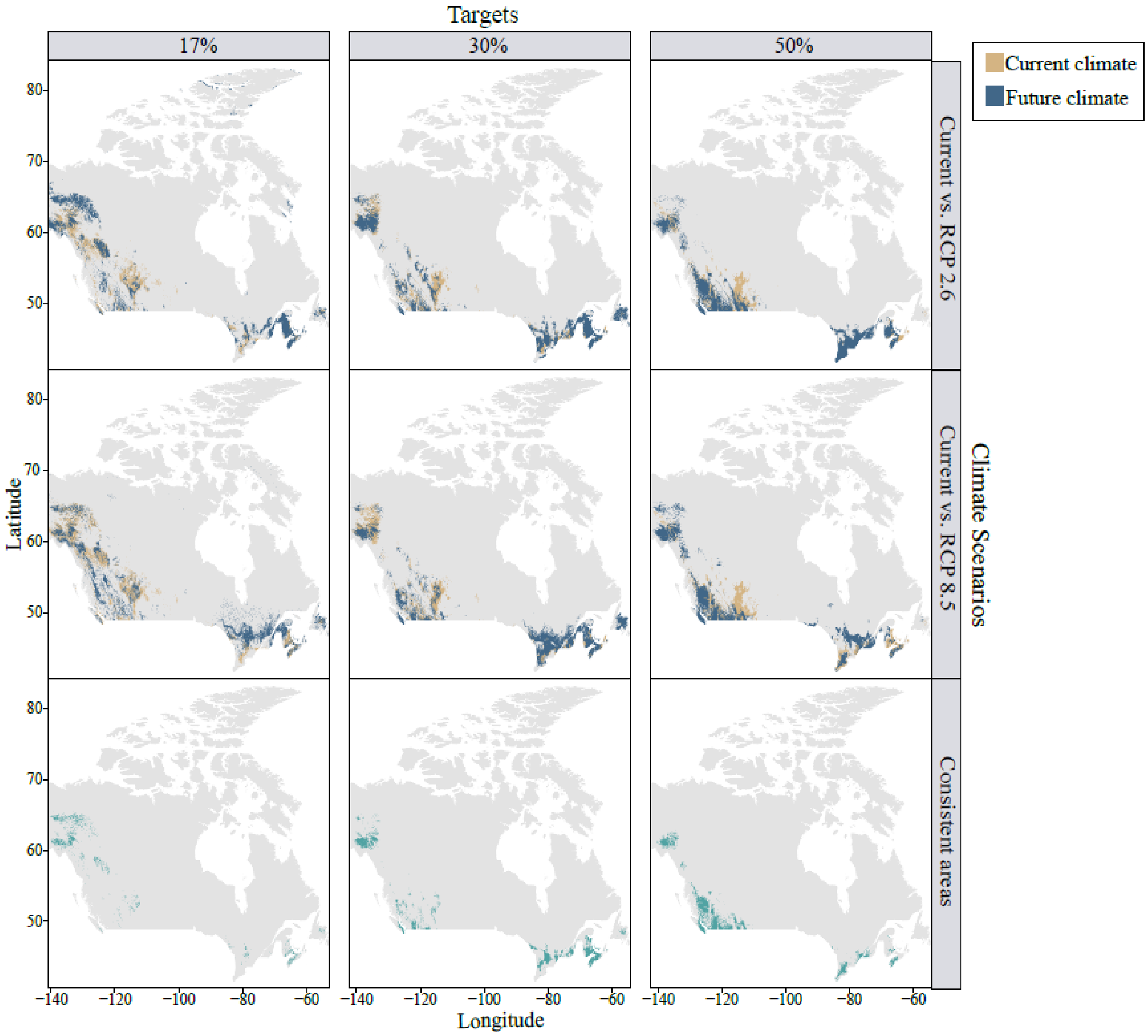

Figure 3: Bumble bee conservation priority areas using the maximize phylogenetic diversity objective (conserves the most phylogenetic diversity without going over budget) under current and future (RCP 2.6 and RCP 8.5) climate projections, and three conservation targets (the amount of area of each species' distribution to be conserved). Weights were added to species at risk according to IUCN criteria. The first two rows show the current climate and the two climate projections. The final row shows the areas that are consistently identified as conservation priority areas for all three climate scenarios.

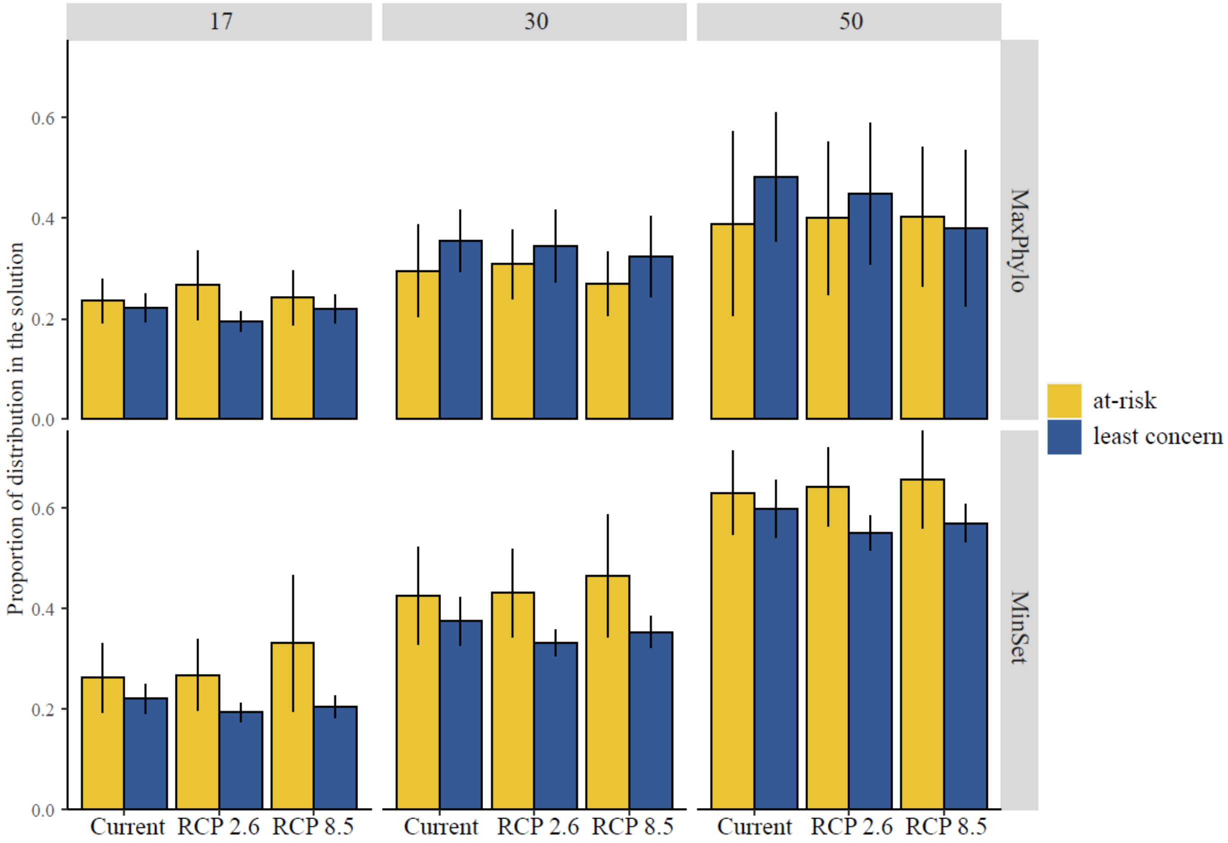

Figure 4: The proportion of distributions within conservation priority areas for at-risk and least-concern bumble bee species' in the maximize phylogenetic diversity (top row) and minimize cost (bottom row) conservation priority solutions under current and future climate (RCP 2.6 and 8.5 scenarios). The three targets used in both objective functions (17% left, 30% middle, 50% right) are also shown.

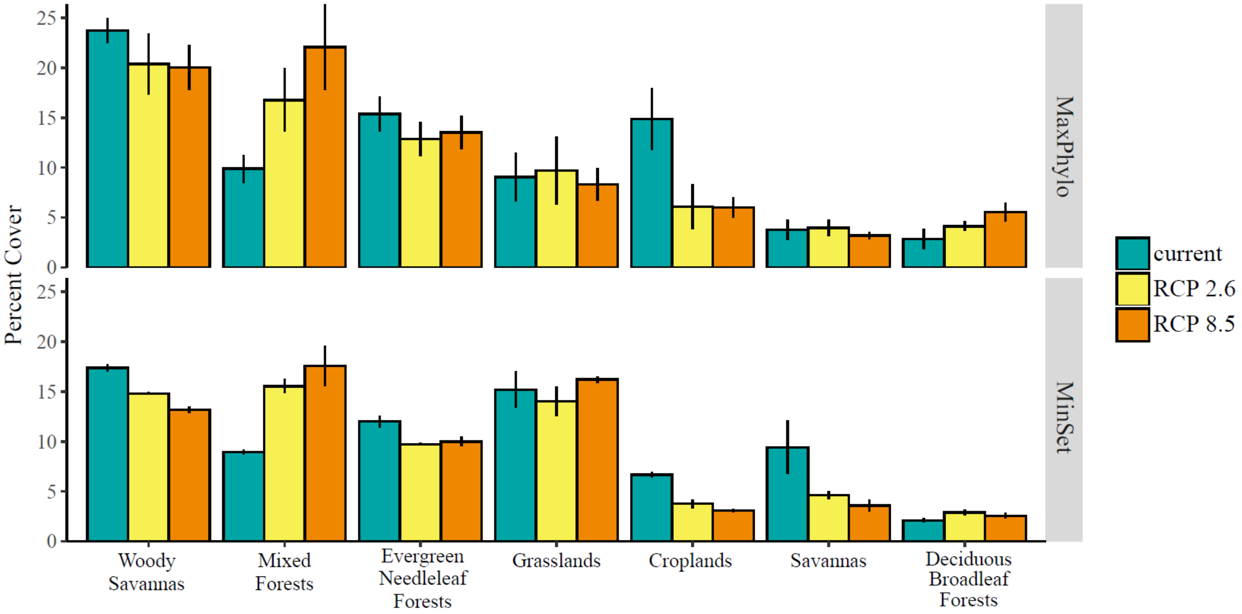

Figure 5: The percent cover of different land cover classes within conservation priority areas for both conservation prioritization objectives and the three climate scenarios. MaxPhylo = maximize phylogenetic diversity objective. MinSet = minimize cost objective. Values shown are means ± SE.

Chapter 4: Training and usage of detection dogs to better understand bumble bee nesting habitat: Challenges and opportunities

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Author’s Contributions

ARL and SRC conceived the ideas and secured funding. ARL, DW, NR and SRC designed the methodology, and all authors collected the data. ARL developed the social media campaign and recruitment of community science participants. VJM facilitated community science participant field work. DW and NR analyzed the data. ARL led the writing of the manuscript. All authors edited the manuscript and gave final approval for inclusion into the dissertation and eventual publication to a peer-reviewed journal article.

We intend to submit this manuscript for publication in the journal PLOS ONE

Summary

Bumble bees are among the most-at-risk group of pollinators. However, bumble bee habitat usage and selection, especially nesting requirements, remain relatively unknown as nests are often concealed making them difficult to locate. Methods that efficiently locate nests are invaluable to better understand habitat requirements and count wild populations. We report the constraints and possibilities observed while seeking to train detection dogs to locate bumble bee nests, building on previous studies' findings. Three conservation detection dogs were initially trained to bumble bee nest material from three species, first within a lineup, then placed in the open or partially concealed as an area search. The next intended step was to expose the dogs to known wild nests located by community science volunteers. Despite significant effort with humans visually searching (> 250 hrs), few wild nests were confirmed and deemed viable for dog training purposes (n = 2). Although no formal surveys were ultimately conducted, broader insight was gained relative to training detection dogs for this application, and for searching with them in various habitats. The maximum observed detection distance for bumble bee nest material for the dogs in the initial, controlled training setting was 15 m. However, this detection distance decreased significantly (< 1 m) once training progressed to exposure or buried samples, and natural nests. The three main considerations around future training and usage of detection dogs to locate bumble bee nests are: 1) dogs might benefit from receiving a more concerted transitional training step of multiple exposures to known, naturally occurring nests (regardless of bumble bee species) but this may not be feasible, given the difficulty finding wild nests, and might not be necessary, given their prior demonstrated ability to generalize and find wild nests after testing to human-buried nest material; 2) confirming a nest find made by a dog, via resident bee presence, is nuanced; and, 3) future study design and objectives must harness strengths and

reflect limitations of detection dog surveys and search strategies. Certain landscape types may not be conducive for using detection dogs to search for bumble bee nests.

Introduction

Approximately 25% of all bumble bee species globally are in decline [1,2] with habitat loss acknowledged as one of the main threats [2,3]. Agricultural intensification and development have decreased floral resources [4,5] and little is known about the loss of available nesting and overwintering sites through increasing impermeable surface cover, loss of grasslands and a reduction of forest cover. Permeable surfaces are needed for overwintering and nesting sites. Queens overwinter by burying themselves underground, often at the base of trees [6–8] and many bumble bee species also nest underground [9]. Conserving and ensuring the availability of viable bumble bee nesting habitat is important for mitigating declines in bumble bee populations.

Most bumble bee conservation efforts and habitat studies have focused on increasing floral resource availability [e.g. 10,11] while nesting and overwintering resources have received comparatively little consideration. This may be because bumble bee nests are often well-concealed and thus difficult to locate [9]. The entrances to bumble bee nests are usually very small and typically only detected when bees are frequently observed entering/exiting an opening or openings [e.g. of multiple entrances 12,13; S. O’Connor pers. comms. 2019]. Bumble bee habitat studies should focus on nesting requirements because it is an important determinant of a species’ critical habitat, a parameter which must be defined for legal protections, and locating bumble bee nests allows monitoring demographic responses of individual species for determining habitat quality [14]. To manage declining bumble bee species, it is important that we increase our knowledge of nesting habitat and our ability to locate nests.

Despite wild pollinator conservation being an active area of research, wild bumble bee nests remain difficult to locate; and there is an urgent need to develop approaches to increase our understanding of habitat selection. Previous studies investigating bumble bee nesting habitat have used various methods from inferential to observational to locate nests [9]. Examples of inferential strategies include: molecular methods to identify the number of colonies in a habitat [15], statistical modelling of foragers to predict nest location [16], and the presence of nest-searching queens as a proxy for nest locations [17]. These methods offer relatively quick and easy identification of the number of colonies within the landscape but often cannot locate the actual nest site. Without actual nest locations identified, valuable information including small scale habitat characteristics, ecological requirements, bee activity, and demographic information, will be missed. Observational methods usually involve slowly walking transects within a site to locate nests [18] or opportunistic sightings of bumble bee nests [19]. Although these methods are time and labour intensive and usually result in few nests being located without substantial effort, they do offer an advantage of directly finding bumble bee nests. There remains a need for efficiently and effectively locating bumble bee nests.

Potentially complementary approaches that could facilitate direct searches for bumble bee nests have been explored in pursuit of better describing nesting habitat preferences. Two previous studies in the United Kingdom, assessed the viability of using dogs to locate bumble bee nests [20,21]. Detection dogs have successfully been used to facilitate or optimize the finding of numerous wildlife species and targets deemed to be of high or urgent conservation value, or which help inform assessment of habitat quality and selection [e.g. 22 Table 10.1]. Detection dog-assisted searches of a location can often be conducted rapidly, while offering comprehensive survey coverage of an area [e.g. 23]. The use of detection dog-handler teams as a survey tool may also introduce less bias than human-only efforts, potentially leading to exploration and finds in unexpected areas that might not otherwise have been searched [e.g. river otter and American mink fecal matter along river banks, 24]. Similarly, the use of detection dogs may increase the likelihood of finding a target that is more difficult for humans to visually detect or discern [e.g. invasive/noxious weeds as part of latter stage eradication efforts, 25].

As part of broader bumble bee conservation research, and building from prior work [20,21], we had an objective of training then fielding (i.e. conducting surveys in targeted areas to gather data to meet study objectives) conservation detection dog-handler teams to locate wild bumble bee nests in southern Ontario, Canada in the summer of 2019. Here, we attempted to apply a transitional (and previously found to be effective) training step [26], of offering the dogs multiple exposures to located wild nests. We also examined previously reported parameters [e.g. find rate 20,21] and conferred with authors of prior bumble bee studies on unreported variables (e.g. detection distances; S. O’Connor, pers comm. 2019). Although the specific objective of formally surveying with the dogs for wild nests could not ultimately be fulfilled, invaluable new insight was acquired on whether, and how, to best proceed with this type of work. Herein we describe the primary challenges that the professionally trained conservation detection dogs, and that the humans tasked with searching for wild nests in conjunction with those efforts, encountered during the process and discuss potential opportunities for further research going forward.

Materials and Methods

Initial bumble bee nest sample acquisition and dog training

A conservation dog organization (Working Dogs for Conservation; WD4C, Missoula, Montana) conducted initial dog training in a controlled environment in Montana [26-29] (June 3 to 19, 2019), followed by *in-situ* training and exploratory ground-truthing near our designated study sites (Silver Creek Conservation Area (43.691917, -79.966178), and Terra Cotta Conservation Area (43.721493, -79.959306)) in southern Ontario, Canada (June 24, to July 5, 2019). The training samples were from seven nests of three bumble bee species (Table 1). Samples consisted of wax cells constructed by the bees (honey pots with and without nectar, brood cells) and some deceased worker bees. Nests were initiated by wild-caught queens that were reared in lab conditions at the University of Illinois. Samples were stored in a freezer at -20 °C until shipment to WD4C in a Styrofoam cooler on ice packs. Upon arrival, they were separated into three distinct subsamples (to offer as training samples to the dogs), placed in fresh, labeled Ziploc bags (sandwich-size) and stored in a freezer at -20 °C until training began. Once used for training each subsample was subsequently stored in sealed glass jars, further detailed below.

Three conservation detection dogs (two male border collies and a female Belgian Malinois) received introductory exposure to bumble bee nest material, using standard and previously established conservation dog training methods [26–29]. All dogs had previous experience training to, and surveying for, other conservation detection targets prior to this study. Briefly, during the initial sessions in Montana, the dogs were first presented with an array of cinder blocks within which a training sample was concealed in some of the blocks in a sealed, 8 oz glass canning jar (Ball ®, regular mason) with a perforated lid. Training then proceeded to dogs free searching off-leash within a fenced area (approximately 250 m^2) for samples that were placed in low lying vegetation (mowed grass, garden). Approximately 2 g of nesting material was used in each training exposure. Each dog indicated recognition of the bumble bee nest material (i.e. the target scent) by giving a passive “alert” (i.e. sitting or lying beside it), for which they were rewarded through a play session with the handler. Each dog received 126 individual exposures to training nest samples from all three bumble bee species before departing for Ontario. The purpose of training conducted in Montana was for the dogs to gain familiarity to the scent of bumble bee nest materials and to generalize to multiple species. In this application, generalization refers to dogs being able to detect bumble bee nests of additional species not presented to them during initial training. Whenever observed, the detection distances – from where the dogs exhibited a “change in behaviour” indicating they had picked up the scent relative to the placed sample, were noted. Other noteworthy elements (e.g. nest ID and subsample number used relative to training session) were also recorded at the different progressions reached during this initial phase of training.

Human wild nest searches for in-situ dog training

The bumble bee nest detection work previously undertaken with dogs [20,21], and direct communication with one of the primary authors of those studies (S. O’Connor, pers. comm. 2019), revealed that dogs have the ability to generalize (i.e. being trained using nest samples from certain bumble bee species and detecting placed nest material and wild nests from other bumble bee species during field testing, and once formally fielded, respectively). In parallel, WD4C trainer-handlers have found that even if/when dogs have shown an ability to generalize to a particular target, offering exposure opportunities to known/confirmed targets *in-situ* can be beneficial to dogs prior to fielding. As such, *in-situ* exposure has become an additional, warranted training process for many targets to which dogs have shown they can generalize, but which produce multiple scent variants and/or a smaller amount of scent. Therefore, while the dog team training was underway in Montana, a concerted attempt was being made in southern Ontario to locate wild bumble bee nests so that the dogs could undergo the transitional training stage and be promptly reinforced for finding *in-situ* wild nests that were not handled or placed by humans. Researchers and volunteers searched for nests within the designated study sites. A social media campaign was also launched to increase the chances of finding bumble bee nests across southern Ontario, particularly on private property.

ARL began searches for bumble bee nests on May 1, 2019 between 9:30 am to 3:30 pm until July 5. VJM, SRC and 17 community science volunteers also searched for bumble bee nests from June 17 until July 5. Searches were conducted two to three times per week on warm, sunny days from 10:00 am to 4:00 pm. Prior to beginning nest searches, all volunteers were instructed on how to identify a bumble bee from other bees, what bumble bee nesting behaviour could look like (orientation flights, queens with pollen entering a potential nest site, worker bees entering/exiting a potential nest site), common nesting locations [7,30–32] and the distinction between bumble bee foraging and nest-searching behaviour. Community scientists were not required to have any prior experience with field work or bumble bee identification. For the first two weeks, volunteer nest searches at the study site were conducted by slowly walking the trail system throughout the conservation areas searching on either side of the trail for signs of nesting activity. Previous studies looking for bumble bee nests slowly walked pre-designated transects through study sites [i.e. 18], however, we were required to stay on the established trail system as much as possible by the conservation authority as sites were within protected areas. Locations within the study areas with many nest-searching queens were noted as areas to concentrate nest searches as high nest-searching queen activity has been previously associated with bumble bee nest activity [33]. The second two weeks were spent searching targeted locations for between 30-90 minutes as the average foraging duration for workers (which we assumed to be similar for queens) has been measured for at least 31 minutes [34]. These target areas either had high nest-searching queen activity or suspected nest activity (e.g. queen disappearing into a hole or debris, exhibiting an orientation flight). Any potential nest sites were flagged and georeferenced for researcher confirmation. A researcher (ARL, VJM, SRC) would then wait at the entrance for a minimum of 30 minutes to confirm if the site was a bumble bee nest. A nest would only be confirmed if workers were observed entering/exiting or a queen bumble bee was observed entering with pollen (which can be an indication of nest initiation) [7,30].

The social media campaign consisted of blog posts, a dedicated web page, email newsletters, posts to relevant Facebook groups, Twitter and Instagram. The campaign included a description of where to look for nests and common indicators of nesting activity. The submission of nest sightings was encouraged, through a dedicated webform or to the Bumble Bee Watch community science program. We also monitored Bumble Bee Watch separately for any uploaded nest sightings in southern Ontario.

On site and in-situ training with the dog-handler teams

Once the dog teams arrived in Ontario, on site and *in-situ* training was carried out from June 24-July 5, 2019. The weather conditions and start time for training sessions are shown in Appendix J. This date range was selected as it generally overlaps with the colony cycles for most bumble bee species found in southern Ontario [35]. New nest training materials were provided in

Ontario, to supplement prior dog training and to maintain both continuity and momentum, since no wild nests had yet been confirmed when the dog teams first arrived. These additional samples (2 g) were from two commercial colonies of *Bombus impatiens* Cresson (Table 1), purchased from BioBest (https://www.biobestgroup.com/), and were housed in a laboratory at York University (Toronto, Ontario). These nest training materials were stored in a freezer and kept in a cooler with ice packs when used in training.

On two separate days, training was conducted at Silver Creek Conservation Area and a residential area approximately 2.5 km away. Freshly thawed training samples were placed on the surface of the ground, under vegetation/debris, or buried to a depth of approximately 5 cm using a designated hand trowel. In each case, samples were left in place for approximately 30 minutes to 1 hour to ensure enough scent dispersal before the dogs were exposed to them. An equal number of control scent areas were created using the same methods described above, using a separate hand trowel when mimicking the burial process, but without placing a nest sample. Controls ensured that the dogs would locate the bumble bee nest scent rather than cueing in on human or other non-target scents arising from sample handling and disturbed ground. The detection distance, nest ID, and vegetation surrounding the training samples (if applicable) are summarized in Table 2.

In-situ training and exploratory ground-truthing sites

The sites selected for survey (but where *in-situ* training and ground-truthing was ultimately carried out instead) were Silver Creek Conservation Area and Terra Cotta Conservation Area. Both conservation areas are predominantly deciduous forest, but with distinct terrain. Silver Creek Conservation Area is more variable topographically than Terra Cotta, with limestone ridges, steep cliffs, rocky boulders, and crevices. The ground vegetation is often dense with underbrush, leaf litter, downed trees, and moss, and the substrate is often rocky. Terra Cotta has a relatively more open understory of deciduous forest, with less vegetation, rocks and downed trees than Silver Creek. The ground was often covered with leaf litter. Terra Cotta also has more open areas for recreation (such as picnic areas), and an open reclaimed campground dominated by grasses, forbs and shrubs. There is also an area with discarded straw, and mounds of dirt, rocks, and other debris from past construction activities.

Results

Human wild nest searches and social media nest observations

Human survey efforts at Silver Creek and Terra Cotta Conservation Areas identified six areas with high potential nest activity (i.e. high nest-searching queen activity or where queens/workers were observed entering a potential nest site). Beginning in May, ARL searched for bumble bee nest activity for 75 hours prior to volunteer involvement. In total, 17 volunteers plus three researchers searched for bumble nests for a combined 250.25 hours. During these surveys, the bumble bee species observed were *Bombus borealis* Kirby, *B. bimaculatus* Cresson, *B. citrinus* Smith, *B. griseocollis* DeGeer, *B. impatiens*, *B. perplexus* Cresson, *B. rufocinctus* Cresson, and *B. vagans* Smith. Bumble bees were identified according to Williams et al [35]. All six areas with presumed high probability of containing a nest were observed intensely by volunteers and bumble bee experts for a minimum of 30-90 minutes. However, it is thought that the late, cool, and rainy spring experienced in southern Ontario (Table 3) caused a corresponding delay in the emergence of queens, and they continued to be observed nest searching until June, with very few workers present in the area, suggesting few colonies were established at this time. This significantly limited our ability to locate bumble bee nests. We were unsuccessful in finding/confirming any established wild nests via human nest searches prior to the arrival of the dog teams in southern Ontario to facilitate the transition to *in-situ* training (which would have ensured immediate availability of promptly rewardable nests for the dogs).

There were 14 respondents to our social media campaigns (excluding submissions to Bumble Bee Watch). Of these, six submitted nest locations outside of southern Ontario or that were not bumble bee nests (in one case, the nest of a carpenter bee, *Xylocopa sp.*). Of the remaining eight respondents, we were able to confirm that three had indeed sighted bumble bee nests, two of which were deemed suitable for training purposes. The other five respondents were excluded either due to limitations on gaining access to their private residence, or because we could not confirm the presence of a nest during a site visit. The rationale for excluding one of the three confirmed nests from training is explained below.

In-situ training with the dog-handler teams

During their initial introduction in controlled settings in Montana, each of the three dogs received 126 exposures to human-placed bumble bee nest material, which they readily recognized (i.e., change of behaviour from up to 15 m away, moving towards the sample and alerting) by the end of this training period. In Ontario, they subsequently were each provided with 18 exposures to human-placed samples in residential and conservation areas (concealed or buried at a maximum depth of 5 cm), at which point, observed detection distances were narrower (< 1 m; Table 2). Note that the field-testing component described in Waters et al. [20] and O’Connor et al. [21] comprised exposure to 20 nest samples buried at depth of 10 cm. As a pre-fielding, interim training step (as described above), significant effort was invested in locating wild nests, as a way for handlers to both reinforce and confirm the ability of these individual dogs to find, and generalize to, wild nests prior to fielding. Here, we further expand upon the process of exposing the dogs to two wild nests, and their responses.

Wild nest #1 (*B. impatiens* with presence of cuckoo parasite, *B. citrinus*)

The first wild nest, located at a resident's property in Norwich, Ontario (Table 2), was not immediately recognized by any of the dogs. The entrance to the nest was located within the step of a patio made of flag stones, gravel, landscape fabric and railroad ties (i.e. long, chemically treated wooden beams). A few *B. impatiens* workers were observed approximately every 10 minutes entering and leaving through a gap above one of the railroad ties. After excavating the nest by lifting the flag stones, removing some gravel and landscaping fabric, and providing reinforcement, all three dogs were able to independently find and then alert to the nest. Reinforcement involved verbally encouraging the dogs as they investigated the area, presenting places to sniff and finally, immediately delivering a toy to them when they got their noses over the exposed nest material. Once the nest material was exposed, a distance of 0.61 m could be measured from the observed entrance (from which bees were seen entering and exiting, and the closest point the dogs could have gotten their noses to it) relative to where the nest material itself was actually situated. Excavation of the nest revealed approximately 30 brood cells intact and 2 opened; two dead *B. impatiens* queens and one dead *B. citrinus* female, plus one live *B. citrinus* female, and one live *B. impatiens* queen. Approximately eight *B. impatiens* workers were collected from the nest or as they returned to the nest. This nest (comprising approximately 1.5 – 2 g of material) was removed for further training purposes.

Wild nest #2 (*B. bimaculatus*)

Two of the three dogs showed immediate recognition to this wild nest. Reported in a suburban backyard in Guelph, Ontario, the nest was adjacent to a water feature/mini artificial pond (~ 0.4 m^2). The nest was underground within a raised soil bed, enclosed on one side with wooden slats next to the water feature with the entrance concealed by dense vegetation. Worker bees were seen very frequently entering and leaving the nest (one every 2-5 minutes). All bees (approximately 150, mainly workers with a few gynes and males) were either netted as they exited the nest or as they returned to the nest area. Once the bees were removed the dogs searched the area by the nest. As described above for nest #1, this nest was similarly reinforced to the dogs, after which they all detected the nest scent within 0.15 m from the nest entrance (measured post nest excavation) when brought back around to the area.

Wild nest # 3 (*B. bimaculatus*)

The third nest was within the foundation of a house in Georgetown, Ontario and only accessible through an opening that was both elevated from the ground (~80 cm) and angled at approximately 45°. This limited air flow and potential nest scent availability for the dogs. Additionally, although bees could be seen entering and exiting one area, the entrance to, and location of, the nest itself was difficult to pinpoint, preventing the handlers-trainers from being certain they would in fact be reinforcing and rewarding the dogs to scent emanating from that nest. Due to these factors, this nest was determined not to be suitable for dog training and we opted not to use it.

Ultimately, we did not move ahead to surveying because we sought to offer exposure to naturally occurring nests as the transitional training step, and the WD4C handler-trainers concluded that nearing the end of the period allocated to them, the dogs were not yet field-ready.

Detection distances and search strategy

The detection distances observed during controlled area searches (i.e. for samples placed on the surface of the ground or buried/concealed within vegetation to a maximum depth of 5 cm) are summarized in Table 2. Detection distances to naturally occurring wild nests were much shorter (less than 1 m). At our study's conclusion, we followed up with authors of the prior nest detection work in order to compare findings during training, testing and fielding. Previously observed detection distances spanned up to a couple of meters (S. O'Connor, pers. comm. 2019). Locating a target that, for various reasons, emits a faint scent within its natural environment requires an intensive "detail search". This is conducted with dogs on leash to maintain slow movement and coverage of an area, their noses kept close to the ground, and with handlers repeatedly pointing out appropriate habitat features (in this case, downed logs, the base of trees, leaf debris) to search. Ensuring that dogs have ample opportunities to access important features within a given landscape type takes a long time relative to the amount of area that can be covered. The use of this search strategy relative to small scent targets (e.g. blunt-nosed leopard lizard (*Gambelia sila*) scat) is further described in Statham et al. [36] and Filazzola et al. [37]. The need for detailed searching in relation to bumble bee nests, and the landscape within which they may occur, was also encountered in the prior bumble bee nest search and detection work. S. O'Connor (pers. comm. 2019) revealed that the dog in the Waters et al. [20] study worked on leash and, although the dog in the O'Connor et al. [21] study worked off-leash, the handler did direct the dog to search certain areas by pointing to the ground or to habitat features. It was also learned that the dog successfully located nests previously identified by humans, but also at times was unable to find a nest that had been recognized or missed a known entrance (where bees had just been seen leaving) several times after sniffing it directly (S. O'Connor, pers. comm. 2019). Detailed searches for small scent targets are mentally exerting for dogs, which in turn requires pacing on the handler's part, and remaining vigilant for signs of fatigue in the dog. After observing the behaviour of each individual dog during searches, the WD4C trainer-handlers estimated that each dog could search for no more than 20 minutes (consecutively) before requiring a break, during which time a different dog could be brought out to resume searching. A similar result was reported by O'Connor et al. [21], where 25-minute search sessions were conducted. Once a dog was given a break, they were able to continue searching for another one or two sessions, but for increasingly shorter increments.

Opportunistic ground-truthing with the dog-handler teams

In the absence of confirmed wild nests for training, ground truthing with the dog-handler teams was conducted within six of the target areas identified by human surveys. Field exercises were devised by the handler-trainers, who are also seasoned field biologists, to gather complementary information around the feasibility of surveying for bumble bee nests and addressing the knowledge gap of which North American habitats may be most conducive to detailed searching.

On June 27, three of these targeted areas were visited at Silver Creek Conservation Area. The first was a relatively open area with many downed trees, the second was another relatively open site with dense vegetation litter and a drain pipe, and the third was a heavily vegetated location where a queen was observed to disappear (potentially indicating a nest site) and workers had been seen in the area. One of the dogs showed pronounced interest at a drainpipe and at an animal burrow, at the second and third ground truthing sites, respectively. Researchers (VJM and ARL) observed the drainpipe (60 minutes) and animal burrows (90 minutes) and inspected the two areas with endoscope cameras but could not confirm the presence of a bumble bee nest. On July 3, the dog teams visited another area at Silver Creek Conservation Area with moderately dense vegetation, many downed trees, moss, and rocky terrain. The difficult and dense terrain made it challenging for dogs and handlers to move together through the area. None of the dogs showed interest at this location. On July 4 and 5, the dogs were brought to three separate parts of Terra Cotta Conservation Area. On July 4, search grids (3 x 3 m) were conducted in an open, sloped area with downed logs and an abundance of leaf debris at one of the targeted locations. On July 5, an area with straw bales and discarded landscaping materials (soil, gravel, tree branches) was visited by the dog teams as well as an open grassy area with some dirt mounds and downed utility poles. Previous studies have indicated that bumble bees nest in straw/hay and in rock piles [e.g. 38,39] so these seemed like appropriate/promising areas to explore. No nests were detected at any of these search locations.

Discussion

Efficiently and effectively locating bumble bee nests is critical for ensuring adequate protection of bumble bee habitat and to help understand population dynamics. In this study, we aimed to train detection dogs to search for bumble bee nests in southern Ontario. To build on prior work with dogs to this target, and mindful of insight gained during the course of that work [20,21 S. O’Connor, pers. comms. 2019] we sought naturally occurring nests on which to train dogs and lay a solid foundation of scent recognition and bumble bee nest/species generalization prior to surveying. We declined to proceed with formal surveys because the core part of our approach was to offer naturally occurring nests as the transitional training step, and we did not consider that the dogs reached the point of field-readiness within the allotted timeframe. However, our experiences and observations during training may be valuable to researchers who are considering implementing this methodology within their future conservation or monitoring efforts. The three major challenges/constraints we observed are: i) prior to fielding, and given their proven ability to generalize to numerous species in prior studies, we predict dogs would likely benefit from exposure to naturally-occurring, wild bumble bee nests; however this may neither be a realistic or feasible training scenario, certainly not as a standalone transitional training step, given the difficulty in finding wild nests, ii) whether found by a human or by a dog, confirming a wild nest find established through the presence of a resident individual in the nest is labour-intensive, time-consuming, and nuanced, and iii) Study design, including selection of survey sites, must reflect the realities and limitations of dog search strategies and capabilities.

Constraint 1: Dogs might benefit from exposure to naturally occurring bumble bee nests prior to fielding, but this may not be viable given the difficulty in finding wild nests

Knowing that dogs can progress from finding human-handled, placed nests, to naturally occurring nests, we sought to examine whether wild nest exposure could serve as a standalone transitional training step instead. Based on their own experiences and observations in the field of conservation detection, the handler-trainers at WD4C have found this to be an important training steppingstone with many targets [26]. As such, giving dogs an interim training scenario where they are exposed to known wild nests could be formative. It is also worth noting that any additional exposure to wild/naturally occurring nests helps further refine the dog handler’s search image, which only assists them in better directing the dog during detailed searches. However, bumble bee nests are difficult to locate as they are often underground or in other hard to observe locations [9]. Indeed, subterranean targets offer their own inherent challenges [as discussed in

40,41]. Finding bumble bee nests often requires large time investments and/or vast number of volunteers [i.e. 39, and demonstrated here]. Recruiting, organizing, and training volunteers (especially for conducting searches at field sites vs. backyard garden searches) can be challenging if there is not already an established volunteer network. We found that volunteers often flagged potential nest sites, but after bumble bee researchers observed the location and discussed the volunteer's observations, we discovered that most of these potential nests were locations of interest for nest-searching queens. We suspect this may be due to the varying behaviour observed between different individual nest-searching queens where some move quickly and do not spend a great deal of time investigating aspects of the environment while others could spend upwards of 30 minutes inspecting a single feature.

Home residents with access to green space may be an invaluable resource for locating bumble bee nests for the purpose of training detection dogs. They may be more likely to locate bumble bee nests within their property compared to volunteers at study sites in natural areas as they would be frequent observers of their property and are more likely to notice high bee activity in an area. Indeed, all the contacts we received from community members with suspected nests were due to the residents observing a lot of bee traffic in the area. Future studies looking to identify wild nests for the purposes of training and transitioning the dogs to naturally occurring nests may wish to focus their attention on home residents' surveys of their properties. Lye et al. [39] successfully located many nests using surveys directed at home residents in the UK. One way that the involvement of detection dogs may uniquely advance bumble bee outreach and conservation efforts is in increasing public attention and engagement, potentially boosting nest site reporting. The residents we visited were very excited by the involvement of the dogs, which we also suspect generated an increased response via social media. The unique ambassador role that conservation detection dogs can play is further described in Sawchuk et al., [42] and Woollett (Smith) et al. [22].

Constraint 2: Confirming a nest find (via presence of resident bumble bees) is labour-intensive, time-consuming and nuanced

Researchers involved in prior work noted (S. O’Connor, pers. comm. 2019), and we observed, that confirming a dog has correctly located a nest and giving them a reward within a timely manner has proven difficult with bumble bee nests. To confirm a nest, a bee must be seen leaving or entering the nest, or the nest must be excavated. It can take a long time for a queen or workers to exit/return to a nest after foraging (e.g. minutes to hours) especially for small colonies, in cool weather, and in early spring. Similarly, nest excavation may be detrimental or counter to conservation objectives. Disrupting and altering vegetation at a nest entrance can prove disorienting to its inhabitants and in some cases could lead to nest failure (S. O’Connor, pers. comm. 2019). There can be multiple entrances to bumble bee nests [12,13] so if a dog alerts at one entrance but the bees are using a different entrance, this might hinder accurately confirming the presence of a bumble bee nest (i.e., missing a nest that is occupied) (S. O’Connor, pers. comm. 2019). Some bumble bee species will nest above-ground in tree cavities or bird boxes [9]. To our knowledge, all training of detection dogs to locate bumble bee nests have focused on underground or surface nests. Above-ground nests in general are likely under-detected for most bumble bee nesting studies due to the difficulties in observing them [9]. Confirming nest identification is still a challenging part of this methodology that has not been well addressed particularly for underground nesting bee species but may not be as much of an issue for surface nesting species. It is important to note that nest detection studies have thus far focused on dogs finding (or being rewarded to) active, i.e., occupied, bumble bee nests. However, dogs can find nests at a variety of phases and activities, including failed and vacated nests (S O'Connor, pers. comm. 2019), which may also yield useful perspectives in habitat selection and preferences. Researchers may wish to consider enlarging their survey scope accordingly and explore the viability of "proxy" targets of high conservation value. These proxy targets would occur in association with bumble bee nests, might be easier for dogs to locate (and be rewarded for), and could provide complementary information about nesting habitat selection and preferences. For example, dogs were trained to threatened Kincaid's lupine (*Lupinus sulphureus* ssp. *Kincaidii*), a primary host plant to the endangered Fender's blue butterfly (*Icaricia icarioides fenderi*), thereby allowing information about habitat quality to be gathered without having to train the dogs to the scent of the rare butterfly itself [43]. No proxy targets have yet been identified for bumble bee nests. Potential appropriate and viable proxy targets can be considered in consultation with working dog professionals to determine if they are feasible from a detection dog perspective, and, if so, to develop appropriate methodologies, and estimate effectiveness of these targets relative to the study objectives.

Constraint 3a: Study designs and objectives must match the requisite search strategy using dogs

We found that the typically faint scent and concealed nature of naturally occurring bumble bee nests requires short, intensive searches. As such, it is impractical to have dog-handler teams search entire large areas for bumble bee nests. We observed this firsthand during ground-truthing exercises. Grid or subset/small-scale transect searches may be a more effective use of dogs in pre-selected locations where nest activity is suspected such as areas with: high nest-searching queen activity, previous nest sites, high traffic zones with workers (away from foraging sites) and bees performing orientation flights. Statham et al. [36] outlined important considerations around the intensive strategy used to find the diminutively scented scats of endangered blunt-nosed leopard lizards, including a search strategy which focused on shrubs that Filazzola et al. [37] determined to be positively associated with the occurrence of blunt nosed leopard lizard scats. Possible visual cues, i.e. landscape features known to be ecologically relevant to bumble bee nests, such as fence lines, hedgerows, or field margins [38,44] can also be singled out to increase the chances of finding nests. For the aim of finding bumble bee nests using dogs, open sites without dense or tall vegetation, few downed woody debris and other litter appear to be optimal study sites [20,21]. Field site options that match these site descriptions and are known to have bumble bee nests include open deciduous forests, short grass meadows, agricultural fields and field margins, sand dune, and open shrubland [9]. Similarly, bumble bees have been known to nest in distinct features and landmarks such as farm (out)buildings, discarded machinery and old vehicles and around drainage ditches (E. Venturini, pers. comm. 2020) which represent potentially more readily accessible and less ambiguous search areas for the dogs.

Here, we found that the dogs could perform continuous detailed searches for about 20 minutes before they needed a break, which is similar to the 25-minute search period reported by O’Connor et al., (2012). The requirement for breaks would need to be factored into the study timeline and possibly consider increasing the number of dogs deployed to allow for more continuous searching.

Constraint 3b: Survey timing, nesting timeframes and accurately determining presence/absence

An added challenge we experienced in our study was the unusually wet and late spring in southern Ontario in 2019. In 2018, we planned to conduct *in-situ* field training and surveys in late June and early July 2019 to overlap with the phenology of many bumble bee species, assuming an “average” year [35]. The late spring meant that bumble bee workers were only just starting to be observed in the vicinity immediately before the dog teams were scheduled to arrive. This likely meant few colonies had been established and those that had were likely small with few workers and therefore less worker traffic than if colonies were larger in size. However, it cannot be inferred from this that, correspondingly, less scent would be available for the dogs. Indeed, prior studies showed that the dog was able to detect tiny fragments of nest material left in the field [21]. Additionally, the dog in that study occasionally detected nests which were deemed to have been inactive for a period of months, and found some nest material that was very small, or almost entirely consumed by wax moths (S. O’Connor, pers. comm. 2019). Accordingly, researchers may wish to incorporate not only occupied nests but also previously occupied locations into their study design and objectives. Future studies must consider the best survey timeframe to maximize the likelihood of finding different species, and/or the most nests.

Constraint 3c: Study sites must be selected with an understanding of the limitations that certain habitats may pose to dog-handler searches

The effectiveness of detection dogs varies according to the environment in which they operate. This has specifically proven to be the case with bumble bee nests, where prior work showed varying levels of nest detection success within several different types of habitats in which surveys were conducted [20, 21, and see Table 4]. Note especially the difference in reported find rate success and searching relative to effort in Waters et al. [20] working in open island habitat versus by O’Connor et al. [21] in certain more vegetated, challenging terrains. Bumble bees may nest in a plethora of different habitats, not all of which can readily be traversed or efficiently searched by dog-handler teams. Numerous studies have reported on the role of vegetation in find rates [see for examples, 45,46] and it was specifically described as a limiting factor in bumble bee nest detection by both Waters et al. [20] and O’Connor et al. [21]. Researchers wishing to explore the incorporation of detection dogs for future bumble bee nest related efforts should carefully consider whether their focal location and the habitat therein would lend itself well to the use of this monitoring method.

Conclusion

Detection dogs can find bumble bee nests in the wild [20,21]. However, it appears that as a monitoring tool, using detection dogs as a method of locating bumble bee nests for ecological studies has limited applications with significant drawbacks to its implementation, especially regarding nest confirmation and timely reward. Some of these constraints could perhaps be mitigated to a certain degree, with careful considerations of study question and design. This includes developing research questions that can feasibly be answered with this method, designing studies around directed and detailed searches, locating wild bumble bee nests for training dogs prior to their deployment, choosing study sites that are conducive to dog search strategy and capabilities, determining how nest confirmation will occur, and potentially incorporating inactive/former bee nests (versus, or in addition to, occupied ones). It may be possible to pair some modeling and projection with a selection of small subplots to detail search within a larger area. Additionally, the ability of dogs to generalize across bumble bee species, and the potential greater facility in finding proxy targets important to bumble bees, remains worthy of consideration in exploring the future viability of any applications.

The exposure of dogs in training to wild nest *in-situ*, the inherent olfactory challenges posed by nests and the nuances and difficulties posed to people for confirmation purposes all make this a difficult target on which to train and field dogs. Bearing in mind these now well-established limitations of using detection dogs to further bumble bee research and conservation objectives, at least in terms of seeking naturally occurring nests in certain types of habitats, further exploratory work is nonetheless warranted to explore the potential ways that the involvement of detection dogs could uniquely help researchers gain insight into bumble bee habitat selection, occupancy or nesting preference.

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Tables

Table 1: A description of the training bumble bee nest samples the dogs were exposed to in Montana and Ontario. Training nest samples were bits of nesting material separated from the rest of the nest (approximately 2 g of material) containing wax cells, honey pots, and brood cells.

Training Location	**Type of training**	**Species**	**Number of nests**	**Captive or wild nest**	**Source**
Montana	Lineup and area search (placed in mowed grass, garden)	*B. impatiens*	4	Captive colony founded by wild-caught queens	Dr. Benn Sadd, University of Illinois
Montana	Lineup and area search (placed in mowed grass, garden)	*B. terricola*	2	Captive colony founded by wild-caught queens	Dr. Benn Sadd, University of Illinois
Montana	Lineup and area search (placed in mowed grass, garden)	*B. occidentalis*	1	Captive colony founded by wild-caught queen	Dr. Benn Sadd, University of Illinois
Ontario	Area search, placed (on ground, in vegetation, buried max 5 cm)	*B. impatiens*	2	Commercial colony	BioBest
Ontario	Area search	*B impatiens/ citrinus*	1	Wild colony	Reported by private citizen, Norwich, Ontario
Ontario	Area search	*B. bimaculatus*	1	Wild colony	Reported by private citizen, Guelph Ontario.

Table 2: Detection distances observed for human-placed material and wild bumble bee (*Bombus* sp.). Square brackets for detection distances indicate the more common detection distances within the range. The survey date and a description of each site is also provided.

Date	**Species**	**Human-placed or wild?**	**Observed detection distance range (m)**	**Site description**
June 24, 2019	*B. impatiens*	Human-placed	0 – 2 [0.5 - 0.75]	Georgetown, Ontario, suburban residential area, lawns/gardens
June 25, 2019	*B. impatiens*	Human-placed	0 - 5 [0.25]	Silver Creek Conservation Area, deciduous forest, densely vegetation understory, downed trees
June 26, 2019	*B. impatiens*	Human-placed	0 – 2 [0.25 - 0.27]	Georgetown, Ontario, suburban residential area, lawns/gardens
June 27, 2019	*B. impatiens*	Human-placed	0 – 0.25	Silver Creek Conservation Area, deciduous forest, densely vegetation understory, downed trees
June 29, 2019	*B. impatiens/ citrinus*	Wild	0.61	Norwich, Ontario, suburban residence, lawn/garden
July 2, 2019	*B. bimaculatus*	Wild	0.15	Guelph, Ontario, suburban residence, lawn/garden
July 3, 2019	*B. impatiens/ citrinus*	Human-placed	0 – 0.15	Silver Creek Conservation Area, deciduous forest, rocky, many logs, moss, dense leaf litter
July 4. 2019	*B. impatiens/ citrinus*	Human-placed	0 – 0.25	Terra Cotta Conservation Area, open deciduous forest, few logs, dense leaf litter

July 5, 2019	*B. impatiens/ citrinus*	Human-placed	0 – 0.15	Terra Cotta Conservation Area, open area with straw, hay bales, dirt mounds and debris

Table 3: A comparison of historical average (1981-2010) and 2019 temperature and precipitation data for a weather station located in Georgetown, Ontario (WWTP ID= 6152695, 43.640005, -79.879172, ~8 km away from Silver Creek Conservation Area, https://climate.weather.gc.ca/).

Month	**Temperature daily average 1981-2010 (°C)**	**Temperature daily average 2019 (°C)**	**Precipitation average 1981-2010 (mm)**	**Precipitation average 2019 (mm)**
April	6.0	4.8	76.5	93.4
May	12.3	11.2	79.3	97.6
June	17.4	17.4	74.8	108.6

Table 4. Summary of study aims, parameters, findings and outlooks from prior published bumble bee nest detection dog work. Text in square brackets denote author notes from the study.

Study	**Aim**	**Location**	**Timing of study**	**Finding(s)**	**Outlook:**
Waters et al. (2011)	Train dog to find nests of rare bee species Ultimate goal: Assessing nest density for estimate of effective population size	Hebridean Island of Tiree (Scotland) [Tiree described as having an 'unusually high' density of bumble bee species]	August and September (2006) Coinciding with the end of the nesting cycle	Dog find rate: 33 nests over 30 ha 4 spp. represented Recorded nest densities: *B. muscorum*: 1.86 nests/ha (machair) [a] *B. distinguendus*: 0.533 nests/ha (dunes) *B. lapidarius*: 0.267 nests/ha (machair) *B. jonellus:* 0.133 nests/ha, single nest find (lowland heath) Patterns of habitat preferences could be discerned according to species [Nests of *B. muscorum*, most abundant species, was found at highest rate]	The technique has great potential, but using a dog in dense vegetation limits the effectiveness [Testing and searches were all executed in open habitat The island of Tiree is almost entirely devoid of densely vegetated habitat]
O'Connor et al. (2012)	Comparison of: 1) efficiency of two detection dogs trained to bumble bee nests	Rural and woodland habitats in the United Kingdom:	May to August (2008)	Dog find rate: In rural habitats: 9 nests of 4 species *B. terrestris* *B. pascuorum* *B. lucorum* (n = 2) *B. hortorum*	Detection dogs are not a cost-effective method for locating bumble bee nests, especially relative to volunteers Fixed searches are appropriate for an aim of estimating bumble bee nest density

	based on the performance of the dog in the current study and those study conditions relative to that of the dog in Waters et al. (2011) 2) ability of a dog to locate nests when carrying out repeat searches of agricultural habitats through the season 3) efficiency of a dog compared with human volunteers at finding nests in woodland	Farmland near Stirling, Scotland – hedgerow fence line bank (i.e., steeply sloping earth bordering lanes and ditches) long grass (>15 cm), short grass (<10 cm) woodland edge (within 10 m of) Open woodland habitat		Overall rate: 1 nest/19 h 24 min search time However, on a bee nest per habitat basis: 3 nests in woodland edge habitat 3 nests within hedgerows 1 nest in short grass 1 nest in long grass 1 nest in bank habitats 0 nests detected along fences Free searches - human volunteers vs dog in woodland Similar find rates = 1 nest / 1 h 20 min Fixed search by humans in woodland 4 nests found by volunteers 3 x *B. terrestris* + 1 x *B. pratorum*). 1 nest for 3 h 20 min of searching Free search by humans in woodland 10 nests found by volunteers 7 x *B. terrestris* + 2 x *B. lucorum* + 1 x *B. pratorum* 1 nest for 1 h 20 min The dog located 10 nests during woodland searches of the same area as the volunteers	Free searches are a better method for finding many nests Novice volunteers performed as well as experienced ones

				7 x *B. terrestris* + 1 *x B. lucorum* + 1x *B. hortorum* + 1 x *B. lapidarius*	
Goulson et al. (2018)	Find wild nests to install cameras and record activity, detect gyne production, and record visits by vertebrate predators Screened workers for internal parasites, providing a detailed account of the factors affecting the fates of 47 bumblebee nests.	See O'Connor et al. (2012)	See O'Connor et al. (2012)	47 wild nests found by a detection dog[b] *and* volunteers. This study made use of nests found during work conducted by, and described in, O'Connor et al. (2012)	Peripheral/opportunistic use of already trained detection dog, i.e., no specific conclusions relative to their efficacy. [But worth highlighting the detection dog tangibly contributed in this way to bumble bee nest detection efforts and conservation/research efforts].

a. rare habitat confined to west Scotland and Ireland, consists of flat coastal plain of species-rich grassland growing on wind-blown shell sand.

b. same dog that participated in O'Connor et al. (2012)

Waters J, O'Connor S, Park KJ, Goulson D. Testing a detection dog to locate bumblebee colonies and estimate nest density. Apidologie. 2011 Mar;42(2):200–5.

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Chapter 5: Summary and future directions for bumble bee habitat research

Amanda R. Liczner

Summary of PhD research

The objective of my PhD research was to characterize the habitat for bumble bees with a focus on Northeastern North American species. To do this I conducted a systematic literature review (chapter 1), quantified the habitat for two at-risk bumble bee species in southern Ontario (chapter 2), identified priority areas for bumble bee conservation under current and future climate scenarios (chapter 3) and attempted to use detection dogs to locate bumble bee nests (chapter 4).

The literature review was conducted to get a sense of the published literature on bumble bee habitat. This review included search terms for the three main resources needed by bumble bees namely floral, nesting and overwintering. It was apparent from the results of the search terms that nesting and overwintering resources were greatly understudied and in need of more research. We therefore decided to focus on the nesting and overwintering studies for our review. Although there were few studies on overwintering habitat (ten), generally, bumble bee queens overwinter by burying themselves underground at a depth between 2-15 cm in shaded banks, and/or near trees, often with moss covering the ground or in bare-ground patches. However, overwintering habitat was not systematically surveyed in these studies. Therefore, these results likely do not represent a full description of bumble bee overwintering habitat. Bumble bee nests were most often reported underground, but whether this is because bumble bees prefer to nest underground or because most survey methods are biased towards detecting underground (and surface) nests is undetermined. Bumble bees will nest in a variety of landscapes (grasslands, agriculture and forest landscapes are most common) and the entrances are usually concealed (e.g. by vegetation). However, more studies are needed for specific generalizations to be made about these aspects of bumble bee habitat, such as species or subgeneric specific differences in nesting behaviour.

Specific quantifications of bumble bee habitat are often unknown but are important for accurately managing bumble bee habitat especially for declining bumble bee species. To address this knowledge gap, we conducted habitat surveys for two at-risk bumble bee species (*Bombus terricola* and *B. pensylvanicus*) across southern Ontario in spring, summer and late-summer for local variables and flowering plant species cover. Landscape variables were extracted from raster and agricultural census data. Here, we showed that both species had specific habitat requirements for landscape variables, local variables and flowering plant species cover. *B. terricola* was associated with forested landscapes and flowering plant species common in forested landscapes, as well as areas with high coarse woody debris. *B. pensylvanicus* was associated with open/grassland habitats and flowering plant species common in open/grassland areas. Landscape variables explained more variation in habitat for both species compared to local variables and flowering plant species. Floral resources were consistently the most important variable across the season (spring, summer and late-summer) followed by floral structure, nesting and overwintering resources. However, we must caution the interpretation of these results as our study was analyzed using correlational methods, and we did not capture the uncertainty in our observations. As our results are based on correlations, the variables measured might not be distinct, and thus the habitats of *B. terricola* and *B. pensylvanicus* habitats may not be distinct. Similarly, as we did not measure the uncertainty surrounding our observations there may be limited confidence in our conclusions. Future studies would benefit from implementing hypothesis testing and experimental designs to further test these trends. These results may have important implications for managing the habitat for these two declining species (and possibly other related species) within southern Ontario and similar regions.

In addition to habitat loss, bumble bees are threatened by climate change. Effective conservation plans for declining bumble bees will not only need to consider how to manage limited resources effectively, but also plan for changing future climatic conditions. We identified conservation priority areas for bumble bee species across Canada under current and two future climate scenarios (RCP 2.6 and RCP 8.5) for the year 2050. These priority areas were also identified for two different objectives: one which aims to minimize the costs of conservation actions, and the second which aims to maximize phylogenetic diversity while remaining within a budget for conservation actions. Conservation priority areas for bumble bees are in western Canada, southern Ontario, southern Quebec and the Maritimes, and most often contain woodland savannas, mixed forests, and evergreen needleleaf forests. Bumble bee conservation priority areas are predicted to shift northward and upwards in elevation with climate change and mixed forests will become more common in priority areas while croplands, savannas and woodland savannas become less common. Bumble bee priority areas are currently not well represented within Canadian protected areas. However, as bumble bees are generalist species, it may be possible to effectively conserve species within properly managed private lands. This study has important implications for the effective management of limited conservation resources while protecting bumble bee species.

One of the most likely reasons that the nesting habitat for bumble bees is understudied is due to the difficulty in locating bumble bee nests. Bumble bee nests are often underground, or in concealed locations, and are usually only detected in large numbers after massive survey efforts are deployed. We aimed to test a relatively novel method for efficiently identifying bumble bee nests using trained detection dogs to locate the scent of bumble bee nests. The detection dogs were successfully able to detect the scent of bumble bee nests within a controlled indoor training environment and when training samples were hidden by humans outdoors. The final stage of training involves exposing the dogs to multiple instances of wild nests, however, despite our best efforts to locate wild nests, only two nests could be confirmed for dog training purposes. We used this opportunity to assess the strengths and challenges for using detection dogs to locate bumble bee nests in future studies. We found that locating wild nests requires a large effort before the dogs can begin searching, and this effort may be deemed too challenging to employ this method. Additionally, the distance where dogs can detect the scent of bumble bee nests is quite narrow, meaning dogs must be kept on leash and conduct detailed searches of the study site. This limits the types of study sites and objectives that would be useful for use with detection dogs. Any dog detections must be confirmed to be a nest. For bumble bees, this would be the presence of a worker bee entering/exiting a nest, a queen entering with pollen, or visualization of the nest (through excavation or endoscope camera viewing of the nest). A further complication is that bumble bee nests can have multiple entrances and it can be difficult to pinpoint the location of the nest to confirm if the dog has made a correct identification. Using detection dogs to locate bumble bee nests for ecological studies is challenging and may be a viable methodology in only certain instances.

Future work

Here, I present some possible avenues of future research either directly related to characterizing bumble bee habitat, or that would benefit habitat studies but are not directly investigating bumble bee habitat. The future work proposed is to review the published scientific literature on forage resources for bumble bees, improve bumble bee survey efforts, increase our knowledge of bumble bee movement and dispersal, and continue to identify nesting and overwintering habitat.

To my knowledge, there is no extensive literature review on the foraging resources for bumble bees. This review should consider addressing forage habitat at multiple scales from the landscape (i.e. forest, vs. grassland, gradients of urbanization or intensity of agriculture (e.g. Jönsson et al., 2015; Schochet et al., 2016)), to the patch (floral abundance, diversity, richness, density etc. (e.g. Bowers, 1985; Carvell et al., 2015; McCracken et al., 2015)) to the species (i.e. resource selection (e.g. Heinrich, 1976; Tuell et al., 2014)) as resource selection occurs as a hierarchical process (Gaillard et al., 2010). It would also be interesting to review the effects forage quality (protein, lipid and carbohydrate content (e.g. Harmon-Threatt and Kremen, 2015; Kriesell et al., 2017)) of nectar and pollen provided by different flower species as forage quality has been shown to influence bumble bee foraging behaviour (Vaudo et al., 2016). The results of this review can help managers select species to seed and design plots in habitat restoration projects.

Improved bumble bee survey efforts would greatly benefit future bumble bee habitat studies. The large Bumble Bees of North America Database (Richardson, 2020) managed by Dr. Leif Richardson is a compilation of survey efforts from many sources, but most of these records are from haphazard searches and can only infer presence data. The same location is not necessarily surveyed in multiple years making it difficult to determine the effects of changing landscapes, or loss (or gain) of species to an area. Presence only data also limits the types of analyses that can be conducted. Survey efforts would be improved by having standardized searches over multiple years of many sites. Surveys should also be extended to areas that are unreported, such as the arctic and alpine in Canada, which can help Data Deficient species and monitor population trends. These types of survey efforts could allow similar studies as my chapter 2 but to model the effects of landscape or local-scale habitat changes that have caused changes in bumble bee occupancy at sites and infer the importance of certain habitat features. It could also improve the accuracy of future climate models and conservation priority area determinations.

By investigating bumble bee movement and dispersal, we can improve our understanding of habitat selection (Mola and Williams, 2019). This can be done by tracking bumble bee movement (through radio-tracking, or harmonic radar for example), and observing the types of habitat features bumble bees select, and perhaps those that act as barriers to movement (Kriesell et al., 2017; Woodgate et al., 2016). Experimentally manipulated landscapes, or restoration areas would be excellent study sites to examine bumble bee habitat selection through observations in bumble bee movement. This is because specific hypothesis and predictions can be tested with regards to different landscape and/or patch features. It may also be important to test for possible differences in movement and dispersal between bumble bee castes especially for the reproductive castes (males and gynes) and spring queens as they represent the only opportunity for dispersal and gene flow (i.e. Makinson et al., 2019). Dispersal information for bumble bees is especially important for studies investigating the impacts of climate change on bumble bees and their ability to track their shifting climatic envelopes. Accurate dispersal information would greatly improve predictive ability for climate change modelling such as those conducted in chapter 3. Determining the features (if any) bumble bees are resistant to moving through would be important for designing effective corridors so bumble bees can track their shifting climate.

Overwintering and nesting sites for bumble bees are still not well understood but they are extremely important to identify. There have been more efforts to date looking into overwintering sites for bumble bees (Williams et al., 2019) including a community science campaign to help increase observations of overwintering queens (https://www.queenquest.org/). Although detection dogs may not be as efficient at locating bumble bee nests as we had hoped, other methods such as radio-tracking could be another method to locate bumble bee nests without requiring massive search effort. It is important that our understanding of nesting and overwintering resources is improved to ensure that all critical resources are available for bumble bees. Most bumble bee conservation efforts focus on increasing forage for bumble bees (Carvell et al., 2007; Dicks et al., 2015; Moquet et al., 2017) but it is unclear if forage or possibly other resources are limiting bumble bee survival.

There are still many opportunities to improve our understanding of the habitat of Northeastern North American bumble bee species. Additional reviews are required to synthesize knowledge to date on habitat characteristics, such as the proposed review on foraging habitat. Continued field surveys are required to improve our ability to track population trends, accurately assess species, and increase confidence in modelling. Avenues of future research include examining bumble bee movement to infer habitat selection and determine bumble bee dispersal especially for tracking climate change. Nesting and overwintering also remain understudied aspects of bumble bee habitat that require further consideration. Future studies focusing on these areas will greatly improve our understanding of bumble bee habitat and conservation efforts.

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Appendices

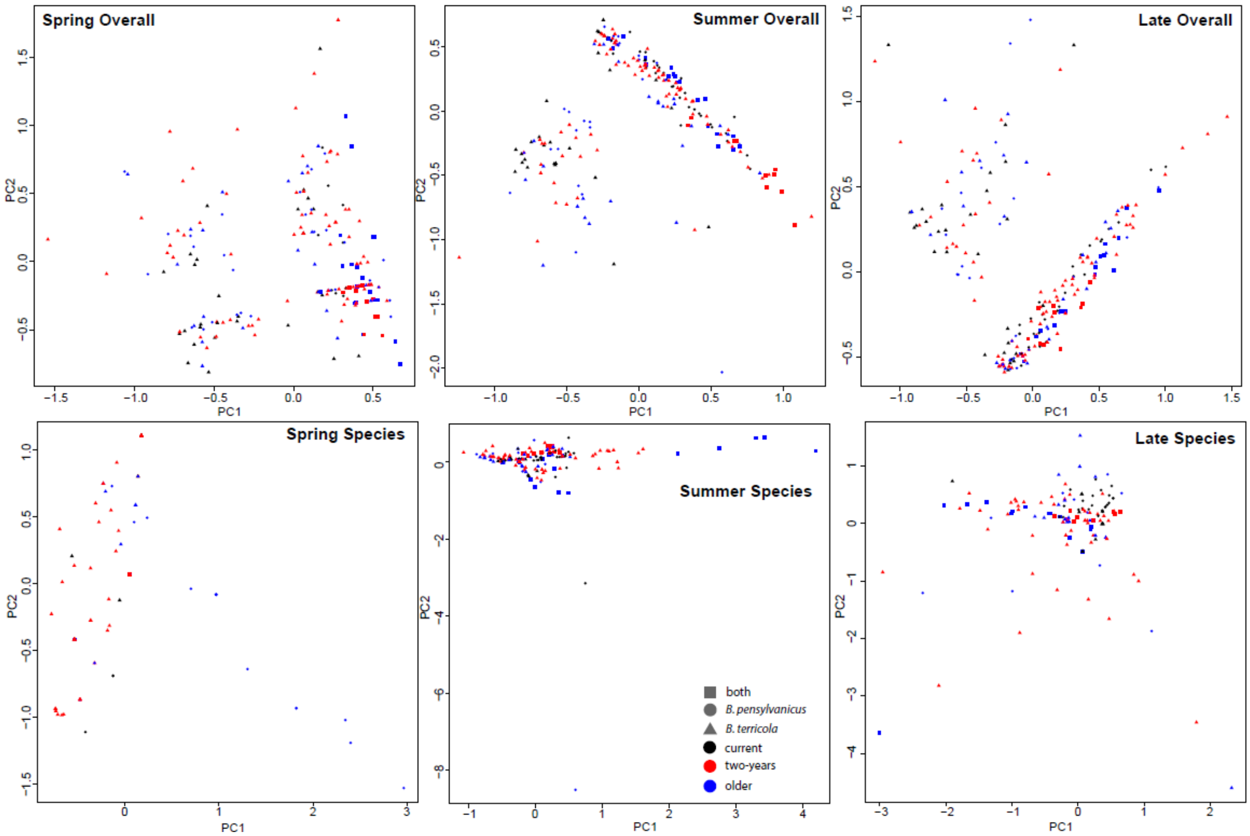
Appendix A: Chapter 2 study site locations

The name and GPS location of the 25 sites used in the chapter 2 habitat analyses**.** The bumble bee species that was recently observed at each site is also given and the number of times each site was surveyed over the 2017 season. Surveys took place in spring, mid-summer, and late-summer when possible. The last know occurrence for each species at each site is given in brackets

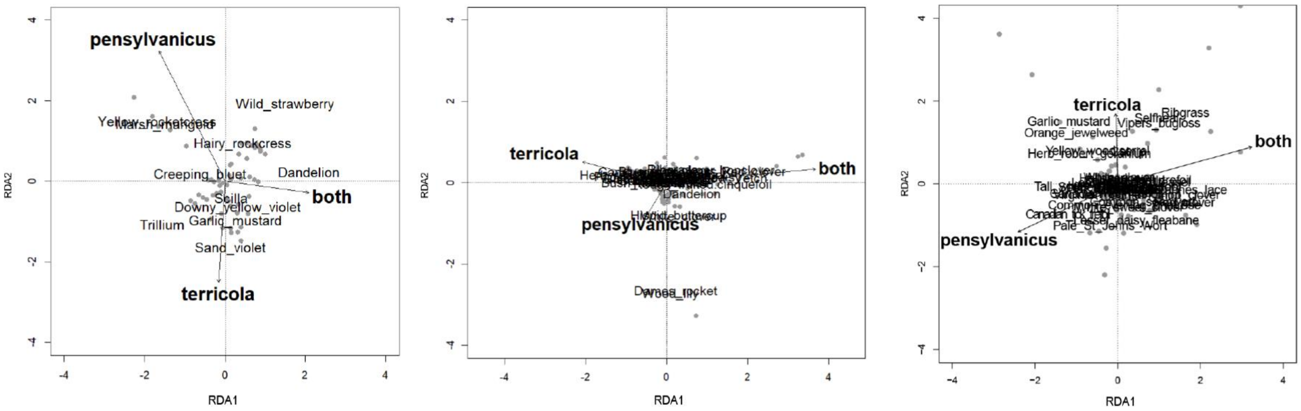
Site Name	**Latitude**	**Longitude**	**Species**	**Surveys**
Arrowhead Provincial Park	45.3917	-79.1978	*B. terricola* (2015)	3
Awenda Provincial Park	44.84871	-80.0194	*B. terricola* (2017)	3
Backus Woods	42.68102	-80.4739	*B. pensylvanicus* (2017)	2
Bass Lake Provincial Park	44.60251	-79.4867	*B. terricola* (2015)	3
Bayview Park	44.38811	-79.6869	Both (*terricola* = 2015, *pensylvanicus* = 2013)	3
Beausoleil Island	44.84754	-79.8605	*B. terricola* (2015)	1
Black Creek Provincial Park	44.96836	-81.3625	*B. terricola* (2015)	3
Bruce Peninsula National Park	45.2128	-81.4895	*B. terricola* (2017)	3
Bruce Trail Caledon	43.8015	-79.99	*B. pensylvanicus* (2002)	3
Central Big Creek Block	42.64919	-80.5604	*B. pensylvanicus* (2016)	3
Forks of the Credit Provincial Park	43.8249	-80.004	Both (*terricola* =2014, *pensylvanicus* = 2017)	3
Guelph Lake Conservation Area	43.596	-80.252	*B. terricola* (2015)	3
Harris Park	42.983	-81.25	*B. pensylvanicus* (2005)	3
Inverhuron Provincial Park	44.2987	-81.5944	*B. terricola* (2017)	3
MacNaughton Trail	43.35	-81.483	*B. pensylvanicus* (2005)	3
Mara Provincial Park	44.58661	-79.3571	*B. terricola* (2015)	3
Matchedash Bay	44.75084	-79.646	*B. terricola* (2015)	3
Pinery Provincial Park	43.2734	-81.8183	*B. pensylvanicus* (2017)	2
Pollinators Park	43.57776	-80.2331	Both (*terricola* =2015, *pensylvanicus* =2015)	3
Scotsdale Farm	43.69024	-80.0051	*B. terricola* (2014)	3
Singing Sands	45.1912	-81.5776	*B. terricola* (2014)	3
Sulphur Springs Conservation Area	44.11729	-81.0035	*B. terricola* (2015)	3
Turkey Point Provincial Park	42.7279	-80.3369	*B. pensylvanicus* (2005)	3
University of Guelph Arboretum	43.54205	-80.2115	*B. terricola* (2015)	3
Waubaushene Beaches	44.75054	-79.7209	*B. terricola* (2015)	3

Appendix B: Effect of survey year on chapter 2 analyses

Some of our study sites had older (i.e. >2 years) records of occurrence, mainly for *B. pensylvanicus* (see description of site selection). To determine if these older sites have different local scale conditions compared to sites with more recent occurrence records, we performed a Principle Components Analysis (PCA) and a partial Redundancy Analysis. The PCAs and partial RDAs were performed for local and flowering plant species cover variables over the three survey periods. For the PCA, we coded sites as current (2017 occurrence), two years (occurrence record within two years), and older (records older than two years). Here we aimed to see if sites with different occurrence ages ordinated with each other (i.e. mixed) which would indicate no difference with occurrence age, or if sites ordinate in groups by occurrence age indicating a difference between sites of different occurrence ages. We found that sites were mixed for local variables, but there was some grouping of older sites for flowering plant species. We investigated the impact of this grouping effect of older sites for the flowering plant species analyses further using partial Redundancy Analysis. The partial Redundancy analysis removed the variation explained by occurrence age, and then plot the resulting RDAs. We found that the results of the partial RDAs of flowering plant species generally matched the original RDAs for flowering plant species (Figure 4). Although the position of the bumble bee species and some of the flowering plant species shifted, each bumble bee species was still correlated with the same species. We therefore decided that occurrence age of our sites is not having a large effect on our study results.



Appendix B1: Principal Components Analysis (PCA) for local scale and flowering plant species variables in the spring, summer and late-summer.



Appendix B2: Results of a partial Redundancy Analysis (RDA) for flowering plant species in spring (left), summer (center) and late-summer (right).

Appendix C: Chapter 2 raster file names and descriptions

Names of all raster files for chapter 2 and a description of the classes and resolutions within each raster file. Description of each variable taken from data source specifications.

Variable name	*Description*
Southern Ontario Land Resource Inventory 2011 (resolution 30 m)[1]	
Open Beach/Bar	Unconsolidated mineral substrates. Subject to active shoreline processes: ice scour, wave energy, erosion and deposition. Tree cover < 25%; shrub cover <25%.
Treed Sand Dune	Exposed sands formed by extant or historical shoreline or aeolian processes. Subject to active processes. 25% < tree cover < 60%.
Open Alvar	Level, unfractured limestone (carbonate) bedrock/ patchy mosaic of bare rock pavement and shallow substrates (< 15 cm) over bedrock/ vegetative cover < 25%
Shrub Alvar	Level, unfractured limestone (carbonate) bedrock/ patchy mosaic of bare rock pavement and shallow substrates (< 15 cm) over bedrock/ tree cover < 25%; shrub cover => 25%.
Treed Alvar	Level, unfractured limestone (carbonate) bedrock/ patchy mosaic of bare rock pavement and shallow substrates (< 15 cm) over bedrock/ 25% < tree cover < 60%
Tallgrass Woodland	Ground layer dominated by prairie graminoids; variable cover of open-grown trees/ 35% < tree cover < 60%.
Forest	Tree cover > 60%. Upland tree species > 75% canopy cover > 2m in height.
Coniferous Forest	Tree cover > 60%. Upland coniferous tree species > 75% canopy cover > 2m in height.
Mixed Forest	Tree cover > 60%. Upland coniferous tree species > 25% and deciduous tree species > 25% of canopy cover > 2m in height.
Deciduous Forest	Tree cover > 60%. Upland deciduous tree species > 75% canopy cover > 2m in height.
Treed Swamp	Treed communities. Water table seasonally or permanently at, near, or above substrate surface/ Tree cover > 25%. Dominated by hydrophytic tree and shrub species.
Thicket Swamp	Open and shrub communities. Water table seasonally or permanently at, near, or above substrate surface/ Tree cover <= 25%; hydrophytic shrubs > 25%. Dominated by hydrophytic tree and shrub species.
Marsh	Open and shrub communities. Water table seasonally or permanently at, near or above substrate surface – tree and shrub cover <=25%. Dominated by emergent hydrophytic macrophytes.
Open Water	Water depth > 2 meters. Lake trophic status. No macrophyte vegetation, trees or shrub cover.
Plantations – Tree Cultivated	Tree cover > 60%, (trees > 2m height), linear organization, uniform tree type.
Hedge Rows	Tree cover > 60%, (trees > 2m height), linear arrangement, minimum 10 meters width, maximum 30 meters width.
Tilled	Agricultural fields managed as continuous annual row crops inferred from 3 observed sequential time periods over a 10-year

	time period. There can be as many as 2 time periods where fields are rotated with perennial crops. (e.g., hay, improved pasture)
Transportation	Highways, roads.
Built-Up Area - Pervious	Urban recreation areas. (i.e., golf courses, playing fields)
Built-Up Area - Impervious	Residential, industrial, commercial and civic areas.
Extraction Aggregate	Pits, quarries
Undifferentiated	Includes some agricultural features not included in tilled (i.e. orchards, vineyards, perennial crops and idle land > 10 years – out of agricultural production) as well as urban brown fields, hydro and transportation right-of-way's, upland thicket and openings within forests.
Statistics Canada Agricultural Census 2011 (summarized per census boundary)	
NmbrHBC[2]	The number of honey bee colonies in usage
NmFRHBC[2]	The number of farms reporting colonies of honey bees
NmFRGOB[2]	The number of farms reporting gallons of other bees
AcrsfHr[3]	Acres of herbicides applied
NmbrFRH[3]	Number of farms reporting herbicide application
AcrsfFn$_3$	Acres of fungicide applied
NmbrFRF[3]	Number of farms reporting fungicide application
AcrsfIn	Acres of insecticide applied
NmbrFRI	Number of farms reporting insecticide application
NmbFRGV[4]	Number of farms reporting greenhouse vegetables
SqrMtGV[4]	Square meters of vegetable greenhouses
Ontario Agricultural Resource Inventory 2005 (25 m resolution)	
BERRIES	Strawberry, raspberry, blueberry, or other bush-berry production, including associated fallow or plough-down crops. Does not include berries interplanted with fruit trees.
BUILT.UP	Urban related uses, including churches, cemeteries, rural strip developments of four or more houses, transformer stations, sewage lagoons or water treatment facilities
CONTINUOUS.ROW.CROP	Single intensive crop type i.e. corn or beans. Also includes any combination of corn, white beans, soybeans or any other varieties of beans in rotation. The entire area except for topographically limited portions and non-systems use must be row crop. Corn dryers and elevator storage systems are good indicators. Often barns are absent except for a machine shed(s). There must be no grain crops or hay. Usually very large fields often with no fence boundaries
CORN.SYSTEM	A rotational system in which corn and/or beans occupy more than 40%, but less than 100% of the area. The remainder is composed of grain and hay. A small proportion (less than 10%) may be pasture. Usually silos and corn cribs are good indicators of this system. There may also be a complex of barns which indicate feeding of dairy, beef or hogs.
EXTRACTION.PITS. AND.QUARRIES	Sand and/or gravel pits and quarries
GRAIN.SYSTEM	A combination of sod crops and grains in which grain is predominant, occupying more than 85% of the area and in some cases as much as 100%. The field sizes are usually large with fences often absent. A lower intensity cash cropping system. There are no row crops; good quality hay or pasture may compose up to 15% of the area.

GRAZING.SYSTEM	Native grass pasture where topography precludes the use of machinery. Usually on poorer land where slopes, river valleys, rock outcrops or shallow soils occur. Most often seen in association with another system.
HAY.SYSTEM	A rotational system in which good quality hay and pasture predominate. Very small amounts of corn may be present, typically less than 5 acres. Hay must be the largest proportion of the system
IDLE.AGRICULTURAL. LAND.5.10. YEARS	Land idle for 5-10 years and in a state of reversion to natural vegetation. Small alders, willows or thorn bushes are several feet in height
IDLE.AGRICULTURAL. LAND.OVER.10. YEARS	Land idle for more than 10 years and supporting native vegetation. The re-growth is larger than that which can normally be cleared by farm machinery
MIXED.SYSTEM	A rotational system composed of grain, corn or beans and hey in roughly equal proportions. No crop dominates the system and field are generally small. Barns are usually the older type and silos are smaller and less numerous. Associated with a traditional farming system. Sod crops cover more than 20% of the area. Corn and beans together occupy less than 40% of the area.
NURSERY	Intensive production of trees, shrubs, vines or flowers for transplant or sale. Includes fallow or plough-down crops.
ORCHARD	Primarily hardy fruit production, usually with a combination of pears, plums, and apples dominant. Orchard must occupy more than 90% of the are. If peaches and/or cherries occur, they must occupy less than 50% of the area
PASTURE.SYSTEM	Sod crops constitute the whole area with little or no rotation. Poor quality weedy hay and/or pasture cover more than 50% of the area. Associated with extensive or unconfined grazing of livestock. There should be minimal evidence of recent cultivation
RECREATION	Recreation facilities such as parks, picnic areas, campgrounds, drive-in theatres and conservation areas. Recreation facilities inside urban areas not to be identified
REFORESTATION	Land supporting a stand of artificially stocked trees
SWAMP.MARSH.OR.BOG	Swamp, marsh, or boggy areas supporting vegetation characteristic of a poorly drained area.
TOBACCO	Tobacco occupies more than 50% of the area, but corn rotation may occur. Includes associated plough-down or fallow crops.
WATER	Large bodies of flowing or standing water sufficiently large to require mapping. In general, these bodies should occupy three hectares or more
WOODLAND	Forest cover with a minimum of 54% crown closure density and not less than one hectare in area
WorldClim (1 km resolution)[6]	
PrecipitationSeasonality	The annual range in precipitation (mm)
MeanTempWarmQuart	Mean temperature (°C) of the warmest quarter
PrecipDriestMonth	Precipitation (mm) of the driest month
altitude	The altitude in m. a. s. l
MinTempColdMonth	Minimum temperature (°C) of the coldest month
PrecipDriestQuart	Precipitation (mm) of the driest quarter
PrecipWarmQuart	Precipitation (mm) of the warmest quarter
MeanTempColdestQuart	Mean temperature (°C) of the coldest quarter

MeanDiurnalRange	Mean temperature range between the daily maximum and minimum temperatures (°C)
TempSeasonality	The annual range in temperature (°C)
MaxTempWarmMonth	The maximum temperature (°C) of the warmest month
PrecipColdQuart	Precipitation (mm) of the coldest quarter
AnnualPrecip	Annual precipitation (mm)
AnnualMeanTemp	Annual mean temperature (°C)
PrecipWetQuart	Precipitation (mm) of the wettest quarter
Soil Survey Complex of Ontario 2003 (1 km resolution)[7]	
Clayloam	Clay loam soil texture
FineSandyLoam	Fine Sandy Loam soil texture
Loam	Loam soil texture
LoamySand	Loamy sand soil texture
Organic	Organic soil texture
Sand	Sand soil texture
SandyLoam	Sandy loam soil texture

1. SOLRIS version 2. Data Specifications; 2. Statistics Canada Table 32-10-0432-01 Bees on Census Day; 3. Statistics Canada Table 32-10-0409-01 Land inputs in the year prior to the census; 4. Statistics Canada Table 32-10-0019-01 Estimates of specialized greenhouse operations, greenhouse area, and months of operation; 5. Ontario Agricultural Resource Inventory metadata; 6. WorldClim Bioclimatic variables http://worldclim.org/bioclim 7. Land information Ontario Soil Survey Complex metadata

Appendix D: Chapter 2 collinear variables

List of collinear variables that were removed prior to step-wise regression and ordination analysis. Collinear variables were identified using Variance Inflation Factor.

Collinear Variables	***R^2 value***	***Variable Removed***
	Spring	
Vegetation cover, bare-ground cover	-1	Bare-ground cover
Manitoba maple (Acer negundo) cover, Scilla (Scilla siberica) cover	1	Manitoba maple cover (*Acer negundo*)
Floral cover, floral patchiness	0.97	Floral patchiness
	Summer	
Vegetation cover, bare-ground cover	-1	Bare-ground cover
Cardamine (Silene noctiflora) cover, motherwort (Leonurus cardiaca) cover	1	Cardamine *(Silene noctiflora)* cover
Queen-Anne's lace (Daucus carota) cover, Butterflyweed (Asclepias tuberosa) cover	1	Queen-Anne's Lace *(Daucus carota)* cover
Snapdragon (Antirrhinum sp.) cover, catnip (Nepeta cataria) cover	1	Snapdragon *(Antirrhinum sp.)* cover
Motherwort (Leonurus cardiaca) cover, Wood avens (Geum urbanum) cover	0.97	Motherwort *Leonurus cardiaca)* cover
Viper's bugloss (Echium vulgare) cover, Bull thistle (Cirsium vulgare) cover	0.94	Viper's bugloss *(Echium vulgare)* cover
	Late-summer	
Vegetation cover, bare-ground cover	-1	Bare-ground cover
Purple-flowering raspberry (Rubus odoratus) cover, Enchanter's nightshade (Circaea lutetiana) cover	1	Purple-flowering raspberry *(Rubus odoratus)* cover
Pale touch-me-not (Impatiens pallida) cover, Tickseed cover	1	Pale touch-me-not *(Impatiens pallida)* cover
Dwarf Canadian primrose (Primula mistassinica var. intercedens) cover, Mistassini primrose (Primula mistassinica) cover	1	Dwarf Canadian primrose *(Primula mistassinica var. intercedens)* cover
Downy-skullcap (Scutellaria incana) cover, Boneset (Eupatorium perfoliatum) cover	0.98	Boneset *(Eupatorium perfoliatum)*
Large-leaved aster (Eurybia macrophylla) cover, Wormseed mustard (Erysimum cheiranthoides)	0.94	Large-leaved aster *(Eurybia macrophylla)*
	Landcover	
Shrub alvar cover, open alvar cover	1	Shrub alvar cover
Treed alvar cover, open alvar cover	1	Treed alvar cover
Tallgrass woodland cover, treed sand dune cover	1	Tallgrass woodland cover
Precipitation in the warmest quarter, precipitation in the driest quarter	1	Precipitation in the warmest quarter
Extraction pits and quarries cover with extraction aggregates cover	1	Extraction pits and quarries cover

Number of farms reporting fungicide, insecticide, herbicide	0.95-0.97	Number of farms reported fungicide, herbicide
Acres of farms applying fungicide, insecticide, herbicide	0.91	Acres of farms applying fungicide, herbicide
Pasture system cover, extraction aggregates cover	0.96	Extraction aggregate cover
Maximum temperature of the warmest month, Coniferous forest cover	-0.91	Maximum Temperature of the warmest month
Temperature seasonality, diurnal range	0.91	Temperature seasonality
Annual precipitation, Precipitation of the driest month	0.91	Annual precipitation

Appendix E: Chapter 2 final models based on AIC criteria

Variables selected for inclusion in the flowering species cover RDAs (spring, summer, and late-summer) and landcover RDA by stepwise logistic regression using AIC criteria using both directions. Step = the variable removed and the model AIC after removing the variable. Full model = all variables. Final model includes all variables selected from the stepwise regression. Descriptions of landscape variables are found in Appendix D.

Step	**AIC**
Spring – B. terricola	
Full model	251.93
- Spring beauty (*Claytonia caroliniana*)	249.93
- Bog yellowcress (*Rorippa palustris*)	247.93
- False violet (*Dalibarda repens*)	245.93
- Bittercress (*Cardamine sp.*)	243.93
- Forget me not (*Myosotis sylvatica*)	242.02
- Two leaved toothwort (*Cardamine diphylla*)	240.21
- Wild strawberry (*Fragaria virginiana*)	238.42
- Yellow trout lily (*Erythronium americanum*)	236.68
- Dandelion (*Taraxacum officinale*)	235.01
- Sharp lobed hepatica (*Anemone acutiloba*)	233.97
- Bloodroot (*Sanguinaria canadensis*)	232.92
- Bear berry (*Arctostaphylos uva-ursi*)	231.86
- Apple tree (*Malus sp.*)	230.79
- Spring cress (*Cardamine bulbosa*)	229.71
- Squirrel corn (*Dicentra canadensis*)	228.63
- Wild black currant (*Ribes americanum)*	227.54
- Gaywings (*Polygaloides paucifolia*)	226.44
- False Solomon seal (*Maianthemum stellatum*)	226.01
- Pin cherry (*Prunus pensylvanica*)	225.77
- Herb Robert geranium (*Geranium robertianum*)	225.49
- Mistassini primrose (*Primula mistassinica*)	225.18
- Early blue cohosh (*Caulophyllum giganteum*)	225.15
Final model: Creeping bluet *(Houstonia serpyllifolia*), scilla (*Scilla siberica*), marsh marigold (*Caltha palustris*), yellow rocketcress (*Barbarea vulgaris*), garlic mustard (*Alliaria petiolata*), trillium (*Trillium grandiflorum*), hairy rockcress (*Arabis hirsute*), sand violet (*Viola affinis*), downy yellow violet (*Viola pubescens*)	**225.15**
Spring – B. pensylvanicus	
Full model	183.77
- Bog yellowcress (*Rorippa palustris*)	181.77
- False violet (*Dalibarda repens*)	179.77
- Spring beauty (*Claytonia caroliniana*)	177.77
- Bittercress (*Cardamine sp.*)	175.77
- Sharp lobed hepatica (*Anemone acutiloba*)	173.82
- Two leaved toothwort (*Cardamine diphylla*)	171.97

- Bear berry (*Arctostaphylos uva-ursi*)	170.33
- Apple tree (*Malus sp.*)	168.69
- Bloodroot (*Sanguinaria canadensis*)	167.04
- Squirrel corn (*Dicentra canadensis*)	165.39
- Gaywings (*Polygaloides paucifolia*)	163.74
- Spring cress (*Cardamine bulbosa*)	162.08
- Downy yellow violet (*Viola pubescens*)	160.62
- Wild black currant (*Ribes americanum)*	159.21
- Mistassini primrose (*Primula mistassinica*)	157.86
- Forget me not (*Myosotis sylvatica*)	156.59
- Pin cherry (*Prunus pensylvanica*)	155.35
- False Solomon seal (*Maianthemum stellatum*)	154.23
- Early blue cohosh (*Caulophyllum giganteum*)	153.21
- Herb Robert geranium (*Geranium robertianum*)	152.61
- Trillium (*Trillium grandiflorum*)	151.99
- Yellow trout lily (*Erythronium americanum*)	151.33
Final model: Creeping bluet (*Houstonia serpyllifolia*), scilla (*Scilla siberica*), marsh marigold (*Caltha palustris*), yellow rocketcress (*Barbarea vulgaris*), garlic mustard (*Alliaria petiolata*), wild strawberry (*Fragaria virginiana*), hairy rockcress (*Arabis hirsute*), Dandelion (*Taraxacum officinale*), sand violet (*Viola affinis*)	**151.33**
Spring – Both	
Full model	187.14
- Two leaved toothwort (*Cardamine diphylla*)	185.14
- Bog yellowcress (*Rorippa palustris*)	183.14
- Bittercress (*Cardamine sp.*)	181.14
- False violet (*Dalibarda repens*)	179.14
- Spring beauty (*Claytonia caroliniana*)	177.14
- Herb Robert geranium (*Geranium robertianum*)	175.14
- Wild black currant (*Ribes americanum*)	173.14
- Garlic mustard (*Alliaria petiolata*)	171.59
- Pin cherry (*Prunus pensylvanica*)	170.03
- Scilla (*Scilla siberica*)	168.47
- Squirrel corn (*Dicentra canadensis*)	166.91
- Bloodroot (*Sanguinaria canadensis*)	165.33
- Apple tree (*Malus sp.*)	163.76
- Gaywings (*Polygaloides paucifolia*)	162.18
- Spring cress (*Cardamine bulbosa*)	160.60
- Yellow rocketcress (*Barbarea vulgaris*)	159.01
- Bear berry (*Arctostaphylos uva-ursi*)	157.42
- Early blue cohosh (*Caulophyllum giganteum*)	155.82
- False Solomon seal (*Maianthemum stellatum*)	154.22
- Hairy rockcress (*Arabis hirsuta*)	153.01
- Mistassini primrose (*Primula mistassinica*)	151.78
- Sharp lobed hepatica (*Anemone acutiloba*)	150.80

- Yellow trout lily (*Erythronium americanum*)	149.93
- Marsh marigold (*Caltha palustris)*	149.38
- Forget me not (*Myosotis sylvatica*)	148.96
Final model: Creeping bluet *(Houstonia serpyllifolia*), scilla (*Scilla siberica*), wild strawberry (*Fragaria virginiana*), dandelion (*Taraxacum officinale*), sand violet (*Viola affinis*), downy yellow violet (*Viola pubescens*)	**148.96**
Summer – B. terricola	
Full model	287.42
- Common knapweed (*Centaurea nigra)*	285.42
- Prairie fire (*Castilleja coccinea*)	283.42
- Virginia waterleaf (*Hydrophyllum virginianum*)	281.42
- Canada mayflower (*Maianthemum canadense)*	279.42
- Lyre leaved rockcress *(Arabidopsis lyrata)*	277.42
- Milkweed (*Asclepias syriaca*)	275.42
- Field pennycress (*Thlaspi arvense*)	273.42
- Wood lily (*Lilium philadelphicum*)	271.42
- Pink pyrola (*Pyrola asarifolia*)	269.42
- Field sow thistle (*Sonchus arvensis*)	267.42
- Marsh bedstraw (*Galium palustre*)	265.42
- Gaywings (*Polygaloides paucifolia*)	263.42
- Tall buttercup (*Ranunculus acris*)	261.42
- Canada avens (*Geum canadense*)	259.42
- Yellow ladyslipper (*Cypripedium calceolus var parviflorum*)	257.42
- Celandine (*Chelidonium majus*)	255.42
- Eastern field bedstraw (*Galium mollugo*)	253.43
- Hop clover (*Medicago lupulina*)	251.45
- Goat’s beard (*Tragopogon dubius*)	249.55
- Red osier dogwood (*Cornus stolonifera*)	247.64
- Crown vetch (*Securigera varia*)	245.78
- Butterfly weed (*Asclepias tuberosa*)	243.95
- Yellow bedstraw (*Galium verum*)	242.16
- Balsam ragwort (*Senecio pauperculus*)	240.41
- Hawkweed (*Hieracium umbrellata*)	238.75
- Venus looking glass (*Triodanis perfoliata*)	237.12
- New Jersey tea (*Ceanothus americanus*)	235.48
- Beach pea (*Lathyrus japonicus*)	233.89
- Virginia mountain mint (*Pycnanthemum virginianum*)	232.36
- Robin Plantain (*Erigeron pulchellus*)	231.06
- Dandelion (*Taraxacum officinale*)	229.81
- St John’s wort (*Hypericum perforatum*)	228.39
- Garlic mustard (*Alliaria petiolata*)	227.15
- Wild strawberry (*Fragaria virginiana*)	226.17
- Bull thistle (*Cirsium vulgare*)	225.17

- Downy wood mint (*Blephilia ciliata*)	224.39
- Catnip (*Nepeta cataria*)	223.71
- Mouse ear chickweed (*Cerastium fontanum*)	223.15
- Seneca snakeroot (*Polygala senega*)	222.63
- Forget me not (*Myosotis sylvatica*)	222.11
- Philadelphia fleabane (*Erigeron philadelphicus*)	221.62
- Wild sarsaparilla (*Aralia nudicaulis*)	221.47
- Enchanter's nightshade (*Circaea lutetiana*)	221.25
- Bunchberry (*Cornus canadensis*)	220.99
Final model: Herb Robert geranium (*Geranium robertianum*), prickly wild rose (*Rosa acicularis*), hispid buttercup (*Ranunculus hispidus*), blue-eyed grass (*Sisyrinchium montanum*), ox-eye daisy (*Leucanthemum vulgare*), field bindweed (*Convolvulus arvensis*), showy lady slipper (*Cypripedium reginae*), low hopclover (*Trifolium campestre*), bird's foot trefoil (*Lotus corniculatus*), cherry (*Prunus sp.*), thyme -leaved speedwell (*Veronica serpyllifolia*), cow vetch (*Vicia cracca*), bladder campion (*Silene vulgans*), red clover (*Trifolium pratense*), white clover (*Trifolium repens*), field chickweed (*Stellaria graminea*), rough-fruited cinquefoil (*Potentilla recta*), bush honeysuckle (*Diervilla lonicera*), petunia (*Petunia sp.)*, musk mallow (*Malva moschata*), wood avens (*Geum urbanum*), leafy spurge (*Euphorbia esula*), ribgrass (*Plantago lanceolata*), white sweet clover (*Melilotus albus*), wild bergamot (*Monarda fistulosa*), sumac (*Rhus sp.*), dame's rocket (*Hesperis matronalis*), hairy puccoon (*Lithospermum caroliniense*)	**220.99**
Summer – B. pensylvanicus	
Full model	228.23
- Red osier dogwood (*Cornus stolonifera*)	226.23
- Virginia mountain mint (*Pycnanthemum virginianum*)	224.23
- Butterflyweed (*Asclepias tuberosa*)	222.23
- Canada avens (*Geum canadense*)	220.23
- Venus looking glass (*Triodanis perfoliata*)	218.23
- Beach pea (*Lathyrus japonicus*)	216.23
- Virginia waterleaf (*Hydrophyllum virginianum*)	214.23
- Canada mayflower (*Maianthemum canadense)*	212.23
- Field sow thistle (*Sonchus arvensis*)	210.23
- Yellow ladyslipper (*Cypripedium calceolus var parviflorum*)	208.23
- Prairie fire (*Castilleja coccinea*)	206.23
- Gaywings (*Polygaloides paucifolia*)	204.23
- Marsh bedstraw (*Galium palustre*)	202.23
- Pink pyrola (*Pyrola asarifolia*)	200.23
- Field bindweed (*Convolvulus arvensis)*	198.23
- Dame's rocket (*Hesperis matronalis*)	196.23
- Yellow bedstraw (*Galium verum*)	194.23
- Goat's beard (*Tragopogon dubius*)	192.23
- Musk mallow *(Malva moschata)*	190.23
- Common knapweed (*Centaurea nigra)*	188.23
- Celandine (*Chelidonium majus*)	186.23

- Lyre leaved rockcress *(Arabidopsis lyrata)*	184.23
- Balsam ragwort (*Senecio pauperculus*)	182.23
- Wood avens (*Geum urbanum*)	180.23
- Field pennycress (*Thlaspi arvense*)	178.23
- Tall buttercup (*Ranunculus acris*)	176.23
- Low hopclover (*Trifolium campestre*)	174.23
- Field chickweed (*Stellaria graminea*)	172.23
- New Jersey tea (*Ceanothus americanus*)	170.23
- Downy wood mint (*Blephilia ciliata*)	168.23
- Milkweed (*Asclepias syriaca*)	166.23
- Rough-fruited cinquefoil (*Potentilla recta*)	164.23
- Forget me not (*Myosotis sylvatica*)	162.23
- Eastern field bedstraw (*Galium mollugo*)	160.23
- Petunia (*Petunia sp.)*	158.99
- Seneca snakeroot (*Polygala senega*)	157.73
- Mouse ear chickweed (*Cerastium fontanum*)	156.46
- Philadelphia fleabane (*Erigeron philadelphicus*)	155.17
- Crown vetch (*Securigera varia*)	153.88
- Bull thistle (*Cirsium vulgare*)	152.57
- White clover (*Trifolium repens*)	151.39
- Bush honeysuckle (*Diervilla lonicera*)	150.71
- Showy lady slipper (*Cypripedium reginae*)	149.98
- Enchanter's nightshade (*Circaea lutetiana*)	149.21
- Catnip (*Nepeta cataria*)	149.04
Final model: Garlic mustard (*Alliaria petiolata*), wild strawberry (*Fragaria virginiana*), dandelion (*Taracacum officinale*), herb Robert geranium (*Geranium robertianum*), prickly wild rose (*Rosa acicularis*), hispid buttercup (*Ranunculus hispidus*), blue-eyed grass (*Sisyrinchium montanum*), ox-eye daisy (*Leucanthemum vulgare*), hawkweed (*Hieracium umbrellata*), robin plantain (*Erigeron pulchellus*), bird foot trefoil (*Lotus corniculatus*), cherry (*Prunus sp.*), thyme -leaved speedwell (*Veronica serpyllifolia*), cow vetch (*Vicia cracca*), bladder campion (*Silene vulgans*), red clover (*Trifolium pratense*), hop clover (*Medicago lupulina*), wild sarsaparilla (*Aralia nudicaulis*), St. John's wort *(Hypericum perforatum),* leafy spurge (*Euphorbia esula*), ribgrass (*Plantago lanceolata*), white sweet clover (*Melilotus albus*), wild bergamot (*Monarda fistulosa*), sumac (*Rhus sp.*), wood lily (*Lilium philadelphicum*), hairy puccoon (*Lithospermum caroliniense*)	**149.04**
Summer - Both	
Full model	198.60
- Venus looking glass (*Triodanis perfoliata*)	196.60
- Virginia waterleaf (*Hydrophyllum virginianum*)	194.60
- Showy lady slipper (*Cypripedium reginae*)	192.60
- Pink pyrola (*Pyrola asarifolia*)	190.60
- Field sow thistle (*Sonchus arvensis*)	188.60
- Canada mayflower (*Maianthemum canadense)*	186.60
- Goat's beard (*Tragopogon dubius*)	184.60

- Wood avens (*Geum urbanum*)	182.60
- Wild strawberry (*Fragaria virginiana*)	180.60
- Milkweed (*Asclepias syriaca*)	178.60
- Butterflyweed (*Asclepias tuberosa*)	176.60
- Lyre leaved rockcress *(Arabidopsis lyrata)*	174.60
- Cherry (*Prunus sp.*)	172.60
- Wood lily (*Lilium philadelphicum*)	170.60
- Yellow bedstraw (*Galium verum*)	168.60
- Wild bergamot (*Monarda fistulosa*)	166.60
- Balsam ragwort (*Senecio pauperculus*)	164.60
- Common knapweed (*Centaurea nigra)*	162.60
- New Jersey tea (*Ceanothus americanus*)	160.60
- Celandine (*Chelidonium majus*)	158.60
- Yellow lady slipper (*Cypripedium calceolus var parviflorum*)	156.60
- Beach pea (*Lathyrus japonicus*)	154.60
- Thyme-leaved speedwell (*Veronica serpyllifolia*)	152.60
- Virginia mountain mint (*Pycnanthemum virginianum*)	150.60
- Marsh bedstraw (*Galium palustre*)	148.60
- Canada avens (*Geum canadense*)	146.60
- Tall buttercup (*Ranunculus acris*)	144.60
- Field pennycress (*Thlaspi arvense*)	142.60
- Bunchberry (*Cornus canadensis*)	140.60
- Ribgrass (*Plantago lanceolata*)	138.63
- Downy wood mint (*Blephilia ciliata*)	136.68
- Bladder campion (*Silene vulgans*)	134.73
- Field bindweed (*Convolvulus arvensis)*	132.84
- Seneca snakeroot (*Polygala senega*)	130.95
- Bull thistle (*Cirsium vulgare*)	129.06
- Sumac (*Rhus sp.*)	127.16
- Hairy puccoon (*Lithospermum caroliniense*)	125.27
- Gaywings (*Polygaloides paucifolia*)	123.37
- Wild sarsaparilla (*Aralia nudicaulis*)	121.47
- Blue-eyed grass (*Sisyrinchium montanum*)	119.64
- Enchanter’s nightshade (*Circaea lutetiana*)	117.83
- Garlic mustard (*Alliaria petiolata*)	116.03
- White sweet clover (*Melilotus albus*)	114.22
- Mouse ear chickweed (*Cerastium fontanum*)	112.45
- Bush honeysuckle (*Diervilla lonicera*)	110.71
- Herb Robert geranium (*Geranium robertianum*)	108.97
- Robin Plantain (*Erigeron pulchellus*)	107.24
- Philadelphia fleabane (*Erigeron philadelphicus*)	105.50
- Prairie fire (*Castilleja coccinea*)	103.87
- Red osier dogwood (*Cornus stolonifera*)	102.27

- Dame’s rocket (*Hesperis matronalis*)	100.72
- Red clover (*Trifolium pratense*)	99.29
- Forget me not (*Myosotis sylvatica*)	97.94
- Hispid buttercup (*Ranunculus hispidus)*	96.49
- Prickly wild rose *(Rosa acicularis)*	95.21
- Musk mallow (*Malva moschata*)	93.97
- Eastern field bedstraw *(Galium mollugo)*	93.09
- Crown vetch (*Securigera varia)*	92.41
- Hop clover (*Medicago lupulina*)	91.68
- White clover (*Trifolium repens*)	90.81
- Bird’s foot trefoil (*Lotus corniculatus)*	90.03
- St John’s wort (*Hypericum perforatum*)	89.30
- Hawkweed (*Hieracium umbrellata*)	89.11
Final model: Dandelion (*Taraxacum officinale*), ox-eye daisy (*Leucanthemum vulgare*), low hopclover (*Trifolium campestre*), cow vetch (*Vicia cracca*), field chickweed (*Stellaria graminea*), rough-fruited cinquefoil (*Potentilla recta*), catnip (*Nepeta cataria*), petunia (*Petunia sp.*), leafy spurge (*Euphorbia esula*)	**89.11**
Late-summer – B. terricola	
Full model	303.51
- Pitcher plant (*Sarracenia purpurea*)	303.51
- Bladder campion (*Silene vulgans*)	301.51
- Hoary vervain (*Verbena stricta*)	299.51
- Harebell (*Campanula rotundifolia)*	297.51
- Sticky false asphodel (*Triantha glutinosa*)	295.51
- Mouse ear chickweed (*Cerastium fontanum*)	293.51
- Dames’ rocket (*Hesperis matronalis*)	291.51
- Spotted Joe pyeweed (*Eupatorium maculatum*)	289.51
- Hairy bush clover (*Lespedeza hirta*)	287.51
- Butter and eggs (*Linaria vulgaris*)	285.51
- Small flowered gerardia (*Agalinis paupercula)*	283.51
- Enchanter’s nightshade (*Circaea lutetiana*)	281.51
- Wormseed mustard (*Erysimum cheiranthoides*)	279.51
- White camas (Zigadenus elegans)	277.51
- New England aster (*Symphyotrichum novae-angliae*)	275.51
- Mistassini primrose (*Primula mistassinica*)	273.51
- Red osier dogwood (*Cornus stolonifera*)	271.51
- Bur marigold (*Bidens laevis*)	269.51
- Cardamine (*Silene noctiflora*)	267.51
- Crown vetch (*Securigera varia*)	265.53
- Cow vetch (*Vicia cracca*)	263.55
- Ribgrass (*Plantago lanceolata*)	261.60
- St. John’s wort (*Hypericum perforatum*)	259.65
- Hispid buttercup (*Ranunculus hispidus*)	257.76
- Catnip (*Nepeta cataria*)	255.89

- Bird's foot trefoil (*Lotus corniculatus*)	254.01
- Wild bergamot (*Monarda fistulosa*)	252.49
- Dandelion (*Taraxacum officinale)*	251.22
- Common mullein (*Verbascum thapsus*)	249.97
- Eastern field bedstraw *(Galium mollugo)*	248.82
- Field bindweed (*Convolvulus arvensis)*	247.66
- Northern water horehound *(Lycopus uniflorus)*	246.49
- Low hopclover (*Trifolium campestre*)	245.31
- Tickseed (*Coreopsis lanceolata*)	244.11
- Ox-eye daisy (*Leucanthemum vulgare*)	243.14
- Lesser daisy fleabane (*Erigeron strigosus*)	242.18
- Bittersweet nightshade (*Solanum dulcamara*)	241.42
- Goldenrod sp. (*Soldago sp.*)	240.36
- Cowcockle (*Vaccaria hispanica)*	239.62
- Hog peanut (*Amphicarpaea bracteata*)	239.01
- Brown knapweed (*Centaurea jacea*)	238.56
- Queen Anne's lace (*Daucus carota*)	238.04
- Hawkweed (*Hieracium umbrellata*)	237.75
- Common yarrow (*Achillea millefolium*)	237.47
- Tall white rattlesnake root (*Prenanthes altissima*)	236.55
- Bull thistle (*Cirsium vulgare*)	236.35
Final model: Garlic mustard (*Alliaria petiolata*), herb Robert geranium (*Geranium robertianum*), red clover (*Trifolium pratense*), white clover (*Trifolium repens)*, hop clover (*Medicago lupulina*), viper's bugloss (*Echium vulgare*), leafy spurge (*Euphorbia esula*), white sweet clover *(Melilotus albus*), Virginia mountain mint (*Pycnanthemum virginianum*), goat's beard (*Tragopogon dubius*), milkweed (*Asclepias syriaca*), helleborine (*Epipactis helleborine*), downy skullcap (*Scutellaria incana*), chicory (*Cichorium intybus*), common evening primrose (*Oenothera biennis*), spotted knapweed (*Centaurea maculosa*), silverweed (*Potentilla anserina)*, yellow wood-sorrel (*Oxalis stricta*), pale St. John's wort (*Hypericum ellipticum*), common soapwort (*Saponaria officinalis*), tall white aster (*Symphyotrichum lanceolatum)*, wild teasel (*Dipsacus fullonum*), Canada thistle (*Cirsium arvense*), Canadian tick trefoil (*Desmodium canadense*), flat topped aster (*Aster umbellatus)*, orange jewelweed (*Impatiens capensis*), selfheal (*Prunella vulgaris*)	**236.35**
Late-summer – B. pensylvanicus	
Full model	265.19
- Pitcher plant (*Sarracenia purpurea*)	265.19
- Goat's beard (*Tragopogon dubius*)	263.19
- Common soapwort (*Saponaria officinalis*)	261.19
- Butter and eggs (*Linaria vulgaris*)	259.19
- Hairy bush clover (*Lespedeza hirta*)	257.19
- Ox-eye daisy (*Leucanthemum vulgare*)	255.19
- Common mullein (*Verbascum thapsus*)	253.19
- Mouse ear chickweed (*Cerastium fontanum*)	251.19
- Sticky false asphodel (*Triantha glutinosa*)	249.19

- Bladder campion (*Silene vulgans*)	247.19
- Wormseed mustard (*Erysimum cheiranthoides*)	245.19
- White camas (Zigadenus elegans)	243.19
- Spotted Joe pyeweed (*Eupatorium maculatum*)	241.19
- Harebell (*Campanula rotundifolia)*	239.19
- New England aster (*Symphyotrichum novae-angliae*)	237.19
- Bur marigold (*Bidens laevis*)	235.19
- Viper's bugloss (*Echium vulgare*)	233.19
- Enchanter's nightshade (*Circaea lutetiana*)	231.19
- Field bindweed (*Convolvulus arvensis)*	229.19
- Small flowered gerardia (*Agalinis paupercula)*	227.19
- Wild teasel (*Dipsacus fullonum*)	225.19
- Dandelion (*Taraxacum officinale)*	223.19
- Cardamine (*Silene noctiflora*)	221.19
- Dame's rocket (*Hesperis matronalis*)	219.19
- Wild bergamot (*Monarda fistulosa*)	217.19
- Mistassini primrose (*Primula mistassinica*)	215.19
- Hoary vervain (*Verbena stricta*)	213.19
- Eastern field bedstraw *(Galium mollugo)*	211.19
- Hog peanut (*Amphicarpaea bracteata*)	209.19
- Yellow wood-sorrel (*Oxalis stricta*)	207.19
- Red osier dogwood (*Cornus stolonifera*)	205.19
- Queen Anne's lace (*Daucus carota*)	203.20
- St John's wort (*Hypericum perforatum*)	201.30
- Lesser daisy fleabane (*Erigeron strigosus*)	199.44
- Cow vetch (*Vicia cracca*)	197.59
- Northern water horehound *(Lycopus uniflorus)*	196.26
- Low hopclover (*Trifolium campestre*)	194.92
- Tickseed (*Coreopsis lanceolata*)	193.57
- Leafy spurge (*Euphorbia esula*)	192.21
- Cowcockle (*Vaccaria hispanica)*	191.11
- Goldenrod sp. (*Soldago sp.*)	189.85
- Virginia mountain mint (*Pycnanthemum virginianum*)	188.52
- Brown knapweed (*Centaurea jacea*)	187.79
- Herb Robert geranium (*Geranium robertianum*)	187.12
- White clover (*Trifolium repens)*	186.43
- Downy skullcap (*Scutellaria incana*)	185.70
- Silverweed (*Potentilla anserina)*	184.95
- Hawkweed (*Hieracium umbrellata*)	184.17
- Milkweed (*Asclepias syriaca*)	183.36
- Hispid buttercup (*Ranunculus hispidus*)	182.53
- Hop clover (*Medicago lupulina*)	181.67
- Bittersweet nightshade (*Solanum dulcamara*)	180.80

- Common yarrow (*Achillea millefolium*)	180.44
Final model: Garlic mustard (*Alliaria petiolata*), bird's foot trefoil (*Lotus corniculatus*), red clover (*Trifolium pratense*), catnip (*Nepeta cataria*), bull thistle (*Cirsium vulgare*), ribgrass (*Plantago lanceolata*), white sweet clover *(Melilotus albus*), crown vetch (*Securigera varia*), helleborine (*Epipactis helleborine*), chicory (*Cichorium intybus*), common evening primrose (*Oenothera biennis*), spotted knapweed (*Centaurea maculosa*), pale St. John's wort (*Hypericum ellipticum)*, tall white aster (*Symphyotrichum lanceolatum)*, Canada thistle (*Cirsium arvense*), Canadian tick trefoil (*Desmodium canadense*), flat topped aster (*Aster umbellatus)*, tall white rattlesnake root (*Prenanthes altissima*), orange jewelweed (*Impatiens capensis*), selfheal (*Prunella vulgaris*)	**180.44**
Late-summer – Both	
Full model	215.4426
- Pitcher plant (*Sarracenia purpurea*)	215.4426
- Ribgrass (*Plantago lanceolata*)	213.4426
- Wild bergamot (*Monarda fistulosa*)	211.4426
- Northern water horehound *(Lycopus uniflorus)*	209.4426
- White camas (Zigadenus elegans)	207.4426
- Mouse ear chickweed (*Cerastium fontanum*)	205.4426
- Garlic mustard (*Alliaria petiolata*)	203.4426
- Hairy bush clover (*Lespedeza hirta*)	201.4426
- Silverweed (*Potentilla anserina)*	199.4426
- Enchanter's nightshade (*Circaea lutetiana*)	197.4426
- Tall white rattlesnake root (*Prenanthes altissima*)	195.4426
- Brown knapweed (*Centaurea jacea*)	193.4426
- Wormseed mustard (*Erysimum cheiranthoides*)	191.4426
- Eastern field bedstraw *(Galium mollugo)*	189.4426
- Red osier dogwood (*Cornus stolonifera*)	187.4426
- Dame's rocket (*Hesperis matronalis*)	185.4426
- Hoary vervain (*Verbena stricta*)	183.4426
- Mistassini primrose (*Primula mistassinica*)	181.4426
- New England aster (*Symphyotrichum novae-angliae*)	179.4426
- Bur marigold (*Bidens laevis*)	177.4426
- Wild teasel (*Dipsacus fullonum*)	175.4426
- Cardamine (*Silene noctiflora*)	173.4426
- Yellow wood-sorrel (*Oxalis stricta*)	171.4426
- Common soapwort (*Saponaria officinalis*)	169.4426
- Common mullein (*Verbascum thapsus*)	167.4426
- Sticky false asphodel (*Triantha glutinosa*)	165.4426
- Pale St. John's Wort (*Hypericum ellipticum)*	163.4426
- Bladder campion (*Silene vulgans*)	161.4426
- Common yarrow (*Achillea millefolium*)	159.561
- Tall white aster (*Symphyotrichum lanceolatum)*	157.6776
- Downy skullcap (*Scutellaria incana*)	155.7926

- Low hopclover (*Trifolium campestre*)	153.9059
- Tickseed (*Coreopsis lanceolata*)	152.0177
- Spotted Joe pyeweed (*Eupatorium maculatum*)	150.1279
- St. John’s wort (*Hypericum perforatum*)	148.2533
- Small flowered gerardia (*Agalinis paupercula)*	146.4334
- Virginia mountain mint (*Pycnanthemum virginianum*)	144.6137
- Bittersweet nightshade (*Solanum dulcamara*)	142.8093
- Common evening primrose (*Oenothera biennis*)	141.0189
- Butter and eggs (*Linaria vulgaris*)	139.2689
- Field bindweed (*Convolvulus arvensis)*	137.5079
- Chicory (*Cichorium intybus***)**	135.903
- Bird’s foot trefoil (*Lotus corniculatus*)	134.2724
- Goldenrod sp. (*Soldago sp.*)	132.6784
- Flat topped aster (*Aster umbellatus)*	131.0361
- Cowcockle (*Vaccaria hispanica)*	129.4322
- Hawkweed (*Hieracium umbrellata*)	127.8258
- Harebell (*Campanula rotundifolia)*	126.2353
- Cow vetch (*Vicia cracca*)	124.7104
- Orange jewelweed (*Impatiens capensis*)	123.2367
- Herb Robert geranium (*Geranium robertianum*)	121.8607
- Hispid buttercup (*Ranunculus hispidus*)	120.4789
- Ox-eye daisy (*Leucanthemum vulgare*)	119.453
- Dandelion (*Taraxacum officinale)*	118.6334
- Helleborine (*Epipactis helleborine*)	118.2693
- Spotted knapweed (*Centaurea maculosa*)	117.9973
- Selfheal (*Prunella vulgaris*)	117.8988
- Catnip (*Nepeta cataria*)	117.7847
Final model: Red clover (*Trifolium pratense*), white clover (*Trifolium repens)*, hop clover (*Medicago lupulina*), viper’s bugloss (*Echium vulgare*), bull thistle (*Cirsium vulgare*), leafy spurge (*Euphorbia esula*), white sweet clover *(Melilotus albus*), goat’s beard (*Tragopogon dubius*), milkweed (*Asclepias syriaca*), crown vetch (*Securigera varia*), Queen Anne’s lace (*Daucus carota*), lesser daisy fleabane (*Erigeron strigosus*), Canada thistle (*Cirsium arvense*), hog peanut (*Amphicarpaea bracteata*), Canadian tick trefoil (*Desmodium canadense*)	**117.7847**
Landcover – B. terricola	
Full model	50
- Water soil	50
- Sandy Loam	50
- Sand	50
- Organic	50
- Loamy Sand	50
- Loam	50
- Fine Sandy Loam	50
- Clay Loam	50

- Built Up	50
- PrecipWetQuart	50
- AnnualMeanTemp	50
- PrecipColdQuart	50
- MeanDiurnalRange	50
- MeanTempColdestQuart	50
- PrecupDriestQuart	50
- MinTempColdMonth	50
- altitude	50
- PrecipDriestMonth	50
- MeanTempWarmQuart	50
- PrecipitationSeasonality	50
- AgDiversity	50
- Woodland	50
- waterari	50
- Tobacco.system	50
- Swamp.marsh.or.bog	50
- Reforestation	50
- Recreation	50
- Orchard	50
- Nursery	50
- Mixed.system	50
- Idle.agricultural. land.over.10. years.	50
- Idle.agricultural. land.5.10. years.	50
- Hay.system	50
- Grazing.system	50
- Grain.system	50
- Corn.system	50
- Continuous.row.crop	50
- Built.up	50
- Berries	50
- SqrMtGV	50
- NmbFRGV	50
- AcrsfHr	50
- Forest	48
- Extractionaggregate	46
- OpenBeach.Bar	44
- OpenWater	42
- PlantationsTreeCultivated	40
- HedgeRows	38
- Marsh	36
- HabDiversity	34
- DeciduousForest	32

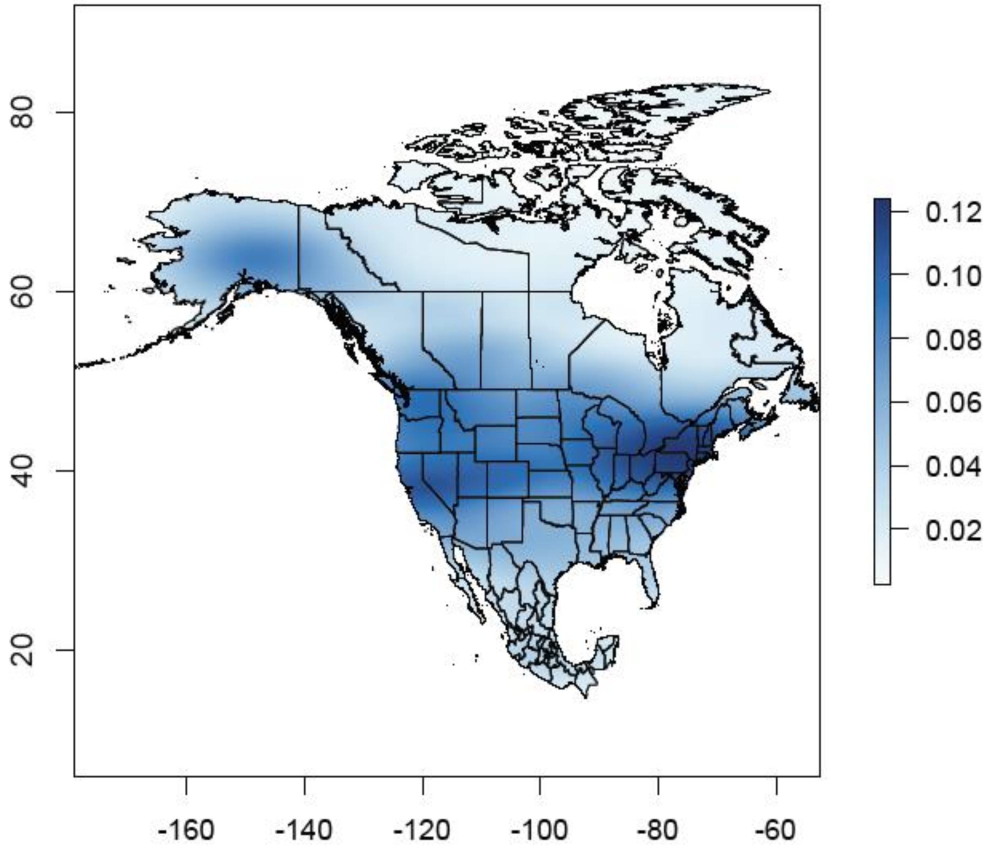
- NmFRHBC	30
- MixedForest	28
- OpenaAlvar	26
- NmFRGOB	24
- TreedSwamp	22
- ThicketSwamp	20
- Tilled	18
- NmbrFRI	16
- ConiferousForest	14
- Undifferentiated	12
- BuildUpAreaPervious	10
Final model: TreeSandDune, Transportation, Number of honey bee colonies, Acres of insecticide	**10**
Landscape – B. pensylvanicus	
Full model	50
- Water soil	50
- Sandy Loam	50
- Sand	50
- Organic	50
- Loamy Sand	50
- Loam	50
- Fine Sandy Loam	50
- Clay Loam	50
- Built Up	50
- PrecipWetQuart	50
- AnnualMeanTemp	50
- PrecipColdQuart	50
- MeanDiurnalRange	50
- MeanTempColdestQuart	50
- PrecupDriestQuart	50
- MinTempColdMonth	50
- altitude	50
- PrecipDriestMonth	50
- MeanTempWarmQuart	50
- PrecipitationSeasonality	50
- AgDiversity	50
- Woodland	50
- waterari	50
- Tobacco.system	50
- Swamp.marsh.or.bog	50
- Reforestation	50
- Recreation	50
- Orchard	50

- Nursery	50
- Mixed.system	50
- Idle.agricultural.land..over.10.years.	50
- Idle.agricultural.land..5.10.years.	50
- Hay.system	50
- Grazing.system	50
- Grain.system	50
- Corn.system	50
- Continuous.row.crop	50
- Built.up	50
- Berries	50
- SqrMtGV	50
- NmbFRGV	50
- AcrsfHr	50
- HabDiversity	48
- TreedSwamp	46
- HedgeRows	44
- Tilled	42
- PlantationsTreeCultivated	40
- OpenaAlvar	38
- TreeSandDune	36
- OpenBeach.Bar	34
- AcrsfIn	32
- Marsh	30
- NmFRHBC	28
- MixedForest	26
- Forest	24
- ThicketSwamp	22
- BuildUpAreaPervious	20
- Extractionaggregate	18
- Transportation	16
- NmFRGOB	14
- NmbrHBC	12
- OpenWater	10
- DeciduousForest	8
Final model: ConiferousForest, Undifferentiated, Number of farms reporting insecticide	**8**
Landscape - both	
Full model	50
- Watersoil	50
- SandyLoam	50
- Sand	50
- Organic	50

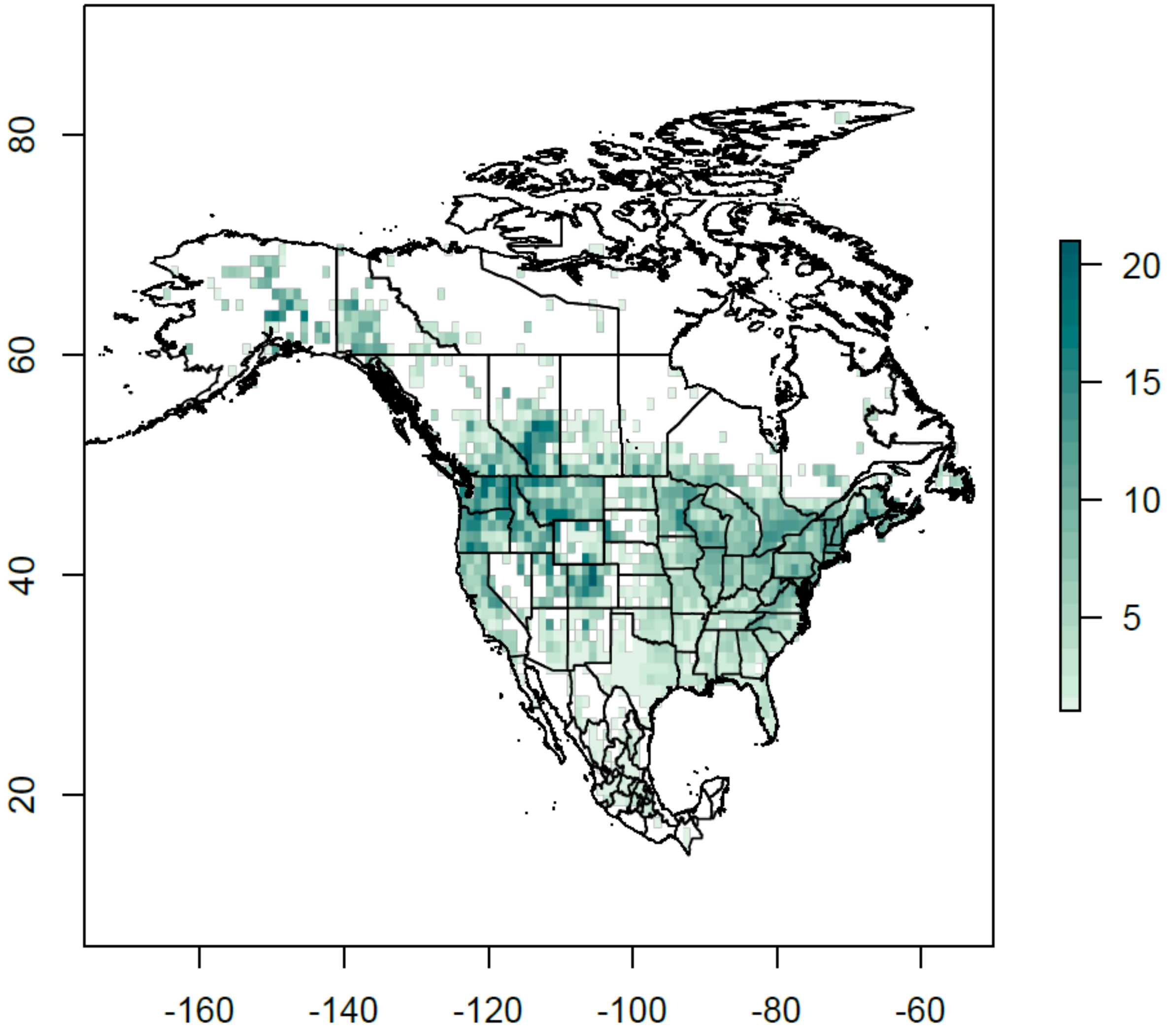
- LoamySand	50
- Loam	50
- FineSandyLoam	50
- ClayLoam	50
- BuiltUp	50
- PrecipWetQuart	50
- AnnualMeanTemp	50
- PrecipColdQuart	50
- MeanDiurnalRange	50
- MeanTempColdestQuart	50
- PrecupDriestQuart	50
- MinTempColdMonth	50
- altitude	50
- PrecipDriestMonth	50
- MeanTempWarmQuart	50
- PrecipitationSeasonality	50
- AgDiversity	50
- Woodland	50
- waterari	50
- Tobacco.system	50
- Swamp.marsh.or.bog	50
- Reforestation	50
- Recreation	50
- Orchard	50
- Nursery	50
- Mixed.system	50
- Idle.agricultural.land..over.10.years.	50
- Idle.agricultural.land..5.10.years.	50
- Hay.system	50
- Grazing.system	50
- Grain.system	50
- Corn.system	50
- Continuous.row.crop	50
- Built.up	50
- Berries	50
- SqrMtGV	50
- NmbFRGV	50
- AcrsfHr	50
- NmFRHBC	48
- ThicketSwamp	46
- OpenWater	44
- OpenaAlvar	42
- Undifferentiated	40

- Forest	38
- Marsh	36
- ConiferousForest	34
- DeciduousForest	32
- NmFRGOB	30
- TreeSandDune	28
- OpenBeach.Bar	26
- HabDiversity	24
- HedgeRows	22
- AcrsfIn	20
- NmbrFRI	18
- TreedSwamp	16
- Tilled	14
- PlantationsTreeCultivated	12
- Extraction-aggregate	10
- NmbrHBC	8
Final model: Mixed Forest, Transportation, Built-up area pervious	**8**

Appendix F: The density of occurrence records and number of bumble bee species in the bumble bee occurrence dataset used in chapter 3.



Appendix F1: Kernel-density of bumble bee occurrence records across North America from the Bumble Bees of North America database



Appendix F2: The number of bumble bee species from the Bumble Bees of North America Dataset (2008-2018) within 1° grid cells.

Appendix G: The number of occurrences and IUCN status for each bumble bee species included in chapter 3 species distribution models

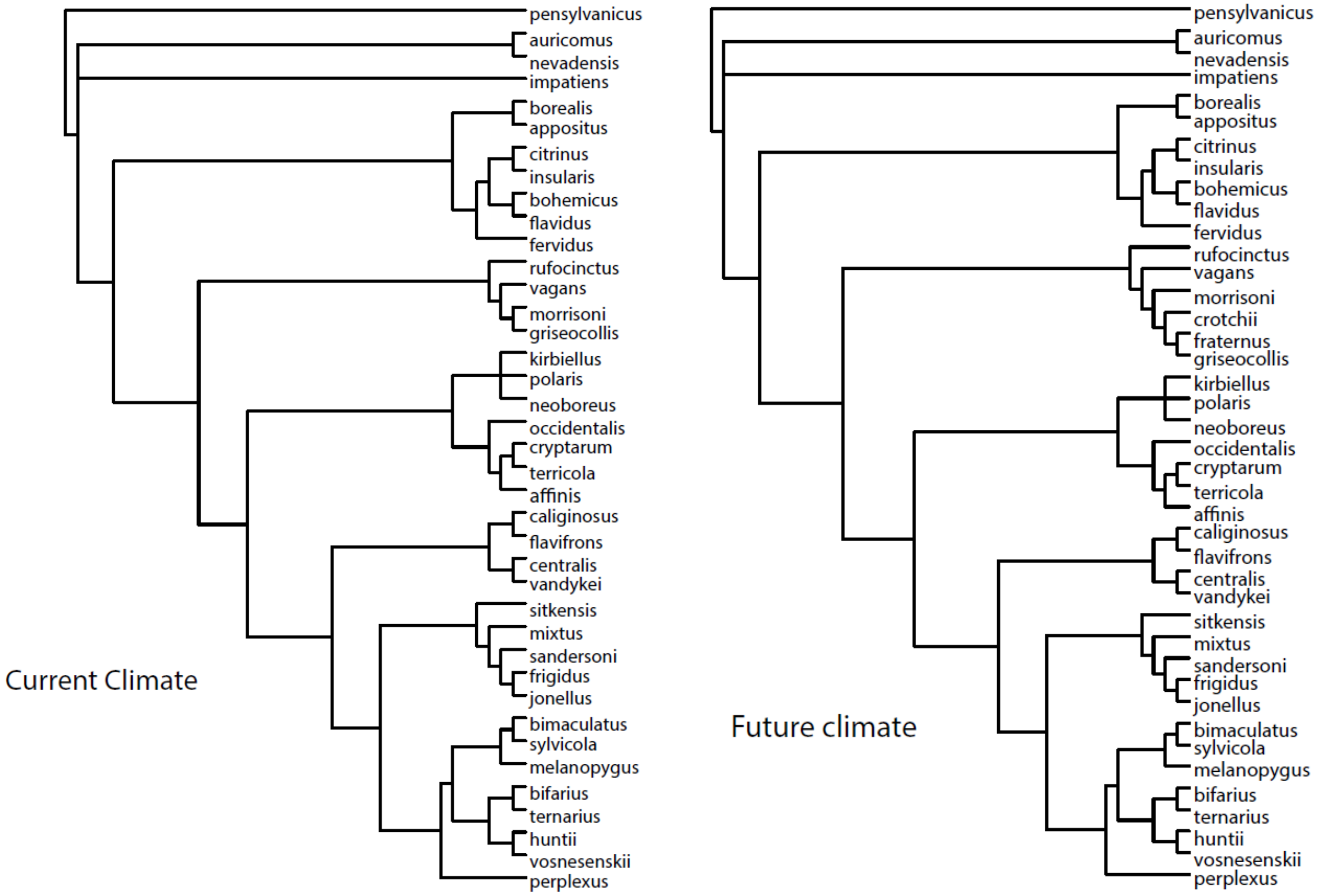
Bumble bee species, number of occurrences and IUCN Red List status for each bumble bee species included in the species distribution models.

***Bombus* Species**	**Number of occurrences**	**IUCN Status**
affinis	707	Critically Endangered
appositus	455	Least Concern
auricomus	1050	Least Concern
bifarius	1413	Least Concern
bimaculatus	4650	Least Concern
bohemicus	59	Critically Endangered
borealis	1432	Least Concern
caliginosus	80	Vulnerable
centralis	729	Least Concern
citrinus	701	Least Concern
crotchii	77	Endangered
cryptarum	328	Data Deficient
fervidus	2503	Vulnerable
flavidus	539	Data Deficient
flavifrons	1278	Least Concern
fraternus	266	Endangered
frigidus	490	Least Concern
griseocollis	6629	Least Concern
huntii	1590	Least Concern
impatiens	17083	Least Concern
insularis	512	Least Concern
jonellus	151	Data Deficient
kirbiellus	148	Data Deficient
*kluanensis**	10	Not Assessed
melanopygus	1739	Least Concern
mixtus	1325	Least Concern
morrisoni	165	Vulnerable
*natvigi**	18	Data Deficient
neoboreus	39	Data Deficient
nevadensis	807	Least Concern
occidentalis	1018	Vulnerable
pensylvanicus	5004	Vulnerable
perplexus	1741	Least Concern
polaris	93	Data Deficient
rufocinctus	1923	Least Concern

sandersoni	447	Least Concern
sitkensis	234	Least Concern
*suckleyi**	18	Critically Endangered
sylvicola	561	Least Concern
ternarius	4361	Least Concern
terricola	1495	Vulnerable
vagans	3722	Least Concern
vandykei	130	Least Concern
vosnesenskii	2444	Least Concern

* Species that modelled poorly with MaxEnt and were excluded from the prioritization analyses

Appendix H: Chapter 3 phylogenetic trees for current and future climate scenarios



Phylogenetic trees of the bumble bee species included in the conservation prioritization analysis for the current and future climates. The phylogenetic trees were produced from The Open Tree of Life API package "*rotl*". There future tree includes two additional species (*Bombus crotchii, and B. fraternus*) that are predicted to occur in Canada in the future, but do not currently occur.

Appendix I: Proportion of each bumble bee species' distribution within a conservation solution in chapter 3

Proportion of each bumble bee species' distribution retained in the conservation prioritization solution for each objective, climate scenario and target/budget. NA = the species was not predicted to be within Canada

Minimize cost objective	**Targets**								
	Current climate			**RCP 2.6**			**RCP 8.5**		
Bumble bee species	**17%**	**30%**	**50%**	**17%**	**30%**	**50%**	**17%**	**30%**	**50%**
B. affinis	0.538	0.829	0.936	0.287	0.450	0.681	0.329	0.490	0.787
B. appositus	0.180	0.300	0.500	0.218	0.344	0.541	0.194	0.362	0.615
B. auricomus	0.280	0.500	0.717	0.170	0.300	0.607	0.255	0.433	0.653
B. bifarius	0.193	0.322	0.529	0.178	0.300	0.500	0.194	0.352	0.607
B. bimaculatus	0.281	0.504	0.716	0.178	0.303	0.500	0.205	0.364	0.588
B. bohemicus	0.170	0.300	0.500	0.170	0.300	0.500	0.200	0.309	0.500
B. borealis	0.170	0.302	0.519	0.170	0.304	0.523	0.182	0.328	0.543
B. caliginosus	0.235	0.322	0.651	0.170	0.300	0.565	0.170	0.300	0.500
B. centralis	0.206	0.345	0.542	0.170	0.310	0.555	0.170	0.300	0.534
B. citrinus	0.322	0.530	0.741	0.266	0.459	0.692	0.239	0.429	0.656
B. crotchii	NA	NA	NA	NA	NA	NA	1.000	1.000	1.000
B. cryptarum	0.277	0.467	0.691	0.297	0.504	0.739	0.356	0.498	0.700
B. fervidus	0.264	0.435	0.663	0.246	0.419	0.661	0.253	0.425	0.655
B. flavidus	0.184	0.333	0.537	0.197	0.360	0.579	0.230	0.399	0.624
B. flavifrons	0.256	0.441	0.664	0.195	0.354	0.594	0.267	0.408	0.614
B. fraternus	NA	NA	NA	0.171	0.300	0.500	0.170	0.300	0.500
B. frigidus	0.170	0.300	0.500	0.170	0.315	0.500	0.215	0.322	0.500
B. griseocollis	0.220	0.441	0.665	0.170	0.300	0.504	0.170	0.300	0.500
B. huntii	0.170	0.300	0.500	0.170	0.300	0.500	0.170	0.300	0.500
B. impatiens	0.314	0.541	0.747	0.272	0.468	0.732	0.273	0.475	0.715
B. insularis	0.214	0.370	0.586	0.192	0.363	0.601	0.252	0.411	0.625
B. jonellus	0.315	0.489	0.716	0.354	0.557	0.760	0.338	0.470	0.637
B. kirbiellus	0.435	0.656	0.783	0.532	0.723	0.835	0.685	0.813	0.910
B. melanopygus	0.233	0.387	0.586	0.170	0.300	0.500	0.170	0.300	0.500
B. mixtus	0.258	0.440	0.642	0.185	0.338	0.558	0.279	0.425	0.627
B. morrisoni	0.170	0.300	0.500	0.409	0.596	0.811	0.304	0.496	0.750
B. neoboreus	0.394	0.612	0.814	0.469	0.698	0.882	0.568	0.721	0.883
B. nevadensis	0.170	0.300	0.538	0.170	0.300	0.539	0.170	0.300	0.514
B. occidentalis	0.170	0.300	0.511	0.170	0.305	0.541	0.174	0.311	0.548
B. pensylvanicus	0.170	0.300	0.501	0.199	0.344	0.564	0.170	0.300	0.500

B. perplexus	0.185	0.319	0.524	0.187	0.324	0.538	0.198	0.354	0.573
B. polaris	0.170	0.300	0.500	0.170	0.300	0.500	0.170	0.300	0.500
B. rufocinctus	0.184	0.316	0.526	0.170	0.300	0.502	0.170	0.300	0.500
B. sandersoni	0.170	0.300	0.500	0.183	0.327	0.546	0.216	0.391	0.642
B. sitkensis	0.170	0.300	0.500	0.170	0.300	0.500	0.170	0.300	0.500
B. sylvicola	0.243	0.424	0.646	0.220	0.370	0.531	0.170	0.300	0.500
B. ternarius	0.170	0.300	0.500	0.170	0.300	0.505	0.173	0.310	0.519
B. terricola	0.170	0.300	0.500	0.170	0.300	0.500	0.170	0.300	0.500
B. vagans	0.201	0.347	0.547	0.190	0.333	0.557	0.202	0.366	0.602
B. vandykei	0.241	0.310	0.680	0.262	0.322	0.559	0.236	0.390	0.602
B. vosnesenskii	0.308	0.399	0.843	0.216	0.333	0.573	0.170	0.300	0.500
Maximize phylogenetic diversity objective	**Targets**								
	Current climate			**RCP 2.6**			**RCP 8.5**		
	17%	**30%**	**50%**	**17%**	**30%**	**50%**	**17%**	**30%**	**50%**
B. affinis	0.293	0.723	0.929	0.307	0.386	0.514	0.341	0.413	0.500
B. appositus	0.175	0.438	0.869	0.212	0.518	0.930	0.296	0.612	0.949
B. auricomus	0.207	0.431	0.869	0.170	0.377	0.690	0.267	0.506	0.430
B. bifarius	0.207	0.393	0.737	0.181	0.495	0.789	0.302	0.511	0.821
B. bimaculatus	0.255	0.524	0.509	0.185	0.296	0.280	0.209	0.300	0.162
B. bohemicus	0.171	0.130	0.102	0.170	0.159	0.129	0.172	0.156	0.163
B. borealis	0.171	0.283	0.231	0.170	0.243	0.175	0.188	0.196	0.096
B. caliginosus	0.256	0.300	0.635	0.170	0.377	0.718	0.171	0.364	0.703
B. centralis	0.235	0.358	0.575	0.173	0.336	0.573	0.220	0.347	0.536
B. citrinus	0.279	0.551	0.500	0.267	0.446	0.386	0.240	0.349	0.186
B. crotchii	NA	NA	NA	NA	NA	NA	0.223	0.337	0.559
B. cryptarum	0.283	0.226	0.172	0.299	0.278	0.213	0.252	0.163	0.259
B. fervidus	0.247	0.468	0.604	0.251	0.419	0.500	0.263	0.378	0.297
B. flavidus	0.196	0.242	0.259	0.197	0.280	0.245	0.228	0.235	0.197
B. flavifrons	0.284	0.319	0.402	0.198	0.363	0.502	0.294	0.316	0.547
B. fraternus	NA	NA	NA	0.170	0.300	0.500	0.170	0.300	0.500
B. frigidus	0.170	0.141	0.130	0.171	0.155	0.149	0.170	0.105	0.167
B. griseocollis	0.206	0.481	0.573	0.183	0.300	0.363	0.173	0.271	0.171
B. huntii	0.174	0.300	0.583	0.170	0.300	0.511	0.179	0.300	0.500
B. impatiens	0.278	0.575	0.576	0.282	0.460	0.439	0.272	0.415	0.232
B. insularis	0.252	0.300	0.385	0.192	0.325	0.404	0.259	0.300	0.334
B. jonellus	0.260	0.216	0.089	0.349	0.292	0.192	0.236	0.150	0.256
B. kirbiellus	0.381	0.279	0.212	0.527	0.311	0.377	0.450	0.307	0.460
B. melanopygus	0.262	0.343	0.513	0.170	0.300	0.500	0.197	0.302	0.504
B. mixtus	0.293	0.360	0.479	0.187	0.313	0.500	0.304	0.307	0.489
B. morrisoni	0.176	0.399	0.995	0.387	0.577	1.000	0.325	0.416	0.965
B. neoboreus	0.367	0.224	0.118	0.467	0.300	0.233	0.363	0.300	0.466
B. nevadensis	0.196	0.300	0.488	0.170	0.300	0.500	0.201	0.300	0.421
B. occidentalis	0.195	0.300	0.500	0.170	0.373	0.571	0.241	0.313	0.559

B. pensylvanicus	0.170	0.300	0.574	0.198	0.312	0.615	0.192	0.300	0.429
B. perplexus	0.180	0.300	0.262	0.190	0.300	0.232	0.203	0.266	0.142
B. polaris	0.135	0.063	0.028	0.170	0.053	0.042	0.071	0.030	0.051
B. rufocinctus	0.187	0.317	0.359	0.170	0.278	0.265	0.178	0.217	0.152
B. sandersoni	0.170	0.238	0.173	0.183	0.273	0.147	0.217	0.252	0.102
B. sitkensis	0.170	0.300	0.500	0.171	0.300	0.501	0.184	0.300	0.500
B. sylvicola	0.215	0.194	0.186	0.219	0.172	0.191	0.130	0.074	0.126
B. ternarius	0.170	0.279	0.231	0.170	0.229	0.150	0.178	0.179	0.091
B. terricola	0.170	0.261	0.222	0.170	0.215	0.152	0.170	0.156	0.086
B. vagans	0.192	0.313	0.274	0.192	0.291	0.233	0.206	0.254	0.147
B. vandykei	0.267	0.432	0.770	0.274	0.706	0.925	0.235	0.638	0.897
B. vosnesenskii	0.335	0.393	0.870	0.212	0.538	0.865	0.176	0.459	0.792

Appendix J: Chapter 4 detection dog session parameters during on-site and *in-situ* training in Ontario, Canada (June 24 – July 5, 2019)

Detection dog session parameters during on-site and *in-situ* training in Ontario, Canada (June 24 – July 5, 2019)

Start times	6 – 7 am
Starting temperatures	11 – 17.2°C
End times	10 am – 1 pm
Ending temperatures	24 – 27.8°C

Appendix J summarizes work session parameters of the dog-handler teams, which depended on the prevailing weather conditions, to capitalize on cooler temperatures and reduce heat stress opportunities on the dogs. The session timeframes and temperatures also coincided with reduced bumble bee activity to minimize dogs coming into contact with bees and reducing potential harm to the bumble bees themselves.

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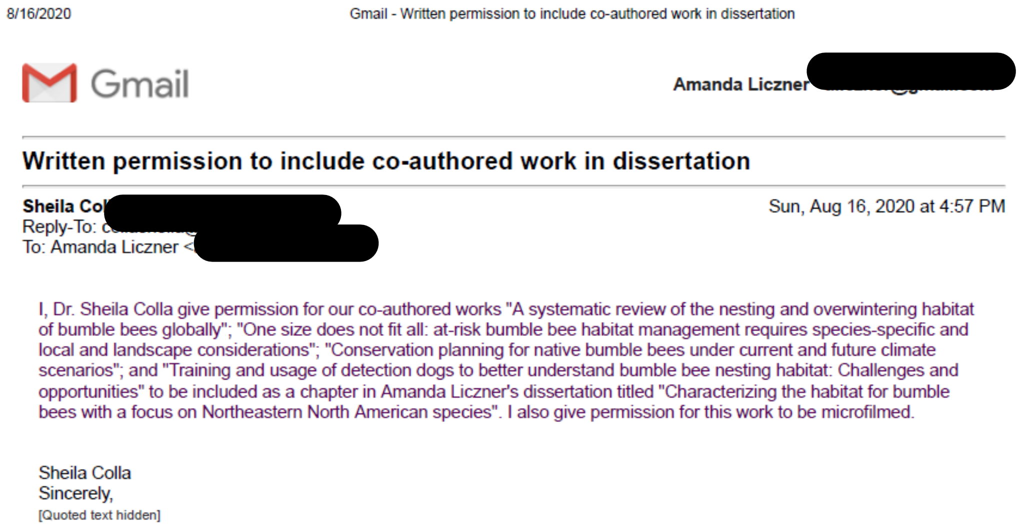
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Sheila Co[redacted] Sun, Aug 16, 2020 at 4:57 PM
Reply-To:
To: Amanda Liczner <[redacted]

I, Dr. Sheila Colla give permission for our co-authored works "A systematic review of the nesting and overwintering habitat of bumble bees globally"; "One size does not fit all: at-risk bumble bee habitat management requires species-specific and local and landscape considerations"; "Conservation planning for native bumble bees under current and future climate scenarios"; and "Training and usage of detection dogs to better understand bumble bee nesting habitat: Challenges and opportunities" to be included as a chapter in Amanda Liczner's dissertation titled "Characterizing the habitat for bumble bees with a focus on Northeastern North American species". I also give permission for this work to be microfilmed.

Sheila Colla
Sincerely,
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Amanda Liczner[redacted]

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Leif Richardson [redacted] Mon, Aug 17, 2020 at 9:17 AM
To: Amanda Liczne
Cc: Richard Schuster[redacted]

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Leif

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Leif Richardson
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--
Leif Richardson
[redacted]
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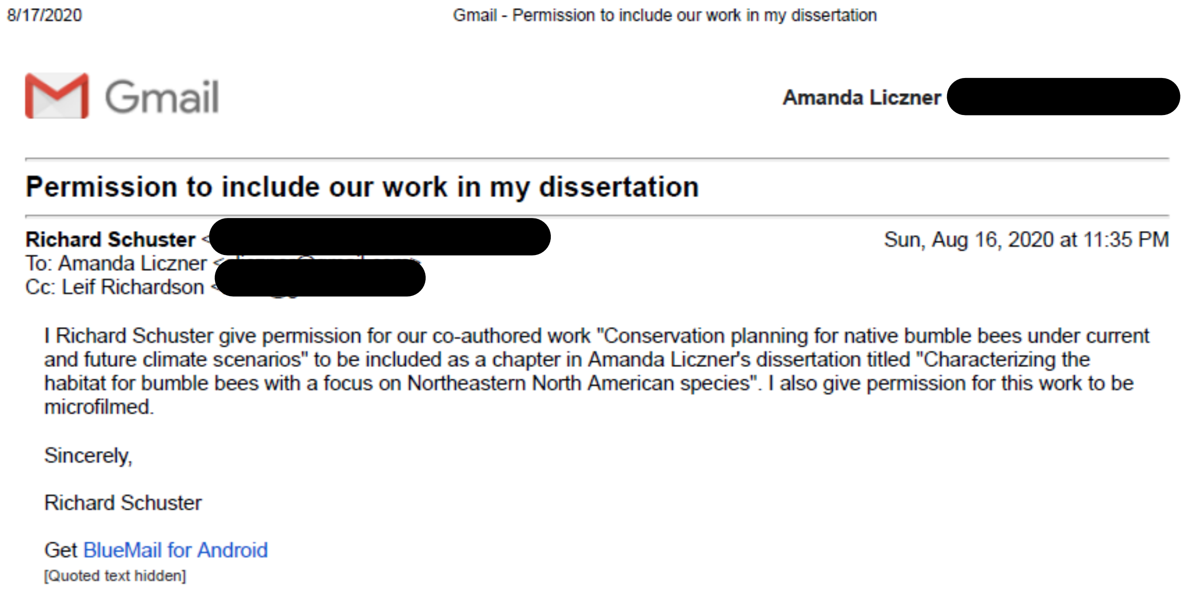

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Sincerely,

Richard Schuster

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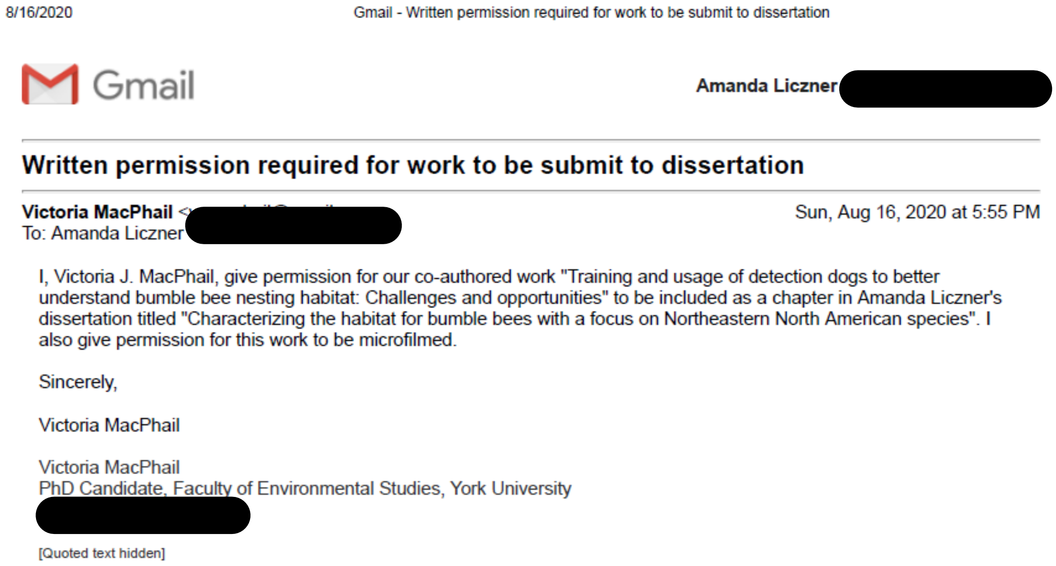

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Sincerely,

Victoria MacPhail

Victoria MacPhail
PhD Candidate, Faculty of Environmental Studies, York University
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NGAIO RICHARDS, Forensics & Field Specialist
mobile: [redacted]
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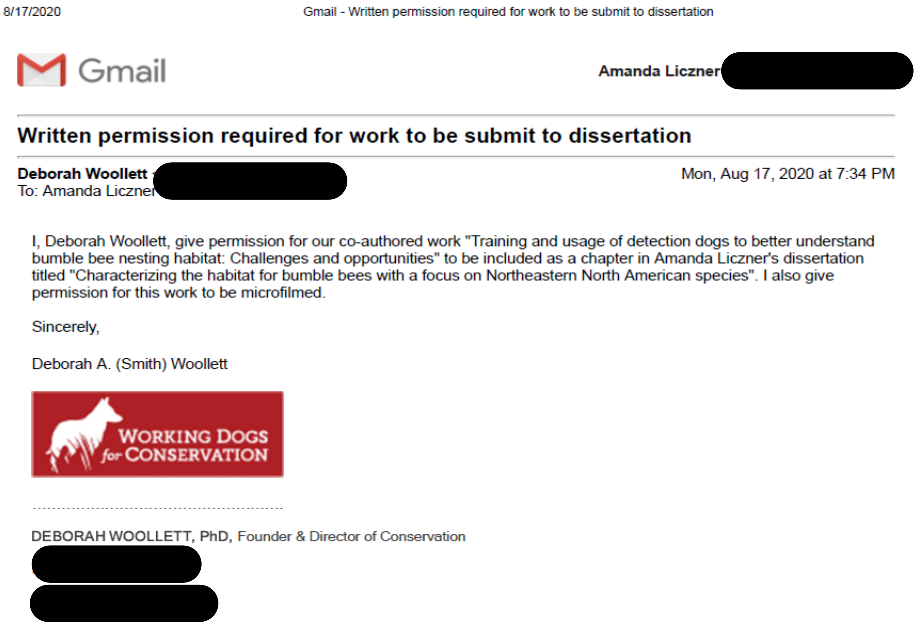

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Sincerely,

Deborah A. (Smith) Woollett

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DEBORAH WOOLLETT, PhD, Founder & Director of Conservation
[redacted]

Appendix N: Candidate's contribution to co-authored work

Statement of Amanda Liczner's contribution to co-authored works included in the dissertation titled "Characterizing the habitat for bumble bees with a focus on North American bumble bee species"

Chapter 1: A systematic review of the nesting and overwintering habitat of bumble bees globally

Amanda R. Liczner, Sheila R. Colla

Published in the Journal of Insect Conservation as: Liczner, A.R. and Colla, S.R., 2019. A systematic review of the nesting and overwintering habitat of bumble bees globally. Journal of Insect Conservation, pp.1-15.

Candidates contribution:
Amanda Liczner and Dr. Colla conceived of the idea for the literature review and developed the search terms to be used to find relevant published journal articles to incorporate in the review. Amanda Liczner conducted the literature review using Web of Science, processed all articles (i.e. read the article for relevance), extracted data related to bumble bee nesting and overwintering variables from the relevant articles to be included in the review, summarized and analyzed the data, and produced the manuscript figures. Amanda Liczner led the writing of the manuscript, and both Amanda and Dr. Colla contributing to editing the article. Both Amanda Liczner and Dr. Colla gave approval for publication and incorporation of this manuscript into the dissertation.

Chapter 2: One size does not fit all: at-risk bumble bee habitat management requires species-specific and local and landscape considerations

Amanda R. Liczner, Sheila R. Colla

This chapter has been accepted for publication in the journal Insect Conservation and Diversity as Liczner, A.R. and Colla, S.R., 2020. One-size does not fit all: at-risk bumble bee habitat management requires species-specific local and landscape considerations. Insect Conservation and Diversity.

Candidate contribution:

Amanda Liczner and Dr. Colla developed the ideas and methodology for this manuscript. Amanda Liczner selected the survey sites, devised the survey schedule, recruited research assistants, conducted the field work, input the data, managed the data including data cleaning, completed all analyses and produced the figures used in the manuscript. Amanda Liczner led the writing of the manuscript. Both Amanda Liczner and Dr. Colla edited the manuscript and gave approval for the manuscript to be published in the Journal of Insect Conservation and Diversity and for inclusion in the dissertation.

Chapter 3: Conservation planning for native bumble bees under current and future climate scenarios

Amanda R. Liczner, Richard Schuster, Leif L. Richardson, Sheila R. Colla[4]

This chapter will be submitted for publication to the Journal of Applied Ecology

Candidate contribution:

Amanda Liczner and Dr. Colla came up with the idea for the project. All authors contributed to designing the methodology, but it was led by Amanda Liczner. The bumble bee occurrence data used in the analyses of this project were provided by Dr. Richardson. Amanda Liczner obtained the climate, landcover and protected areas data. Amanda Liczner compiled all datasets and cleaned the data for analyses. Amanda Liczner led the analysis with Dr. Schuster who aided in debugging coding issues and helped develop functions to improve the efficiency of running models. Amanda Liczner produced all the figures used in the manuscript. Amanda Liczner led the writing of the manuscript. All authors contributed equally to editing the manuscript and gave permission for this manuscript to be submit to the Journal of Applied Ecology (at a future date) and for inclusion in the dissertation.

Chapter 4: Training and usage of detection dogs to better understand bumble bee nesting habitat: Challenges and opportunities

Amanda R. Liczner, Victoria J. MacPhail, Deborah A. (Smith) Woollett, Ngaio L. Richards, Sheila R. Colla

This chapter will be submitted to the journal PLOS ONE

Candidates contribution:
Amanda Liczner and Dr. Colla conceived of the ideas for this manuscript and secured the funding needed to complete the fieldwork. Amanda, Dr. Colla, Dr. (Smith) Woollett and Dr. Richards developed the methodology. All authors collected the data in the field. Amanda Liczner developed the social media campaign to locate bumble bee nests throughout southern Ontario. Victoria MacPhail facilitated data collection by the community science participants. Dr. (Smith) Woollett and Dr. Richards analyzed the data. Amanda Liczner led the writing of the manuscript. All authors contributed equally to editing the manuscript and gave permission for the article to be submit to PLOS ONE (at a future date) and for inclusion of the manuscript in the dissertation.

August 18, 2020

Amanda Liczner
PhD Candidate
Department of Biology,
York University

August 18, 2020

Dr. Sheila Colla
Assistant Professor
Faculty of Environment and Urban
Change, York University