A manual on bumble bee field methods: Finding and monitoring bumble bees across cryptic stages of their life cycle.

This manual was conceived to share and collate methods and ideas introduced during the session '*2023 Nesting (& Diapause) Biology Session at the Building Our Methods Using Sound Science (BOMBUSS 3.0)*' in Chiapas, Mexico (2023). We were inspired by the 'The Very Handy Manual: How to Catch and Identify Bees and Manage a Collection' and are hoping this manual can fill a similar niche within the world of bumble bee research. As such, this document is intended to serve as an open, continuous, and collaborative assortment of methods and ideas where researchers spanning disciplines and entities can contribute methods they are using to find and monitor bumble bees across the more cryptic stages of their life cycle e.g., nesting or overwintering.

While everyone who attended and shared their ideas at our session contributed to this document, the individuals below took on a more substantial role in developing some of these methods, organizing and editing this document, and ensuring it was disseminated to the broader research community. We also include specific emails in case you have a more pointed question that might be helpful about the methods included here. If you would like your email to be included, please reach out! We hope this document can be shared beyond those in attendance at BOMBUSS 3.0, 2023, so if you would like to be more involved, please reach out. In addition, if you contribute to this document further and would like to be acknowledged, please add your name to this list!

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Why do we need this manual?

Why do we need a manual just for the methods used to study bumble bees in the field? For any researcher who has spent hours, if not days, trying to find a single bumble bee nest in the field, the answer is obvious: bumble bees are a valuable yet incredibly challenging field system to work with. Their annual colony life cycle (**Figure 1**) means that most of their life cycle is hidden away from view, spent underground as mated gynes in the fall and winter months, or as ephemeral nest-searching spring queens disappearing into holes and crevasses in the ground. This annual life cycle also means that research on bumble bees is often limited to activity occurring during the social period of their life cycle e.g., foraging workers, in part, due to the difficulty in finding wild bumble bee nests. While studying foraging workers is valuable for understanding questions about behavior, there is limited knowledge to be gained regarding bumble bee colony processes, like growth and reproduction.

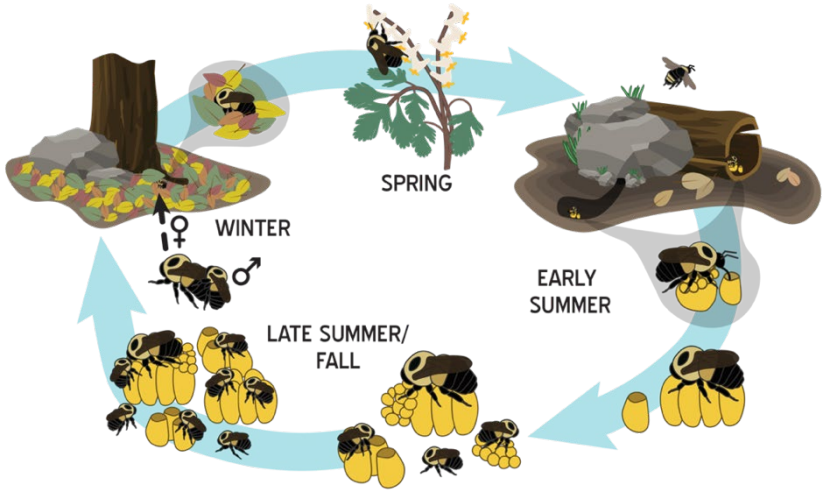

Figure 1. In seasonal environments, the bumble bee colony goes through solitary and social phases that are synchronized with the environmental conditions (**Graphic credit**: Dr. Jeremy Hemberger (https://jhemberger.github.io/graphic_art/).

This is problematic for several reasons, one being that colonies are a more representative unit of the population rather than the individual worker, making accurate estimates of wild populations challenging. Colony development and survival under natural conditions cannot be studied without adequate methods for finding sufficient nests across diverse landscapes. More broadly, understanding the habitat requirements of a species is a critical piece of information for federal protections, both during the listing and follow-up status reviews. As one's motivation for studying bumble bees in the field may differ, the methods described below are intended to be used by bumble bee researchers in any capacity.

While we don't go into extreme detail for the methods and rather outline each step, this is done intentionally for a few reasons. Most research systems, either the species present or landscape type, will differ at least in some way. Similarly, the timing of a particular species' activity and the

research question will determine how these methods are used. We also cite the paper where these methods were originally used. We encourage researchers who have experience with a highlighted method to reach out if they can contribute additional feedback, or if they have an additional method that should be included.

Studying springtime nest-searching/colony-founding queens

Understanding bumble bee nesting habitat preferences can begin by observing queen nest-searching behavior. While the presence of nest-searching queens doesn't necessarily guarantee nests will be found in a specific area, previous research has shown a weak correlation between nest-searching queen abundance and nest densities (O'Connor, Park, and Goulson 2017). However, recent research examining movement patterns of nest searching queens across habitat types shows the potential utility in measuring bumble bee queen nest-searching movement and behavior (Pugesek and Crone 2022). Below we highlight some of these methods in-detail, how they were used, and when they may be useful.

Tracking movement of nest searching queens

Tracking animal movement patterns to understand resource distribution and quality is a commonly employed method in wildlife biology, but one that has been less frequently employed in insect research. Movement ecology suggests that slower movement and an increased number of turns or 'steps' indicates a greater amount of time inspecting a habitat, and likely also increased resource quality and quantity. Here is a basic summary of the methods used in (Pugesek and Crone 2022):

Helpful background

The methods below were used to study *B. impatiens* queen movement during nest searching over a three-week span in 2018, which aligns with peak queen activity periods in the northeastern United States (April 26th-May 16th). Nest searching queens are easy to identify, displaying a unique, stereotypical, zig-zag movement that can be observed in this video **(Figure 3)**. In this study, these methods were used to collect flight path data on individual queens across landscape types, e.g., hayfield, forest, and meadows, and determine if there were differences in their flight paths as they moved through different habitat types. **Figure 2** demonstrates what a flight path and the associated 'steps' would look like. Surveys were conducted from 9:00-15:30, when the weather was clear or overcast but not rainy. It's typically best to avoid tracking bumble bee queens when the temperature is below 50F/10C, or on particularly windy days, as this may affect queen movement.

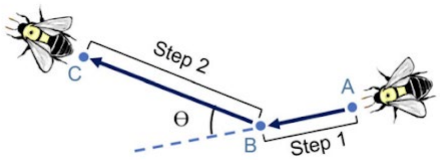

Figure 2. An example of a bumblebee flight path with each letter representing a location where the focal individual inspected a potential nesting site. Each letter represents a stopping point and is considered a "step". The turning angle between the two steps can then be calculated and represented by the letter theta (Θ) (Diagram from (Pugesek and Crone 2022):

Suggested supplies

- Numbered flags
- Timer or watch
- GPS device, e.g., Trimble Geo7x or phone app capable of GPS waypoints
- Non-toxic paint marking pen (e.g. Testors™)
- Queen marking vial, such as a honey bee queen marking vial
- Insect net
- Personal field equipment
    - Sunscreen, water, food, hydration tablets, Benadryl, etc. (this will vary by researcher and region)

Figure 3. A marked *B. impatiens* queen as she looks for an appropriate nesting site, displaying stereotypical zig-zig nest searching behavior (Photo Credit: Erin Treanore).

Step-by-step methods

1) Individual **nest-searching** queens were followed from a distance of >1 m and a numbered flag was dropped on the ground at locations where the queens landed on the ground to inspect an area as a potential nest site. Depending on the study system, it is possible that dropping the numbered flag at a time interval (ex. Every 10 seconds) might be better.

2) Time at which the queen began inspecting a site, duration, and termination were all recorded. Queens were no longer recorded when they stopped nest searching and flew away or the length of time inspecting a potential nest site exceeded five minutes. Most queens were followed for a period of 1.5 – 7 minutes.

3) Following a tracking event, gps location of all flags were noted, and two sequential locations were recorded as a single "step" (**Figure 2),** as such, a flight path with fewer than two steps cannot be recorded as there is insufficient information.

4) To avoid repeated sampling, queens were marked whenever possible with a colored and non-toxic paint marker (Sharpie paint, oil-based marker) after flight paths ended.

5) On a given day, between 1-13 individuals were monitored in total with 2-3 individuals typically monitored in a given landscape type before switching to a new landscape type.

Transect and site walks to gather counts of queens

Transect walks or "bee walks" have frequently been used to understand questions ranging from nesting habitat quality to phenology of different bumble species. While these transect walks can be used to study bumble bees at all life stages, for this section we will focus on them in the context of bumble bee queens. Depending on the question of interest, transects (or sites) ranging in size are repeatedly walked over the activity period to document when, where, and how many bumble bee queens are present. Additional information is often gathered on queen behavior, e.g., nest-searching versus foraging, host plant (if foraging), or nectar versus pollen foraging (as pollen foraging is often used as an indicator of colony establishment (Alford 1978; Tripodi and Strange 2019).

Numerous studies on bumble bees have relied on this survey type (Lye et al. 2009; Lanterman et al. 2019; Svensson, Lagerlöf, and Svensson 2000; O'Connor, Park, and Goulson 2017), and they require relatively few supplies to be conducted effectively. Rather, the main limitation here is finding the best locations to establish the transects, and the time and personnel it takes to survey them on a regular basis. Because these methods are more established, below we highlight general methods that have been used in several studies.

Helpful background

Like collecting flight path data, transect and site walks are best conducted on days when the ambient air temperature is at least 10°C/50°F, with little wind and no precipitation. The timeframe

when queens are most active will vary by region, but transect walks are the most helpful when they capture a majority of queens' activity period. In the northeastern United States, for example, this can begin in mid-April and last until mid-June, but the exact timeframe should be based on region, species(s) of interest, and weather patterns each year.

Transect length can vary widely depending on the system, region, and question. The *BeeWalk* protocol, which is a well-established community science program in Great Britain run by the Bumblebee Trust, has volunteers walk a transect length of approximately 1 mile and report the number of bumble bees seen over the course of their walk. Other research groups have tried much shorter transects and a greater number of replicates, for example, Lye et al. 2009 reported using 6 x 100 meter transects in their system, which other researchers reporting similar approaches with mixed success[2]. These are just a few examples, and the below methods are meant to provide a general summary of these methods with modifications made for specific study systems.

Suggested supplies

- 100-meter tape and flags (if measuring and marking transects)
- Insect net
- Clear plastic vials
- Camera
- Non-toxic paint or powder- if marking queens, e.g., Testors non-toxic paint pens
- Datasheets/Clipboards
- Kestrel Weather Meter or access to date from a nearby well-monitored weather station
- Personal field equipment
    - Sunscreen, water, food, hydration tablets, benadryl, etc. (this will vary by researcher and region)
- GPS Unit or GPS Mobile application (e.g. Gaia or Avenza) for marking walking routes that don't follow a straight line

Step-by-step (general) methods

[2] *Elizabeth Crone*-We have done queen transects in four systems: Napa Valley, CA, Ipswich MA, Bodega Marine Reserve, CA and Sagehen Creek Reserve, CA. In Napa, queens were too rare to detect in meaningful numbers in the transects we walked, at least at the length and survey frequency we tried (5, 100 m transects, walked 1-2x/week). In all the other study systems transects were longer and less formally laid out, following 1-2 km walking routes rather than short transects. These led to meaningful data for the most common species. "We" = E. Crone, G. Pugesek, N. Rosenburger & N. Williams in Napa, E. Crone & E. Treanore in Ipswich, and S. Finn's unpublished dissertation research for the California sites

1. At the beginning of spring queen activity period, seek out days that are dry, calm, and with temperatures of at least 10C/50F. Surveys should occur during a predetermined time interval e.g., 8:00-17:00.

    a. It can be helpful to record the temperature, cloud conditions (sunny, partly sunny, or cloudy), average ground wind speed, and length of survey for each transect walked.

2. Begin walking predetermined transect route at a slow steady pace, for example, ~3 km/h, and looking for queens flying within your "recording box".

    a. Rather than scanning the entire landscape, determine how far to each side and in front of you that you'll be looking for queens; this is your "recording box". For context, the BeeWalk protocol suggests 4m on either side of the transect and 2m in front of you.

3. Upon observing a queen, note her behavior, e.g., flying, foraging for pollen or nectar, or nest searching.

    a. Several studies have helpful information on what each of these categories look like, or how they've classified the queens' behavior. For example, (Lye et al. 2009) states the following in their methods:

    "*Flying queens made fast and mostly linear flights, usually 0.2 m above the ground. Foraging queens were those observed visiting flowers, and the species of plant was recorded. Nest seeking queens were recognized by their behavior; they mostly flew slowly and low to the ground, frequently changing direction and stopping to investigate crevices and cavities. For foraging and nest seeking queens, it was noted whether or not each queen was carrying pollen in her corbiculae, an indicator that she had already founded a nest.*"

4. For each queen, netting and getting a clear photograph of her in a clear plastic vial can be helpful in identifying her to species (but not always!). Researchers interested in doing mark-recapture studies can use this opportunity to mark her with either a unique identifier or a distinct color.
    a. Whether you choose to anesthetize the queen prior to marking her, e.g., with cold or CO_2, is often guided by marking protocols. However, queens narcotized with CO_2 can experience a suite of short-term and long-term behavioral and physiological shifts ((Amsalem and Grozinger 2017), so keep this in mind if you use this method!

5. Depending on the research question, additional information on habitat type, time of day, species of foraging, etc. can also be helpful to collect for each observed queen.

Radio Telemetry (Nest Searching spring queens)

General helpful background

Liczner at al. have radio-tagged spring bumble bee queens as a part of four studies. In the first (spring 2022), *Bombus impatiens* spring queens were radio tagged as a way to locate nest sites. We mainly tried to locate queens with pollen as that indicates nest initiation, however, as they are less common, we also tagged queens that were observed performing nest-searching flights (flying low to the ground in a zig-zag pattern, landing frequently), and queens that were foraging for nectar. We then wanted to compare how queens move between agricultural and natural areas (spring 2023). Here, we tagged *B. impatiens* queens at two sites while foraging or nest-searching to compare their movement behaviour. In spring 2024, we tagged queens as they woke up from hibernation (the hibernation spot was detected the previous fall) to track their dispersal. Queens were hibernating in close proximity to each other. When first emerging from hibernation, they are quite groggy, clumsy fliers and do not fly very far, stopping often for long rest periods. After about 3 days of dispersing no further than 30 m from the hibernation site, queens then begin further flights. In spring 2024, we also tagged queens with pollen to track their location to a nest. We noticed that *B. impatiens* queens appear to have a small window where we could find many queens collecting pollen, after which point, they become less numerous and appear to only collect nectar. We tracked queens to a possible nest site but we believe the nest failed as summer surveys did not find evidence of a nest.

Other spring projects included tagging commercial queens after being exposed to different systemic pesticides to observe their impacts on queen movement. For all Liczner at al. projects, queen movement was tracked using an array of autonomous radio towers and handheld yagi antennas. Much of the effort for these projects was to study the movement of spring queens, and unfortunately we were unsuccessful in locating any nests.

Suggested supplies

- Cooler filled with crushed ice (ice packs *can* work but take much longer to chill queens)
- Falcon tubes
- Net
- Super glue
- Curved tweezers
- Radio tags and related equipment (may vary by supplier, i.e., device to turn tags on)
- Receiver

Step-by-step (general) methods

1) For locating queens from the hibernation site (at least for *Bombus impatiens*): these queens do not move far for about 3 days. If you are interested in tagging queens from hibernation, but do not know where hibernation sites are, if you locate queens acting very sleepy in early spring, you are more than likely within 30 m from a hibernation site. If that level of error is okay for your study objectives, you might be okay with using that location

an an approximate hibernation spot
For locating queens with pollen (at least for *Bombus impatiens*): There is a small window where queens appear to collect pollen, after which it becomes much less feasible (in Eastern North America, if the first round of dandelion bloom has ended you are probably too late). We found most dandelions and autumn olives in our study sites.

2) Once queens are located, place them in a falcon tube (or similar) and place in a cooler with crushed ice to chill. Depending on the weather conditions this can take anywhere from 5 - 30 minutes.

3) Turn on the radio tag. Note: tags can be ordered with shorter antennas. Lengths are recommended to be about 5 cm. A smaller tag antenna will reduce detection distances, however, longer antennas get tangled in vegetation impacting queen flight.

4) Once queens are sufficiently chilled (no longer moving, in a semi-curled up position), remove the queen from the tube and place it on a surface for tagging. Use the curved tweezers to separate the wings and hold the wing out of the way for tagging. The handle of the tweezers should be towards the head of the queen, and the pointy side of the tweezers should be into the tagging surface.
**other groups have shaved queens to glue the tag right to the exoskeleton. I find this takes up time and doesn't improve adhesion. The hairs when mixed with the glue become hard and might help the tag stay on in some cases but this may vary with species/regions with varying temperatures and humidity.*

5) Place a small dot of glue on the tag and drag the dot lengthwise down the tag. It is important to put the right amount of glue to ensure the tag sticks - not too much that it won't dry and not too little that there isn't enough. Quickly place the tag in the center of the abdomen on the back half of T1. Gently press the tag into the abdomen to get good adhesion, taking care not to let the tag stick to your fingers - using the cap of the glue instead of your fingers here is a good trick. After a few seconds of pressing, release the tag and let it dry without touching it - playing with the tag too much increases the drying time

6) Once the glue seems tacky (mostly dry) carefully remove the tweezers without bumping the radio tag. The queen should still be asleep at this point. You may want to place "bumpers" to keep her upright while drying. The lids of flacon tubes or the glue tubes can be used to prop up the queen by placing them on either side of her. When the queens wake they tend to roll over, potentially disrupting the tag

7) As the queen wakes, keep an eye on the tag to ensure it remains attached. If the glue hasn't had enough time to dry, it may fall off, or the queen can rip it off herself. Eventually she will decide to fly off, when she does this seems to be different by individual. Some will clumsily take off immediately, not quite warm enough to fly properly yet, while others will crawl all over your workstation for a while looking for a great place to nap. It can take anywhere from 1 min - > 30 min for her to fly away. They will often groom and attempt to remove the tag - they can be distracted from doing this (sometimes) by offering flowers or placing them on a flower.

8) To track the queens, it is helpful to have at least two observers. One can hold a receiver while another helps keep track. They like to all of a sudden take off in a direction such as straight up and zoom out of sight, so two pairs of eyes are better than one. Track the

queen for as long as possible before leaving the field site as they are very difficult to locate between days. Spring queens are less likely to visit the same floral areas repeatedly especially in early spring for more than a couple days (at least at our study site in and for *Bombus impatiens*).

Outstanding questions and novel ideas

Outstanding questions-

- Are field domiciles a useful way to identify queen abundance and nesting preferences?- *Nelson Pomeroy*
- How can we account for differences in our ability to detect and follow queens in different habitats?
- Do parasites e.g. Sphaerularia., nematodes modify queen nest searching behavior?

Novel ideas-

Finding established nests

Finding bumble bee nests has been one of the major challenges of field work, given the inconspicuous nature of their nests and the relatively low-level of activity at the entrance (Goulson 2010). Some species, such as *B. impatiens*, predominantly nest belowground in old rodent burrows or crevasses, while other species, such as *B. griseocollis*, nest aboveground in grassy tufts and debris (Pugesek and Crone 2021; Liczner and Colla 2019). Still, for many species we have limited data on their nesting habitat requirements, which stems, in part, from the difficulty in finding nests. While numerous studies and articles contain anecdotal information on bumble bee nesting habitat and requirements, fewer studies include methods using standardized and systematic approaches for finding nests. Collating the methods from these studies highlights similarities in the approaches that rely on personnel-based efforts, while canine-based approaches employ a slightly different set of techniques. A summary of the methods used can be found below.

Standardized nest searching with personnel

Finding nests requires personnel that are attuned to the environment around them, noting bumble bees flying vertically rather than the stereotypical foraging movement between flowers, or noting a sequence of individuals leaving and entering the same area. A gentle but persistent humming or buzzing can sometimes be heard on hot summer days for some aboveground species as they

thermoregulate their nests, or a more pronounced buzzing if a nest is stepped on during a transect walk. Noticing these cues relies on careful and systematic surveying of a given space, especially during the period when nests are at their peak.

One of the earlier studies (Harder 1986) that examined bumble bee nesting relied on weekly transect walks from May-September throughout a 3.4 ha field, which ultimately yielded a total of 35 nests across 7 species. Other approaches have relied on volunteers trained on the same protocol who visually surveyed a set area e.g., a garden between 20-36 square meters, for a 20-minute period; an approach that also yielded a substantial number of nests (Osborne et al. 2007). methods described below utilize a similar approach as (Harder 1986), surveying the same piece of land on a weekly basis but limiting the survey period to the peak activity of the period, as was done by (Osborne et al. 2007).

Helpful background information

Searching for nests is best done during the peak activity period of a species, or when a colony is expected to have a significant foraging presence. The methods below were developed in Massachusetts, so the surveying tended to occur in July and August, the peak activity for most Massachusetts bumble bees. Surveying occurred 1x per week at each of the sites (30 in total) for a 5-week period, a period that captured when most colonies would be at their most active. In the weeks prior to the surveys, the landscape of interest was surveyed to identify 30 appropriate plot locations (1500^2 m, 30 x 50 m) that were evenly divided across three habitat types. It's important to keep in mind how this land is managed, e.g., *will it be mowed over the survey period*, when deciding if an area is appropriate for a plot. We've had great success with these methods working in flat and more-open landscapes, but they may be less fruitful in mountainous regions, dense forests, or scrubby and windy coastal habitat, where flight behavior in and out of the nest is harder to distinguish from foraging behavior. To read a more in-depth description of the methods, see (Pugesek 2021).

For the surveys themselves, like collecting flight path data, nest density surveys are best conducted on days when the ambient air temperature is at least 10 C/50 F, with little wind and no precipitation. Surveys were completed with 1-person per plot rather than splitting the workload between several people and this is what we encourage-multiple plots can be surveyed simultaneously though. Each plot was surveyed using a slow and deliberate walking pattern (**Figure 4**) designed to fully cover the plot multiple times per survey period.

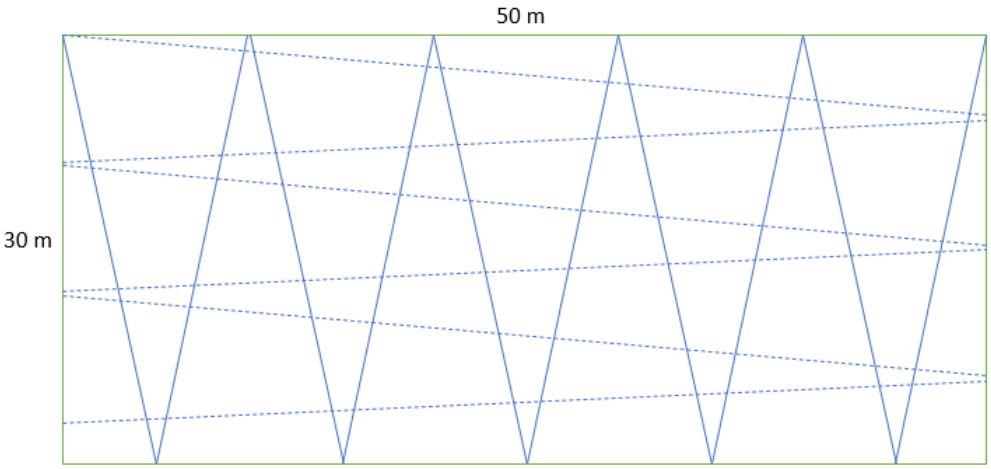

Figure 4. Example of a complete pass of the plot, within the hour survey period, one normally completes 2-4 complete passes of the whole plot.

Suggested supplies

- 100-meter tape (*setup only*)
- Flagging tape and colored flags (~2 feet tall)
    - Fewer flags needed for plot surveys themselves
- Plastic bobby pins
- Numbered round vegetation tags
- Insect net
- Clear plastic vials
- Camera or phone for pictures
- GPS device, e.g., Trimble Geo7x or phone app capable of GPS waypoints
- Timekeeping device
- Clipboard, writing material, datasheets
- Personal field equipment
    - Sunscreen, water, food, hydration tablets, benadryl, etc. (this will vary by researcher and region)

Step-by-step (general) methods

Plot setup:

1. Identify the area of interest you are interested in surveying-make sure you have permission from the landowner or land manager if not your own and be sure to highlight the length of time the flags/flagging tape will be up.

a. If working on agricultural land that is currently in production, it's helpful to have frequent communication with the land manager and make sure they're aware of seemingly minute details. For example, we only use plastic bobby pins in hay fields managed for cattle grazing, as a metal bobby pin could be fatal if ingested.
b. This goes both ways, and make sure you're informed of any practices they're using that could affect you, *e.g.,* pesticide use.

2. Given the question of interest, select a plot or transect that will capture a diversity of habitat features- our plots were 1500 meters^2, non-uniform in shape and haphazardly selected within a specific landscape type.

3. Mark the corners of the plots or transects with a brightly colored flag-we used flagging tape was used to mark trees in the forest as boundaries, whereas flags were used in meadows. When the area was managed for agricultural use, flags were setup and taken down on a per-survey basis using saved GPS points.
    a. Be sure to save the GPS coordinates for each corner as flags can disappear. It's much easier to place a flag back in the same location than starting from scratch.

Plot surveying:

1. Prior to plot surveys, record data on the time start, weather conditions, and anything noteworthy about the land management, e.g., recently mowed.

2. Set a timer for 60 minutes and begin walking slowly around the plot, employing a meandering pattern that zigzags tightly across the plot, as seen in the diagram above.
    a. It's helpful to carry 1-2 flags with you at a time and keep a few others in a corner of the plot (in addition to water).

3. Stop frequently to observe the surrounding area, noting any bumble bee workers that are flying vertically or that appear to be heading towards a hole in the ground.

4. If you find a nest, or believe you may have, mark the location with a flag and continue to survey. At the end of the 60-minute period, return to the potential nest site and observe for 15-minutes to see if workers continue to enter/exit the nest. If this is the case, place a vegetation tag next to the nest entrance and hold it in place with a bobby pin. It can be helpful to record additional details about the nest, we collected the following:

    a. Tag number and plot ID
    b. GPS coordinates of nest
    c. Date and time of survey
    d. Species ID
    e. Nesting above or below ground?
    f. Ground cover assessment of area approximately 0.5m x 0.5m above the nest entrance (approximate ground cover by percent)

g. Distance to nearest forest edge (can either measure with tape measure or take GPS point of forest edge and calculate)
h. Note whether this is a 'recapture' (is there already a red tag, or is this a newly found nest)
i. If two nests are found in one plot during one survey, enter a second line in the data sheet for that survey data

5. At the end of the survey, remove any flagging around the nest if the intention is to assess how likely a nest is to be *recaptured* in a plot without noticeable flagging. If the goal is only to find bumble bee nests, flags might be useful to delineate where each nest is located as the vegetation tags don't stand out in the landscape. This decision can be guided by the research question.

6. Using this approach is helpful as it captures personnel effort and can be done by 1-person full-time, or multiple people part-time. However, relying on one-person to complete all the surveys will likely exceed a 40-hour work week, so keep this in mind when selecting site locations, proximity to each other and travel logistics.

Non-standardized nest searching with personnel

In addition to standardized nest searches, nests may also be found using "free searches", which can occur as a supplement to the systematic approach or as a standalone. Free searches are much more haphazard, employing a similar meandering movement through a landscape but not over the same region repeatedly. Similar visual or auditory cues will lead a surveyor to a bumble bee nest, but the infrequent nature of these walks means that they're more likely to be fruitful when nests have produced several generations of workers and have a more pronounced foraging effort.

This method was first mentioned (by this name) by (O'Connor, Park, and Goulson 2017) as a way of comparing human versus canine-based search efforts. (Pugesek and Crone 2021) used a similar approach as a way of maximizing the number of nests found when combined with systematic searching for nests. Many of the supplies and techniques are similar to those used in systematic nest searching, therefore we provide an abbreviated version of supplies and methods. Similarly, the searches themselves are best conducted on days when the ambient air temperature is at least 10 C/50 F, with little wind and no precipitation.

Suggested supplies

- Insect net
- Clear plastic vials
- Camera or phone for pictures
- GPS device, e.g., Trimble Geo7x or phone app capable of GPS waypoints
- Clipboard, writing material, datasheets
- Personal field equipment

- Sunscreen, water, food, dehydration tablets, benadryl, etc. (this will vary by researcher and region)

Step-by-step (general) methods

1. Identify the area of interest for surveying and make sure you have permission from the landowner or manager.

2. Prior to plot surveys, record data on the time start, weather conditions, and anything noteworthy about the land management, e.g., recently mowed.

3. Walk haphazardly but slowly through the area of interest, searching for indicators of a bumble bee nest entrance.

    a. It often makes sense to record how much time was spent in different habitat types to better estimate the amount personnel/time was invested and the number of nests that were found.

4. If a potential nest is spotted, it can be helpful to confirm by waiting for the entrance/exit of at least 2 workers, or a queen with pollen baskets.
    a. Collect the same information as described above on the nest itself.

Canine-based search efforts

Dogs can be trained to find many things (ranging from food items to drugs to animal scat to butterfly larvae) by smell. The idea of using scent-trained dogs to find bumble bee nests has come up in the scientific literature from time to time (Waters et al. 2011; Liczner et al. 2021; O'Connor, Park, and Goulson 2012). To our knowledge, the use of scent dogs has never been more effective than using humans to find bumble bee nests (see (O'Connor, Park, and Goulson 2012) for a formal comparison of humans vs. dogs, and discussion by (Liczner et al. 2021)).

One feature that makes use of scent dogs problematic is that they need to be trained to detect bumble bee nests before they can successfully find them. Early attempts to train scent dogs to find bumble bee nests tried using buried nest material as a training tool (Waters et al. 2011; Liczner et al. 2021; O'Connor, Park, and Goulson 2012). This method was successful at training dogs to find bits of buried nest material, but not necessarily to find nests of wild bumble bee colonies ((Goulson 2016)-“A Sting in the Tale”). One reason that buried nest material might not be analogous to actual nests is that the nests themselves may be located underground, far from and not necessarily below the entrance tunnels. Liczner et al. (2021) supplemented training with wild nests, but only two nests were located for training, and this was not sufficient to provide them with enough reinforcement. One additional feature noted by Liczner et al (2021) is that the conservation scent dog program they worked with was based in Montana (far from their field sites

in Ontario), and much of the training was done remotely in Montana. For other targets (food, drugs, animal scat, insect larvae), it would be possible to realistically send material to a remote training site. This would be much harder to implement for bumble bee nests.

In the process of researching scent-training methods, one of us (E. Crone) consulted with a regional center for scent-dog training (Kate Bigger at High Fidelity Dogs in Everett MA, spring/summer 2017). Bigger noted several additional reasons why bumble bee nests would be harder to detect than butterfly larvae or animal scats: One concern was that, unlike mammals or even caterpillars, there is no scent trail left by a flying bumble bee. In addition, the bumble bee nest itself is not necessarily close to the entrance hole at the ground surface. Finally, as also noted by Liczner et al. (2021), it takes time to verify that bumble bee nests identified by the dog are actual nests, e.g., by watching for nest traffic. This makes it difficult to reward scent dogs for successful nest detection in the wild.

With these caveats in mind, one way forward to improve success and the ease of assessing nest detection would be using scent dogs in an area where some parts of the landscape are easy for humans to search (e.g., flat meadows with high nest densities) but other areas of interest are hard for humans to search but feasible for dogs (e.g., rugged terrain). One option would be to train the dogs at the site with nests, then bring them back to the site regularly to reinforce training in cases where the nest sites are known. This approach would still involve a full-time human field crew to find nests at and monitor the status of the reference site, as well as the dogs and their handlers. Another approach could be to use visual cues to guide dog searches (Liczner et al 2021). An example of this could be a fence line where dogs could be trained to search along the fence for bumble bee nests. This may increase the speed of sampling and make the search effort easier on the dog-handler team. Of course, this comes with the drawback of not being able to search an entire field site but may be useful for some study questions. There are likely other creative paths forward that can improve the likelihood of success using a canine-based method - we encourage researchers interested in canine-based nest searching to share with the broader community any successes they have.

Serendipitous discovery-now what?

Often, bumble bee nests are discovered serendipitously, by homeowners who are gardening and reach out to the nearest biology department, or by graduate students who stumble upon one while setting up another field experiment. Similarly, nests in agricultural systems are often discovered during harvest season[3]. Regardless of how a nest is discovered, collecting a standard set of information is crucial in continuing to characterize bumble bee nesting habitat. Adding this information (assuming it is not being published in an upcoming study) to an established public data repository is critical to ensuring its longevity and value for the wider research community. One of the most established databases is bumblebeewatch.org, which requires to create an

[3] Oscar Martinez-Agricultural landscape in Tacana Volcano, Chiapas, Mexico / Guatemala. One way to find nests is in the season when people are cleaning their fields (usually maze, squash, beans) and start again sowing their fields. It can also be found when picking up coffee fruit in coffee plantations.

account but then allows you to add sightings bumble bees and any nests you find. Here is a suggested list of information to try and collect if you come across a bumble bee nest:

1) *Date that the nest was first noticed*
2) *How much traffic was at the nest?*
3) *Where was the nest located?*
4) *Describe any additional details about the nesting sight that might be of interest.*
5) *How much sun does the nest receive?*
6) *How many entrances does the nest have?*
7) *What direction does the primary nest entrance face?*
8) *List any observed nest materials that are being used.*
9) *Have you ever noticed this area being used by bumble bees before?*
10) *If you answered yes to the previous question, how often have you noticed this nesting site being used by bumble bees?*
11) *Have you ever noticed this area being used by another animal?*

After collecting this information, make sure to upload your sighting under the 'submit a sighting' tab on the website. Taking pictures can supplement the above information and provide a more detailed record of the nesting habitat.

Observing nests: What can be learned?

Once a nest is found, it can be useful to monitor nests and track colony growth and production of sexuals (gynes and males). We have done this successfully with *Bombus impatiens and B. griseocollis*. These methods are highlighted in Treanore et al. (*in prep*). We have also observed nests of *B. vosnesenskii*, *B. californicus, B. bifarius,* and *B. flavifrons*, though not at a frequency that yielded meaningful results.

Suggested supplies:

- Insect net
- Clear plastic vials
- Camera or phone for pictures
- Clipboard, writing material, datasheets
- Personal field equipment
- Sunscreen, water, food, dehydration tablets, benadryl, etc. (this will vary by researcher and region)

Step-by-step (general) methods:

1. Locate nest by one of the above methods
2. Depending on how long the species colony is active, the frequency of observation may need to change to capture accurate nest growth data. Nests should be observed at minimum twice a week for creating growth curves, though more frequent observations will always build a clearer picture.
3. Sit approximately 1 meter away from nest entrance with clear view of nest entrance; close enough to distinguish caste, but not so close as to interfere. This may vary from nest to nest depending on aggression levels.
4. Set timer for 30 minutes
5. Note individuals entering and exiting, other helpful information to collect depending on study goals includes foraging specialization and caste

Outstanding questions and novel ideas

Outstanding questions-

- Improving our understanding of bumble bee nesting behavior in the tropics *(Oscar Martinez).*

- Currently what is known about tropical bumble bee nesting is quite limited, with this comment from BOMBUSS 2023 capturing it well:

"*For the Mesoamerican region, there are only two studies that described bumblebee nests that were found below ground for two species: B. medius and B. ephippiatus (Michener and Laberge 1954; Laverty and Plowright 1985). The nest of B. medius was found in San Luis Potosí in Mexico on the edge of an acahual in 1954, and it was four inches below the ground in a site that was probably previously occupied by a mouse according to Michener and Laberge. Inside the nest they found pieces of leaves and small branches. In Costa Rica near Monteverde and the Irazú Volcano, two nests of B. ephippiatus were found in 1985 according to Laverty and Plowright. Both nests were found underground near acahuales and pastures*."

- How to scale these methods and is there a way to automate somehow to make methods for make nest detection more accessible for those without full time people to do the surveys?-*BOMBUSS 2023 Subgroup*

Novel ideas

Can we pair RFID with these wild nests to detail foraging patterns and the timeline of sexual dispersal from their natal nest?-*EDT*

Studying overwintering and hibernating queens

Studying the overwintering stage of the bumble bee lifecycle may be the most challenging aspect of the entire colony life cycle. Gynes eclose in their natal colonies and then consume pollen and nectar to sequester sufficient nutrients for the upcoming winter diapause. Prior to entering diapause, gynes will mate (but see ((Mullins, Strange, and Tripodi 2020), search for a suitable hibernaculum, and then seemingly disappear until springtime. How long each of these individual phases lasts in natural conditions is not entirely clear and requires additional study using innovative field approaches.

This limited gyne activity period means that finding overwintering gynes is easiest with knowledge of the location of a natal colony. In some species and systems, gynes may overwinter near their natal colony and can be seen digging their hibernaculum, allowing for studies on natural diapause conditions to occur with systematic searching. Furthermore, new technology aimed at radio-tracking where gynes overwinter may provide insight into where gynes that don't overwinter near their natal colony go. Less frequently, overwintering queens are occasionally stumbled upon by the lucky gardener or researcher who happens to pick the right location to dig up late-season potatoes or search for overwintering queens, respectively. All these methods can provide valuable information on habitat requirements for overwintering and are discussed in more detail below.

Systematic Searching

Systematically searching for overwintering queens can feel like searching for a needle in a haystack, but there are some tips that make it a little bit easier. Queens from some species, such as *B. impatiens*, will sometimes nest near their natal nest[4] (Pugesek, Thuma, and Crone 2023; Liczner and Colla 2019; Plath 1927), which makes a systematic approach a lot more targeted. For example, Pugesek et al. 2023 found 111 queens around 4 natal nests by looking for displaced soil and evidence of digging near the natal nest. Evidence for this in other species, such as *B. affinis*, *B. fervidus, and B. griseocollis,* is lacking ((Plath 1927; Pugesek, Thuma, and Crone 2023). When the location of the natal nest is **unknown**, focusing on habitat and groundcover types that have been anecdotally linked to queen overwintering may be helpful. For example, the community science initiative QueenQuest, which sought to find overwintering queens includes the following suggestion:

"From anecdotal reports and published literature, it seems most likely that bumble bee queens may be found in loose soils, in the accumulated litter under conifers and other trees, and near structures such as tree trunks, root masses, walls and other human-created structures."

The most recent and comprehensive summary of queen overwintering research by (Liczner and Colla 2019) summarizes what is known in the ten published studies (at that time) on queen

[4] Elaine Evans, BOMBUSS 2023- *Monitored survival, temps, and took some super cooling points for overwintering impatiens at a few different clusters of gynes digging near their natal nests. Found areas from people calling the lab. In one area there were lots of false starts, half dug hibernacula. A difficulty was that we had to poke around to confirm there was a bee in there, but didn't want to disturb them.*

overwintering habitat. While queens have often been suggested to overwinter in north-facing slopes in (4/10 studies), other studies found queens in flat areas or slopes facing other directions. Ground cover was the most shared across the studies, with queens said to avoid areas with dense vegetation and instead selecting areas with bare ground, under tree litter, moss, or within exposed areas in grass (Liczner and Colla 2019). Of the few studies on queen overwintering that have been published since this review came out, (Williams et al. 2019) found 10 queens (all western bumble bee species) overwintering under cypress litter in the duff layer between the cypress needles and the mineral soil, which further supports these suggested habitat preferences.

Using this information, it can be easier to narrow down an area for surveying for overwintering queens systematically. After selecting a promising spot to search for queens, it's also important to consider the depth at which queens may be overwintering. The previous review of literature suggests queens most frequently are found at depths between 2-15 cm, but this will vary by species and region (Liczner and Colla 2019). The QueenQuest protocol suggests surveying to around 10cm, which might be a safe bet in most regions/species. With that in mind, if you find yourself struggling to move the soil because of how compact it is, you probably won't find queens at that depth.

Helpful background information

Natal colonies are easiest to find during their peak activity period, so those interested in studying queen overwintering near the natal nest may be better off searching for nests a month or two prior to this life stage. The nests used in the study where these methods were developed were discovered much earlier in the season (Pugesek, Thuma, and Crone 2023), and weekly nest observations happened to reveal gynes that appeared to be digging into the nearby soil. A few other important things to note, these methods have only been used in *B. impatiens*, which is known to overwinter in aggregations (Plath 1927); whether other species also overwinter near their natal colonies is unclear. The groundcover surrounding the natal nest is also important to consider when studying overwintering queens, as some ground cover types may make it challenging to find evidence of hibernacula. The methods employed below were only used for queens in forests as the dense vegetation in grasslands presented a challenge. Finally, here the researchers looked for evidence of queen hibernaculum in September and October and waited until November and March to excavate for queens; this makes sense for this region (Massachusetts, United States) and species.

***Please note**: Searching for overwintering queens in regions where the Rusty Patched Bumble Bee may be disturbed is NOT encouraged and is a violation of the Endangered Species Act of 1973 if done without a permit. The following map available at QueenQuest.org highlights areas of high concern: https://www.queenquest.org/*

Suggested supplies

Surveying:

- Quadrat, measuring tape, or meter stick
- Uniquely numbered vegetation tags

Excavation:

- Gardening Gloves
- Trowel, spoons, paintbrush or small brush, small hand tools for digging
- Measuring tape or meter stick
- Camera (smartphone or other camera)
- Tarp, bin, or other vessel for holding removed soil
- Cooler with ice packs
- Clean 50 mL falcon tubes

Step-by-step methods (natal nest):

1. Identify natal nests that have been observed producing gynes or that were healthy enough gyne production may have occurred (if nest observations did not occur).

2. Soon after gyne production begins, visit the natal nest sites approximately 1x per week.

3. During each visit, measure out quadrats (this study used $16m^2$) in varying distances from the natal nest. Here the farthest quadrat was 10m from the colony entrance, with 7-9 quadrats measured per visit.

4. Search each quadrat thoroughly for indicators of a hibernaculum; this may be fresh earth near a small hole or a queen digging into the ground.

5. At each potential overwintering site, mark the location with a uniquely numbered plant vegetation tag.

Excavation:

6. To verify whether queens are overwintering in the marked location, return to the overwintering sites ~ 2 months after their initial discovery, e.g., early November and December.
    a. Rather than every overwintering site, select a subset of the marked overwintering sites to excavate as queens will be disturbed regardless of how much care is taken. This study selected ~25% of the sites to excavate.

7. Gently dig around the marked entrance with a spoon or small trowel, depending how close they are to one another.

8. In *B. impatiens*, a majority of the queens were found within the first few cm of soil, but they may be up to 10 cm down.

9. If you find a queen, verify whether the queen is alive or dead (Pugesek, Thuma, and Crone 2023) has detailed methods and photos on how this can be determined, but generally queens will be fully intact, with pristine coats and may awaken upon disturbance.

10. Measure the depth at which the queen is buried and immediately place her in a tube on ice to avoid completely disturbing her from diapause. Queens can be uniquely marked with plastic queen tags (Opalithplattchen), measured, etc. depending on the research goals.

11. Return queens to their original locations as soon as possible, gently reburying and placing metal tags over the queens on the top of the soil to mark her location.

12. To check overwintering survival, revisit queens closely before emergence in the springtime, repeating this entire process. The above study revisited queens in March due to fieldwork restrictions, but originally aimed to check queens in early-April. Obviously, this will vary by region, and these are just suggestions, but this method has worked previously.

Step-by-step methods (random location):

These methods are guided by the QueenQuest protocol and additional published studies ((Williams et al. 2019; Pugesek, Thuma, and Crone 2023) and intended to be used in situations where the location of the natal nest is unknown, but rather the habitat suggests this could be potential overwintering site. QueenQuest and personal experiences suggest approaching this with a small field crew, as this is a bit labor-intensive, low-reward, and more fun with friends!

1. Select a small quadrat in a location which, based on what is known about bumble bee overwintering habitat, might harbor overwintering bumble bee queens. One square meter is suggested as a good starting size!

2. Slowly begin removing the surface substrate, e.g., plant litter, a few cm at a time. Place the substrate on a tarp or a bin and gently sift through it as you go, in case any queens are tucked away.

3. Slowly and methodically continue removing plant litter until you reach a depth of about 10 cm deep (or until the soil becomes too compact!).

4. If you find a queen, you can employ the same process as above if you'd like to continue monitoring overwintering survival. Alternatively- a one-off discovery can still be valuable; the most important data to document can be found in the '*Serendipitous discovery-now what*?' section.

5. Once you finish surveying your quadrat, return the displaced plant material, trying to replicate the initial conditions as closely as possible. Measure the amount of land surveyed, the depth, and record the amount of time/personnel involved. This information is valuable to estimate the effort involved and can be reported on the bumble bee watch website with the reported information in the notes section on http://bumblebeewatch.org/[5].

Radio Telemetry fall gynes

Liczner et al. have radio tagged *Bombus impatiens* gynes in late-summer to track their movements prior to hibernation. To find gynes, it is best to find a highly rewarding dense floral patch (we use a large *Monarda* patch) and survey this area to find gynes. Gynes are not very common, with only a couple usually caught per day, some days no gynes were found. Individual gynes frequently return to the same floral patch (even the same flower they were caught on), and often sleep on flowers making them somewhat easier to track than spring queens. After a few days to a week, the gyne does not return, presumably she has found a hibernation site. Liczner et al. have also tagged gynes at a nest site. Gynes sporadically leave and return to the nest over a period of weeks. Liczner et al. have also used commercial gynes to test the impact of pesticides on gyne movement.

Suggested supplies

(same as spring queen telemetry)

Step-by-step (general) methods

(same as spring queen telemetry

[5] The previous effort to collect this data, QueenQuest, is no longer being actively monitored but if someone was looking to take over this program, John Mola mentioned he'd be happy to hand over whatever bits of data and info exists.

Serendipitous discovery-now what?

On occasion, overwintering queens are discovered serendipitously by a gardener or a researcher who happens to lift the right log on a hike. Regardless of how a queen is discovered, collecting a standard set of information is crucial in characterizing bumble bee overwintering habitat. Similarly, adding this information to an established public data repository is critical to ensuring its longevity and value for the wider research community. Reporting this information can be done on bumblebeewatch.org. Here is a suggested list of information to collect if you come across an overwintering bumble bee queen:

1) *Date of discovery*
2) *GPS coordinates of location*
3) *Species*
4) *Number of queens*
5) *Surrounding landscape type (e.g., on the perimeter of a forest next to a hayfield)*
6) *Depth of queen*
7) *Soil characteristics, e.g., compact, leaf litter, sandy*
8) *Groundcover type and/or nearby vegetation*
9) *Queen behavior/your behavior, e.g., did you see the queen excavating or did you stumble upon her?*
10) *Was there anything obscuring the queen, such as a log or stone?*
11) *Did the queen appear alive or dead? See (Pugesek, Thuma, and Crone 2023) for photos.*
12) *Anything else interesting? For example, covered in a white mold…*
13) *Take photos!*

Outstanding questions and novel ideas

Outstanding questions-

How can we better understand the behavior of bumble bee queens in tropical environments, after eclosion and to the point of colony foundation is a relatively understudied/unknown area of research *(Oscar Martínez)*.

Is diapause not present in tropical bumblebees and how can we study this (*Oscar Martínez*)

In high elevations, gynes are often not observed on the landscape at all. Do some species, or some species in certain environments, overwinter inside their natal nests? (*Sylvie Finn*)

Relevant Publications

Studying springtime nest-searching/colony-founding queens

1. Movement of nest-searching bumblebee queens reflects nesting habitat quality (Pugesek and Crone 2022)

2. Location of bumblebee nests is predicted by counts of nest-searching queens (O'Connor, Park, and Goulson 2017)

3. Assessing the value of Rural Stewardship schemes for providing foraging resources and nesting habitat for bumblebee queens (Hymenoptera: Apidae) (Lye et al. 2009)

4. Habitat preference and phenology of nest seeking and foraging spring bumble bee queens in northeastern North America (Hymenoptera: Apidae: Bombus)'(Svensson, Lagerlöf, and Svensson 2000)

5. Habitat Preference and Phenology of Nest Seeking and Foraging Spring Bumble Bee Queens in Northeastern North America (Hymenoptera: Apidae: Bombus) (Lanterman et al. 2019)

Finding established nests

1. Humans versus dogs; a comparison of methods for the detection of bumble bee nests (O'Connor, Park, and Goulson 2012)

2. Accounting for imperfect detection in species with sessile life cycle stages: a case study of bumble bee nests (Iles et al. 2019)

3. Influences on the density and dispersion of bumble bee nests (Hymenoptera: Apidae) (Harder 1986)

4. Contrasting effects of land cover on nesting habitat use and reproductive output for bumble bees (Pugesek and Crone 2021)

Studying overwintering and hibernating queens

1. First field-based estimates of bumblebee diapause survival rates showcase high survivorship in the wild (Pugesek, Thuma, and Crone 2023)

2. Fantastic bees and where to find them: locating the cryptic overwintering queens of a western bumble bee (Williams et al. 2019)

3. A study of the hibernation of bumble bees (Hymenoptera: Bombidae) in Southern England (Alford 1969)

4. Notes on the hibernation of several North American bumblebees (Plath 1927)

5. The humble-bee, its life-history and how to domesticate it (Sladen 1912)

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