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Bombus Microcolonies as a Tool for Biological Understanding and Pesticide Risk Assessment

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Abstract

Bumble bees provide valuable pollination services to many wild and agricultural plants. Populations of some bumble bee species are in decline, prompting the need to better understand bumble bee biology and to develop methodologies for assessing the effects of environmental stressors on these bees. Use of bumble bee microcolonies as an experimental tool is steadily increasing. This review closely examines the microcolony model using peer-reviewed published literature identified by searching three databases through November 2018. Microcolonies have been successfully used for investigating a range of endpoints including behavior, the gut microbiome, nutrition, development, pathogens, chemical biology and pesticides/xenobiotics. Methods for the initiation and monitoring of microcolonies, as well as the recorded variables were catalogued and described. From this information, we identified a series of recommendations for standardizing core elements of microcolony studies. Standardization is critical to establishing the foundation needed to support use of this model for biological response investigations and particularly for supporting use in pesticide risk assessment.

Keywords

bumble bee; hazard assessment; pesticide; methodology; insect behavior; sociobiology

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Introduction

Honey bees (*Apis mellifera*) have long been considered the most important pollinator of many agricultural plants (Allen-Wardell et al. 1998, Kevan 1999, Delaplane and Mayer 2000). The value of these pollination services provided by non-*Apis* bees is now widely recognized (Klein et al. 2007, Breeze et al. 2011, Garibaldi et al. 2013, Klatt et al. 2014). Bumble bees (*Bombus* spp.), for example, are important pollinators of many wild plants and provide essential auxiliary and novel crop pollination services valued as high as $963 per hectare (Kleijn et al. 2015).

Disconcertingly, populations of some bees are in decline (Potts et al. 2010, Cameron et al. 2011, Burkle et al. 2013, Koh et al. 2016, Meeus et al. 2018). Many studies have illustrated the effects of environmental stressors on the health and performance of honey bees including pesticides (Johnson et al. 2010, Henry et al. 2012, Whitehorn et al. 2012), pathogens (Cox-Foster et al. 2007), parasites (Le Conte et al. 2010) and poor nutrition (Huang 2012, Di Pasquale et al. 2013). While it is tempting to apply these findings to bumble bees, there are many notable differences between honey bees and bumble bees that complicate extrapolation of honey bee specific data (Stoner 2016, Gradish et al. 2018). For example, bumble bees form annual colonies in contrast to perennial honey bee colonies. These taxa prefer to forage on different plants, have unique parasite and pathogen communities, and can have different responses to pesticide inputs (Thompson and Hunt 1999, Besard et al. 2011).

Recognizing the importance of bumble bees to managed and natural landscapes, there is a pronounced need for bumble bee-specific methodologies to develop a better understanding of their biology and how these bees respond to various stressors. Recently, the Office of Economic Cooperation and Development (OECD) validated two acute toxicity testing protocols for adult bumble bees housed in isolation (OECD 2017b, a). In addition, the International Commission for Plant-Pollinator Relationships (ICPPR) is working to develop a chronic oral toxicity test for bumble bees (OECD 2017b). While these testing formats reduce experimental complexity, they limit investigations to acute exposures in adult bees and ignore important effects related to colony health and production of new progeny. In contrast, microcolonies can facilitate completion of a wide array of investigations and take advantage of bumble bee social plasticity to maintain some elements of colony-level dynamics.

Microcolonies are formed when a group of bumble bee workers are isolated in a queenless environment (Figure 1). Separation from the queen stimulates one of the workers (usually the largest one with the most developed ovaries) to establish dominance and begin laying eggs (Free 1955). These eggs are unfertilized and, due to the haplodiploid reproductive system in bees, result in the production of male offspring (i.e., drones). Using microcolonies, investigators can evaluate the effects of chronic exposures to various factors not only on adult bees but also on brood development. Importantly, these investigations can be conducted under defined laboratory conditions on standardized colonies, and, because large numbers of microcolonies can be maintained simultaneously, experimental sample size and replication capacity are robust. Given these attributes, experimental use of microcolonies

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has been rapidly increasing (Figure 2). However, there are no published evaluations of microcolony development or performance. Here, we sought to provide a comprehensive review of the microcolony model and discuss how this model has been applied. In addition, we provide suggestions for microcolony protocol standardization for risk assessment that are needed to maximize the utility of this tool across a broad array of investigations.

Methods

Peer-reviewed research articles were identified by searching Google Scholar, Web of Science, and ProQuest Agricultural and Environmental Science Database for publications through November 2018 using the search string (“Bombus” or “bumblebee” or “bumble-bee” or “bumble bee”) and (“microcolony” or “micro-colony” or “queenless” or “queen-less” or “queenless colony”). An additional 8 articles were identified by reviewing the reference section of these papers. For inclusion in this review, microcolonies had to be queenless, housed in an artificial container that was not the originating colony container, and could not be initiated in the presence of live brood from a queenright colony.

Results and Discussion

A total of 75 peer-reviewed articles were identified. To maximize the utility of this review, microcolony design parameters were extracted from all selected studies (Supp. Table S1). In the sections that follow we catalogued and critically reviewed the approaches applied to establishing microcolonies, the various endpoints assessed in these studies and provide recommendations for methods standardization.

Microcolony composition

Although the basic components of a microcolony (i.e., worker bees, food provisions and the nest chamber) appear straightforward, numerous approaches for establishing microcolonies have been applied (Suppl. Table S1). Since these differences can significantly impact microcolony formation and potentially study outcome, we categorized these design variables and discuss the significance of different configurations.

***Bombus* species —**The majority of published studies used *Bombus terrestris* (60/75) to establish microcolonies and a smaller number used *Bombus impatiens* (13/75) (Suppl. Table S1). Both species are commercially available in their respective continents, providing a reliable source of worker bees for microcolony studies year-round (Velthuis and van Doorn 2006, Winter et al. 2006), *B. impatiens* is sold in North America (importation of other species is restricted) and *B. terrestris* is available in most other locations. Seven studies utilized microcolonies composed of wild-caught bees (e.g., *B. terrestris* (Larrere and Couillaud 1993, Regali and Rasmont 1995, Tasei et al. 2000, Tasei and Aupinel 2008b, Moerman et al. 2016), *B. impatiens* (Sibbald and Plowright 2014), *B. hypnorum* (Moerman et al. 2016) and *B. pratorum* (Free 1955, Moerman et al. 2016).

Age of workers —Microcolonies can be initiated with either workers of unknown age or newly emerged workers (i.e., callow workers). Using older, non-age matched workers allows for access to more individuals, thereby enabling researchers to setup more microcolonies

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at one time. However, under this circumstance, worker deaths due to old age may be mistaken for treatment-related effects. Consequently, age-unknown microcolonies are best suited for producing drones for mating or for creating pathogen cultures needed for other purposes. Newly emerged bees are the best choice for studies investigating the effects of experimental treatments. Within the literature, the age of workers used to initiate microcolonies was not always clearly disclosed, confounding study interpretation (Suppl. Table S1). Seeding microcolonies with newly emerged bees creates a uniform age group, promoting experimental reproducibility, and minimizes mortality from aggression between foreign nestmates (Bloch and Hefetz 1999, Doums et al. 2002, Rutrecht et al. 2007). Furthermore, newly emerged bees may carry fewer pathogens than older workers exposed to nestmates longer; but, there may be reductions of gut microbiomes in naïve bees that could have potential downstream effects on worker survival and microcolony development (Meeus et al. 2013, Kwong et al. 2014).

Number and source of bees — Published studies used anywhere from 1 to 20 worker(s)/microcolony, but use of five workers was most common, followed by three workers (Figure 3; Suppl. Table S1). Seeding microcolonies with more workers dilutes the brood tending responsibilities across more individuals and elevates the microclimate temperature (Plowright and Jay 1968, Cameron 1985, Goulson 2010, Mommaerts et al. 2010b). Under these favorable conditions, more male offspring can ultimately be produced (Gradish et al. 2013). The number of nestmates can also affect worker dominance behavior, ovary development and oocyte length, with some species differences (Cnaani et al. 2007, Amsalem and Hefetz 2011). In a study with *B. impatiens*, Cnaani et al. (2007) showed that oocytes were smaller in microcolonies containing fewer workers, but this effect was not seen between 2- and 4-member microcolonies in *B. terrestris* (Cnaani et al. 2002). Understanding these potential sources of variation is important when conducting comparative studies.

Although *B. terrestris* and *B. impatiens* workers can be obtained from commercial vendors (e.g., Koppert and Biobest), it can be challenging to obtain enough newly emerged workers at one time for large experiments. To meet demands, some researchers collected newly emerged workers directly from multiple queenright colonies (Meeus et al. 2013). In other cases, investigators isolated and artificially incubated pupal cocoons by extraction from the queenright colony and allowing the adults to emerge (Billiet et al. 2016). In all cases, the selection, source and handling of newly emerged adult bees may have downstream impacts on microcolony development and progression (Laycock et al. 2012, Laycock et al. 2014, Amsalem et al. 2015).

Artificial microcolony container structure — Microcolonies can be housed in containers composed of wood, plastic, Styrofoam and metal (Figure 4; Table S1). Compared to wood, plastics offer more versatility allowing the investigator to create a custom nesting habitat. However, plastic can be more expensive than wood and cannot be heat-treated for sterilization. Plastic microcolony chambers can be either purpose-made for bumble bee rearing (i.e., "queen boxes") or repurposed from common containers (Figure 4A–D). Using disposable plastic "deli containers" can be a relatively inexpensive way to house microcolonies while eliminating pathogen carry over between experiments. Depending on

the duration of the experiment, it may be necessary to clean the containers or transfer the microcolony to a new, clean chamber. Some chamber designs incorporate a pass-through bottom to allow bee waste to be removed without causing stress to the bees (Figure 4C–D; (Malone et al. 2007, Gradish et al. 2013, Richardson et al. 2015).

Single chamber designs are the most common, but some study designs incorporated separate feeding and nesting chambers (Mommaerts et al. 2010a, Ruedenauer et al. 2016). When size of chamber was reported, the volume of the nest chamber in reviewed studies ranged from ~127.2 cm^3 to 10,304 cm^3 (Table S1). Although investigators tend to use larger chambers when seeding microcolonies with more workers (Figure 3; Table S1), the relationship between the number of workers and microcolony container size has not been investigated.

Duration of experiment —The duration of microcolony investigations is dictated by the purpose of the experiment and limited by worker bee mortality. Study duration ranged from 3 to 100 days with studies evaluating drone production requiring the most time for completion (Table S1).

Sugar source —Microcolonies must be provisioned with sugar solution *ad libitum*. Many researchers rely on commercial sugar syrups (e.g., Biogluc®, Attracker, Apiinvert and Invertibee; Table S1). These syrups are convenient, but the formulation may not be disclosed to the investigator. Other researchers make their own sugar solutions that frequently contain only sucrose (Table S1). Occasionally, a mixture of honey and water is used(e.g. Elston et al. 2013, Gradish et al. 2013, Ramanaidu and Cutler 2013), but honey may contain pathogens and pesticides potentially confounding results (Bogdanov 2006, Kujawski et al. 2014). The composition of sugars in the syrup can be important for the establishment of the gut microbiome (Billet et al. 2016). Bees provisioned with syrups containing suboptimal sucrose content (i.e., 15 – 29%) consumed less syrup and experienced higher rates of mortality and infections than those fed syrup containing 30% sucrose (Conroy et al. 2016). High fructose syrups can impact gut microbiome development (Billet et al. 2016). *Bombus terrestris* microcolonies composed of 10 workers utilized an average of 300–400 μL Biogluc® solution per bee per day (Meeus et al. 2013). For some investigations, such as those evaluating pesticides, evaporation of syrup should be monitored to correct consumption estimates. Development from egg to emerged drone for *B. terrestris* required on average 128 mg of sugar (eho et al. 2014).

Pollen source —Since an effective artificial protein source is not available for bumble bee microcolonies, microcolonies must be fed honey bee-collected pollen. The floral composition of the pollen will depend upon the bee foraging environment and, for that reason, exact replication of pollen sources is not possible. Further, long-term storage of pollen has been shown to affect pollen quality (Pernal and Currie 2000). Restricting access to high-quality pollen was shown to increase larval and pupal development times and ultimately impacted drone production (Sutcliffe and Plowright 1990, Regali and Rasmont 1995, Génissel et al. 2002). Bees in microcolonies prefer some species of pollen over others, and pollen choice can have downstream effects on egg deposition, oophagic behavior and drone production (Aupinel et al. 2001, Moerman et al. 2015, Billiet et al. 2016, Ruedenauer et al. 2016, Dance et al. 2017). Pollen quality can also have an undesired effect on bee

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immune responses (Roger et al. 2017a). Worker *B. terrestris* pollen needs are estimated to be 25 – 30 mg/day (Meeus et al. 2013).

In some cases, microcolonies were established using sterilized pollen to control the pathogen load. Gamma irradiation (16.9 kGy) has been shown to effectively sterilize pollen (Graystock et al. 2016); however, elimination of microbes in pollen is known to affect the gut microbiome of bees (Meeus et al. 2013). Consequently, the effects of pollen sterilization methods on pollen preference, palatability and microcolony development and productivity require additional investigation.

Nesting material —Bumble bees combine pollen with wax to produce the scaffold of their nest (Goulson 2010). Pollen composition, which varies greatly, and quantity are known to impact bumble bee colony and offspring size (Moerman et al. 2015). Consequently, the source of pollen, timing of pollen provisioning and the quantity of pollen provided all have the potential to impact microcolony initiation, progression and ultimately productivity. In some studies, microcolonies were provided with artificial nesting material or emerged pupal cells collected from other colonies at the time of nest initiation (e.g. Besard et al. 2011, Munday and Brown 2018). Although these materials appeared to stimulate nest initiation, there is the possibility that providing additional nesting material may introduce pathogens into the microcolony.

Temperature, relative humidity and light regimes —Microcolonies have been maintained under a variety of environmental conditions including a temperature range of 23 – 30°C, relative humidity range of 20 – 80% and varying amounts of light. Each *Bombus* species may have an ideal nesting temperature and deviations from that temperature may impact brood development (Yoon et al. 2002, Gurel et al. 2008). Humidity levels can affect foraging rates and pollen collection by *B. terrestris* species in the wild, with bees collecting more pollen in low humidity environments (Peat and Goulson 2005). Thus, experimental relative humidity may affect microcolony productivity and pesticide exposure levels when delivered in food. Most microcolonies are held in total darkness, but some studies incorporate light:dark cycles (e.g. Génissel et al. 2002, Arnold et al. 2014). Generally, drone offspring masses (larvae, pupae and on the day of adult eclosion) decreased when photoperiods were adjusted to include increasing exposure to light (Amin et al. 2007).

Parameters measured in microcolony experiments

Microcolonies have been used to investigate responses to various treatments including behavior, gut microbiome, nutrition, development, pathogens, chemical biology and pesticides/xenobiotics (Table 1). Depending on the experimental purpose, investigators collected data from various parameters described below. While there are specific data captured under each of these categories, many of these factors are interrelated (Figure 5).

Worker mortality —The number of worker bees remaining after an experimental treatment can be easily quantified, providing information about the effect of experimental treatments on workers (Babendreier et al. 2008, Mommaerts et al. 2010b, Ramanaidu and Cutler 2013, Smagghe et al. 2013, Wang et al. 2016). Remembering that microcolony

productivity is influenced by the number of workers (Gradish et al. 2013), loss of workers can impact brood development and ultimately drone production. Consequently, brood effects must ultimately be evaluated in the context of worker bee mortality. For these reasons, monitoring worker mortality is an important core element of microcolony study design.

Duration of lifecycle stages —The amount of time required to deposit eggs and complete various lifecycle stages (i.e., egg hatching, larval development and pupation) can be monitored in microcolonies (Figure 5). While the number of establishing workers has not been directly shown to be correlated with the time to first oviposition, worker age, and the quality and amount of the pollen are known to impact egg laying activity (Génissel et al. 2002, Gradish et al. 2013, Meeus et al. 2014, Amsalem et al. 2015). In addition, some pesticides, plant toxins and bacteria have been shown to lengthen the time to first oviposition (Ramanaidu and Cutler 2013, Richardson et al. 2015). Prolonging the timeline for microcolony progression can result in increased resource demands and may affect drone production.

Offspring production —Drone production is a key metric of microcolony productivity and can be easily quantified. Drone production is affected by experimental treatments, including pesticides (Tasei et al. 2000, Mommaerts et al. 2006, Besard et al. 2011, Laycock et al. 2012, Elston et al. 2013, Smagghe et al. 2013, Laycock et al. 2014, Ceuppens et al. 2015), pathogens (Ramanaidu and Cutler 2013, Meeus et al. 2014) and nutritional modifications (Laycock et al. 2012, Moerman et al. 2015, Ruedenauer et al. 2016, Billiet et al. 2017, Dance et al. 2017). The relationship between the number of eggs laid, number of larvae, and number of drones produced is obviously related (Figure 5). However, bumble bee nests are stratified with new structures built directly on top of old structures which hinders our ability to accurately monitor the number of eggs and larvae. One study showed that treatment-related effects on the number of eggs and drones are not always evident when evaluating numbers of larvae and pupae (Moerman et al. 2015). Oophagy and larval ejection may contribute to observed inconsistencies; mortality before the adult stage should be partitioned accordingly between mortality due to treatment effects or due to these behaviors (which may also be treatment induced).

Mass/body size —It is essential to record initial body weight of the worker bees and to seed microcolonies with bees of similar mass (Roger et al. 2017a, Roger et al. 2017b). Worker size can directly affect the time required to establish dominance in the microcolony and, potentially, the amount of time required for first oviposition (Amsalem and Hefetz 2011). Increased food consumption by larger workers (Peat and Goulson 2005, Couvillon and Dornhaus 2010) may affect consumption/exposure rates in pesticide exposure studies.

Treatment effects may also be detected by weighing the brood. Changes in brood mass will alter the nectar needs of the colony, with heavier brood requiring more sugar (Pendrel and Plowright 1981, eho et al. 2014). Poor quality pollen has been shown to affect larval and occasionally pupal weights (Tasei and Aupinel 2008a, Moerman et al. 2017, Roger et al. 2017a, Roger et al. 2017b, Vanderplanck et al. 2018). Other variables may also influence larval mass, such as light:dark regimes, species of bumble bee used, and relative humidity (Peat and Goulson 2005, Amin et al. 2007, Moerman et al. 2015).

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While assessing brood may be difficult and destructive, determining the weight of drones is common and non-destructive. Since the mass of larvae and pupae should correlate with the drone size, collection of larval and pupal masses is not essential for all investigations. Drone size has been shown to be affected by food quality and availability, the presence of antibiotics, toxicants introduced into pollen, light:dark regimen and pathogens (Sutcliffe and Plowright 1988, Amin et al. 2007, Malone et al. 2007, Meeus et al. 2013, Arnold et al. 2014, Meeus et al. 2014, Barbosa et al. 2015, Ceuppens et al. 2015, Dance et al. 2017).

Behavior —Feeding behavior is integral to microcolony success and, for that reason, quantifying food consumption is common. Pollen and nectar consumption vary between different *Bombus* species and fluctuate day-to-day (Manson and Thomson 2009, eho et al. 2014, Moerman et al. 2015, Piiroinen et al. 2016, Dance et al. 2017). Larger workers and larger brood require more food (eho et al. 2014, Schaeffer et al. 2017). Experimental treatments may alter food consumption, potentially impacting the size and number of the drones produced (Laycock et al. 2012, Moerman et al. 2015, Ruedenauer et al. 2016). If the experimental design allows for increased foraging movement (i.e., a second chamber), food uptake will be increased to satisfy energetic demands associated with foraging (Ceuppens et al. 2015). It is important to also note that experimental treatments given in sugar syrup may have a delayed effect if existing honeypots are being utilized in a microcolony (Barbosa et al. 2015).

In addition to monitoring feeding activity, investigators can monitor other worker behaviors such as: aggressiveness associated with the establishment of dominance, sluggishness and paralysis (Vandoorn 1989, Wang et al. 2016); nest construction (i.e., honey pots and brood clumps) (Elston et al. 2013); and oophagy and larval ejections (Tasei et al. 2000, Génissel et al. 2002, Munday and Brown 2018, Vanderplanck et al. 2018).

Ultrastructure —Microcolonies have proven a useful tool for deciphering the role of specific Dufour's gland signals in reproductive division of labor in queenright colonies (Amsalem and Hefetz 2011, Amsalem et al. 2015) and for investigating treatment effects on worker bee ovarian development and oocyte size (Cnaani et al. 2007, Manson and Thomson 2009, Laycock et al. 2012, Barbosa et al. 2015). Investigators should be aware that Dufour's gland signals as well as ovarian development can be affected by the group size and age of the microcolony workers, which can impact microcolony initiation and progression (Amsalem and Hefetz 2011, Amsalem et al. 2015).

Gut microbiome—The bee gut microbiome plays a vital role in metabolism, immune function growth and development (Mockler et al. 2018, Raymann and Moran 2018, Rothman et al. 2019). Studies investigating the impacts of treatments on the composition of the gut microbiome on bee health are becoming increasingly common. For studies of this type, it is important to recognize that the gut microbiome may be affected by social interactions with bees from the maternal colony (Meeus et al. 2013, Kwong et al. 2014, Kwong and Moran 2015, 2016, Billiet et al. 2017) and on the diet fed to the nascent microcolony (Billiet et al. 2017). The gut microbiome may also be affected by exposure to antibiotics and diets high in fructose, as well as the use of sterilized pollen as a food source

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(Meeus et al. 2013, Billiet et al. 2017), and thus care must be taken to separate microcolony effects from treatment effects.

Recommendations for standardization for pesticide risk assessment: To protect bees, many regulatory authorities require that pesticides be subjected to a risk assessment process to identify potential risks to bees. Traditionally, the honey bee has served as the model organism for this process and results observed in honey bees are assumed to be predictive of outcomes in all other bees (Gradish et al. 2018). Acknowledging differences in life histories, phenology and pesticide sensitivity between *Apis* and non-*Apis* bees (Brittain and Potts 2011, Cresswell et al. 2012, Arena and Sgolastra 2014, Stoner 2016, Thompson 2016, Heard et al. 2017), reliance on the honey bee as a surrogate species for pesticide risk assessment has been questioned (Stoner 2016, Rortais et al. 2017), prompting the development of protocols specifically designed for assessing the effects of pesticides on non-*Apis* bees.

Microcolony protocols can be readily adapted to address a broad range of experimental questions; however, because risk assessment of pesticide impact requires a high level of reproducibility, we recommend adoption of standard protocols. Because we currently do not fully understand the impact of all design parameters on microcolonies, we cannot provide a definitive protocol for all bumble bee species, but we can provide a framework to begin the much-needed work of methods standardization. The following recommendations are derived from a consensus of studies carried out to date using *B. terrestris* and *B. impatiens* for the purpose of pesticide risk assessment.

Microcolony chambers —Microcolony chambers should be well-ventilated and either sanitizable or disposable. The chambers should be maintained in darkness, and if not, the chambers should be made of light-obstructing material. To eliminate the need to clean microcolony chambers during experiments and cause stress to the colony, the chambers should include a pass-through floor to isolate bee waste. Unless the experimental design requires measuring foraging behavior, only one chamber should be used. For microcolonies composed of 5 workers, we suggest chambers like those pictured in Figure 4A – D, but remind the reader that no comparative studies have been conducted to determine the optimal microcolony chamber size.

Microcolony composition —To maximize study consistency, microcolonies should be established using five newly-emerged workers of similar mass. To account for source colony differences (such as genetics or basal pathogen infections), the workers from multiple colonies should be randomized prior to seeding microcolonies; however, where colony-level differences are of interest, single source microcolonies can be used. Investigators should consider the potential impact of interactions between newly-emerged workers and mature workers occurring in the queenright colony on the establishment of gut microboiota in naïve bumblebees which can lead to downstream effects in microcolony development and progression (Koch and Schmid-Hempel 2011, Kwong and Moran 2016). Because the number of workers in a microcolony impacts microcolony productivity, microcolonies with worker mortality occurring within the first 24 hours of seeding should be discarded and further mortality should be recorded and used to standardize results. Likewise, treatments should not be applied within the first 24 hours of microcolony establishment so that pre-

treatment mortality can be detected and to allow the workers to acclimate to the chamber and their nest mates.

Food provisioning—Microcolonies should be provisioned with fresh sugar syrup *ad libitum*. Sugar syrup should be composed of 30% sucrose content (sucrose should be the dominant sugar). Additives (e.g., vitamins, essential oils) have limited research on effectiveness and should be minimally added and always reported (Gosterit and Oytun Cicek 2017). Fresh sugar syrup should be provided every 2 – 3 days.

Microcolonies should be provisioned with fresh (or fresh-frozen) honey bee-collected pollen *ad libitum*. At the time of microcolony initiation, the bees must be given a pollen ball (i.e., ground pollen (2.5–4 parts by weight) mixed with sugar syrup (1 part by weight), typically 1 – 2 g, to facilitate nest building (Table S1). After a 1-week period, the bees should be given fresh pollen every 2–3 days. Ideally, pollen species and pre-existing pathogen and pesticide load in the pollen should be captured and the source kept constant through an experiment, if possible. This can be accomplished by thoroughly homogenizing all the pollen prior to the initiation of an experiment. While sterile pollen may be required in some cases, researchers should be aware that sterilization may alter pollen palatability. When applicable, pollen sterilization methods should be reported.

Delivery of test material —When investigating the effects of pesticides on microcolonies, the test material can be delivered in either the sugar syrup or the pollen. The exposure mechanism should be thoughtfully considered since delivery in the pollen will likely target the developing brood whereas syrup delivery could impact both the brood and the workers. Since microcolony development hinges on the presence of healthy workers, we advise investigators to conduct a range-finding study to identify doses that are not acutely toxic to the workers, unless information on chronic toxicity in adult bumble bees is already available. Also, when conducting pesticide exposure studies, evaporation controls should be incorporated into the study design to correct consumption estimates.

Study duration —Although the duration of microcolony studies will be determined by the specific needs of the investigator, study duration is ultimately limited by worker bee viability. When focusing on drone production, a minimum of 35 – 40 days from the time of nest initiation is required. Since it is difficult to understand the impact of resultant drones on brood tending, resource utilization and crowding, we recommend removing drones from the microcolony as they emerge unless subsequent drone mortality is a measured parameter. Due to the potential of founding worker mortality in microcolonies we recommend, for pesticide risk assessment, conducting studies for a minimum of 42 days and tracking worker mortality.

Microcolony monitoring —Although the parameters recorded will be influenced by investigator needs, we recommend collecting a core set of measurements including: 1) initial worker weight, 2) worker mortality, 3) syrup/pollen utilization, 4) days to 1st oviposition, 5) days to drone emergence and 6) the number and weight of drones produced. These measures are needed to establish parameters for microcolony control performance, the baseline for exposure-effects studies. Other measures of microcolony maturation are possible (i.e., eggs

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hatching, development of larvae, development of pupae); but microcolonies are structurally amorphous with newer structures obstructing older structures hidden beneath. Consequently, attempting to quantify various nest structures may be laborious and of limited utility.

Reporting —To facilitate data interpretation and to maximize study reproducibility, investigators are strongly encouraged to report the formulation of the syrup used for feeding, the pollen source and age, any pollen sterilization, the exact age and source of the worker bees and environmental conditions (i.e., temperature, relative humidity and light regimen) used. Start date, end date and specific dates of data collection for endpoints should be recorded and reported.

Data extrapolation and application in pesticide risk assessment

Bumble bee species vary in their nesting behaviors (Hobbs et al. 1962, Macfarlane et al. 1994, Knight et al. 2005), basic reproductive parameters (Asada and Ono 2000, Cnaani et al. 2002) and life expectancy (Goldblatt and Fell 1987). Within the same species, pre-experiment commercial-animal husbandry practices and colony quality (e.g., queen status, colony age, colony strength, colony life stage, disease status) vary, potentially impacting data interpretation and study reproducibility. Microcolonies are more easily standardized and provide a mechanism to study treatment effects on brood development while preserving elements of colony structure. This tool also has utility for investigating the effect of different routes of exposure (i.e., pollen vs. nectar) on larval development. Data from well-designed studies comparing outcomes observed in microcolonies and queenright colonies are essential to determining the suitability of microcolonies for hazard assessment.

Use of microcolonies for risk assessment also requires a better understanding of how species selection impacts study outcomes. The overwhelming majority of microcolony studies have been performed using *B. terrestris.* This species is native to Europe and is not available to North American investigators. *S*pecies differences have prompted some investigators to question whether, or not *B. terrestris* is a suitable surrogate for risk assessment performed on other bumble bee species (Gradish et al. 2013, Moerman et al. 2015). There are only two published studies comparing the sensitivity of different bumble bee species to pesticides (Baron et al. 2017, Wu-Smart and Spivak 2018), but the recent development of acute toxicity tests (OECD 2017b, a) for bumble bees and ongoing development of a chronic toxicity test enhance our ability to make important comparisons between species to inform risk assessment approaches.

Concluding remarks

Microcolonies are a useful tool for studies of bumble bee biology and for assessing the effects of stressors on these bees. Using bumble bee microcolonies, it is possible to conduct chronic exposure studies under defined laboratory conditions with the capacity to evaluate sublethal effects on adults and brood. However, researchers must exercise care by standardizing protocols (especially for risk assessment) as much as possible, fully reporting study design parameters, and collecting data on standardized microcolony endpoints. Additional investigations are needed to better understand the suitability of methods for each bumble bee species. Only then will the true value of this model be understood.

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Supplementary Material

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Abbreviations:

OECD	Office of Economic Cooperation and Development

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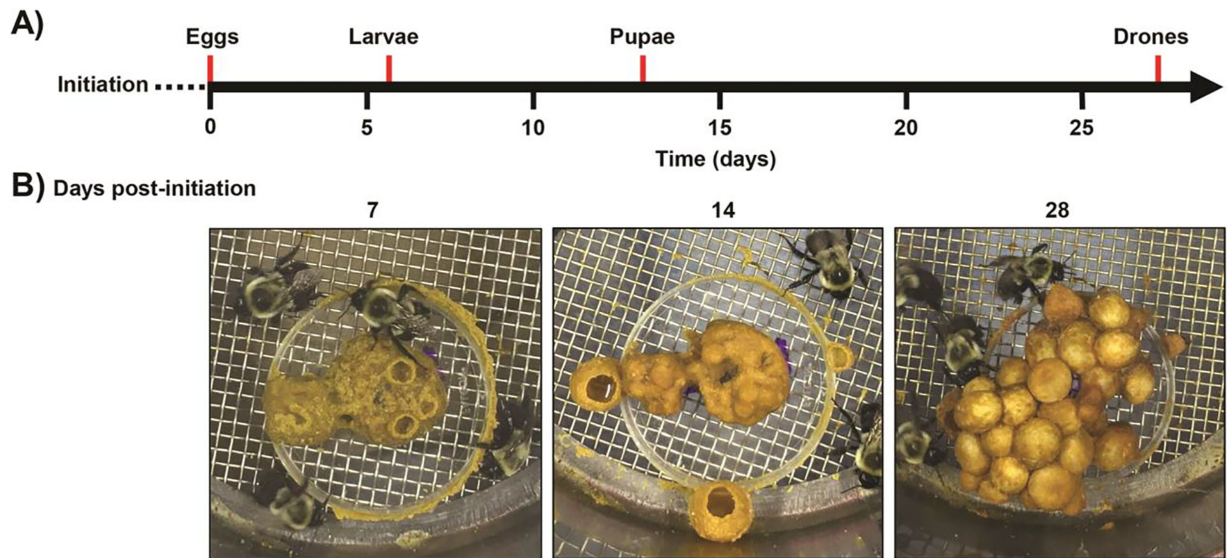

Figure 1. Microcolony development timeline.

A) Microcolonies were initiated with five callow *Bombus impatiens* workers and provisioned with a pollen patty (2 g) and syrup to promote nest building. After 7 days, microcolonies were fed fresh pollen paste and syrup every Monday, Wednesday, and Friday. **B)** Starting from nest initiation, photos show microcolony progression from uncapped egg chambers to pupal cells.

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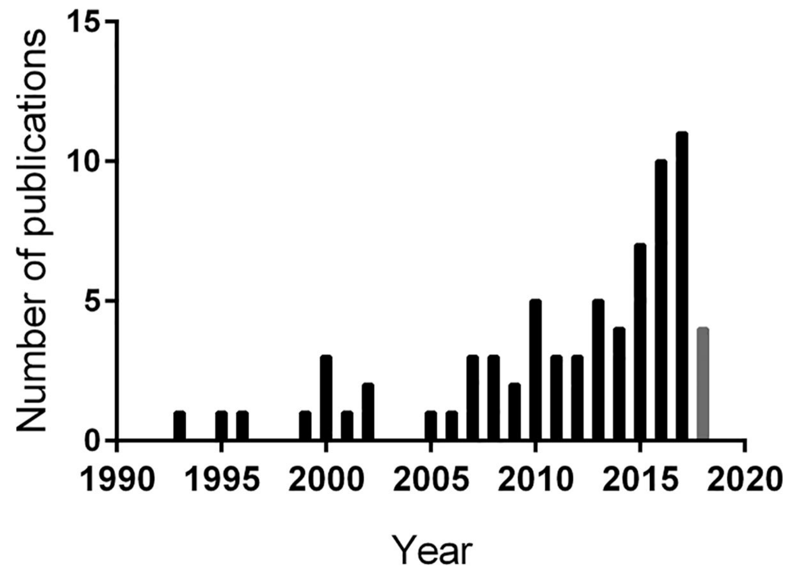

Figure 2. Number of microcolony publications per year.
Google Scholar, Web of Science and the Pro Quest Agricultural and Environmental Science Database were searched through November 2018 using a common search string. All results were compiled and organized by year of publication. Given that our search was completed before all publications for 2018 were available, the year 2018 is captured with a grey bar.

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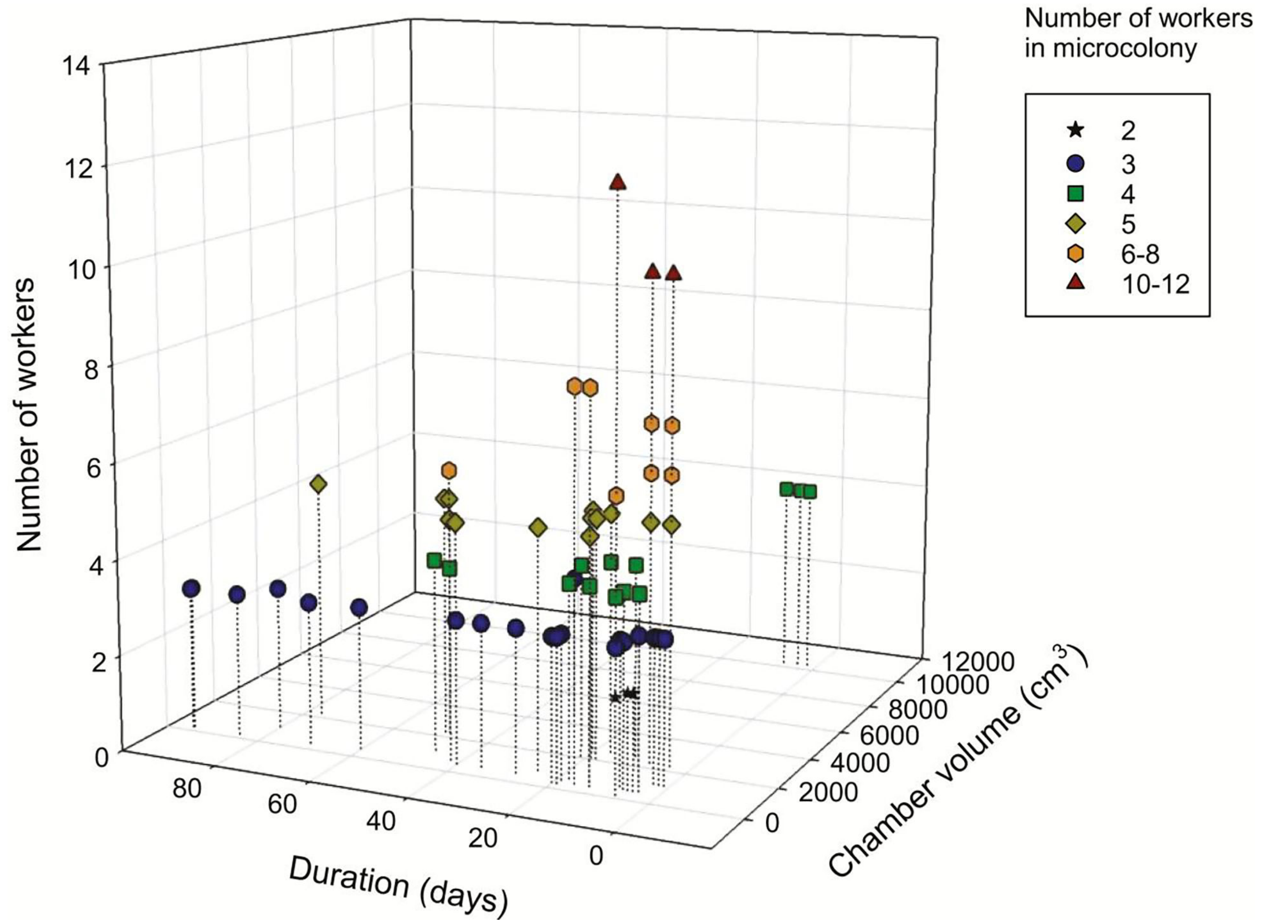

Figure 3. Microcolony design parameters.

Microcolony design parameters were extracted from research articles published through November 2018 (Table S1) and plotted to show the relationships between the number of workers, microcolony container volume and experimental duration. Only studies reporting data for all three parameters were included (i.e., 52 of the 75 published articles). When a study included microcolonies composed of different numbers of workers, each configuration was counted individually. If study duration indicated a specific number of days after egg laying, we assigned a value of 6 days for egg initiation.

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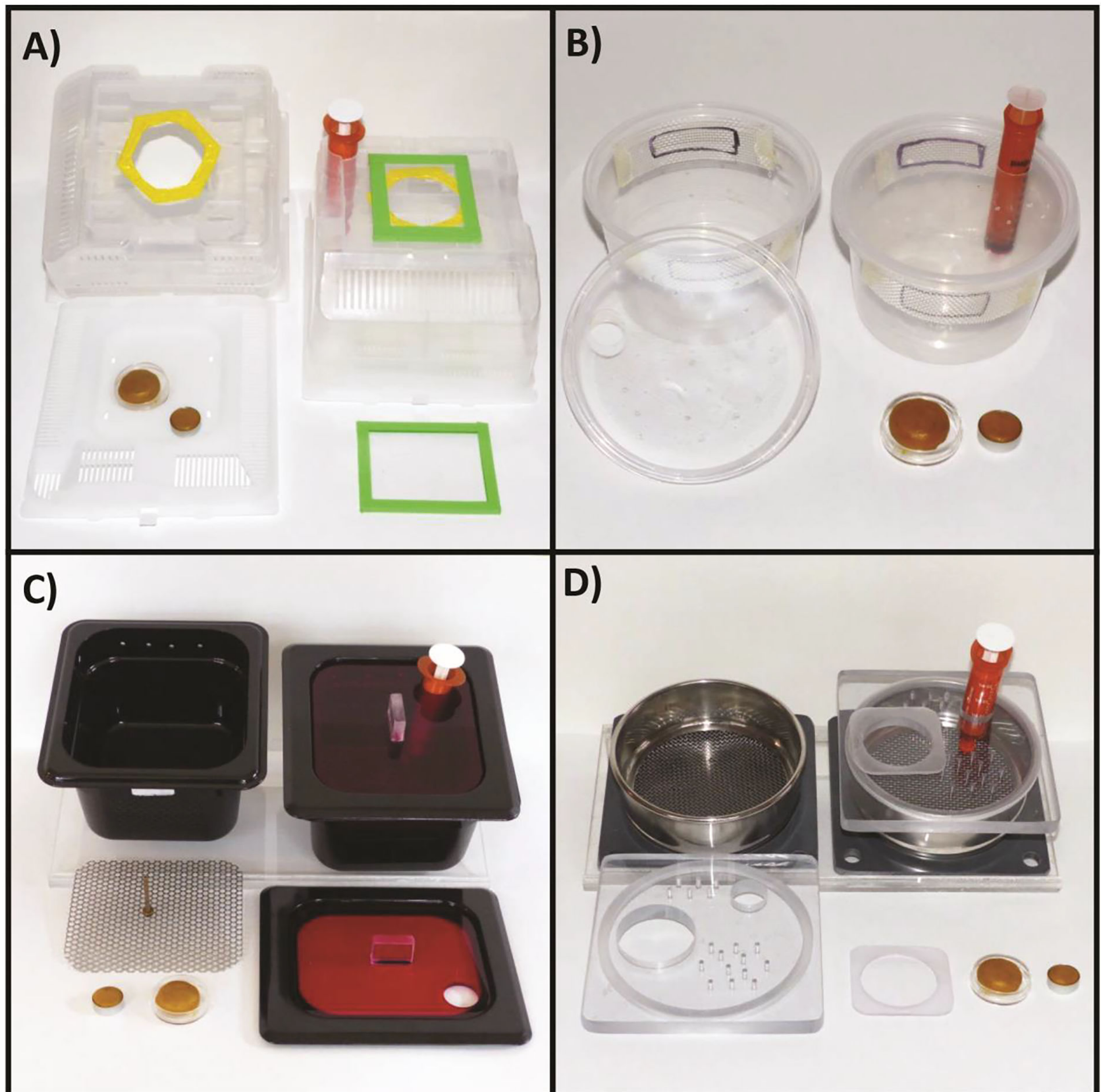

Figure 4. Example microcolony chambers.

A) "Queen box" (17.8 × 15.2 × 10.1 cm) used by commercial bumble bee vendors (i.e., Koppert, Biobest) with syringe feeder (USDA-ARS Pollinating Insect Research Unit; North Logan, UT). **B)** Disposable "deli cup" (16 oz, 11.4 cm tall × 7.6 cm diameter) modified with mesh to improve ventilation and to accommodate a syringe feeder (USDA-ARS Pollinating Insect Research Unit; North Logan, UT). **C)** Food pan (1/4 size, 12.7 × 11.4 × 10.1 cm) modified to include a raised perforated stainless-steel mesh floor to separate bee waste from the nest area, see-through red plexiglass lid, syringe feeder and holes for additional ventilation (US EPA, RTP, NC). **D)** Stainless steel geology sieve (4 cm tall × 12.7 cm diameter) with pass-through floor, ventilated baseplate, see-through lid and syringe feeder (US EPA, RTP, NC; design adopted from Bayer CropSciences). All container designs are

shown with petri dish containing a pollen ball for nest initiation and a separate dish for pollen feeding.

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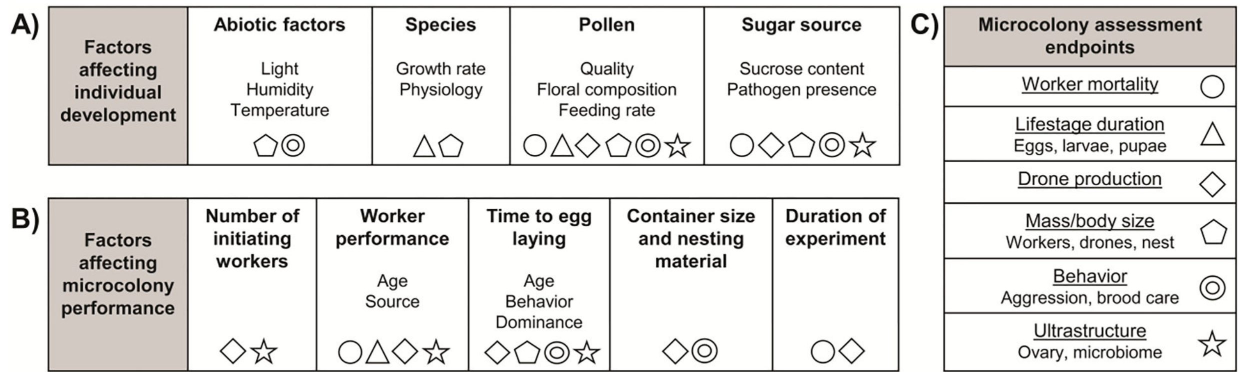

Figure 5. Relationship between microcolony endpoints.

Factors (in bold) known to affect individual bumble bee development **(A)** and microcolony performance **(B)** with some examples shown below. Symbols indicate microcolony assessment endpoints **(C)** impacted by these factors.

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Table 1.

Types of studies performed using microcolonies and number of publications through November 2018.

Primary study type	Number of publications
Behavior	6
Gut microbiome	4
Nutrition	21
Development	6
Pathogen	6
Chemical biology	8
Pesticide/xenobiotic	24