New agent-based risk assessment model for *Bombus*

Deliverable 8.3

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PoshBee

Pan-european assessment, monitoring, and mitigation of stressors on the health of bees

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Preface

To represent wild bees along with honey bees (*Apis mellifera*) in risk assessment modelling for EFSA in deliverable 8.4, deliverable 8.3 has sought to create a new bumblebee species (*Bombus* spp.) agent-based model. We have designed the simulation to be as generic for bumble bees as possible, and then we have initially parameterised for *Bombus terrestris*, the buff-tailed bumblebee (Figure 1) mainly using data from laboratory reared bees. To allow for the simulation to be parameterised against different sorts of data, we have split the simulation into three versions. Version one, bumble bees fed in the lab, version two, bumble bees placed in the field and version 3, wild bumble bees making their own way. This report will report on version one of the simulations.

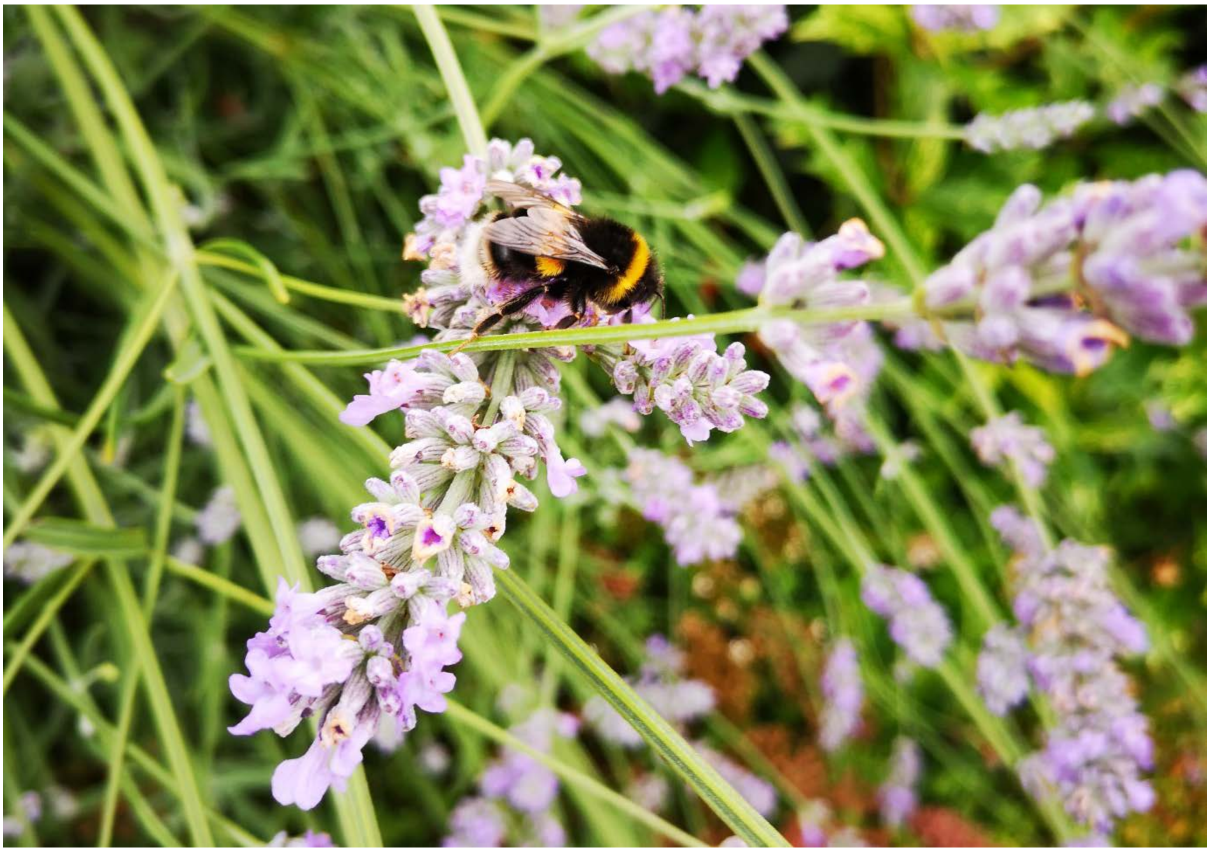

Figure 1. Buff-tailed bumble bee (*Bombus terrestris*) resting during rain in Hampshire, England.

Summary

There are approximately 250 known species of bumble bee (*Bombus* spp.) worldwide, which are most abundant in, although not restricted to, northern temperate zones, but with one species ranging naturally as far south as the southern tip of South America. There are 68 known species in Europe (Williams, 2000), and 65 within the European Union (Polce *et al.*, 2018). Of the 68 species in Europe, 10 are cuckoo bumble bees (*Bombus* (*Psithyrus*) spp.), and one a brood parasite *B. hyperboreus* (Gjershaug, 2009). In the rest of the species a sexually reproductive female (gyne) forms a social colony (eusocial) and becomes a queen (Free and Butler, 1959; Michener, 1974 as cited in Kells and Goulson, 2003; Goulson, 2010). The new *Bombus* agent-based simulation focuses on the eusocial bumble bee species and in the first instance will be parameterised for the common and widespread species *Bombus terrestris*.

The bumble bee simulation is an individual-based model that simulates the actions and interactions of every individual at any stage or age within a bumble bee colony. Bee individuals in this model are considered as software agents with the ability to sense their local environment and other entities in the simulation, and using this information take decisions altering their behaviour to fulfil their own agenda (e.g. survival, reproduction). All versions of the bumble bee simulation are built within the Animal, Landscape and Man Simulation System (ALMaSS), which is a landscape scale simulation system agent-based model framework (Topping et al., 2003). Using this framework will allow future versions of the bumble bee model to use simulated landscapes including nectar and pollen resources developed for the honey bee and solitary bee ALMaSS sister simulations.

Version one of the simulations takes place entirely within bumble bee colonies kept at known temperatures and provided with known quantities of nectar and pollen daily. The simulation has seven individual agent bumble bee life-stages: egg, larva, pupa, worker, gyne, queen and male. Gynes are young female bumble bees with the potential to become queens once they have mated, hibernated, and founded colonies. We define gynes as separate life-stages within the simulation to prevent gynes performing queen tasks within the colony of their birth or queens accidentally leaving the colony and hibernating for a second winter. The simulation also has clusters of juveniles and the colony as agents within the simulation. Having these two added agents has allowed for group mortality as well as actions to be performed on groups.

Individual agents have different behaviours which they can perform within a 10-minute time step. Each individual decides what behaviour it is performing based on their own context within the colony. Adults can affect themselves, each other, juveniles, and the colony. This includes changing the temperature of the colony or cluster of juveniles, supplying food, and dominating each other. Both the queen and the workers can lay eggs. The queen can lay female (diploid) and male (haploid) eggs, while the workers can only lay male eggs under certain colony, physiological and developmental conditions. The queen initially lays female eggs, which can develop into workers or gynes. The first eggs of the colony are normally destined to develop into workers. Later female eggs can develop into gynes if the queen does not switch to producing males first. Version one of the simulations is complete once these reproductive gynes and males appear, as these individuals cannot leave the colony and complete the species life cycle. Version one can continue to be further parameterised and tweaked as part of the iterative development cycle typically used to create complex models such as this. For

example, one such modification might be to allow colonies to only produce male reproductive adults, as currently the simulation can only produce males after the switch to gyne production.

1. Introduction

The ALMaSS bumble bee model is a relatively complex and detailed model and is developed using general approaches used for ecological models of this type. These approaches are based on an iterative modelling cycle (see Topping et al, 2010). In this specific case the modelling cycle (Fig. 2) is used to develop three versions of the *Bombus* model, each one providing the basis for the next.

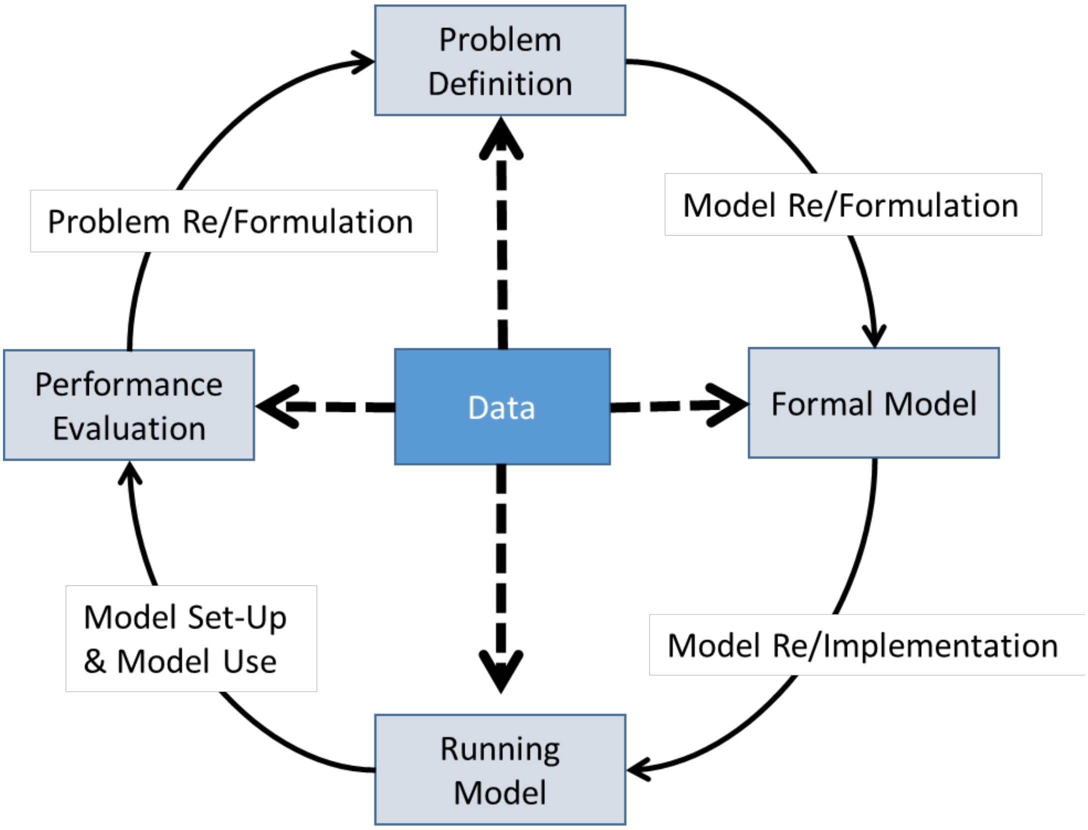

Figure 2: The modelling cycle, an iterative process for model development driven by data.

Hence, our problem definition changes with each model version and we iteratively move towards a final version, which can be used for risk assessment. For the reasons described below the transition from laboratory or wild colonies increases uncertainty with each step, hence our initial focus on version 1 of the model.

Collecting data on wild bumble bee colonies is difficult. Colonies are hard to find (Kells and Goulson, 2003), are often below ground (Liczner and Colla, 2019), are defended by stinging bumble bees and are often destroyed by being dug into. Wild populations of bumble bees are difficult to assess as workers do not represent individual reproductive units and therefore proxy methods such as genetics (to identify individuals from the same colony), modelling or using nest-searching queens are used to estimate population size (Liczner *et al.*, 2021). Observations of the inner workings of wild colonies are rare, with most having taken place during the early to mid-20th century (Lindhard, 1912; Sladen, 1912; Free and Butler, 1959). These problems are both a reason to use agent-based models to do risk assessment as well as a challenge when parameterising the simulation.

Scientists study bumble bee colonies in the laboratory and in artificial field colonies placed in the field which they have initiated in the laboratory. These studies are facilitated by the use of certain bumble bee species, notably *Bombus terrestris*, which are raised and used for commercial agricultural pollination both within and outside of its native range (Velthuis and Van Doorn, 2006; Rasmont *et al.*, 2008; Lecocq *et al.*, 2016). Due to this use and availability, we know the most about *B. terrestris*, which is why it is the first species being parameterised within our simulation. To allow us to best match the data from studies of *B. terrestris* in the laboratory (both wild collected and commercial), and artificial field colonies, we have split the simulation into three phased versions. Version one simulates colonies in the laboratory alone, matching laboratory colony experiments. Version two, which will place simulated colonies in known locations in the landscape to try and emulate artificial field colony boxes, including those from PoshBee work package one (see Deliverable D1.7). Then finally, version three will take what we have learnt in versions one and two and apply this to wild colonies. Version three can potentially use population level observations at the field scale to try and tweak the calibration of wild colonies away from commercial colonies, which are known to perform differently (Ings, Raine and Chittka, 2005; Ings, Ward and Chittka, 2006; Velthuis and Van Doorn, 2006).

2. Version 1 – simulated lab colonies

The simulated lab colonies start with a gyne who at once founds a colony with a defined number of workers and becomes a queen. The ambient temperature in the simulation emulates the room colonies are kept at in laboratory experiments. This is either defined as a single value, or as a value for each day, with the last day with a defined temperature repeated until the end of the simulation. So, for example, if provided with 28, 28, 22.5, 20, then colony would be 28 °C for the first two days, then 22.5 °C, and then 20 °C for the rest of the simulation run. These ambient temperatures should not be confused with the temperature in the colonies themselves, which are increased or decreased by the simulation based on the number of adults in the colonies. The colonies are provided with either a defined amount of nectar and pollen daily or, if trying to emulate *ad libitum* feeding, with very high quantities of each. The simulation duration can be set by the user.

2.1. Temperature

Adults and larvae can be set as either poikilotherm (“cold blooded”) or homeotherm (“warm blooded”). We usually run the simulation with both adults and larva as poikilotherms, which use less maintenance energy, and then adults are able to raise their body temperature with flight muscles if they have large enough energy reserves to raise their temperature above that of the surroundings. Maintenance energy usage for poikilotherms and homeotherms are calculated using allometric equations from Peters (1983). The simulation uses the mass of adults to scale approximately to surface area and then combines this with adult heat capacity to calculate how much the individual would cool towards the temperature of their surroundings within a time step. The energy that would be needed to raise the temperature back to the optimum for an adult is then calculated and requested from the energy stores of the individual. The proportion of the energy requested that is received is then used to work out how warm the individual is at the end of that 10-minute time step.

The colony temperature is calculated simplistically using the temperature of the colony on the previous day, a coefficient representing how insulated the colony is, the number of adults and a coefficient representing how much each can warm the colony. The simulation calculates the new temperature, and, if this temperature is over 26.5 °C, calculates the number of adults fanning to cool the colony. This results in very few workers fanning at temperatures only a little over 26.5 °C and does not affect colony temperature until the colony is warmer than the optimum colony temperature. The simulation assigns workers to the task of fanning, with the largest assigned first, as larger workers respond more immediately to raised temperatures (Oyen, Giri and Dillon, 2016; Oyen and Dillon, 2018). With enough workers fanning, the temperature is either optimum or below. If there are not enough workers, the temperature is reduced proportionally based on how many there are, but the colony is then above the colony optimum.

The queen and the workers inspect clusters of juveniles. If the temperature of the cluster is below optimum for juvenile development, the probability of the adult incubating that cluster is calculated. The more the temperature is below optimum, the higher the probability of incubating. The adult raises the temperature based on their own temperature, and the size, mass and heat capacity of the cluster. If the time it would take to warm the cluster is less than the 10-minute time step of the simulation, then the cluster is warmed to the optimum development temperature. If it would take longer, the temperature is warmed to the temperature that could be achieved within 10 minutes. Mortality of adults and juveniles is increased both below and above the optimum temperature. Temperature affects development time with development happening faster in warmer clusters.

2.2. Mass and energy

All bumble bee individuals have a mass. We have subdivided this into lean and fat mass, although larval individuals do not store fat within the simulation. Larvae and adults also have a stomach which defines how much and how quickly individuals can consume food. When ‘fed’, the larvae contain the food which they digest over time. Larvae and adults are hungry once their stomach is empty. They empty their stomach based on a digestion rate. This digestion rate is lower for energy rich food with a higher sugar content or amount of protein. Nectar has a volume, mass of sugar and an amount of energy. Pollen has energy and volume, and has a mass of the total, sugar, protein and fatty acids. The queen and workers are assumed to be able to sense if a larva is hungry when they inspect a cluster. If they find hungry larvae, they get food from the colony stores which the simulation refills daily in version one of the simulations and feed the larvae. The simulation can be parameterised so that adults feed the largest larvae first to evaluate different feeding strategies. Adults go and consume food if they have an empty stomach or if their lean mass is below the maximum that it has previously been, as this suggests they have been unable to get food in the past and have lost lean mass to maintenance and other essential task. Adults can refuse certain tasks if they have no fat stored, which we are using as a proxy for any stored energy over that of their lean mass. Chossat’s rule states that an animal dies when its weight loss approaches half its initial body mass (Peters, 1983 citing Kleiber 1961), therefore, if a juvenile or adult ends up with a lean mass that is less than or equal to half their maximum lean mass, they starve and die.

2.3. Mortality

Every bumble bee life stage and the colony have a probability of dying within a 10-minute time step, which is independent of direct known causes, such as starvation or, in later versions, direct depredation. The simulation can increase this probability of dying in any step based on any stressors, such as non-optimal temperature. In the future we may use this to increase mortality through disease and pesticides if direct mechanisms are not known. An example of direct mechanisms would be workers reducing their foraging activity, which could lead to starvation of themselves or others within the colony. If all individuals in the colony die, then the colony dies. In version one, the colony only dies if a death date is set to end the simulation. Within the simulation, clusters can also be destroyed, killing all the juveniles with it. In version one, a cluster of juveniles is only destroyed if the queen or workers decide to eat the young eggs in a cluster. The queen will do this if she recognises that the eggs are not her eggs and workers will do the same after the competition point. In both cases, this only happens in the first quarter of the development time of the eggs, which is approximately a day.

2.4. Juvenile development

The development times of juveniles and callow individuals are governed by the temperature of their immediate surroundings, that of the cluster or colony respectively. A number of degree minutes are needed to reach the next developmental stage. Individuals accumulate degree minutes over the minimum development temperature within each time-step. Once the individual has accumulated the amount of heat needed, the individual transitions to the next developmental stage (egg, larva, pupa, and callow adult). The callow adults are not a separate life-stage, but are adults that do nothing but eat and sleep. Using this approach allows egg development to vary between three and five days depending on the temperature, and callow individuals to take approximately a day to harden into adults. Larva degree minutes have been programmed so that they can vary over the lifetime of the colony to facilitate increased worker size as the colony ages. This increase in development time can then be used to have the females size tipping over a threshold into that of gynes. The degree minutes can be varied in three mutually exclusive ways: 1) an equation based purely on the number of workers in the colony, 2) an equation based on pheromone signals in the colony which are diluted by additional workers, and 3) by the queen directly reducing the degree minutes of larvae she directly interacts with. The result of all three is that as the number of workers in the colony increases, the development of larvae takes longer, and they can potentially get larger if there is enough food. Gyne larvae are female larvae that remain close to the maximum degree minutes for the first number of days, also defined by degree minutes accumulated. Male larvae have a fixed number of degree minutes needed. An individual’s pupal degree minutes are half what the degree minutes of that larva were.

2.5. Wax and colony growth

The queen and the workers add wax to the colony by converting energy reserves to wax. They use this wax to build the first nectar pots as well as to contain the juvenile clusters, initially and as the larvae in the cluster grow. Currently the queen and the workers do not recycle the wax within the simulation, but we consider this a minor energetic inaccuracy. Limitations in the energy reserves of the queen and workers limits wax production and also reduces the number of eggs that can be laid within a day to below the maximum that is possible. The energy needed for each egg is taken from body fat. Eaten eggs return calories and protein to an adult.

2.6. Switching and competition point

Currently the switching point of the colony happens only when gynes appear, with male eggs being laid once the queen encounters a gyne larva. We acknowledge this cannot be the only way the switching point happens, as some colonies can produce only workers and males. The current plan is for an additional switch to make male only production possible, independent of any switch to gyne production. If the males appear very early, the females will remain small and no gynes will appear, and the colony will only produce workers and reproductive males. If male production happens later, a mix of workers, gynes, and males will appear in the colony. If the colony starts to fail before males appear, irrespective of whether gynes have been produced, very few or no males will appear. In the current version without the independent male switch, gynes naturally appear when the number of workers is high enough. The queen and workers can detect when they have encountered gyne larvae. Each individual may notice gyne larvae at different times. Once the queen notices, she will switch to male eggs. The workers will do nothing until they have not encountered anymore gyne larvae for a defined number of steps or days. At this point, if the worker has developed ovaries, they will compete with the other females of the colony by attempting to lay their own male eggs and they will eat the young eggs of other females. Presumably, if the queen starts producing males in a colony without gynes, the workers will also then begin to compete, but this remains to be modelled.

2.7. Ovary development

Every time step the queen and workers interact with a set number of other females. They dominate the individuals that are less aggressive than they are, using proportional ovary size as a proxy for aggression. With negligible ovaries, the larger individuals dominate. If both individuals are equally aggressive, neither is dominated. In most cases, the queen will always dominate any individual she encounters, but later in the colony life, when workers are equally aggressive, it is dangerous for the queen to provoke confrontation, therefore neither will be dominated. If a worker has not been dominated enough times within a 10-minute time-step and has excess protein, they can add mass to their ovaries proportionally to the excess protein up to a maximum amount per time step. Workers can also lose ovary mass if they are dominated or if there is an energy deficit.

2.8. Sleep

Adults need at least four hours sleep and preferably eight hours (Nagari *et al.*, 2019). The simulation increases the probability of sleeping when the day is getting closer to ending and the individual still needs sleep. Workers and the queen within the colony sleep on and off throughout the day, sleeping and waking based on probability, with no sustained sleep period. Workers and the queen can get by on only four hours sleep, callow adults, gynes and males always sleep eight hours at night-time. Workers and the queen can sleep while they incubate and, if clusters need incubating, they will incubate if they need sleep. If there are no tasks to do, the workers and the queen will get some extra sleep up to the amount wanted, but not needed.

3. Calibration of version one of the simulations

Version one of the simulations has been configured using data from several sources. There are over one hundred variables that can be changed within the simulation. Many parameters can be set using values from the literature, but other values will need to be calibrated based on the best fit within realistic range limits. Cedric Alaux (INRAE) has provided data of increasing worker numbers as the colony ages at 28°C and we have data at 20°C and 25°C from Holland et al. (2015). Some of these data give different initial temperatures that the colonies were kept at once the colonies were received from suppliers, although we do not know temperatures during transportation. This is one of the reasons the simulation can vary temperature by day. We also have data on the size distribution of individuals within colonies kept in the labs at Trinity College Dublin by Róisín Barrett. The data from Cedric Alaux also provides information on development times that we can attempt to emulate.

Calibration followed the human-guided *ad-hoc* process for pattern-oriented modelling described by Topping, Høye, Olesen (2010). This process requires an initial variation of the value of all parameters individually to see which have an effect on which output. This is also essential as it allows parameters that may work against each other or in conjunction to be found, to ensure that we do not move one output value away from the test value while trying to improve the fit of another test value. In this case it may be possible to use a third parameter to move one back towards a desired output value without influencing or countering the effects of fitting the first parameters. Once initial tests are carried out parameter values are determined by an iterative process of changing the values and evaluating the output responses. This is aided by creation of target precision values for multiple outputs at once and creating a least-squares fit statistic (weighted towards the least uncertain targets). Fitting continues until a satisfactory fit is obtained and then all parameters are varied across their reasonable range to create a measure of sensitivity.

The results of the calibration process and the full description of the model are available as a living document at https://almass_test_group.gitlab.io/ALMaSS_all/_bombus_page.html This documentation is in ODdox format (Topping, Høye, Olesen 2010) allowing not only a description of the processes and variables but also providing access to the program code. This facilitates access to complex model codes. Full code is available from the ALMaSS gitlab project https://gitlab.com/ChrisTopping/ALMaSS_all/-/tree/bombus_10minStep (access requires that you create a free GitLab account).

In addition, a formal model description is under preparation for publication in the Food and Ecological Systems Modelling Journal. The formal model documentation is a more formal way of describing equations used for processes in the model, parameter values, and the literature support from them.

4. Future version

The future versions of the simulations will take the modelling beyond pristine colonies of bumble bees in the laboratory. Pesticides, parasites and disease will be included. This can take place in the laboratory version of the simulation, or in the initial move out of the laboratory. Moving the colonies out of the laboratory version will first be into artificial boxes at known locations in the field. These boxes will then be subject to changes in the air temperature and bumble bees will be able to forage for nectar and pollen within the landscape. Work package one of PoshBee (A site network for assessing exposure of bees to chemical, nutritional, and pathogen stressors) collected data on artificial colony boxes in known locations. We can use these data and other similar data to parameterise the second version of the simulations. Following this, the third version will turn the bumble bees loose. The simulation will start with gynes that will be hibernating underground, and will awake and emerge once the temperature rises. The gynes will then forage and find a location for, and then found their colony. They may alternatively attempt to steal a colony from a conspecific queen. The colony will then run similarly to versions one and two, before the gynes and males will leave the colony. It will be assumed in version three that all gynes will find a mate, without the need for gynes and males to interact. The mated gynes will prepare and search for a hibernation site and sleep through the winter. At this stage the simulations can be run for multiple years, with the cycle of colony and hibernation repeating.

Beyond these initial simulation development versions, the simulation could be expanded to other species, but also to different questions. The rudiments of a record of parenthood already exist within the simulation, with each individual knowing the ID of their parents. This could be expanded to look at simulated pseudo-genetics. This would also allow for inbreeding and the consequent result that eggs that should develop into workers, emerge instead as inbred males. The simulation can also be expanded to look at different species, potentially including cuckoo bumble bees (*Bombus* (*Psithyrus*) spp.).

5. Conclusion

Version one of the simulations includes the complicated eusocial phase of the colony, and the majority of the complexity in all three versions of the simulation. This version is valuable in its own right as it allows for analysis and increased understanding of bumble bees. As such it could be used for other bumble bee species kept in the laboratory, even where we do not have a landscape data or data on bumble bees in the wild. Having matched laboratory data on *B. terrestris* in the first instance, and other species in the future, the later versions can then extend these species into landscapes even where data are lacking. This would give an approximation of what may happen to different species in the wild based on different landscape management. However, the version used for PoshBee Tasks 8.4 & 8.5 (‘Integrative analysis of bee health and production of tools for risk assessment’ and ‘Develop a multi-species Environmental Risk Assessment (ERA) tool for evaluating the potential effects of agrochemicals or farming practices on bees’ respectively) will use the *B. terrestris* parameterisation of the model to represent bumble bees generally.

6. Acknowledgements

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