

PROTOCOL			
Title of protocol:	Rearing captive research colonies of the bumble bee, <i>Bombus terrestris</i>		
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<p>Protocol:</p> <p>Note: You must have read and signed the bumble bee-handling risk assessment form before following this protocol, and use/wear safety equipment and/or clothing as directed by the risk assessment.</p> <p><u>A. Receiving colonies and preparing nest-boxes</u></p> <p>1. The colonies are generally supplied by commercial suppliers as a nest of adults and brood each housed in a plastic box enclosed within a cardboard box (with several colony boxes being enclosed in a bigger cardboard box). The brood mass will be surrounded by cotton wool unless the supplier is contacted beforehand and asked not to include it. Cotton wool is recommended for orders delivered in winter and early spring because it keeps the bees insulated during transport from the supplier; however, because it can be fiddly to remove, it adds to the time of processing colonies when received. After bringing the colonies (housed in this way) from the point of delivery into the culture facility, do the following. (At UEA, the culture facility is generally the Controlled Environment Facility [CEF], a suite of CT [constant temperature] rooms, or one of the CT rooms on the BIO 01 corridor.)</p> <p>a. Unpack the colonies from the bigger cardboard box(es) and move the colonies into a constant temperature (CT) room, with conditions of temperature, humidity and lighting as in point C1 below.</p> <p>b. Check each colony is alive, queenright, and in good condition, and fits what was requested in the order. If the colony does not contain cotton wool, then the bees and the brood can be observed by opening the cardboard lid (without opening the plastic lid). If the colony does contain cotton wool, you will also need to lift this to examine the colony underneath.</p> <p>c. Remove the caps on the nectar tanks if necessary. The plastic box sits on a plastic container filled with artificial nectar that is attached to the colony via a 'wick' that the bees drink from; however, access to the artificial nectar is usually prevented during transport from the supplier via a plastic cap, so it may be necessary to remove this cap when the colonies arrive to allow the bees access to the artificial nectar.</p> <p>d. If there are any defective colonies, take photographs as evidence and then notify them to the supplier for return/replacement.</p>			

2. Raise the lid on each cardboard box (for ventilation) and leave the bees in the CT room without further disturbance for a period of 24 hours.

3. Prepare a supply of clean, wooden nest-boxes (UEA ones are of internal dimensions, 17 cm × 27.5 cm × 16 cm high) and fit each with a clear acetate rim (to form a barrier to bees trying to escape by climbing up the interior walls), a clear Perspex lid and a clean plastic wick holder. The wick holder should be snugly pre-fitted into the hole at the base of the nest-box, and can be drawn from a store of clean ones collected from the artificial nectar containers supplied in previous deliveries of colonies.

An effective way to fit the wick holder is to tap it in by using a mallet applied to the end of a length of wood (e.g. headless hammer handle) the opposite end of which is held resting on the wick holder. Once this is done, stand each wooden nest-box on a container of artificial nectar (generally a sealed plastic flatpack provided for each colony by the supplier) and ensure the wick is securely protruding through the floor into the interior of the nest-box (via the hole in the floor). When preparing the container with the nectar in advance, and using a clean, dry wick, soak the wick in artificial nectar before using, squeezing any air out with forceps, as it will not deliver the artificial nectar to the nest-box interior if it has not been soaked first. When using a container of artificial nectar supplied with the order, first remove it from the colony by taking the plastic box containing the colony out of its enclosing cardboard box and then detaching the artificial nectar container. Then remove the plastic wick holder supplied with the container and either discard it or retain it for cleaning and adding to the store of clean wick holders for future use. Use clean forceps to transfer the soaked wick to the wooden nest-box. Then use a clean Stanley knife to carefully remove the protruding moulded rim that housed the wick and wick holder, which will allow the wooden nest-box to sit flat on top of the nectar container. If using this method, ensure that the wick and artificial nectar container are taken from the same colony that will later be transferred into a given wooden nest-box.

4. Fill a small Petri dish (deep wall and 2.5 cm diameter is best) with dried pollen, and place this within the wooden nest-box.

5. Number both the external wall of the nest-box and its Perspex lid with an identifying number or code, e.g. written on pieces of masking tape.

B. Transfer of colonies into rearing boxes (wooden nest-boxes)

1. For transferring colonies from the supplier's boxes into the prepared wooden nest-boxes, wear beekeeping gloves and a beekeeping suit and veil. Do the transfers in the CT room under red light, as the bees are likely to fly in normal white light. Remove the plastic box in which the bees are contained from within the enclosing cardboard box. Slightly open the plastic lid to access the bees. If the colonies came with cotton wool, use a pair of long forceps to gently remove this first, also using the forceps to pick off any workers that are on the cotton wool and transferring them temporarily into a separate container, e.g. a glass conical flask sealed with a foam stopper.

When working with open nest-boxes, whether during the transfer process or at any other time, avoid as far as possible exhaling over the adult bees, as on detecting a person's breath they can become agitated.

2. Transfer the remainder of the colony in the order: all remaining workers; the queen; and the brood mass. The queen will generally remain on the brood during the worker transfer, and should be the last adult to be transferred. Handle each adult bee by catching it by a rear leg

using long forceps. Workers may alternatively be handled with the forceps gently clasping the thorax, but this is not advisable for the queen.

a. Transferring workers: When transferring the workers, watch out for escapes. It will usually also be necessary to keep a count of the number of workers transferred so that colony size can be recorded. (Transferring the workers can also be combined with marking them -- see below -- if required, though in general workers should not be kept separate from one another or the brood mass for too long.) Catch each worker one by one and place it in the conical flask.

b. Transferring the queen: Once all workers have been transferred out of the supplier's box, catch the queen (by holding her by a rear leg with the forceps) and then transfer her to either: (i) a separate conical flask; (ii) the flask with the workers, especially if the colony is a small one; or (iii) straight to the wooden nest-box, where she can be reunited shortly afterwards with the brood mass. If method (iii) is used, care must of course be taken not to damage the queen when adding the brood mass (whether on the plastic platform or in sections).

c. Transferring the brood mass: Once all adult bees have been removed from the supplier's box, remove the lid from this box to give yourself easy access to the brood. Either lever out the entire plastic platform (that has the brood attached to its upper surface) and place it on the floor of the wooden nest-box, or carefully lever off the brood from the platform in sections using a spatula and transfer it section by section to the floor of the wooden nest-box. If the former method is used, then use a clean Stanley knife to remove the moulded plastic tabs on the bottom of the platform, along with any adhering polystyrene, to allow it to sit flush to the floor of the nest-box.

There are advantages and disadvantages with each of these methods. In orders provided by some suppliers, the brood platform is quite wide, and so there will be only narrow gaps between its margins and the interior faces of the side walls of the wooden nest-box (of dimensions stated above). Such gaps provide places for the bees to hide or get stuck in, and can be harder to keep clean. In addition, with all sizes of platform, there is a risk that bees access the underside of the platform and become stuck, and/or lost. While these problems are avoided by levering the brood from the platform in sections and transferring it to the nest-box, this latter method inevitably causes disruption of the brood mass. It is rarely possible to transfer the mass in one piece, and transferring it in sections necessitates reassembling it, a process during which some larvae may fall out of the comb. These larvae suffer mortality, because workers will deposit them in a midden in a corner of the nest-box. Losses of brood from this cause may be substantial if particular care is not taken, and for this reason the method of brood mass transfer used may depend on the type of experiment being undertaken.

d. Reuniting the adults with the brood: Once the brood mass has been completely transferred, slide back the Perspex lid of the wooden nest-box and, by gently tipping the conical flask(s), add the queen (if she was in a separate flask and not already added), queen + workers (if in a flask together and again if the queen was not already added) or, once the queen is in the nest-box, the workers alone.

3. Checking the colony: Over the next 24 h, check that the queen (and most of the workers) quickly return to the brood and that the colony appears to be behaving normally. The workers should be taking pollen from the pollen dish (the queen may join them in this) and accessing the artificial nectar via the wick. If the artificial nectar is properly accessible, it should be possible to see some stored in the brood-cells used as honey pots (they will be half-full of liquid with a glistening surface). The queen should start building egg-cells, often in a linked chain. These indicators will confirm that the transfer has been successful and the colony is in good condition.

C. Colony Maintenance

1. Each nest-box should be housed on a table or shelf within a CT room, maintained at standard conditions of 26-28°C and 60% relative humidity. Nest-boxes can be housed in darkness (with the researcher switching on red-light lamps when performing maintenance or experimental activities as necessary), or under constant red light. (In the UEA CEF, check the indicator board in each room's lobby regularly to ensure that these conditions are maintained.)
2. Check pollen dishes at least every 2 days, or daily for large colonies, and top up as necessary. All checks should be done under red light if possible (using red-light head torches); however, white light can be used to check the food status, as brief periods of low-intensity white light do not disturb the bees greatly. Keep disturbance to a minimum when replenishing the pollen. If checking intervals are greater than every 2 days (e.g. over a weekend), then provide additional dishes of pollen. The supplier's containers of artificial nectar usually each hold enough artificial nectar to keep a colony fed for the duration of the colony cycle, but periodically check that sufficient artificial nectar remains in the container and in the colony's own stores (within the cells used as honey pots). Unless otherwise required, aim to ensure an *ad libitum* supply of pollen and artificial nectar throughout a given rearing cycle or experiment.
3. The bees will use the corners of the nest-box as latrines and middens (e.g. for dead adults and larvae). Keep these areas clean by using a spatula and/or forceps. Clean equipment after cleaning out each colony to prevent cross contamination. For excessively wet waste, use sawdust to soak up the moisture before cleaning as described, or wipe away with a ball of paper towel held in forceps. It is usually sufficient to conduct a full colony clean once a week.
4. Check the colony regularly for the appearance or build-up of pests, e.g. booklice (Psocoptera), mites (Acari) and moths (a common species in captive colonies is *Plodia interpunctella*, but be vigilant for other species too). If any pests are found to be contaminating a colony, remove and destroy them and clean the nest-box interior as thoroughly as possible. Likewise, regularly check the inner walls and fittings of the CT room housing the nest-boxes for pests (especially the corners of the room and the benches), including adult, pupal or larval moths, and destroy them.
5. Once the colony begins to produce adult sexuals (i.e. there is eclosion of new, adult queens and males), remove these individuals from the colony regularly (unless the experimental protocol stipulates otherwise), as in nature they would leave the colony after a few days.

If fed well and regularly cleaned, and if adult sexuals are removed, colonies should remain healthy until the end of their colony cycle, with the workers maintaining a clean comb themselves. Note, however, that in older colonies the wax of the comb may darken in colour.

D. Individual marking of workers

1. Use a wooden toothpick or cocktail stick to push a set of numbered paper disks (as required) from the grid of numbered disks onto the desk surface so that they rest with the numbered face upwards.

The easiest numbers to read are in black font on a pale background such as white or yellow. Avoid white font on a red background, as the numbers will be unreadable under red light. Disks made of card (as opposed to plastic) seem to stick better to the bees and are easier to read (because they are less reflective) during playbacks of digital film of marked bees.

If the intention is to mark newly-eclosed (callow) workers, note that callows retain their silvery coat colour (before the full adult coat colours appear) for only 12-24 h after eclosion.

2. Use a pair of long forceps to remove an unmarked worker from its colony's nest-box and place it in a honey bee queen-marking cage. (This is a commercially available device consisting of a clear plastic tube with a removable, broad plastic mesh cap at one end and a foam-lined plunger at the other.) Manipulate the cage and the worker within it so that the worker's dorsal surface is held in position against the inner face of the mesh. This can be achieved in two ways: (a) by gently agitating the worker to make it adopt a threat posture in which it lies on its back on the mesh, then restraining it in that position by slowly inserting the plunger; or (b) by allowing the worker to stand on the foam of the plunger and then slowly inserting the plunger with the worker on it until the worker's dorsal surface contacts the mesh. The dorsal side of the worker's thorax should be poking up slightly through the mesh.

3. While holding the worker in place in the cage with one hand, use your free hand to open a bottle of marking glue and then to dip one tip of the toothpick/cocktail stick in the glue. Next apply (by gently rubbing) a small amount ($< 5 \mu\text{l}$) of glue onto the part of the worker's thorax that is poking through the mesh. This works best when the hairs are gently brushed out of the way (or even scraped off with a scalpel).

4. Working swiftly, use the other tip of the toothpick/cocktail stick (i.e. the tip without glue on it) and wet it in a small container of water. Pick up the numbered disk with the wet tip of the cocktail stick (the disk will adhere to it). Use the cocktail stick, transfer the paper disk to the still-wet glue on the thorax of the worker. Ensure the numbered part of the disk is still pointing upwards and position the disk on the worker's body so that the numbers are orientated relative to the bee's body in a way that makes them easy to distinguish from one another, e.g. so that 6's are distinguishable from 9's. Use the stick to hold the disk in place for at least a minute for the glue to dry.

5) Slide the plunger away from the worker and transfer the worker back to its nest-box (using long forceps if required). If you pressed too hard on the plunger this can temporarily immobilise the worker; however, it will usually become active again after a few minutes of being back in the colony.

6) Repeat this process until all bees in the colony have been marked, or as a given experiment demands. Ensure that no two bees have the same number, or if this is unavoidable, ensure that different coloured disks are used to distinguish them. If required, record the total number of marked bees, and the identity of each marked bee, in a given colony.

Main equipment/consumables needed:

Colony rearing

- A4 acetate sheets (for making nest-box rims)
- Artificial nectar container (plastic flatpack from commercial suppliers)
- Beekeeping gloves
- Beekeeping suit
- Beekeeping veil
- Conical flask, glass (for temporary housing of bees)
- Forceps, long (for handling bees)
- Mallet and separate length of wood, e.g. wooden hammer handle (to insert wick holders)

- Nest-boxes, wooden
- Nest-box rim template (Perspex frame for use in cutting out acetate rims to correct size)
- Perspex lids (for nest-boxes)
- Petri dish, small, deep
- Pollen, dried (from commercial suppliers)
- Rubber bungs (for sealing any holes in nest-boxes)
- Scalpel
- Spatula
- Stanley knife (for cutting acetate etc.)
- Tape, i.e. duct tape (for sealing any gaps/holes in nest-boxes)
- Wicks and wick holders (for delivering artificial nectar from container to nest-box)
- Wire gauze (for covering ventilation holes in nest-boxes)

Individual marking of workers (additional equipment)

- Disks for marking, individually numbered
- Glue (for affixing disks)
- Honey bee queen-marking cage
- Toothpick / cocktail sticks, wooden (for applying glue to bees when marking them)

Further information:

Carnell JD, Page S, Goulson D, Hughes WOH (2020) Trialling techniques for rearing long-tongued bumblebees under laboratory conditions. *Apidologie* 51: 254-266.

Velthuis HHW, Van Doorn A (2006) A century of advances in bumblebee domestication and the economic and environmental aspects of its commercialization for pollination. *Apidologie* 37: 421-451.