Received: 11 July 2024 | Accepted: 7 February 2025

DOI: 10.1002/2688-8319.70052

BRITISH ECOLOGICAL SOCIETY Ecological Solutions and Evidence

PRACTICAL TOOLS

The BumbleBox: An open-source platform for quantifying behaviour in bumblebee colonies

August Easton-Calabria | **James D. Crall**

Department of Entomology, University of Wisconsin-Madison, Madison, Wisconsin, USA

Correspondence
August Easton-Calabria
Email: eastoncalabr@wisc.edu

James D. Crall
Email: james.crall@wisc.edu

Funding information
National Institute of Food and Agriculture, Grant/Award Number: 2019-67012-33596, 2022-67013-36275 and WIS04062; Winslow Foundation; Feir Fellowship; Wisconsin Alumni Research Foundation

Handling Editor: Natalia Escobedo Kenefic

Abstract

1. Bumblebees (Apini: *Bombus*) are important pollinators globally and an emerging model system for studying the ecology and evolution of social behaviour and effects of environmental stressors on bees.
2. Behavioural studies of bumblebees have conventionally relied on labour and time-intensive manual observations. While recent years have seen rapid advances in automated behavioural tracking in social insects, these tracking technologies are often expensive and require extensive programming experience, limiting accessibility and widespread adoption.
3. Here we introduce the BumbleBox, an open-source system for the tagging, automated tracking and behavioural quantification of individual bumblebees that can be built using low-cost consumer components, DIY fabrication (i.e. 3D-printing and laser-cutting) and printed 'Augmented Reality University of Cordoba' (ArUco) markers. We provide an integrated pipeline for data collection and analysis, including nest arena design, software for automated collection of video data and the quantification of individual behaviour.
4. *Practical implication*: The BumbleBox system is designed to be (a) *accessible*, requiring no prior experience with programming or hardware design to operate; (b) *scalable*, allowing long-term, automated tracking across many units in parallel at low cost; and (c) *modular*, allowing for flexible adoption to unique applications in bumblebees and other systems. We validate the use of this system in a widespread bumblebee species (*B. impatiens*) that is both commercially and ecologically important. Finally, we highlight widespread potential applications in quantifying behaviour and pollinator health in bumblebees and other social insects, including screening impacts of pesticides and other environmental stressors on social behaviour.

KEYWORDS
automated behavioural tracking, low-cost, open-source, pollinator health, social insect behaviour

1 | INTRODUCTION

Social bees are critically important for the functioning of ecosystems and the delivery of pollination services across the globe (Klein et al., 2006). Bumblebees (Apini: *Bombus*) are a diverse group of generalist pollinators that are among the most abundant pollinators globally [~260 species (Cameron & Sadd, 2020)]. Bumblebees are economically important for crop pollination (Fijen et al., 2018) and an emerging model system for studying social behaviour and the effects of environmental stressors on bees (Amsalem et al., 2014; Baer, 2003; Easton-Calabria et al., 2023; Gill et al., 2012; Woodard et al., 2015).

Automated tracking and behavioural quantification within social insect colonies has advanced rapidly in recent years (Boenisch et al., 2018; Crall, Switzer, et al., 2018; Mersch et al., 2013; Sclocco et al., 2021), providing fundamental insight into social interaction networks (Richardson et al., 2017), disease dynamics (Stroeymeyt et al., 2018) and effects of anthropogenic stress (Easton-Calabria et al., 2023). However, methods for automated tracking are often expensive and computationally intensive, requiring substantial experience in hardware design and computer programming. The emergence of open-source, inexpensive and low-power electronics, however, is making scalable quantification of social behaviour feasible and accessible, especially for remote fieldwork. This creates new opportunities for standardized, reproducible methods of automated behavioural quantification to address key knowledge gaps in bumblebee behaviour, ecology and health. Making use of these new technologies could particularly benefit bumblebee behavioural research in parts of the world where species are understudied due to resource constraints relative to species in the United States and Europe.

Here, we introduce the BumbleBox, an automated, open-source hardware and software imaging system for automated tracking and behavioural quantification of uniquely identified bumblebees within the nest. The BumbleBox system is designed to be (a) accessible, including open-source design and low cost, (b) scalable, with capacity for parallel, automated data analysis across multiple units and extended time periods, and (c) modular, with adaptability for different experimental contexts and applications. We first describe the basic structure, function and operation of the system and then describe the integrated pipeline from arena design and experimental collection of video data to the quantification of individual behaviour. Finally, we demonstrate the application of this system for automated tracking of individual behaviour within bumblebee colonies and explore its use in different experimental contexts and applications (e.g. across multiple species in the laboratory and field).

2 | MATERIALS AND METHODS

The BumbleBox is designed to provide automation of experimental tasks such as video recording, tracking and behavioural quantification within bumblebee colonies on the Raspberry Pi (Saunders et al., 2022). A primary goal of this system is accessibility for researchers and practitioners across fields, and a detailed guide for construction, installation and use is provided at https://github.com/Crall-Lab/BumbleBox/tree/master. The following sections review the BumbleBox's operation and capacity.

2.1 | Design and hardware

The BumbleBox consists of a nest arena and imaging module for automated recording (Figure 1a). The nest arena (154 × 154 × 275 mm) houses the bumblebee (*Bombus* spp.) colony, allows for viewing and feeding of the colony and accommodates imaging under controlled infrared lighting conditions. It is fabricated from 3D printed and laser cut components because of (a) the increasing availability of these fabrication techniques (e.g. open-access maker spaces or online fabrication services), (b) cost-effectiveness (total material costs: ~$350 USD) and (c) modularity and customizability.

Imaging within BumbleBoxes occurs via the Raspberry Pi high-quality camera (RPi HQ, 12 megapixels, 4056 × 3040 pixels), which is mounted on top of the device (Figure 1a) and provides recording of the colony viewed from above. Within the nest, illumination is provided by infrared LEDs (840 nm), as bees cannot perceive near-infrared light (Martínez-Harms et al., 2010). To provide imaging in the near-IR spectrum, the RPi HQ camera is modified to remove the IR-blocking filter.

2.2 | Individual bee tracking

The BumbleBox uses ArUco (an OpenCV module, implemented in Python) markers to capture the position of individual bees within the nest. ArUco marker detection software is open-source and community-maintained, and the markers can be printed on paper and detected at small sizes within images (Garrido-Jurado et al., 2014). The ArUco software tracks tags from predefined tag dictionaries and offers dictionaries of different marker sizes and tag numbers (ranging from 50 to 1000 tags). The BumbleBox Github repository (https://github.com/Crall-Lab/BumbleBox/tree/master) includes high-quality image files of each standard tag dictionary for printing and allows users to easily change the tag dictionary being tracked.

2.3 | Experimental workflow

The BumbleBox software supports (1) setup, (2) data acquisition and (3) behavioural analysis. The BumbleBox uses custom Python scripts to automate data capture and analyse behavioural data, but previous knowledge of Python is not required. For example, users start collecting data by customizing a single script (setup.py) (Table 1). The default recording values have been tested using the standard nest arena designs for the BumbleBox described here (imaged with

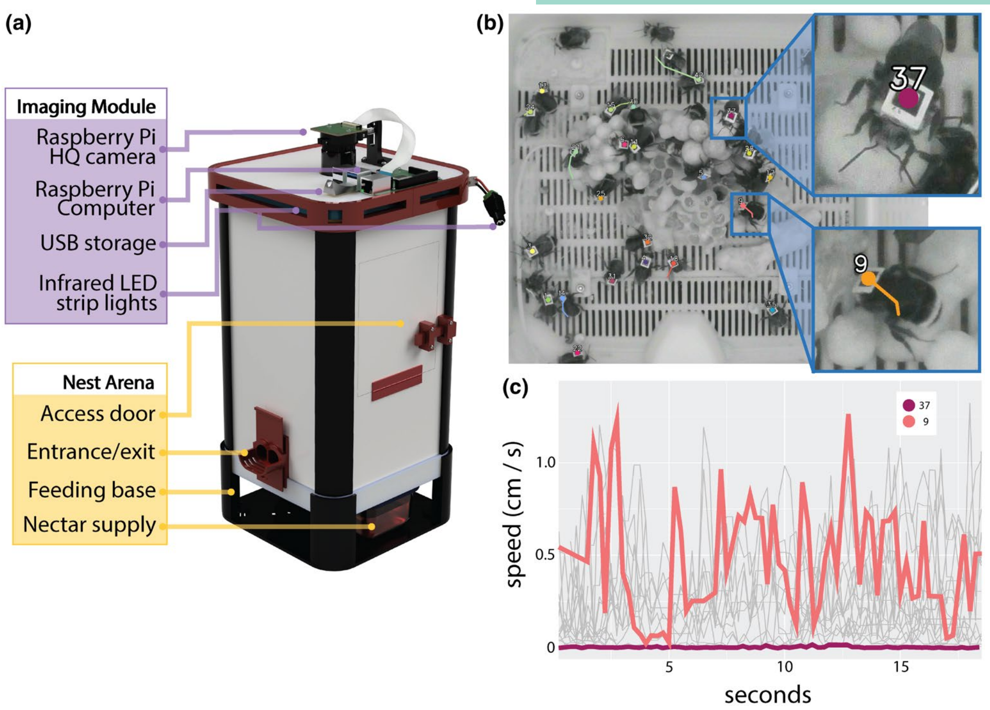

FIGURE 1 Visualization of the BumbleBox tracking systems and example data output. (a) A 3D render of the custom BumbleBox design. (b) Example tracking within the BumbleBox, with insets showing two example individuals with locations tracked using ArUco tags. (c) The speeds of two individuals [same individuals as in (b)] plotted over the course of a single 20-second recording period.

the Raspberry Pi High Quality Camera, and infrared 840 nm LEDs). Users then start the automation of data capture by simply running a single script (start_automated_recording.py).

2.3.1 | Setup

Before initiating data collection, users can ensure optimal imaging conditions (i.e. confirm the nest arena is well lit and tags are visible and in focus) using a script (rpi4_preview.py) that provides a preview of the nest floor. Users can then customize data collection parameters in the setup script (setup.py), including recording time, frame rate and whether automatic behavioural quantification should take place after each video (see Table 1 for a description of recording parameters and Table 2 for a description of available behavioural metrics). Then, the test tracking script (test_tracking.py) can be run to confirm tags are being read accurately. Once the preview footage and tracking look correct, the user can begin collecting data by running the automated recording script (start_automated_recording.py, also described above). Data collection begins automatically and will continue whenever the Raspberry Pi is powered.

2.3.2 | Data acquisition and storage

The main function of data acquisition in the BumbleBox is to capture video of the nest arena, run ArUco detection on video frames to detect the location of tagged bees and save video and tracking data. Additionally, composite images of the nest without bees (using median background estimation) are stored for later labelling of different nest components, which are useful for a variety of downstream data analyses (e.g. brood care rates).

The user decides how often data acquisition occurs via the setup.py script. There are two types of data outputs: (1) CSV files containing tag coordinate data and behavioural quantification and (2) video files. Each row of the CSV file after tracking contains the detected tag ID, the frame of the recording it was detected within and the *x*, *y* pixel coordinates of the tag centre. This is the raw tracking file, and an updated file is generated from it that interpolates missing data and stores the behavioural metrics of interest (see Section 2.3.4).

Available data storage can become limited as the generated video files can be large (e.g. ~40 MB for a full-resolution 20-s video at 4 frames per second [fps]). Tracking occurs immediately after recording, which allows users to discard videos to reduce the number of videos saved. While it is possible to collect data using the BumbleBox without saving videos at all, saving periodic video files allows a user to check for errors and assess tracking quality and are useful for manual ground-truth observations and additional downstream analyses (such as pose tracking; Smith et al., 2022; Wolf et al., 2023). Since tags are tracked with and without storing the video data, the user can set different frequencies for tag tracking with and without video recording depending on experimental needs.

TABLE 1 Adjustable recording parameters for the BumbleBox in the setup.py script.

Parameter name	Description	Possible values in setup.Py
Recording length (seconds)	The length of individual recording periods. Default: 20 s	1–300 were tested
Tag tracking (true/false)	Track Aruco tags from frames immediately after capture and store tag data in a text file, without saving a video	True or false
Data capture frequency (once every X minutes, see possible values)	How often data are captured without saving video (tag tracking must be true)	1–10, 15, 20, 30, 60
Video recording frequency (once every X minutes, see possible values)	How often videos are saved. Overrides data capture frequency if tag tracking is turned off (so every recording is saved as a video)	1–10, 15, 20, 30, 60
Video width, height (pixels)	Choose the output video size in pixels. You should maintain a 4:3 pixel ratio if downscaling from full resolution to avoid distortion. Default: 4056 × 3040	Width = 1–4056 Height = 1–3040
Video file type (MP4 or MJPEG)	Higher framerate can be achieved with the MP4 file type. Default: MP4	'mp4' or 'mjpeg'
Shutter speed (microseconds, or μs)	Default: 2500 (2.5 ms)	Minimum: 571 (at full resolution)
Frame rate (1/X frames per second, see maximum values)	Use MP4 video compression for higher framerates, but requires shorter videos when at full resolution (tested on 8 GB RAM RPis). Default: 5	MP4: max. ~8 (max. ~140 frames per video) MJPEG: max. ~4.5
Video quality (for MJPEG videos only)	Determines the level of MJPEG compression on each frame Higher values yield better image quality and larger file sizes. Default: 95	0–100
Infrared recording (true/false)	If true, frames will be processed using an algorithm that accounts for IR light	True or false
Noise reduction mode	Choose amount of digital noise reduction. Default: Auto	'Auto': 'Fast': 'HighQuality': 'Off'
Digital zoom	Record from a smaller portion of the camera's sensor. Example records only 400 × 300 pixels from the sensor, starting at (1000, 800) from the upper left corner. Useful for removing light glare in non-BumbleBox arenas. Default: None	None or (top-left *x*-coordinate, top-left *y*-coordinate, width, height) Ex. (1000, 800, 400, 300)
Aruco dictionary	Select the Aruco dictionary size that is being used. Default: 4 × 4_50	'4 × 4_50', '4 × 4_100', '4 × 4_250', '4 × 4_1000'
Aruco detection parameters	Rather than setting Aruco detection parameters individually, choose to use a preselected set of detection parameters tailored to either the custom or the Koppert-adapted BumbleBox. Default: custom	'custom': 'koppert': none
Interpolate missing data	Interpolate tag position data between frames when a tag is tracked, lost, and then tracked again. Set the maximum gap in data to interpolate between. Default: True, maximum 3 s gap between tracked frames	True or false. If true, 'max_seconds_gap' must be set to a value less than the recording length (seconds)

TABLE 2 Behavioural metrics currently calculated by the BumbleBox software.

Metric	Description
Speed	A bee's speed (when moving) calculated across tracked frames, in pixels per second
Activity	The proportion of time a bee spends moving vs. at rest per recording period
Distance from social center	Each tracked bee's distance from the average *x*, *y* position of all tracked bees in a given video
Distance to other bees	Each tracked bee's distance to every other tracked bee in a given frame
Contacts	Records contacts between tracked bees in a frame based on a threshold contact distance
Distance to nest components	The distance of each tracked bee in a frame to each labelled nest component

2.3.3 | Overview of alternative data acquisition and processing approaches

The BumbleBox offers two options for data acquisition and tag tracking:

Option 1: *Non-continuous video capture with immediate on-device tag tracking* (default): Using this approach, video data are recorded and then automatically paused to allow for image processing (i.e. tag tracking) on the Raspberry Pi before the next video is collected (on a pre-determined schedule). This approach reduces data storage and subsequent processing requirements necessary and is ideal for long-term monitoring with the Raspberry Pi, or other applications where continuous behavioural quantification is not needed. Tracked videos can optionally be stored [e.g. for integration with additional analyses, such as SLEAP or NAPS (Pereira et al., 2022; Wolf et al., 2023)].

Option 2: *Continuous video capture with subsequent, post-hoc tracking*: This approach foregoes tracking and image processing during data capture and instead performs continuous video recording and storage for subsequent processing. After data capture, tag tracking can then be performed using the BumbleBox (using the 'track_prerecorded_videos.py' script) either on the Raspberry Pi or on a separate machine. This approach can provide continuous recording for applications where necessary but quickly yields large datasets using the full resolution (required for ArUco tracking), so will generally be more feasible for shorter experiments.

While the most computationally intensive data acquisition method, combining *continuous* video capture with *real-time* image tracking, can be important for certain applications (e.g. real-time analysis for closed-loop experimentation and robotics integration; Rüegg et al., 2024), this approach is not currently feasible with the processing capabilities of the Raspberry Pi system in 2025. Raspberry Pi computation, memory and bandwidth have increased dramatically with each new generation, so it seems possible that the computational requirements could be met by the Raspberry Pi in a matter of years.

2.3.4 | Behavioural quantification

The locations of tagged bees within the nest are used to quantify behaviour of uniquely tracked individuals (Table 2). For example, position within the nest, speed, movement, proximity to other bees and nest components, and interactions between individuals can be used to quantify task performance and division of labour within bumblebee colonies (Figure 2) (Crall, Gravish, et al., 2018). Missing tag data can be optionally linearly interpolated between two discontinuous tracking instances (frames) within a video, and the maximum number of seconds between tag instances can be set by the user in the setup.py script. Behavioural quantification can take place automatically after instances of data capture or can be calculated on previously collected data, depending on parameters defined in the setup script. Core behavioural quantification functions are included, but experienced users can easily extend the toolkit with additional metrics—such as individual orientation, angular velocity or detection of direct social interactions (e.g. head-to-head encounters)—and are encouraged to share their contributions on GitHub for use by the broader community. Users can turn on automatic behaviour quantification and choose which metrics are to be calculated in the setup script (setup.py).

Location and movement of bees relative to nest structure (e.g. brood and wax pots) can be informative for understanding within-nest behaviour of bumblebees (Crall, Gravish, et al., 2018; Jandt & Dornhaus, 2009). The BumbleBox software includes a GUI-based script to label nest structures (e.g. larvae, pupae, wax pots, etc.) and other important features like food sources.

Background images for labelling are first generated by median background estimation as in Crall, Gravish, et al. (2018) (Figure 2c). After labelling, the resulting nest maps can be used to quantify additional behavioural metrics such as minimum distance to wax pots or brood (Figure 2e).

3 | RESULTS

3.1 | Validation

We created a microcolony by tagging 16 bumblebee (*Bombus impatiens* Cresson, 1863) workers with ArUco tags (2.6 mm side lengths, printed on TerraSlate paper using a laserjet printer) and imaged them using the BumbleBox system over a period of 24 h (Figure 2). We briefly anaesthetized bees with carbon dioxide and used cyanoacrylate gel superglue to adhere the tags to the thoraxes of the bumblebees. Bees were allowed to recover for 48 h before being tracked over a 24-h period.

Two hundred and forty videos (10 s long, ~5 fps, 12,464 total frames) were recorded, and 107,242 bee positions were captured. The bees were identified using tags 16 tags (tags 0–15 out of a 50 tag dictionary, 0–49), but there were seven instances of tracked tags that were among the 34 tags not used (ID 17: 3 instances, ID 26: 1 instance, ID 34: 1 instance, ID 37: 2 instances), and thus considered 'detectable' false positives. To estimate the 'undetectable' false positive rate (i.e. false positives among the 16 tags used in the experiment), we can assume an equivalent false positive rate across tags, then extrapolate to estimate the number of false positives occurring within tags 0–15 across this recording period [i.e. (detectable false positives)/([# used tags]/[# unused tags])]. This yields an estimate of ~3.29 false positives (7 detectable false positives × [16/34]), or a 0.003% false positive rate and estimated accuracy of >99.99%. This error rate will likely change if any major changes to the BumbleBox design are made. An average of 8.6 tags were tracked per frame (median: 9, minimum: 3,

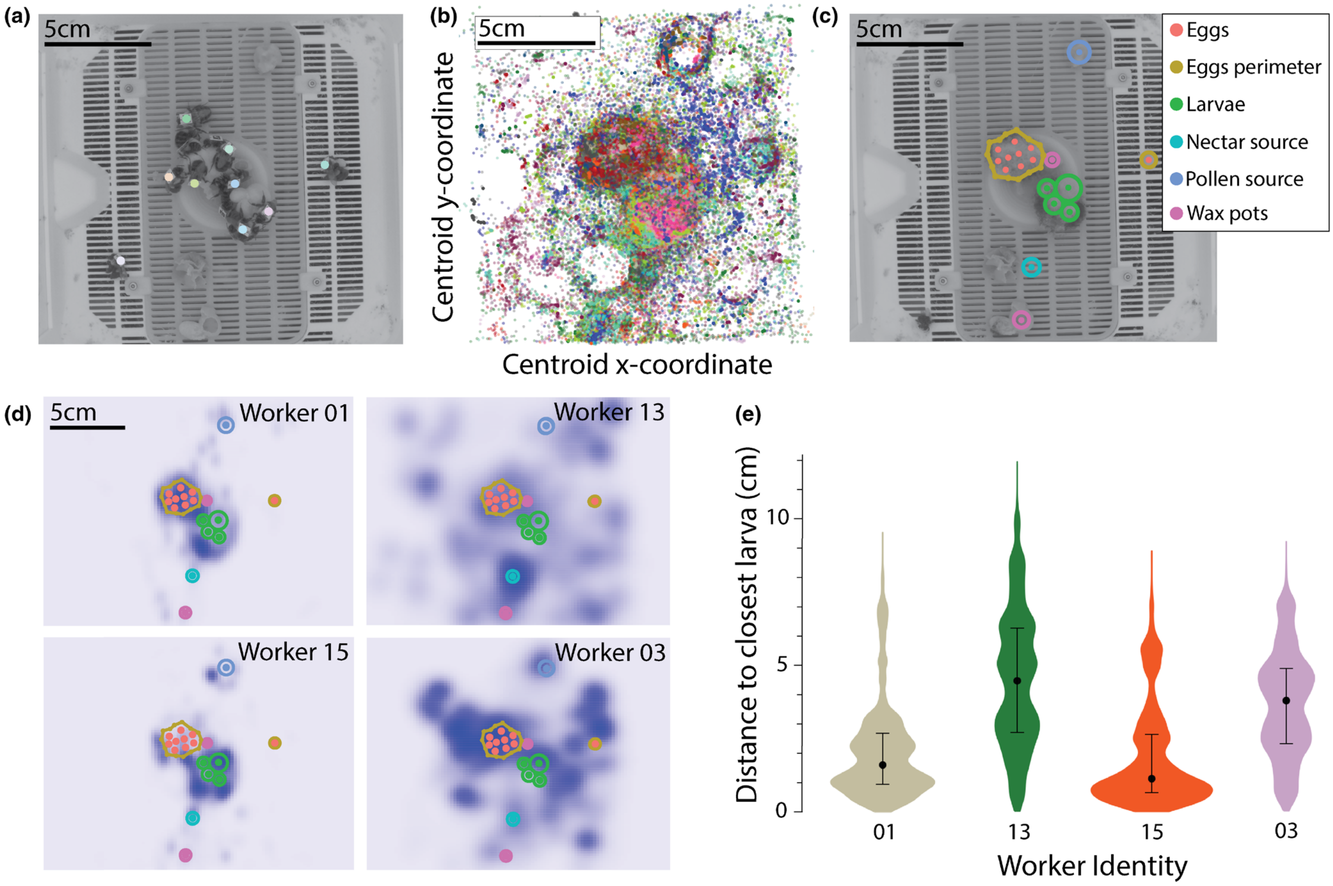

FIGURE 2 Example quantification of behaviour in the BumbleBox. (a) Image of a tracked *B. impatiens* microcolony from the BumbleBox camera. Solid makers show locations of individually tracked bees. (b) Plot showing location data for the microcolony over the course of a single day; each colour represents a separate individual. (c) Different nest components plotted over a composite nest image generated using the graphical user interface available with the BumbleBox software. (d) 2D Gaussian kernel density estimation plots demonstrating the spatial distribution probability of four different individuals within the nest over the course of a single day, with the nest component data overlaid. (e) Violin plots depicting probability distributions of each bee's distance to the closest larva over the course of a single day. Black points indicate median values and vertical lines represent the interquartile range.

lower-quartile: 7, upper-quartile: 10, maximum: 14). See Figure S3 and text in Supporting Information for further discussion of tracking performance.

3.2 | Example applications and modularity

The modularity and scalability of the BumbleBox system allow for diverse applications in the study of bumblebee behaviour and health. For example, the BumbleBox system can be adapted for use in field or semi-field conditions: a recent greenhouse experiment modified the BumbleBox to include thermocouples (to quantify brood temperature), as well as the addition of an automated foraging monitoring system (Figure 3a–c, unpublished data). The BumbleBox system is also scalable: a version of the BumbleBox system was used to study the combined effects of thermal stress and neonicotinoid exposure in *B. impatiens* microcolonies (Easton-Calabria et al., 2023), using 12 units in parallel in the laboratory (Figure 3d). Finally, the BumbleBox has been used across multiple species of *Bombus*, including *B. impatiens*, *B. griseocollis* and *B. bimaculatus* from Wisconsin, USA, *B. vosnesenskii* from Oregon, USA, and *B. ephippiatus* (Figure 3e) from Chiapas, Mexico.

The design also (optionally) allows users to move the nest from a separate, smaller rearing chamber (145 × 76 × 50 mm) into the floor of the nest arena (Figure S1, design files available on GitHub). This integration is useful for experiments using microcolonies that need to be established (Klinger et al., 2019) or long-term tracking of colonies reared from wild-caught *Bombus* queens throughout the entire colony cycle (Christman et al., 2023; Rowe et al., 2023).

An additional prototype BumbleBox design acts as an attachment to commercially available Koppert™ bumblebee colony boxes, using the Koppert box itself as the imaging arena. This design replaces the top of the Koppert box and raises it to image the colony from above (Figure S2, prototype design files available on GitHub). It is intended to tag and image mature Koppert commercial colonies, with applications for both laboratory and field studies.

It is important to be able to modify the BumbleBox beyond this set of designs to accommodate different research questions (e.g.

BRITISH ECOLOGICAL SOCIETY Ecological Solutions and Evidence

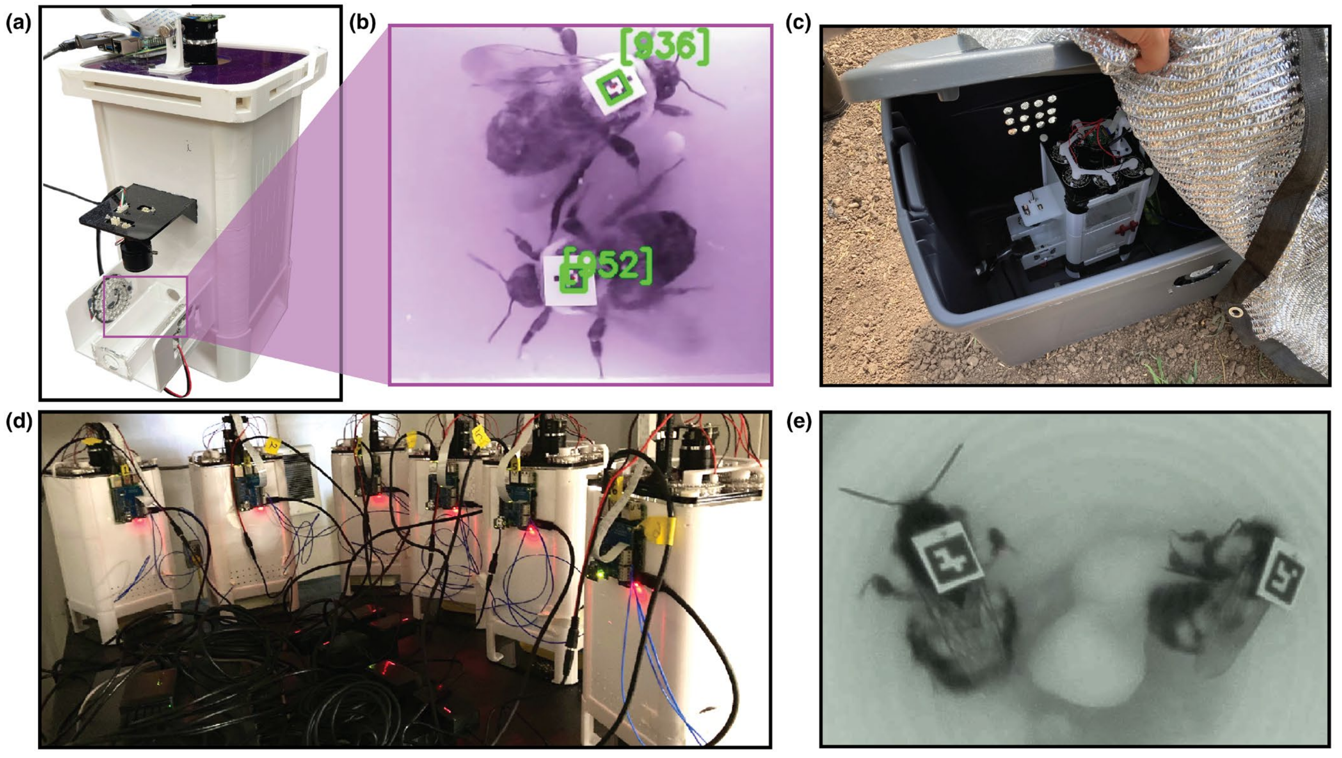

FIGURE 3 Examples of modular experimental applications of the BumbleBox system. (a) BumbleBox with one wall panel modified as a forage tunnel and outfitted with a second camera (USB) to track foraging activity (b). (c) Modified BumbleBox deployed in the field to monitor foraging activity and in-nest behaviour of free-foraging *B. impatiens* colonies. (d) Multiple ($n=6$) BumbleBoxes outfitted with thermocouples to quantify behavioural responses to thermal stress. (e) A close-up image of two *Bombus ephippiatus* workers from behavioural experiments conducted by collaborators in Chiapas, Mexico.

modified imaging arenas, camera heights or lighting conditions). When doing so, it is important to find the ArUco tracking parameters that provide optimal tracking performance in the new design. The BumbleBox includes a custom tracking optimization script (tracking_optimization.py) in the BumbleBox repository to allow users to optimize tracking performance in modified imaging setups.

Finally, the BumbleBox is applicable to behaviour research in other social insect models and model organisms more generally. The imaging module is removable and can be used separately from the arena; the arena can be adapted to house other animal models, and ArUco markers have already been used to study behaviour in other animal models, such as in ants and chickens (Alindekon et al., 2025; Sclocco et al., 2021). The BumbleBox is complementary to other behavioural tracking methods (e.g. integrating tag tracking with pose estimation using SLEAP or NAPS; Smith et al., 2022; Wolf et al., 2023) to capture poses, positions and identities of individuals.

4 | DISCUSSION

The BumbleBox is a low-cost, open-source and modular system for quantifying behaviour of uniquely tracked individuals within a bumblebee colony. The system is designed specifically with accessibility to researchers in mind. In particular, it is designed to be usable without modification by users with limited prior knowledge in computer programming or hardware design, while providing flexibility and modularity for experienced users, and has broad research applications in bumblebees and potentially other social insect species (Guo et al., 2024).

The standardized, automated behavioural tracking provided by the BumbleBox has diverse potential applications in bumblebee behaviour, ecology and health. First, scalable and standardized behavioural tracking could help elucidate impacts of agrochemicals and other stressors in bumblebees, particularly in light of interactive effects that necessitate testing under multiple conditions (Fisher et al., 2023; Siviter et al., 2021). Second, quantifying how these stress responses vary across species and populations could help bring insight into the differential success of *Bombus* species. Bumblebee declines in North America and Europe have been well documented (Cameron et al., 2011), but population trends vary substantially across species (Jackson et al., 2022); this highlights the need to understand mechanisms underlying species-level differences in behaviour and colony performance. In addition, research has focused disproportionately on North American and European *Bombus*. Developing low-cost tools that are accessible to researchers in low-income countries—often a result of colonial or imperialist wealth extraction—is imperative to correct this imbalance and fill critical knowledge gaps in the ecology, evolution and diversity of social behaviour in bumblebees.

AUTHOR CONTRIBUTIONS

August Easton-Calabria created the BumbleBox and wrote the system software. James D. Crall contributed significantly to conceptual design, provided feedback, discussed results and interpreted data with August Easton-Calabria. Both authors contributed to the preparation of the manuscript.

ACKNOWLEDGEMENTS

We thank Rémy Vandame, Alejandra Martínez de Castro, and Andres Castro-Siller for their collaboration in testing and using the earlier iterations of the BumbleBox. We also thank Brett Graham of the Harvard Neurotechnology Core for his assistance in the early stages of development of the BumbleBox software, and to Michael Dillon, Emily Bick, and members of the Crall lab for feedback on initial drafts of this manuscript. This work was supported by the USDA National Institute of Food and Agriculture (NIFA) Postdoctoral Fellowship to J.D.C. (grant no. 2019-67012-33596), the Agricultural and Food Research Initiative grant no. 2022-67013-36275 from the USDA National Institute of Food and Agriculture to J.D.C., a USDA NIFA Hatch Project (grant no. WIS04062), a Winslow Foundation grant to J.D.C., a UW-Madison Research Forward grant to J.D.C., and the UW-Madison Lillian and Alex Feir Wisconsin Distinguished Fellowship in Entomology to A.E.-C. Koppert supported this work by providing bumblebee colonies at a discounted rate.

CONFLICT OF INTEREST STATEMENT

The authors declare no competing interests.

PEER REVIEW

The peer review history for this article is available at https://www.webofscience.com/api/gateway/wos/peer-review/10.1002/2688-8319.70052.

DATA AVAILABILITY STATEMENT

All data are available at DOI: 10.5061/dryad.x3ffbg7x8 (Easton-Calabria & Crall, 2025), including raw tracking output, nest map data, and summary data used in subsequent analyses. See the Dryad README for details.

ORCID

August Easton-Calabria https://orcid.org/0000-0002-3373-3302
James D. Crall https://orcid.org/0000-0002-8981-3782

REFERENCES

Alindekon, S., Deutsch, J., Rodenburg, T. B., Langbein, J., Puppe, B., & Louton, H. (2025). Tag "n" Track: Tackling the validation challenge in animal behaviour studies through automated referencing with ArUco markers. *Computers and Electronics in Agriculture*, *229*, 109812. https://doi.org/10.1016/j.compag.2024.109812

Amsalem, E., Malka, O., Grozinger, C., & Hefetz, A. (2014). Exploring the role of juvenile hormone and vitellogenin in reproduction and social behavior in bumble bees. *BMC Evolutionary Biology*, *14*(1), 45. https://doi.org/10.1186/1471-2148-14-45

Baer, B. (2003). Bumblebees as model organisms to study male sexual selection in social insects. *Behavioral Ecology and Sociobiology*, *54*(6), 521–533. https://doi.org/10.1007/s00265-003-0673-5

Boenisch, F., Rosemann, B., Wild, B., Dormagen, D., Wario, F., & Landgraf, T. (2018). Tracking all members of a honey bee colony over their lifetime using learned models of correspondence. *Frontiers in Robotics and AI*, *5*, 35. https://doi.org/10.3389/frobt.2018.00035

Cameron, S. A., Lozier, J. D., Strange, J. P., Koch, J. B., Cordes, N., Solter, L. F., & Griswold, T. L. (2011). Patterns of widespread decline in North American bumble bees. *Proceedings of the National Academy of Sciences of the United States of America*, *108*(2), 662–667. https://doi.org/10.1073/pnas.1014743108

Cameron, S. A., & Sadd, B. M. (2020). Global trends in bumble bee health. *Annual Review of Entomology*, *65*(1), 209–232. https://doi.org/10.1146/annurev-ento-011118-111847

Christman, M. E., Barkan, N. P., Campion, C., Heraghty, S. D., Keaveny, E. C., Verble, K. M., Waybright, S. A., Dillon, M. E., Lozier, J. D., & Strange, J. P. (2023). It is buzziness time: Rearing, mating, and overwintering *Bombus vosnesenskii* (Hymenoptera: Apidae). *Journal of Insect Science*, *23*(5), 18. https://doi.org/10.1093/jisesa/iead089

Crall, J. D., Gravish, N., Mountcastle, A. M., Kocher, S. D., Oppenheimer, R. L., Pierce, N. E., & Combes, S. A. (2018). Spatial fidelity of workers predicts collective response to disturbance in a social insect. *Nature Communications*, *9*(1), 1201. https://doi.org/10.1038/s41467-018-03561-w

Crall, J. D., Switzer, C. M., Oppenheimer, R. L., Ford Versypt, A. N., Dey, B., Brown, A., Eyster, M., Guérin, C., Pierce, N. E., & Combes, S. A. (2018). Neonicotinoid exposure disrupts bumblebee nest behavior, social networks, and thermoregulation. *Science*, *362*(6415), 683–686.

Easton-Calabria, A. C., & Crall, J. A. (2025). The BumbleBox: An open-source platform for quantifying behavior in bumblebee colonies [Data set]. *Dryad*. https://doi.org/10.5061/dryad.x3ffbg7x8

Easton-Calabria, A. C., Thuma, J. A., Cronin, K., Melone, G., Laskowski, M., Smith, M. A. Y., Pasadyn, C. L., de Bivort, B. L., & Crall, J. D. (2023). Colony size buffers interactions between neonicotinoid exposure and cold stress in bumblebees. *Proceedings of the Royal Society B: Biological Sciences*, *290*, 20230555. https://doi.org/10.1098/rspb.2023.0555

Fijen, T. P. M., Scheper, J. A., Boom, T. M., Janssen, N., Raemakers, I., & Kleijn, D. (2018). Insect pollination is at least as important for marketable crop yield as plant quality in a seed crop. *Ecology Letters*, *21*(11), 1704–1713. https://doi.org/10.1111/ele.13150

Fisher, A., Tadei, R., Berenbaum, M., Nieh, J., Siviter, H., Crall, J., Glass, J. R., Muth, F., Liao, L.-H., Traynor, K., DesJardins, N., Nocelli, R., Simon-Delso, N., & Harrison, J. F. (2023). Breaking the cycle: Reforming pesticide regulation to protect pollinators. *BioScience*, *73*(11), 808–813. https://doi.org/10.1093/biosci/biad088

Garrido-Jurado, S., Muñoz-Salinas, R., Madrid-Cuevas, F. J., & Marín-Jiménez, M. J. (2014). Automatic generation and detection of highly reliable fiducial markers under occlusion. *Pattern Recognition*, *47*(6), 2280–2292.

Gill, R. J., Ramos-Rodriguez, O., & Raine, N. E. (2012). Combined pesticide exposure severely affects individual- and colony-level traits in bees. *Nature*, *491*(7422), 11585. https://doi.org/10.1038/nature11585

Guo, X., Hasenjager, M. J., Fefferman, N. H., & Pinter-Wollman, N. (2024). *Social interactions are impacted by food availability, food type, and group size*. bioRxiv. 2024.03.07.584014. https://doi.org/10.1101/2024.03.07.584014

Jackson, H. M., Johnson, S. A., Morandin, L. A., Richardson, L. L., Guzman, L. M., & M'Gonigle, L. K. (2022). Climate change winners and losers among north American bumblebees. *Biology Letters*, *18*(6), 20210551. https://doi.org/10.1098/rsbl.2021.0551

Jandt, J. M., & Dornhaus, A. (2009). Spatial organization and division of labour in the bumblebee *Bombus impatiens*. *Animal Behaviour*, *77*(3), 641–651. https://doi.org/10.1016/j.anbehav.2008.11.019

Klein, A.-M., Vaissière, B. E., Cane, J. H., Steffan-Dewenter, I., Cunningham, S. A., Kremen, C., & Tscharntke, T. (2006). Importance of pollinators in changing landscapes for world crops. *Proceedings of the Royal Society B: Biological Sciences*, *274*(1608), 303–313. https://doi.org/10.1098/rspb.2006.3721

Klinger, E. G., Camp, A. A., Strange, J. P., Cox-Foster, D., & Lehmann, D. M. (2019). *Bombus* (Hymenoptera: Apidae) Microcolonies as a Tool for Biological Understanding and pesticide risk assessment. *Environmental Entomology*, *48*, 1249–1259. https://doi.org/10.1093/ee/nvz117

Martínez-Harms, J., Palacios, A. G., Márquez, N., Estay, P., Arroyo, M. T. K., & Mpodozis, J. (2010). Can red flowers be conspicuous to bees? *Bombus dahlbomii* and South American temperate forest flowers as a case in point. *Journal of Experimental Biology*, *213*(4), 564–571. https://doi.org/10.1242/jeb.037622

Mersch, D. P., Crespi, A., & Keller, L. (2013). Tracking individuals shows spatial Fidelity is a key regulator of ant social organization. *Science*, *340*(6136), 1090–1093. https://doi.org/10.1126/science.1234316

Pereira, T. D., Tabris, N., Matsliah, A., Turner, D. M., Li, J., Ravindranath, S., Papadoyannis, E. S., Normand, E., Deutsch, D. S., Wang, Z. Y., McKenzie-Smith, G. C., Mitelut, C. C., Castro, M. D., D'Uva, J., Kislin, M., Sanes, D. H., Kocher, S. D., Wang, S. S.-H., Falkner, A. L., … Murthy, M. (2022). SLEAP: A deep learning system for multi-animal pose tracking. *Nature Methods*, *19*(4), 486–495. https://doi.org/10.1038/s41592-022-01426-1

Richardson, T. O., Liechti, J. I., Stroeymeyt, N., Bonhoeffer, S., & Keller, L. (2017). Short-term activity cycles impede information transmission in ant colonies. *PLoS Computational Biology*, *13*(5), e1005527. https://doi.org/10.1371/journal.pcbi.1005527

Rowe, G., Hagadorn, M. A., Lindsay, T.-T. T., Malfi, R., Williams, N. M., & Strange, J. P. (2023). Chapter 20 – Production of bumblebees (Hymenoptera: Apidae) for pollination and research. In J. A. Morales-Ramos, M. G. Rojas, & D. I. Shapiro-Ilan (Eds.), *Mass production of beneficial organisms* (2nd ed., pp. 559–579). Academic Press. https://doi.org/10.1016/B978-0-12-822106-8.00004-X

Rüegg, M., Motes-Rodrigo, A., Tuleu, A., Stroeymeyt, N., Richardson, T. O., Sakar, M. S., & Keller, L. (2024). Precise tactile stimulation of worker ants by a robotic manipulator reveals that individual responses are density- and context-dependent. *Methods in Ecology and Evolution*, *15*, 117–129. https://doi.org/10.1111/2041-210X.14244

Saunders, J. L., Ott, L. A., & Wehr, M. (2022). *AUTOPILOT: Automating experiments with lots of Raspberry Pis (807693)*. bioRxiv. https://doi.org/10.1101/807693

Sclocco, A., Ong, S. J. Y., Pyay Aung, S. Y., & Teseo, S. (2021). Integrating real-time data analysis into automatic tracking of social insects. *Royal Society Open Science*, *8*(3), 202033. https://doi.org/10.1098/rsos.202033

Siviter, H., Bailes, E. J., Martin, C. D., Oliver, T. R., Koricheva, J., Leadbeater, E., & Brown, M. J. F. (2021). Agrochemicals interact synergistically to increase bee mortality. *Nature*, *596*(7872), 389–392. https://doi.org/10.1038/s41586-021-03787-7

Smith, M. A.-Y., Easton-Calabria, A., Zhang, T., Zmyslony, S., Thuma, J., Cronin, K., Pasadyn, C. L., de Bivort, B. L., & Crall, J. D. (2022). Long-term tracking and quantification of individual behavior in bumble bee colonies. *Artificial Life and Robotics*, *27*(2), 401–406. https://doi.org/10.1007/s10015-022-00762-x

Stroeymeyt, N., Grasse, A. V., Crespi, A., Mersch, D. P., Cremer, S., & Keller, L. (2018). Social network plasticity decreases disease transmission in a eusocial insect. *Science*, *362*(6417), 941–945. https://doi.org/10.1126/science.aat4793

Wolf, S. W., Ruttenberg, D. M., Knapp, D. Y., Webb, A. E., Traniello, I. M., McKenzie-Smith, G. C., Leheny, S. A., Shaevitz, J. W., & Kocher, S. D. (2023). NAPS: Integrating pose estimation and tag-based tracking. *Methods in Ecology and Evolution*, *14*(10), 2541–2548. https://doi.org/10.1111/2041-210X.14201

Woodard, S. H., Lozier, J. D., Goulson, D., Williams, P. H., Strange, J. P., & Jha, S. (2015). Molecular tools and bumble bees: Revealing hidden details of ecology and evolution in a model system. *Molecular Ecology*, *24*(12), 2916–2936. https://doi.org/10.1111/mec.13198

SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

Figure S1. The queen/microcolony starter box.

Figure S2. The Koppert©-fitted BumbleBox.

Figure S3. Tracking coverage across 16 workers over 24 h.

How to cite this article: Easton-Calabria, A., & Crall, J. D. (2025). The BumbleBox: An open-source platform for quantifying behaviour in bumblebee colonies. *Ecological Solutions and Evidence*, *6*, e70052. https://doi.org/10.1002/2688-8319.70052