Bee markings with colored or fluorescent flour and digital detection with different camera systems

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Abstract: Es werden neuartige Methoden vorgestellt, mit denen Honigbienen (*Apis mellifera*, Apidae) markiert und an Trachtpflanzen bzw. Bienenbeuten detektiert werden können. Versuche dazu wurden am Lehr- und Forschungsbienenstand der Hochschule für nachhaltige Entwicklung in Eberswalde (HNEE), auf landwirtschaftlichen Versuchsflächen und am Forstbotanischen Garten in Eberswalde durchgeführt. Bei den Markierungen wurde mit herkömmlichen Markierungsfarben und mit neuartigen Möglichkeiten durch buntes bzw. fluoreszierendes Mehl experimentiert. An künstlichen Futtertränken und an verschiedenen Bienenbeuten wurden die Applikationen der Farben auf die Sammelbienen mit verschiedenen Geräten und die Detektion der markierten Bienen mit verschiedenen Kamerasystemen getestet. Mit studentischen Gruppen wurde zeitgleich an verschiedenen Trachtpflanzen im Forstbotanischen Garten und auf den landwirtschaftlichen Versuchsflächen blütenbesuchende Honigbienen markiert. Andere Studierendengruppen bzw. online verschaltete Kamerasysteme an und in den Bienenbeuten nahmen die Ankunft der markierten Bienen auf. Zusätzlich wurden die Beobachtungen zu den Trachtpflanzen, auch bezüglich weiterer Blütenbesucher wie Wildbienen, in eine georeferenzierte Onlinedatenbank eingegeben. Diese nutzen Studierende noch im Gelände auf ihren mobilen Endgeräten oder Tablets. Die bisher erfolgreichsten Methoden und Geräte bei den Farbmarkierungen und Detektionen werden vorgestellt.

Keywords: Honigbiene, *Apis mellifera*, Markierungen, Versuche, Sammelbienen, Didaktik, Trachtpflanzen, georeferenzierte Datenbanken, Kamerasysteme, Bienenverhalten, Empfehlungen

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Through the research of K. v. Frisch, the color marking of honey bees has become famous. He marked individual bees with color dots on the bee thorax in the 1920s to decipher the bee dances (see e.g. v. Frisch 1923). Since then, other marking experiments have been developed with mostly ethological or sociobiological questions, and meanwhile, state-of-the-art techniques have been used for this purpose (e.g. Ai & Takahashi 2021, Hagler & al. 2011). These include, for example, the automatic tracking system of Boenisch & al. (2018), in which individually coded platelets are glued to the bees' thoracic tergites and automatically recognized. Much simpler, non-individual marking systems work with flour dusting. In traditional beekeeping, for example, a trick has been used for some time to determine the origin of a departed swarm. For this purpose, normal household flour is dusted on the resting swarm cluster and then it is observed at the potential hives of origin whether flour-dusted bees fly back (cited e.g. in Meyerhoff 1985). In this work, simple flour dustings are also used to mark honey bees without having to catch the bees individually. In this case, different colored flours are used, and the focus is more on didactic aspects and on working with students at an educational apiary and on experimental plots. Another new feature is that honey bees are colorfully marked without contact not only on the hives (as e.g. in Bonoan & Starks 2016), but also on the forage plants. Furthermore, it is new that markings with different colors are carried out simultaneously at several locations in the field, that online camera techniques are used at several locations, and that an online database is used for the area-specific assignment of the forage plants and bee observations. Since this was new territory, the methodological steps had to be tested and evaluated first. This is presented in the following.

Study site

The surveys were carried out at three locations in the Eberswalde urban area (see also Fig. 1):

1) at the educational apiary of the University for Sustainable Development (HNEE),
2) at the botanical garden in Eberswalde (Forest Botanical Garden of HNEE),
3) on agricultural experimental plots of HNEE, which are located island-like in the forest area (see 3 in Fig. 1).

The distance between the apiary (1 in Fig. 1) and the forage plants in the Botanical Garden (2 in Fig. 1) is about 850 m, and the distance between the apiary and the forage plants on the Agricultural Experimental Plots (3 in Fig. 1) is about 1200 m. The educational apiary of the HNEE is located on the forest campus; this is on the edge of the Eberswalde city area. Here, up to five bee colonies are cared for in different hive systems. The hives (type Langstroth 2/3) stand on radio hive scales, which record daily weight changes as well as temperature data and transmit them online. A top bar hive (TBH) from the We4bee project (2023) also records temperature, wind and other data. The flight hole of the TBH and the flight holes of two Langstroth hives are monitored by digitally connected cameras. Inside the TBH there is another camera. The educational apiary is used regularly for bee science lessons and studies.

Material and Methods

At the apiary of the Eberswalde University for Sustainable Development (HNEE), experiments with various marking methods have been carried out since 2020. Due to the contact restrictions during the lockdown phases of the Corona crisis, new - also digital - ways of teaching bee science have been explored. This included evaluations of online camera transmissions of cameras placed in front of the flight holes, but also students going out alone or in small groups to search for foraging plants and bees in the vicinity of the educational apiary. In each case, mapped forage plants and honey bees or wild bees sitting in the flowers were entered into a georeferenced database (newly established "Bloom-ing & Bee-ing" database for students using ArcGIS Online 2023) with as much detail as possible regarding abundance, species, and behavior. Clues to preferentially approached forage plants in the vicinity of the apiary have been obtained previously by pollen analyses from evaluated honey (e.g., in Rademacher 2018) and by joint areal mapping. Markings of honey bees were initially done only at the apiary with marker pens otherwise used for bee queens from 2020 onwards (see below: A). In spring and summer 2022, honey bees were marked with colored flour both at the apiary and on the forage plants (see below: B).

Regarding A.: At the apiary, the first and second authors caught collecting honey bees with nets in front of the flight holes of three hives (2 x Langstroth, 1 x Top Bar Hive) and cooled them down in an adjacent cold room of the HNEE with an average room temperature of 5 degrees Celsius over a few minutes so that they could be picked up by hand and individually marked on the thorax in the colors red, yellow and white, depending on the hive. For this purpose, marking pens for queen breeding (from the company Posca) were used (see also Eckhardt 2020). Subsequently, the marked honey bees were placed in the sun and released. Student groups attempted observations of the marked bees at different forage plants in the vicinity and on their return with pre-installed camera systems at the flight holes. The determined and mapped forage plants and the abundances of honey bees landing there were entered into the georeferenced online database "Bloom-ing & Bee-ing" (ArcGIS Online 2023) by students using mobile devices (smartphones, tablets). Continuous film recordings of a fixed outdoor camera ('IB836BA-HAT' from Vivotek) aimed at the flight holes of two Langstroth hives at the apiary and the recordings of the outdoor and indoor camera in the Top Bar Hive (TBH) of we4bee (2023) were evaluated. The TBH outdoor and indoor images are automatically transmitted online every 15 min. They were recorded, assembled and edited with the software program Animotica and analyzed with VLC media player (see https://animotica-video-editor.de.softonic.com and https://www.vlc.de).

Regarding B.: Based on the previous experiments, we again worked with student groups, but they not only marked honeybees at the apiary, but also at different forage plants with various types of colored flour. This was preceded by several experiments by Lara (2022) to test the use of different cameras and flour applicators. Additionally, the influence of flour dusting on the bees' behavior was observed. As material for the flour dusting corn starch flour dyed with food coloring was used. This so-called

"Holi powder" is a pure natural product, free of harmful chemicals, with a reduced amount of fine dust and biodegradable (see Polvoholi (2023). The powder is available in different colors. Red, blue, green, yellow, and orange were used for the experiments. In addition, experiments were conducted with fluorescent corn flour from the company H2i (2023).

Various tools were tested for the application of the powder to the bees. At the beginning a flour-filled cup covered with a silk stocking was used like a sugar sprinkler. Subsequently, single-handed sprayers, plastic sprayers, and pump-spray sprayers for hair powder, as they are available in cosmetics stores, were tested (the latter see top right in Fig. 2). In order to test the different flour applications and the detectability of the markings with different camera systems, an artificial feeding site at a distance of about 100 m from the apiary was used. This was a set-up poultry drinker filled with 1.5 l sugar liquid (50% sugar, 50% water), to which a drop of anise oil was added for bee attraction, seen in Seeley (2017). The experiments were documented using various camera techniques and subsequently evaluated. The following cameras were tested: two 'Panasonic HC-V777' camcorders, an outdoor 'IB836BA-HAT' network camera from Vivotek, an 'outdoor Wi-Fi nesting box camera' from Green-Backyard, a 'Hero11' action camera from the supplier GoPro, and a full-frame 'Alpha 7 III' camera from Sony. Camcorders and full-frame camera were mounted on tripods, pointed at the hives, while the action camera was fixed to the hives itself with a goose-neck mount. The Wi-Fi nesting box camera was fixed to a self-constructed mount and placed on changing locations. These camera systems were considered mobile setups, limited by battery capacities and memory space. The network camera was part of a fixed setting, constantly delivering online footage to a server at the HNEE (see above). When the fluorescent special flour was used, an LED UV spotlight was used at an approximate distance of 2 m from the hives.

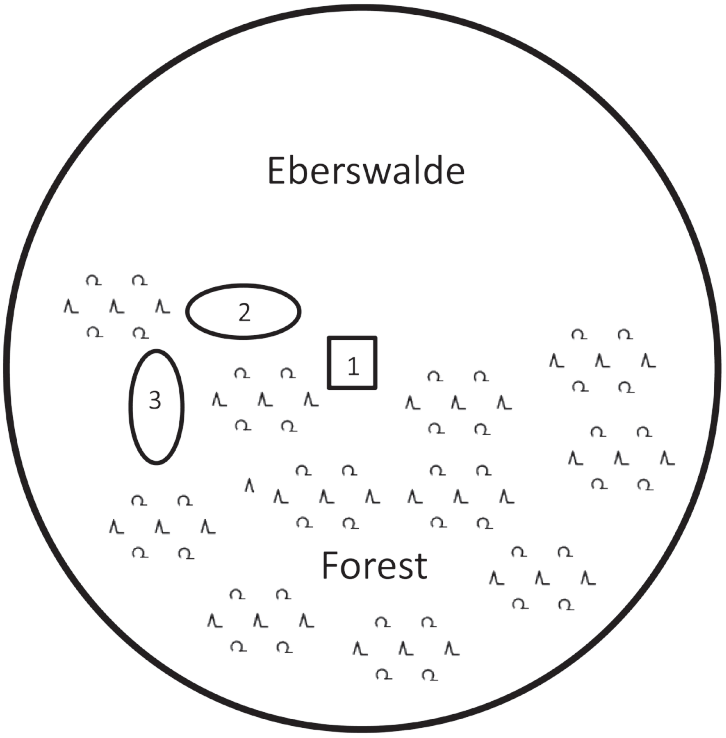

Fig. 1: Locations where honey bees were marked with flour in different colors by student groups: 1 = apiary, 2 = botanical garden, 3 = agricultural experimental plots; the surroundings of the apiary with a 2 km radius are shown. Distance between 1 and 2 approx. 850 m, between 1 and 3 approx. 1200 m; drawing by U. Schulz after presentation in Eckhardt (2020).

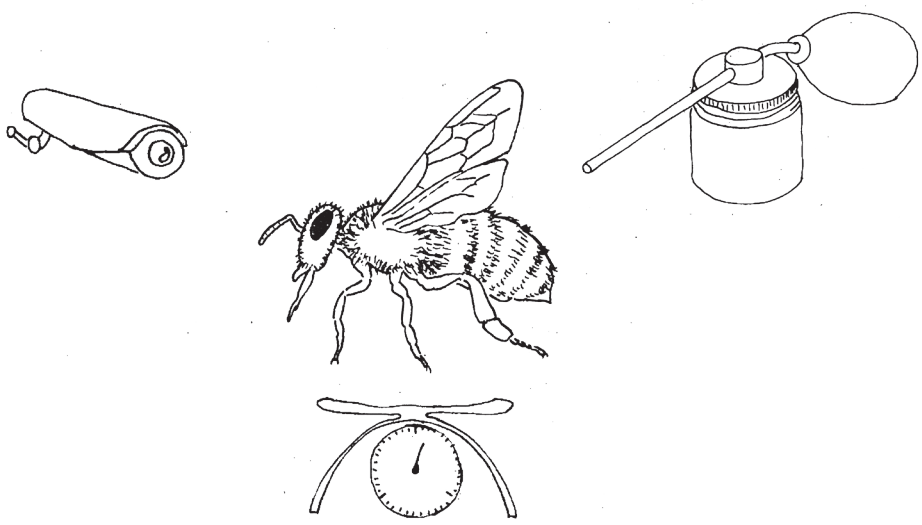

Fig. 2: At the apiary (see 1 in Fig. 1), outdoor and indoor cameras allow online observation and marker recognition of arriving honey bees; other parameters measured at or in the hives include hive weight, outdoor/indoor temperature, and precipitation (see also www.we4bee.com); a pump-sprayer for application of colored flour is shown in the upper right. Drawings by U. Schulz modified after Schmidt in Schulz & al. (2021) and after Frings (1994).

Results and Discussion

Comparison of marking pens and flour dusting as marking strategy

Marking with flour turned out to be significantly more effective than marking with bee queen pens. With both, individual marking with different colors is possible (eight colors with Holi powder, five colors with bee queen pens). However, marking with bee queen pens requires prior cooling of the worker bees or at least some experience in injury-free handling of bees. In addition, this type of marking is significantly more time-consuming and therefore fewer individuals can be marked within the same time frame (cf. Eckhardt 2020 and Lara 2022). Thus, the probability of meaningful observations increases when flour pollination is used. On the online cameras (outdoor camera 'IB836BA-HAT' from the company Vivotek and indoor camera of the Top Bar Hive from we4bee) the color dots of the bees marked with bee queen pens are clearly visible (shown in more detail in Eckhardt 2020). Among other things, this allowed an online demonstration of the phenomenon that honey bees do not always return to their hive of origin, but also embed themselves with other colonies (see also v. Frisch 1923). The detectability of flour dusted bees is also very good but depends on various factors (see below). In the following, the overall more successful marking with colored flour is discussed.

Experiments focusing on honeybee behavior in context of flour dusting as marking strategy

Preliminary experiments on marking with flour gave indications that honeybees are not strongly affected in their behavior by the dusting. Initially, honeybees were excessively dusted with flour by the "powdered sugar method" often used in practice (described e.g. in Poker & al. 2011). They then started cleaning behavior on themselves and the other bees, but still flew off after a maximum of six minutes. Despite the cleaning activities, the marking flour remained well visible. Exterior and interior cameras showed no aggressive behavior by guard bees or other bees toward the flour-marked bees, and there was no conspicuously high death rate among these. This could be explained by the fact that the Holipowder is an untreated material with food dyes.

Complementary it could be adding to the explanation that ticking of small particles in their hair coat is part of the natural behavior of the bees (but with the difference that pollen grains can range from 7 to 100 µm depending on plant species and the grain sizes of cereal- or also corn flour range to 180 µm; Delort & Amato 2018).

Experiments on flour application

During the trials at the artificial feeding station single-handed sprayers, plastic sprayers, and pump-spray sprayers for hair powder, as they are available in cosmetics stores, were tested. In the course of the trials, it was found that the application with a pump-spray sprayer for hair powder (for illustration, see top right of Fig. 2) was most favorable. This allowed the colored powder to be applied to the bees' bodies intermittently and at specific points. With this application method it is easiest to control dosages thus there is less waste of the colored powder than with the other methods.

Comparison of used camera techniques

Not all camera systems proved equally successful. For example, the video data analysis of the outdoor Wi-Fi nesting box camera did not achieve profitable images, as the images were not focused and not displayed free of interference. Similarly, the focus and image quality of the network camera were not adequate for bees marked with color flour but were still suitable for detecting markings with fluorescent flour. The images from the camcorders and the GoPro action camera, on the other hand, all clearly showed flour markings. However, the mobile camera systems were susceptible to strong sunlight and automatically switched off if they became too hot. In front of the hives, they therefore had to be partly protected with sunshades. The markings with fluorescent special flour were not only visible when the UV lamp was used, but also viewable in sunshine.

A special evaluation option results from the analysis of the TBH outdoor and indoor camera shots. Since these are single images, which can be brought into a photo slideshow by e.g. the program Animotica (2023), it is possible to apply special filter settings concerning different colors e.g. with the program VLC-Media-Player or to extract all colors except the searched one (e.g. red marking). Thus, it was possible to quickly search thousands of individual images for color-marked bees. This is very labor-saving, since results show that only comparably low amounts of pictures are showing marked bees. For example, from the inside camera of the TBH out of over 1800 single photographs (to varying marking times of 27.5.2020 – 10.06.2020) only 0.3% showed marked bees, with the outside camera at least 1.5% (which corresponds to 40 marked honeybees; see also Eckhardt 2020).

Comparison of different marking strategies

Overall, marking the bees with colored flour and especially with fluorescent flour was successful. These markings thus lend themselves to further investigations and for bee didactic purposes. For example, honeybees that had been marked by students on canola plants (*Brassica napus*) in the experimental agricultural plots using blue flour could be observed by other students within the online cameras at the apiary after about five minutes (more detailed at Lara 2022). From this, an approximate flight speed of 15 km/h minimum could be estimated, which is lower than the approx. 30 km/h usually given by v. Frisch & Lindauer (1955). In general, using these observations can give first simple indications of which forage plants in which areas are approached by the collecting bees of the apiary, even if these are located island-like in the forest area (see 3 in Fig. 1).

The advantage of flour marking over conventional bee marking methods lies in its simplicity. The bees do not have to be caught and held, the colored flour can be obtained easily and applied at any bee on the finding site. Furthermore, applied flour with food coloring can be expected to have a lesser impact on the bees and their behavior than, for example, dabbing on paint colors or sticking on platelets. Dabbing pigments on the thorax as in v. Frisch (1923) or on the abdomen as in Beutler (1954), the use of bee queen pens (see above) or even sharpie pens as in Tiesler & Englert (1989) is more laborious and more likely to affect the bees, as it can lead to smearing of dyes or glues e.g. on wings and facet eyes (Eckhardt 2020).

Recommendations for honey bee marking and online detection in apiology education

The present experiments and methodological explorations lead to the following initial conclusions. For further marking experiments or use in apiology teaching, the following is recommended:

1) use of colored and fluorescent flour,
2) application with a precision applicator and
3) use of the following cameras: camcorder 'Panasonic HC-V777' as well as a GoPro action camera depending on the sunshine, if necessary with an ultraviolet lamp.

Regarding flour markings it is important to take seasonal and regional pollen colors into account. During the trials, for example, it became clear that red-marked honey bees were sometimes difficult to distinguish from other honey bees that carried in reddish pollen from horse chestnuts (*Aesculus hippocastanum* L.). Although pollen is carried in at the pollen pouches, it can also be distributed over the body depending on the flower, in the case of pollen from *Impatiens glandulifera* Royle even clearly visible on the thorax. Therefore, before applying the flour color, it is worthwhile to observe the honey bees and to take into account the respective regional pollen carried in different colors. Depending on this, a different, contrasting color must be selected for clear marking with colored flour. Overviews of seasonal pollen colors (e.g. Kirk 2017) can be helpful.

It is advised, from a didactic standpoint, that all students, who are distributed in the field or at different positions of the study plots, independently search for potential forage plants, determine them and enter them into the georeferenced database, regardless of the marking results. In this study, there were *Phacelia*, oil radish, white mustard, white clover and summer rapeseed on the agricultural test plots, as well as various wild herbs in the botanical garden (see Fig. 1). In this way, the students not only learn to distinguish between honey bees and different wild bees, but also become familiar with their various cultivated and naturally occurring forage plants. Thereby, there is even a learning success independent of how many marked bees are finally sighted.

Acknowledgment

We would like to thank all students of the BSc courses Landscape Use and Conservation or Organic Agriculture and Marketing for their dedicated work at the apiary and on the experimental plots. We would also like to thank Andreas Thun and Mirella Zeidler (DILeLa/HNEE) for setting up the equipment, cameras, tablets, and the respective software. We thank the we4bee team around Prof. Tautz and Dr. Leikam for providing the online networked Top Bar Hive with sensors and online cameras.

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