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ORIGINAL RESEARCH ARTICLE

Standard methods to estimate strength parameters, flight activity, comb construction, and fitness of *Apis mellifera* colonies 2.0

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ABSTRACT

This paper describes methods for estimating honey bee (*Apis mellifera*) colony strength by which mean population measures of adult bees and brood are obtained. Additionally, secondary measures, such as the quantity of stored honey and pollen, brood pattern, flight activity, comb construction, and the expression of visible disease or parasite symptoms are addressed. There are generally two contexts in which an investigator wishes to measure colony strength: (1) at the beginning of a study as part of manipulations to produce uniform colonies and reduce experimental error, and (2) as a response variable during or at the end of an experiment. Moreover, there are two general modes for measuring colony strength: (1) an objective mode that uses quantitative measures, and (2) a subjective mode that relies on visual estimates by one or more observers. Other parameters that do not directly measure colony strength are described because they give important indicators of colony state. These parameters include flight activity at the hive entrance, comb construction, and two proxy measures of colony fitness: production of queen cells and drone brood.

Métodos estándar para estimar parámetros sobre la fortaleza, actividad de vuelo, construcción de panales y aptitud de colonias de *Apis mellifera*

Este artículo describe métodos para estimar la fortaleza de colonias de abejas melíferas, es decir, mediciones de la población de abejas adultas y de cría. Además, se describen mediciones secundarias como la cantidad de miel y polen almacenados, el patrón de la cría, actividad de vuelo, construcción de panales y signos visibles de enfermedades o parásitos. En general hay dos contextos en los que un investigador desearía medir la fortaleza de una colonia: (1) al comienzo de un estudio como parte de las manipulaciones para producir colonias homogéneas y reducir el error experimental, y (2) como variable de respuesta durante o al final de un experimento. En general hay dos maneras de medir la fortaleza de las colonias: (1) una manera objetiva usando mediciones cuantitativas, y (2) una manera subjetiva que se basa en estimaciones visuales de uno o más observadores. También se describen parámetros que no miden directamente la fortaleza de una colonia, porque son indicadores importantes de la condición de la misma, como la actividad de vuelo en la piquera, la construcción de panales y dos mediciones de aptitud de la colonia: producción de celdas reales y cría de zánganos.

评估西方蜜蜂蜂群群势、飞行活动、造脾和适合度的标准方法

本文介绍了估算西方蜜蜂(*Apis mellifera*)群势的方法，通过该方法可以获得成蜂和子脾的计量。此外，还介绍了几个次要指标，如储存蜂蜜和花粉的数量、子区模式、飞行活动、造脾以及疾病或寄生虫可见症状的表现。通常在两种情况下，研究人员希望能测量蜂群群势：第一，在启动某项研究时为获得均等蜂群、减少实验误差而进行操作；第二，在实验期间或实验结束后，将其作为一个响应变量。此外，测量蜂群群势有两种通用模式：一是使用定量测量的客观模式；二是依赖于一个或多个观测人员目测的主观模式。本文也介绍了其它不直接测量蜂群群势的重要指标，这些指标可以指示蜂群的状态，包括巢房入口处的飞行活动、造脾，以及蜂群适合度的两个间接指标：王台和雄蜂蛹的出现。

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1. Introduction

Herein, methods for estimating honey bee (*Apis mellifera* Linnaeus) colony strength are described as per Delaplane et al. (2013), with revisions and new information on methods to assess flight activity at the hive entrance using Doppler radars (section Flight activity at hive entrance using Doppler radar) and video monitoring techniques (section Flight activity at the hive entrance using video monitoring). Also, an update on procedures to measure colony strength at the end of experiments using the subjective method is provided. Improvements in procedures to reduce bias between observers are emphasized (section Subjective mode). The main difference between the last version of the manuscript and the present version are revisions based on current literature and the inclusion of novel technological tools to assist researchers in measuring colony strength and other related parameters.

The methods described here for estimating honey bee colony strength primarily include population measures of worker adult bees and brood. Methods for secondary measures, such as the quantity of stored honey and pollen, brood pattern (the degree of worker brood solidity or contiguity), and the expression of visible disease or parasite symptoms are also included. Strictly speaking, these measures are not indicators of a colony's immediate state as they are legacy effects or predictors of future conditions (Budge et al., 2015; Dainat et al., 2012; Jevtić et al., 2009; Lee et al., 2019).

For research purposes, there are two contexts in which an investigator wishes to measure colony strength: (1) at the beginning of a study as part of manipulations to produce uniform colonies and reduce experimental error, and (2) as response variable during or at the end of an experiment. Moreover, there are two general modes of measuring colony strength: (1) an objective mode that uses quantitative measures, such as weight (mg, g, or kg) or area (cm^2), covered in sections Objective mode by computer-assisted digital image analysis and Objective mode, and (2) a subjective mode that relies on visual estimates by one or more observers, covered in sections Subjective mode and Subjective mode. The objective mode is the more accurate of the two, but it is also invasive and disruptive to the honey bees, constituting in some cases the complete deconstruction and reassembly of colonies with disruption to any social cohesion formerly intact. For this reason, we consider the objective mode best suited to the beginning and end of experiments. In addition, computer-assisted digital image analysis, covered in section Objective mode by computer-assisted

digital image analysis, is minimally invasive and automatically generates archival images for data traceability and verification; it also provides objective quantitative data. Its main disadvantages are cost and dependence on technology. However, it is the opinion of some that the speed and ease of visual estimates surpass the advantages of objectivity and archival properties of digital methods. Nevertheless, we will probably see technical improvements and increasing use of this mode soon. The subjective mode is less accurate but far less disruptive to the honey bee colony. Therefore, it is appropriate for collecting response variables during the experiment when the investigator has an interest in preserving the social cohesion of experimental colonies. One exception to this would be if the sampling intervals are sufficiently distanced (2–3 times per year) to justify the objective mode throughout the experiment. Nevertheless, with safeguards in place, such as we describe below, the subjective mode is an acceptably robust and reproducible technique. Thus, it is probably the mode that can be applied in most cases.

Sections Indirect indicators of colony strength and state and Proxy measures of honey bee colony fitness cover methods that indirectly measure colony strength and state. These include measuring flight activity at the hive entrance (visual observations using electronic devices, or with Doppler radar), comb construction, and two proxy measures of colony fitness: production of queen cells, and drone brood.

A note is warranted here on a couple of omissions from this chapter: gross colony weight, X-ray tomography, and infrared imaging. Gross colony weight is a useful metric in the context of seasonal changes in forage availability. Hive-scale data have long interested beekeepers for their usefulness in tracking local nectar flows. More recently, these kinds of data have been used to monitor flowering phenology in the context of climate change, environmental effects, and landscape on colony health and productivity (Lecocq et al., 2015; Meikle et al., 2018; Nightingale et al., 2008). As a measure of colony strength *per se*, however, gross colony weight is ambiguous and unreliable, owing to the fact that workers from health-compromised colonies may express precocious foraging with the result that weights of colonies may vary in response to disease or other disorders (Mayack & Naug, 2009).

X-ray tomography offers what is probably the most empirically quantifiable, thorough, and non-invasive means of monitoring the colony strength of colonies of honey bees or other social insects (Greco, 2010). Although it sets a gold standard, its enormous technical requirements keep this method out of reach of most honey bee researchers. Additionally, infrared thermal imaging has been proposed as a non-invasive method to assess colony strength and viability (Shaw et al., 2011), and has been shown to have promising advantages. It is a rapid method, it does not compromise the queen, and it could be used to assess overwintering colonies (Bromenshenk, 2015; Ducsharm & Eccles, 2019). However, more studies are needed to correlate the data generated from infrared imaging with data from validated methodologies and consider the effect of variables that can affect the infrared measurements (e.g., solar heat).

2. An optimal colony configuration

When establishing honey bee colonies for experiments, it is useful to have some guidance on how colony population size can be expected to affect colony growth, behaviour, and survivorship. Additionally, factors, such as season, nectar and pollen availability, and colony health can influence colony population growth (Guzman-Novoa et al., 2010; Taha, 2014), and thus need to be considered as well. The best guidance on this matter comes from Harbo (1986) who, in a study conducted in Baton Rouge (LA, USA), compared worker brood production, worker survival, and honey gain in colonies initiated with a queen, no worker brood, as well as 2300, 4500, 9000, 17,000, and 35,000 adult bees, while fixing worker bee density at *ca*. 230 per 1000 cm^3 of hive space. The authors assigned hives of different sizes (10, 20, 39, 64, and 124 L) to the initial experimental populations to provide a similar worker density for the experimental colonies. The experiment was repeated in each of the months of February, April, June, August, and October, and terminated 19 days after the queens were released, which allowed measuring egg-laying rates, number of eggs laid on the first day, number of larvae after 96 h of the queens being released, and cm^2 of capped brood. Worker survival (after a 22-day trial) was significantly higher in colonies with 2300–9000 bees than in colonies with 35,000 bees during the months of June, August, and October. Larger populations tended to store more nectar per adult bee during times of nectar flow and consumed less during times of nectar dearth. However, smaller populations produced more brood per adult bee. Harbo (1986) concluded that the optimal colony size is 9000 worker bees, as colonies of this size are between the honey hoarding efficiency of large populations and the brood rearing efficiency of small colonies. Colonies that are significantly larger than this are costly and labor-intensive to set up, and less suitable for measures of population growth because they are already near their maximum. Colonies significantly smaller than this may do well at the height of the season, but they are more vulnerable to winter and summer

stress. Thus, researchers should consider colony size and the likelihood of colony survival in the region where the experiment will be conducted. Honey bees are normalized for colony-source genetics and parasite loads if colonies are established with a common pool of workers as described in section Classical objective mode. If colony growth is a measure of interest, the investigator can invite a greater range of expansion if colonies are started with no brood. But if one prefers to provide colonies with brood, it is reasonable to stock colonies of 9000 bees with no more than two combs of brood of various ages, allowing plenty of open cells to accommodate growth.

3. Establishing experimental colonies of uniform strength

This section describes two variations of an objective mode for setting up uniform colonies for experiments. The first (Classical objective mode), which we call the classical objective mode, and a variation (Shook swarm objective mode), the so-called "shook swarm" method. Table 1 highlights the pros and cons of each.

3.1. Classical objective mode

One of the recurring pitfalls of honey bee research involving colonies is a large experimental error, which can hinder the investigator's attempts to discriminate statistical differences among effects of interest. One of the best ways to minimize this problem is to begin experiments with colonies as uniformly as possible in regard to comb space, food resources, and populations of adult bees and brood. It is the job of the investigator to distribute these resources equitably among experimental colonies. The number of colonies per treatment will also influence the statistical discrimination of treatments or factors of interest. Therefore, more colonies per treatment reduce the experimental error.

The following synthesis draws from methods pioneered by Harbo, who was mainly interested in reducing environmental variation in honey bee breeding programs (Delaplane & Harbo, 1987; Harbo, 1983, 1986, 1988, 1993), and adapted later by investigators who recognized the utility of these methods for field research on the mite, *Varroa destructor* (Berry et al., 2010; Delaplane & Hood, 1997, 1999; Ellis et al., 2001; Sinia & Guzman-Novoa, 2018; Strange & Sheppard, 2001), bee behaviour (Giray et al., 2000; Guzman-Novoa et al., 2002), colony growth (Berry & Delaplane, 2001), selective breeding (De la Mora et al., 2020; Guzman-Novoa, 2007), and construction of colony population models (Khoury et al., 2013; Russell et al., 2013; Torres et al., 2015). Additionally, the classical objective mode to assess colony strength has been incorporated into monitoring strategies that include parameters of colony population dynamics and ecological data to assess the effect of stressors on colony health (Odoux et al., 2014).

The goal is to have field colonies equalized in regard to adult bees, brood, mites, and food resources within units of higher-order experimental replication (i.e., blocks or whole plots), usually based on geography. To accomplish the above, proceed as follows:

1. Pre-stock empty hives with brood, empty combs, syrup feeders, and a caged queen in advance of receiving worker bees (Figure 1). All hives should receive the same amount/number of these items. Minimize variation due to bee genetics by providing each colony a sister queen reared from the same mother and open-mated in the same

Table 1. Pros and cons of two variations of an objective mode for establishing honey bee (*Apis mellifera*) colonies of uniform initial strength.

Method	Pros	Cons
Classical (section Classical objective mode)	1. Results in colonies with initial populations of adult bees normalized for genetics and pathogen load. 2. Results in colonies with brood of all stages, accelerating colony growth. 3. Results in maximized colony uniformity regarding initial adult bee populations.	1. Disease and parasite legacy effect in brood, although normalized, is nevertheless sustained into the experimental period. 2. Because brood of all stages is present, progeny turnover from new experimental queens is correspondingly delayed.
Shook swarm (section Shook swarm objective mode)	1. Does not require use of a customized cage to house common pool of bees. 2. Sustains colony-specific identity from pre-experimental to experimental period. 3. Because of #2, drift is not a concern and it is not necessary to move experimental colonies from the source apiary. 4. Because all brood is removed and replaced with frames of foundation, disease and parasite legacy effect is minimized. 5. Because of #4, if *Varroa destructor* control is an element of experimental design, the initial broodless period provides an ideal opportunity to treat for the mites.	1. Because adult bees are not normalized, between-colony variation in genetics and pathogen load remains at pre-experiment levels. 2. Because colonies begin broodless, colony growth is delayed.

Figure 1. Investigators pre-stocking experimental hives with equal numbers of brood combs, honey combs and caged honey bee (*Apis mellifera*) queens in preparation for receiving worker bees from a common cage.

Figure 2. A frame with a grid in dm^2 ($1dm^2 = 0.01\ m^2$) is used to visually sum the surface area of honey bee (*Apis mellifera*) brood. Photo credit: Benoît Droz, Swiss Bee Research Centre, Agroscope.

vicinity. A more robust option is to instrumentally inseminate sister queens (Cobey et al., 2013) with the same pool of mixed drone semen.

2. Staple (or strap later) together bottom boards and hive bodies to prepare them for moving.
3. Screen hive entrances to trap bees temporarily. This is done for two reasons: (a) experimental colonies often need to be moved to a permanent site and away from the source colonies from which workers are collected, and (b) a period of in-hive confinement, usually overnight, seems to help bees orient to their new hive and queen.
4. Collect brood for incipient experimental colonies from the same source colonies used to collect adult bees.
5. Perform an initial measure of the quantity of brood available in donor colonies by:

5.1. overlaying on each side of every brood comb a transparent plastic or wire grid pre-marked in cm^2 or dm^2.

5.2. Visually sum the number of squares of the total brood area (Figure 2).

5.3. Calculate number of brood cells (if required) by multiplying brood area (cm^2 or dm^2) by the

average cells per cm^2 or dm^2. This value varies by geography and honey bee genotype (Table 2); the investigator can determine local average cell density by counting the number of cells directly in a square equaling 1 cm^2 or 1 dm^2 and using the mean of at least 10 measurements.

6. Assign a near-equal quantity of brood to experimental colonies. We do not prescribe "random" brood assignment because the investigator should place a higher priority on equalizing the quantity of brood over concerns of non-random assignment of brood. Efforts should be made to equalize the relative quantity of sealed *vs.* open brood.
7. Derive and equalize the initial number of cells of honey or pollen or even empty cells.

   Depending on one's standards for strict uniformity, it may be simpler to provide nothing but brood or empty cells and to provide uniform nutrition across the experiment by using sucrose syrup and protein supplements.

7.1. If the investigator is using nucleus hives small enough to weigh them in the field (Figure 3), the intermediate step of a colony-specific cage is not necessary and the investigator can scoop bees from the common cage (a cage used to contain worker bees shaken from different source colonies; Figure 4) directly into the pre-weighed or tared hive. The net weight (kg) of bees is recorded. Then, the initial population was determined the same way as in step 11.

8. Collect adult bees for experimental set-up by shaking workers from a diversity of source colonies into one large, common, ventilated cage, allowing workers (and diseases and parasites) to mix freely. When working with bees of African ancestry, it is helpful to spray the bees on the comb with water mist before shaking them into the cage to prevent them from flying away and losing them. The weight of worker bees collected (kg) should exceed the target weight of bees needed for the study by at least 2 kg, or at least by a third in the case of African subspecies, to account for adult bee loss through death or flight. Honey bee survival in the cage is greatly improved if the investigator designs it to accommodate 4–6 brood combs to provide a clustering surface (Figure 4). Maintain the cage in cool conditions for at least 24 h to prevent bee death from over-heating, and allow thorough admixing of bees. This will result in a uniformly heterogeneous mixture.
9. To equalize initial colony populations, it is preferable to make colony-specific caged cohorts. Empty screened cages, ideally made to fit on top of an empty hive, are each pre-weighed or tared with a balance in the field. The large common cage is opened and the adult bees are sprayed with water to reduce flight. Then, adult bees are transferred from the common cage into the smaller colony-specific cages with the aid of cups or scoops (Figure 5). Adult bees are added or removed from each colony-cage until the target weight (preferably $\geq$2 kg) is achieved and recorded.
10. A sample of *ca.* 300 workers is collected from each incipient colony into a pre-weighed or

Table 2. Surface area of some regionally common frame types and expected honey bee (*Apis mellifera*) density when a frame is fully occupied by worker bees.

Region	Local frame type	Number bees per fully-occupied side	Surface (cm^2) per side of frame	Bees (cm^2)	Ref	Worker cells (cm^2)
North America	Deep Langstroth	1215	880	1.38	[a]	3.7[c]–3.9[d]
North America	3/4s	910	655	1.39	[a]	
North America	Western	785	565	1.39	[a]	
North America	Shallow	640	461	1.39	[a]	
Europe	Swiss	1200	930	1.29	[b]	4.0[e]
Europe	Dadant	1400	1130	1.24	[b]	4.0[e]
Europe	German normal	900	720	1.25	[b]	4.0[e]
Europe	Langstroth	1100	880	1.25	[b]	4.0[e]
Europe	Zander	1000	810	1.23	[b]	4.0[e]
South and Central America	Jumbo for brood chamber (modified Dadant)	1980	1130	1.75	[f]	4.1–4.7[g]
South and Central America	Jumbo for super (modified Dadant)	920	520	1.77	[f]	4.1–4.7[g]
Africa	We are not aware of published methods for determining bee numbers and cell density with *Apis mellifera* in Africa. However, these bees are ca. 3% smaller than African bees in South America[h], so it is reasonable to apply this conversion to the values given above for South and Central America.					

[a]Burgett and Burikam (1985).
[b]Imdorf and Gerig (2001).
[c]Harbo (1993).
[d]Harbo (1988).
[e]Imdorf et al. (1987).
[f]Gris (2002).
[g]Guzman-Novoa et al. (2011).
[h]Buco et al. (1987).

Figure 3. Nucleus honey bee (*Apis mellifera*) colonies are small enough to be weighed directly in the field, bypassing the need for intermediate hive-specific cohort cages.

Figure 4. A ventilated cage made to hold a large common heterogeneous mixture of honey bees (*Apis mellifera*) for starting experiments.

tared container and weighed fresh. Then, workers from the jar are counted in the lab to derive a colony-specific measure of the average fresh weight of individuals (mg per bee). To count worker bees, it is necessary to immobilize them first. This can be done either by freezing them or non-sacrificially with CO_2 narcosis (Human et al., 2013). Dividing the initial colony cohort size (kg, from step 7) by the average fresh weight of individuals (mg) gives the initial bee population for the colony.

11. If initial measures reveal outliers in terms of the number of adult bees and the amount of brood, honey, pollen, and empty cells, corrective action

Figure 5. Honey bees (*Apis mellifera*) are transferred from the common cage to hive-specific cohort cages by use of cups or scoops.

should be taken. In general, corrections aimed at minimizing experimental error are permissible until the point at which treatments are begun.

12. Move equalized colonies to their permanent apiary site. Over-heating is a risk, and hives must be kept as cool as possible. There is a special advantage to setting up colonies late in the day and moving hives to the experimental apiary at night. Not only is it cooler, but once hives are unloaded and entrances opened, the bees do not fly because of the darkness and this protracted period inside the hive seems to help them orient to the new queen and reduces drifting. Colonies can be given sucrose syrup after they are unloaded or 24 h later, after bees have settled.
13. Arrange apiaries to limit worker drift between colonies. This can be done by "complicating" the visual field of bees with orienting landmarks near their nest entrances. This can be as simple as using rocks or trees or more deliberate, such as painting varying geometric shapes on hive fronts. Arranging hives in a strongly linear arrangement is not good because hives at the ends tend to accumulate bees. For this reason, some investigators place hives in a circle (Dynes et al., 2019).
14. Control colonies for non-target diseases and disorders, queen conservation, swarm prevention, and feeding as necessary. Of these, queen loss and swarming tend to be the most disruptive to colony populations. Cutting out queen cells, adding honey supers, marking queens, and regular inspections reduce these problems. If honey supers are added, it is best to add them above a queen excluder to limit the range of the queen's egg-laying activity. The goal of these manipulations is to decrease experimental residual error. Creating 2–3 more colonies per treatment than originally needed is desirable in case colonies are lost during the experiments.

3.2. Shook swarm objective mode

With this method, it is assumed that investigators will use a pre-existing apiary and modify it for the experiment's purposes. It is important that the experiment starts at a time of year during which the bees can easily draw out foundation into comb.

1. In the days leading to set-up, locate, cage, and return the queens back to their colonies to save time on set-up day. Although caging queens seems to be a safe procedure, additional queens to replace possible losses should be considered.
2. Remove colonies from the apiary if they are expressing disease symptoms, significantly

under-performing, or otherwise showing excessive between-colony variation.
3. Import new colonies if needed to reach the target colony number and treat them similarly.
4. Bring several empty hives equal to the target number of colonies to the apiary, each stocked with brood frames of new foundation, honey supers with frames of foundation if the nectar flow warrants, and sugar syrup feeders.
5. If affordable, it is good to start new colonies on factory-new woodenware to avoid confounding issues of any disease or chemical residue legacy effects. If new hives and/or frames are not available, the woodenware can be scraped and flamed before use. Chemical residues have been found in wax foundation, thus, researchers could consider the use of plastic foundation or submitting foundation samples for chemical analysis to discard potential sources of contamination (Lozano et al., 2019; Morales et al., 2020).
6. Move aside each hive from its stand. The hive can be placed temporarily on the ground, next to its original stand.
7. Set an empty hive in its place.
8. Remove roughly half of the frames of foundation to create space in the new hive.
9. Suspend a caged queen between the two center-most frames of foundation in the new hive.
10. Remove frames from the original hive sequentially.
11. Shake the adult bees off the frames into the new hive. One to 2.5 kg of adult bees is enough to start a colony. This is equivalent to about 7000–17,000 worker bees (Lee & Winston, 1987; MAAREC, n.d.; Smith et al., 2014).
12. Bounce or brush the adult bees out of the supers into the new hive.
13. Return the bee-free combs to the original hive.
14. Cover the original and the new hives to discourage robbing behaviour.
15. Return the frames of foundation initially removed to the new boxes. Proceed gently to avoid injuring bees that may be heaped on the floor.
16. Feed experimental colonies sugar syrup to encourage comb construction on the new foundation, unless there is a strong nectar flow in progress.
17. Remove the old bee-free chambers from the experimental apiary. Use the combs from those boxes elsewhere as supplemental brood or feed. None of these combs should be placed in the new hives.
18. After 1 day, release the caged queens in the experimental colonies.
19. Monitor colonies for queen performance and colony development.
20. Replace poor-performing queens as needed to minimize within-apiary experimental error.
21. Apply treatments/begin experiment once colonies reach a development state consistent with the experiment's objectives.

The expected outcome of this maneuver is to have a reduced within-apiary variability in the colony developmental state.

4. Measuring brood, honey, pollen, and other strength parameters during the experiment

4.1. Objective mode by computer-assisted digital image analysis

Computer-assisted digital image analysis can be used to measure surface area of comb occupied by honey bees or other colony resources, such as eggs, larvae, sealed brood, nectar, or pollen. There are two kinds of data output: (1) direct surface measurements (cm^2 or dm^2) of target parameter (Alves et al., 2020; Cornelissen et al., 2009; van Dooremalen et al., 2018) (section Direct surface measurements of target), and (2) ratio of target surface relative to total comb surface (Yoshiyama et al., 2011) (section Ratio of target surface relative to total comb surface area). In the case of 1., it is possible to convert surface to units of bees or cells using conversion values in Table 2. Freely available software, like *DeepBee*© (Alves et al., 2020), can be used to process comb images automatically and export the results for analyses into spreadsheets. Additionally, Jeker et al. (2012) developed a computer-assisted method to analyze parameters associated with brood development, food storage, and queen fertility, using images from brood frames. The software in turn allows us to classify and trace the cells under study. This is of particular value when periodical colony development assessments are necessary (e.g., every three weeks throughout the season).

4.1.1. Technology and photographic considerations

- A high-resolution camera (3648 × 2736, 10 megapixels, or higher) is preferred. A digital single-lens reflex camera (DSLR) or similar camera is recommended. Compact cameras will work fine too, but it is unlikely that eggs and young brood will be visible when using this camera.
- Use image formats with the least amount of compression (resulting in the larger file size). For DSLR cameras, this will be either RAW or TIFF format, and for compact cameras, this will be JPEG format. As Image J software (Schneider et al., 2012) does not support the use of RAW images, conversion to either TIFF or JPEG files

(uncompressed) is required. This can be done using free-ware, such as Irfan-View (Boze, 2001).

- Use of a tripod with a fixed distance to the frames is recommended. This makes image analysis easier and pictures more comparable.
- Ensure that the object (comb frame) completely covers the picture. This will result in the highest resolution and optimal lighting conditions.
- It is advisable to use Shutter speed priority (indicated with an "S" on camera) with a setting of 1/125. Lower shutter speeds can result in blurred bees as their movement is caught on camera. In low light conditions, use a flash or adjust the ISO values.
- Aperture settings are dependent on the type of lens used. For DSLR, it is better to use a fixed 50 mm lens. These generally are affordable, sharp, fast (low *f*-value, e.g., *f*1:1.4), and have little distortion. Using these lenses, the f-value should be above *f*4.5 for sharp pictures. For zoom lenses, aim at an *f*-value between *f*6.7 and *f*13.
- Cloudy conditions can create low light levels; likewise, the sun can obscure details due to high contrasts. Optimal results are possible with a shaded location and a flash, but this is not practical due to terrain difficulties and limited battery life. Cloudy weather is no problem when using a fast lens. When it is sunny, it is best to take pictures with one's back to the sun.

4.1.2. Direct surface measurements of target

1. Photographic records of honey bees on combs will vary according to the time of day and honey bee foraging activity. For this reason, it is important to control for this effect, either by limiting observations to a narrow time window on successive days, randomly assigning time of inspection such that the day effect is equitably and randomly distributed across treatments, or closing hive entrances in the early morning until bees are counted. This constraint does not apply to cell-based resources, such as brood, honey, or pollen.
2. Hives are lightly smoked, opened, and frames permanently labeled: frame 1 side A or B, frame 2 side A or B, and so forth.
3. Each frame is removed and photographed on each side in such a way that colony and frame labeling are recorded. It is preferable to use a custom-built holding mount where each comb is placed in a holder and the distance between the comb and camera is fixed (Figure 6).
4. Combs are first photographed with bees. If additional comb resources are of interest, then the bees are brushed into a holding box, and the comb photographed again to expose brood, honey, or pollen. It is important to avoid brushing bees back into the hive because this will affect the photographic bee record of subsequent

Figure 6. Mount where a comb is placed in a holder and the distance between the comb and camera is fixed to photograph its sides.

frames. Eggs and 1–3 day old larvae may be hard to see and if these brood stages are the objective of the study, it is preferable to apply digital cell/location recognition software, such as *CombCount* (Colin et al., 2018) and *DeepBee*© (Alves et al., 2020), or the commercially available *HoneybeeComplete* (WSC Regexpert, 2021).

5. The digital photos are analyzed using a computer program, such as ImageJ (Schneider et al., 2012), available free at http://rsbweb.nih.gov/ij/. Post-hoc, the photos are uploaded to a computer and analyzed as diagrammed in Figure 7.
6. The results of the calculated area are in dm^2 or cm^2 depending on the scale that has been set. To finish the analysis, the number or bees or cells are derived with a spreadsheet (e.g., Excel; Microsoft Corporation, 2018) or a similar spreadsheet program using the expected density of bees per cm^2 or cells per cm^2 given in Table 2. Surface data from this digital analysis could be inserted into Column G or H in Figures 8–10.

4.1.3. Ratio of target surface relative to total comb surface area

This method, also applying ImageJ (Schneider et al., 2012), yields the ratio between the selected area and the total comb area. Before computer analysis, each digital image of a comb side is digitally edited by the investigator to delineate comb areas of the target resource (e.g., capped brood area, open brood, pollen, or honey with unique identifying colours). Figure 11 and Table 3 outline the application and the colour codes, respectively. Alternatively, the digital analysis of comb areas of target resources can be done with image processing software (e.g., ImageJ or Adobe Photoshop) (Faulkner & Chavez, 2017; Jeker et al., 2012; Schneider et al., 2012).

4.2. Subjective mode

This section describes a subjective mode for reporting the quantity of any kind of colony resource stored in cells: open brood, sealed brood, honey, or pollen. The methods are similar to those described for measuring colony populations subjectively in section Subjective mode. The only difference concerns whether the investigator wants to report the resource in units of area (cm^2) or number of cells. Authors have also reported resources in units of "frames," but this is unnecessarily ambiguous and makes it harder to compare data to other studies (Chabert et al., 2021). As mentioned before, honey is traditionally reported as weight (kg), and it is best to use queen excluders and pre-weighed honey supers as described in section Objective mode. However, if the investigator wants to report honey occurring in combs alongside brood, it may be necessary to report it in units of cm^2 or cells as described in this section. These methods for measuring brood, honey, or pollen are fundamentally the same for African honey bees, given that the investigator uses the region-specific multipliers from Table 2.

1. Estimates are performed by no fewer than two observers, one of them being a dedicated

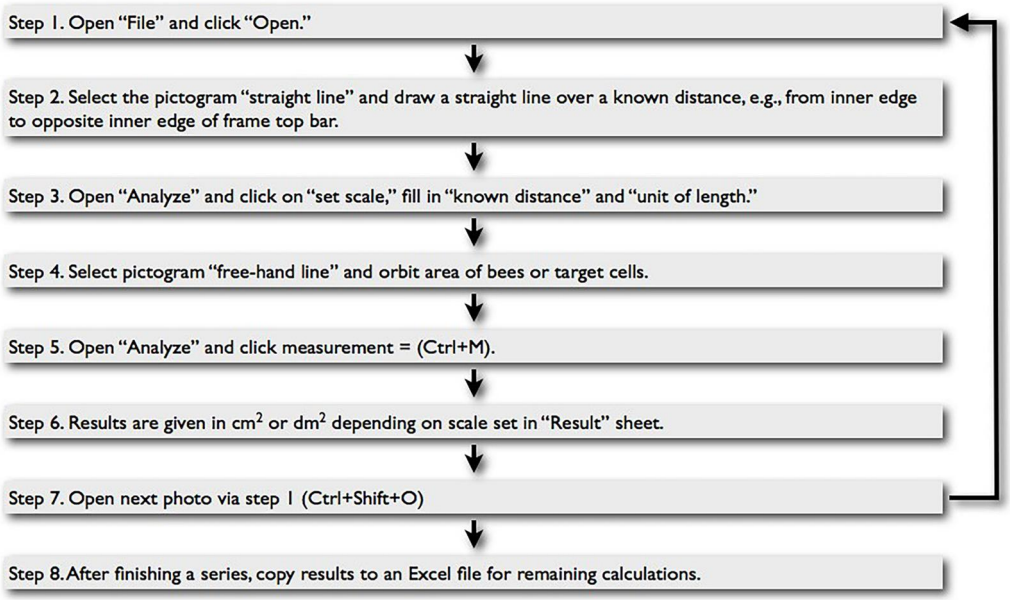

Figure 7. Outline of the method of Cornelissen et al. (2009). Flow chart of computer assisted image analysis applying ImageJ software. Step 2 can be skipped by making the photos in a fixed position at which the distance between camera and frame is constant.

G2 =(F2*880)

	A	B	C	D	E	F	G	H
1	colony	frame	side	observer 1	observer 2	mean of observers	cm2 covered by bees	bees per side
2	1	1	A	0.2	0.3	0.25	220	303.6
3	1	1	B	0.3	0.5	0.4	352	485.76
4	1	2	A	0.4	0.4	0.4	352	485.76
5	1	2	B	0.5	0.6	0.55	484	667.92
6	1	3	A	0.7	0.8	0.75	660	910.8
7	1	3	B	0.7	0.7	0.7	616	850.08
8	1	4	A	0.5	0.4	0.45	396	546.48
9	1	4	B	0.3	0.3	0.3	264	364.32
10	1	5	A	0.4	0.2	0.3	264	364.32
11	1	5	B	0.1	0	0.05	44	60.72
12							colony bee population	5039.76
13	2	1	A	0	0	0	0	0
14	2	1	B	0.2	0.3	0.25	220	303.6
15	2	2	A	0.4	0.4	0.4	352	485.76
16	2	2	B	0.5	0.6	0.55	484	667.92
17	2	3	A	1	0.9	0.95	836	1153.68
18	2	3	B	0.9	0.8	0.85	748	1032.24
19	2	4	A	0.7	0.8	0.75	660	910.8
20	2	4	B	0.5	0.5	0.5	440	607.2
21	2	5	A	0.3	0.4	0.35	308	425.04
22	2	5	B	0	0	0	0	0
23							colony bee population	5586.24

Figure 8. Example of a datasheet for converting raw observer data into colony honey bee (*Apis mellifera*) population.

G12 =SUM(G2:G11)

	A	B	C	D	E	F	G
1	colony	frame	side	observer 1	observer 2	mean of observers	cm2 covered by open brood
2	1	1	A	0.1	0.2	0.15	132
3	1	1	B	0.3	0.4	0.35	308
4	1	2	A	0.6	0.4	0.5	440
5	1	2	B	0.7	0.6	0.65	572
6	1	3	A	0.8	0.8	0.8	704
7	1	3	B	1	1	1	880
8	1	4	A	0.9	1	0.95	836
9	1	4	B	0.8	1	0.9	792
10	1	5	A	0.5	0.4	0.45	396
11	1	5	B	0.1	0	0.05	44
12						sum cm2 of colony open brood	5104
13	2	1	A	0	0	0	0
14	2	1	B	0.1	0.3	0.2	176
15	2	2	A	0.5	0.4	0.45	396
16	2	2	B	0.5	0.6	0.55	484
17	2	3	A	0.9	0.9	0.9	792
18	2	3	B	1	0.8	0.9	792
19	2	4	A	0.7	0.7	0.7	616
20	2	4	B	0.3	0.4	0.35	308
21	2	5	A	0	0.1	0.05	44
22	2	5	B	0	0	0	0
23						sum cm2 of colony open brood	3608

Figure 9. Example of a datasheet for converting raw observer data into cm^2 of open honey bee (*Apis mellifera*) brood cells (see section Subjective mode).

note-taker who writes down numbers, or each fitted with an audio recorder.

2. A colony is opened and combs of honey bees sequentially removed. Each observer looks at one side of a comb or alternatively one observer looks at both sides of a comb while the other takes notes. The observer visually estimates the percentage of the comb surface occupied by the target resource, and records the number with the secretary or audio recorder. It is convenient to label frames 1-X, with each side indicated A or B. As described in the previous section, the observer

H2 =(G2*3.7)

	A	B	C	D	E	F	G	H
1	colony	frame	side	observer 1	observer 2	mean of observers	cm2 covered by open brood	cells open brood
2	1	1	A	0.1	0.2	0.15	132	488.4
3	1	1	B	0.3	0.4	0.35	308	1139.6
4	1	2	A	0.6	0.4	0.5	440	1628
5	1	2	B	0.7	0.6	0.65	572	2116.4
6	1	3	A	0.8	0.8	0.8	704	2604.8
7	1	3	B	1	1	1	880	3256
8	1	4	A	0.9	1	0.95	836	3093.2
9	1	4	B	0.8	1	0.9	792	2930.4
10	1	5	A	0.5	0.4	0.45	396	1465.2
11	1	5	B	0.1	0	0.05	44	162.8
12							sum cells of colony open brood	18884.8
13	2	1	A	0	0	0	0	0
14	2	1	B	0.1	0.3	0.2	176	651.2
15	2	2	A	0.5	0.4	0.45	396	1465.2
16	2	2	B	0.5	0.6	0.55	484	1790.8
17	2	3	A	0.9	0.9	0.9	792	2930.4
18	2	3	B	1	0.8	0.9	792	2930.4
19	2	4	A	0.7	0.7	0.7	616	2279.2
20	2	4	B	0.3	0.4	0.35	308	1139.6
21	2	5	A	0	0.1	0.05	44	162.8
22	2	5	B	0	0	0	0	0
23							sum cells of colony open brood	13349.6

Figure 10. Example of a datasheet for converting raw observer data into number of open honey bee (*Apis mellifera*) brood cells.

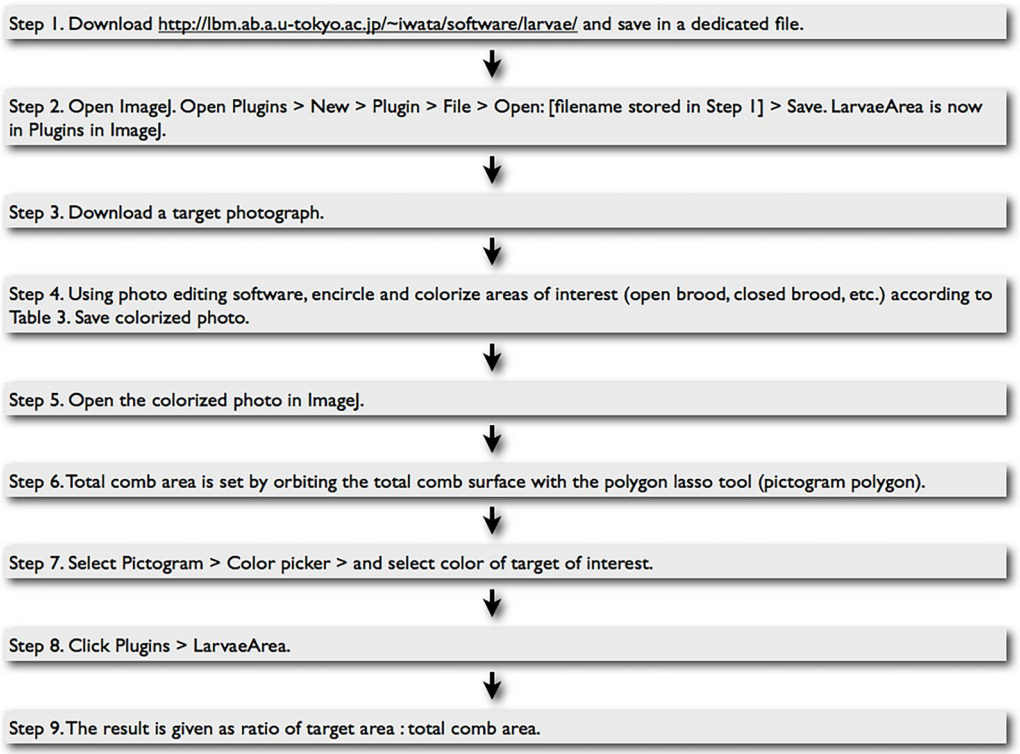

Figure 11. Outline of method of Yoshiyama et al. (2011) for determining ratio of target surface: total comb surface. It is used with the OECD colour codes in Table 3.

is imaginatively sorting the resource into one contiguous mass and making a decision on the percentage surface area of the comb the contiguous resource occupies. This can be difficult in cases of spotty brood where widely separated cells must be imaginatively grouped together. It is to be expected that the accuracy of this mode is best when target resources are massed together in convenient contiguous patches.

3. Figure 9 is a screenshot of a datasheet (e.g., Excel; Microsoft Corporation, 2018) demonstrating the conversion of raw data from two observers

Table 3. Colour and number-coding of cell contents of honey bee (*Apis mellifera*) combs according to the Organism for Economic Cooperation and Development (OECD, 2007).

Brood stages/stores	Colour	Number
Empty cells	Brown	0
Eggs	Blue	1
Young larvae (1–3 days)	Green	2
Older larvae (4–6 days)	Red	3
Pupae (capped brood)	Yellow	4
Nectar	Orange	5
Pollen/bee bread	Deep pink	6
Dead larvae/pupae	Dark salmon	7
Not characterized (nc)	White	8

into cm^2 of target resource, in this example open cells of brood. There are two fictional colonies, each with five North American deep frames, each with two sides. Columns D and E show the respective visual estimates of two observers for the proportion of comb surface occupied by open brood, and column F is the mean of the two. Column G converts the mean proportion of the surface occupied by open brood into area (cm^2), using the surface area for one side of a North American deep frame from Table 2 (880 cm^2). This is done by multiplying the mean proportion in column F by 880 cm^2. Rows 12 and 23 sum the area of open brood for each colony.

4. If investigators use colonies with different sized supers and frames, it will be necessary to adjust calculations for the one-side surface area unique to each comb type. This would affect the area conversion factor used in Figure 9, column G.
5. To report a resource in units of cells, it is necessary to multiply the cm^2 of resource by the average cell density per cm^2. This value varies by geography; conversion factors range from 3.7 to 4.7 (Table 2). It is advisable for investigators to determine this value for their local conditions. Figure 10 shows a modification of Figure 9 taking the data from cm^2 of open brood to the number of cells of open brood, using a conversion factor of 3.7.

4.3. Indirect indicators of colony strength and state

4.3.1. Flight activity at the hive entrance

Fight activity can be assessed visually by observers. It can also be recorded and assessed with the aid of technological tools (Odemer, 2021), including infrared detectors (Struye et al., 1994), video-monitoring (Campbell et al., 2008; Kale et al., 2015), and Doppler radar (Cunha et al., 2020). The following protocol describes the visual monitoring of flight activity, which could be used as an indicator of colony strength and state.

1. Bee flight activity can be monitored visually at hive entrances to gain a relative measure of colony foraging effort. To control for between-colony variation due to time of day, the investigator should: (1) limit observations to days and time of day with good flight conditions, (2) randomize the numeric order in which colonies are measured, (3) measure all colonies within a relatively narrow window of hours, and (4) limit colony observations to the same time window over successive days.
2. Two observers sit at the side of a hive, both positioned well enough to the side to avoid obstructing the flight of the bees. Each observer has a hand-held counting device, and one keeps time.
3. For one 15-min counting episode, both observers count and record the number of bees exiting the colony (but see step 5 below). Exiting bees are simpler to count because returning foragers land with less predictability, some directly into the entrance, others onto the front of the hive.
4. The mean of the two observers is derived and the data is reported as exiting foragers per min.
5. Investigators may want to focus on returning foragers instead of exiting bees, especially if pollen foraging is a parameter of interest. In these cases, observers need to count foragers returning with and without corbicular pollen loads to derive the proportion of foragers collecting pollen.

4.3.2. Flight activity at hive entrance using Doppler radar

The following protocol describes a method of recording flight activity using a Doppler radar (Cunha et al., 2020).

1. Place a beehive activity monitor in front of the hive above the entrance (Figure 12; Cunha et al., 2020). The beehive activity monitor consists of a Doppler radar (HB100; with an Industrial Scientific Measurement-ISM band of 10.5 GHz), a signal conditioning amplifier (peak gain of 72 dB at a frequency of 300 Hz and 60 db/dec roll off for the upper frequencies, and lower poles placed at 5 and 50 Hz), a fifth order active band-pass filter for signal conditioning, a microcontroller (such as a 32-bit ATSAMD21G18®; Microchip Technology, 2021) on an Adafruit Feather board®; Adafruit Industries, n.d.) for data acquisition and processing, a real-time clock, a microSD card, and a power management block (such as a 3.7 V 2000 mAh lithium polymer battery, Adafruit PowerBoost 500 battery charger, and a 1 W solar panel) (Adafruit Industries, n.d.).

2. Assess flight activity with the beehive activity monitor set at a frequency of 10.5 GHz; the microcontroller activates the Doppler sensor every 5 min for 30 s to measure bee forager activity.
3. Use an analog-to-digital converter to digitalize the raw data generated by the Doppler sensor.
4. Process the digital raw data (WAV format) with a programming computer platform (such as MATLAB®; MATLAB, 2010) to generate intensity plots, which are used to analyze flight activity, including bees leaving and entering the hive, and to generate a dataset. To interpret the digitalized data, the investigator should consider that: (1) the highest measured frequency of departing forager bees is 250 Hz, and (2) returning bees can be identified with a frequency close to 50 Hz (Cunha et al., 2020).
5. Lastly, correlate flight activity with colony strength to complement the analysis using an appropriate statistical method, such as a general linear model.

4.3.3. Flight activity at the hive entrance using video monitoring

The following protocol describes a method of recording flight activity using the assistance of video monitoring (Campbell et al., 2008; Kale et al., 2015).

1. A digital board camera (such as Unibrain Fire-™; UNIBRAIN, 2021) with a wide-angle lens ($f = 2.1$ mm, 80° horizontal view angle) should be installed with a holder above the entrance of the hive (with the lens facing downwards, and 20 cm above the landing platform) (Supplementary Video 1; Tashakkori et al., 2021). To facilitate the evaluations, a white landing platform should be used at the hive entrance.
2. Flight activity at the hive entrance is measured by detecting bees and tracking their motion through a sequence of video frames (e.g., 600–1800 frames; 30 frames/s). The researcher may want to consider variables that can affect observations, including the resolution of the camera, lighting conditions, artifacts (e.g., shadows of moving bees and foliage), quick movements of bees that impede following individuals through different frames, and clustering of bees that obstruct the view of incoming and outgoing bees (Tashakkori et al., 2021).
3. The researcher would be able to distinguish different behaviours, including loitering (bees standing idly), crawling, fanning, flying out, and flying into the hive. It should be considered that: (1) the movement of crawling bees between frames is not significant, (2) bees entering the hive tend to present a lateral motion (they are looking for a place to land), (3) Bees flying away from the hive exhibit a pronounced forward motion in each frame. The researcher should also consider that a bee may change its behaviour rapidly, which can hinder tracking the bees throughout the video frames.

Figure 12. Beehive activity monitor (including a Doppler radar) located above the entrance of a hive, with solar panels as energy sources (from Cunha et al., 2020 with permission).

4. Alternatively, to detect the bees in the video, the observer can include a background subtraction and contour calculation of the bees' images, which consists of the classification of the pixels in a frame as foreground or background using programming functions, like OpenCV© (Bradski, 2000). This system allows the detection of bees in the frame by examining individual contours, although it can fail to detect bees that are overlapped or bees that remain stationary for long periods.
5. The investigator could visually detect and track the bees' motion through the sequence of video frames (to classify their behaviour) and create a manually annotated dataset or could use automated processing techniques (Tashakkori & Ghadiri, 2015). The dataset could be used for the analysis of flight activity.

4.3.4. Comb construction

This section draws upon methods of Mattila and Seeley (2007) to measure comb construction during times of rich nectar flow or when artificial feeding (i.e., sugar syrup) is provided to stimulate bees to draw out new comb (Szabo, 1977).

1. Colonies are each provided a hive body provisioned with 10 new frames, five combless and five with wax foundation, alternating. The use of alternating frames of foundation encourages bees to build combs in compliance with the removable-frame parallel orientation of Langstroth hives. A 2–3 cm strip of foundation could be attached with molten wax underneath the top bars of the combless frames to provide an anchor for the bees to build new comb.
2. Measures of the area of comb constructed (both natural and on the foundation) by each colony can begin two days after establishment and weekly thereafter until all comb is finished, or the nectar season is over. The comb area on both sides of every frame that was originally combless is determined and summed by the colony, either with the Objective mode or Subjective mode. Inexperienced observers will need to be trained to discriminate differences between the natural comb and the imprinted beeswax foundation.

4.4. Proxy measures of honey bee colony fitness

4.4.1. Queen cell production

This measure can be determined while the colony is being opened and measured for other strength metrics. It should be done after bee population measures have been taken. Every brood comb is shaken free of bees and examined carefully for the presence of queen cells provisioned with royal jelly and a larva. The cells are counted and then might be cut out for two reasons: (1) to prevent swarming (unless swarming is a variable of interest), and (2) to prevent redundant duplicate observations on subsequent samples. For each block of the experiment, this variable can be reported as the sum of queen cells constructed per colony.

4.4.2. Drone brood production

This measure is best taken in spring or early summer when drones are being actively reared. It is nothing more than an extension of the Objective mode or Subjective mode, limiting observations to drone cells filled with larvae or capped with pupae. Values for drone cells per cm^2 for European honey bees range from 2.3 (J.A. Berry, University of Georgia, USA, pers. obs.) to 2.6 (Dadant, 1963); a good estimate for African subspecies is 3.0 (Buco et al., 1987; Hepburn, 1983).

5. Measuring colony strength at the end of the experiment

5.1. Objective mode

This section is derivative of the references cited in section Classical objective mode.

1. The day before the experiment is set to end, each queen is found, caged with attendants, and returned to her colony. This will save a great deal of time the next day. Additionally, any hive cracks or gaps are sealed with duct tape to prevent bee loss.
2. The night or early morning before colonies are dismantled, the entrance of each hive is securely closed with ventilated screen to trap workers inside the hive.
3. Adult honey bee population at the end of an experiment is derived from net colony weight (kg) and average fresh individual bee weight (mg). Each screened whole hive is weighed in the field with a calibrated balance capable of a precision of 0.001 kg, then opened, all bees brushed off every comb and surface (usually into a temporary holding hive), and the hive re-weighed without honey bees. The difference in before/after bee removal weight is the net weight of bees. A sample of *ca*. 300 live bees is collected into a pre-weighed or tared container, weighed, the bees frozen or narcotized with cold or CO_2 as per Human et al. (2013), and counted to determine the average fresh weight (mg) per bee. Net colony bee weight is divided by the average fresh weight per bee to derive

colony honey bee population. If the fresh bee sample is frozen or stored in alcohol, it can be used to determine adult loads of diseases, *V. destructor*, or other parasites of the investigator's choice.

4. Combs are labeled to preserve colony-specific identity and moved to the laboratory for further measures.
5. The number of brood cells is derived as described in section Classical objective mode, using a grid pre-marked in cm^2 or dm^2, the area of brood is added (Figure 2), and this area (cm^2 or dm^2) is converted to number of brood cells by multiplying cm^2 or dm^2 by the average cell density per cm^2 or dm^2 appropriate to one's locality. This same method can be used to derive the cell number of any comb resource of interest to the investigator: honey, pollen, or empty cells.
6. Brood solidness is determined by placing a grid or a piece of cardboard that delimits 100 cells over a section of sealed brood and subtracting empty cells to estimate the percentage brood solidness (Figure 13). This measure is repeated on different patches of brood to derive a mean of at least 10 observations per colony.
7. Alternatively, to reporting comb resources as the number of cells, many investigators report these resources empirically as total area (cm^2 or dm^2).
8. In the case of honey, it is traditional to report this variable by weight (kg). In these cases, the investigator is aided with the use of queen excluders that restrict the brood to the lower hive bodies. If supers are pre-weighed before adding to hives, the investigator can determine honey yield by simply weighing bee-free honey supers at the end of the experiment.
9. Visible brood disorders can be quantified by first selecting a relatively contiguous patch of brood in the late larval/capped stage (stage more likely to express visible symptoms), and overlaying on the patch a 10-cm horizontal transect and a 10-cm vertical transect intersecting at the center (Figure 14). Along each transect, every cell of brood is examined under strong light and magnification for visible disorders (e.g., symptoms typical of American foulbrood, European foulbrood, sacbrood, chalkbrood, and *V. destructor*) (de Graaf et al., 2013; de Miranda et al., 2013; Dietemann et al., 2013; Forsgren et al., 2013; Jensen et al., 2013). The parameter is reported as the percentage of brood expressing visible disorders.

5.2. Subjective mode

This section describes the subjective mode of measuring colony strength. Table 4 mentions the pros and cons of the subjective and the objective modes of measuring colony strength. This method employs human observers to visually estimate the surface area of a comb covered by a target, such as bees, brood, honey, pollen, or other variables; if necessary, the target's comb surface can be converted to appropriate units, like number of honey bees, cm^2, or number of cells. The syntheses draw from the work of Burgett and Burikam (1985) and subsequent papers from North America (Skinner et al., 2001, Delaplane et al., 2005, 2010), Europe (Dainat et al., 2020; Imdorf et al., 1987; Imdorf & Gerig, 2001), and Central America (Gris, 2002; Guzman-Novoa et al., 2011, 2020). More recently, Hernandez et al. (2020) proposed a method (ColEval) that complements the existing protocols (Dainat et al., 2020; Delaplane et al., 2005, 2010) by using a reference image bank for learning and training to correct for biases of different observers, and to calculate variables related to the evaluation of 25 colonies or more (i.e., number of adult workers or open and capped brood cells, and area with honey, nectar or pollen). Additionally, to correct for human bias and determine inter-observer error, each of *i-N* observers individually evaluates

Figure 13. A piece of cardboard with a square equal in size to 10 × 10 cells is laid over a patch of honey bee (*Apis mellifera*) brood. Percentage brood solidness is measured directly as (100 cells screened minus the number of empty cells).

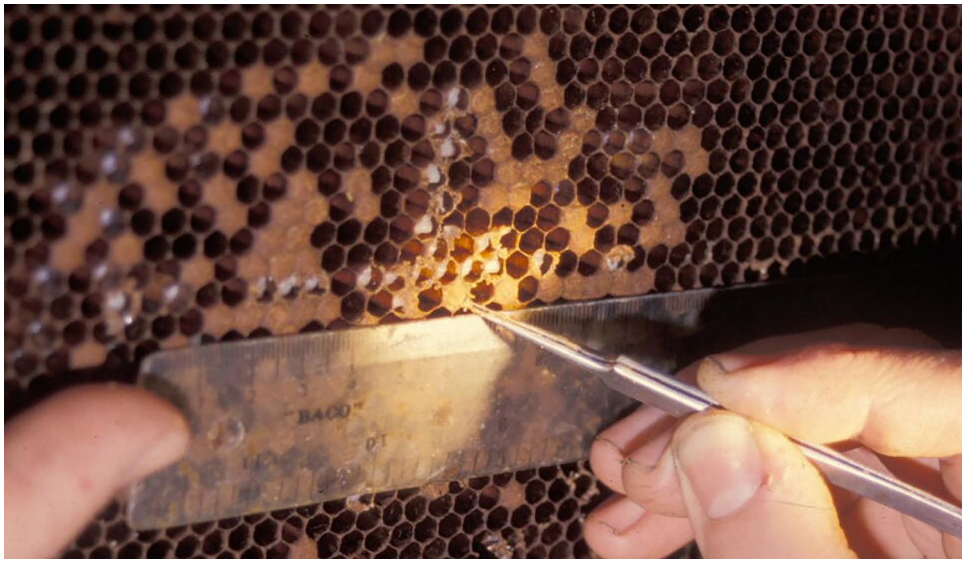

Figure 14. A cross-shaped 10 × 10 cm transect intersects in the middle of a patch of contiguous honey bee (*Apis mellifera*) brood, and every cell along the transect is opened and assessed for visible disorders.

Table 4. Pros and cons of the objective and subjective modes for measuring honey bee (*Apis mellifera*) colony strength.

Method	Pros	Cons
Objective (section Objective mode)	1. More accurate. The method allows for high repeatability and reproducibility.	1. Labour intensive. The method requires of more steps, time, and resources. 2. Invasive. The method can disrupt the colony; the complete deconstruction and reassembly of colonies disrupts social cohesion. 3. The method requires movement of hives and colonies to a new location.
Subjective (section Subjective mode)	1. It requires less personnel compared to the Objective method. However, experienced observers are needed. 2. Less disruptive than the objective method (section Objective mode). Allows for the maintenance of the adhesive cohesion and health of the honey bee colony. 3. No relocation of the colonies is required.	1. It is less accurate, as it relies on visual observations. However, the repeatability and reproducibility of the method is acceptable.

one third of the same colonies. A correction coefficient *C* is then applied to the data of each observer *i* taking the mean of all counts as the reference as follows:

$$Ci = \text{mean observer}/\text{mean total}$$

The subjective mode for visual estimates of worker bees on combs will vary according to the time of day and worker bee foraging activity. For this reason, it is important to control for this effect, either by limiting observations to a narrow time window on successive days, randomly assigning time of inspection such that the day effect is equitably and randomly distributed across treatments, or closing hive entrances in the early morning until bees are counted.

1. Estimates should be performed by at least two persons, with one observer and one note-taker, who writes down numbers. Alternatively, the information can be saved using an audio recorder. However, the investigator should balance accuracy and convenience and decide if more observers and/or note-takers are necessary. In that case, the average of the values recorded by multiple observers should be calculated.
2. A colony is opened and combs of bees are sequentially removed. Each observer looks at one side of a comb, visually estimates the percentage of the comb surface covered by bees, and records the number with the note-taker or audio recorder. It is convenient to label frames 1-X, with each side (X) indicated as A or B. For beginners, it is advisable to "calibrate the individual" with estimates made by an experienced observer. Observers describe the process as a kind of mental "resorting" the honey bees, such that the bees are imaginatively moved into a contiguous mass on the comb surface, at which point the reader estimates the percentage surface of the comb they cover. It is important to sort the bees visually into a contiguous mass that approximates their density if the frame were fully covered because the bee densities given in Table 2 (1.23–1.77 honey bees per cm^2) apply to combs at full carrying capacity.

3. Investigators can use the values in Table 2 or calculate the comb side surface area unique to their hive. Figure 8 is a screenshot of a datasheet (Excel; Microsoft Corporation, 2018) demonstrating the conversion of raw data from two observers into a colony bee population. There are two fictional colonies, both with five North American deep frames, each with two sides. Columns D and E show the respective visual estimates of two observers for the percentage comb surface covered by bees, and column F is the mean of the two. Column G converts the mean percentage surface covered by bees into area (cm^2) covered by bees, using the surface area for one side of a North American deep frame from Table 2 (880 cm^2). Column H converts cm^2 of bees to the number of bees with the appropriate bee density (1.38 honey bees/cm^2). Finally, rows 12 and 23 sum the bees of each frame and side to yield colony honey bee population.
4. If investigators use colonies with different sized supers and frames, it will be necessary to adjust calculations for the one-side surface area unique to each comb type. Honey bee density at full carrying capacity is consistent within North America or Europe, so it should be adequate to pick a density value from Table 2 that best fits one's local situation.

5.3. Special considerations for measuring adult honey bee populations of African descent

For African or Africanized honey bees, the methods described in section Subjective mode must be modified to account for the fact that these bees immediately fly when disturbed.

1. Two observers plus a dedicated data recorder are recommended.
2. The key difference is that each observer makes a visual estimate of the percentage surface of the comb occupied by bees immediately as one of them withdraws it from the hive. Each observer is responsible for only one side of a comb.
3. To minimize the loss of bees, it is necessary to keep the hive undisturbed as much as possible, working downward, removing the lid, and then first measuring bees in honey supers, and immediately afterwards, bees in the brood chambers.
4. Raw data are converted into colony bee population using Table 2 and the methods given in Figure 8.

An alternative to estimating bees on combs visually is to remove each frame rapidly, and immediately shake the bees into a large plastic bag (or a net bag). The bag is weighed to determine the total net weight (kg) of bees, and then a fresh sample of *ca*. 20 g of bees is collected. The bees in the bag are returned to the colony and the sample is taken to the lab where it is weighed, frozen, and the bees counted, to determine g per bee. Dividing total net weight by g per bee gives colony bee population.

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