

REVIEW ARTICLE



Standard methods for estimating strength parameters of *Apis mellifera* colonies

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Summary

This paper covers measures of field colony strength, by which we mean population measures of adult bees and brood. 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 response variables during or at the end of an experiment. Moreover, there are two general modes for measuring colony strength: 1. an objective mode which uses empirical measures and; 2. a subjective mode that relies on visual estimates by one or more observers. There is a third emerging mode for measuring colony strength; 3. computer-assisted digital image analysis. A final section deals with parameters that do not directly measure colony strength yet give important indicators of colony state: 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 de las colonias de *Apis mellifera*

Resumen

Este trabajo trata sobre mediciones de campo de la fortaleza de colonias de abejas, es decir, mediciones de la población de abejas adultas y de cría. 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 uniformes y reducir el error experimental y; 2. como variables de respuesta durante o al final de un experimento. Además, en general hay dos maneras de medir la fortaleza de las colonias: 1. una manera objetiva usando mediciones empíricas y; 2. una manera subjetiva que se basa en estimaciones visuales de uno o mas observadores. Hay una tercer forma más reciente de medir la fortaleza de una colonia; 3. análisis computacional de imágenes digitales. La sección final trata sobre parámetros que no miden directamente la fortaleza de la colonia pero que son importantes indicadores del estado de ésta: actividad de vuelo en la piquera de la colmena, construcción de panales y dos mediciones equivalentes de aptitud de la colonia: producción de celdas reales y de cría de zánganos.

评估西方蜜蜂蜂群群势的标准方法

本文综述了多种测量蜂群群势的方法，主要包括成年蜂和幼虫的群势。通常研究者在两种情况下需要测量蜂群群势：1 在实验开始时，作为处理的一部分，希望获得统一的群势以减少实验误差；2 群势是实验中或实验结束时的反应变量。此外，测量蜂群群势有两种基本的模式：1 客观模式，应用实验技术测量；2 主观模式，依赖于一个或多个观察者的视觉估计。还有第三种新兴的模式，3 计算机辅助的数字图像分析。最后一部

分是关于利用重要的指示参数，开展非直接测量评估，如：巢门口的飞行频率、群内巢脾的建造数度，以及王台和雄蜂的产生和发展，这两个指示蜂群适应度指标的测定。

Keywords: bee population, brood area, brood pattern, honey stores, disease symptoms, parasite symptoms, flight activity, queen cells, drone brood, digital image analysis, honey bee, *BEEBOOK*, COLOSS

1. Introduction

Herein, we cover measures of honey bee field colony strength, by which we primarily mean population measures of adult bees and brood. We will also talk about secondary measures such as: quantity of stored honey and pollen; "brood pattern" by which is meant the degree of worker brood solidity or contiguity; and the expression of visible disease or parasite symptoms. Strictly speaking these secondary measures are not so much indicators of a colony's immediate state as they are legacy effects or predictors of future condition.

For our 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 variables during or at the end of an experiment. Moreover, there are two general modes of measuring colony strength: 1. an objective mode which uses empirical measures such as weight (mg, g, or kg) or area (cm²), covered in sections 3 and 4.1. and; 2. a subjective mode that relies on visual estimates by one or more observers, covered in sections 4.2. and 5.1. The objective mode is the more accurate of the two, but it is also invasive and disruptive to the 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 contrast, the subjective mode is less accurate, but far less disruptive to the bees and therefore appropriate for collecting response variables during the experiment when the investigator has an interest in preserving the social cohesion and health 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. Nevertheless, with safeguards in place such as we describe below, the subjective mode is an acceptably robust technique.

There is a third emerging mode for measuring colony strength; 3. computer-assisted digital image analysis, covered in sections 5.2. – 5.5. This method is minimally invasive, automatically generates archival images for data traceability and verification, provides objective empirical data, and can be done moderately quickly. Its chief disadvantages are cost and dependence on technology. Moreover, 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 in the near future. Computer-assisted digital image analysis is useful for experiments that call for measures

of bee health or development but fall short of field-scale colony strength assessment, chief examples being laboratory studies in environmental toxicology or nutrition.

The sections 5.6. – 5.9. cover methods that do not fit neatly into the other sections. These include: measuring flight activity at the entrance; comb construction; and two proxy measures of colony fitness: production of queen cells; and drone brood.

A note is warranted here on a couple omissions from this chapter; gross colony weight and X-ray tomography. 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, and more recently these kinds of data have been used to monitor flowering phenology in the context of climate change (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 increase, not decrease, in response to disease or other disorders (Mayack and Naug, 2009). X-ray tomography offers what is probably the most empirically quantifiable, thorough, and non-invasive means of monitoring colony strength of colonies of honey bees or other social insects (Greco, 2010). Although it sets a gold standard, its formidable technical requirements keep this method out of reach of most honey bee researchers.

2. An optimal colony configuration

In establishing 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. The best guidance in this matter comes from John Harbo (1986) who compared brood production, worker survival, and honey gain in colonies begun with no brood and 2,300, 4,500, 9,000, 17,000, or 35,000 bees while fixing bee density at *ca.* 230 per 1000 cm³ hive space. The experiment was repeated in each of the months of Feb, Apr, Jun, Aug, and Oct and terminated before brood emerged. Worker survival (22 days) was significantly higher in colonies with 2,300-9,000 bees than in colonies with 35,000 bees. Larger populations tended to store more nectar per bee during times of nectar flow and consume less during times of nectar dearth. However, smaller populations produced more brood per bee. Harbo concluded that colonies established with 9,000 bees are a good optimum between the honey hoarding efficiency of large populations

Table 1. Pros and cons of two variations of an objective mode for starting up colonies of uniform initial strength.

Method	Pros	Cons
Classical 3.1.	1. Results in colonies with initial populations of adult bees normalized for genetics and pathogen load.	1. Disease and parasite legacy effect in brood, although normalized, is nevertheless sustained into the experimental period.
	2. Results in colonies with brood of all stages, accelerating colony growth.	2. Because brood of all stages is present, progeny turn-over from new experimental queens is correspondingly delayed.
	3. Results in maximized colony uniformity in regards to initial adult bee populations.	
Shook swarm 3.2.	1. Does not require use of a customized cage to house common pool of bees.	1. Because adult bees are not normalized, between-colony variation in genetics and pathogen load remains at pre-experiment levels.
	2. Sustains colony-specific identity from pre-experimental to experimental period	2. Because colonies begin broodless, colony growth is delayed.
	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 control is an element of experimental design, the initial broodless period provides an ideal opportunity to treat for mites.	

and the brood rearing efficiency of small colonies. Colonies that are significantly larger than this are costly and labour-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 stresses. Bees are normalized for colony-source genetics and parasite loads if colonies are set up with the common pool of bees as described in section 3.1. If colony growth is a measure of interest, the investigator can invite a greater range of expansion if colonies are begun with no brood. But if one prefers to provide colonies with brood, it is reasonable to stock colonies of 9,000 bees with no more than two combs of brood of various ages, allowing plenty of open cells to accommodate growth.

3. Setting up experimental colonies of uniform strength

This section describes two variations of an objective mode for setting up uniform colonies for experiments. The first (3.1.), which we call the classical objective mode and a variation (3.2.), the so-called "shook swarm" method. Table 1 explains the pros and cons.

3.1. Classical objective mode

One of the recurring pitfalls of honey bee field research is large experimental error which handicaps 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 uniform 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 following synthesis draws from methods pioneered by John Harbo who was interested chiefly in reducing environmental variation in honey bee breeding programs (Harbo, 1983, 1986, 1988, 1993; Delaplane and Harbo, 1987) and adapted later by workers who recognized the utility of these methods for field research on *Varroa destructor* (Delaplane and Hood, 1997, 1999; Ellis *et al.*, 2001; Strange and Sheppard, 2001; Berry *et al.*, 2010) and colony growth (Berry and Delaplane, 2001).

1. The goal is field colonies equalized with regard to bees, brood, mites, and food resources within units of higher-order experimental replication, i.e. blocks or whole plots, usually based on geography.
2. Empty hives are pre-stocked with brood, empty combs, syrup feeders, and a caged queen in advance of receiving worker bees (Fig. 1). Bottom boards and hive bodies are stapled together to prepare them for moving. Hive entrances are screened to temporarily trap bees; this is done for two reasons: 1. experimental colonies often need to be moved to a permanent site and away from the source colonies from which workers are collected and; 2. a period of in-hive confinement, usually overnight, seems to help bees orient to their new hive and queen.
3. Brood for incipient experimental colonies can be collected from the same source colonies used to collect adults. A near-equal quantity of brood is then assigned to experimental colonies without regard to source. We do not prescribe



Fig. 1. Investigators pre-stocking experimental hives with equal numbers of brood combs and honey combs and caged queens in preparation for receiving worker bees from a common cage.

“random” brood assignment because the investigator should place a higher priority on equalizing quantity of brood over concerns of non-random assignment of brood. Efforts should be made to equalize the relative quantity of sealed versus open brood. A measure of beginning quantity of brood is done by overlaying on each side of every brood comb a grid pre-marked in cm^2 and visually summing the area of brood (Fig. 2) The area (cm^2) of brood can be converted to cells of brood by multiplying cm^2 by the average cells per cm^2 . This value varies by geography and bee genetics (Table 2), or the investigator can determine local average cell density per cm^2 by counting the number of cells directly in a square equalling one cm^2 and using the mean of at least ten measurements.

4. In a similar fashion the investigator can derive and equalize the beginning number of cells of honey or pollen or even cells that are empty. 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 use of sugar syrup and protein supplements.
5. Variation due to bee genetics is minimized by providing each colony a sister queen reared from the same mother and open-mated in the same vicinity. A more robust option is to

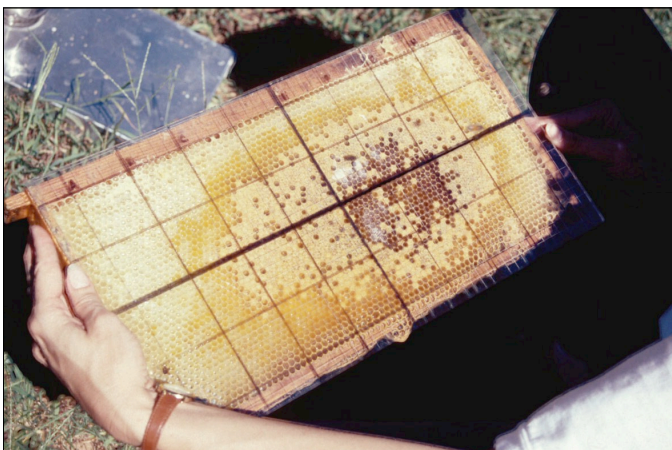


Fig. 2. A transparent plastic grid etched in square centimetres is used to visually sum the surface area of brood.

instrumentally inseminate sister queens (see the *BEEBOOK* paper on instrumental insemination of queens (Cobey *et al.*, 2013)) with the same pool of mixed drone semen.

6. Adult bees are collected 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 freely mix. With African subspecies, it helps minimize loss from flight to first spray bees on the comb with water mist. The cage is maintained in cool conditions to prevent bee death from over-heating for at least 24 hours to allow thorough admixing of bees, resulting in a uniformly heterogeneous mixture. The weight of 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 bee loss through death or flight. Bee survival in the cage is greatly improved if the investigator designs it to accommodate 5-6 Langstroth sized brood combs to provide clustering surface (Fig. 3).



Fig. 3. A ventilated cage made to hold a large common heterogeneous mixture of bees for starting experiments.

7. In order 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, the bees sprayed with water to reduce flight, then bees transferred from the common cage into the smaller colony-specific cages with the aid of cups or scoops (Fig. 4). Bees are added or removed from each colony-cage until the target weight is achieved and recorded, preferably ≥ 2 kg.

Table 2. Surface area of some regionally common frame types and expected bee density when 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 <i>A. mellifera</i> 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.					

^aBurgett and Burikam (1985), ^bImdorf and Gerig (2001), ^cHarbo (1993), ^dHarbo (1988), ^eImdorf *et al.* (1987), ^fGris (2002), ^gGuzman-Novoa *et al.* (2011),

^hBuco *et al.* (1987).

8. A sample of ca. 300 workers is collected from each incipient colony into a pre-weighed or -tared screw-top container, weighed fresh, then the number of bees counted in the lab to derive a colony-specific measure of average fresh weight of individuals (mg per bee). To count bees it is necessary to first immobilize them, either by freezing them or non-sacrificially with CO₂ narcosis. Dividing initial colony cohort size (kg, from step 7) by average fresh weight of individuals (mg) gives initial bee population for the colony.
9. A variation of steps 7 and 8 is available if the investigator is using nucleus hives small enough to weigh entire in the field (Fig. 5). In these cases, the intermediate step of a colony-specific cage is not necessary and the investigator can scoop bees from the common cage directly into the pre-weighed or



Fig. 4. Bees are transferred from the common cage to hive-specific cohort cages by use of cups or scoops.

- tared hive. The net weight (kg) of bees is recorded, then initial population determined the same way as given in step 8.
10. If initial measures reveal outliers in the amount of bees, brood, honey, pollen, and empty cells, corrective action should be taken. In general, corrections aimed at minimizing experimental error are permissible until the point at which treatments are begun.
11. After bees and all resources are placed inside hives (with entrances screened, but without sugar syrup which can spill in transit), hives are then moved 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



Fig. 5. Nucleus colonies are small enough to be weighed directly in the field, bypassing the need for intermediate hive-specific cohort cages.

help them orientate to the new queen and reduces drifting. Colonies can be given sugar syrup after they are unloaded or 24 hours later after bees have settled down.

12. Apiaries should be arranged to limit worker drift between colonies. This can be done by "complicating" the visual field of bees with orientating 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.
13. Colony maintenance should include control of 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 can 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.

3.2. Shook swarm objective mode

1. 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 start at a time of year that the bees can draw out foundation into comb.
2. In the days leading up to set-up, queens in each colony are caged and returned to the colony to save time on set-up day. Colonies are removed from the apiary if they are expressing disease symptoms, significantly under-performing, or otherwise causing excessive between-colony variation. New colonies are imported if needed to reach the target colony number and treated similarly.
3. A number of empty hives equal to the target number of colonies is brought to the apiary, each stocked with brood chamber frames of new foundation, including honey supers with frames of foundation if the nectar flow warrants, and sugar syrup feeders. If affordable, it is good to start new colonies on factory-new woodenware to avoid confounding issues of any disease legacy effects.
4. Each hive in the apiary is moved aside and an empty hive set in its place. Roughly half of the frames of foundation are momentarily removed to create space, then the caged queen is suspended between two centre-most frames of foundation.
5. Combs of bees from the original colony are then sequentially removed and the adult bees shaken off the combs into the new box. Bees are bounced or brushed out of the supers and the bee-free combs returned to them and covered to discourage robbing behaviour.

6. Once all bees are shaken into the new hive, the frames of foundation initially removed are now returned to the new boxes, gently to avoid injuring bees which may be heaped on the floor. Unless there is a strong nectar flow in progress, it is advisable to feed experimental colonies sugar syrup to encourage drawing out the new foundation.
7. The old bee-free boxes of combs are then removed from the experimental apiary and the combs used as needed elsewhere as supplemental brood or feed.
8. After one day, the caged queens in experimental colonies are released. Colonies are subsequently monitored for queen performance and normal colony development. Poor-performing queens are replaced as needed to minimize within-apiary experimental error. Once colonies reach a development state consistent with the experiment's objectives, treatments may be applied and the experiment begin.
9. The expected outcome of this manoeuvre is a high degree of within-apiary consistency in colony developmental state.

4. Measuring colony strength at end of experiment

4.1. Objective mode

This section is derivative of the references cited in section 3.1.

1. The day before the experiment is ended, 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 colony is securely closed with a ventilated screen to trap workers inside.
3. Ending colony adult bee population is derived from net colony bee weight (kg) and average fresh bee weight (mg). Each screened whole hive is weighed in the field, then opened, all bees brushed off every comb and surface (usually into a temporary holding hive), and the hive re-weighed without bees. The difference in 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 CO₂, and counted to determine average fresh weight (mg) per bee. Net colony bee weight is divided by average fresh weight per bee to derive colony bee population. If the fresh bee sample is frozen or stored in alcohol, it can be used to later determine adult loads of diseases, varroa mites, or other parasites of the investigator's choice.
4. Combs are labelled to preserve colony-specific identity and moved to the laboratory for further measures.
5. Number of brood cells is derived as described in section 3, using a grid pre-marked in cm², visually summing the area of



Fig. 6. A piece of cardboard with a square equal in size to 10 x 10 cells is laid over a patch of brood. Percentage brood solidness is measured directly as (100 - no. empty cells).

brood (Fig. 2), and converting area of brood (cm^2) to cells of brood by multiplying cm^2 by the average cell density per cm^2 appropriate to one's locality (Table 2). This same method can be used to derive 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 that delimits 100 cells over a section of sealed brood and subtracting empty cells to estimate percentage brood solidness (Fig. 6). This measure is repeated on different patches of brood to derive a mean of at least ten observations.
7. Alternatively to reporting comb resources as cells, many investigators report these resources empirically as total area (cm^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 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



Fig. 7. A cross-shaped 10 x 10-cm transect intersects in the middle of a patch of contiguous brood, and every cell along the transect is opened and assessed for visible disorders.

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 centre (Fig. 7). Along each transect every cell of brood is examined under strong light and magnification for visible disorders, i.e., symptoms typical of American foulbrood, European foulbrood, sacbrood, or chalkbrood. The parameter is reported as percentage of brood expressing visible disorders.

4.2. Subjective mode

This section and the next describe the subjective mode of measuring colony strength and are thus best suited for collecting response variables while the field experiment is in progress. The gist of the method is the use of human observers who visually estimate the surface area of a comb covered by a target; bees, brood, honey, pollen, etc., and if necessary convert comb surface to target-appropriate units, i.e. bees, cm^2 , or cells. The syntheses in sections 4 and 5 draw from the work of Burgett and Burikam (1985) and derivative papers from North America (Skinner *et al.*, 2001, Delaplane *et al.*, 2005, 2010), Imdorf *et al.* (1987) and Imdorf and Gerig (2001) from Europe, and Gris (2002) and Guzman-Novoa *et al.* (2011) from Central America.

1. Visual estimates of bees on combs will vary according to time of day and 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 day effect is equitably and randomly distributed across treatments, or closing hive entrances in the early morning until bees are counted.
2. Estimates should be carried out by no fewer than two human observers, preferably each with a dedicated secretary who writes down numbers, or each fitted with an audio recorder.
3. A colony is opened and combs of bees 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 secretary or audio recorder. It is convenient to label frames 1-X, with each side indicated 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 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 visually sort the bees 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 bees per cm^2) apply to combs at full carrying capacity.
4. Investigators can use the values in Table 2 or calculate the comb side surface area unique to their equipment. Fig. 8 is a

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

Fig. 8. Example Excel worksheet for converting raw observer data into colony bee population. See 4.2., paragraph 4.

screenshot of an Excel datasheet demonstrating the conversion of raw data from two observers into colony bee population. There are two fictional colonies, each with 5 North American deep frames, each with two sides. Columns D and E show the respective visual estimates of two observers for 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 (cm²) covered by bees, using the surface area for one side of a North American deep frame from Table 2 (880 cm²). Column H converts cm² bees to number of bees with the appropriate bee density (1.38 bees / cm²). Finally, rows 12 and 23 sum the bees of each frame and side to yield colony bee population.

- 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. 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.

4.3. Special considerations for measuring adult bee populations in African bees

When it comes to African bees, the methods described in 4.2. must be modified to account for the fact that these bees immediately fly when disturbed.

- Two observers plus a dedicated data recorder are recommended as before.
- 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.
- To minimize loss of bees it is necessary to keep the hive intact as much as possible, working downward, removing the lid

then first measuring bees in honey supers, then bees in the brood chambers.

- Raw data are converted into colony bee population using Table 2 and the methods given in Fig. 8.
- An alternative to visually estimating bees on combs is to rapidly remove each frame and immediately shake the bees into a large plastic bag. The bag is weighed to determine total net weight (kg) of bees, then a fresh sample of *ca.* 20 g bees collected. The bees in the bag are returned to the colony and the sample 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.

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

5.1. Subjective mode

This section describes a subjective mode for reporting 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 bee populations subjectively in section 4.2. The only difference concerns whether the investigator wants to report the resource in units of area (cm²) 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. 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 paragraph 8, section 4.1. However, if the investigator wants to report honey occurring in combs alongside brood it may be necessary to report it in units of cm² or cells as described in this section. These methods for measuring brood, honey, or pollen are fundamentally the same for African bees, given that the investigator uses the region-specific multipliers in Table 2.

- Estimates are carried out by no fewer than two observers, preferably each with a dedicated secretary who writes down numbers, or each fitted with an audio recorder.
- A colony is opened and combs of bees sequentially removed. Each observer looks at one side of a comb, 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 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

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

Fig. 9. Example Excel worksheet for converting raw observer data into cm² open brood. See 5.1., paragraph 3.

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.

- Fig. 9 is a screenshot of an Excel datasheet demonstrating the conversion of raw data from two observers into cm² of target resource, in this example open cells of brood. There are two fictional colonies, each with 5 North American deep frames, each with two sides. Columns D and E show the respective visual estimates of two observers for percentage comb surface occupied by brood, and column F is the mean of the two. Column G converts the mean percentage surface occupied by open brood into area (cm²), using the surface area for one side of a North American deep frame from Table 2 (880 cm²). Rows 12 and 23 sum the area of open brood for each colony.
- 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 Fig. 9, column G.

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

Fig. 10. Example Excel worksheet for converting raw observer data into cells open brood. See 5.1., paragraph 5.

- To report a resource in units of cells, it is necessary to multiply the cm² of resource by the average cell density per cm². This value varies by geography; conversion factors range from 3.7 - 4.7 (Table 2). It is advisable for investigators to determine this value for their local conditions. Figure 10 shows a modification of Fig. 9 taking the data from cm² open brood to cells of open brood, using a conversion factor of 3.7.

5.2. Computer-assisted digital image analysis

Computer-assisted digital image analysis can be used to directly measure surface area of comb occupied by bees or other colony resources such as open brood, sealed brood, or pollen. There are two kinds of data output: 1. direct surface measurements (cm² or dm²) of target (Cornelissen *et al.*, 2009) (section 5.4.) and; 2. ratio of surface target relative to total comb surface (Yoshiyama *et al.*, 2011) (section 5.5.). In the case of 1., it is possible to convert surface to units of bees or cells using conversion values in Table 2.

5.3. Technology and photographic considerations

- A high-resolution camera (3648 x 2736, 10 megapixels, or higher) is preferred. We recommend a DSLR or similar camera. Compact cameras will work fine too, but it is unlikely that eggs and young brood will be visible.
- 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 JPEG. As Image J software doesn't 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 (<http://www.irfanview.com/>).
- Use of a tripod with a fixed distance to the frames is recommended. This makes image analysis easier and pictures more comparable.
- Make sure 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 ISO values.
- Aperture settings are dependent on the type of lens used. For DSLR we suggest using a fixed 50 mm lens. These generally are affordable, sharp, fast (low f-value, e.g. f1:1.4), and have little distortion. Using these lenses the f-value should be above f4.5 for sharp pictures. For zoom lenses aim at an f-value of between f6.7 and f13.
- 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 in our experience 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.

5.4. Direct surface measurements of target

1. Photographic records of bees on combs will vary according to time of day and 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 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 labelled: 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 labelling 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 fixed.
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 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.

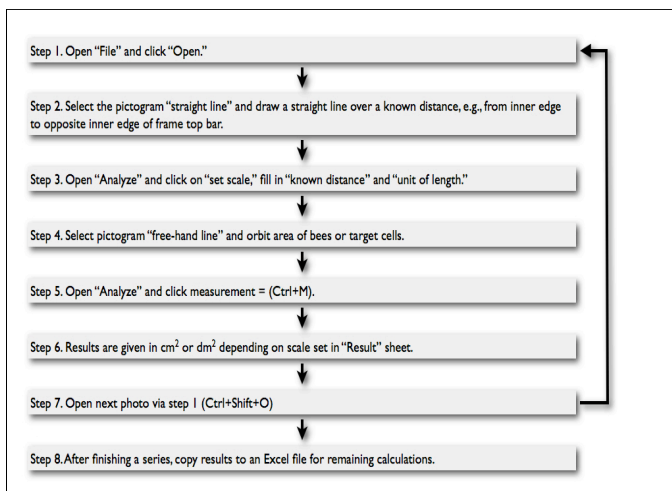


Fig. 11. Outline of the method of Cornelissen *et al.* (2009). Flow chart of computer assisted image analysis applying ImageJ software. Step 2 can be skipped by making the photos in a fixed position at which the distance between camera and frame is constant.

Table 3. Colour- and number-coding of cell contents according to 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

5. The digital photos are analysed using a computer program such as ImageJ, available free at <http://rsbweb.nih.gov/ij/>. Post-hoc, the photos are uploaded in the computer and analysed as diagrammed in Fig. 11.
6. The results of the calculated area is in dm² or cm² depending on the scale that has been set. To finish the analysis, the number of bees or cells are derived with Excel or a similar spreadsheet program using the expected density of bees per cm² or cells per cm² given in Table 2. Surface data from this digital analysis could be inserted into Column G in Figs 8-10.

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

This method, also applying ImageJ, yields the ratio between selected area and the total comb area. Prior to computer analysis, each digital image of a comb side is digitally edited by the investigator to delineate comb areas of target resource, e.g. capped brood area, open brood, pollen, or honey with unique identifying colours. In Fig. 12, this application is outlined.

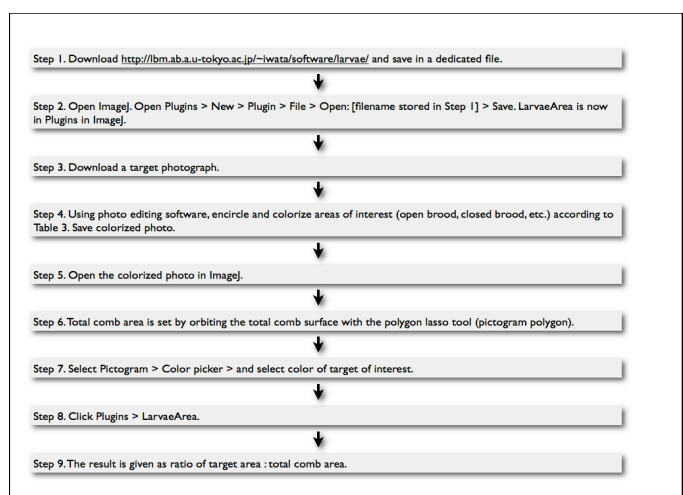


Fig. 12. Outline of method of Yoshiyama *et al.* (2011) for determining ratio of target surface : total comb surface. It is used with the OECD colour codes in Table 3.

5.6. Flight activity

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 to the side of a colony, each 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, each observer counts and records the number of bees exiting the colony (but see 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 reported as exiting foragers per min.
5. Investigators may want to focus on returning, instead of exiting foragers, especially if pollen foraging is a parameter of interest. In these cases observers need to count foragers returning with, and without, corbicular pollen loads in order to derive proportion of foragers collecting pollen.

5.7. Comb construction

This section draws upon methods of Matilla and Seeley (2007).

1. This metric can be collected only during times of rich nectar flow when bees can draw out new comb.
2. Colonies are each provided a hive body provisioned with ten new frames; 5 combless and 5 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 equipment.
3. Measures of 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. Comb area on both sides of every frame is determined and summed by colony, either with the Objective mode (4.1.) or Subjective mode (5.1.). Inexperienced observers will need to be trained to discriminate differences between natural comb and the imprinted beeswax foundation.

5.8. 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 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 sum of queen cells constructed per colony.

5.9. 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 (4.1.) or Subjective mode (5.1.) limiting observations to drone cells filled with larvae or capped with pupae. Values for drone cells per cm² for European bees range from 2.3 (J A Berry, University of Georgia, USA, pers. obs.) and 2.6 (Dadant, 1963); a good estimate for African races is 3.0 (Buco *et al.*, 1987; Hepburn, 1983).

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