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# The COLOSS *BEEBOOK*, a Manual of Standard Methods for Honey Bee Research

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## The Vision

The *BEEBOOK* project arose from discussions at early COLOSS (Prevention of Honey Bee Colony LOSSes) meetings. The vision was, and still is, to develop a definitive inventory of standard techniques and methods in honey bee research to ensure that studies performed by different laboratories around the world would be directly comparable. The manual, titled the COLOSS *BEEBOOK*, was inspired by publications with similar purposes for *Drosophila* fruit fly research (Williams et al., 2012). The previous lack of standards had made, for example, establishing whether honey bee colony losses had indeed increased, or were greater in some countries than in others, exceptionally difficult. The *BEEBOOK* is not meant to standardize the research itself, but focusses solely on the methods for which standardization is useful to enable reproducibility as a cornerstone of science. The *BEEBOOK* is a tool for all who want to conduct research on honey bees. It has been written in such a way that those new to honey bee research can use it to begin studies in fields with which they may not be familiar.

## Creation

Production of the *BEEBOOK* began after recruiting international experts to lead the compilation of each research domain. These senior authors (first in the author list) were tasked with recruiting a suitable international team of contributors to select methods to be used as standards and then to report the methods in a user-friendly manner (Williams et al., 2012).

The initial *BEEBOOK* was divided into three volumes: The COLOSS *BEEBOOK*, Volume I: Standard methods for *Apis mellifera* research; The COLOSS *BEEBOOK*,

Volume II: Standard methods for *Apis mellifera* pest and pathogen research; and The COLOSS *BEEBOOK*, Volume III: Standard methods for *Apis mellifera* product research (Figure 1). The first two volumes of the COLOSS *BEEBOOK* were published in 2013 (Figure 2). The final chapters of Volume III were published in 2021 and a hard copy volume will be published shortly.

## A Global Enterprise

The *BEEBOOK* is a truly international collaboration. In Volume I, 167 international scientists from 29 countries collaborated to produce 18 chapters including over a thousand protocols related to studying honey bees and their colonies. For Volume II, 98 international scientists from 22 countries collaborated to produce 12 chapters including over 500 protocols. Volume III represents the work of 133 authors from 26 countries spanning five continents.

## One Book, Multiple Forms

Each *BEEBOOK* chapter has been published as an open access peer-reviewed article in the *Journal of Apicultural Research* (JAR). The .pdf papers can be downloaded free to view directly from the Taylor & Francis online platform for JAR and through the COLOSS website: <https://coloss.org/>. They are also available as hard copy books (Dietemann et al., 2013a, 2013b), and can be purchased from the IBRA bookshop: <https://ibra.org.uk/shop> or from Amazon.

## A Standard beyond Others

The reader may wonder about the difference between the *BEEBOOK* and existing standards provided by the Office International des Epizooties (OIE) and the European Organization for Economic Co-operation and Development (OECD).

In the *BEEBOOK*, we often refer to OIE, OECD, and other standards, since they describe methods to diagnose pests and diseases (OIE) or to perform, for example, routine analyses for toxicity tests (OECD). The *BEEBOOK*, however, goes well beyond diagnosis and routine analyses by describing the methods to perform research on the honey bee, its colony, hive products, and associated organisms. Where necessary, the *BEEBOOK* recognizes existing standards such as those provided by the OIE and OECD, and presents a harmonized compendium of research methods, written and reviewed by an international team of scientists.

## User Acceptance

With the completion of the original three volumes of the COLOSS *BEEBOOK*, we now explore whether it has, as hoped, been fully adopted by the bee science community as an aid to collaborative science and the development of our understanding of honey bees. There are several measures of its use. One is the number of citations of the chapters in other scientific papers. The CrossRef database in August 2021 showed a total of 2,300 citations to *BEEBOOK* chapters. The citations are accumulating at a rate of approximately 50 per month. The rate of citation has increased greatly in the last two years, and the most highly cited chapters are those on *Nosema* (Fries et al., 2013; 196 citations), *Varroa* (Dietemann et al., 2013c; 177 citations) and viruses (de Miranda et al., 2013; 156 citations). Another measure of adoption by the scientific community is the number of times that individual chapters are downloaded. The chapters from Volumes I and II had 2,986 downloads from the IBRA website between January 2013 and September 2014, before JAR was transferred to the Taylor & Francis Online platform in April 2015. Since that time, there have been nearly 180,000 downloads

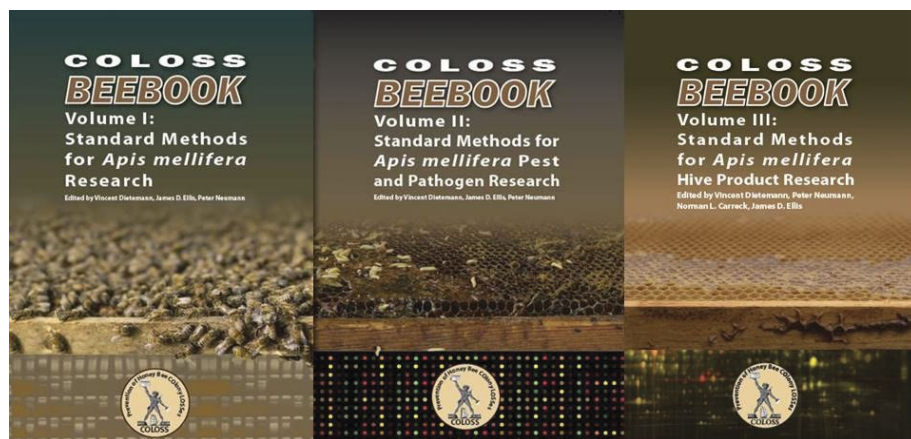


Figure 1. The first three COLOSS BEEBOOK volumes.



Figure 2. The launch of the first two volumes of the COLOSS BEEBOOK, Kiev, Ukraine, September 2013. L to R: Norman Carreck, Jamie Ellis, Peter Neumann and Vincent Dietemann.

of BEEBOOK chapters, including the Volume III chapters published more recently. This is roughly 6,500 downloads per month, or an average of 170 per chapter per month. The propolis chapter (Bankova et al., 2019) has been especially popular, having been downloaded over 20,800 times since its original online publication in September 2016, now making it the most heavily downloaded paper from the more than two thousand published in *JAR* over its 60-year history.

## Beyond *Apis mellifera*

The field of bee science has much to gain from the development of research on the Eastern honey bee, *Apis cerana*. Correspondingly, experts on this species from the COLOSS network have collaborated to share technical experiences on specific techniques for *A. cerana* research in Volume IV, “Standard methods for *Apis cerana* research”, which is currently being

prepared. This will provide standard methods for general research on *A. cerana* and research on its pests, pathogens, and hive products. More information on Eastern honey bees will provide greater insight into the evolution and host-parasite relationships of *Apis* species.

## Not Only for Scientists!

The unique BEEBOOK project has been fully embraced by the honey bee research community, and the numbers of citations that the papers receive are encouraging. Although many COLOSS members are research scientists, and the first volumes of the BEEBOOK concentrated mainly on scientific techniques, many other members work in agricultural extension services and have the task of disseminating scientific knowledge to beekeepers. Therefore BEEBOOK Volume V, “Standard methods for sustainable beekeeping”, is in the planning stages and will follow the

format of earlier volumes. Global principles will be summarized by international contributors who will detail regional variations in beekeeping techniques due to local conditions.

## An Evolving Tool

The BEEBOOK was, from the outset, intended as an evolving tool, keeping pace with the development of new methods and the improvement of existing ones. The field of molecular methods for honey bee research has experienced a rapid evolution since the first publication of Evans et al. (2013), especially with the development of “omics methods” namely: genomics, the study of the structure, function, evolution and mapping of genomes (the genetic makeup of an organism); proteomics, the study of genes coding for proteins and their function; and metabolomics, the study of the genetic basis of an organism’s metabolism (the chemical processes within an organism that sustain life). Since it is the COLOSS BEEBOOK’s purpose to compile the most up to date methods, we are currently including a chapter focused on “Standard methods for *Apis* ‘omics research” in Volume IV. This chapter will cover the immense changes in sequencing and analytics related to full-genome analyses of honey bees and functional insights into bee health, development, reproduction, and behavior. It will also cover new work comparing the genomes and biologies of multiple *Apis* species, including *A. cerana*, and the emergent field of metabolomics, which can use protein and biochemical insights to describe the multitude of important chemical processes that occur inside bees. This chapter will provide methods, resources, and a roadmap to online genomic and genetic databases that will be useful for researchers.

It was recognized at the start of the BEEBOOK project that new threats to bees and new areas of interest would emerge and would need to be added. For example, the yellow legged Asian hornet *Vespa velutina* was at the BEEBOOK’s inception not considered of significance to western honey bees. However, in the last few years, this view has changed as many parts of Europe now experience its harmful effects. Correspondingly, a new chapter on *V. velutina* is being written. This provides an example of how the BEEBOOK can evolve to include new areas of research not previously considered relevant to honey bees.



Inevitably, as new techniques are developed, some of the *BEEBOOK* chapters will become outdated. Thus, several of the original chapters are now being updated. Following contact with the initial author teams, we can report that interest has been expressed updating several of the original chapters. In some cases, the original lead author will be coordinating the revision, but in many cases, newer and younger authors have now joined the teams. In this way, the *BEEBOOK* project will continue to develop and evolve for the foreseeable future.

In conclusion, the *BEEBOOK* is one of COLOSS's most successful, impactful, and enduring outputs to date. We believe it will continue to impact honey bee research globally for decades to come. We also believe it is a fitting testimony to the accomplishments made when scientists work together with common purpose.

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## Disclosure statement

No potential conflict of interest was reported by the authors.


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