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OPINION ARTICLE

The COLOSS *BEEBOOK* – an example of standard methods in insect research

E. Facchini[1]*, *V. Dietemann*[2,3], *J.D. Ellis*[4], *J.D. Evans*[5], *P. Neumann*[2,6] *and N.L. Carreck*[7,8]

[1]Hendrix Genetics Research, Technology & Services B.V., Boxmeer, The Netherlands; [2]Swiss Bee Research Center, Agroscope, Bern, Switzerland; [3]Department of Ecology and Evolution, University of Lausanne, Lausanne, Switzerland; [4]Entomology and Nematology Department, University of Florida, Gainesville, FL, USA; [5]USDA ARS, Bee Research Laboratory, Beltsville, MD, USA; [6]Institute of Bee Health, Vetsuisse Faculty, University of Bern, Bern, Switzerland; [7]Carreck Consultancy Ltd, Shipley, West Sussex, UK; [8]University of Sussex, Falmer, East Sussex, UK; *elena.facchini@hendrix-genetics.com

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Abstract

Honey bees play an essential role in modern agriculture as farm animals and crop pollinators, and they contribute to one third of our diet. Over the last few decades, managed honey bees have faced large-scale losses worldwide. Various causes include the spread of pathogens and parasites, habitat loss and loss of forage, pesticide use, and climate change. Many scientists investigated these issues worldwide separately and independently, often using different methodologies, and this approach might lead to conflicting and sometimes erroneous findings. To provide global and sustainable solutions, a group of bee scientists established COLOSS, a non-profit association for the prevention of honey bee COlony LOSSes. Its mission is to investigate the causes of declining bee health and find effective means to improve the well-being of bees. COLOSS comprises various Core Projects and Task Forces focusing on specific topics identified by the association to receive priority attention. Among the core projects, the COLOSS *BEEBOOK* is a unique venture aiming to provide a standardised methods manual for studying the honey bee. The project's goal has been to create a comprehensive collection of established methods and techniques for honey bee research, with the aim of making studies conducted by different groups across the world more comparable. The resulting practical manual contains over 2,000 standardized methods across all fields of honey bee research. It is definitive, but evolving, research manual. There is a strong need for standardisation in the fast-growing field of edible insect farming and research. The COLOSS *BEEBOOK* stands as a testament to the achievements that can be made when researchers collaborate towards a common goal, and it can serve as an inspiration for the establishment of standardized methods for the mass rearing of edible insects.

Keywords

insect research – standardisation – edible insects – COLOSS – *BEEBOOK*

Introduction

COLOSS (Prevention of honey bee COlony LOSSes) is an international non-profit research association established in 2008 (Brodschneider *et al.*, 2022) with the mission to investigate the causes of declining bee health and find effective means to improve the well-being of bees. In the early 2000s, managed honey bee colonies showed unprecedentedly low winter survival (Neumann and Carreck, 2010). Honey bee researchers began investigating the phenomenon, but soon, the lack of stan-

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dardised research methods seriously hindered their ability to compare the data on colony losses obtained worldwide. Therefore, in its second year of activity, the COLOSS team conceived the idea of organising honey bee research methods in a single manual, the COLOSS *BEEBOOK* (Williams *et al.*, 2012). The concept took inspiration from publications with similar purposes for fruit fly research (Lindsley and Grell, 1968).

This opinion paper aims to introduce the COLOSS *BEEBOOK* to the wider insect research community, to promote international networking and collaboration for the collection and description of standard methods. We hope this will encourage networking and collaboration to support the standardisation of edible insect research methods.

1 The edible insect industry and the need for standardisation

The commercial mass rearing of the black soldier fly (*Hermetia illucens*) and other edible insect species for food and feed has grown rapidly in the past ten years. The reason for the growing interest in farmed insects is their remarkable ability to transform low-quality organic materials into valuable food, feed, and technical products. This has led to a surge in both academic and industrial interest in the mass rearing of edible insects, with the sector experiencing exponential growth. The mass rearing of insects for animal feed and pet food has received investments exceeding €1 billion since its establishment and is forecast to generate potentially 25,000 jobs by 2030 in Europe alone (IPIFF, 2021). Regarding academic attention, Van Huis (2022) reported that more than 80% of all relevant articles were published after 2017 when searching published articles with the terms 'edible insects' and 'black soldier fly'.

As with the increased attention of the bee research community in the 2000s, several research teams recently started to study the most common farmed insect species worldwide. During the 70th annual meeting of the European Federation of Animal Science in Ghent, the lack of basic knowledge and reference values and the difficulty in comparing published studies from different laboratories were presented and discussed. Indeed, it was highlighted that standardisation was needed for comparability and reproducibility. Bosch *et al.* (2019) indicated some areas that would benefit from having a collection of standardised methods, such as genetics and strain of origin, establishing a reference diet to understand better nutrition, standardise the calculation of larval performance parameters, rearing methods and environmental conditions, chemical analyses of the products, the biology of the animal and the impact that different life stages can have on the quality of the products, and more in general experimental designs. On that occasion, the COLOSS association and the *BEEBOOK* were highlighted as examples of international coordination and collaborative effort.

2 The COLOSS *BEEBOOK*

The project's main objective has been to create a comprehensive and reliable collection of standardized techniques and methodologies for honey bee research, with the ultimate aim of enabling studies conducted by different laboratories around the world to be compared easily and directly. It is a practical manual divided into volumes compiling more than 2,000 standard methods in all research fields.

The creation of the *BEEBOOK* was organised by assigning each chapter to a senior international expert in each domain, tasked with recruiting an appropriate team of scientists who would contribute to the collection, the selection, and the description of standard methods for each relevant field. The writing style conforms to a broad audience, from scientists to beekeepers.

The original *BEEBOOK* initiative was comprised of three separate volumes: The first volume contains standardized methods for conducting research on *Apis mellifera*; the second volume outlines standardized methods for studying pests and pathogens affecting *Apis mellifera*; and the third volume focuses on standardized methods for researching hive products produced by *Apis mellifera* (Figure 1).

Volume I of the COLOSS *BEEBOOK*, Standard Methods for *Apis mellifera* Research, focused on protocols related to studying the organism itself and the honey bee colony in a broader sense. In Volume I, 167 international scientists from 29 countries collaborated to produce 18 chapters, including over a thousand protocols for studying honey bees and their colonies. These chapters include research protocols associated with honey bees in the fields of anatomy, artificial rearing of *A. mellifera* larvae, behaviour, cage studies, cell cultures, characterisation of subspecies and ecotypes, chemical ecology, estimation of colony strength parameters, endosymbionts, geographic information systems, instrumental insemination, miscellaneous methods,

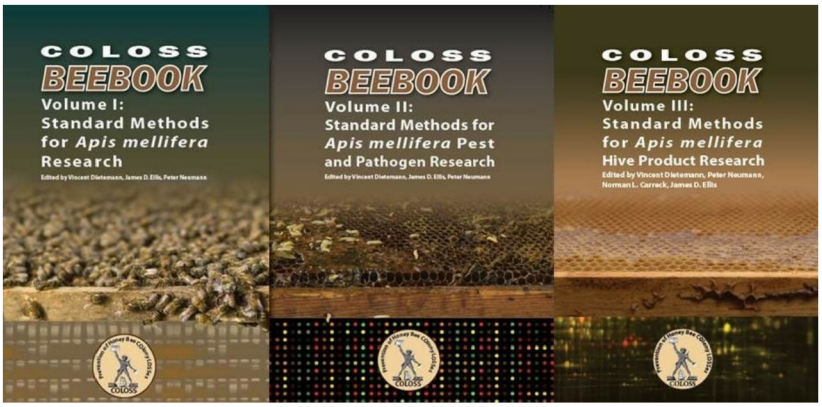

FIGURE 1 The first three COLOSS *BEEBOOK* volumes.

molecular biology, physiology and biochemistry, pollination, rearing and selecting queens, statistics, and toxicology.

As the title suggests, Volume II of the COLOSS *BEEBOOK*: Standard methods for *Apis mellifera* pest and pathogen research focused on the globally relevant methods to study the significant pest and pathogens of the western honey bee and the impact on their well-being. A team of 98 scientists from 22 different countries collaborated to create 12 chapters, which collectively contain over 500 protocols focused on studying pests and pathogens affecting honey bees. The chapters are organized into three sections. The first section deals with protocols related to honey bee epidemiology, which includes epidemiological research and survey methods for estimating colony losses. The second section contains chapters focused on honey bee pests, including small hive beetles, tracheal mites, *Tropilaelaps* mites, varroa, and wax moths. The third and final section contains chapters listing protocols for studying honey bee pathogens. This section includes chapters on American foulbrood, European foulbrood, fungi, nosema, and viruses.

The COLOSS *BEEBOOK* Volume III focused on researching *Apis mellifera* hive products. Volume III contains papers on royal jelly, beeswax, propolis and brood as human food (Jensen *et al.*, 2019), honey, pollen, and venom. These seven papers were written by 133 authors representing 26 countries.

The *BEEBOOK* recognises existing international standards where they exist and presents a harmonised compendium of research methods. It contains references to the Office International des Epizooties (OIE) to describe the diagnosing of pests and diseases and to the European Organization for Economic Co-operation and Development (OECD) for routine toxicity test analyses. The added value of the *BEEBOOK* is that it expanded the collection of standard methods by including research methods on the honey bee, its colony, hive products, and associated organisms.

3 An evolving tool

Right from the start, the *BEEBOOK* was designed to be a dynamic and constantly evolving resource that would keep up with advancements in technology and improvements in existing research methods. The field of molecular biology techniques in 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, proteomics, and metabolomics. As the goal of the COLOSS *BEEBOOK* is to provide the most current and up-to-date research methods, a new chapter focused on 'Standard methods for *Apis* 'omics' research' is currently being developed for Volume IV. This chapter will cover the latest advancements in sequencing and analytics for full-genome analysis of honey bees, providing functional insights into their health, development, reproduction, and behaviour. It will also include new research on the comparison of genomes and biologies across multiple *Apis* species, including *A. cerana*. This chapter will provide researchers with methods, resources, and a roadmap to online genomic and genetic databases to aid in their research. Other chapters of Volume IV will cover the eastern honey bee *A. cerana* highlighting where its different biology requires different techniques to those appropriate to *A. mellifera* in Volumes I–III.

Moreover, since the first publication of the first three *BEEBOOK* volumes, new threats to bees have emerged, promoting new areas of interest. The most striking example regards the yellow-legged Asian hornet, *Vespa velutina*, which was not considered of significance to

western honey bees until only a few years ago. The harmful effects of *V. velutina* on pollinators are now being observed in many parts of Europe, which has led to the creation of a new chapter on the topic that will be added to Volume II of the *BEEBOOK*. This serves as an example of how the *BEEBOOK* can adapt and expand to include new research areas that were not previously considered relevant to honey bees. As new techniques and methods are developed, some of the existing *BEEBOOK* chapters have become outdated and require updating. The original lead authors are coordinating revisions in some cases, but newer and younger authors have also joined the teams in many instances. This will enable the *BEEBOOK* project to continue evolving and developing in the foreseeable future.

4 Dissemination and user acceptance

Every chapter of the *BEEBOOK* has been published as a peer-reviewed article that can be accessed for free through the *Journal of Apicultural Research* (*JAR*). These papers can be downloaded as a PDF from either the Taylor & Francis Online platform for *JAR* or the COLOSS website, and hard copies of the books can be purchased from the International Bee Research Association (IBRA) bookshop or Amazon.

As a measure of the *BEEBOOK* acceptance and adoption by the scientific community, two metrics can be considered: the number of citations in scientific papers and the number of downloads. The CrossRef database in August 2022 showed 3,000 citations to *BEEBOOK* chapters. The citations are accumulating at a rate of approximately 50 per month. The rate of citation has significantly increased in the last three years, and the most highly cited chapters are those on varroa (Dietemann *et al.*, 2013; 248 citations), pollination (Delaplane *et al.*, 2013a; 243 citations) and colony strength (Delaplane *et al.*, 2013b; 243 citations).

Between January 2013 and September 2014, the IBRA website recorded 2,986 downloads of the chapters from Volumes I and II. However, since *JAR* moved to the Taylor & Francis Online platform in April 2015, there have been nearly 240,000 downloads of the *BEEBOOK* chapters, including those from the more recent Volume III. This equates to an average of around 8,000 downloads per month or roughly 270 downloads per chapter per month. The propolis chapter (Bankova *et al.*, 2019) has been especially popular, having been downloaded almost 26,000 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.

5 Conclusion

The authors aspire for the *BEEBOOK* to be widely recognized as a comprehensive resource for honey bee research and to serve as a model for other fields of research. They hope that like the original fruit fly book, which developed into a journal publishing updates and new methods, the edible insect research community will adopt and enhance the concept. The *BEEBOOK* is regarded as one of COLOSS's most successful and long-lasting achievements with a far-reaching impact on honey bee research. The authors anticipate that it will continue to be relevant for decades to come and view it as a testament to the remarkable achievements that can be made when scientists collaborate towards a common goal. They believe that the *BEEBOOK* has the potential to inspire other global research communities.

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Conflict of interest

The authors declare that there is no conflict of interest.

OECD disclaimer

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