A Protocol for Monitoring Populations of Free-Living Western Honey Bees in Temperate Regions

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Abstract: Despite their ecological significance, wild *Apis mellifera* populations remain critically understudied. Addressing this research gap requires the study and monitoring of free-living colonies to identify potential self-sustaining populations. However, a lack of standardized methodologies has hindered these efforts. To address this challenge, Honey Bee Watch, an international coalition dedicated to studying free-living honey bees, has developed a comprehensive monitoring protocol. This protocol offers a consistent methodology for researchers and citizen scientists to collect vital data on colony survival, activity, and environmental conditions. It emphasizes monitoring at five key phenological stages throughout the year to ensure the collection of scientifically robust data. Additionally, an illustrated guideline is provided to help users accurately identify and track colonies, enhancing data accuracy. The adoption of this protocol by both the scientific community and citizen scientists will not only strengthen research efforts and foster public engagement, but also help close existing knowledge gaps regarding the distribution and density of wild *A. mellifera* populations, ultimately guiding more effective conservation strategies.

Keywords: Wild honey bees; Apis mellifera; population monitoring; self-sustaining population; free-living honey bees; wild-living honey bees

Introduction

The Western honey bee, *Apis mellifera*, is commonly recognized for its importance as a managed pollinator, yet its role as a wild species is often neglected (Requier et al., 2019). Originally *A. mellifera* thrived in wild populations in its native habitats across Europe, the Middle East, and Africa (Ruttner, 1988). While the species is now distributed almost globally, with significant populations thriving in the wild in the Americas and Australia (Chapman et al., 2008; Guzman-Novoa et al., 2024; Oldroyd et al., 1997; Pinto et al., 2005; Seeley, 2007), wild colonies are rare in temperate regions, such as in Europe (Jaffé et al., 2010; Moritz et al., 2007; Requier et al., 2019). This is due to numerous challenges, including the spread of *Varroa destructor* and the deadly viruses it transmits (Traynor et al., 2020), threats imposed by other pests and novel predators (e.g., *Vespa velutina*), as well as many concurring factors such as deforestation and habitat loss, which lead to decreasing foraging and nesting sites (Rutschmann et al., 2022), and beekeeping practices that favor introgressive hybridization (Requier et al., 2019). Yet, despite the consensus on the urgent need to protect wild pollinators in general, wild *A. mellifera* colonies remain largely understudied, detailed data on their current distribution are lacking, and their conservation status is still undefined (De La Rúa et al., 2014).

Until a few decades ago, the majority of colonies found nesting freely without human intervention in temperate areas would have been considered part of a wild population. Such populations would have been comprised of self-sustaining groups of unmanaged colonies exposed to natural selection, with their self-sustainability dependent upon the reproduction of colonies within the population, rather than from the influx of external swarms. Due to the spread of *Varroa*, coupled with increasing pressures imposed by anthropogenic and beekeeping activities, it is now widely considered improbable that colonies found nesting in the wild in most parts of the temperate range descend from self-sustaining wild populations. Instead, they are assumed to be mostly escapees from managed apiaries, incapable of surviving unaided. While such a hypothesis has been proven for some locations (e.g., Kohl et al., 2022), it remains largely unexplored.

As the number of studies on colonies found in the wild has increased in recent years, terms like "free-living" and "wild-living" have emerged to describe unmanaged colonies of unknown origin, i.e., those nesting in places of their own choosing (Browne et al., 2020; Figure 1, Kohl & Rutschmann, 2018; Kohl et al., 2022; Requier et al., 2020; Rutschmann et al., 2022; 2024; Visick & Ratnieks, 2023). By studying the survival of these colonies, population demographics data

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Figure 1. Examples of nests of free-living *A. mellifera* colonies. Honey bees can take residence within natural (e.g., a tree trunk, left side of the picture) or artificial cavities (e.g., roof spaces, within walls, right side of the picture). Photos by Arrigo Moro and Keith Browne.

can be inferred, and, with that, self-sustaining groups potentially identified (Kohl et al., 2022; Oldroyd et al., 1997; Seeley, 2017). This approach opens the possibility of understanding whether any isolated self-sustaining wild populations still exist, as well as whether local managed honey bees have lost their adaptive capacity to survive under natural conditions.

Challenges in Methodology and Data Collection

The International Union for Conservation of Nature (IUCN) Red List still classifies the status of wild *Apis mellifera* in Europe as "Data Deficient" (De La Rúa et al., 2014), reflecting a substantial lack of information on their density, distribution, and survival. This significant knowledge gap emphasizes the need for extensive research on the subject.

Previous studies based on data from repeated nest observations have analyzed free-living colonies' population dynamics, winter survival, and self-sustaining capacity (Kohl et al., 2022; Oldroyd et al., 1997; Seeley, 2017). Furthermore, there has been an increase in citizen-science initiatives by independent groups aiming to replicate such studies with the help of volunteers (Browne et al., 2020; Cordillot, 2024; Lang & Albouy, 2022; Rutschmann et al., 2024). These emerging initiatives aim to collect scientifically sound data; however, the success of their endeavors will ultimately depend on how well the monitoring methodologies used in previous scientific peer-reviewed studies are applied and replicated.

Unfortunately, the methodology for collecting nest observation data differs across studies and is often not easily accessible to non-scientific users. Since citizen-science volunteers are often inexperienced with bees, supporting materials such as simple guidelines and references need to be provided to ensure accuracy and data quality. Moreover, Recent findings highlight significant monitoring biases in citizen-science projects, underscoring the importance of stringent validation protocols to ensure reliable data (Rutschmann et al., 2024).

Development of a Standardized Protocol

Honey Bee Watch (HBW, www.honeybeewatch.com, Moro et al., 2021; Dall'Olio et al., 2021) is an international coalition of bee researchers, bee experts, and citizen scientists, tasked with studying free-living colonies to better understand whether they survive via natural selection and, if so, how and why. Motivated by the absence of standard reference methods for studying *A. mellifera* in the wild, a dedicated Task Force of HBW members (the authors herein) developed a standardized protocol for monitoring nests of free-living colonies. During development, previous research publications on the topic were reviewed and methodologies currently used by similar initiatives in different countries analyzed. The resulting protocol can be accessed in the supplementary materials of this article (Supplementary files S1 and S2) as well as online at www.honeybeewatch.com/resources.

This open-access, participatory protocol is designed for year-round use in temperate climates by researchers and citizen scientists alike. It gathers essential data about colonies and their habitats, including bee activity, survival rates, weather conditions, and other pertinent environmental factors. Collecting this valuable information on free-living colonies is crucial for addressing the unresolved Red List status of *A. mellifera* in Europe and is expected to inspire future research efforts around the world.

It is important to note that wild honey bees in the tropics, e.g., in Africa, are as much understudied as those living in temperate regions, so collecting data on their population dynamics is arguably urgent too (Dietemann et al., 2009). However, the use of the protocol presented here will be impractical when applied to those populations. Since tropical honey bees periodically migrate while tracking floral resources, an abandoned cavity once occupied by a colony is not indicative of colony death; therefore, the rationale of monitoring specific nesting sites to determine colony survival rates (see below) will not necessarily work within tropical conditions.

The Protocol and How to Implement It

To comprehensively study free-living colonies—and determine their self-sustaining capacity—reports on their locations and nesting habits should be integrated with data describing colonies' statuses at regular intervals. As such, the developed protocol is based on a questionnaire to be completed during monitoring inspections of individual free-living colonies. Questions and answers have been

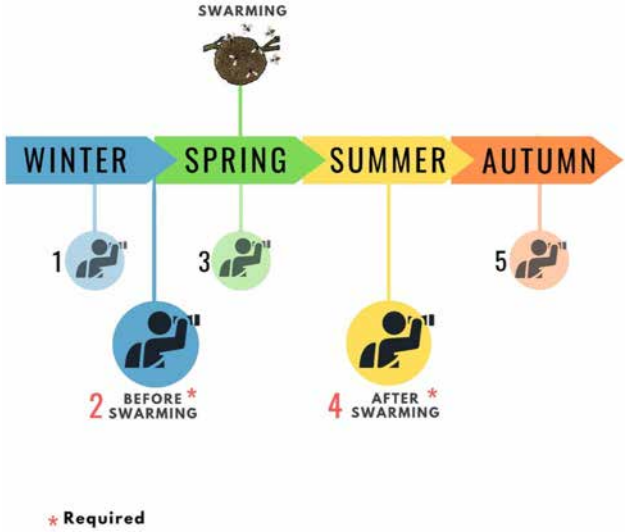

Figure 2. Example of a yearly monitoring schedule within a temperate region: Where possible, we recommend that five monitoring inspections be carried out during the following periods: (1) end of winter, (2) between end of winter and before swarming, (3) during swarming, (4) between end of swarming and beginning of autumn, (5) between end of autumn and beginning of winter.

standardized to ensure comparability across studies using the same protocol. The data collected can be integrated with genetic testing to confirm continued nest occupancy by the same colony or its descendants (Kohl et al., 2022), as with other metadata on climatic and environmental factors to understand their impact on colony survival.

The questionnaire aims to collect status reports following key phenological events in honey bee colonies. As such, the timing of monitoring inspections have been scheduled around calendar periods that significantly impact colony survival, development, and fitness (Figure 2).

While we encourage users to follow this protocol and fill out the questionnaire as frequently as possible to reduce inaccuracies and catch any unforeseen activities (like swarming or a die-out), we recommend completing it at least once during each of these five periods (Figure 2):

1. **End of winter**: Start the monitoring cycle by recording the status of colonies at the end of winter. This marks the end of the most challenging season for honey bees in temperate regions, when colonies may have succumbed to high parasite and pathogen loads and/or lack of food reserves.
2. **Between end of winter and beginning of swarming**: A second monitoring during this period is fundamental to determine whether the colonies that have successfully overwintered are able to reach the reproductive season.
3. **Swarming season**: Capturing colony activity at least once during the swarming season is crucial to assess whether a colony is reproducing.
4. **Between end of swarming and beginning of autumn**: Perform at least one inspection between the end of swarming and the beginning of autumn since this period can be very challenging and colonies may dwindle or die out due to forage shortages.
5. **Between end of autumn and beginning of winter**: Conduct at least one final monitoring inspection during late autumn. High *V. destructor* infestations during this time can lead to pre-winter collapse and nests could also be destroyed or depredated.

The beginning and end of every period can be inferred by observing local managed colonies or by asking local beekeepers or honey bee researchers. In those cases where monitoring a nest site five times per year is not feasible, we require that users complete the monitoring questionnaire a minimum of twice annually, right before and right after the swarming season (Figure 2). Capturing these two monitoring moments will, at the very least, provide some scientifically viable data, which can potentially be supplemented with others' nearby observations. If a nest was found inactive during the previous inspection, we request that it still be visited in subsequent periods, unless the nest had been completely removed, i.e., the tree containing its cavity fell. This is because the nest could have been reoccupied by a swarm since the last visit.

It is also important that users of this questionnaire be (or become) well acquainted with honey bees to ensure data validity. At minimum, users should be able to distinguish honey bees from other look-alike hymenopterans and recognize important events such as swarming. To assist with this, an illustrated Guideline was created to accompany the protocol (see Supplementary Material S2, www.honeybeewatch.com/resources).

Concluding Remarks

The repeated use of this monitoring protocol will enable the tracking of changes in population demographics and swarming events, as well as the identification of elements affecting the survival of *A. mellifera* in the wild. Its widespread use will also enable the collection of comprehensive data that are critical to addressing current knowledge gaps regarding the distribution, density, and survival of wild honey bee colonies and self-sustaining populations. These and other data will ultimately support the re-emerging field of research on honey bees living in the wild.

We therefore encourage future researchers and independent citizen-science initiatives to adopt this standard, open-access methodology when monitoring free-living colonies. We believe that the fruits of our collaborative approach will not only improve overall scientific understanding but also promote public engagement in the sciences and increase awareness of the importance of wild honey bees, pollinators in general, and their conservation.

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