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Review

Emerging technologies for pollinator monitoring^{*} Toke T Høye^{1,2}, Matteo Montagna^{3,4}, Bas Oteman⁵ and David B Roy^{6,7}



Efficient tools for monitoring pollinator populations are urgently needed to address their reported declines. Here, we review advanced technologies focusing on image recognition and DNA-based methods to monitor bees, hoverflies, moths, and butterflies. Insect camera traps are widely used to record nocturnal insects against uniform backgrounds, while cameras studving diurnal pollinators in natural vegetation are in early stages of development. Depending on context, insect camera traps can assess occurrence, phenology, and proxies of abundance for easily recognisable and common species. DNAbased techniques can drastically decrease the costs of sample processing and speed of specimen identification but strongly depend on the completeness of reference DNA databases, which are continually improving. Molecular analyses are becoming more affordable as uptake increases. Image-based methods for identification of dead specimens show promising results for some invertebrates, but image reference databases for pollinators are far from complete. Building image reference databases with expert entomologists is a priority. Lidar and acoustic sensors are emerging technologies although it is still uncertain which insect taxa can be separated in data from these sensors and how well. By improving accessibility to novel technologies and integrating them with existing approaches, monitoring of pollinators and other insects could deliver richer. more standardised and possibly cheaper data with benefits to future insect conservation efforts.

Addresses

¹Department of Ecoscience, Aarhus University, 8000 Aarhus C, Denmark

² Arctic Research Centre, Aarhus University, 8000 Aarhus C, Denmark ³ Department of Agricultural Sciences, University of Naples Federico II, 80055 Portici, Italy

⁴ Interuniversity Center for Studies on Bioinspired Agro-Environmental Technology (BAT Center), University of Naples Federico II, 80138 Naples, Italy

⁵ Dutch Butterfly Conservation, Wageningen, the Netherlands

⁶ UK Centre for Ecology & Hydrology, Wallingford OX10 8BB, United Kingdom

⁷ Centre for Ecology and Conservation, University of Exeter, Penryn TR10 9EZ, United Kingdom

Corresponding author: Høye, Toke T (tth@ecos.au.dk)

Current Opinion in Insect Science 2025, 69:101367

This review comes from a themed issue on Global change biology

Edited by Toke Høye and Eliza Grames

For complete overview about the section, refer "Global change biology (2024)"

Available online 12 March 2025

https://doi.org/10.1016/j.cois.2025.101367

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Assessing emerging technologies

In this paper, we evaluate emerging technologies for insect monitoring by assessing to what extent they can deliver data on pollinator abundance and species richness as this is currently requested by the European Commission as part of plans to install an EU Pollinator Monitoring Scheme [1]. We base the review on expert and stakeholder input to the EuropaBON and MAMBO projects on technology for biodiversity monitoring [2,3]. In addition, we have compiled recently published papers, websites, and services supporting the monitoring of pollinators. We focus on emerging technologies that could mature sufficiently to contribute to continentalscale monitoring within the next 5-10 years. The techniques either perform nonlethal monitoring in the field or taxa identification of specimens in the lab based on imaging and DNA-based methods.

There are additional technologies developing of relevance to pollinator monitoring. These include LiDAR [4], spectral analysis of thin-film wing interference signals [5], miniature tags [6], malaise traps with automatically interchanging vials, and moth freezers for conserving specimens after trapping [7,8]. It also remains to be demonstrated that the soundscape can effectively be translated into observations of pollinators at the species level or even higher taxonomic units, but see [9,10].

^{*} Given the role as Guest Editor, Toke Høye had no involvement in the peer review of the article and has no access to information regarding its peer-review. Full responsibility for the editorial process of this article was delegated to Eliza Grames.

Insect camera traps for *in situ* pollinator monitoring

Description

Insect camera trap technologies are maturing rapidly [11–13]. To monitor pollinators, camera traps can focus on natural vegetation or introduced floral resources as socalled phytometer plants to standardise the floral resource across study sites [14,15]. Other baits or even artificial flowers can also be introduced to attract and monitor pollinators. The FAIR device integrates a camera into a malaise trap [16], which avoids the use of attractants and could be particularly relevant for monitoring hoverflies. The most mature insect camera traps involve recording images against standardised backgrounds (either sticky or nonsticky). These usually comprise a uniform background such as a yellow sticky trap [17], a screen [18] or an illuminated white sheet and a UV light [19], or the inside of a plastic pheromone trap [20]. Already, such systems are delivering season-wide and very rich monitoring data for moths at the species level [21].

Key advantages and drawbacks

Insect camera traps can collect data at unprecedented temporal resolution over long timescales in an automated and standardised way [11,22]. High-frequency imaging can record foraging behaviour, such as floral preference, diurnal patterns, as well as the sensitivity of pollinator activity to short-term weather fluctuations [15,17,23,24]. Insect camera traps are nonlethal and enable observation of elusive insect species while minimising labour costs of monitoring [25]. Field sensors deployed across entire seasons can capture detailed insect phenology data, which is valuable context when comparing abundance or the occurrence of specific species monitored using other means (e.g. line transects or light trapping). Image-based approaches are often simple to validate as experts can immediately check relevant morphological characters from images if images are of sufficient quality. Insect camera traps also reduce the expertise required for fieldwork.

With camera traps, it is possible to record a single individual multiple times, which makes estimating abundance challenging. Also, some traps will attract pollinators (e.g. when fitted with a yellow screen or artificial flowers), and the strength of the attractant may depend on the dynamics of similar resources in the surroundings (e.g. flower abundance). For instance, pan traps relying on attraction have been reported to attract more insects in landscapes with few flowers [26]. Systems for nocturnal insects (e.g. moths) use a light as the attractant and may be less affected by bias from flower availability although are sensitive to changes in local artificial light at night levels [27]. The performance of deep learning models is increasing rapidly and performs well for moths in Europe and North America [21,28] but are much less developed for other regions and other pollinator taxa. While image quality in these systems can be improved, tests with higher quality cameras still have limitations regarding costs, durability and automation [29]. Finally, theft and vandalism of equipment might be a problem for field equipment fitted with solar panels and batteries or lights to attract and record insects as this makes them more conspicuous.

Future research needs

To process images from insect camera traps, efficient localisation algorithms are needed as insects are typically small compared to the image size [13]. Classification models also need to cope with the high diversity and large proportion of undescribed species. Bees and hoverflies are particularly difficult to identify from images, although new results show how images of wing patterns can enhance existing training data sets [30]. It will be important to evaluate how representative the data from insect camera traps are compared to other methods in terms of visitation rates and taxonomic bias. For traps with attractants, it is important to test the effect of different recording schedules on the data collected. This is because the attractant may prevent the insects from carrying out their normal behaviour and since predation risk may increase with the duration of trapping.

Systems for monitoring pollinators in natural vegetation need further development, although some success has been achieved on low stature vegetation [15,31] or even constructed standardised flower beds for pollinator monitoring [32,33]. Insect camera traps with onboard processing are developing slowly as the available data sets to train deep learning models to locate and identify pollinators are still small and covers only small subsets of common species. Such functionality is needed as the systems are capable of generating very large volumes of data (e.g. [18,21]). This also calls for user-friendly interfaces to ensure efficient uptake.

DNA-based approaches for pollinator monitoring Description

Unlike *in situ* observations, DNA-based methods separate insect sampling (which is typically a lethal procedure, except in the case of airborne DNA) from identification. Identification requires specimen processing in a laboratory, appropriate facilities to store the collected specimens, and basic bioinformatic analyses. Pollinators can be collected through netting or trapping; the obtained bulk samples are then identified by DNA-based methods. DNA barcoding and metabarcoding leverage short, selected, and standardised DNA sequences (barcodes) to identify organisms [34]. DNA barcoding requires four main steps: (i) DNA extraction; (ii) DNA barcode amplification by polymerase chain

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| Overview of the main Costs of equipment a | emerg | jing technologies for pollinator moni A methods are estimated by the auth | itoring, their technology readiness levels (TRL) hors and are likely to decrease in the future. | , advantages and disadva | ntages, costs and future research needs. |
| Name | TRL | Advantages | Disadvantages | Costs | Future research needs |
| In situ insect camera traps | 5-7 | Nonlethal sampling High temporal resolution Validation possible, images stored Requires limited expertise | No single solution for all pollinators Not all individuals can be identified to species Risk of theft The same individual can be recorded multiple times | €3000–€5000 per camera | Moth camera traps are ready for pilot testing. For diumal pollinators, attraction dependence needs further study. |
| DNA-based methods | ω | Collection requires limited expertise Only a small part of specimen required Samples can be processed quickly Pollen DNA can also be extracted | Lethal sampling Time consuming collection of field samples Expertise required to interpret results (metabarcoding) Strongly dependent on reference databases Primers-induced bias | €2-€10 per barcode, €18-€80 per meta- barcode sample | Reference databases currently cover only 33–60% of known species, depending on the taxonomic group. Potentially pair with expert identification to expand databases and verify identifications. |
| Image recognition of preserved specimens | Ŋ | Collection requires limited expertise Reduces taxonomic expertise and potentially reduced costs Likely to identify most individuals to species | Requires expertise in mechanical and electrical and software engineering for maintenance Strongly dependent on reference databases | €5000-€20 000 per machine | Large potential, further testing and development required. Next step is to build a pollinator reference library and test image recognition performance |

reaction (PCR); (iii) sequencing of the PCR product using Sanger or Oxford Nanopore Technology; (iv) taxonomic assignment by comparing the obtained barcode with reference databases of orthologous sequences (their completeness influences the accuracy of the taxonomic assignment).

The primary marker for animal DNA barcoding is a fragment of 658 bp at the 5' region of the mitochondrial gene cytochrome c oxidase subunit I (COI) [35]. Once the barcode sequence is generated, the organism can be identified using tools such as the Barcode of Life Data (BOLD) system (https://v4.boldsystems.org/). BOLD currently includes ~14 million insect COI sequences, corresponding to 264 301 species (June 2024). Among them, COI sequences of 105 572 Lepidoptera, 2643 Syrphidae, and 51 169 Hymenoptera species, covering 60.5%, 44%, and 33% of the described species for these groups, respectively. Importantly, reference barcodes are missing for a large number of pollinator species in many regions of Africa, Asia, and South America limiting the potential of this approach for species identification, globally. DNA metabarcoding extends DNA barcoding allowing the simultaneous identification of multiple taxa in samples containing DNA from more than one organism. The method combines DNA-based identification and high-throughput next-generation sequencing (NGS) technologies. The DNA sequences generated using DNA barcoding and metabarcoding can also provide useful information on the intraspecific genetic diversity of selected taxa (e.g. [36,37]).

Volunteers with basic training can collect and preprocess the samples. However, DNA extraction and PCR or library assembly require a molecular biology lab. The analyses of the results of Sanger sequencing do not require specific bioinformatic expertise, while the analyses of NGS data do. Nonetheless, user-friendly apps are available to analyse NGS output (e.g. mBRAVE, https:// mbrave.net/). Additionally, well-curated insect barcode databases enhancing the taxonomic identification of NGS data are being developed, for example, COIns [38]. However, the adoption of DNA metabarcoding will be constrained in regions where reference databases poorly represent the local insect fauna. The interpretation of DNA barcoding and metabarcoding results needs minimal effort.

Key advantages and drawbacks

A key advantage of DNA-based tools is their flexibility in collection methods. Pollinators can be collected by volunteers with moderate training or through expert networks; however, it is crucial to standardise data collection protocols. Additionally, DNA-based methods enable the identification of pollinators at any developmental stage, even starting from partial specimens, something that is typically not possible through morphological examination. A promising approach relies on the characterisation of specimens collected by pan traps or Malaise traps starting from the DNA they released in the preservative solution (e.g. absolute ethanol) [39]. Airborne DNA can be utilised to identify insects inhabiting a specific environment [40]. One significant advantage of DNA-based methods is that they do not require expert taxonomists for their application in monitoring programmes, although such expertise remains essential for developing the reference DNA sequence database. Notably, the costs associated with these approaches are currently moderate (Table 1) and expected to decrease further in the future. The entire process of data generation and analysis is likely to become more efficient, user-friendly, and require less personnel effort.

DNA metabarcoding (PCR-based) is effective for determining species presence or absence; however, selecting appropriate PCR primers is crucial to ensure accurate detection of target species. Despite its effectiveness, DNA metabarcoding is not reliable for estimating species abundance, also due to potential biases introduced during the PCR process [41]. Nonetheless, a correlation between the number of NGS sequences obtained and the organism biomass in the initial bulk sample has been observed [42]. These limitations must be carefully considered to avoid drawing erroneous conclusions [43,44]. At present, DNA barcoding and metabarcoding remain promising approaches primarily for species presence-absence monitoring. A further challenge is represented by reference database completeness. Well-curated reference databases are essential for the accurate taxonomic assignment of DNA sequences, yet they do not currently include all described species. In particular, rare species are often absent, and the gaps are much bigger in the most diverse regions globally. However, initiatives at the national and EU levels, such as iBOL Europe, and global initiatives, including the iBOL Consortium, have been established to address these gaps. Finally, for DNA barcoding and metabarcoding to be effectively implemented in largescale monitoring programmes, it is essential to standardise sample collection and processing procedures (e.g. [45,46]).

Future research needs

We envision three main areas of development to implement DNA-based methods in pollinator monitoring programmes and overcome their current limitations. First, eDNA metabarcoding, along with innovative and promising PCR-free approaches such as sequence capture [47], must be adopted in large-scale pollinator pilot monitoring programmes and compared with the current state-of-the-art monitoring approaches. Second, the integration of DNA-based methods with image-based technologies, such as BIODISCOVER or DiversityScanner [48–50], could allow for the automation of specimen sorting and accelerate the identification process. Moreover, if a newly obtained DNA sequence does not match any in the reference databases, either the specimen or high-quality images of it can be shared with expert taxonomists for morphological identification, helping to improve the reference databases. Finally, it is vital to develop targeted programmes aimed at expanding the reference sequence databases for pollinator species, with a special focus on rare species and underrepresented regions.

Image-based approaches for studying preserved pollinator specimens Description

Imaging of pinned specimens and individuals in bulk insect samples can facilitate rapid counting and identification of pollinators in historical collections or collected as part of monitoring programmes. Technologies include systems for bulk photography of museum collection drawers [51], petri dishes or trays [48,52]; photography of parts of insects, such as their wings [30,53,54] or multiangle imaging of insects in a liquid medium [50] or 3D imaging [53,55]. Specimen imaging can generate highresolution images for potential identification to specieslevel taxonomic resolution.

Among the image-based tools, the BIODISCOVER system [50] allows for detailed close-up images of individual specimens using a robotics-enabled framework, where bulk samples are processed individually with a collaborative robot and imaged. The DiversityScanner [48] offers principally the same functionality but is currently not relevant for pollinator samples as it only takes presorted specimens smaller than 3 mm length. Recently, the Entomoscope has been presented as a lowcost solution for close-up photography of specimens [56]. This system is mainly proposed for species discovery but could potentially be adapted for imaging specimens after collection and submitting image data to a central database for subsequent species identification. There are also commercially available microscopes, but these are currently prohibitively expensive for most organisations and do not integrate any robotics for automation of specimens handling [57].

Key advantages and drawbacks

Specimens-based imaging solutions can facilitate the rapid identification of specimens in wet insect bulk samples. Such samples are typically collected with pan, vane, or malaise traps. Bees and hoverflies are the most common pollinator taxa in such samples. With automation, it is conceivable that insect bulk samples can be brought to centralised processing labs, where specimens can be identified, counted, and sorted. Hard-to-identify specimens could then identified by taxonomic experts or potentially through DNA barcoding. In this way, automated imaging can allow experts to concentrate on identification of the most challenging species. Such imaging systems are relatively expensive, technically sophisticated, and require maintenance by people with different skills to those traditionally involved with pollinator species identification.

Future research needs

Traditional identification or nondestructive DNA barcoding can contribute to building reference collections for image-based identification. However, more work is needed to design workflows that leverage different technologies for cost-efficient and accurate identification of pollinator specimens. While image classification models for butterflies and moths are quite well developed, more image data are needed for bee and hoverfly species. Robot-enabled image-based identification of bulk samples also need to be further developed and evaluated for their cost-efficiency and complementarity to DNA barcoding and metabarcoding.

Conclusions and recommendations

Emerging technologies are rapidly developing and can potentially have a large effect on future pollinator monitoring schemes. Statistical models to integrate data types from multiple monitoring approaches can maximise their combined value [58]. Insect camera traps for nocturnal insects attracted to UV lights and DNA-based species identification show greatest promise. However, their usefulness strongly depends on the availability of image reference databases. These are rapidly being developed but have pronounced gaps in the most speciesrich regions worldwide. Citizen science portals have an important role in developing reference collections of images that have been labelled by entomologists [59]. Insect camera traps for diurnal pollinators need further study into the visitation rates at the traps and the effects of temporally dynamic floral resources in the surroundings of the trap. Establishing how repeatedly counting individuals affect abundance estimates is also important. The lab-based specimen identification based on image recognition could drastically decrease the costs of pan, vane, or malaise trap bulk sample processing. Gap filling in image and DNA reference databases for pollinators will support specimens-based identification in the lab. Already identified specimens in collections are critical resources in this respect. Capacity building and the development of user-friendly interfaces, will help ensure that these emerging technologies can be applied at scale to deliver standardised monitoring data on pollinators.

Data Availability

No data were used for the research described in the article.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgements

This research was funded by Aage V. Jensen Naturfond grant number N2024-0013 to TTH and the European Union's Horizon Europe Research and Innovation programme under Grant Agreement No. 101060639 (MAMBO) to TTH and DBR. MM is partially supported by the European Union Next-GenerationEU [PIANO NAZIONALE DI RIPRESA E RESILIENZA (PNRR)—MISSIONE 4 COMPONENTE 2, INVESTIMENTO 1.4—D.D. 1032 17/06/2022, CN00000022]. This manuscript reflects only the authors' views and opinions; neither the European Union nor the European Commission can be considered responsible for them.

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