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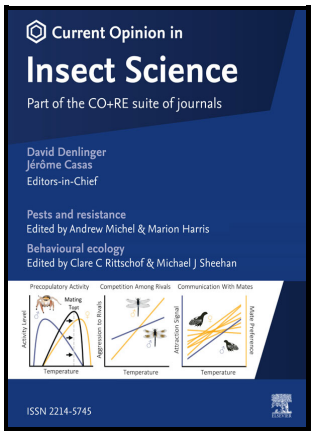

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Emerging technologies for pollinator monitoring

Short Title: Emerging technologies for pollinator monitoring

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Abstract

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 studying 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 recognizable and common species. DNA-based 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 which insect taxa can be separated in data from these sensors and how well is still uncertain. By improving accessibility to novel technologies and integrating them with existing approaches, monitoring of pollinators and other insects could deliver richer, more standardized and possibly cheaper data with benefits to future insect conservation efforts.

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 continental-scale monitoring within the next five to ten years. The techniques either perform non-lethal 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].

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 so-called 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 relevant for monitoring hoverflies in particular. The most mature insect camera traps involve recording images against standardized backgrounds (either sticky or non-sticky). 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 expose foraging behaviour

such as floral preference, diurnal patterns as well as the sensitivity to short-term weather fluctuations in pollinator abundance [15,17,23,24]. Insect camera traps are non-lethal and enable observation of elusive insect species, while minimizing 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 datasets [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 because 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 datasets 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 standardized DNA sequences (barcodes) to identify organisms [34]. DNA barcoding requires four main steps: (i) DNA extraction; (ii) DNA barcode amplification by 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, 2,643 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 pre-process the samples. However, DNA extraction and PCR or library assembly require a molecular biology lab. The analyses of the results of Sanger sequencing does not require specific bioinformatic expertise, while the analyses of NGS data do. Nonetheless, user-friendly apps are available to analyze NGS output (e.g., mBRAVE, https://mbrave.net/). Additionally, well-curated insect barcode databases enhancing the taxonomic identification of NGS data are being developed e.g., 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 standardize 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 characterization 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 utilized 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 programs, 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 large-scale monitoring programs, it is essential to standardize 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 programs 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 programs 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. Lastly, it is vital to develop targeted programs 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 programs.

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 multi-angle imaging of insects in a liquid medium [50] or 3D imaging [53,55]. Specimen imaging can generate high-resolution images for identification potential to species level 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 pre-sorted specimens smaller than 3mm length. Recently, the Entomoscope has been presented as a low-cost 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 hover flies are the most common pollinator taxa in such samples. With automation, it is conceivable that insect bulk samples can be brought to centralized 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 non-destructive 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 is 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 species-rich 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 additional study into the effects of temporally dynamic floral resources as their attractive nature causes them to compete with local floral resources. 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 standardized monitoring data on pollinators.

Table 1 Overview of the main emerging technologies for pollinator monitoring, their technology readiness levels (TRL), advantages and disadvantages, costs and future research needs. Costs of equipment and DNA-methods are estimated by the authors and are likely to decrease in the future.

Name	TRL	Advantages	Disadvantages	Costs	Future research needs
In situ insect camera traps	5-7	• Non-lethal 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	€3,000-€5,000 per camera	Moth camera traps are ready for pilot testing. For diurnal pollinators, attraction dependence needs further study.
DNA-based methods	8	• Collection requires limi	• Lethal sampling • Time consuming	€2-€10 per barcode, €18-€80 per meta-barcode sample	Reference databases currently cover only 33-60% of known species,

		ted expertise • Only a small part of specimen required • Samples can be processed quickly • Pollen DNA can also be extracted	collection of field samples • Expertise required to interpret results (metabarcoding) • Strongly dependent on reference databases • Primers-induced bias		depending on the taxonomic group. Potentially pair with expert identification to expand databases and verify identifications.
Image-recognition of preserved specimens	5	• Collection requires limited expertise • Reduces taxono	• Requires expertise in mechanical and electrical and software engineering for maintenance	€5,000-€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

	mic exp erti se and pot enti ally red uce d cost s • Like ly to iden tify mos t indi vidu als to spe cies	• Strongl y depend ent on referen ce databa ses	

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Recommended papers

(*) Alison, J., S. Payne, J. M. Alexander, A. D. Bjorkman, V. R. Clark, O. Gwate, M. Huntsaar, E. Iseli, J. Lenoir, H. M. R. Mann, S.-L. Steenhuisen, and T. T. Høye. 2024. Deep learning to extract the meteorological by-catch of wildlife cameras. Global Change Biology 30:e17078.

This paper demonstrates a novel approach to extract critical micro-climatic data directly from images used to monitor animals such as pollinators.

(**) Bjerge, K., Mann, H. M. R., Høye, T. T. & Karstoft, H. A deep learning pipeline for time-lapse camera monitoring of floral environments and insect populations. Ecological Informatics 84, 102861 (2024). https://doi.org/10.1016/j.ecoinf.2024.102861

Quantifying the floral resources seen by an insect camera trap provides important context for the number and duration of insect visits to flowers.

(*) Geissmann, Q., P. K. Abram, D. Wu, C. H. Haney, and J. Carrillo. 2022. Sticky Pi is a high-frequency smart trap that enables the study of insect circadian activity under natural conditions. PLoS Biology 20.

The Sticky Pi trap is a prime example of a low cost and highly performing insect monitoring system for studying the diurnal and seasonal dynamics of flying insects.

(*) Høye, T. T., M. Dyrmann, C. Kjær, J. Nielsen, M. Bruus, C. L. Mielec, M. S. Vesterdal, K. Bjerge, S. A. Madsen, M. R. Jeppesen, and C. Melvad. 2022. Accurate image-based identification of macroinvertebrate specimens using deep learning—How much training data is needed? PeerJ 10:e13837.

For the species recognition of images of preserved specimens, it is critical to know how many specimens are needed in order to train accurate deep learning models. This paper provides an important benchmark for this question.

(*) Leigh, D. M. *et al.* Opportunities and challenges of macrogenetic studies. *Nature Reviews Genetics* 22, 791-807 (2021). https://doi.org/10.1038/s41576-021-00394-0

The emergent field of macrogenetics, which aims to exploit information stored in publicly accessible DNA bioprojects to explore intraspecific genetic variation, is reviewed in this paper. The review highlights knowledge gaps and outlines future directions necessary for macrogenetics to fully realize its potential in biodiversity monitoring and conservation.

(**) Littlefair, J. E. et al. Air-quality networks collect environmental DNA with the potential to measure biodiversity at continental scales. Curr. Biol. 33, R426-R428 (2023). https://doi.org/https://doi.org/10.1016/j.cub.2023.04.036

This study is the first to exploit environmental DNA captured by routine ambient air quality monitoring stations to assess the local biodiversity of vertebrates, arthropods, plants, and fungi. Air quality monitoring filters present the best opportunity to date for detailed monitoring of terrestrial biodiversity using an existing, replicated transnational design that is already in operation.

(*) Magoga, G. et al. Curation of a reference database of COI sequences for insect identification through DNA metabarcoding: COins. Database 2022 (2022). https://doi.org/10.1093/database/baac055

In this paper, a database of 5' region cytochrome c oxidase subunit I sequences of insects was developed through a combination of automated and manually curated steps. The database includes over 532,000 representative sequences from more than 106,000 species, specifically formatted for the QIIME2 software platform. It is useful for DNA-metabarcoding analyses.

(**) Potts, S. et al. Refined proposal for an EU pollinator monitoring scheme. (Publications Office of the European Union, 2024).

This report presents a proposal for an EU Pollinator Monitoring Scheme (EU-PoMS), based on the findings of an expert group from across Europe. Among many topics, the report evaluates the potential of emerging technologies for pollinator monitoring, the capacity required to implement EU-PoMS across all EU Member States and proposals for biodiversity indicators to assess pollinator populations.

(*) Rodriguez, A., C. Desjonquères, V. Hevia, M. Llorente, J. Ulloa, and D. Llusia. 2024. Towards acoustic monitoring of bees: wingbeat sounds are related to species and individual traits. Philosophical Transactions of the Royal Society B 375:20230111.

This paper is an important reference for acoustic monitoring of bees. Although many species cannot be separated it outlines important future research directions.

(**) Kohlberg, A. B., Myers, C. R. & Figueroa, L. L. From buzzes to bytes: A systematic review of automated bioacoustics models used to detect, classify and monitor insects. *J. Appl. Ecol.* 61, 1199-1211 (2024). https://doi.org/https://doi.org/10.1111/1365-2664.14630

This excellent review provides a much needed overview of which insect taxa can be monitored via recordings of the sounds they make. The paper is supported by a rich set of appendices.

(*) Roy, D.B. et al. 2024. Towards a standardized framework for AI-assisted, image-based monitoring of nocturnal insects. Philosophical Transactions of the Royal Society B, 379(1904), p.20230108.

The paper describes a framework for automated, image-based monitoring of nocturnal insects—from sensor development and field deployment to workflows for data processing and publishing. The paper proposes priorities for future research and development to realise the potential of automated monitoring of nocturnal insects as an important group of pollinating insects.

(**) Sittinger, M., J. Uhler, M. Pink, and A. Herz. 2024. Insect detect: An open-source DIY camera trap for automated insect monitoring. PLOS ONE 19:e0295474.

The paper provides valuable instructions for building a sophisticated insect camera trap and associated image analysis pipeline. A good example of open science research.

(**) Spiesman, B. J., C. Gratton, E. Gratton, and H. Hines. 2024. Deep learning for identifying bee species from images of wings and pinned specimens. PLOS ONE 19:e0303383.

For bees, image-based identification methods are challenging. This paper describes a novel approach to focus on wing patterns for improved taxonomical resolution.

(**) van Klink, R. et al. 2022. Emerging technologies revolutionise insect ecology and monitoring. Trends in Ecology & Evolution 37:872-885.

This paper appraises emerging tools and technologies that provide unprecedented opportunities for insect ecology. These technologies can enhance spatial, temporal, and taxonomic coverage of monitoring, although have some limitation and no single approach can monitor all insects. The paper demonstrates the strengths and weaknesses of different approaches to monitoring and analysis of insect populations and many of the insights are highly relevant to the subset of insects that have a role in pollination.

(*) Wührl, L. et al. DiversityScanner: Robotic handling of small invertebrates with machine learning methods. Mol. Ecol. Resour. 22, 1626-1638 (2022). https://doi.org/10.1111/1755-0998.13567

This paper proposes a robot, DiversityScanner, designed to handle specimens from insect bulk samples, such as those collected from Malaise traps, for DNA barcoding analyses. Once labelled with taxonomic information from DNA barcodes, these images can be used as training data for machine learning, enabling convolutional neural networks to identify the specimens.

Declaration of interests

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.