Using environmental DNA (eDNA) to elucidate the role of habitat upon the transmission of pollinator disease.

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Insect pollinators are an integral component of global ecosystems, and vital to future food security [1]. UK and Worldwide pollinator populations are declining [1-3]. Declines are widely attributed to agricultural intensification, the establishment of invasive species, disease and climate change [4, 5]. However, the direction, strength and causes of insect declines are highly variable, and evidence is needed to quantify the mechanisms driving these processes [6-8]. One key topic is the role of emerging infectious diseases (EIDs) and potential disease spill over from managed to wild populations. The honey bee (*Apis mellifera*) is the dominant managed pollinator species worldwide, and is additionally used for the production of honey, bees wax and royal jelly. Since honeybees forage over large distances, they integrate information on habitat resources over vast spatial scales, and interact with *c.* 250 native wild bee species. Beekeeping as a hobby in the UK is rapidly growing with over 29,000 beekeepers managing around 126,000 colonies [9]. Consequently, beekeepers represent a valuable national network for the study of this species, a resource currently being utilised by the UKCEH National Honey Monitoring Scheme (NHMS), https://honey-monitoring.ac.uk/. This citizen science scheme applies molecular techniques to determine the floral composition of honey, thereby measuring pollinator resources at landscape scales[10, 11]. Combing archived data with the sampling of wild pollinators this studentship will develop new techniques to investigate honeybee interactions with their landscape, wild pollinator competition, and exposure to inter-species disease transmission.

The study of environmental DNA (eDNA) utilises molecular technologies to investigate the genetic material contained within a given environment. When specific taxonomically relevant genes are studied it's possible to discern the plant species from which a sample is composed - a technique known as plant metabarcoding. Currently, the accepted methodology for the identification of the floral composition of honey is through the microscopic analysis of pollen. Metabarcoding is a significant advance on this approach in terms of speed and accuracy [10, 12-15]. However, such technologies require the development of new assays and bioinformatics pipelines, to compare the validity of this technique to existing technologies and to increase taxonomic resolution and capacity.

In addition to taxonomic information eDNA also contains genetic material from bees and any pathogens to which they may have been exposed [16]. Pilot work at UKCEH was able to successfully detect DNA in honey derived from known bee pathogens: *Nosema apis, Melissococcus plutonius* and *Paenibacillus larvae*. The developed assays require that honey is screened for each pathogen separately, and therefore is costly and time consuming. However, the sequencing of eDNA holds the potential to simultaneously screen for multiple pathogens and further, allows for the discovery of additional pathogens which would otherwise be undetected.

Through a combination of cutting edge field and laboratory techniques this project will explore the use of emerging sequencing technologies to investigate key questions within the field: How do emerging and existing technologies compare as a record of pollinator foraging activity? Can common bee

pathogens be detected in eDNA? Are honeybees a good sentinel species to explore managed and wild pollinator interactions? Do any common plant signatures occur between pollinators, and are these linked to potential disease transmission sites? This studentship will be able to draw upon the experience of the UKCEH, Reading and BBKA teams and has access to a large network of highly experienced citizen scientist beekeepers. This unique placement ensures that the student is able draw on additional existing metadata and samples which would otherwise be impossible for a student to generate over the duration of a single PhD. This PhD investigates its aims through a combination of laboratory work and in depth field experiments set up in partnership with experts from the BBKA. The overall aim is to understand how honeybees interact with other pollinators and their environment, thereby identifying potential routes of disease transmission, and how this influences trends in pollinator decline.

Training opportunities:

Through UKCEH the student will receive practical onsite training in molecular biological techniques, such as DNA extraction and next generation sequencing as well as skills in field sampling, statistics and ecological modelling. Additionally, the student will be encouraged to attend a wide range of learning and development activities, including presentational skills and paper writing. CEH offers training for students and supervisors based on the well-received Vitae Researcher Development Framework, using this as a tool to plan, promote and support the personal, professional and career development of PhD researchers. Additionally, the student will be exposed to in-depth bioinformatics and statistical knowledge and expertise from the supervisory team based in the University of Reading who have developed world-class informatic tools for analysing next generation sequencing data. Through working with the CASE-partner the student will attend training in bee husbandry, microscopy of pollen grains and bee disease assessment, with the aim of gaining BBKA national certification in these skills. The student will attend both national and local BBKA events. This close working partnership with BBKA members means that the student will gain skills and experience in presenting their work to non-scientists and in running a national scale citizen science project.

Student profile:

The project is suitable for a suitable for students with a good degree (first class or 2:1) or M.Sc. in molecular biology, ecology, or zoology. We would like a student who is enthusiastic and keen to learn new skills in the field, lab and bioinformatics. The candidate will be working closely with non-scientists therefore must have good interpersonal skills, and be able to clearly convey their work to others. The candidate would also be expected to work conscientiously and independently both in the laboratory and

field. Desirable attributes would include skills in molecular biology, ecology, bioinformatics, and entomology.

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