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Standardized protocol for collecting bee samples to generate molecular data

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Abstract. This protocol provides guidance on the appropriate collection of bee specimens or tissue samples for molecular analysis, with an emphasis on generating genetic and genomic data while ensuring tissue integrity. Specifically, the protocol focuses on tissue collection and storage methods, including relevant specimen metadata recording and reporting, but does not cover any downstream handling or analyses, which vary depending on the aims of a given project or study. This protocol is specifically designed for freshly collected, individual bee specimens intended for genetic, genomic, or other molecular analyses. While molecular approaches to bee monitoring are not the primary focus, we emphasize their promising role for future applications. This protocol is part of a series developed in association with the U.S. National Native Bee Monitoring Network to standardize bee monitoring practices.

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INTRODUCTION

Molecular data provide critical information for bee conservation and monitoring efforts (Zayed, 2009; López-Uribe *et al.*, 2017; Lozier & Zayed, 2017; Kelemen & Rehan, 2021). Characterizing genetic variation in individuals and populations enables the estimation of colony density for social species (McGrady *et al.*, 2021), effective population sizes (Lozier *et al.*, 2023), adaptive processes (Theodorou *et al.*, 2018; Jaffé *et al.*, 2019; Pope *et al.*, 2023), levels of genetic diversity and inbreeding (López-Uribe *et al.*, 2019; Mola *et al.*, 2024), signatures of environmental stressors (Tsvetkov *et al.*, 2021), and other types of information that are cornerstones of population management and conservation. These types of information are essential for identifying how bee populations and species have responded to past environmental changes, whether they are adapting to ongoing changes, and if there is genetic variation available for them to adapt (López-Uribe *et al.*, 2025). Additionally, DNA-barcoding is a standard approach for molecular-based bee identification (Packer *et al.*, 2009). New methods that implement non-lethal collections of tissue (Herrera-Mesías *et al.*, 2022) or environmental DNA (eDNA; Thomsen & Sigsgaard, 2019) hold promise for a future role in bee monitoring, although at present they are still in the earlier stages of testing and implementation. Molecular data also aid in discovering and delimiting previously uncharacterized cryptic species and lineages, as well as distinguishing between intra-specific (*i.e.*, polymorphic) and inter-specific variation (Gueuning *et al.*, 2020; Andrade *et al.*, 2022; Sandoval-Arango *et al.*, 2023). This helps to establish and differentiate species and subspecies, which are the fundamental units of conservation action.

With increasing bee survey and monitoring efforts, and the resulting increase in the number of bee collections (Woodard *et al.*, 2020; Tepedino & Portman, 2021), it is critical to establish standards for how to preserve biological material to maximize its utility for genetic, genomic, and other molecular analyses. Although most bee monitoring efforts do not explicitly address the characterization of genetic or genomic diversity at the moment, this has been proposed as a critical component of more comprehensive future monitoring, especially given the accelerated rate of species and genetic loss across taxa worldwide (Sánchez-Bayo & Wyckhuys, 2019; Exposito-Alonso *et al.*, 2022; van Klink *et al.*, 2022). The ways that samples are collected and stored directly impact whether they can be used for molecular analyses, with the general pattern that the more effort that is devoted to handling samples, the greater the number of molecular analyses that can be subsequently performed. Although samples that were not collected using this protocol, or any specific tissue preservation methods, can still be used to acquire molecular data (*e.g.*, through museomics), some limitations (outlined below) cannot be overcome if samples are not collected appropriately.

Here, we provide general guidelines for the collection of material (including tissue samples or entire organisms, referred to as tissue hereafter) intended for genetic, genomic, transcriptomic, and other molecular types of datasets. This protocol is designed to address specifically how tissues should be collected, rather than downstream handling or analyses, the specifics of which depend on the aims of a given project or study (see Schweizer *et al.*, 2021). The protocol is focused specifically on freshly collected, individual bee specimens and associated data that are collected with the intention of use for genetic, genomic, or other molecular analyses. We provide examples of studies that used different types of methods and molecular markers to answer questions about population structure, demography, inbreeding, and colony abundance.

We recognize that many other methods are used to generate molecular data to answer some of the above questions (*e.g.*, use of museum specimens, bees collected from passive traps), and that there is considerable interest in the future role of molecular methods of monitoring through eDNA and DNA metabarcoding (Levenson *et al.*, 2024). Therefore, we offer some general recommendations for best practices outside of our specific protocol, such as collection methods if specimens will be pinned and stored in collections. We also provide a brief treatment of the future role of molecular methods of bee monitoring that are on the horizon.

SAMPLING DESIGN

The sampling and experimental design employed for generating different types of molecular data is dependent on the goals of a study or project. Before carrying out any sampling, we highly recommend consulting with the specific research groups performing subsequent bioinformatic and statistical analyses to design a sampling scheme that will best support your monitoring or other goals. Some aspects to take into consideration include establishing a well-defined question that determines whether neutral or adaptive (or both) DNA markers are needed, or whether expression data based on RNA are required. The genetic marker needed for the study will determine what tissue sampling protocol is necessary. We also urge investigators to consider potential future uses of the tissues, because the samples could have value well beyond the focal study if extra effort to appropriately collect and store the tissue is taken. Currently, a variety of genetic markers—defined as sequences in DNA or RNA that can be used to identify specific regions of a genome or transcriptome—are available (Schiebelhut *et al.*, 2024) and their suitability will depend on the question and budget available for the study (Table 1).

Similarly, the choice of sampling effort related to the number of individuals per population, and number of populations, depends heavily on the specific questions of a study or project. There is generally a tradeoff between the number of samples and the number of markers that must be generated for genetic analyses (Schweizer *et al.*, 2021). For example, if you are using a dozen microsatellite markers to determine population genetic structure, you will need a larger number of samples per population, whereas if you are using thousands of markers across the genome, fewer than 10 individuals per population may be sufficient (see Case Study I). Likewise, if you need to screen a very large number of individuals (*e.g.*, many hundreds) for in-depth parentage or colony density analyses, screening fewer markers per individual may be a more economical approach (see Case Study II). If the objective of a study is to examine lineage survival through time for social species such as bumble bees (*Bombus* Latreille) (Carvell *et al.*, 2017), note that sample sizes must be particularly large to account for the attempt to recapture the same lineages at multiple time points.

SAMPLE COLLECTION AND STORAGE

Tissue collection methods (and the level of rigor and effort required) are dependent on what type(s) of molecular data the samples will be used to generate and what types of questions will be answered (Fig. 1). Methods for tissue sampling can be categorized by their ability to preserve the highest quality tissue that can be used to recover different types of markers (Table 2). The best available method is to flash-freeze (defined as placing into ultra-cold temperatures < -80 ºC) individuals in liquid

Table 1. Summary of modern genetic markers commonly used for population genetics, phylogenetics, genomics, and transcriptomic analyses for questions related to pollinator monitoring and conservation.

Type of Marker or Method	Descriptions	Examples
Microsatellites	Short, repetitive sequences of DNA, typically consisting of 1-6 base pairs, that are scattered throughout the genome and are highly polymorphic	Jha & Kremen (2013), Mola *et al.* (2024), Pope *et al.* (2023)
Mitochondrial DNA (mtDNA)	Represents the maternal lineage of a species and can be analyzed either by individual loci or by sequencing the entire genome	Landaverde-González *et al.* (2017), Praz *et al.* (2022)
Restriction-site approaches (*e.g.*, RAD-seq)	Approaches that use restriction enzymes to cut DNA at specific sites, allowing for the sequencing of adjacent regions to identify genetic variations across the genome	Lozier (2014), Jackson *et al.* (2018), Theodorou *et al.* (2018), Pope *et al.* (2023), Samad-Zada *et al.* (2023)
Hybridization approaches (*e.g.*, UCEs, AHEs, RAD capture)	Approaches that involve using probes to enrich and sequence specific regions of the genome selectively, allowing for targeted genetic analysis and comparison across individuals and species	Gueuning *et al.* (2020), Andrade *et al.* (2022), Samad-Zada *et al.* (2023), Sandoval-Arango *et al.* (2023)
Whole genome sequencing	Determines the complete DNA sequence of an organism's genome; depending on the sequencing technology used, its quality and completeness can vary from tens of thousands of scaffolds to chromosomal-level resolution	Lozier *et al.* (2023), Pope *et al.* (2023)
Transcriptomics and other gene expression analyses	Involve the study of the complete set of RNA transcripts, or targeted regions, expressed by organisms under specific conditions	Tsvetkov *et al.* (2021), French *et al.* (2024)

nitrogen immediately after collection to preserve the best quality tissue that allows you to recover most molecular markers. Alternatively, individuals can be brought to the laboratory alive for flash freezing. If the recommended flash-freezing cannot happen immediately, then tissue or whole bees can be maintained in a nucleic acid stabilizer such as RNA*later* (Ambion, Invitrogen), DNA/RNA Shield (Zymo Research), or ATL Buffer (Qiagen). These stabilizers preserve both RNA and DNA without ultra-cold storage, although further testing is needed to understand fully how these stabilizers ultimately influence molecular quality through time (*e.g.*, Passow *et al.*, 2019). When using this approach, collect the bee specimens and immediately place them into the solution, ensuring that the sample is completely submerged to stabilize the RNA and DNA in all tissues of the specimen. Follow manufacturers' protocols for specimen storage specific to the stabilizing solution. For example, when using RNA*later*, allow the specimens to incubate at room temperature (20 to 25 °C) for at least 24 hours; after

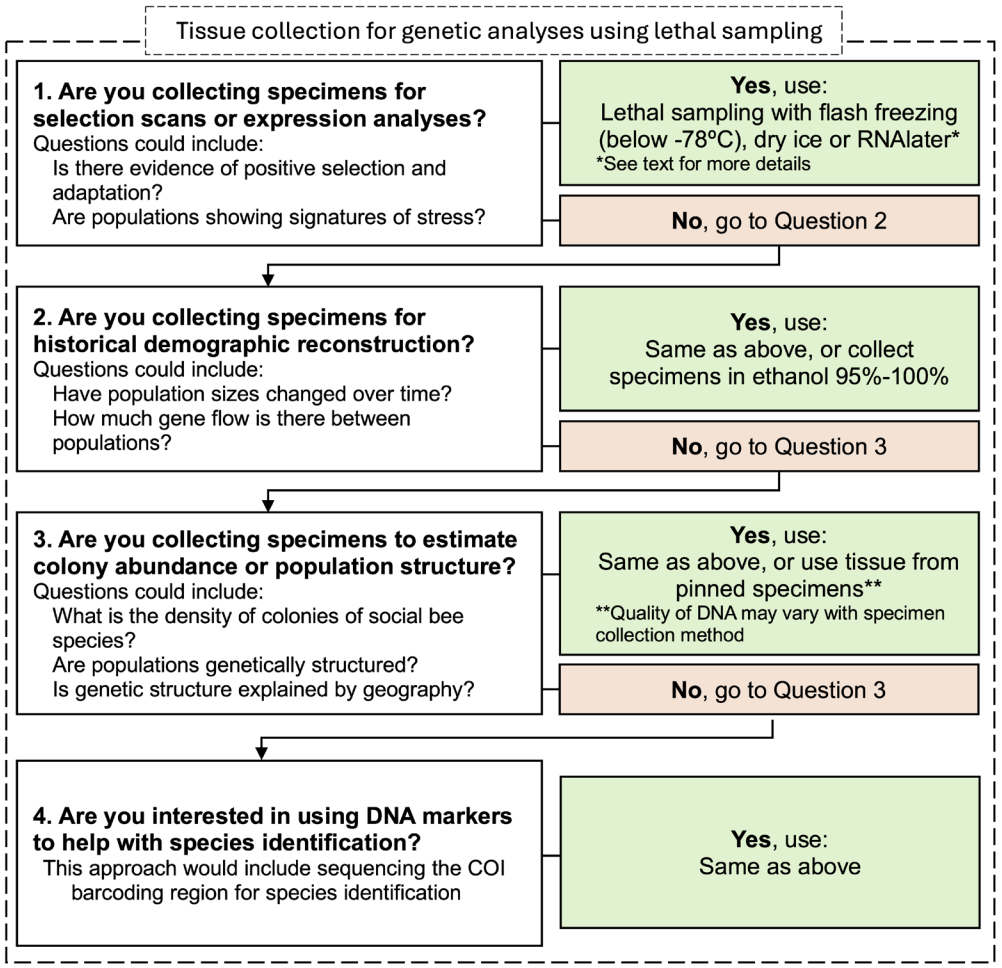

Figure 1. Flowchart guiding the selection of preservation methods based on specific research questions. Note that methods in figure are the minimum effort that can be used for samples intended for a specific purpose.

that, it is recommended to store them at -20 ºC. Specimens can be stored in RNA*later* at room temperature for one week or at -4 ºC for up to one month. Alternatively, tissue or specimens can be preserved in 95–100% ethanol at room temperature for a couple of weeks and then transferred to ultra-cold storage without significantly impacting DNA quality for some applications (Table 2). Note, however, that tissue that is not immediately flash-frozen cannot be used for some molecular analyses, including transcriptomic and proteomic analyses, because RNA and proteins quickly degrade at room temperature unless tissue is preserved in a stabilizing buffer (*e.g.*, RNA*later*).

If you plan to use the samples to generate high-quality genomes (see Case Study III), transcriptomes (Tsvetkov *et al.*, 2021), or proteomes, immediate flash-freezing is necessary for optimal tissue preservation. We also recommend flash-freezing because it minimizes the suffering of the bees collected and, in situations where you are unsure how samples will be used, it preserves high-molecular weight (HMW) DNA that is

Table 2. Summary of tissue preservation methods categorized from best to worst, in terms of quality of the molecules preserved and the type(s) of molecular marker recovered.

Preservation method	Molecule quality	Molecules	Type(s) of marker recovered	Notes
Flash freezing (below -78 ºC)	Best	DNA, RNA, Proteins	All markers	Requires access to liquid nitrogen or dry ice and long-term storage in ultralow freezers
RNA*later*	Very good	DNA, RNA	Microsatellites, mtDNA, genomics, transcriptomics	Less suitable for reference genomes
Ethanol 95–100%	Good	DNA	Microsatellites, mtDNA, genomics	Lower-quality DNA
Ethanol <95% or ethyl acetate	Poor	DNA	Microsatellites, mtDNA, and possibly genomics	Preserves fragmented DNA

usable for any subsequent purpose. It also preserves RNA and proteins and helps maintain DNA structure, which is critical for building reference-quality genomes. In the field, flash-freezing can be performed by placing tubes on dry ice in a cryogenic cooler (~-80 ºC; be sure that tubes come in direct contact with ice) or into an ultra-cold dry shipper charged with liquid nitrogen (-196 ºC).

Samples that will be used for purposes that do not require RNA or higher-quality DNA, such as for the generation of microsatellite data (see Case Study II), ddRAD, ultraconserved elements (UCEs), or whole genome resequencing, can alternatively be stored in a stabilizing liquid such as 95–100% ethanol at room temperature (Marquina *et al.*, 2021) or on ice for an extended period of time (>12 hours) without significant loss of DNA quality. Samples should be stored at -20 ºC or below as soon as possible when using these approaches. Note that repeated freezing and thawing of specimens (or of their extracted DNA) reduces their quality for molecular use (Shao *et al.*, 2012) and should be avoided as much as possible. Other preservatives have also been shown to store high-quality DNA safely (Mulcahy *et al.*, 2016), but these have not been tested extensively in bees.

Passive traps, such as bee bowls, that capture multiple individuals of a species or multiple species may negatively impact the quality of specimens for genetic or genomic studies (Ballare *et al.*, 2019); therefore, we recommend caution when using passive traps for collecting tissue for these types of analyses. We note, however, that the degree of degradation depends on a variety of factors, such as the duration of time until specimens are placed in storage and the preservative used. Additionally, the collection of specimens in propylene glycol or ethyl acetate as a killing agent can result in the degradation of genetic material and thus these substances are not acceptable for use with this protocol (Vaudo *et al.*, 2018). For active collection methods such as netting, cyanide is an acceptable alternative as cyanide-killed bees consistently have higher-quality DNA (inferred from a higher molecular weight) than samples collected in ethyl acetate (Ballare *et al.*, 2019).

A core component of this protocol is that individually captured bees be placed into unique, prelabeled, sterilized tubes for transfer and storage. We recommend using

labeling systems with unique catalog numbers that begin with acronyms of collections or projects followed by consecutive numbers (*e.g.*, USDA-ARS:BBSL0511821 for a specimen that will be deposited in the USDA ARS Bee Lab, Logan, UT, USA). These numbers should be associated with all other metadata required to follow best data management practices (Du Clos *et al.*, 2024a; Du Clos *et al.*, 2024b). When handling specimens, it is recommended to use sterilized forceps, to minimize the accidental transfer of DNA or other materials among samples. It is worth noting, however, that complete sterilization of handling tools may be impractical in some situations. If non-sterilized materials are used, the transfer of contaminant DNA or RNA can be filtered out of data bioinformatically, but it is better to avoid cross-contamination at the time that samples are collected. When possible, we also advise transferring specimens into cryogenic vials, as these are specially designed to withstand ultra-cold conditions (< -80 ºC), and direct exposure to liquid nitrogen, for long-term storage. Regardless of their intended use, once samples are collected and transferred to the laboratory, we advise storage in a -80 ºC freezer. DNA, RNA and proteins are well-preserved at this ultra-cold temperature for long periods of time. Note also that specimens stored in ethanol or RNA*later* may require additional processing if the remaining specimen is to be pin mounted and deposited in a museum collection as a voucher specimen (see Strange *et al.*, 2024 for additional information).

ADDITIONAL BEST PRACTICES

Non-lethal tissue collection should be considered as an alternative to lethal collection when possible (Oi *et al.*, 2013; Scriven *et al.*, 2013). Tissue samples, such as parts of individual legs (Holehouse *et al.*, 2003), hairs (Rongstock *et al.*, 2024), and fecal samples (Scriven *et al.*, 2013), can be used to generate genetic and genomic data (see Case Study IV). Antennal tissue can also be used (Oi *et al.*, 2013) but is not preferred above leg or fecal samples because it may be more likely to impact bee flight and foraging. Overall, additional testing is needed to characterize the effects of non-lethal tissue sampling on bee survival and fitness (Mola *et al.*, 2021). Additionally, most studies that have tested these approaches have focused on bumble bees and orchid bees (Apidae: Euglossini), which are larger-bodied; additional testing may be needed to determine whether enough DNA can be extracted from specific tissue types from other bee species. If the quality and quantity of DNA is not sufficient for the intended application, it may be necessary to implement lethal techniques, but this should be determined on a case-by-case manner. For the implementation of these non-lethal sampling approaches, live bees can be briefly immobilized on ice or with CO_2, tissue can be dissected or removed, and the tissue can be treated and stored in the same ways described above for entire bee specimens. We recommend wiping dissection tools with 70-95% ethanol between specimen handling to avoid transferring DNA (or parasites or pathogens) between specimens, although contaminant DNA can be bioinformatically filtered out from samples if needed. Swabbing specimens or washing plant surfaces (*e.g.*, eDNA) may be another way to sample bees non-lethally and detect the presence of pathogens (see Strange *et al.*, 2024), but these approaches are largely untested and mainly yield very low quality DNA or RNA that has more limited utility (but see Johnson *et al.*, 2023; Newton *et al.*, 2023; Avalos *et al.*, 2024).

Vouchering is important to corroborate the species identity of the samples being analyzed (Monckton *et al.*, 2020). As such, we discuss several ways to voucher specimens properly depending on the collection method. For each of these methods,

we recommend storing extracted DNA as a secondary voucher for determining species identity.

If samples are collected non-lethally (see Case Study IV), photographic vouchers can be used instead. In this case, we advise collecting photographs of the entire individual and diagnostic parts of the body that can be used to confirm identification. If diagnostic characters are uncertain, we recommend taking face, lateral, dorsal, and ventral images as standard.

If specimens are sampled lethally but non-destructively (*e.g.*, a single body part or tissue type is removed and used for molecular analysis, but the remainder of the sampled specimen is maintained), the remaining specimen can become the voucher to confirm species identification (see Case Study II). When possible, vouchers should be pinned and clearly associated with the tissue sample used in molecular analysis through a clear labeling system. Imaging the voucher is also valuable in case of specimen damage.

If the protocols require destructive sampling of entire specimens, we recommend collecting an additional series of individuals of the same putative species, to be maintained as voucher specimens that are representatives of the particular collection event and that can be associated with downstream analysis (see Case Study III). If the species is social, vouchers can be collected by taking individuals from the same nest. Otherwise, vouchers that are putatively the same species should ideally be from the same location and date as the destroyed specimen.

We also encourage the sequencing and deposition of DNA barcodes as molecular vouchers, especially for non-lethal samples. The COI barcode gene is a standard marker for species ID in bees and is likely to be a more useful tool for verifying species than other markers, like microsatellites. There are limitations, however, to the broad applicability of DNA barcodes for species identification including a lack of existing reference libraries and absence of genetic divergence among evolutionary units with clear morphological differentiation (*e.g.*, Janko *et al.*, 2024).

METADATA SPECIFIC TO THIS PROTOCOL

Core metadata to record for samples that will be analyzed for molecular data include information about sample handling conditions prior to final storage, time until final storage, final storage conditions, and final storage location. It is valuable to record this type of information at the time samples are collected. This allows you to know, later, whether they can be used for different molecular analyses, even if these analyses were not originally considered at the time of collection. There are ways to test the quality of samples, such as DNA fragmentation analysis, so that decisions can be made about whether samples can and should be used for different molecular analyses, regardless of whether metadata about sample handling conditions were recorded. We note, however, that it is more efficient to record these metadata at the time of collection, so this information is readily available. We are also making these recommendations so that more formal sampling schemes, such as monitoring programs, are aware of the value of preserving samples for molecular analysis even if they are not originally integrated into the program design, and have the information in-hand for doing so in the most efficient and effective ways possible.

The final storage conditions, particularly the temperature at which the sample is stored, are reported in the Darwin Core protocol field **dwc:preparations**. All other information on how the sample was handled is reported together in the term **dwc:materialEntityRemarks**. Genetic sequence identifiers can be provided in the term **dwc:associatedSequences** (Table 3). When providing multiple pieces of information for one Darwin Core term, separate them with a vertical bar; for example: "sample stored on ice between collection and final storage | 0.5 hour between collection and final storage | stored in the USDA ARS Bee Lab, Logan, UT, USA" is an appropriate entry for **dwc:materialEntityRemarks**. Although it may seem counterintuitive to provide this much text in one spreadsheet cell, adhering to these practices aligns with the Darwin Core standard (Wieczorek *et al.*, 2012; Du Clos *et al.*, 2024a) and promotes data reproducibility and utility. Lastly, please be sure to cite this protocol in **dwc:samplingProtocol**. Full details on using these Darwin Core terms and templates to enter this information into a spreadsheet or workbook are provided with *The Wild Bee Data Standard* (Du Clos *et al.*, 2024a; Du Clos *et al.*, 2024b). Optionally, provide any online repository (*e.g.*, GenBank submission number) or other identifying information for the sequence data generated from the samples.

Table 3. List of core metadata to be recorded from specimens collected for genetic data to adhere to The Wild Bee Data Standard (Du Clos *et al.*, 2024a).

Core Metadata	Description	Darwin Core Term
Sample conditions prior to final storage	Report how the specimen was handled between collection and final storage	dwc:materialEntityRemarks
Time until final storage	Report duration between specimen collection and final storage	dwc:materialEntityRemarks
Final storage conditions	Report how the specimen is stored, particularly the temperature	dwc:preparations
Final storage location	Report where the specimen is stored	dwc:materialEntityRemarks
Genetic sequence identifiers	Provide one or more means to locate genetic sequence information, including publications, URLs, or any other type of unique identifier	dwc:associatedSequences

EXAMPLES OF MOLECULAR METHODS AND MARKERS IN BEE RESEARCH

Case Study I. Demographic History and Signatures of Adaptation of an Agricultural Pollinator

Pope *et al.* (2023) reconstructed the demographic history of the squash bee *Xenoglossa pruinosa* (Say) (formerly *Eucera pruinosa*) (Apidae: Eucerini), which is a pollen specialist and important agricultural pollinator of *Cucurbita* L. (Cucurbitacae)

crops (Fig. 2). This study used a combination of microsatellites, ddRAD markers, and whole genome resequencing to characterize the population structure of the species, reconstruct its historical demography, and investigate signatures of positive selection. For the investigation of population structure, 938 individuals from 26 populations were genotyped for five microsatellite loci, and a subset of 142 individuals were genotyped for >110k SNPs from a ddRAD library. After characterizing population structure, a subset of 44 individuals from the main five lineages of the species were selected for whole genome sequencing (about eight individuals per lineage). This smaller dataset was used for demographic inference using ancestral recombination graphs, and inference of purifying and positive selection. With this hierarchical sampling scheme, the study identified five lineages within the species, a recent superexponential demographic expansion across lineages during the past 2,000 years, and signatures of positive selection in 20% of the protein coding genes.

Case Study II. Colony Density Quantification of an Agricultural Pollinator

To estimate accurately the abundance of social species, it is necessary to calculate the number of colonies instead of using the abundance of individual bees as a proxy. McGrady *et al.* (2021) quantified the number of colonies of the bumble bee *Bombus impatiens* Cresson (Apidae: Bombini), which provide pollination services to cucurbit crops (Fig. 3). To estimate this, ~6,000 individuals were collected from 30 cucurbit fields (with an average of 200 worker bees per field) and genotyped for eleven microsatellite markers. All specimens were collected and pinned, and then a mesothoracic leg was removed from each individual and used for DNA extractions to multiplex microsatellite genotyping. Using sibship analysis reconstruction (Jones & Wang, 2013), which assesses individual specimen relatedness, the study concluded that, on average, workers from an estimated 540 colonies were providing pollination services to each cucurbit field.

Case Study III. Reference Genome of a Specialist Pollinator of Conservation Concern

Schweizer *et al.* (2024) built a high-quality reference genome for *Perdita meconis* Griswold (Andrenidae), commonly known as the Mojave poppy bee (Fig. 4). This desert bee specializes in the pollen collection of plants in the genera *Arctomecon* Torrey & Frémont and *Argemone* L. (Papaveraceae). The primary host plant for this bee in Utah (USA) is *Arctomecon humilis* Coville, which is a federally protected plant under the U.S. Endangered Species Act. As a result of habitat loss and the decline of the host plant, *P. meconis* is also a species of conservation concern. The development of a reference genome for *P. meconis*, combined with resequencing data, will facilitate an understanding of the levels of genetic diversity, genetic structure, and isolation of the remaining populations of this species of conservation concern. To collect specimens for this study, the team sampled a small series of individuals of this bee and kept them chilled but alive while transported to the lab. The bees were warmed up to keep a photographic record of the specimens. After that, specimens were kept in a -20 ºC freezer. DNA was successfully extracted from a single male individual (whole-body) despite its small body size, which ranges between 5 to 7 mm in length. Despite the lack of flash freezing after collections, the quality of this genome was high with a N50 of

17.5 and a BUSCO score of 95.5% for the Hymenoptera genes. A reference genome was successfully generated and annotated from the specimen.

Case Study IV. Population Structure and Inbreeding of an Endangered Bee Pollinator

Bombus affinis Cresson (Apidae: Bombini), commonly known as the rusty patched bumble bee, was the first Federally Endangered bee species in the continental United States (Fig. 5). This species began declining in abundance during the late 1990s and has lost an estimated ~70% to 90% of its historical range. Mola *et al.* (2024) investigated the population structure and patterns of extant genetic diversity in this Federally Endangered species. To avoid lethally collecting specimens of this endangered species, the team opportunistically collected tarsal tissue from 470 individuals from 59 sites that corresponded to 13 extant populations. A total of 15 microsatellite loci were amplified in two multiplex sets. The study identified three main genetic clusters that are now being distinctly prioritized for conservation. The estimated number of colonies per site varied between 4 and 70, indicating that the abundance of this species is significantly lower than the observed abundance of common species such as *B. impatiens* (see Case Study II).

Figures 2–5. Bees featured in case studies using molecular methods and markers. **2.** Squash bee, *Xenoglossa pruinosa* (Say). Photo credit: Nash Turley. **3.** Common eastern bumble bee, *Bombus impatiens* Cresson. Photo credit: Laura Jones. **4.** Mojave poppy bee, *Perdita meconis* Griswold. Photo credit: Collen Meidt. **5.** Rusty patched bumble bee, *Bombus affinis* Cresson. Photo credit: Clay Bolt.

DISCUSSION

Although the preservation of tissue for molecular data analyses is beyond the current scope of many wild bee monitoring efforts, it has been proposed as a critical component of more comprehensive future monitoring (van Klink *et al.*, 2022). Outside of monitoring, genetic, genomic, and other molecular approaches are also routinely used in bee research, but to the best of our knowledge, there are no standardized methods for these approaches that are available for the wild bee research community. Standardized protocols and data standards will help to ensure that samples are collected and stewarded properly for subsequent use for molecular analyses. This protocol is especially timely because technology is continuing to advance, resulting in improved molecular capabilities, while also reducing its cost. With these advances, members of the bee research, monitoring, and conservation communities will likely become increasingly interested in adding these types of analyses to their work. Thus, it is critical to develop standards for how tissue is collected, processed, and stored to ensure high-quality genetic, genomic, transcriptomic and other types of molecular data.

Molecular approaches not only aid in understanding historical population dynamics but also provide insights into the evolutionary responses of species to past environmental changes (*e.g.*, López-Uribe *et al.*, 2014; Černá *et al.*, 2017). As such, these methods are invaluable for guiding current and future conservation strategies, ensuring the preservation of biodiversity in the face of ongoing environmental challenges. Traditionally, genetic data have been used to address critical conservation issues such as effective population sizes (Lozier *et al.*, 2023), adaptive processes (Theodorou *et al.*, 2018; Pope *et al.*, 2023), and levels of genetic diversity and inbreeding (López-Uribe *et al.*, 2019; Mola *et al.*, 2024); issues that would otherwise be unanswerable. Genomic and transcriptomic tools are particularly valuable in assessing the recent demographic history of declining and endangered bee species (Kent *et al.*, 2018; Mola *et al.*, 2024). One of the most exciting avenues in this field is the development of protocols that use tissue from pinned museum specimens (Grewe *et al.*, 2021; Brasil *et al.*, 2023; Samad-Zada & Rehan, 2023). Advances in sequencing technologies are enabling the extraction of valuable genetic information from even the smallest and oldest specimens, further expanding the scope of genetic studies (Blaimer *et al.*, 2016). The proper storage of tissue for genetic analysis will open the potential to answer future questions not yet imagined (Nachman *et al.*, 2023) and aid efforts like the Earth Biogenome Project (Lewin *et al.*, 2018), which aims to sequence genomes for all species on Earth.

Molecular approaches such as megabarcoding, DNA metabarcoding, and environmental DNA (eDNA) are promising for advancing insect monitoring, limiting lethal collection, and overcoming the bottleneck of taxonomic identification (Piper *et al.*, 2019; Roger *et al.*, 2022; Avalos *et al.*, 2024). DNA megabarcoding relies on the ability to generate molecular data for a large number of samples quickly and cheaply and to use the data to sort specimens into species more quickly than traditional approaches (Caterino & Recuero, 2023). DNA metabarcoding technology allows researchers to pool hundreds of individuals for targeted sequencing of one genomic region (*e.g.*, the barcode region of the cytochrome oxidase I (COI) gene), thus it can be integrated into already ongoing monitoring efforts to facilitate species identification. eDNA approaches aim to detect the presence of DNA from focal taxa in air, soil, or water. Several recent studies have shown that bee DNA deposited on flowers can be extracted

and species reliably identified from the DNA (Thomsen & Sigsgaard, 2019; Harper *et al.*, 2023). How these approaches compare to traditional monitoring efforts is an area of active research, but eDNA approaches show promise for the discovery and monitoring of biodiversity including plant-bee interactions (Ruppert *et al.*, 2019). Additionally, transcriptomes offer valuable insights into gene expression patterns in response to environmental stressors, providing a deeper understanding of mechanisms underlying stress and drivers of insect decline (Tsvetkov *et al.*, 2021). These tools collectively enhance our ability to monitor bee populations more efficiently and accurately, leading to more informed conservation decisions. The integration of these advanced molecular techniques into traditional monitoring frameworks holds great promise for addressing the challenges of biodiversity loss and ecosystem management.

We close with the argument that once the integrity of specimens for molecular analyses is compromised, it is not possible to go back in time to recover it. Therefore, we encourage the wild bee monitoring community to use a forward-thinking approach and consider making, when possible, the additional effort needed to maximize the uses of their collected specimens in the future. Although insect collections are undeniably extremely valuable for conservation, dry specimens collected using traditional approaches have limited use for some of the approaches discussed in this protocol. We argue that the value of molecular data that can be extracted from specimens in the future is worth the extra time and effort associated with the preservation of tissue for genetic markers, particularly for formal monitoring approaches that rely on lethal sampling. Lastly, if storage space or cryogenic facilities are limiting factors, consider reaching out to other researchers or museums who may specifically maintain repositories of genetic material. We hope this protocol will motivate the community to implement these approaches and move forward with bee monitoring efforts in a more holistic manner.

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