Comparison of storage conditions of bee collected pollen: impact on botanical identification as determined by ITS2 metabarcoding

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Introduction

To understanding the floral environment around an area it is crucial to study pollen collected by honey bees (*Apis mellifera*)[1], which contains information on important pollen sources, biodiversity of different landscapes, seasonal variation, or the development and health of adult bees[2,3]. For that reason, the INSIGNIA project (https://www.insignia-bee.eu/) has relied on the collaboration of Citizen Scientists (CSs) to develop an European environmental study by using bee colonies for pesticide monitoring. CSs around Europe collected a large number of pollen samples, which is helping answering scientific questions on a wide temporal and geographical scales that would otherwise be difficult to address. To facilitate storage at the CS sites of a large number of pollen samples collected across the bee season, while at the same time assuring sample integrity for downstream molecular analyses, here we compared four different storage methods, having in mind simplifying future Citizen Science projects by finding cheaper and easier methods for long-term storage of pollen samples.

Results

A total of 15 plant families and 25 species was detected in the pollen samples collected in Austria and 16 and 33 in Denmark. The three most abundant families represented 62.4% ± 9.1% (mean ± SD; calculated across storage methods and sampling dates) of the total abundance in Austria and 56.3% ± 3.9% in Denmark (Fig. 2).

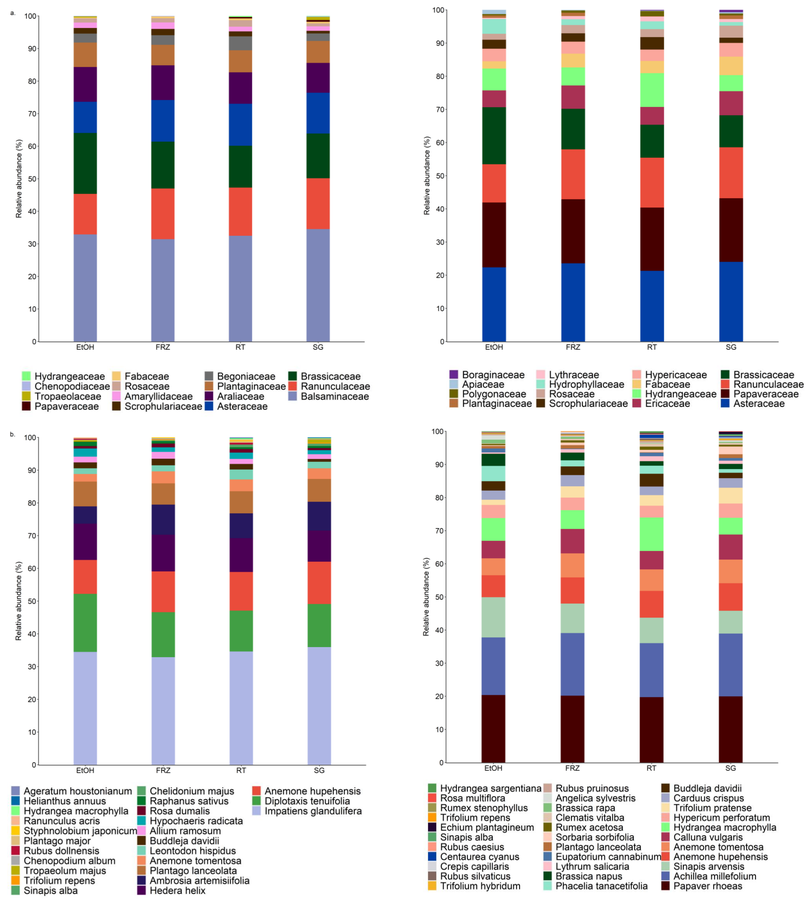

Fig. 4. Mean relative abundances (%) estimated for (a) families and (b) species from classifying sequence reads obtained by ITS2 metabarcoding. Relative abundances were estimated from pollen samples collected in Austria (left) and Denmark (right) and stored in ethanol (EtOH), frozen at -20° C (FRZ), at room temperature (RT), and with 12 g of silica gel (SG).

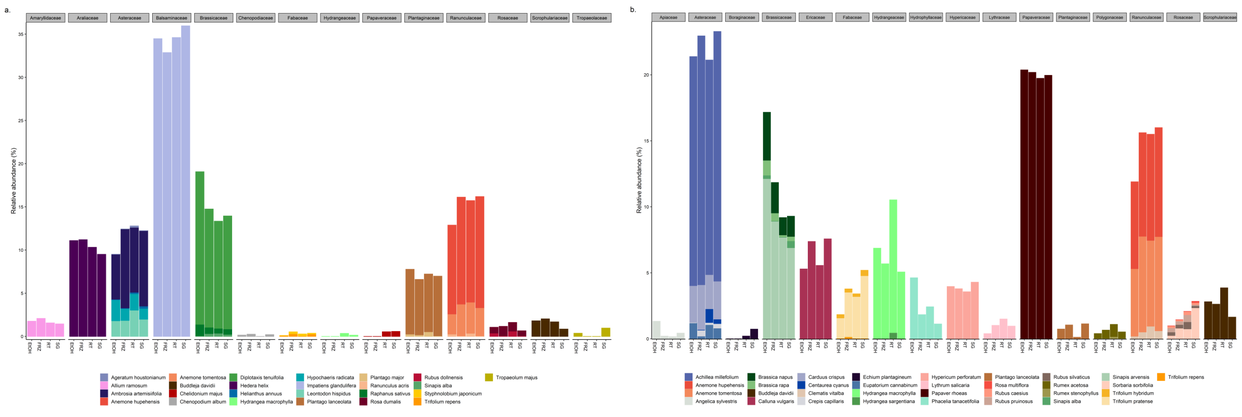

Fig. 2: Mean relative abundances (%) for pollen samples collected in (a) Austria and (b) Denmark and stored in ethanol (EtOH), frozen at -20° C (FRZ) and with 12 g of silica gel (SG). Relative abundances, shown here for species and families, were inferred from sequence reads obtained by ITS2 metabarcoding. In Austria, Chenopodiaceae was only detected in 05/09/20, Hydrangeaceae 10/09/20, Papavaraceae and Tropaeolaceae in 15/09/20.

From the 22 families retrieved, 19 (11 in Austria and 15 in Denmark; Fig. 3) were detected across storage methods, and of the total 51 species, 35 (13 in Austria and 25 in Denmark) were shared amongst storage methods. Unexpectedly, the highest number of species (47) was identified in pollen samples kept at room temperature, with three rare species (relative abundance < 0.19%) uniquely assigned to this storage method (Fig. 3).

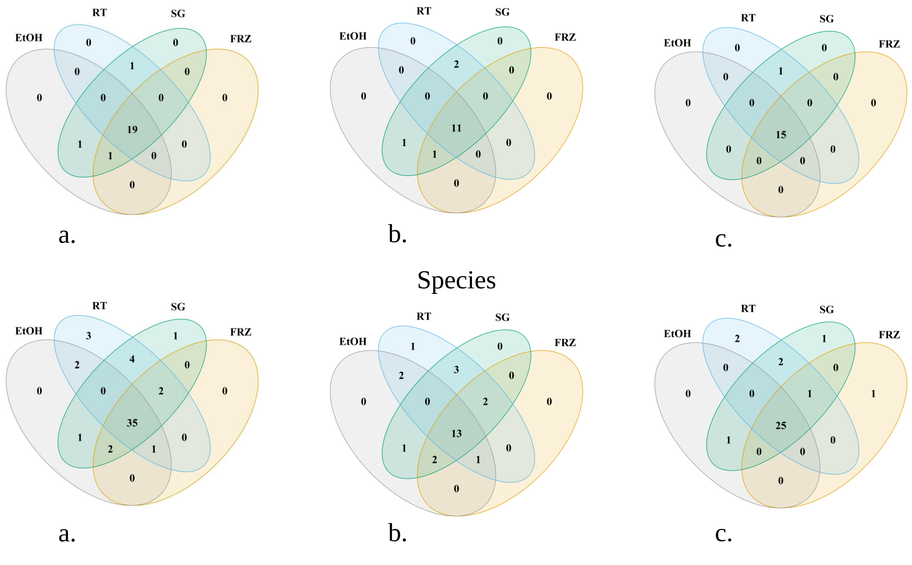

Fig. 3: Venn diagrams representing the number of families and species shared between storage methods for (a) total samples, (b) Austrian samples, and (c) Danish samples.

Conclusions

Overall, botanical identification and relative abundances of the 87 samples suggest that the methods involving desiccation, which are cheaper than ethanol and freezing, can be used by the CS for long-term pollen storage for applications involving DNA metabarcoding. Given that relative humidity at room temperature may vary greatly across countries and seasons, and one sample dried at room temperature was lost due to wax moth infestation, we recommend using the silica storage method. The method is also straightforward for CS to use in practice, and therefore is robust for widespread use in Citizen Science studies.

Methodology

Pollen samples were collected from traps placed in front of beehives in Denmark (N= 40) and Austria(N=47), homogenized and then split into replicates of 5 g each (Fig. 5). The storage methods consisted of:

1. **ethanol:** pollen was immersed in 96% ethanol and stored at room temperature;
2. **silica:** pollen was placed inside a porous tea bag and stored with 12 g of silica;
3. **room temperature:** pollen was placed on a fine gauze/filter-paper and dried at room temperature for one week and then was placed in vials and stored at room temperature;
4. **frozen:** pollen was stored at -20 ºC soon after sampling.

All the samples, apart from the frozen ones, were stored at room temperature in the dark for > 3 months until DNA extraction.

Botanical identification of the 87 samples was performed by DNA metabarcoding using high-throughput sequencing (HTS) with the nuclear barcoding marker ITS2 (internal transcribed spacer 2 regions of nuclear ribosomal DNA). DNA was extracted from ~50 mg of pollen using the NucleoSpin Food Kit (Macherey-Nagel), according to manufacturer's instructions. DNA extracts were PCR-amplified using the universal primers ITS-S2F and ITS-S4R[4], as part of an oligo scaffold that incorporates the MiSeq-specific adapters and the indexes. Library preparation for HTS was performed using a dual-indexing approach. The pollen samples were sequenced on the Illumina MiSeq platform using 2×250 cycles v2 chemistry. Analysis of sequence reads and taxa assignments were performed using an updated ITS2 reference database.

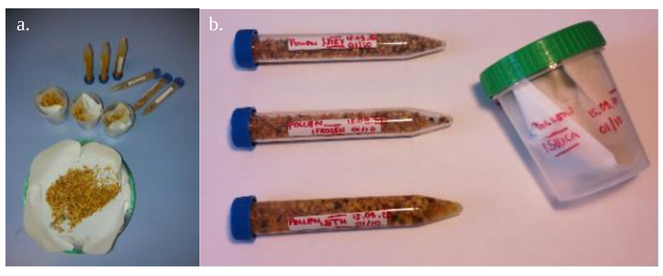

Fig. 5: Different pollen storage methods. (a) Pollen drying at room temperature in a Petri dish, preparation for silica gel drying in three 125 mL vials, three samples in ethanol and in 15 mL tubes for freezing. (b) Final appearance of sample replicates for analysis (from top): pollen dried at room temperature, frozen pollen sample, pollen in ethanol and, in the right, silica-dried pollen sample.

References

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