QUANTIFICATION OF SINGLE-COPY NUCLEAR REGION FOR THE IDENTIFICATION OF POLLEN IN HONEY

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Objective

To develop a simple and cheap genomic testing method to quantify the DNA from the pollen of Jarrah *(E.marginata)* and Marri *(C.calophylla)* in varietal honey.

Project Overview

Validation of the varietal content of honey is traditionally performed by palynology, a difficult, specialised process of identifying species-specific pollens in honey by microscopy. We aimed to develop a DNA test to complement this process. We believe, using methods that target DNA Barcodes; short genetic markers that identify an organism as belonging to a particular species, we can create quantitative Polymerase Chain Reaction (qPCR) assays for both unique species and total plant DNA in honey. These two assays will be used to provide a relative quantity of the specific species in the honey. We had to first develop a generic plant assay from a highly conserved region of DNA that would allow amplification in all plant species used for pollen collection by bees. Selecting a conserved DNA region is difficult due to the intrinsic nature of plant species to spontaneously duplicate or delete genes, or to duplicate their entire genome. After a substantial literature search, Topoisomerase 6(TOPO6), was found to be a potential candidate. The region chosen had the properties of a low-copy nuclear gene with highly conserved regions of both sequence and sequence length.

Materials and Methods

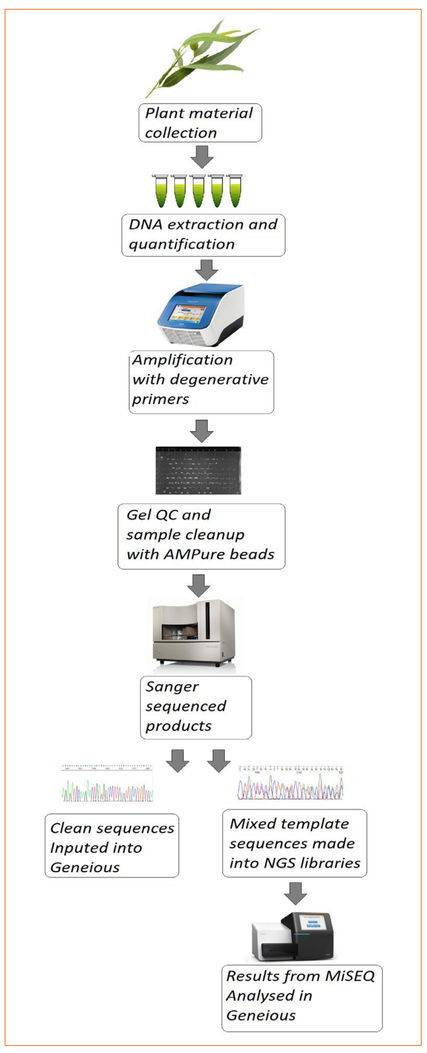

Figure 1. Method Diagram

Three sets of degenerative primers were designed from data generated by Blattner (2016), with a length criteria of about 200bp, a fragment of appropriate size for future use in a qPCR method.

Primers were tested across several plant and honey DNAs. One of the three regions, T6_9Fb10R, produced consistently successful amplifications.

Amplicons for the T6_9Fb10R region were sequenced by SANGER sequencing across a library of DNAs isolated from plants collected from honey collection regions these included samples of Marri and Jarrah.

Amplicons generated from samples with mixed template DNA were analysed by Next Generation Sequencing on an Illumina Miseq.

Sequence data was analysed and a phylogenetic tree was generated in GENEIOUS Version 6.

Results and Discussion

A total of 45 unique sequences of TOP06 fragment T6_9Fb10R were generated from 64 plant DNAs. Analysis of these sequences showed the T6_9Fb10R region was variable between plant species and that the similarity of sequence was greater within families, as shown in the phylogenetic tree below (Figure 2). The size variation(185bp-399bp) of the region between species was small enough to allow the amplicon to be used in a qPCR assay. Analysis of amplification reactions with mixed sequences by NGS showed the sample species content could be determined by the variations of the T6_9Fb10R region and that the region showed multiple alleles in the same plant.

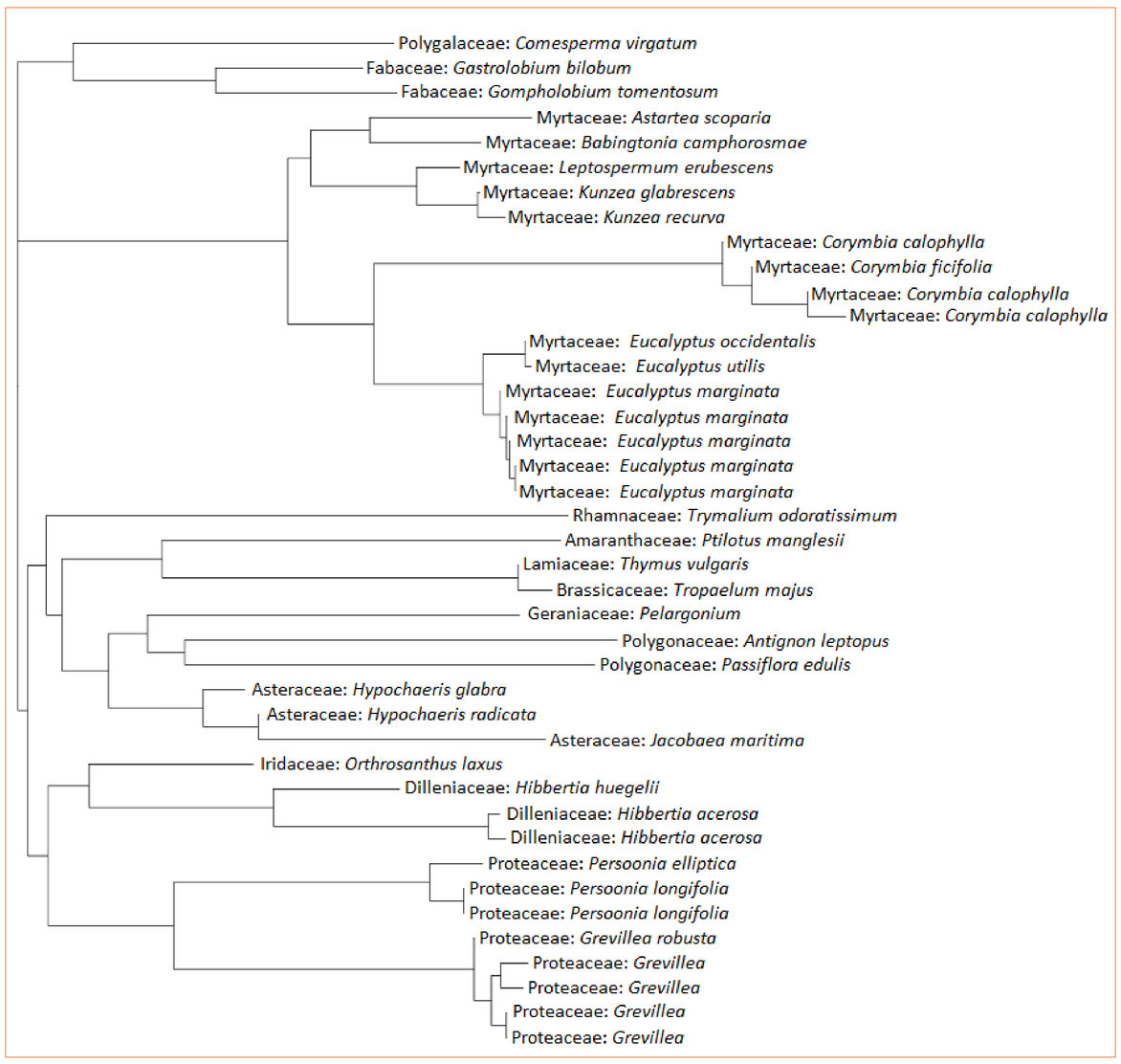

Figure 2. Phylogenetic tree of T6_9Fb10R region in Honey collection area species

Future work

The next stage is the work up of the qPCR assay in order to quantify total plant DNA within a sample. This includes development of a total plant DNA standard and reference standard from pure species DNA. In parallel we will build a database of sequences from several plant species across multiple families, including Marri and Jarrah using Genotyping by Sequencing methodology which should allow us to identify regions for these plants for the species-specific qPCR assays. (In progress)

Acknowledgements

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References

Blattner F. (2016) TOPO6: a nuclear single-copy gene for plant phylogenetic inference. Plant Systematics and Evolution 302:239–244.

Hochback A., Schneider J., Röser M. (2015) A multi-locus analysis of phylogenetic relationships within grass subfamily Pooideae (Poaceae) inferred from sequences of nuclear single copy gene regions compared with plastid DNA. Molecular Phylogenetics and Evolution 87: 14-27.