Genomic Tools to Enable Epidemiological Monitoring of Eukaryotic Pests

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BACKGROUND AND RATIONALE

- Epidemiological comparative genomics techniques are more difficult to deploy on the larger more complex genomes of eukaryotic organisms.
- Larger scale single nucleotide polymorphism (SNP) typing on Next Generation Sequencing (NGS) platforms have the potential to improve the discriminatory power when differentiating between individuals (Reesnas 2016).
- Vespa velutina (Asian hornet) and Møllegæne (false bumble bees) are pest species that are of particular interest in the world of plant health.
- Asian hornets are non-native pest species to the UK and consume native pollinators such as honeybees. There have been 23 confirmed sightings since 2014 (June 2020).
- M. [false bee] cause external galing and internal necrosis on crops such as potatoes, carrots and leeks affecting the yield, marketability, and quality of the crops (EXFRA 2021).

AIMS AND OBJECTIVES

The main aims of the project is to develop genotyping panels for Møllegæne fallax and Vespa velutina that can be used on an outbreak and incursion:
- Identification of highly informative single nucleotide polymorphism (SNP) panels in both study species.
- Design primers to optimise highly multiplex PCR as part of genotyping in thousands sequencing (GT-Seq) assays.
- How rapidly can a generic GT-Seq assay workflow be applied to other species.

METHODOLOGY

- Comparison of sequencing technologies for suitability for the project, e.g., Oxford Nanopore, Pacific and Illumina. Genome assembly and analysis of various sequencing data samples of Møllegæne fallax and Vespa velutina.
- Identification of single nucleotide polymorphism (SNP) panels to establish robustness and specificity of outbreaks and incursions.
- Design primers to optimise highly multiplex PCR as part of the GT-Seq assay and apply assay to samples of V. velutina and M. fallax.
- Extract DNA and sequence amplicons from wax moth larvae or incursions.
- Apply the GT-Seq assay methodology to any other genotypes of interest to plant health to assess the generalisability of the techniques, e.g., Møllegæne Chinese (zebra bee) of M. fallax.

REFERENCES

Background and Rationale

• Epidemiological comparative genomic techniques are more difficult to deploy on the larger more complex genomes of eukaryotic organisms
• Larger scale single nucleotide polymorphism (SNP) typing on Next Generation Sequencing (NGS) platforms have the potential to improve the discriminatory power when differentiating between individuals (Andrews 2018)
• *Vespa velutina* (Asian hornet) and *Meloidogyne fallax* (false Columbia root knot nematode) are pest species that are of particular interest in the world of plant health
Background and Rationale

- Asian hornets are non-native pest species to the UK and consume native pollinators such as honeybees. There have been 23 confirmed sightings since 2016 (Jones 2020)
- *M. fallax* cause external galling and internal necrosis on crops such as potatoes, carrots and leaks affecting the yield, marketability, and quality of the crops (DEFRA 2021)
Aims and Objectives

The main aim of the project is to develop genotyping panels for *Meloidogyne fallax* and *Vespa velutina* that can be used on outbreaks and incursions:

- Identification of highly informative single nucleotide polymorphism (SNP) panels in both study species
- Design primers to optimise highly multiplex PCR as part of genotyping in thousands sequencing (GT-seq) assays
- How rapidly can a generalisable GT-seq assay workflow be applied to other species

![Figure 4. A schematic representation of single nucleotide polymorphisms (SNPs) (University of Utah, 2023)
Methodology

- Comparison of sequencing technologies for suitability for the project, e.g., Oxford Nanopore, PacBio and Illumina.
- Genome assembly and analysis of various resequencing data samples of *Meloidogyne fallax* and *Vespa velutina*.
- Identification of single nucleotide polymorphism (SNP) panels to establish relatedness and origins of outbreaks and incursions.
Methodology

• Design primers to optimise highly multiplex PCR as a part of the GT-seq assay and apply assay to samples of *V. velutina* and *M. fallax*
• Extract DNA and sequence any samples from new outbreaks or incursions
• Apply the GT-seq assay methodology to any other pest species of interest to plant health to assess the generalisability of the technique, e.g., *Meloidogyne chitwoodi* (close relative of *M. fallax*)
References


- University of Utah (2023). Making SNPs Make Sense
Thank You for Listening

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Original thinking... applied