

# 2020 FLYSTRIKE PREVENTION RD&E PROGRAM PROJECT SUMMARY REPORT

AWI PROJECT NO: ON-00624

## INFORMED DEVELOPMENT OF A BLOWFLY VACCINE

### AUTHORS

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### SUMMARY

This research project (2019-2022) focuses on the genetic analysis of Australian sheep blowfly populations across the country. Results will provide critical information to support efforts in identifying and assessing potential blowfly larval proteins for their development as flystrike vaccine candidates. We are assisting CSIRO researchers (ON-00619) to analyse their potential vaccine candidates while also examining our latest results for novel larval vaccine candidates. In addition to vaccine development, results from this study will provide data for cost-benefit assessments of various flystrike control strategies and could also assist with planning their implementation. The genomic analysis of flies from around Australia will be scanned for the presence of genetic changes that could be associated with insecticide resistance against several of the current chemical classes used to treat flystrike.

### PROJECT REPORT

Current blowfly control relies heavily on surgical and chemical treatments. A desire to develop novel options that could replace mulesing and address the risk of insecticide resistance requires a detailed understanding of the fly's biology and an in-depth analysis of its population genetics.

Over the four years of this project we are focussing on two main areas:

1. Using genetic and genomic data to determine the structure of blowfly populations in Australia and assess the level of genetic variation that exists within and between the locations from where they are collected.
2. Understanding the molecular processes that allow the maggots to survive on sheep.

The genomic data will contribute to several different areas related to blowfly control. Knowledge gained from both the population and molecular analyses will be used to identify proteins which have the potential to be developed as a flystrike vaccine. Some of our research is already being used, in collaboration with CSIRO, to help refine the selection and prioritisation of vaccine candidate proteins for vaccine trials (see report on project ON-00619 Vaccine for Control of Flystrike).

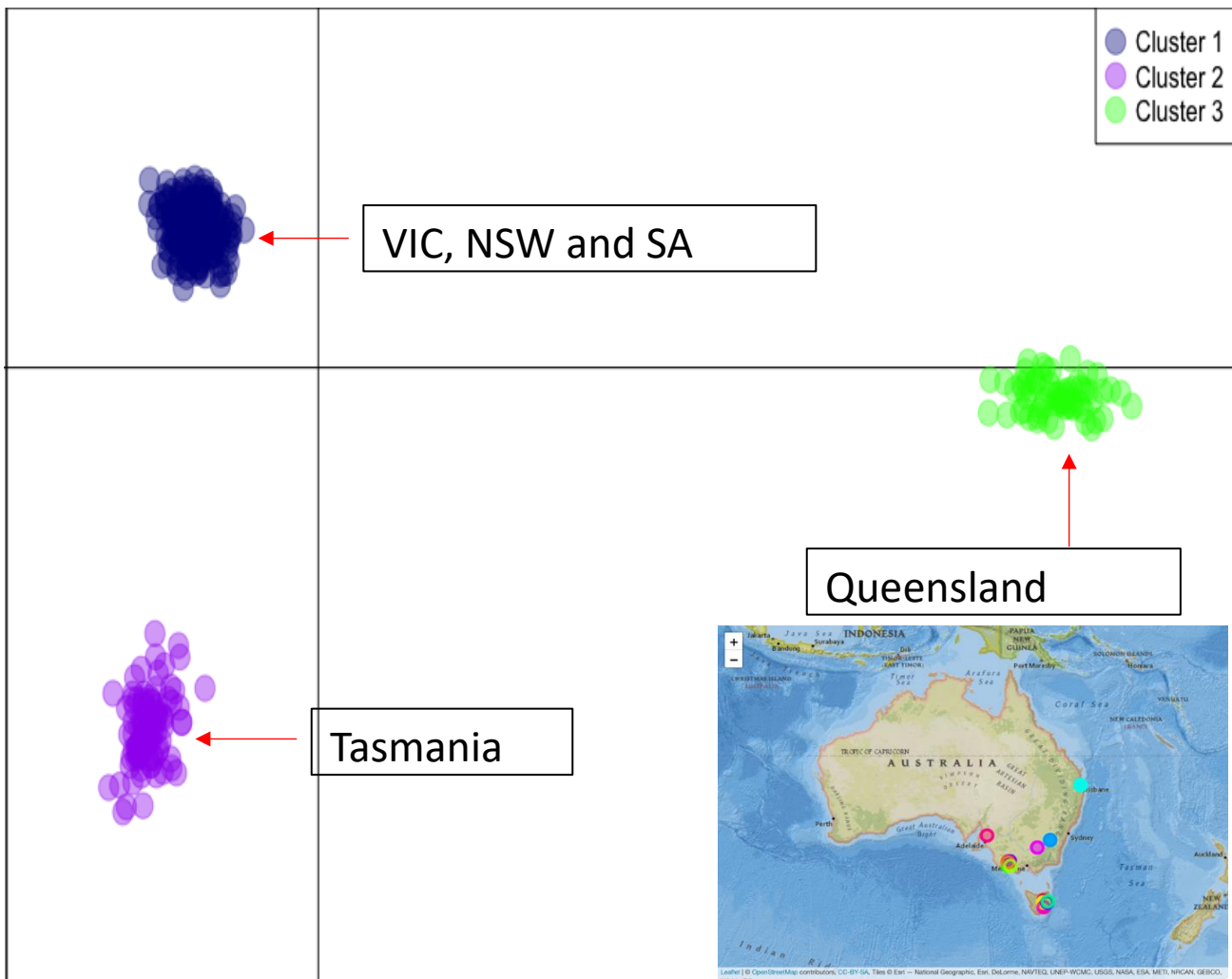
The genomic data will also provide critical information about fly movement around the country which will subsequently help to predict the spread of insecticide resistance outbreaks and assist in informing the development of regional resistance management plans. We are also mining the sequencing results for the presence of insecticide resistance alleles in fly samples. This could provide an early warning of where insecticide resistance may be an issue in the future.



**Figure 1. Dr Anstead out and about delivering and setting up blowfly traps for the 2019/20 season in Western Australia.**

Our first aim to *determine the structure of blowfly populations in Australia and assess the level of existing genetic variation* has progressed well; trapping was conducted over 62 sites in the initial collection season. Flies were collected from properties in QLD, SA, TAS, NSW and VIC and constituted the first of three rounds of collections. The results from the analysis of the initial dataset were very encouraging and genetic markers were identified that will be used to distinguish fly populations from different regions. There were clear distinctions between QLD, TAS and other regions and preliminary analysis suggests that we are also detecting the migration of flies between some regions of VIC, SA and NSW. Future sampling will provide the resolution needed to develop our blowfly population models. There are not enough samples at this stage to

make precise conclusions; however, with the population sampling just completed in the 2019/20 and more to be made in the 2020/21 season, we will be able to provide a clear direction on whether populations are distinct, and which populations are isolated from each other, which has implications for management of resistance outbreaks.



**Figure 2. Discriminant Analysis of Principal Components (DAPC) plot with the blowfly collection sites. Populations of blowflies are clearly divided into three distinct clusters and this type of analysis will help us look at dispersal, gene flow and admixture between the populations in each cluster.**

As part of our collaboration with CSIRO, our genetic data is being applied to help prioritise their vaccine candidate antigens and is also being used in UoM's efforts to identify new potential vaccine candidate genes. We use the genomic data from pooled blowfly samples to check for genetic differences present in different sample areas in order to identify which larval proteins would be best selected for a vaccine that would be effective against all sheep blowfly populations across Australia.

Following ethics approval in the first half of 2019, significant progress towards our second objective, *understanding the molecular processes that allow the maggots to survive on sheep*, has been made. An *in vivo* sheep implant study was conducted in June and July 2019. Blood serum, larval tissue, larval excretions and sheep wound exudate samples were collected. Wound biopsy samples were also collected for subsequent analysis. Proteomic analysis of larval excretion samples has since been conducted on several samples to identify the proteins excreted by maggots during the early stages of wound initiation. We have identified several hundred maggot peptides present in these samples and have been matching these to our data on gene expression that was obtained during the ON-00373 project. Further analysis later this year will continue to build a detailed picture of the early phase of flystrike which will assist in refining our candidate selection and help understand the biological pathways that are involved.

Regular face-to-face meetings with the research team at CSIRO led by Dr Tony Vuocolo (pre-COVID19) have been held since the initiation of the two projects. Both groups have been working together on evaluating and refining the candidate selection and our data has been used to provide input into the results from trials currently being run by CSIRO. We will be continuing to work together in 2020 to further refine the list of proteins that have the potential to be developed as vaccine candidates.

We welcomed Dr Shilpa Kapoor to the research team in September (2019). Dr Kapoor brings highly relevant experience in parasitology, next-generation sequencing and population genetics to the project team. Since joining the UoM team, Shilpa has made an excellent contribution in analysing the 2019 population genomic data, as well as beginning to establish some of the bioinformatics analysis workflows that will be used later in the project. Field sampling of blowflies from the 2019/20 season have also been completed. Hundreds of flies from across the country have been identified and are about to undergo variant analysis. The new data will be integrated with our initial samples to help us build our blowfly population models over the next 12 months.

#### **WHERE TO NEXT/NEXT STEPS**

In the period ahead we will be focussing on our research and look forward to continuing to collaborate with CSIRO to support their vaccine trials. We have been fortunate with timing in that our collection of flies was close to complete prior to the disruption of COVID19 and we have been able to continue our analysis. Our final blowfly collection is planned for September this year and we will again be looking forward to working with growers to assist in obtaining samples from across the country, particularly in areas we have not previously trapped flies. We have greatly appreciated the support and efforts of all of the growers in providing samples for this study.

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