

2020 FLYSTRIKE PREVENTION RD&E PROGRAM PROJECT SUMMARY REPORT

AWI PROJECT NOS: ON-00373 & ON-00516

GENETICS OF BLOWFLY PARASITISM & CRISPR - PHASE 2

AUTHORS

Dr Trent Perry and Professor Philip Batterham, School of BioSciences, Level 4, Bio21 Institute, 30 Flemington Road, The University of Melbourne, VIC 3010

Dr Clare Anstead and Associate Professor Vern Bowles, School of Veterinary Sciences, Cnr Flemington Rd & Park Drive, Building 400, The University of Melbourne, VIC 3010



SUMMARY

Understanding the biology of the Australian sheep blowfly is critical to developing new and effective strategies for its control. The unique parasitic lifecycle of the blowfly means that insect model organisms currently available for study using sophisticated genetic techniques are not necessarily going to provide results about gene function relevant to the blowfly.

The research project, **ON-00373 Genetics of Blowfly Parasitism**, aimed to improve on the genomics resources available in *Lucilia cuprina* (2015-2018) because, while the initial genome was of a high quality when released, new sequencing technologies could be used to improve the *L. cuprina* genome further. This would assist researchers to correctly identify genes, and gene families and provide a blueprint for researchers to analyse the function of genes. To perform such functional analyses, researchers required a way to manipulate genes in the blowfly. A parallel project, **ON-00516 CRISPR, (2018-2019)** used some of the improved genomic resources to develop a method that would efficiently allow researchers to edit the blowfly genome and facilitate the analysis of genes that could help in the search for new blowfly control options.

PROJECT REPORT

In order to understand the biology underlying blowfly parasitism and to assist researchers in their efforts to identify weaknesses in the blowfly that could be targeted to exert control, an improved genomic blueprint and enhanced genetic tools were required. There has been a huge improvement in the technologies available to sequence and assemble genomes and we used one of these to create a higher quality *L. cuprina* genome assembly for researchers to work with. Prior to this project the methods available to investigate the biology of the blowfly and to understand the genes and proteins critical for their capacity to parasitize sheep lacked power. Clustered random interspaced short palindromic repeat (CRISPR) genome editing technology had proved to be a highly efficient and effective strategy for engineering a variety of mutations into genes and was being utilised in several insect pests. Given that no options were available for generating mutations in genes of interest in blowflies, developing CRISPR for use in the blowfly was seen as a priority.

OUTCOME

The major aim for the **ON-00373 Genetics of Blowfly Parasitism** was to assemble and annotate an improved genome. Genome sequencing included use of a new sequencing and assembly technology, Genomics Chicago® library preparation with HiRise™ genome assembly, with one of the initial developers of the method, Dovetail™ Genomics. The table included below outlines the features of the new genome compared to the initial genome published in 2015. The average length of DNA scaffolds is far higher for Freeze 2. This means that the full genome sequence is represented in far fewer large scaffolds, rather than many small fragments. This improved continuity of the genome sequence assists with annotation of complete genes as well as providing information in regards to gene order. In this context, note the N50 and N90 values which show the number of scaffolds that include 50% and 90% of the complete genome assembly, respectively. BUSCO = Benchmarking Universal Single-Copy Orthologues and is a set of genes that is used as a measure of genome quality/completeness and we again see that in the new Freeze 2 genome there are more complete genes and less fragmented or missing genes.

Table 1. Features of the new genome compared to the initial genome published in 2015.

Features of the Genome assemblies	2015 genome (458 Mb)	Freeze 2 genome (465 Mb)
N50 length (bp); Scaffolds required to reach 50% coverage	744,413bp; 165 Scaffolds	6,922,854bp; 18 Scaffolds
N90 length (bp); Scaffolds required to reach 90% coverage	126,471bp; 736 Scaffolds	1,321,550bp; 83 Scaffolds
BUSCO complete genes;	2594;	2704;
BUSCO fragmented genes;	52;	47;
BUSCO missing genes	153	48

N50 = 50% of the entire assembly is contained in contigs or scaffolds equal to or larger than this value

Following assembly of the Freeze 2 genome it was annotated to identify genes on the scaffolds. The number of predicted genes had decreased to 12,933 genes (Table 2). The number of genes without orthologues (counterparts in other species), i.e. genes considered to be unique to the blowfly, has also decreased substantially. This is mainly due to the large number of new insect genomes sequenced since the first release of the blowfly genome in 2015.

Table 2. Gene prediction comparison between the 2015 genome and 2018 genome (Freeze 2).

Gene Prediction Comparison	2015 Genome (14,544 genes)	2018 Genome (12,933 genes)
Single-copy orthologues (4 spp.)	4,106 genes	4,425 genes
Single-copy orthologues (1 sp.)	12,160 genes	11,142 genes
Genes unique to the blowfly	2,062 genes	572 genes

Gene expression was analysed by sequencing RNA from different tissues of the blowfly including odour sensing organs (antennae and maxillary palp). Sequencing was also performed on larvae that had hatched and developed on sheep, prior to initiating a wound. This gene profiling provided support for the gene annotations in the updated genome. We began to use the genomic and transcriptomic resources created in this project to investigate blowfly biology during the detection and invasion stages of blowfly parasitism of sheep. The odourant receptor (OR) genes of *L. cuprina* were extracted from the newly assembled genome. The two tissue specific transcriptomes, one for the antennae and maxillary palp and the other for blowfly wings were sequenced and expression differences in OR expression examined. We found that there are no odourant receptor genes that have expression exclusively in either males vs females or in females that are mated or not. We expanded our analysis

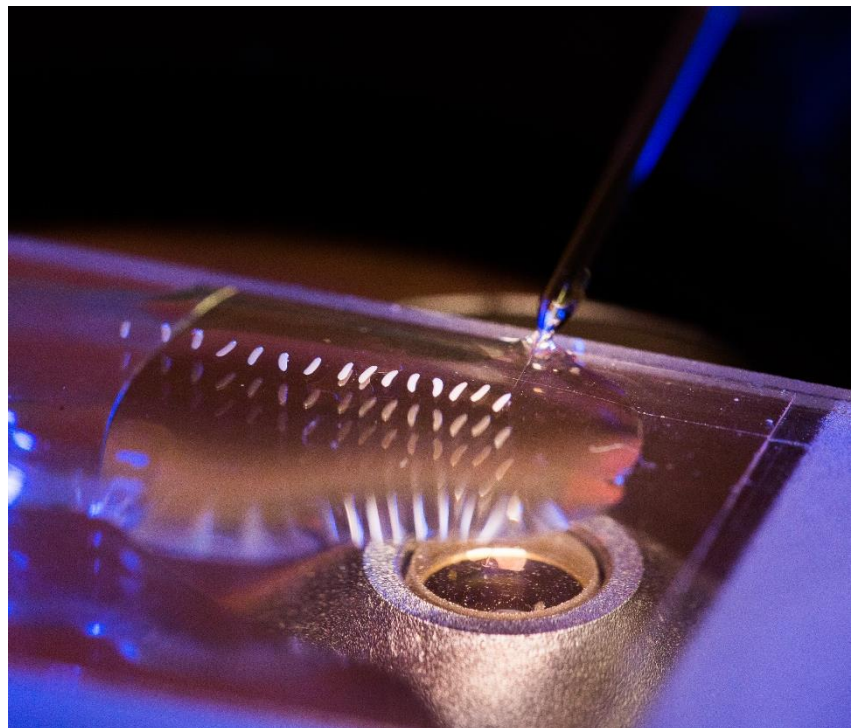
to include chemosensory receptors and some of these did show differences associated with the diet that adult flies were reared on. Follow up work on these genes could include further sampling to test a greater range of conditions helping to validate these findings and work on identifying the odorants and the nature of the responses blowflies have when they are detected (i.e. behaviours such as attraction).

Some of these data generated in this project have led to a new collaboration on olfaction being formed between Prof Coral Warr at UTAS and Prof Batterham and Dr Perry at UOM. This research project examining the evolution of olfactory receptors in *L. cuprina* will run from 2020-2022 and has been funded under the Australian Research Council Discovery Project scheme. It will continue the work looking at odours and odorant receptors that were involved in the attraction of blowflies to sheep. This would not have been possible without the genomic data provided by the ON-00373 project and the development of the CRISPR/CAS9 methodology and is an example of how results from projects funded by AWI have helped attract new researchers, built the capacity of researchers to investigate blowfly biology and led to new projects bringing early career researchers and students to the field.

Another focus of our bioinformatic analyses was to identify genes that were expressed early in the development of larvae on sheep. Assays using gene knockdown highlighted the potential for some genes to halt larval development. These preliminary data were encouraging and can be followed up with the more precisely gene editing technology that we have now developed. Some of this analysis is being used to help identify useful candidates for development of blowfly control options such as a vaccine in the current **ON-00624 Informed Development of a Flystrike Vaccine** project.

Figure 1. Microinjection of fly embryos to introduce the CRISPR/CAS9 components required to modify their genome.

Our results from the CRISPR/CAS9 project (**ON-00516 CRISPR Phase 2**) were promising. We tested multiple methods of microinjection to introduce different combinations of DNA, RNA and Protein into fly embryos which led to the successful creation of targeted deletions. Germline events were identified in two genes, *white* and *Orco* that we then confirmed by sequencing. We were able to establish multiple fly colonies carrying deletions in the *white* gene. The selection of the *white* gene as a target in this project was a strategic decision as this provides an easily identifiable visible marker (flies have white instead of red eyes – pictured below) that we aim to use in future work, including further development of the technology.



While the flies carrying the mutation in the *Orco* gene were less fit, we have also been able to establish a colony of flies homozygous for the *Orco* deletion. These flies are unable to produce a functional *Orco* protein and lack the capacity to smell. These will continue to be studied through the newly funded ARC Discovery project, the foundation of which is based on taking this research forward.



Figure 2. CRISPR/CAS 9 gene modification technology was used to knock out the eye colour gene of the sheep blowfly, producing a fly with white eyes.

PROJECT RECOMMENDATIONS

As reported previously, we have presented the current approach and also future ideas on identifying vaccine candidates to two companies. In both instances it was clear that the project was at too early a stage to attract a commercial partner. There is a need for further biological evidence of a particular gene target being useful as a vaccine candidate to make a compelling case for commercial investment in this area. The vaccine candidate list assembled and analysed in this project was thus at too early a point in the validation process for commercialisation. It does have the potential to be commercially valuable with some further analysis and biological validation that would refine the list to a small number of key candidate genes and this is being pursued as part of the current **ON-00624 Informed Development of a Flystrike Vaccine** project.

The use of CRISPR will only increase in the insect field and we would strongly recommend maintaining a close eye on developments in this space. An additional benefit is that we have also found that being able to perform functional analysis in *L. cuprina*

has increased student interest in pursuing blowfly research projects with our groups which will enhance the ability to recruit new scientists to the field.

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