



# 2018 BREECH FLYSTRIKE RD&E TECHNICAL UPDATE

Fly Genome Research Update

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## Projects and our teams

### Genetics of Blowfly Parasitism



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### Developing Gene Knockout Technology - CRISPR

# Genetics of Blowfly Parasitism - Overview



DNA isolation



Sequence Assembly



Gene Predictions

**GENE SET**  
(n = 12, 933)



Annotation



Blowfly Biology

Features of the draft genomes	2015	2018
	Draft 1 (458 Mb)	Draft 2 (465 Mb)
N50 length (bp); total # >N50 in length	744,413; <b>165</b>	6,922,854; <b>18</b>
N90 length (bp); total # >N90 in length	126,471; <b>736</b>	1,321,550; <b>83</b>
BUSCO (complete; fragmented; missing)	2594; 52; <b>153</b>	2704; 47; <b>48</b>

Measures of genome quality indicate a significant improvement in the last 3 years

Predicted Gene set of *L. cuprina*

Gene Prediction Comparison	Draft 1 (14,544 genes)	Draft 2 (12,933 genes)
Genes supported by expression data	10,121 genes	10,065 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

The number of unique genes in the blowfly genome is now much lower due to the large amount of sequencing of genomes from other organisms over the last three years.

# Genetics of Blowfly Parasitism - Overview



DNA isolation



Sequence Assembly



Gene Predictions



Annotation

Blowfly Biology

Access to a high quality blowfly genome will assist research efforts into this pest

**Mapping genes**

**Investigating biological pathways**

- **Host detection**

- **Parasitism**

**Genetic manipulation of the Blowfly**

## Development of a vaccine against flystrike

### Lessons from previous studies:

#### - need to understand the problem in more detail to develop a vaccine

1. We need to identify better antigens. New methods and the blowfly genome will facilitate this.
2. Require a more detailed knowledge of the biological interactions between blowfly larvae and sheep
3. Have to understand the type of immune response required to provide protection to sheep

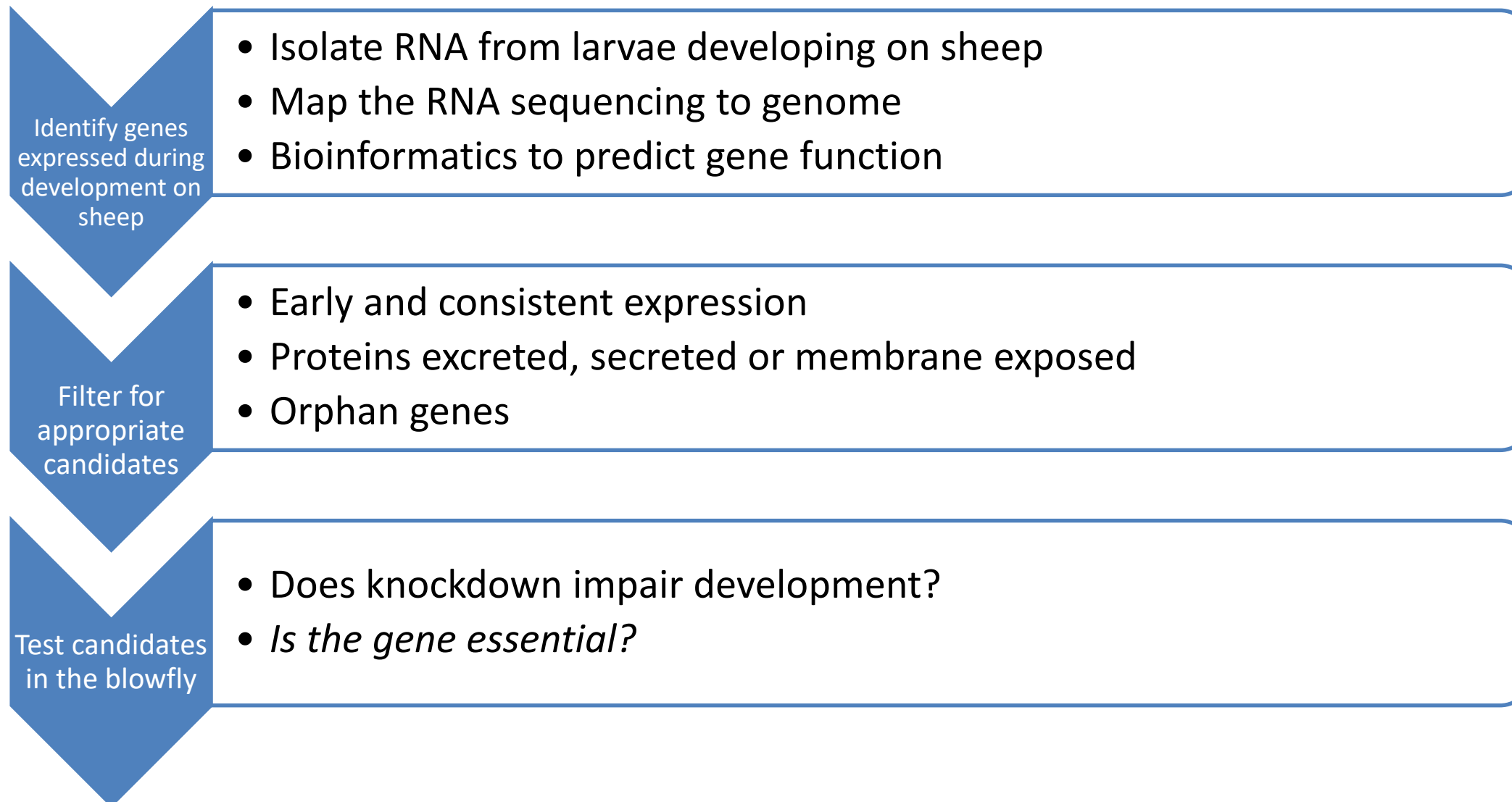
## Development of a vaccine against flystrike

Technology has come a long way

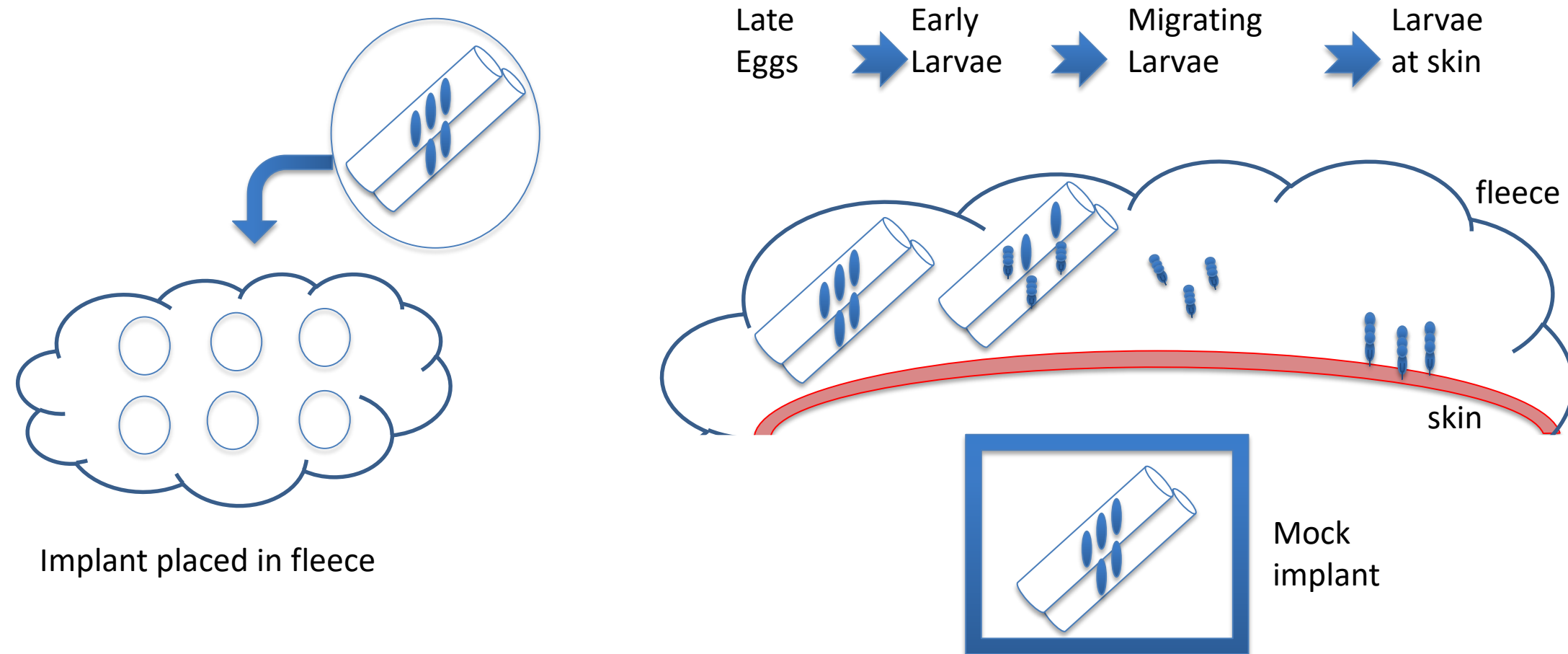
- ✓ Genomics
- ✓ Transcriptomics
- ✓ Proteomics
- ✓ Gene manipulation
- ✓ Immunology
- ✓ Adjuvant chemistry
- ✓ Delivery systems
- ✓ Recombinant technology

**New tools are now  
available to examine and  
dissect the Host/Parasite  
interaction**

## Identification of genes important for larval development



# Identification of genes important for larval development



**Now have a profile of blowfly gene expression during larval development on sheep**  
- this has revealed genes that are present during the life stages we want to target



## Predicting those genes that might be useful vaccine candidates

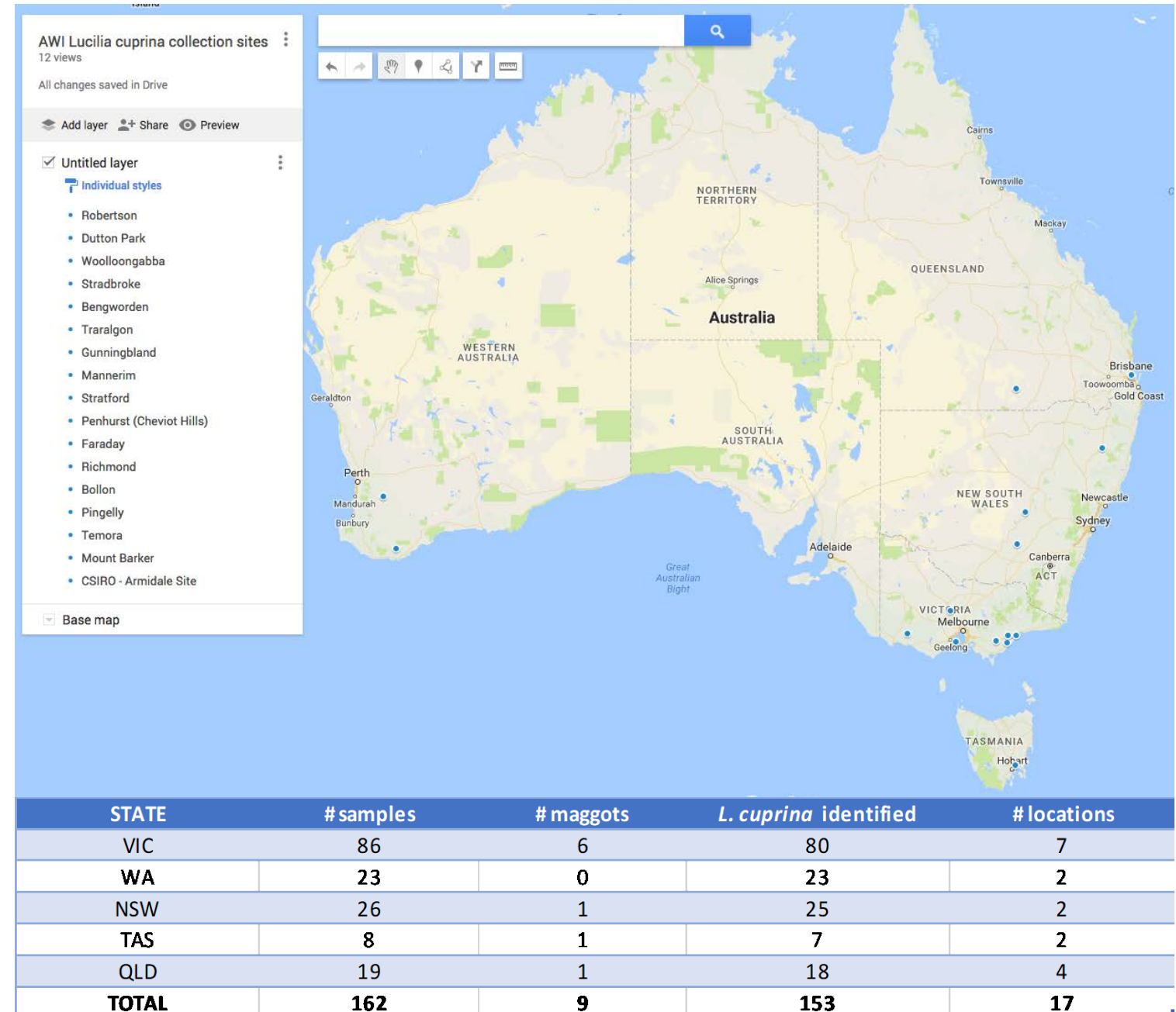
### From the larval gene expression patterns:

- Identifying functional domains and gene orthologues helps us understand the types of pathways that are important for development
- Filter this list for the genes of interest
  - Proteins excreted or secreted from the larvae
  - That have early and consistent expression
  - Blowfly specific genes (function unknown)

	Predicted functions
1	Sterol sensing
2	chitin binding
3	chitin deacetylase
4	extracellular matrix
5	laminin
6	leucine rich repeat
7	midgut cell matrix
8	mucin
9	peptidase
10	protease
11	unknown
12	unknown
13	unknown
14	chitin binding domain
15	cuticle protein
16	unknown
17	orphan
18	orphan
19	orphan
20	orphan

## Natural population sampling

- Blowfly collection conducted in 2017/2018 season
- Sequencing samples from pooled populations
- Will allow us to confirm candidates have conserved protein sequences
- This would be important for widespread effectiveness of any vaccine



# Current work to validate vaccine candidate genes

Look at research from similar genes in other insects and if there are known fitness phenotypes

- Are they likely to be essential?

On-going work to validate vaccine candidate genes

- Gene knockdown (RNAi)
- Evaluating impact of disrupting these on larval growth and development

*Drosophila*  
orthologue

*Lucilia*  
specific

Predicted functions	
Sterol sensing	Yes
chitin binding	Y
chitin deacetylase	Y
extracellular matrix	Y
laminin	Y
leucine rich repeat	Y
midgut cell matrix	Y
mucin	Y
peptidase	Y
protease	Y
unknown	Y
unknown	Y
unknown	Y
chitin binding domain	Partial impact
cuticle protein	P
unknown	P
orphan	? Unknown, as unique to blowfly
orphan	?
orphan	?
orphan	?

If a similar gene from another insect is disrupted does it die?

Yes

Y

Y

Y

Y

Y

Y

Y

Y

Y

Y

Y

Y

Partial impact

P

P

? Unknown, as unique to blowfly

?

?

?

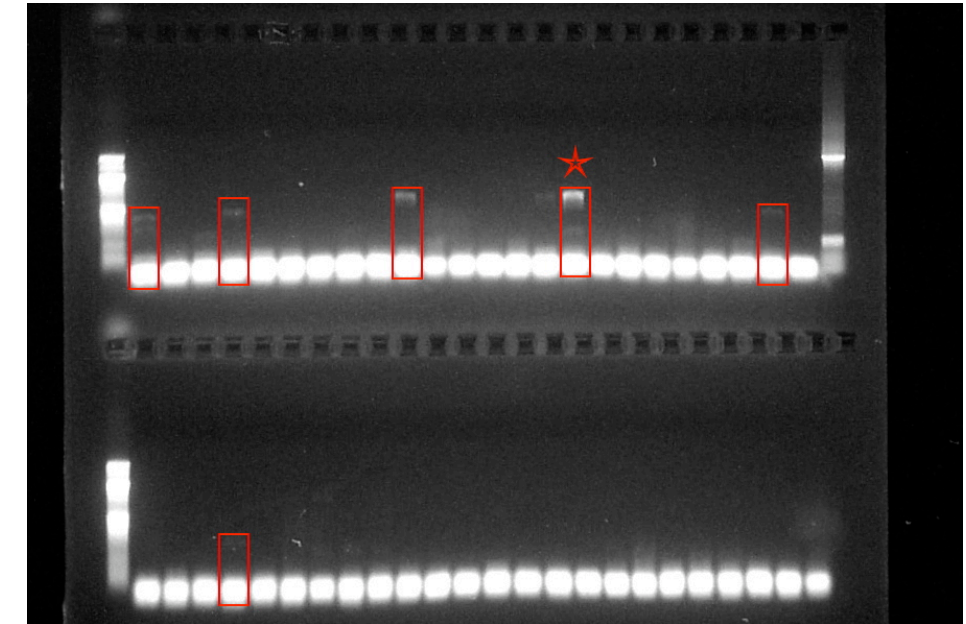


## Developing Gene Knockout Technology - CRISPR

- Model organisms make a significant contribution to our knowledge of gene function
- Analysis in model systems can bridge some of the gaps
  - Conserved gene functions
- In some cases genetic manipulation of *L. cuprina* is most appropriate
  - Orphan genes
  - Establishment of myiasis

## Require a tool to manipulate the blowfly

- This tool exists - CRISPR/CAS9
- A genetic tool adapted across a wide range of organisms
  - Allows editing of genomic DNA
    - The nuclease (CAS9) cuts DNA *in vivo*
    - The sites cut are determined by specific guide RNA sequences
    - We can detect these events using molecular diagnostics



**We can use this to examine any gene of interest in the blowfly**

## Future work to develop new tools and their potential to identify new solutions

To understand the importance of different genes we need to determine the effect when their function is lost or disrupted

Current work has now established a CRISPR/CAS9 technique allowing deletion of specific genes in the blowfly.

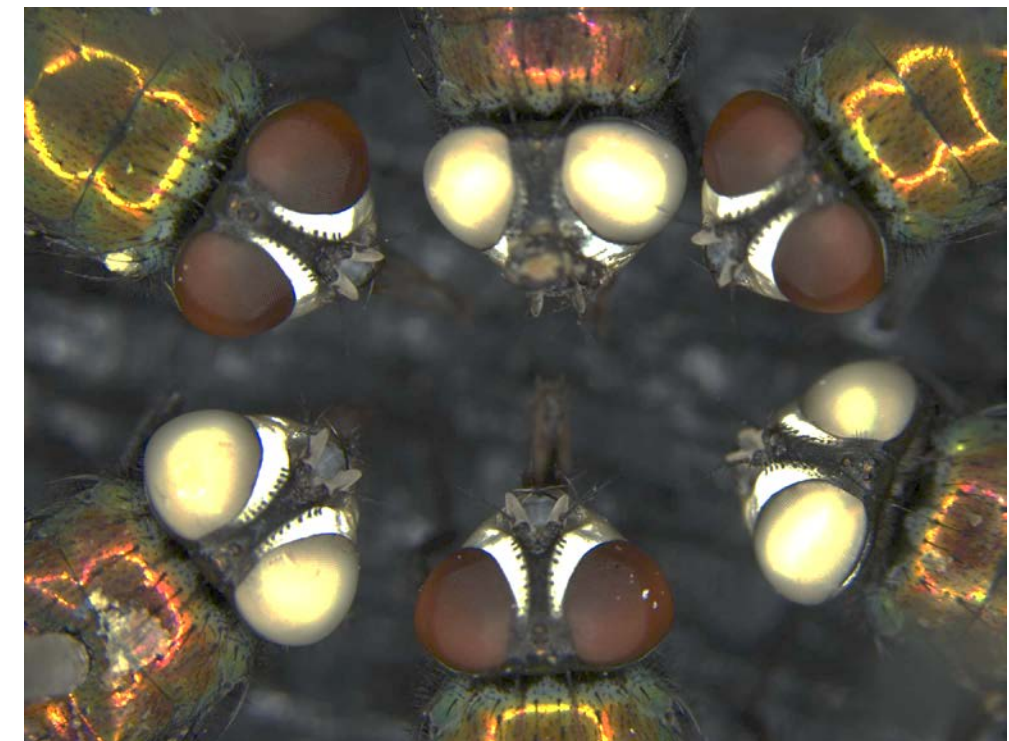
Created a knockout of two genes;

*white* – A “blind” fly

*Orco* – A fly that cannot smell

Next steps are to introduce better genetic tools,

- Provide greater capacity to examine blowfly biology
- Prepare for future control options

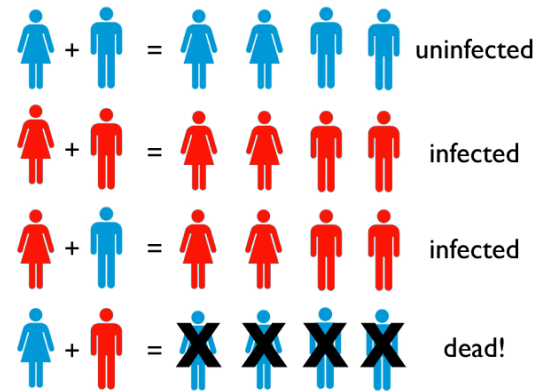




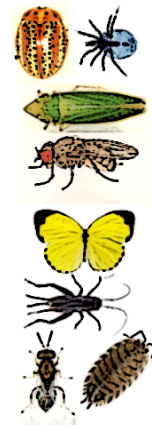
# A biological option for blowfly control?

## Identification of *Wolbachia* in field populations of *L. cuprina*

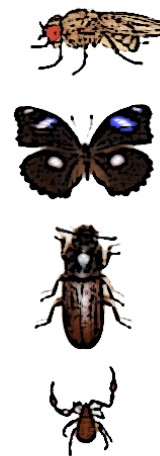
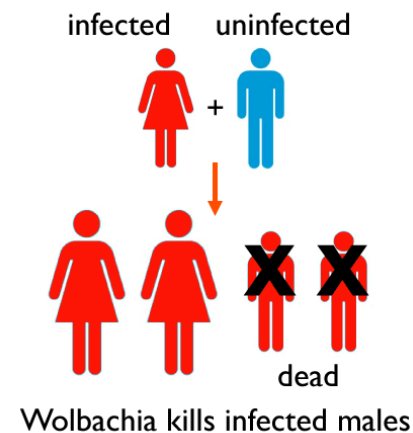
### Cytoplasmic Incompatibility



Infected males are incompatible with uninfected females



### Male Killing



### From 2017/2018 Natural population collection

- 162 samples analysed, 77% had *Wolbachia*
- Present in blowfly samples from all states in the collection
  - WA, NSW, QLD, VIC, TAS



*Wolbachia* in an animal cell

## To come up with methods to control this pest we need to be able to understand and dissect its biology

### Genetics of Blowfly Parasitism

- A reliable, detailed and accessible genome resource
- Greater knowledge of the biology of *L. cuprina* during parasitism and host seeking
- Confirmation this approach can identify genes critical to *L. cuprina* development.

### Development of gene knockout technology – CRISPR

- Created deletions in two genes – have shown the technology works in blowflies.
- Can now use this to better understand a range of other potential gene targets

### **Resources developed provide tools and ideas to stimulate further Blowfly research;**

- MSc and PhD student projects – training the next generation of researchers
- Competitive Australian Research Council project applications

## To come up with methods to control this pest we need to be able to understand and dissect its biology

### Pathway to a vaccine

- This work delivers the initial steps - identifying the genes and establishing methods to examine them in detail.
- Future work over the next 3-5 years should examine the ability of these and further identified genes to provide protection at levels that would be of value to growers.
- This will include testing in blowflies and preliminary trials of promising candidates in sheep to establish sufficient evidence and levels of efficacy to attract investment by an industry partner. We would work with them to deliver a viable, commercial vaccine.



# Acknowledgements

## Australian Wool Innovation

Current and ongoing project funding



## School of BioSciences and Faculty of Veterinary and Agricultural Sciences

Facilities – Molecular labs and equipment, Blowfly rearing rooms, Sheep trial pens

## Collaborators

Dr Clare Anstead, Prof Philip Batterham, Prof Robin Gasser, A/Prof Vern Bowles

Ross Hall, Tinna Yang, Natália Hernandez

Dr Neil Young, Dr Pasi Korhonen, Dr Andreas Stroehlein



## Blowfly collectors

Volunteers across Australia, trapping and returning flies for analysis



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