

2022 FLYSTRIKE RD&E TECHNICAL FORUM

Modelling of blowfly chemical
resistance

Dr Trent Perry – University of Melbourne

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Modelling of Blowfly Chemical Resistance

Blowfly Genetics and Genomics

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Blowfly Genetics And Genomics

Project Team



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Research Funding Support



Australian Wool
Innovation Limited



Australian Government

Australian Research Council

Blowfly genomics

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Blowfly functional genetics

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Blowfly olfaction

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Resources

Volunteer sample collectors

Dr Peter James (UQ)

Dr Jen Smith (CSIRO)

School of BioSciences

Bio21 Institute

UOM Research Focus

1. Blowfly genomic resources
2. Population genomics
3. Early stage myiasis
4. Functional genetics



Images by Casamento photography

ON-00373 (2015-19)
Genetics of Blowfly Parasitism

ON-00570 (2015-19)
Development of gene knockout
technology

ON-00624 (2019-22)
Informed development of a flystrike
vaccine

Research Facilities

- Bio21 Molecular Science and Biotechnology Institute
 - Physical Containment Level 2 (PC2) Insectary
- Faculty of Veterinary and Agricultural Sciences
 - Animal house with Sheep trial pens
- Office of the Gene Technology Regulator
 - Gene Technology and Biosafety Committee
 - Notifiable Low Risk Dealings
- Office of Research Ethics and Integrity
 - Animal Ethics Committee



Images by Casamento photography



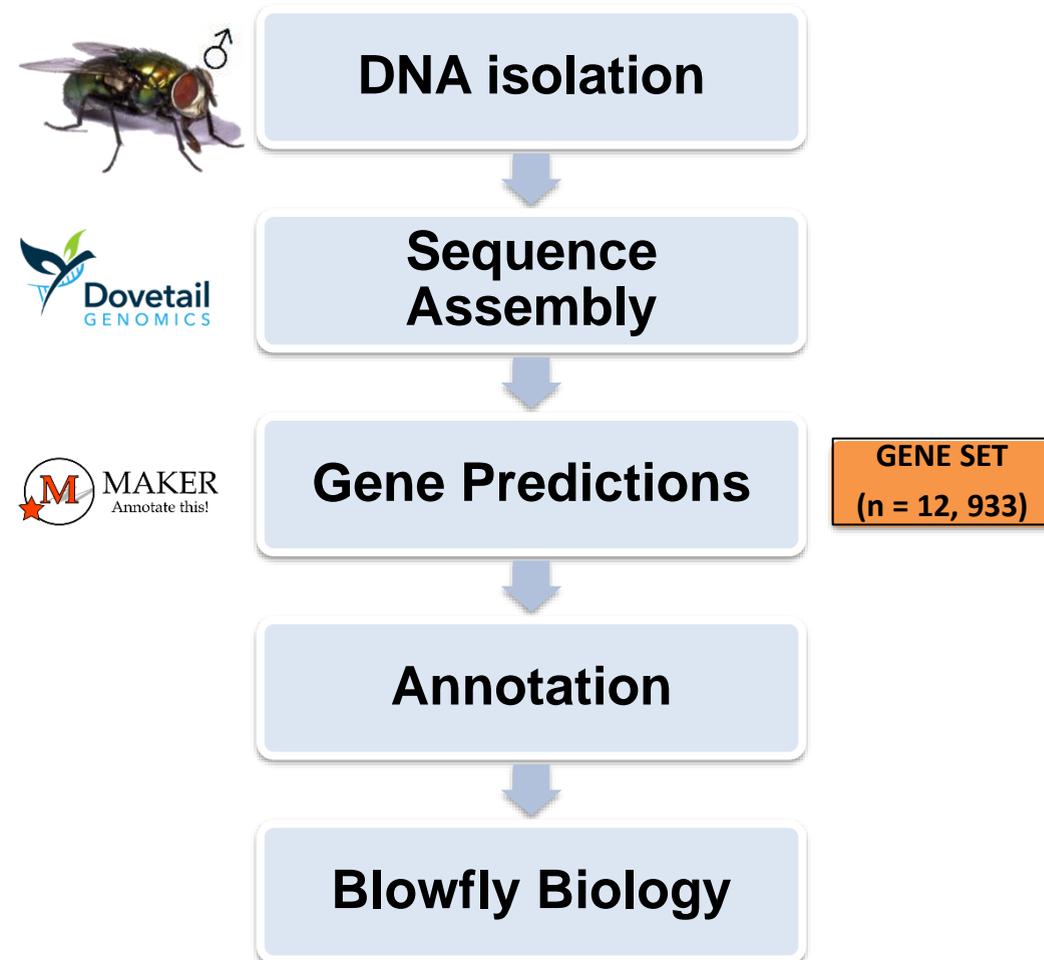
Dr Clare Anstead



Dr Shilpa Kapoor

1. Blowfly Genomics

- Assembly and improvement of the genetic resources available



| 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 |

Foundational research critical for;

- Transcriptomics
- Population genomics
- Functional genetics

2. Population Genomics

ON-00624 Informed development of a flystrike vaccine

Objective 1: Population genetics of *L. cuprina* (2018/19, 2019/20, 2020/21)

- Understand how flies move around the country
- Examine populations for known resistance alleles and frequencies
- Identify protein variation in CSIRO vaccine development program

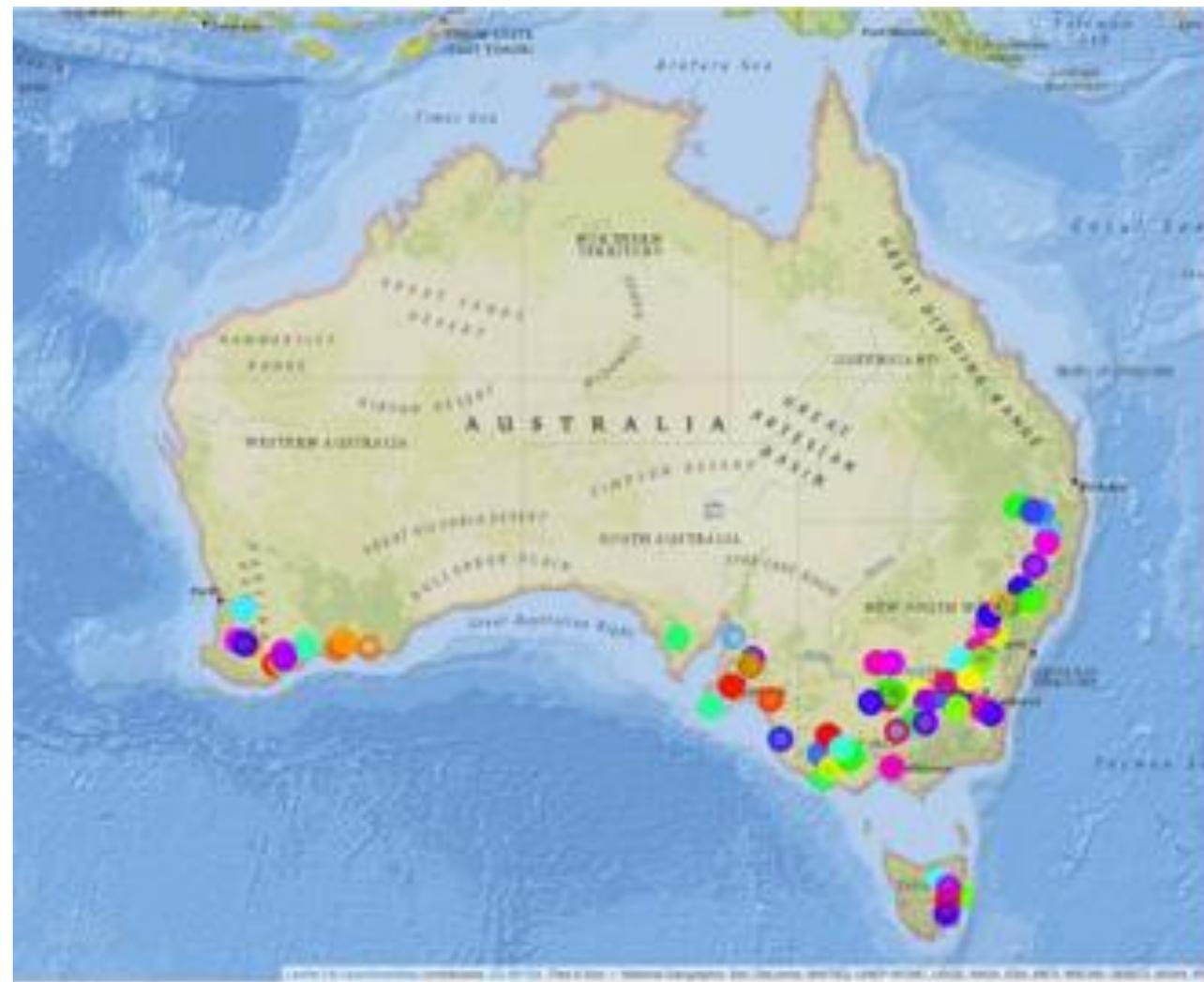
Can be leveraged to support current and future projects to improve fly control

Collection sites

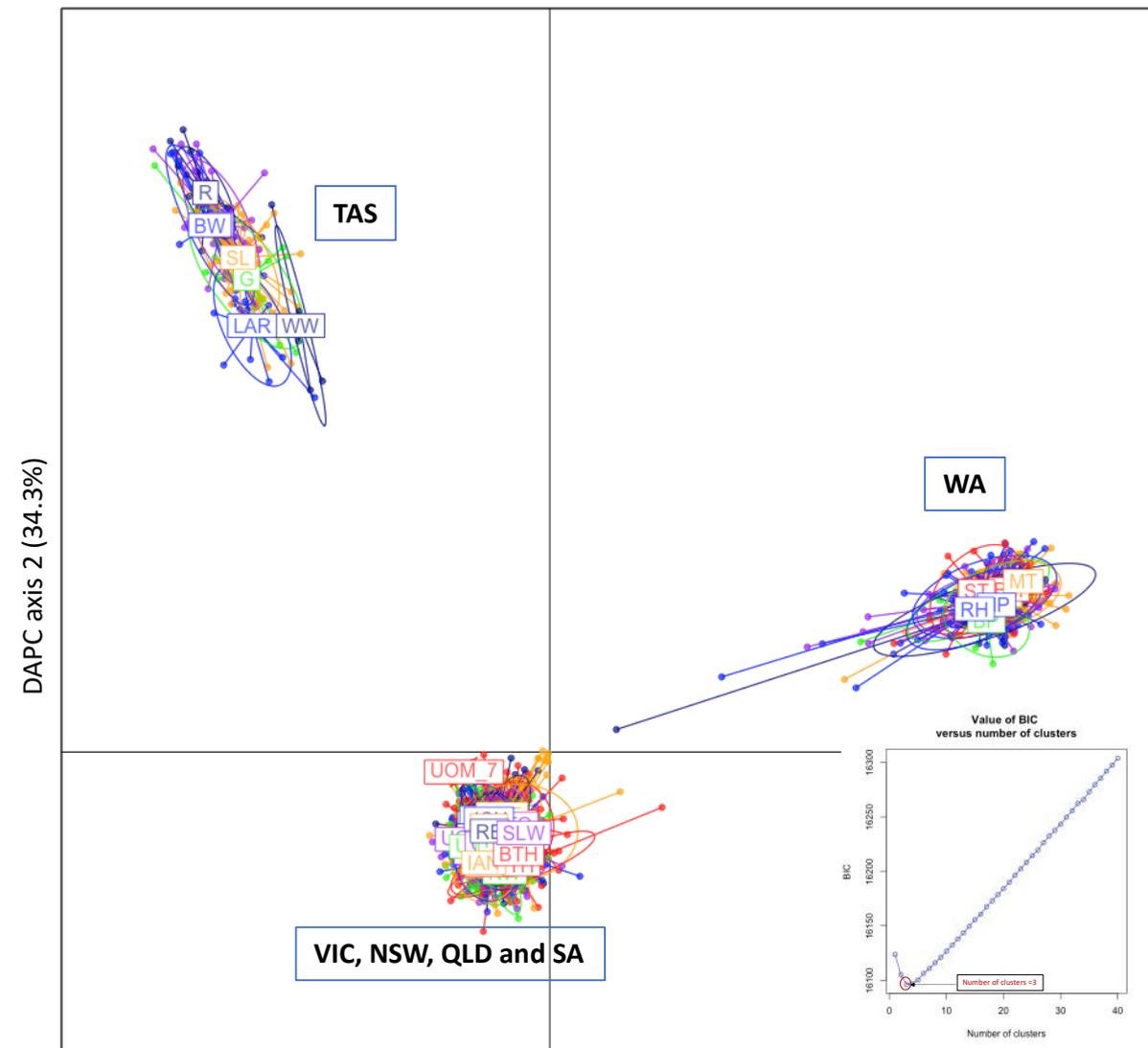
| Year | Sample collections | Number of <i>L. cuprina</i> flies |
|--------------|--------------------|-----------------------------------|
| 2018/2019 | 30 | 413 |
| 2019/2020 | 81 | 1235 |
| 2020/2021 | 49 | 1267 |
| Total | 160 | 2915 |

Samples collected by:

- UOM team
- Growers
- Agronomist networks



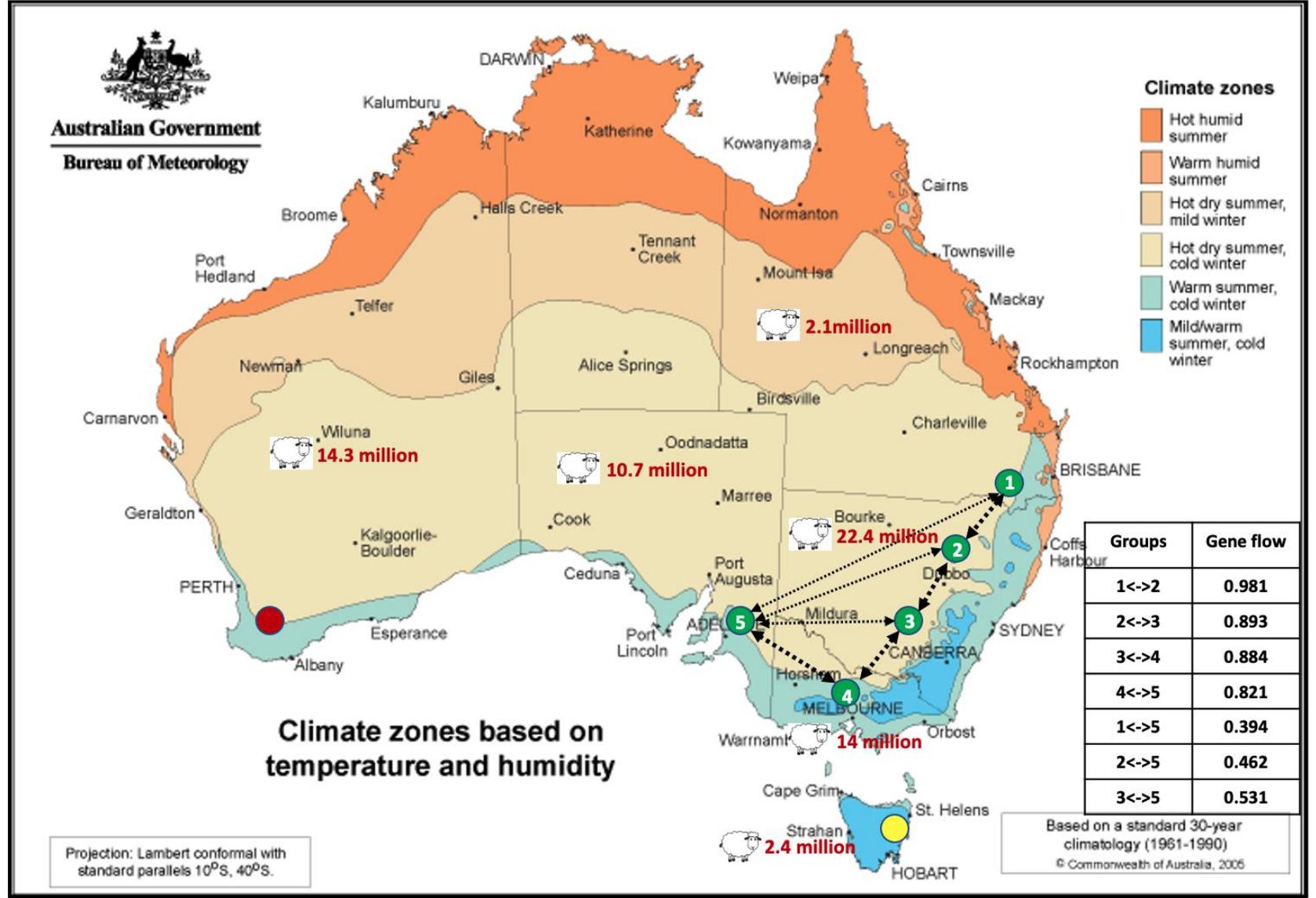
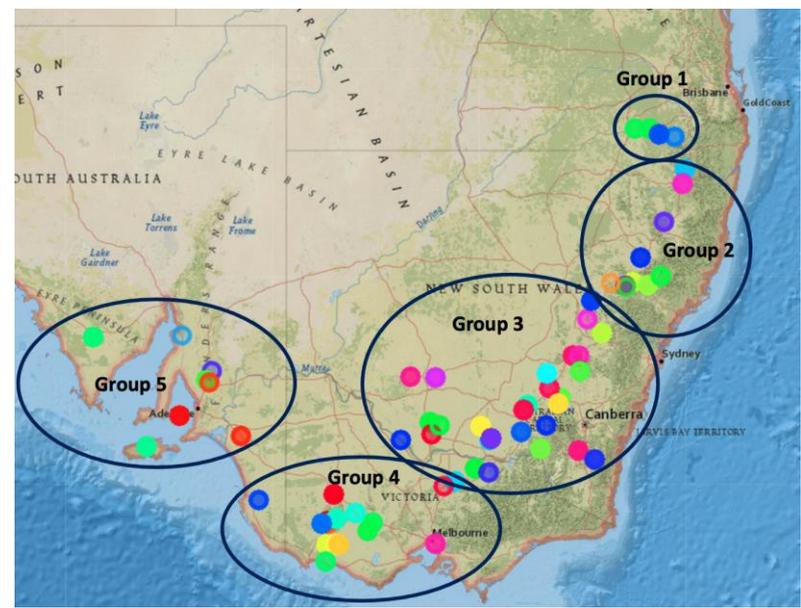
Population Structure Of Australian Sheep Blowfly



- Assigned individuals to groups based on genetic information.
- Single cluster for Eastern Australia
 - VIC, NSW, QLD and SA
- Western Australia and Tasmania form genetically distinct clusters.

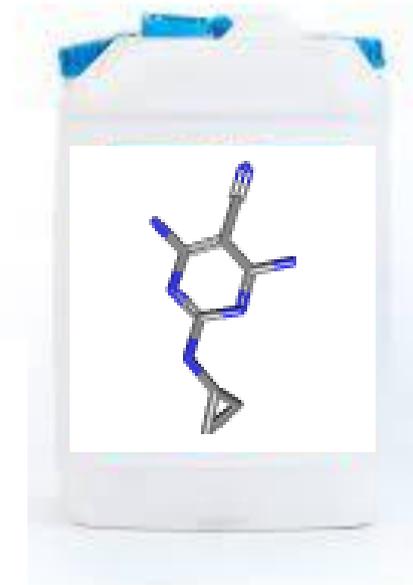
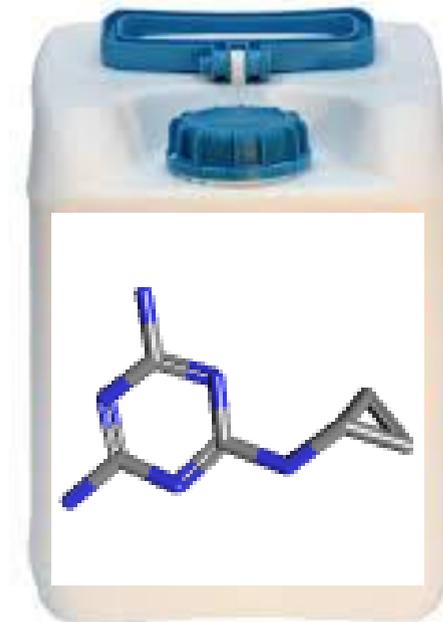
Gene Flow Between Areas

- Within cluster analysis
- 5 groupings of population samples
- Significant gene flow within the cluster proportional to geographic isolation



Insecticide Resistance

- No resistance to **known** target site mutations were detected in our WGS samples for
Spinosad
Neonicotinoids
Macrocyclic lactones
- Protein changes in some fly populations **associated** with resistance identified
Organophosphates
Organochlorines
- Caveat - targets for dicyclanil or cyromazine are **not known**
cannot be determined from the sequence dataset.
Resistance has been reported (Sales et al., 2020)



Insecticide Resistance

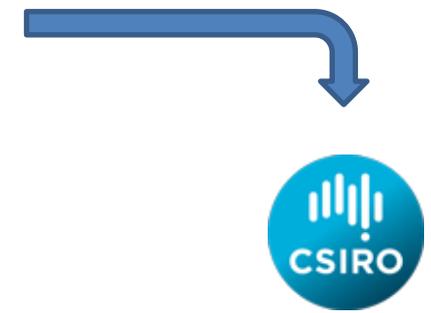
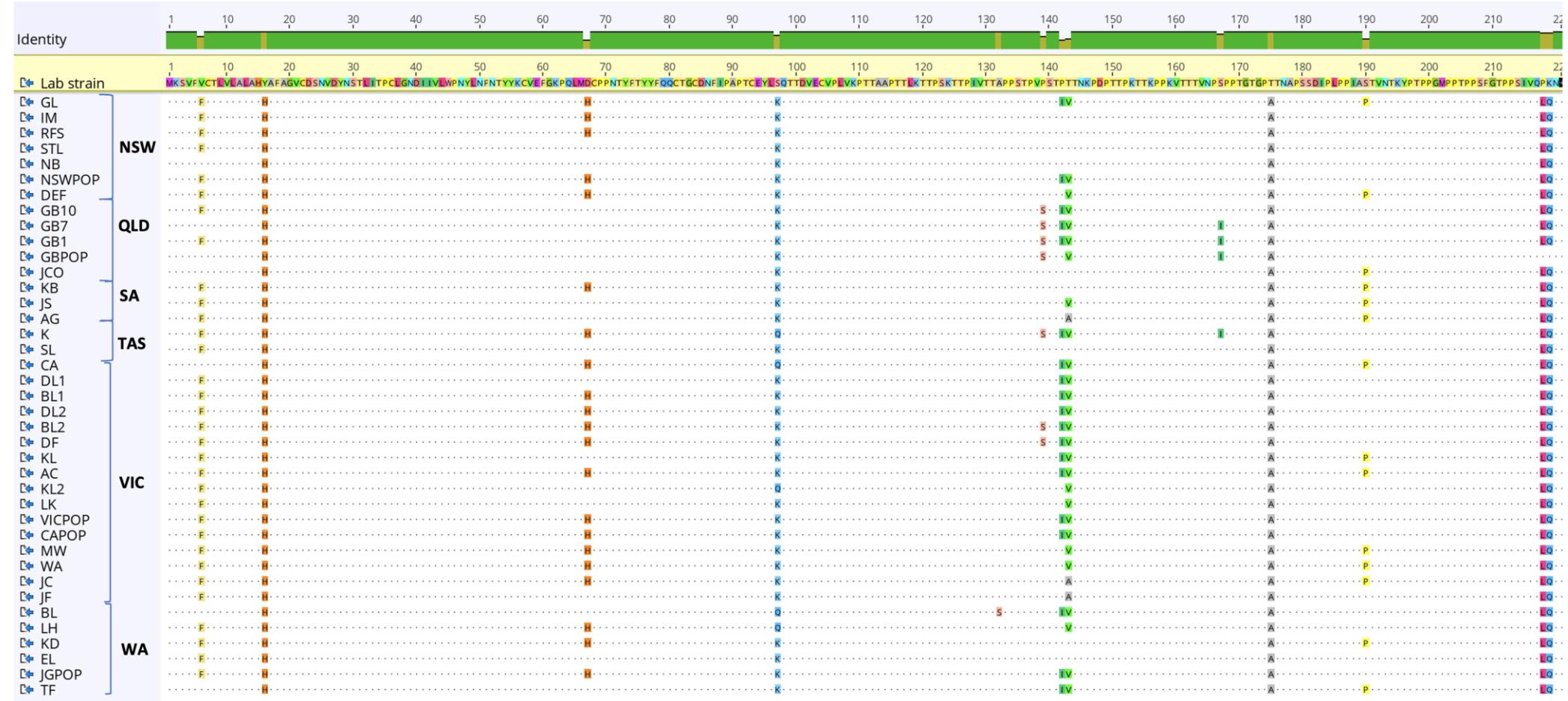


Map of $Lc\alpha E7$ allele frequencies for OP susceptible G^{137} (blue) and resistant flies $Rop-1 D^{137}$ (orange)

| Target | Protein target | Insecticide class | Resistant change detected |
|-----------------------------------|------------------|-----------------------------|---------------------------|
| GABA-gated chloride channel | Rdl | organochlorines | Y |
| Acetylcholinesterase | LcAce | organophosphates | Y ($Lc\alpha E7$) |
| Voltage-gated sodium channel | Kdr/para | pyrethroids | N |
| nAChR receptor subunit $\alpha 6$ | $Lc\alpha 6$ | spinosyns | N |
| nAChR receptor subunit $\alpha 1$ | $Lc\alpha 1$ | neonicotinoids | N |
| nAChR receptor subunit $\beta 2$ | $Lc\beta 2$ | neonicotinoids | N |
| nAChR receptor subunit $\alpha 2$ | $Lc\alpha 2$ | neonicotinoids | N |
| nAChR receptor subunit $\beta 1$ | $Lc\beta 1$ | neonicotinoids/sulfoximines | N |
| Glutamate-gated chloride channel | $LcGlucl1\alpha$ | avermectins | N |

Analysing Variation

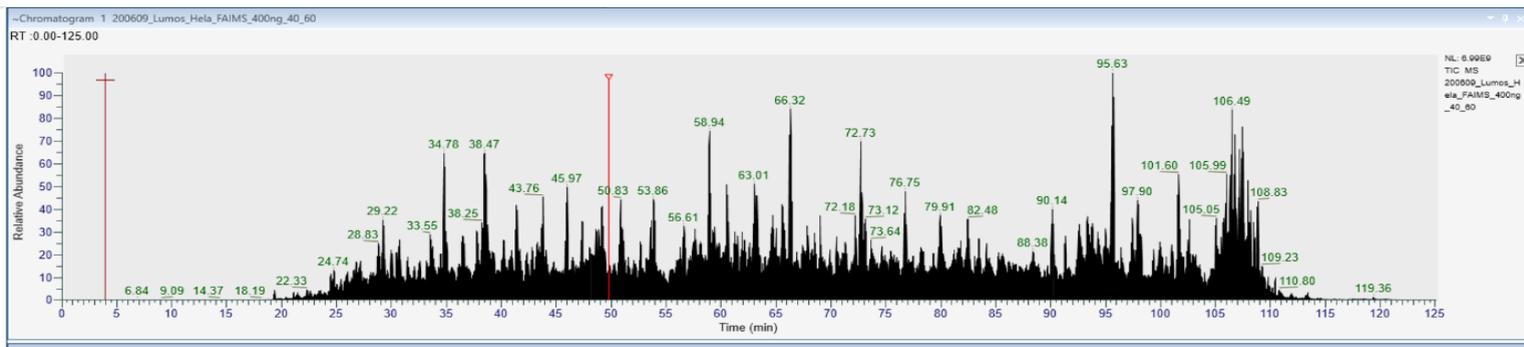
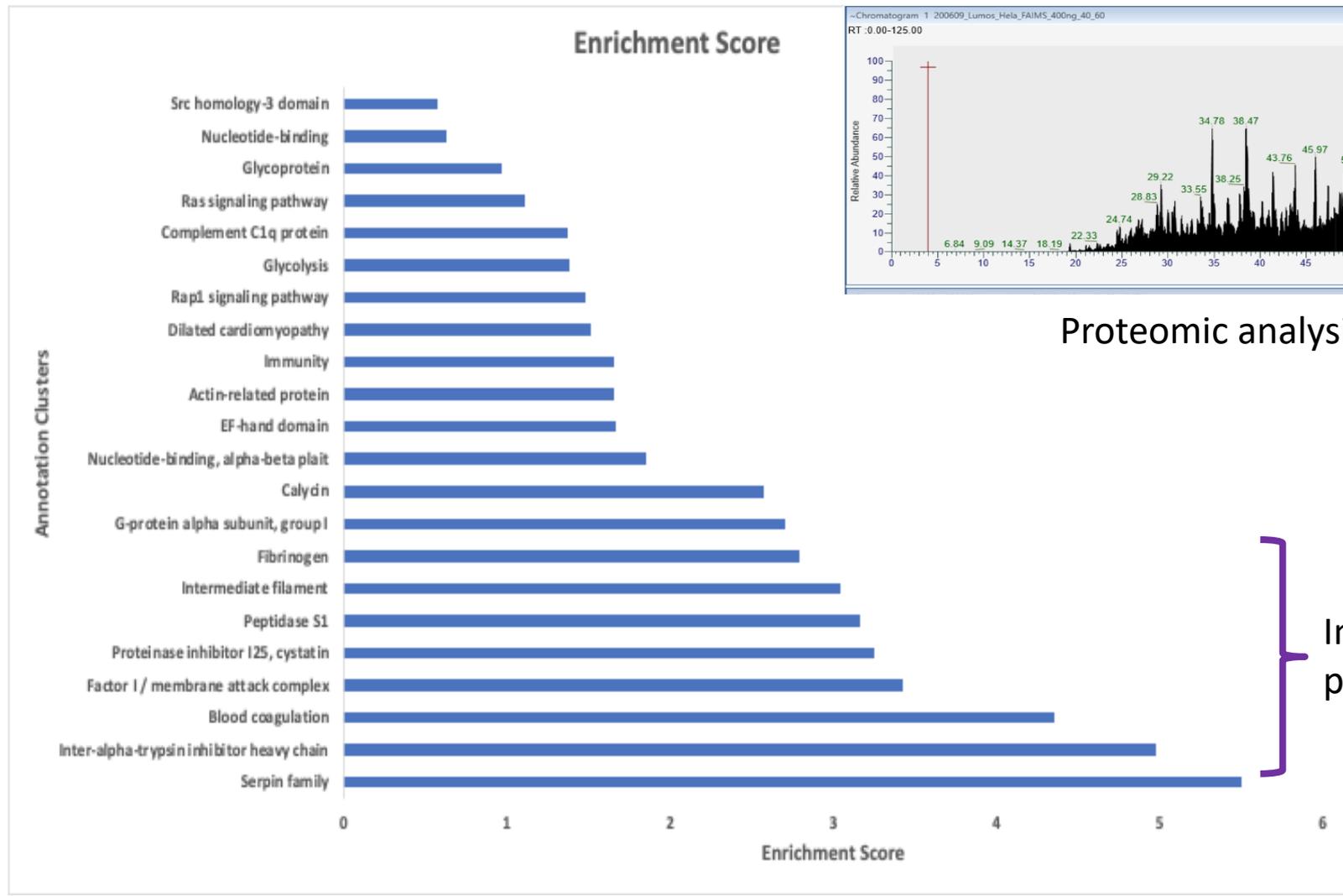
Protein sequences can be compared to look for important changes



3. Early Stage Wound Development

- ON-00624 Informed development of a flystrike vaccine
- **Objective 2:** Analysis of blowfly and sheep proteins in early flystrike.
- Main aims:
 - Identify proteins secreted by developing larvae
 - Consider as new lead proteins for control strategies (Vaccine and novel targets)
 - Provide information on proteins to CSIRO for their vaccine gene candidates
 - Profile how sheep respond to larval infection

The Sheep Response To Flystrike

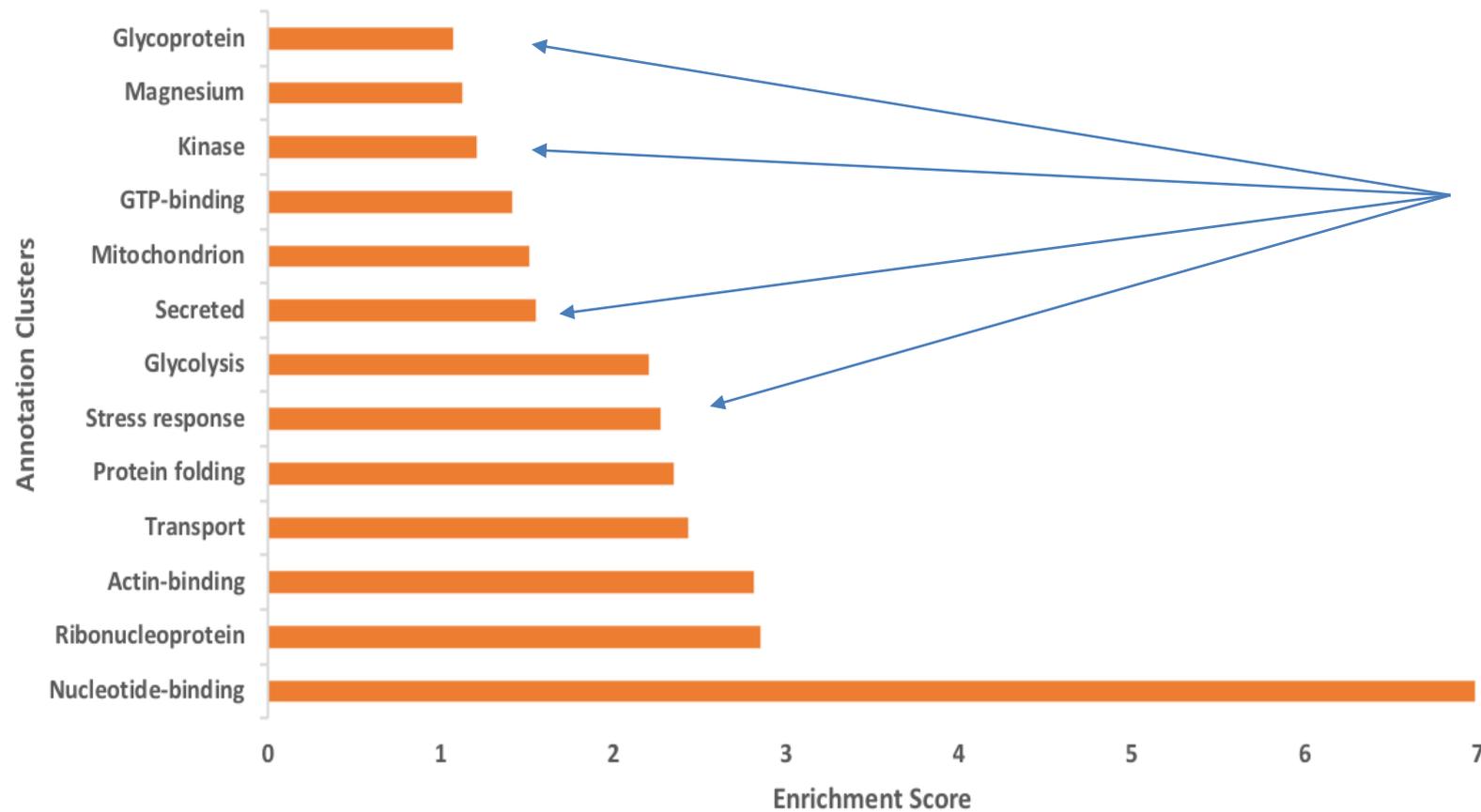


Proteomic analysis of samples from sheep implant trials

Immune response and associated inflammatory proteins enriched in flystrike samples

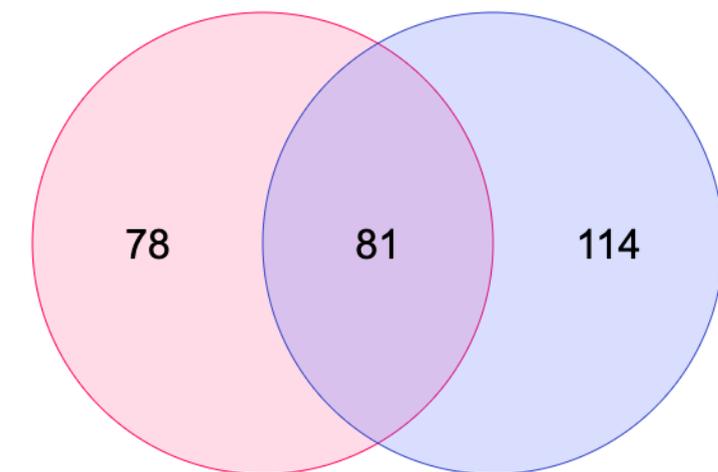
Lucilia cuprina – Proteins Excreted During Flystrike

Enrichment scores for annotation clusters



Larval kinases, secreted proteins, glycoproteins, stress response proteins are enriched in maggot implant samples collected from sheep

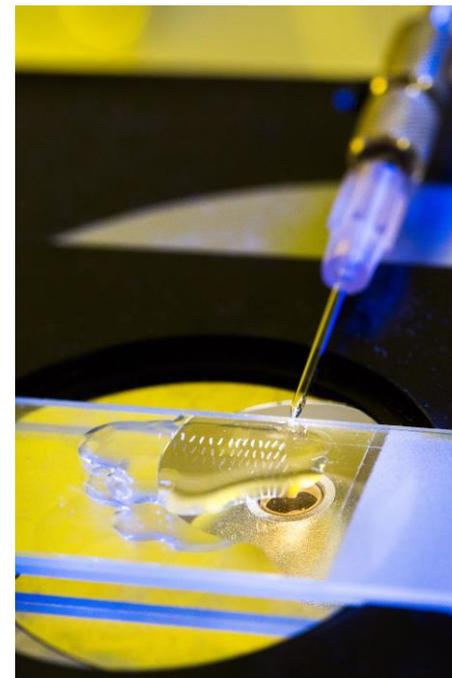
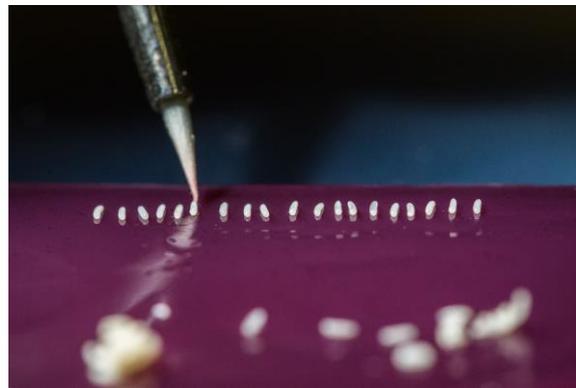
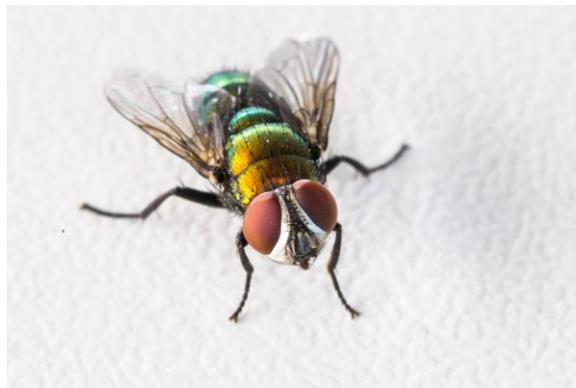
Larval non implant Larval implant



GO enrichment scores for proteins detected in maggot implant samples.

Functional Genetics

ON-00570 Development of gene knockout technology



Images by Casamento photography

Developing tools for

- Interrogating the function of genes of interest (vaccine candidates)
- Identifying genes required for development and survival

Developing tools to examine blowfly biology

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

Initial work established a CRISPR/CAS9 technique to delete specific genes in the blowfly.

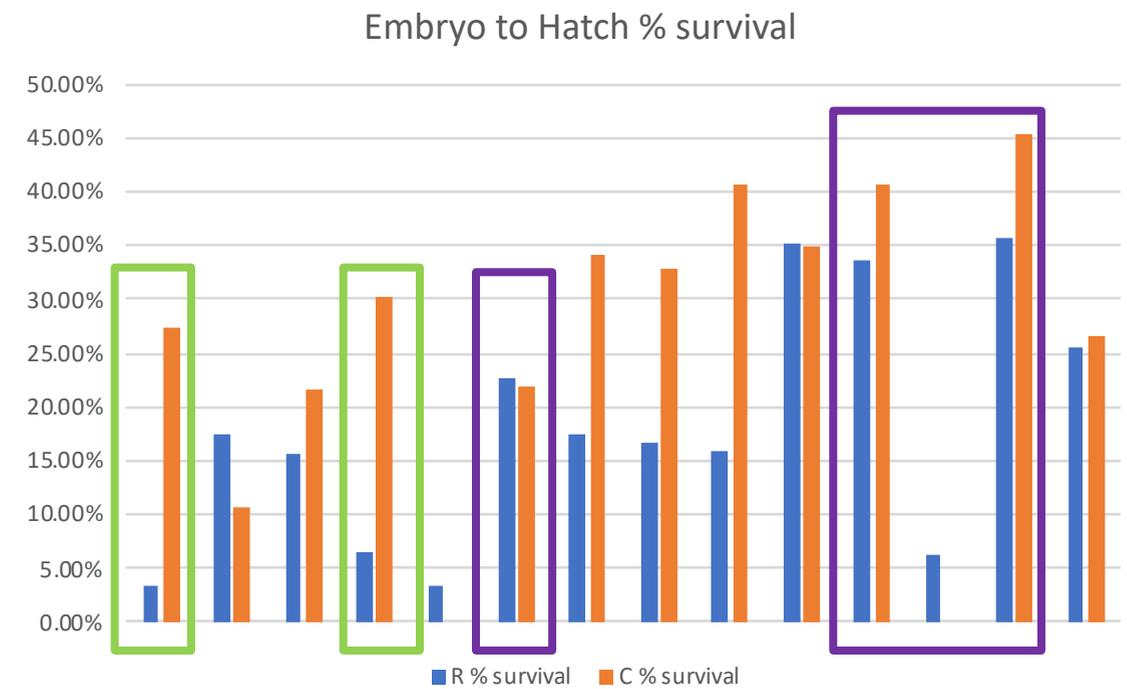
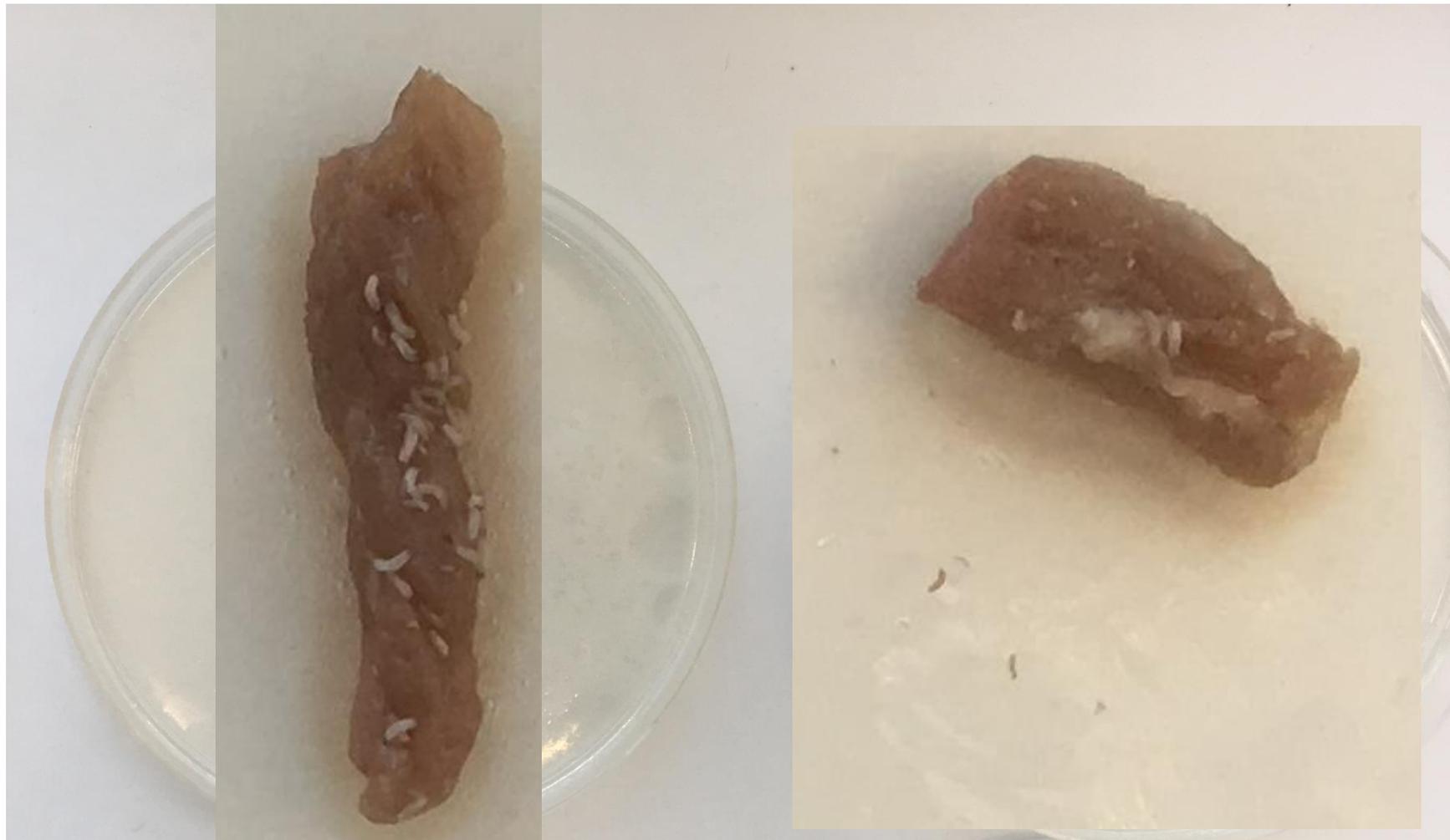
Created genomic knockout of two genes;

white – A “blind” fly (POC)

Orco – A fly that cannot smell

- **Validate gene function**
 - **Vaccine candidate genes (MSc)**
 - **Odorant receptors (ARC DP, Hons)**
 - **Marker genes (Hons)**

Biological Validation In The Blowfly



- Tested the impact of reducing the gene expression of 10 genes of interest.

- Two led to reduced hatch rate

- Two led to mortality of larvae post hatching

Research Outcomes

- Genomic and transcriptomic resources being utilised to support current and future research efforts to enhance control of flystrike
- Identification of a Candidate genes of interest which are important for maggot development
- Technical capabilities to conduct functional genetic experiments on Australian sheep blowfly
- Expansion of research capacity
 - New projects and funding areas (Australian Research Council)
 - New training opportunities (Postdoc, PhD, MSc, Hons students)

Modelling Of Blowfly Chemical Resistance

UOM - Genetic analysis of dicyclanil and cyromazine resistance mechanisms in Australian Sheep Blowfly

- Genetic analysis will help inform modelling work by UTAS
 - Characterise resistance mechanism(s) in a field-collected strain of Australian Sheep Blowfly
 - Provide measurements of the relative fitness of the resistant strain
 - Determine the genetic basis of resistance
- Collaborate with NSW-DPI to support the identification of novel resistance mechanisms detected through NSW-DPI resistance monitoring programs.

Empirical Data For Improved Resistance Modelling

- Help to reduce the number of assumptions underlying the model
 - Is one mechanism or are multiple mechanisms present?
 - Is the same mechanism responsible for the cyromazine and dicyclanil resistance?
 - Is there a fitness cost imposed on flies that are resistant?
- Potential to develop molecular assays to help monitor resistance
 - Lab based analyses
 - On-farm diagnostic kits
- A better model will allow design of the most appropriate resistance management practices.



This publication is based on information presented at the Australian Wool Innovation Limited (AWI) Flystrike RD&E Technical Forum held on 10th August 2022. Some information in this publication has been contributed by one or more third parties and licenced to AWI, and AWI has not verified whether this information is correct. This publication should only be used as a general aid and is not a substitute for specific advice. To the extent permitted by law, we exclude all liability for loss or damage arising from the use of the information in this publication. Except to the extent permitted under Copyright Law no part of this publication may be reproduced by any process, electronic or otherwise without the specific written permission of AWI. Neither may information be stored electronically in any form whatsoever without such permission. AWI is grateful for its funding, which is primarily provided by Australian woolgrowers through a wool levy and by the Australian Government which provides a matching contribution for eligible R&D activities. © 2022 Australian Wool Innovation Limited. All rights reserved.