

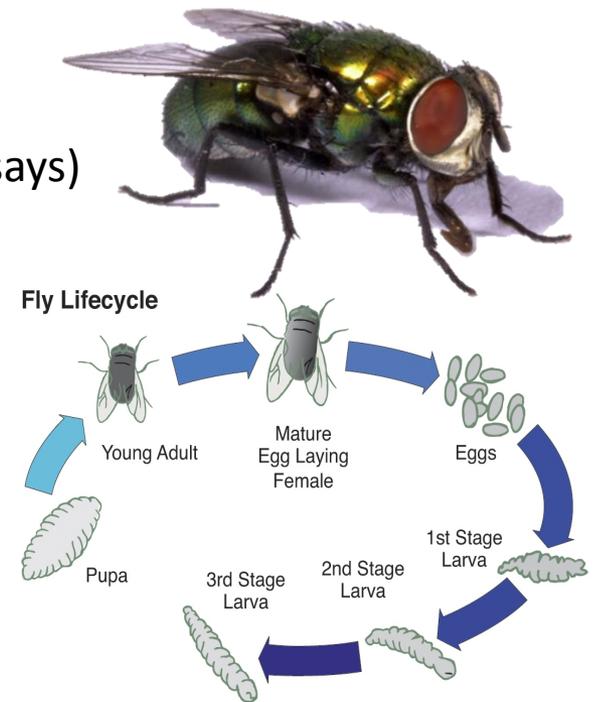
AWI Breech Strike R&D Technical Update
Maritime Museum, Sydney
12th July 2016

T. Perry, C. Anstead, R. Gasser and P. Batterham
The University of Melbourne

Fly Genome: Update of past work and current project

University of Melbourne Team

- Batterham and Perry - School of Biosciences
 - *Lucilia cuprina* colony
 - Assay development (Behavioural and toxicology assays)
 - Developing genomic editing through CRISPR
- Anstead and Gasser - Faculty of Veterinary Science
 - Genome and transcriptome assembly
 - Gene annotation
 - Identification of targets for new control strategies
 - Baits, insecticides and vaccines



Creating awareness of genome resource



ARTICLE

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OPEN

Lucilia cuprina genome unlocks parasitic fly biology to underpin future interventions

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Research review paper

A blow to the fly – *Lucilia cuprina* draft genome and transcriptome to support advances in biology and biotechnology

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- ✓ DNA gene sequence published 2015

Parasites & Vectors
(D_12 June 2016_5,000 words)

REVIEW

New genomic resources underpin future molecular explorations of *Lucilia cuprina* and related flies

Clare A. Anstead^{1*}, Trent Perry², Stephen Richards³, Pasi K. Korhonen¹, Neil D. Young¹, Vernon M. Bowles¹, Philip Batterham² and Robin B. Gasser^{1*}

- ✓ New, valuable resource
- ✓ Promoting awareness to enable and encourage other researchers



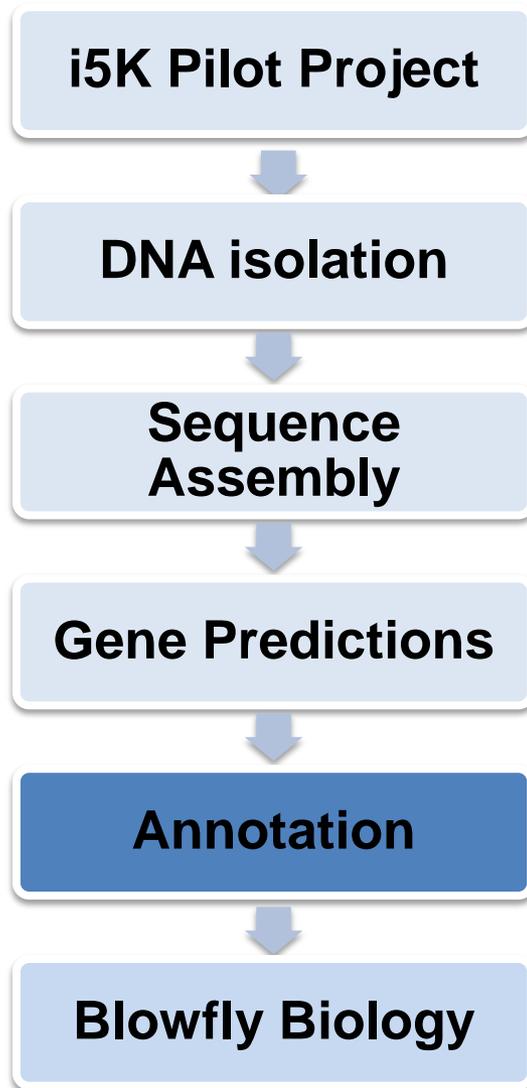
Genome comparison – By the numbers

	<i>Lucilia cuprina</i>	<i>Drosophila melanogaster</i>	<i>Musca domestica</i>
	Blowfly	Vinegar Fly	House Fly
Genome size (Mb)	458	169	750
Chromosomes	5+1	4+1	5+1
N50 scaffold length	744,413	23,011,544	226,573
Coding (%)	6.2	18.3	Not yet known
Number of genes	14,554	15,771	17,508
Repetitive seq. (%)	57.8	36.0	Not yet known
% G&C content in DNA	29	42	35

The Vinegar Fly has been the focus of a large amount of R&D as it is well suited to laboratory studies

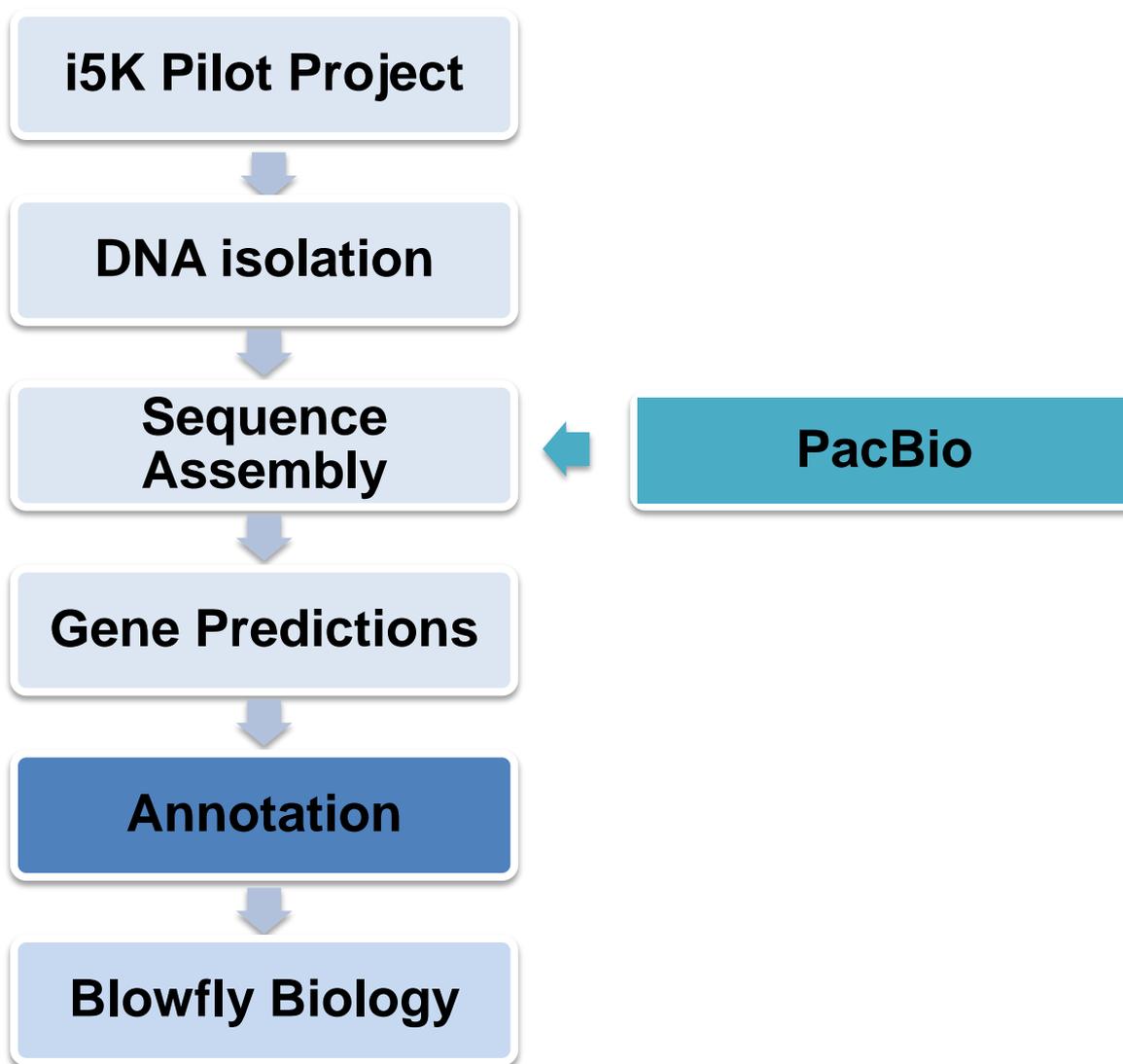


Genome annotation – an ongoing process

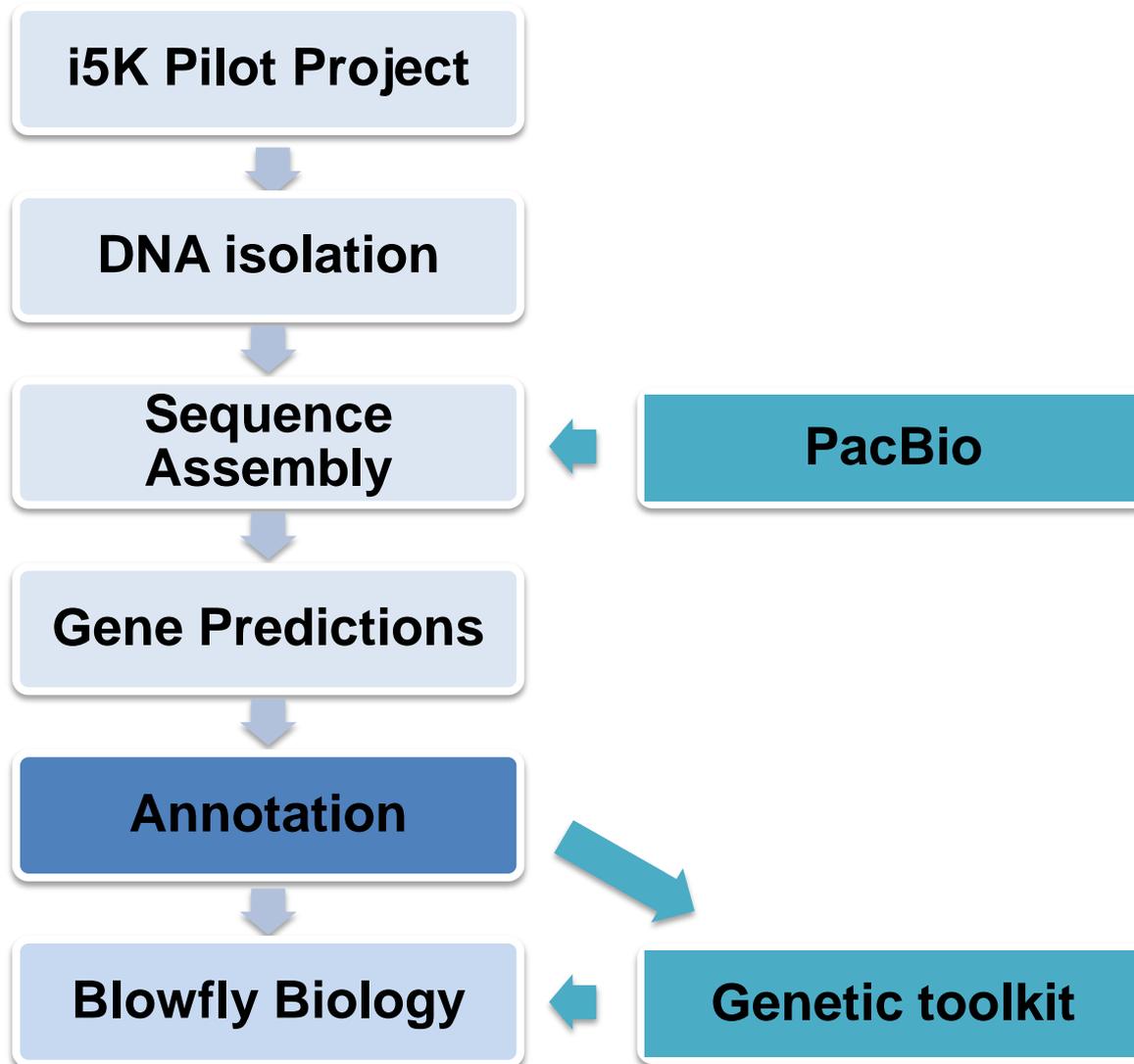


Scaffolds = 4436

Genome annotation – an ongoing process



Genome annotation – an ongoing process



Where to next?

- How do gravid females find sheep?
- What genes are vital for survival and development of *L. cuprina* on sheep? Adult and Larval stages. How can we attack them; Vaccines v Insecticides
- How much migration and variation is there in blowfly populations?
- Can a parasite (*Wolbachia*) assist in the control of this parasite?

.... And how can we resolve these questions?



Creating genetic tools

- Model organisms contribute greatly to our knowledge of gene functions
- Analysis in *D. melanogaster* can bridge some of the gaps
- In some cases genetic manipulation of *L. cuprina* will be required
 - Orphan genes
 - Establishment of myiasis



Genome editing using CRISPR

- CRISPR - Clustered Regularly Interspaced Short Palindromic Repeats
A recent major breakthrough similar to the discovery of DNA
- Involves a nuclease (CAS9) that cuts DNA *in vivo*
- Method successfully adapted across a wide range of species
- Routine in our group using *D. melanogaster* (Vinegar Fly)

Current project

- Create deletion and modification events using CRISPR
- A successful CRISPR event detected in our lab in *L. cuprina*
- Establishing a stable transgenic CAS9 strain of *L. cuprina* to optimize throughput



CRISPR/CAS9 in brief



sgRNAs ▲

CAS9 ◆

Genomic DNA

Desired region for deletion

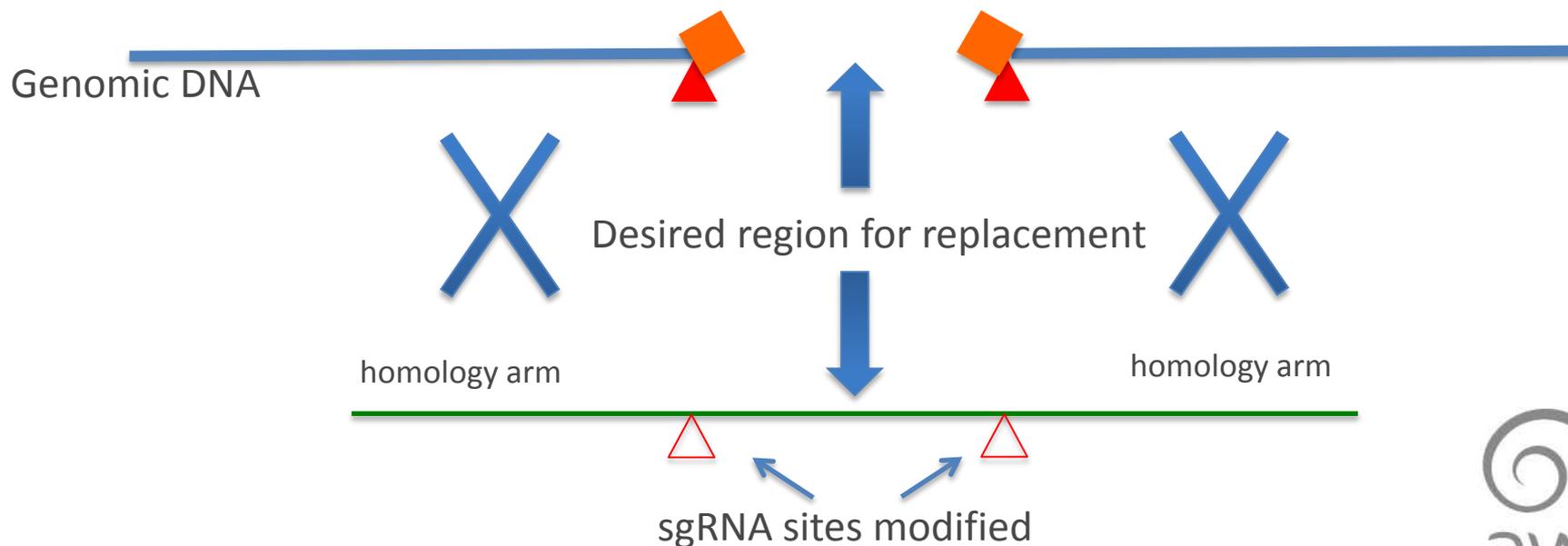


CRISPR/CAS9 in brief



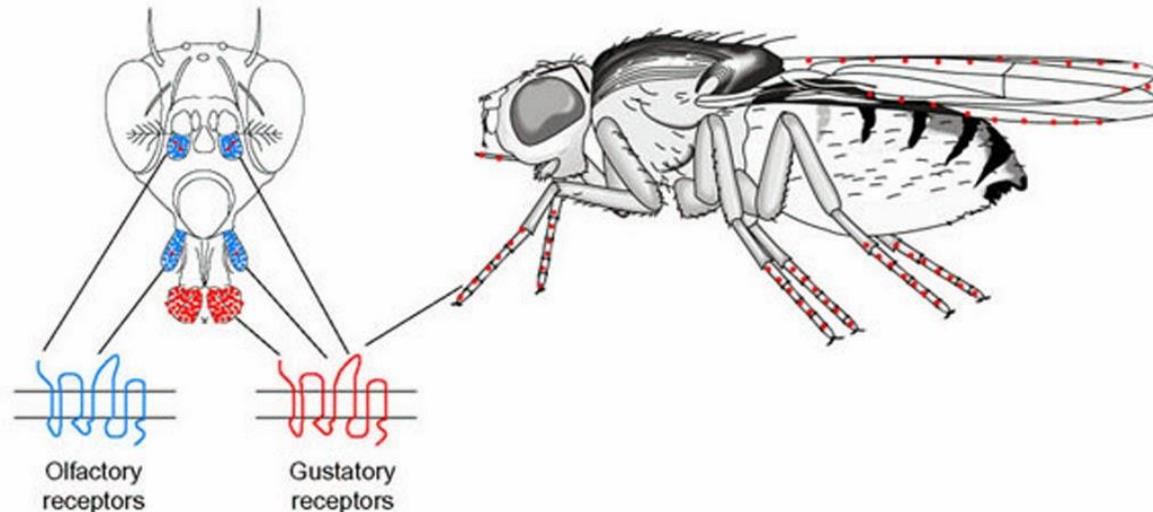
sgRNAs ▲

CAS9 ◆



How do gravid females find sheep?

- Need to identify all olfactory (smell) receptors
- Determine those expressed in gravid female olfactory organs – relative to males and non-gravid females
- Assay olfactory receptors in knockout mutants of blowfly and also in *D. melanogaster* to determine which are involved in responses
- Goal: Development of superior baits for blowfly trapping



What genes are vital for survival and development of *Lucilia* on sheep?

- Developmental time course transcriptomes
- Identify genes important for establishment and maintenance
- Comparison of gene expression differences between growth on live animals (encounter immune response) vs meat
- **Single copy conserved secreted proteins** (vaccine candidates)
- CRISPR validation in blowfly



Single-copy counterparts shared among flies

No genes =
14,554

Sheep Blowfly



Unique
Blowfly
Genes
2,062



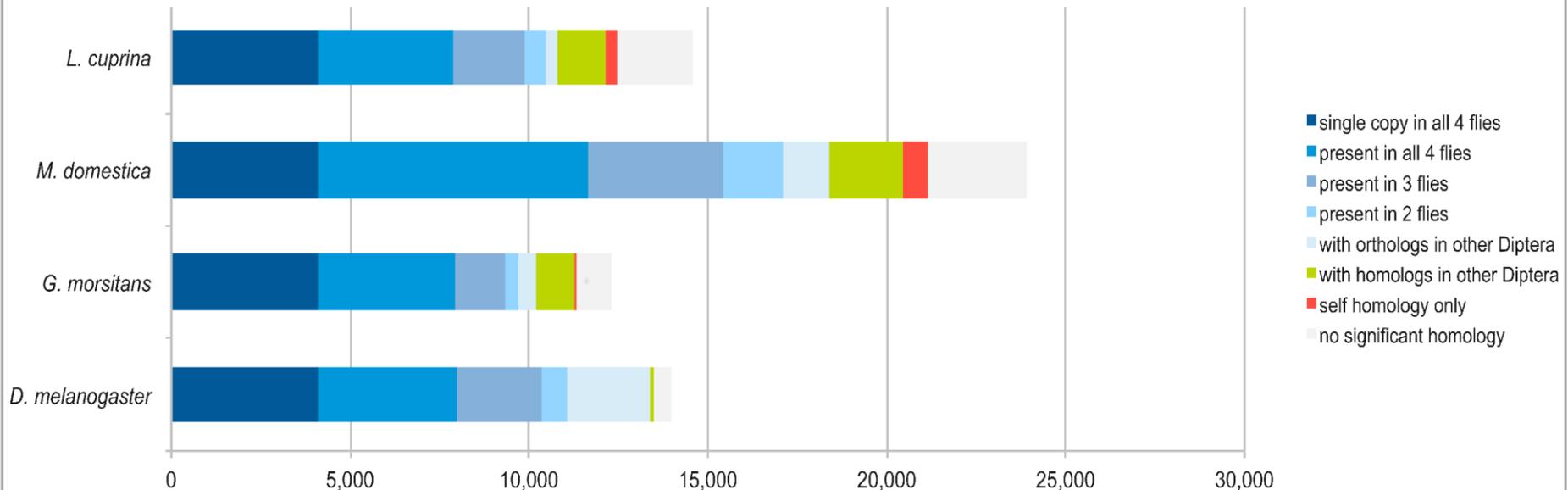
Tsetse Fly
n=6,183



Housefly
n=5,769



Fruit Fly
n=3,222



How much migration and variation is there in blowfly populations?



- Critical information for control
- Vaccine targets must be invariant (always present and do not change)
- One population or many?
- Will test 20 populations, 30 flies each
- Sequence genome from pooled samples
- Assay variation in candidate genes

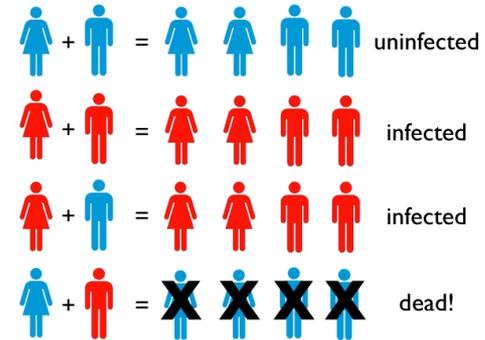


Can a parasite assist in the control of this parasite?

Wolbachia in an animal cell



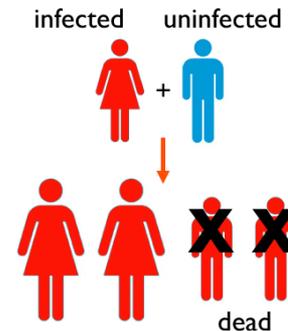
Cytoplasmic Incompatibility



Infected males are incompatible with uninfected females

- Up to 70% of insects species harbor *Wolbachia*
- Does *Lucilia*? (Some evidence to say it might, and lead to pop failure)
- If so, how does it impact fitness?

Male Killing



Wolbachia kills infected males



Summary

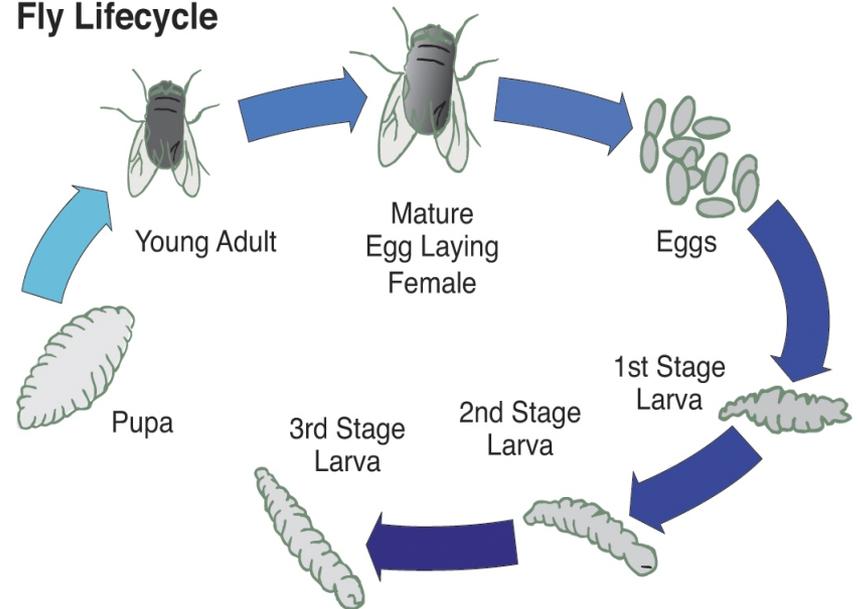
The *L. cuprina* genome will underpin future research

- Establishing genetic methods to manipulate blowfly genes
- Identifying genes critical to establishing a strike
- Characterisation of orphan and critical myiasis maintenance genes
- Unravel population genetics
- Comparative gene analysis

Desirable outcomes:

- New baits, insecticidal therapies and/or an effective vaccine
- Novel intervention strategies (e.g., SIT, CRISPR)

Fly Lifecycle





This publication is based on information presented at the Australian Wool Innovation Limited (AWI) National Wool Research and Development Technical Update on Breech Flystrike Prevention held on 12th July 2016. 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.

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