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Informed Modelling of Sheep Blowfly Chemical Resistance:

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

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EXECUTIVE SUMMARY

Insecticide resistance is a major concern for the sheep industry, which relies on effective applications to control the Australian sheep blowfly, *Lucilia cuprina*, and prevent the onset of myiasis. Dicyclanil and cyromazine are insect growth regulators that may act through interfering with hormones, cuticle formation or aspects of metabolism, although the precise mode of action is unclear. They are long-lasting compounds and have been highly effective at controlling flystrike for several decades. Here we collected a field population of *L. cuprina* from a region of NSW experiencing reduced efficacy of these insect growth regulators. The "GG" strain was 16-fold resistant to dicyclanil and 11-fold resistant to cyromazine, and resistance was incompletely recessive. Fitness assays were performed to determine if resistance carried a cost. Mate competition and overwintering experiments demonstrated the GG strain did not perform worse than control strain, suggesting minimal costs to this level of resistance. Genetic crosses between the GG strain and a control strain were performed, then subjected to multi-generation rounds of selection for dicyclanil or cyromazine resistance. Whole genome sequencing approaches identified two chromosomal regions involved with cyromazine resistance and three regions associated with dicyclanil resistance. Cross resistance was supported at one genetic locus on chromosome 3 and independent regions controlled cyromazine resistance (chromosome 4) and dicyclanil resistance (chromosome 5, 6). This research has determined cyromazine and dicyclanil resistance is polygenic, identified the chromosomal regions that carry major resistance factors for these two chemicals, established a genetic link that may explain one cross resistance factor between dicyclanil and cyromazine, and generated promising results indicating additional and independent chromosomes are involved with resistance to these chemicals. Outcomes of this research can be integrated into informed modelling of sheep blowfly resistance and provide foundational work to identify causal resistance genes.

INTRODUCTION

Recent resistance monitoring research (Sales *et al*, 2020) (ON-00491) has revealed an increasing level and frequency of resistance to dicyclanil and cyromazine in field samples of *Lucilia cuprina (L. cuprina)* in Australia. Resistance is a threat to the continued effectiveness of these two key chemical treatments for the sheep industry, which relies heavily on insect growth regulator chemical treatments for their capacity to provide long-term protection against flystrike. Strategies to slow the spread of dicyclanil and cyromazine resistance through informed flystrike management plans are critical to prolonging their effectiveness. Modelling provides valuable insights into the potential for chemical resistance to spread and advanced computational models are expected to improve the accuracy of the predictions they deliver, and offer better advice based on their outputs.

There are some assumptions required in the current Sheep Blowfly Resistance Model that could have critical effects on predictions of resistance evolution (Benedetti Vallenari *et al*, 2023). Additional genetic research and field studies are expected to enhance these predictions. Recommendations developed under this program, validated by an enhanced Sheep Blowfly Resistance Model, will contribute to consistent and reliable resistance management advice to woolgrowers, helping prolong the effectiveness of existing chemical products, whilst avoiding or delaying the development of resistance of flies to them.

The research questions addressed in this project are linked to an improved understanding of the genetic bases of resistance to the chemicals cyromazine and dicyclanil, and whether there are any associated fitness costs. The outcomes from this project will provide key insights into the blowfly genetics involved in resistance mechanisms and evaluate potential fitness effects on resistant blowflies.

Results from the study of the genetics of resistance to cyromazine and dicyclanil, present in a field collected resistant blowfly strain, will provide information that can be integrated into the Sheep Blowfly Resistance Model. The data generated from this project will support improvements in the modelling of the likely spread, persistence and impact of cyromazine and dicyclanil resistance in *L. cuprina* on current control practices. Evidence based enhancement of the current Sheep Blowfly Resistance Model would determine the key information needed by woolgrowers to resolve critical issues for long-term management of flystrike.

LITERATURE REVIEW

Flystrike has significant impact on the Australian sheep industry (Kotze and James, 2022) and insecticides play a valuable role in pest management. The insecticide cyromazine (N-Cyclopropyl-1,3,5-triazine-2,4,6-triamine) has been an effective chemical control agent against *Lucilia cuprina* and other dipteran pests for more than four decades. It is classed as an insect growth regulator, although the precise mode of action remains unknown (Daborn *et al*, 2000). A second insect growth regulator, dicyclanil, has a chemical structure that is similar to cyromazine and applications have been effective at controlling flystrike for at least 20 weeks (Bowen *et al*, 1999). Resistance to cyromazine and dicyclanil have now been reported in Australian field populations, representing a considerable impact to the sheep industry (Sales *et al*, 2020).

Insecticide resistance is often caused through mutations in genes encoding insecticide receptors, through changing expression levels of receptors (e.g. decreasing abundance) or increasing expression of genes whose protein products can metabolise or sequestering the insecticide. Cross resistance between these two chemicals has previously been demonstrated. A field collected population of *L. cuprina* selected with cyromazine developed ~15-fold resistance, and also showed 20-fold cross-resistance to dicyclanil (Levot and Sales, 2004). Experimental evolution studies have also used the mutagen ethyl methanesulfonate (EMS) on blowflies under laboratory conditions, to create *L. cuprina* strains with 1.5 to 3-fold resistance to cyromazine (Yen *et al*, 1996). Strains with low-levels of cyromazine resistance also showed around 2.5-fold cross resistance to dicyclanil (Magoc *et al*, 2005).

Candidate resistance genes for dicyclanil resistance have previously been identified in the laboratory model, *Drosophila melanogaster*. Transgenic overexpression of two cytochrome P450 genes Cyp6g1 and Cyp12d1 increased resistance to dicyclanil by 2-fold and 1.5 fold, respectively (Daborn *et al*, 2007). Increased abundance of these two enzymes enabled Drosophila to survive higher doses dicyclanil, suggesting mutations that affect regulation of gene expression may cause a level of field resistance in *L. cuprina*. Kotze *et al*. (2022) analysed expression levels of these two P450 genes in *L. cuprina* strain “Walgett”, which was around 20-fold of resistance to dicyclanil (resistance levels varied depending on the generation assayed). Cyp6g1 showed no difference in expression between Walgett and the Laboratory Susceptible strain, but Cyp12d1 had a ~40-fold increase in expression across multiple developmental stages (Kotze *et al*, 2022). Inhibiting or restricting expression levels of P450 genes in the Walgett strain was achieved using the chemical aminobenzotriazole, which reduced dicyclanil resistance from 18-fold to 2.6-fold. This data supported P450 enzymes playing a role in field resistance to dicyclanil. The correlation between Cyp12d1 expression and resistance is of considerable interest.

Random mutagenesis studies in Drosophila have identified candidate resistance genes for cyromazine. The process involved treating strains with chemical mutagen EMS, inducing random changes to the genome, then performing bioassays on isogenic lines. A strain was developed that could survive two-fold higher doses of cyromazine than the parental control strain. Positional cloning and RNAi knockdown experiments identified phosphatidylinositol kinase-like kinase (CG32743, Smg1 on the X chromosome) as the causal gene (Chen *et al*, 2006). Minor levels of cross resistance to dicyclanil were reported for the strain carrying this mutation, rst(1a)cyr, with resistance ratios of 1.58. A second *Drosophila* strain rst(2)Cyr was 3-fold resistant to cyromazine (Daborn *et al*, 2000) and showed 11.6 fold cross resistance with dicyclanil (Magoc *et al*, 2005). Experimental work in *Drosophila* have identified potential candidates for dicyclanil and cyromazine resistance in *L. cuprina* and demonstrated cross-resistance can occur to these chemicals. It should be noted that resistance levels were low in these *Drosophila* studies.

Insecticide resistance is widely assumed to carry a fitness cost in environments without insecticide selection, but recently these ideas have been challenged (ffrench-Constant and Bass, 2017). Mutations in genes that lead to insecticide resistance are expected to perform less well than the wild-type versions. Under stressful environmental conditions such as cool winters, it has been hypothesised that resistance allele frequencies may reduce.

Understanding fitness costs associated with resistance will be useful for informing computational models that predict seasonal fluctuations of pests.

PROJECT OBJECTIVES

The objective of this project was to improve our understanding of the genetic basis of dicyclanil and cyromazine resistance in the Australian sheep blowfly, *Lucilia cuprina*, and provide information that can be integrated into a Sheep Blowfly Resistance Model. We first aimed to characterise the dominance of cyromazine and dicyclanil resistance traits in a field collected blowfly strain with resistance to cyromazine and dicyclanil. Second, we aimed to perform a range of fitness assays to assess whether there is a fitness cost evident in the field collected cyromazine and dicyclanil resistant blowfly strain compared to a susceptible strain. Traits examined were overwintering survival, mating success and detection of the presence of homozygous lethal mutations in the resistant strain. Finally, we sort to identify the gene(s) and mutation involved in the resistance mechanism(s) to cyromazine and dicyclanil present in the field collected resistant blowfly strain.

SUCCESS IN ACHIEVING OBJECTIVES

Number	Description and Summary	
Objective 1 4006896-0020	Characterise the dominance level of dicyclanil and cyromazine resistance traits in a field collected strain.	
1a	Bioassay analysis	The insecticide resistant strain, GG, shows incomplete recessive resistance to dicyclanil (16-fold) and cyromazine (11-fold)
Objective 2 4006896-0030	Perform fitness assays and determine if there is a fitness cost evident in a field collected strain resistant to dicyclanil and cyromazine.	
2a	Overwintering survival	Overwintering assays for 3, 5 and 9 weeks indicate the resistant GG strain did not perform any worse than the laboratory susceptible strain. No fitness cost identified.
2b	Competitive mating assays	Competitive mating assays were performed. No evidence for a fitness cross in the GG strain was found.
2c	Detection of the presence of homozygous lethal mutations in the resistant strain.	Genetic crosses were analysed for homozygous lethal mutations. High levels of polymorphism within LS and GG strains made it difficult to draw strong conclusions from this data.
Objective 3 4006896-0040	Identification of chromosomes, loci and genes associated with dicyclanil and cyromazine resistance.	
3a	Genetic Crosses	Genetic crosses were performed over multiple generations. Two strategies were used, the first to identify potential dominant factors, and the second to identify potential recessive factors, responsible for dicyclanil or cyromazine resistance.
3b	Whole genome sequence analysis	Whole genome sequencing identified three large chromosomal regions associated with dicyclanil resistance and two chromosomal regions associated with cyromazine resistance. One locus is shared and may cause cross resistance. Cyromazine resistance has one recessive factor involved with resistance that appears independent of dicyclanil resistance. Dominant factors, contributing to low levels of resistance, were common to both cyromazine and dicyclanil treated samples.

METHODOLOGY

***Lucilia cuprina* Stains and Culture Conditions.**

Insect population "Laboratory Susceptible", referred to as LS, were obtained from Dr. Peter James (University of Queensland) in 2017, and has been maintained in the laboratory for several decades without insecticide exposure. The LS population has been maintained under laboratory conditions for at least 30 generations at University of Melbourne. Population GG was collected from NSW (-35.8239, 147.9155) in 2020 and was founded from 21 individuals.

Insecticide Preparation

Cyromazine was dissolved in water and desired concentrations produced using serial dilutions. Dicyclanil was first dissolved in 10% DMSO and serial dilutions performed with water. Insecticide was mixed with meat meal and 50 1st instar larvae added per replicate. Sufficient diet was provided to ensure 50 1st instar larvae could reach pupation. Four replicates per insecticide concentration were prepared and six control replicates (without insecticide) were prepared for each assay.

LC_{50} Calculations, Resistance Ratios and Degree of Dominance

Data was analysed using SPSS software, licensed to University of Melbourne. Degree of dominance calculations were obtained using Stone's formula with Log transformed LC_{50} values. $D = (2x\log LC_{RR} - \log LC_{RS} - \log LC_{SS})/ (\log LC_{RR} - \log LC_{SS})$. Values range between –1 (completely recessive) and 1 (completely dominant) (Stone, 1968). Dominance was also calculated using the formula proposed by Bourguet *et al* (1996). Detailed parameter estimates are provided for both dicyclanil (Appendix 2) and cyromazine (Appendix 3). The GG strain was 15.86 fold resistant to dicyclanil and 10.97 fold resistant to cyromazine.

Overwintering Assay

Eggs from each strain were collected by placing a 30 mL plastic cup containing a piece of beef in each cage for approximately 5 hours. Larvae were reared on tinned cat food (Whiskas brand) throughout development, with additional food added as required. Wandering 3rd instars were collected and counted, and then placed in a ventilated box containing vermiculite and stored at 4 degrees Celsius. Following overwintering, boxes were moved to a 27 degree Celsius. Flies that eclosed were counted after three weeks and remaining pupae were assumed to be dead.

Competitive Mating Assay

Competitive mating assays were established in cylindrical vials containing a sugar-yeast diet and beef pieces to stimulate ovipositing. Where possible, eggs were collected from the same female multiple times. Beef and eggs were transferred to small, ventilated plastic containers containing meat meal food source plus vermiculite for pupation. Cages were maintained at 27 degrees Celsius and following emergence, the flies were left to gradually die and were subsequently assessed for phenotypic eye colour to establish paternity.

Homozygous Lethal Mutations

Molecular diagnostic primers were designed using whole genome sequence data generated from the GG strain (based on data from ON-00624). Amplicons were between 100 and 300 bp and contained in silico predicted polymorphisms of variable sizes between the LS and GG strains.

Bioinformatic analysis

Following crossing experiments, flies were stored at -80 °C. Genomic DNA was subsequently isolated from pools of fly heads using the DNeasy Blood and Tissue Kit (Qiagen), with up to 40 flies per pool, representing 80 alleles. DNA from male and female flies were isolated separately. Whole genome sequencing was performed by BGI Tech Solutions

(Hong Kong) (**Table 2**). Data was aligned to Chromosomes 2, 3, 4, 5, 6 and X of the *L. cuprina* genome ASM2204524v1 (NCBI database GCA_022045245), which was originally collected in Los Angeles in 2007 (**Table 1**). Alignments were performed using BWA MEM (version 0.7.17) using default parameters, sorted and index using samtools (version 1.16.1) and duplicate reads removed using picard (version 2.6.0). Genotyping was performed with bctfools mpileup (version 1.15) with the following parameters --redo-BAQ --min-BQ 30 --per-sample-mF. Nucleotide diversity, pi, was then calculated for samples using the vcftools function --window-pi 100000, which determines the level of diversity in 100 kilobase windows. Nucleotide diversity data was imported into the program R for data visualisation using package ggplot2.

Table 1. Chromosome size and gene content of the six *L. cuprina* chromosomes

Chromosome	accession	name	Count	Gene Count	GC %
X	CM039062.1	NC_060949.1	16,485,197	279	30.0
2	CM039057.1	NC_060950.1	71,347,931	2,640	29.5
3	CM039058.1	NC_060951.1	67,696,999	2,996	28.5
4	CM039059.1	NC_060952.1	101,762,404	3,733	28.5
5	CM039060.1	NC_060953.1	71,024,062	2,920	29.0
6	CM039061.1	NC_060954.1	64,449,796	2,979	29.5
Total	-	-	**392,766,389**	**15,547**	-

Table 2. Summary of whole genome sequencing statistics. Clean reads represent the number of high-quality based pairs sequenced. Sequence reads were 150 bp, paired end (PE). Quality is reflected by the Q20(1/100 error) and Q30 (1/1000 error rate). GC% indicates the percentage of G and C bases in the data.

Cross	Sample Description	Pooled Individual	Sample Name	Clean Reads	Read Length	Q20(%)	Q30(%)	GC(%)
Cross A (Dominant)	Cross A Male	1	1_A.MaleParent	200,139,289	150 bp (PE)	97.12	92.48	30.52
	Cross A Female	1	2_A.FemParent	183,963,839	150 bp (PE)	97.17	92.44	30.4
	Control Male pool	15	3_Af3_ConM	199,801,884	150 bp (PE)	97.18	92.43	30.66
	Control Female pool	29	4_Af3_ConF	187,176,943	150 bp (PE)	97.44	93.1	30.61
	Cyromazine Male Pool (0.4 ppm)	40	5_Af3_Cyr0.4M	200,241,352	150 bp (PE)	96.2	90.29	30.95
	Cyromazine Female Pool (0.4 ppm)	40	6_Af3_Cyr0.4F	193,581,167	150 bp (PE)	96.11	90.02	30.84
	Dicyclanil Male Pool (0.06 ppm)	40	7_Af3_Dnl.06M	197,452,624	150 bp (PE)	95.99	89.6	30.78
	Dicyclanil Female Pool (0.06 ppm)	40	8_Af3_Dnl.06F	200,195,191	150 bp (PE)	96.25	90.45	30.83
Cross B (Recessive)	Cross B Male	1	9_B.MaleParent	200,116,504	150 bp (PE)	96.44	90.8	30.77
	Cross B Female	1	10_B.FemaleParent	198,193,974	150 bp (PE)	96.44	90.59	30.64
	Control Male pool	40	11_Bf3_ConM	200,205,676	150 bp (PE)	96.34	90.6	30.97
	Control Female pool	40	12_Bf3_ConF	199,784,814	150 bp (PE)	96.69	91.39	30.8
	Cyromazine Male Pool (1.6 ppm)	40	13_Bf3_Cyr1.6M	163,967,118	150 bp (PE)	95.37	88.74	30.83
	Cyromazine Female Pool (1.6 ppm)	40	14_Bf3_Cyr1.6F	198,380,574	150 bp (PE)	95.37	88.73	30.81
	Dicyclanil Male Pool (0.16 ppm)	40	15_Bf3_Dnl.16M	200,183,691	150 bp (PE)	95.53	89.17	30.88
	Dicyclanil Female Pool (0.16 ppm)	40	16_Bf3_Dnl.16F	184,328,907	150 bp (PE)	95.36	88.68	30

RESULTS

Objective 1: Characterise the dominance level of the dicyclanil and cyromazine resistance in a field collected *Lucilia cuprina* strain.

The Insecticide Resistance Action Committee classifies cyromazine as a Group 17 moulting disrupting insecticide. Dicyclanil is also a moulting disrupting chemical. A larval feeding bioassay was performed using two strains;

Laboratory susceptible strain, "LS"

Field resistant "GG" strain

Hybrid heterozygous strain produced by crossing LS males and GG females

Assays were performed by collecting batches of 50 eggs and placing them on diet containing insecticide. Six insecticide concentrations were screened, with four replicates at each concentration, plus untreated controls. The LC_{50} and LC_{95} values are reported in **Table 3**, and complete dataset is presented in **Appendix 2-3**.

The dicyclanil LC_{50} and LC_{95} resistance ratios were 15.9 and 20.2, compared to the reference strain, LS. For cyromazine, the resistance ratios were 11.0 at LC_{50} and 16.7 at the LC_{95}. Resistance ratios for the hybrid progeny were used to determine the degree of dominance. When applying the lethal concentration expected to kill 50% of the population (LC_{50}), the degree of dominance for dicyclanil was -0.124, and dominance for cyromazine was 0.002. These values indicate resistance is incompletely recessive. A second calculation for dominance, proposed by (Bourguet *et al*, 1996) was also implemented. Here, Dominance, $D = (LD_{50}[RS] - LD_{50}[SS]) / (LD_{50}[RR] - LD_{50}[SS])$. Calculation of the dominance using the above formula to derive values between 0 (completely recessive) and 1 (completely dominant). The dominance value of dicyclanil is (D = 0.159) and cyromazine is (D = 0.233). Both of these values are in agreement with (Stone, 1968) and conclude resistance is within the semi-recessive category, as proposed by (Georghiou, 1969).

Table 3. LC_{50} values, resistance ratios and dominance level for the *L. cuprina* GG strain. Lethal Concentration (LC) values are parts per million (ppm) and dominance (Dom.) was calculated using Stone's formula.

Chemical	Strain	Number	LC_{50} (95% CL)	LC_{50} RR	LC_{50} Dom.	LC_{95} (95% CL)	LC_{95} RR
Dicyclanil	LS	1500	0.014 (0.013 - 0.015)	1.000		0.02 (0.019 - 0.023)	1.000
	GG	1500	0.222 (0.082 - 0.269)	15.857		0.403 (0.318 - 3.11)	20.150
	GGxLS	1050	0.047 (0.024 - 0.063)	3.357	-0.124	0.091 (0.071 - 0.105)	4.550
Cyromazine	LS	1500	0.216 (0.143 - 0.257)	1.000		0.377 (0.317 - 0.577)	1.000
	GG	1500	2.368 (1.188 - 3.219)	10.963		6.284 (4.13 - 87.783)	16.668
	GGxLS	1500	0.717 (0.605 - 0.778)	3.319	0.002	1.046 (0.961 - 1.258)	2.775

Objective 2. Perform fitness assays and determine if there is a fitness cost evident in a field collected strain resistant to dicyclanil and cyromazine

Overwinter Assays

Insecticide resistance traits have long been assumed to have negative fitness costs (ffrench-Constant and Bass, 2017), which may render resistant insects more prone to environmental stress than the wild type, in the absence of insecticide. Overwintering assays were performed to assess the ability of pupae to withstand cold temperature stress. Refrigeration delivered a controlled, sustained temperature to act as a stress from which phenotypic data could be obtained. The pupae were stored at 4 degrees Celsius for 3, 5 or 9 weeks. An untreated control group was not exposed to overwintering conditions, and is categorised as "0 weeks" overwintering.

Assays were performed on three different strains;

LS: the laboratory insecticide susceptible reference strain

GG: Field collected strain with resistance to dicyclanil and cyromazine

HxGG: A hybrid strain generated from crossing female GG flies with heterozygous F_1 males (progeny of GG females and LS males).

Following overwintering treatments, pupae were placed at 27 degrees for a minimum of three weeks. Flies that emerged were counted, providing the emergence rates and pupae that failed to eclose were recorded as dead. A minimum of three replicates were performed for each treatment and strain. Eclosion rates from pupae to fly were recorded for each strain (Figure 1).

In general, increasing the time pupae spent overwintering at 4 degrees increased mortality, and the LS control strain performed worse than GG and hybrid (HxGG) strains at five and nine weeks. To determine if survivorship of the GG strain or hybrid strain were significantly higher than the wild type LS control, two-by-two contingency tables several statistical tests were used.

Odds Ratio: to determine the odds one strain could survive better than the wild type LS strain.

Fisher Exact test: to determine if the odds ratio was statistically significant (p-value)

Chi-squared test: to compare the difference between two strains and provide a significance value (p-value).

The odds ratio determines the odds of the strains of interest surviving (GG and HxGG) compared to the wild type LS strain. For example an odds ratio of 0.50 indicates the test group is half as likely to survive compared to the wild type, and an odds ratio of 4.00 indicates the test group is four times more likely to survive than the wild type. The Fisher exact test determines if the odds ratio is significant. Non-significant results indicate no difference between wild type strain and treatment strain (Table 4, Figure 1, Appendix 4). 95% confidence intervals are provided.

Table 4. Comparison of total pupae survival between wild type (LS) and resistant (GG) strains, and WT versus hybrid strain (HxGG).

Comparison	Weeks	Odds Ratio	95% CI lower	95% CI upper	Fisher Exact	chi-square	chi-square p-value
LS v GG	0	0.89	0.69	1.14	0.3561	0.76395	0.3821
LS v GG	3	1.00	0.68	1.47	1.0	0	1
LS v GG	5	4.12	2.07	8.72	8.53E-06	18.697	1.53E-05

LS v GG	9	Inf.	2.80	Inf.	0.00032	10.189	0.00141
LS v (HxGG)	0	0.88	0.70	1.12	0.3193	0.97568	0.3233
LS v (HxGG)	3	0.76	0.48	1.18	0.2048	1.4142	0.2344
LS v (HxGG)	5	6.59	3.41	13.63	1.42E-10	39.27	3.69E-10
LS v (HxGG)	9	Inf.	7.93	Inf.	2.85E-09	27.632	1.47E-07

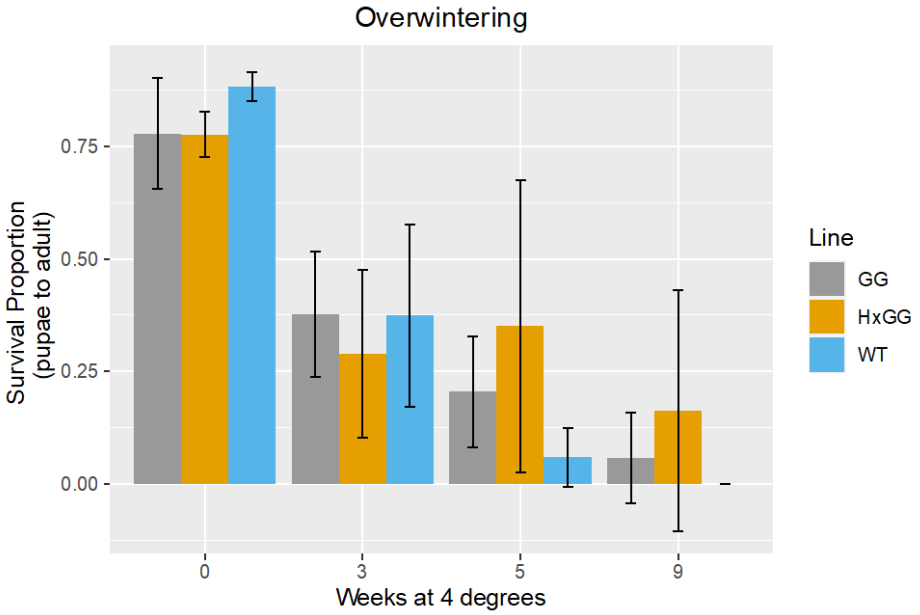

Figure 1. Survivorship from pupae to adult following overwintering stress at 4 degrees for 0, 3, 5 or 9 weeks. LS (WT) is the wild type laboratory reference strain, GG shows resistance to dicyclanil and cyromazine and HxGG are progeny obtained from crossing GG females with heterozygous (GG x WT) males. Error bars are 95% confidence intervals and points represent survivorship proportions of each replicate (adult/pupae). A value of 1.0 indicates all pupae eclosed and a value of 0.0 indicates no pupae survived. Overwintering survival was significantly higher among resistant and hybrid populations at 5 and 9 weeks, relative to the WT laboratory control.

Control treatments survivorship was relatively high for all groups and not significantly different. After three weeks of simulated overwintering, there were no significant differences between strains. However, following five-weeks at 4 degrees Celsius, survivorship of both the GG and HxGG hybrids were significantly higher than WT controls. The longest timepoint assessed was nine-weeks at 4 degrees Celsius. None of the 208 WT pupae survived and as a result, odds ratios could not be calculated (the number is infinite). However, the Fisher exact test and chi-squared test were both significant (Table 4). The LS wild type strain was less able to survive overwintering conditions than GG or HxGG strians. This is probably due the LS strain being maintained in the laboratory over many years in culture. Overwintering simulations therefore do not indicate the GG strain carry a fitness cost.

Competitive Mating Assays

Mate competition can impact allele frequencies in subsequent generations. If insecticide resistant individuals show reduced mating success relative to the wild type, resistance allele frequencies are likely to reduce, in the absence of insecticide selection. Mating competition assays were performed using the following strains:

GG: insecticide resistant

LS: insecticide susceptible wild type

WE: White eye flies, generated from CRIPSR knock out of the gene *white* in the LS strain.

A visible phenotypic marker, white eyes (WE), was used to determine paternity. A previously developed white-eye strain was developed from strain LS by creating a homozygous knockout of the *white* gene, an ATP-binding cassette transporter that is involved with eye pigmentation. The phenotype is completely recessive, as heterozygous individuals for this mutation, with only one *white* allele, cannot be distinguished from the wild type phenotype. If two heterozygotes mate, some of their offspring will have white eyes (25% according to Mendelian laws of segregation) and we can use this to assign paternity. Two males competed for a single heterozygous female in the following crosses (Figure 2).

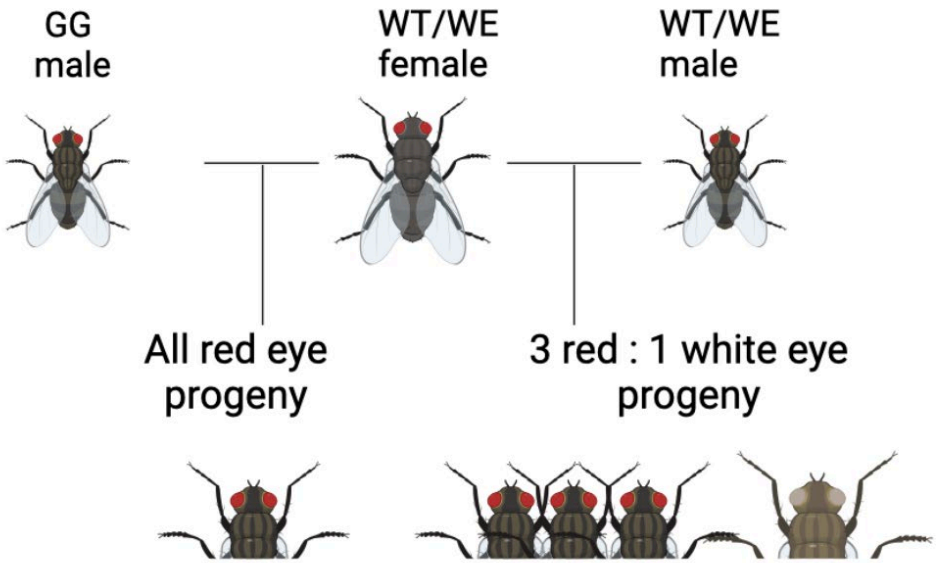

Figure 2. Competitive mating assays highlighting "Cross E" as an example. Paternity can be determined through visual inspection of progeny eye colour. Cages that only produce progeny with normal red eye colour must be sired by the GG male. Cages with some white eye progeny must have been sired by a WT/WE male to produce WE/WE homozygotes.

Three competitive mating strategies were tested and are referred to as Cross E, F and G.

Cross E competed GG males could outcompete LS males for LS females.

Cross F tested if LS males could outcompete GG males for GG females.

Cross G was to determine if there was experimental bias. GG males competed with (GG/WE) males to mate with (GG/WE) females. We expected 50% of the mating assays to have progeny with white eyes and 50% to have progeny with all wild type, normal eyes.

More than 6,000 progeny from crosses were assessed. In most cases, white eye progeny were present in each replicate, which indicates heterozygous male flies (GG/WE or LS/WE) were more competitive than the pure breeding strain (Table 6). These results show most matings occurred between a heterozygous male and female flies, irrespective if the male was GG/WE or LS/WE. The highest number of all-wild type progeny were produced by LS males in Cross F (6/26).

Browne (1958) reported *L. cuprina* virgin females generally mated within 15 minutes when presented with four males. Multiple matings were rare, but could happen. Under rearing conditions at University of Melbourne, timing to copulation of virgin females is highly variable, and can often take multiple days to mate, rather than ~15 minutes. To

improve the likelihood of mating success, we housed two competing males in a 50 mL vial with a single female for up to two weeks. This may have provided the opportunity for females to remate with a second male.

To test whether multiple matings occurred, we analysed deviations from the expected 3 normal:1 white eye ratio. For example, from 100 progeny we would expect 75 normal eye and 25 white eye flies. If we observe 90 normal and 10 white eye progeny, multiple paternity origins may have occurred. Chi-squared tests were performed to determine if the observed ratio of normal:white-eye flies met the expected ratio of 3:1 (Appendix 5). Bonferroni corrections for multiple tests were performed. Cross E did not show any significant deviation (0/21 competitive mating pairs), while evidence from Cross F (3/26) and Cross G (2/15) did support several probable examples of multiple matings.

Table 5. Summary of the competitive crossing strategy, explaining possible outcomes.

Cross Name	Female	Competing males	Possible outcome
E	LS/WE heterozygote	GG males vs. LS/WE males	Progeny with normal eyes have GG father. Progeny white eyes have WT/WE father.
F	GG/WE heterozygote	LS males vs. GG/WE males	Progeny with normal eyes have WT father. Progeny with white eyes have GG/WE father.
G	GG/WE heterozygote	GG males vs. GG/WE males	Progeny with normal eyes have GG father. Progeny with white eyes have GG/WE father.

Cross E: We observed 21/23 replicates had white eye progeny. This result shows LS/WE is more competitive than GG, or the female may have mated with both males. Results indicate wild type LS males were more competitive.

Cross F: High numbers of crosses had white eye progeny; 26/32. This cross suggested GG/WE is more competitive than LS, or multiple female matings occurred. The LS male was the paternal father in 6/32 crosses, which was the highest number of normal eye progeny observed in the experiment.

Cross G: The control cross found 15/16 replicates to have white eye progeny, indicating GG/WE are more competitive than GG, or multiple female matings occurred. We expected 50% of the mating assays to have progeny with white eyes and 50% to have progeny with all wild type, normal eyes. There was a strong bias towards GG/WE matings.

The hybrid males were more competitive among all crosses tested. This may be due to effects such as hybrid vigour, where crossing two outbred individuals together produces progeny that have higher fitness than either of the parental populations. Overall, these crossing experiments do not support a fitness cost (competitive mating success) associated with the GG population.

Table 6. Summary of crossing results show most crossing combinations produced progeny with white eyes. Biological replicates represent individual crosses (one female, two males). Technical replicates (total) include all egg collections from multiple biological replicates.

Cross	Biological Replicates	Technical Replicates (total)	Flies counted	Crosses with normal-eye progeny	Crosses with some white eye progeny	Conclusion
E	22	23	1057	2 (GG male sire)	21 (LS/WE male sire)	LS/WE heterozygotes are more likely to mate than GG homozygotes
F	32	64	3254	6 (LS male sire)	26 (GG/WE male sire)	GG/WE heterozygotes are more likely to mate, LS males are moderate competitors
G	16	38	2137	1 (GG male sire)	15 (GG/WE male sire)	GG/WE heterozygotes are more likely to mate than GG homozygotes

Do Recessive Lethal Mutations affect fitness?

Male dipteran insects have low rates of chromosomal recombination during spermatogenesis (Foster *et al*, 1980; Foster *et al*, 1991). To take advantage of this, genetic crosses were performed to attempt to identify the chromosomes carrying resistant factors, and determine if GG strain carried chromosomes with deleterious recessive lethal mutations that could affect fitness (Figure 3). Mass mating insecticide resistant GG females and LS males, followed by backcrossing the male progeny (F1's) were performed. Backcross larval progeny were screened using insecticidal doses expected to kill ~100% of LS homozygotes, but not LS/GG heterozygotes or GG/GG homozygotes.

Molecular markers were required to assign chromosomes to a parental strain and a series of diagnostic PCR primers were designed for each chromosome. The aim was to determine the origin of chromosomal genotypes (LS/LS origin, LS/GG hybrid or GG/GG). Deviations from expected frequencies indicate the chromosomes carrying resistance factor, as LS/LS carriers are removed at high frequencies, or chromosomes that carry recessive lethal genes, as GG/GG carriers are removed at higher than expected rates.

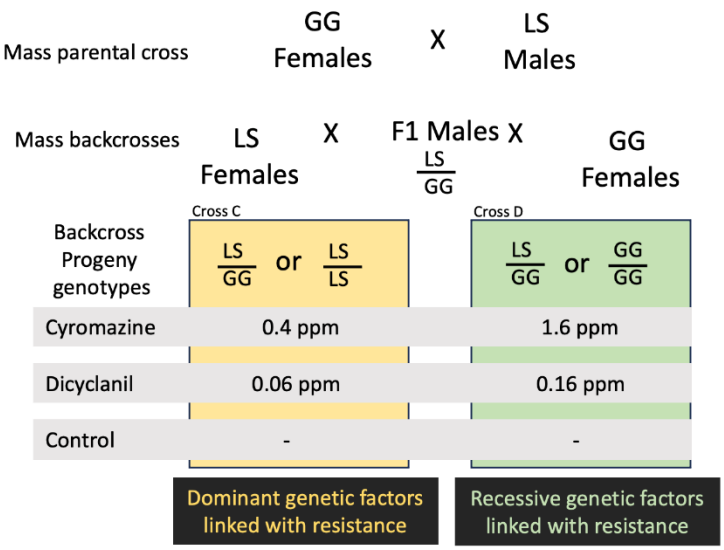

Figure 3. Genetic crosses were performed between GG females and LS males. The F1 male progeny, which were not expected to undergo chromosomal crossing over and recombination, were backcrossed to LS (yellow, cross C) or GG (green, cross D). Backcross progeny were selected using insecticidal doses as indicated. Chromosomes carrying dominant genetic factors were expected to be identified in cross C and recessive genetic factors were expected in cross D.

Ultimately, excessive levels of heterozygosity within both strains meant it was not possible to determine which chromosomes carried resistance factors. It is however unlikely that the GG strain is burdened by recessive lethal mutations. Reasons for this include:

- The strain is robust in laboratory culture
- Overwintering fitness challenges demonstrated the GG strain performed just as well, or better than the lab susceptible strain.
- Crosses between GG/WE males and GG/WE females (Cross G, above) were expected to produce progeny with normal : white eye phenotypes at a ratio of 3 : 1 (1 GG/GG plus 2 GG/WE : 1 WE/WE). This was observed in most cases. If recessive lethal mutations had a significant impact on GG/GG homozygotes and caused death, we would expect to see increased ratios of white-eye progeny among some crosses. This was not observed.

Objective 3. Identification of chromosomes, loci and genes associated with dicyclanil and cyromazine resistance

Despite being maintained in laboratory cultures for several years (GG strain) or several decades (LS strain) considerable levels of genetic variation were present. Crosses were performed between a single male LS and a female GG fly, to minimise the level of genetic variation in a dataset for subsequent genomic analysis. The first filial generation (F_1) hybrid progeny contain chromosomes from both parental strains, and they were used for two crossing strategies:

Cross A: The aim was to identify factors likely to be involved with dominant resistance mechanisms. F_1 progeny were backcrossed to the susceptible LS population to create generation "Backcross 1". Larvae were reared on diet containing 0.06 ppm dicyclanil, 0.4 ppm cyromazine, or no-insecticide controls (**Figure 4**). Survivors were backcrossed to the susceptible LS population two further times, with selection occurring each generation.

Cross B: The aim was to identify the regions of the chromosomes that are contributing to resistance with recessive mechanisms. F_1 progeny were inter-crossed to produce F_2's and these larvae were assayed with 0.16 ppm dicyclanil, 1.6 ppm cyromazine, or no-insecticide controls (**Figure 4**). These doses were greater than the lethal concentration capable of killing >99% of the LS strain (LC_{99}). Surviving progeny of each treatment group were crossed and selection performed for a further two generations.

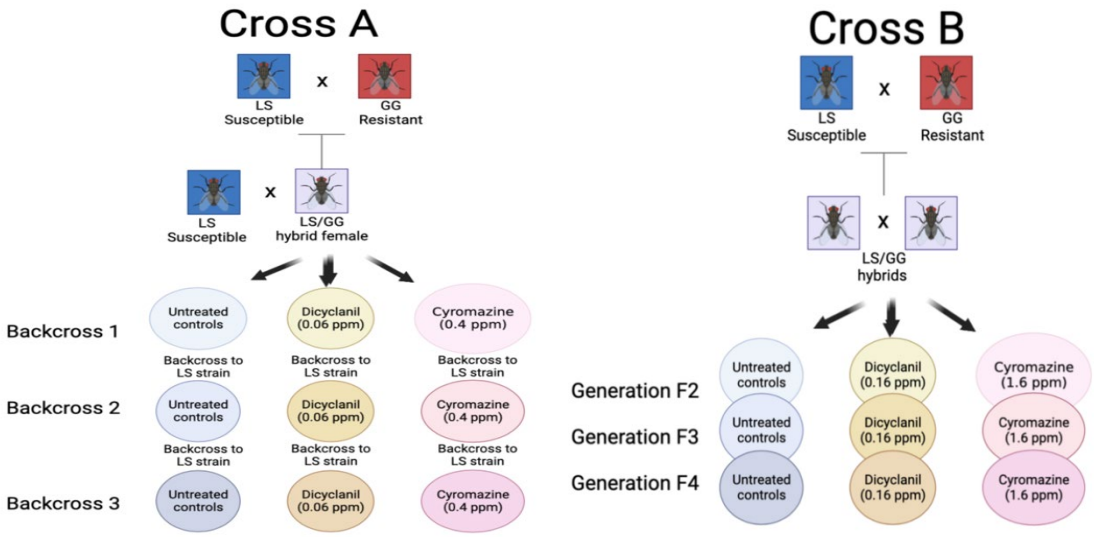

Figure 4. Two different crossing strategies were developed to assess potentially dominant resistance factors (Cross A) or recessive factors (Cross B). Cross A involved repeated backcrossing to the LS strain and reselection with insecticide. Each individual will therefore inherit a maximum of one allele per locus that was derived from the GG strain. Insecticide selection in Cross B was performed at higher concentrations than Cross A, which aimed to identify loci homozygous for GG alleles.

DNA was isolated from pools of forty males and forty females, where possible, following three generations of selection. Fewer than 40 individuals were obtained from the Cross A controls, as survivorship was poor in the final generation (**Table 2**). Whole genome sequencing was performed on pooled DNA and data was aligned to the six *L. cuprina* chromosomes.

Cross A Results

Nucleotide diversity was used as a measure to identify chromosomal regions that may be associated with dominant insecticide resistance factors, which enable heterozygous individuals to survive low doses of cyromazine or dicyclanil (**Table 3**). Crossing strategy A involved repeated backcrossing to the LS strain, followed by selection with low-doses of insecticide. Individuals that survived were expected to have higher than average levels of genetic diversity around resistance loci, as chromosomes from both the GG strain and LS strains should be present in treatment survivors. Untreated control individuals should have lower frequencies of the GG derived chromosomes, and reduced levels of diversity.

Whole genome sequence data was aligned to the *L. cuprina* reference genome, and nucleotide diversity calculated for 100,000 base-pair windows across each chromosome. A calculation to create a ratio was then performed for pairwise comparisons of dicyclanil treated, cyromazine treated and untreated control samples. Nucleotide diversity, π, of treatment *i* was divided by the sum of nucleotide diversity for samples *i* and *j*. $\frac{\pi_i}{\pi_i + \pi_j}$

If there was no difference in nucleotide diversity between two groups, the resulting ratio will be ~0.5. Deviation from a ratio of ~0.5 may indicate insecticide selection is occurring (**Figure 5**).

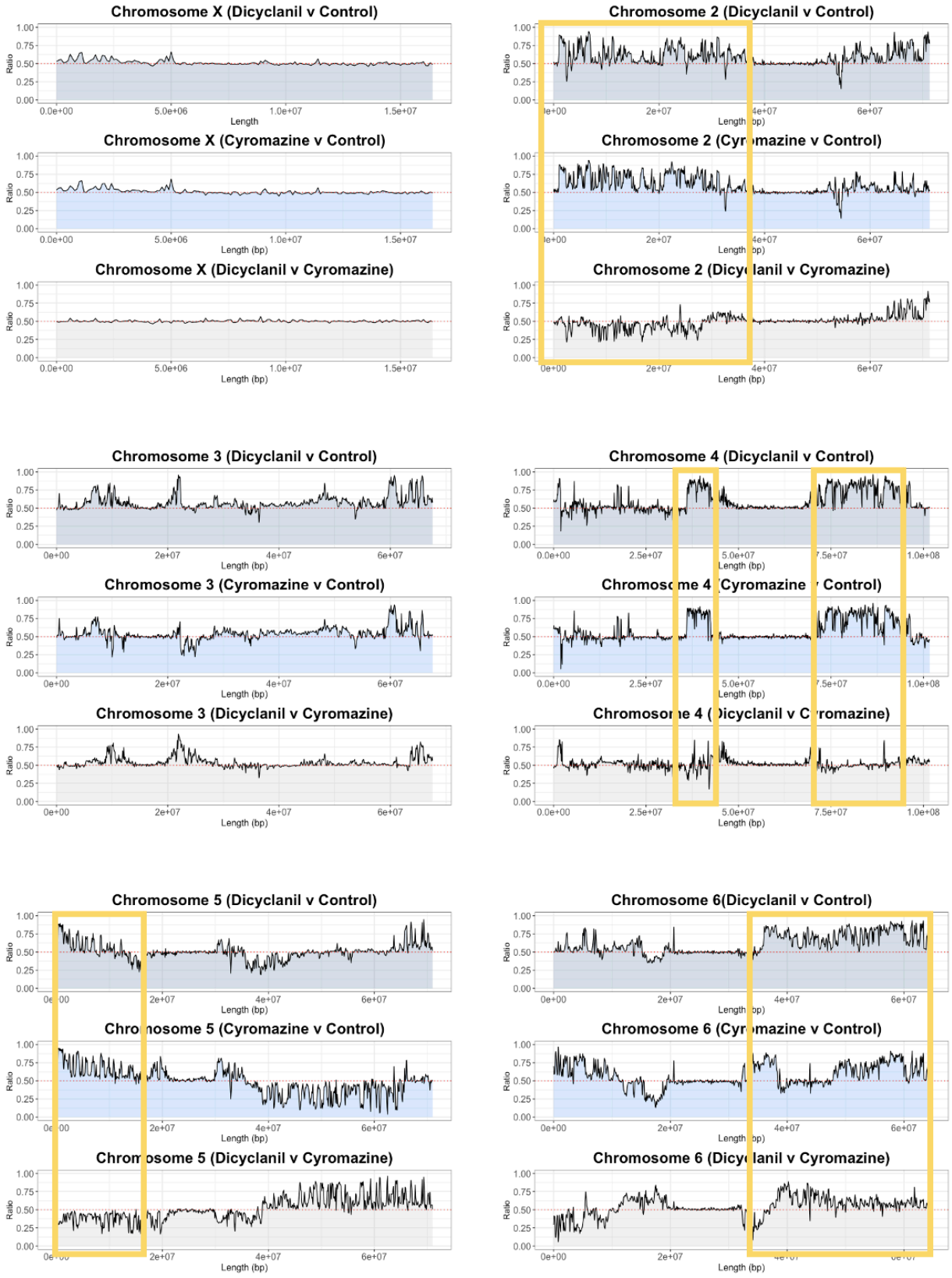

Figure 5. Genomic regions associated with cyromazine resistance and dicyclanil resistance across six *Lucilia cuprina* chromosomes (X, 2, 3, 4, 5, 6) from Cross A (dominant). Yellow boxes indicate elevated levels of genetic diversity, and identify candidate regions for dominant resistance factors that enable heterozygotes to survive low doses of these insecticides. Responses to dicyclanil treatment and cyromazine treatment are similar.

Cross B results

Pools of 40 females from the F_3 generation were sequenced. These flies either had no exposure to insecticide, or three generations of selection on dicyclanil or cyromazine. Following bioinformatic analysis, the nucleotide diversity statistic was used to identify regions of the genome under selection from these chemicals.

Cyromazine resistance loci and dicyclanil resistance loci had different genetic profiles (**Figure 6**). Two regions of the genome were identified from cyromazine selection, including an 8 Mb region of DNA on chromosome 4 plus an 8 Mb region on chromosome 3. Selection for dicyclanil resistance identified three chromosome regions including 14 Mb on chromosome 3, 8 Mb on chromosome 5 and 13 Mb on chromosome 6. Overlap was seen on chromosome 3, suggesting one or more genes in this region may be involved with both cyromazine plus dicyclanil resistance (**Table 7**).

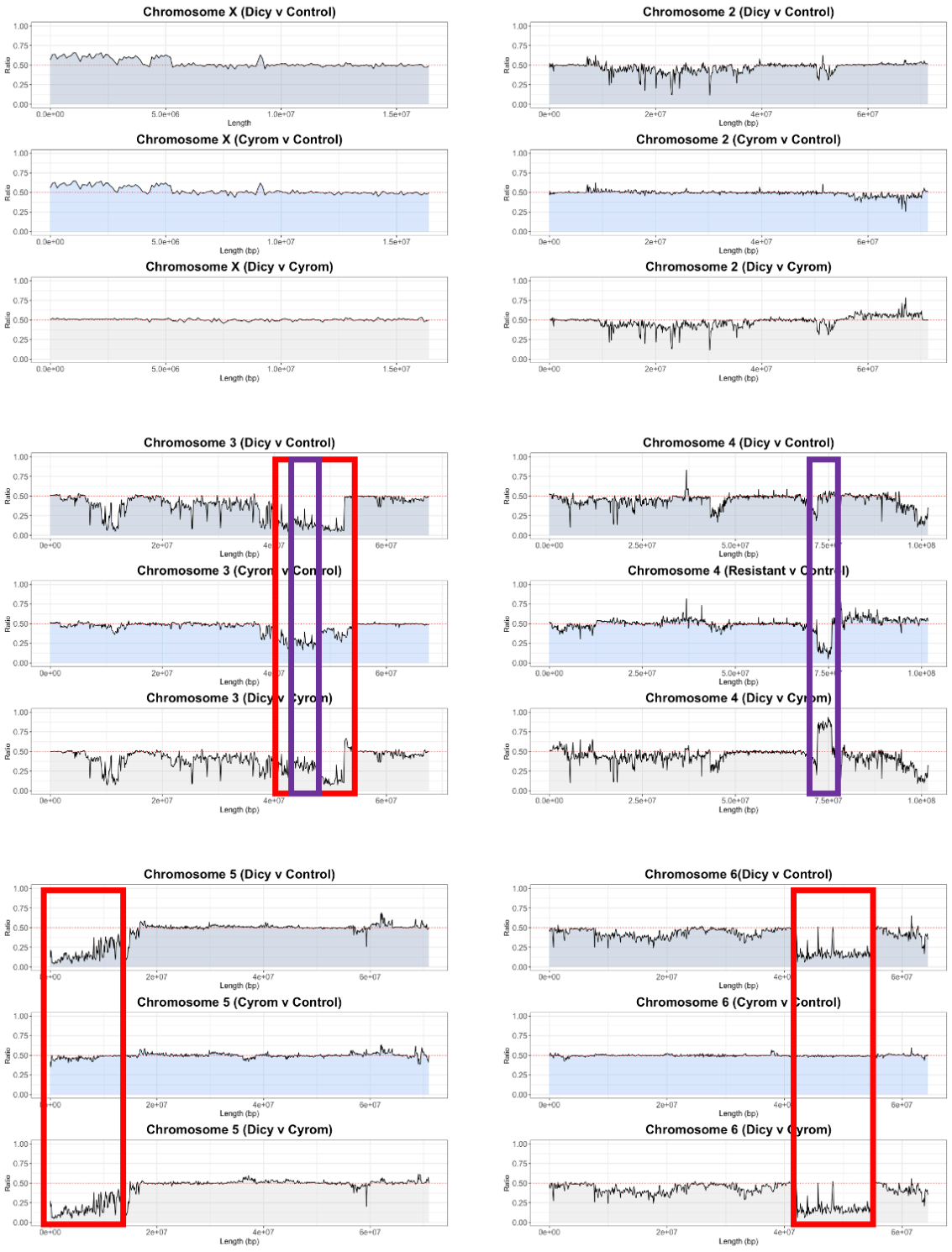

Figure 6. Genomic regions associated with cyromazine resistance and dicyclanil resistance across six *Lucillia cuprina* chromosomes (X, 2, 3, 4, 5, 6) from Cross B (recessive). Cyromazine resistance (purple boxes) or dicyclanil resistance (red boxes) are highlighted. Images are ratios of nucleotide diversity between two groups, for each chromosome (dicyclanil treated vs. control, dark grey; cyromazine treated vs. control, light blue; dicyclanil treated vs. cyromazine treated, light grey).

Table 7. *Genome regions under selection from dicyclanil and cyromazine in the GG population*. Chromosomal regions under selection are summarised from Figure 4 (dominant factors) and Figure 5 (recessive factors). Recessive cyromazine resistance was estimated as a one major and one minor locus based on levels of nucleotide diversity.

Chromosome	Dominant Dicyclanil Resistance	Dominant Cyromazine Resistance	Recessive Dicyclanil Resistance	Recessive Cyromazine Resistance
X	-	-	-	-
2	0 Mb – 35 Mb	0 Mb – 35 Mb	-	-
3	-	-	40 Mb – 54 Mb	40 Mb – 48 Mb (**Minor**)
4	36Mb – 41 Mb 74 Mb - 90 Mb	36 Mb – 41 Mb 76 Mb – 87 Mb	-	70 Mb – 78 Mb (**Major**)
5	0 – 2 Mb	0 – 2 Mb	1 Mb – 8 Mb	-
6	36 Mb – 40 Mb 49 – 64 Mb	33 Mb – 38 Mb 49 – 64 Mb	42 Mb – 55 Mb	-

Comparison of dominant (Cross A) and recessive (Cross B) genomic data.

Dominant genetic factors appear to play a small yet important role in resistance. Heterozygous individuals produced through crossing GG and LS strains are able to tolerate ~3.3-times higher concentrations of cyromazine or dicyclanil than the LS strain. Chromosomal regions under selection for either of these chemicals are broad, yet remarkably consistent.

A single locus on chromosome 4 was associated with recessive cyromazine resistance. The region is approximately 8 million bases and contains many different genes. A second cyromazine resistance locus, on chromosome 3, showed clear overlap with dicyclanil resistance, indicating of cross-resistance to both chemicals may be attributed to one or more genes within this region. Two further regions on chromosome 5 and 6 also produced strong dicyclanil resistance signal.

DISCUSSION

Resistance to cyromazine has been slow to evolve (Baker *et al*, 2014), and resistance to both compounds in the GG strain is incompletely recessive. Low levels of resistance to dicyclanil and cyromazine have previously been selected under laboratory and field conditions, and candidate genes or chromosomal regions subsequently (Chen *et al*, 2006; Daborn *et al*, 2000; Yen *et al*, 1996). Over expression of cytochrome P450 12d1 was found to cause low level dicyclanil resistance in *Drosophila* (Daborn *et al*, 2007). Kotze *et al* (2022) assessed expression of this gene in a field collected *Lucilia cuprina* strain (Walgett) with clear metabolic based resistance. Cyp12d1 was highly expressed in many different life stages of the blowfly. The gene is located on Chromosome 6, at approximately ~59 Mb and falls within a chromosomal region associated with dominant, but not recessive resistance in the GG strain. CYP12d1 was not located in one of the recessive regions, which cause much higher levels of resistance. Over expression of the *L. cuprina* CYP12d1 gene in a controlled genetic background may be required to determine the role this gene plays in resistance, but its contribution is likely to be minor in the GG strain.

Research in *Drosophila* identified a gene, smg1, associated with low levels of cyromazine resistance (Chen *et al*, 2006). This gene is located on Chromosome 3 of *L. cuprina* at position ~55 Mb, and has no genetic association with cyromazine or dicyclanil resistance in the GG strain. A region on chromosome 4 appeared to have a strong association with recessive cyromazine resistance, although there are no predicted cytochrome P450 genes located in this region of the genome.

Genomic analysis has shown resistance to dicyclanil and cyromazine is polygenic, as multiple chromosomal regions show genetic associations with survivorship when blowfly larvae are challenged with these chemicals. One locus on chromosome 3 is common to cyromazine and dicyclanil resistance, supporting a level of cross resistance between the two chemicals (Magoc *et al*, 2005).

Simulated overwintering conditions were applied to determine if the resistant GG strain was likely to carry fitness costs associated with the insecticide resistant phenotype. The strain did not perform worse than the laboratory susceptible control, indicating no clear evidence of a fitness cost. Analysis of fecundity and eclosion rates could potentially be assessed as additional fitness measures, however, comparisons to insecticide susceptible field collected strains would be recommended. The LS strain used in this study has been reared in the laboratory for several decades, and is likely to have undergone selection for these controlled conditions.

This research focused on one *L. cuprina* strain from NSW. Genetic crosses were performed to determine whether the same chromosomal regions, or different chromosomal regions, were involved with dominant and recessive factors. A major assumption of this research is resistance factors carried by the GG strain are also present in other populations. Levels of resistance are likely to vary. Analysis of strains with levels of resistance significantly higher than GG may be useful in identification of additional resistance loci. Investigation of gene expression data is also expected to help identify differentially expressed P450 detoxification genes, or other genetic factors involved with resistance, which could be assigned to chromosomal regions associated with resistance.

IMPACT OF WOOL INDUSTRY – NOW & IN 5 YEARS' TIME

Increasing levels of resistance to dicyclanil and cyromazine will impact the wool industry now, and into the future. Gene flow occurs extensively between *L. cuprina* in the eastern Australian states, and resistance genes could spread relatively quickly. Modelling the outcomes of chemical rotation may improve the efficacy and lifespan of dicyclanil and cyromazine. However, this is likely to involve coordinated efforts among growers to implement consistent spray practices and planned chemical rotations.

Identification of key resistance genes may enable molecular assays to be designed, for use in diagnostic services to woolgrowers with suspected cases of chemical resistant flies. Development of an on-farm diagnostic kit that would provide real-time information to woolgrowers on the resistance status of flies on their property may be useful, but only if resistance remains at low levels. Detection tools, such as loop mediated isothermal amplification (LAMP) kits, may not be particularly useful if resistance is widespread.

Cross resistance is likely to occur between cyromazine and dicyclanil on chromosome 3, producing recessive resistance. A major genetic locus on chromosome 4 is involved with cyromazine resistance. Cross resistance between the two products should not be assumed for all populations. Independent factors may enable both products to be used in rotations, although resistance modelling is required.

CONCLUSION & RECOMMENDATIONS

Cyromazine has been a successful insecticide for more than four decades in Australia, although the precise mechanism of action is unresolved. Resistance to cyromazine and dicyclanil has begun to occur. Here we used fitness assays, genetic crosses and genomic analysis to assess the costs of resistance and identify chromosomal regions involved with resistance to these compounds. The research is based on a single population collected in NSW in 2020, although similar genetic factors are expected to play a role in resistance in other Australian populations.

Extreme overwintering assays did not identify any fitness costs in a resistant population relative to a laboratory reared, insecticide susceptible reference population. Similarly, competitive mating assays did not provide support for fitness costs in the resistant population. Together, lack of a clear fitness cost is not expected to reduce the frequency of resistance alleles when carriers are faced with tough environmental conditions or competition for mating.

Dicyclanil and cyromazine resistance appear to have some common resistance factors, however, a locus on Chromosome 4 is responsible for recessive cyromazine resistance, which is not present in dicyclanil resistant larvae. Identifying the blowfly gene(s) responsible for resistance to dicyclanil and cyromazine will offer crucial insights for the Sheep Blowfly Resistance Model. This includes determining the number of genes involved and confirming whether the resistance mechanisms to these two chemicals are identical, share components, or are entirely independent.

Functional experiments could be performed, by overexpressing *L. cuprina* candidate resistance genes in Drosophila melanogaster, or by using RNAi knockdown experiments in Lucilia.

Once identified, the molecular changes associated with blowfly resistance to cyromazine and dicyclanil would underpin potential next steps for improving the capacity to monitor for the presence and track the spread of resistance in the field.

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LIST OF ABBREVIATIONS AND/OR GLOSSARY

GG: Insect population collected from NSW in 2020 and founded from 21 individuals
LS: Laboratory susceptible population, used as a reference strain

APPENDICES

a. Appendix 1 – AWI Communication Report
b. Appendix 2 – Bioassay results (dicyclanil)
c. Appendix 3 – Bioassay results (cyromazine)
d. Appendix 4 – Overwintering survival
e. Appendix 5 – Competitive mating
f. Appendix 6 – PCR primers
g. Appendix 7 - Research Capacity Building

Appendix 1 – AWI Communication Report

Name of project
Informed Modelling of Sheep Blowfly Chemical Resistance
Name of research body
University of Melbourne
Name(s) of any other project co-funding bodies and funding split
Name(s) of any organisations involved (and specify how they are involved)
NSW-DPI, University of Tasmania. Research generated at University of Melbourne was provided to project collaborators for informed modelling of sheep blowfly chemical resistance
Project start date
16/06/2022
Project end date
30/06/2024
Other key dates (eg key milestones report(s), events , product launch)
Main objectives of the project
The objectives of the project was; • To characterise the dominance level of the dicyclanil and cyromazine resistance traits in a field collected cyromazine and dicyclanil resistant blowfly strain • Performing a range of fitness assays to look at whether there is a fitness cost evident in a field collected cyromazine and dicyclanil resistant blowfly strain compared to a susceptible strainIdentifying the gene(s) and mutation involved in the resistance mechanism(s) to dicyclanil and cyromazine present in a field collected cyromazine and dicyclanil resistant blowfly strain
Project description
The overall research questions addressed in this project are linked to an improved understanding of the genetic bases of resistance to the chemicals cyromazine and dicyclanil, and whether there are any associated fitness costs. The outcomes from this project provided key insights into the blowfly genes involved in the resistance mechanism and evaluated potential fitness effects on resistant blowflies. This research has determined cyromazine and dicyclanil resistance is polygenic, identified the chromosomal regions that carry major resistance factors for these two chemicals, established a genetic link that may explain one cross resistance factor between dicyclanil and cyromazine, and generated promising results indicating additional and independent chromosomes are involved with resistance to these chemicals.

Project (and key milestones) outcomes and outputs
This project has provided information for the Sheep Blowfly Resistance Model. Large genomic datasets have been generated, which will be prepared for research publication. Presentation of data has been made to an Animal Welfare consortium (SWB, 2023) and MerinoLink (Master's student Gregg Wittert, 2023).
Benefits for woolgrowers and wool industry
This project determined resistance to the chemical insecticides cyromazine and dicyclanil occurred within a single NSW population collected in 2020. Resistance is polygenic, meaning multiple genetic factors are involved with resistance to both these chemicals. The same chromosomal region played a role in resistance to cyromazine and dicyclanil and supports a level of cross resistance. However, two independent dicyclanil resistance regions and one independent cyromazine resistant chromosomal regions also were involved with resistance. The outputs of this research can now be modelled to determine the likelihood of resistance occurring, develop strategies that could be used to minimise resistance and model whether both chemicals can be used in rotations.
Is the project related to other AWI-funded or other past/present research
EC249 Data analysis for a decision support model for management of sheep blowfly strike ON-00563 Sheep blowfly resistance strategy ON-00651 Development of a model for flystrike resistance management
Potential/real next steps in the research/project
Several chromosomal regions have been associated with cyromazine and dicyclanil resistance in *L. cuprina*. The next phase of this work would be undertaking further genomic analysis of existing data, including population genetic metrics such as Nei's d_{XY}, assessing allele frequency changes between bioassay survivors and controls and comparing differences between male and female flies. Mining the *L. cuprina* genome across chromosomal regions involved with dicyclanil and cyromazine resistance could be performed to establish a list of candidate genes involved with resistance. Computational analysis using packages could be used to extract protein coding gene sequences from genomic dataset. Genes located at resistance loci, with premature stop codons, major mutations, or under signs of selection could be interpreted as candidate resistance genes. Expression of genes involved with resistance are anticipated to be important. Performing transcriptional analysis of the GG population and LS population will help identify differentially expressed genes at chromosomal regions linked with resistance. Developing molecular markers at resistance loci to link genotype with phenotype among individual flies will provide stronger statistical support for resistance loci. If *L. cuprina* populations arise with considerably higher levels of resistance than the GG population, they could be analysed using genetic approaches, be crossed to the GG strain, to assess complementation (same genes/different genes), and bioassayed using P450 inhibitors to determine if detoxification is a major genetic mechanism Functional analysis of candidate resistance genes could be performed, through CRISPR knock-out of *L .cuprina* genes, or expression of blowfly genes in the model insect Drosophila. Bioassay screening of mutant strains would determine links with resistance.
Names(s)/roles(s)/contact details of the potential spokesperson/people
Dr Simon Baxter | Senior Lecturer School of BioSciences | Faculty of Science BioSciences 4 and Bio21 Institute The University of Melbourne, Victoria 3010 Australia T: +61 3 834 47615 M: 0431 041 082 E: simon.baxter@unimelb.edu.au
Names(s)/roles(s)/contact details of the key personnel in the project that can be contacted for information for communication purposes (if different from above)

Current images/video assets and potential opportunities

Appendix 2 – Bioassay results (dicyclanil)

Parameter estimates for dicyclanil for SPSS

Parameter Estimates							
	Parameter	Estimate	Std. Error	Z	Sig.	95% Confidence Interval	
						Lower Bound	Upper Bound
PROBIT[a]	Conc_GG_Dyc	6.366	.986	6.459	<.001	4.435	8.298
	Intercept	4.156	.573	7.250	<.001	3.583	4.730
a. PROBIT model: PROBIT(p) = Intercept + BX (Covariates X are transformed using the base 10.000 logarithm.)							

Parameter Estimates							
	Parameter	Estimate	Std. Error	Z	Sig.	95% Confidence Interval	
						Lower Bound	Upper Bound
PROBIT[a]	Conc_GGfemxWT_dyc	5.770	1.219	4.734	<.001	3.381	8.159
	Intercept	7.656	1.157	6.617	<.001	6.499	8.813
a. PROBIT model: PROBIT(p) = Intercept + BX (Covariates X are transformed using the base 10.000 logarithm.)							

Parameter Estimates							
	Parameter	Estimate	Std. Error	Z	Sig.	95% Confidence Interval	
						Lower Bound	Upper Bound
PROBIT[a]	Conc_WT_Dyc	10.092	1.706	5.914	<.001	6.747	13.436
	Intercept	18.698	3.036	6.159	<.001	15.662	21.734
a. PROBIT model: PROBIT(p) = Intercept + BX (Covariates X are transformed using the base 10.000 logarithm.)							

Confidence Limits							
	Probability	95% Confidence Limits for Conc_GG_Dyc			95% Confidence Limits for Log(Conc_GG_Dyc)[b]		
		Estimate	Lower Bound	Upper Bound	Estimate	Lower Bound	Upper Bound
PROBIT [a]	.010	.096	.001	.156	-1.018	-3.185	-.807
	.020	.106	.001	.165	-.975	-2.935	-.783
	.030	.113	.002	.171	-.948	-2.777	-.767
	.040	.118	.002	.176	-.928	-2.659	-.755
	.050	.123	.003	.180	-.911	-2.562	-.746
	.060	.127	.003	.183	-.897	-2.480	-.737
	.070	.130	.004	.186	-.885	-2.408	-.730
	.080	.134	.005	.189	-.874	-2.343	-.724
	.090	.137	.005	.192	-.863	-2.285	-.718
	.100	.140	.006	.194	-.854	-2.231	-.712
	.150	.153	.010	.205	-.816	-2.008	-.689
	.200	.164	.015	.214	-.785	-1.832	-.670
	.250	.174	.021	.222	-.759	-1.681	-.653
	.300	.184	.028	.231	-.735	-1.546	-.637
	.350	.193	.038	.239	-.713	-1.422	-.621
	.400	.203	.049	.248	-.693	-1.306	-.605
	.450	.213	.064	.258	-.673	-1.194	-.589
	.500	.222	.082	.269	-.653	-1.087	-.570
	.550	.233	.104	.283	-.633	-.983	-.547
	.600	.244	.131	.303	-.613	-.884	-.519
	.650	.256	.161	.333	-.592	-.792	-.478
	.700	.269	.193	.383	-.570	-.714	-.417
	.750	.284	.222	.472	-.547	-.653	-.326
	.800	.302	.246	.627	-.521	-.609	-.203
	.850	.324	.267	.906	-.490	-.573	-.043
	.900	.354	.289	1.476	-.452	-.539	.169
	.910	.361	.294	1.665	-.442	-.532	.221
	.920	.370	.299	1.898	-.432	-.524	.278
	.930	.379	.305	2.194	-.421	-.516	.341
	.940	.390	.311	2.582	-.409	-.508	.412
	.950	.403	.318	3.110	-.394	-.498	.493
	.960	.419	.326	3.873	-.378	-.487	.588
	.970	.439	.336	5.077	-.357	-.474	.706
	.980	.467	.349	7.283	-.330	-.457	.862
	.990	.516	.370	12.884	-.287	-.431	1.110

a. A heterogeneity factor is used. b. Logarithm base = 10.

Confidence Limits							
	Probability	95% Confidence Limits for Conc_WT_Dyc			95% Confidence Limits for Log(Conc_WT_Dyc)[a]		
		Estimate	Lower Bound	Upper Bound	Estimate	Lower Bound	Upper Bound
PROBIT	.010	.008	.006	.010	-2.083	-2.237	-2.005
	.020	.009	.006	.010	-2.056	-2.197	-1.984
	.030	.009	.007	.011	-2.039	-2.171	-1.971
	.040	.009	.007	.011	-2.026	-2.152	-1.962
	.050	.010	.007	.011	-2.016	-2.137	-1.954
	.060	.010	.008	.011	-2.007	-2.123	-1.947
	.070	.010	.008	.011	-1.999	-2.112	-1.941
	.080	.010	.008	.012	-1.992	-2.101	-1.935
	.090	.010	.008	.012	-1.986	-2.092	-1.931
	.100	.010	.008	.012	-1.980	-2.083	-1.926
	.150	.011	.009	.012	-1.955	-2.047	-1.907
	.200	.012	.010	.013	-1.936	-2.019	-1.892
	.250	.012	.010	.013	-1.920	-1.995	-1.880
	.300	.012	.011	.014	-1.905	-1.973	-1.868
	.350	.013	.011	.014	-1.891	-1.953	-1.857
	.400	.013	.012	.014	-1.878	-1.934	-1.847
	.450	.014	.012	.015	-1.865	-1.916	-1.836
	.500	.014	.013	.015	-1.853	-1.898	-1.826
	.550	.014	.013	.015	-1.840	-1.881	-1.815
	.600	.015	.014	.016	-1.828	-1.863	-1.804
	.650	.015	.014	.016	-1.815	-1.846	-1.792
	.700	.016	.015	.017	-1.801	-1.828	-1.779
	.750	.016	.015	.017	-1.786	-1.810	-1.764
	.800	.017	.016	.018	-1.769	-1.791	-1.745
	.850	.018	.017	.019	-1.750	-1.772	-1.722
	.900	.019	.018	.020	-1.726	-1.749	-1.689
	.910	.019	.018	.021	-1.720	-1.744	-1.681
	.920	.019	.018	.021	-1.714	-1.739	-1.673
	.930	.020	.019	.022	-1.707	-1.733	-1.663
	.940	.020	.019	.022	-1.699	-1.726	-1.652
	.950	.020	.019	.023	-1.690	-1.719	-1.639
	.960	.021	.019	.024	-1.679	-1.710	-1.624
	.970	.022	.020	.025	-1.666	-1.700	-1.605
	.980	.022	.021	.026	-1.649	-1.686	-1.580
	.990	.024	.022	.029	-1.622	-1.665	-1.541

a. Logarithm base = 10

Confidence Limits							
		95% Confidence Limits for Conc_GGfemxWT_Dyc			95% Confidence Limits for Log(Conc_GGfemxWT_Dyc)[a]		
	Probability	Estimate	Lower Bound	Upper Bound	Estimate	Lower Bound	Upper Bound
PROBIT	.010	.019	.005	.032	-1.730	-2.296	-1.493
	.020	.021	.006	.035	-1.683	-2.215	-1.459
	.030	.022	.007	.037	-1.653	-2.165	-1.437
	.040	.023	.007	.038	-1.630	-2.126	-1.421
	.050	.024	.008	.039	-1.612	-2.095	-1.408
	.060	.025	.009	.040	-1.596	-2.069	-1.397
	.070	.026	.009	.041	-1.583	-2.045	-1.387
	.080	.027	.009	.042	-1.571	-2.025	-1.378
	.090	.028	.010	.043	-1.559	-2.006	-1.370
	.100	.028	.010	.043	-1.549	-1.988	-1.363
	.150	.031	.012	.047	-1.507	-1.916	-1.332
	.200	.034	.014	.049	-1.473	-1.859	-1.308
	.250	.036	.015	.052	-1.444	-1.810	-1.287
	.300	.038	.017	.054	-1.418	-1.766	-1.268
	.350	.040	.019	.056	-1.394	-1.726	-1.251
	.400	.043	.021	.058	-1.371	-1.687	-1.234
	.450	.045	.022	.061	-1.349	-1.650	-1.218
	.500	.047	.024	.063	-1.327	-1.613	-1.202
	.550	.050	.027	.065	-1.305	-1.577	-1.186
	.600	.052	.029	.068	-1.283	-1.540	-1.169
	.650	.055	.032	.070	-1.260	-1.501	-1.152
	.700	.058	.035	.073	-1.236	-1.461	-1.134
	.750	.062	.038	.077	-1.210	-1.418	-1.115
	.800	.066	.043	.081	-1.181	-1.370	-1.093
	.850	.071	.048	.086	-1.147	-1.315	-1.066
	.900	.079	.057	.093	-1.105	-1.246	-1.032
	.910	.080	.059	.095	-1.095	-1.230	-1.024
	.920	.083	.061	.097	-1.083	-1.212	-1.015
	.930	.085	.064	.099	-1.071	-1.193	-1.004
	.940	.088	.067	.102	-1.058	-1.172	-.992
	.950	.091	.071	.105	-1.042	-1.148	-.979
	.960	.095	.076	.109	-1.024	-1.121	-.962
	.970	.100	.082	.115	-1.001	-1.088	-.940
	.980	.107	.090	.123	-.971	-1.047	-.909
	.990	.119	.103	.140	-.924	-.988	-.853

a. Logarithm base = 10

Appendix 3 – Bioassay results (cyromazine)

Probit parameter estimates for cyromazine from SPSS

Parameter Estimates							
	Parameter	Estimate	Std. Error	Z	Sig.	95% Confidence Interval	
						Lower Bound	Upper Bound
PROBIT[a]	Conc_GG_Cyr	3.881	.663	5.856	<.001	2.582	5.180
	Intercept	-1.453	.309	-4.707	<.001	-1.762	-1.145
a. PROBIT model: PROBIT(p) = Intercept + BX (Covariates X are transformed using the base 10.000 logarithm.)							

Parameter Estimates							
	Parameter	Estimate	Std. Error	Z	Sig.	95% Confidence Interval	
						Lower Bound	Upper Bound
PROBIT[a]	Conc_WT_Cyr	6.808	.850	8.008	<.001	5.142	8.474
	Intercept	4.530	.478	9.472	<.001	4.052	5.009
a. PROBIT model: PROBIT(p) = Intercept + BX (Covariates X are transformed using the base 10.000 logarithm.)							

Parameter Estimates							
	Parameter	Estimate	Std. Error	Z	Sig.	95% Confidence Interval	
						Lower Bound	Upper Bound
PROBIT[a]	Conc_femGGxWT_Cyr	10.033	1.572	6.384	<.001	6.952	13.113
	Intercept	1.448	.147	9.847	<.001	1.301	1.595
a. PROBIT model: PROBIT(p) = Intercept + BX (Covariates X are transformed using the base 10.000 logarithm.)							

Confidence Limits							
		95% Confidence Limits for Conc_GG_Cyr			95% Confidence Limits for log(Conc_GG_Cyr)[b]		
	Probability	Estimate	Lower Bound	Upper Bound	Estimate	Lower Bound	Upper Bound
PROBIT[a]	.010	.596	.005	1.188	-.225	-2.288	.075
	.020	.700	.010	1.308	-.155	-2.002	.116
	.030	.776	.015	1.390	-.110	-1.821	.143
	.040	.838	.021	1.457	-.077	-1.685	.163
	.050	.893	.027	1.513	-.049	-1.574	.180
	.060	.942	.033	1.564	-.026	-1.480	.194
	.070	.987	.040	1.609	-.006	-1.398	.207
	.080	1.029	.047	1.652	.012	-1.324	.218
	.090	1.069	.055	1.692	.029	-1.257	.228
	.100	1.107	.064	1.729	.044	-1.196	.238
	.150	1.281	.114	1.898	.107	-.942	.278
	.200	1.437	.182	2.051	.158	-.741	.312
	.250	1.587	.269	2.198	.201	-.570	.342
	.300	1.735	.381	2.350	.239	-.419	.371
	.350	1.884	.524	2.513	.275	-.281	.400
	.400	2.038	.703	2.699	.309	-.153	.431
	.450	2.198	.923	2.925	.342	-.035	.466
	.500	2.368	1.188	3.219	.374	.075	.508
	.550	2.552	1.489	3.635	.407	.173	.560
	.600	2.752	1.807	4.265	.440	.257	.630
	.650	2.976	2.116	5.250	.474	.325	.720
	.700	3.232	2.400	6.800	.510	.380	.833
	.750	3.533	2.666	9.273	.548	.426	.967
	.800	3.902	2.932	13.393	.591	.467	1.127
	.850	4.380	3.222	20.895	.641	.508	1.320
	.900	5.065	3.580	37.054	.705	.554	1.569
	.910	5.246	3.667	42.610	.720	.564	1.630
	.920	5.450	3.763	49.616	.736	.576	1.696
	.930	5.684	3.870	58.682	.755	.588	1.769
	.940	5.957	3.990	70.813	.775	.601	1.850
	.950	6.284	4.130	87.783	.798	.616	1.943
	.960	6.691	4.298	113.050	.825	.633	2.053
	.970	7.228	4.511	154.396	.859	.654	2.189
	.980	8.009	4.806	233.878	.904	.682	2.369
	.990	9.415	5.303	450.761	.974	.724	2.654

a. A heterogeneity factor is used. b. Logarithm base = 10

Confidence Limits							
		95% Confidence Limits for Conc_WT_Cyr			95% Confidence Limits for log(Conc_WT_Cyr)[b]		
	Probability	Estimate	Lower Bound	Upper Bound	Estimate	Lower Bound	Upper Bound
PROBIT[a]	.010	.098	.026	.147	-1.007	-1.584	-.834
	.020	.108	.032	.156	-.967	-1.496	-.807
	.030	.114	.036	.162	-.942	-1.440	-.790
	.040	.119	.040	.167	-.923	-1.398	-.777
	.050	.124	.043	.171	-.907	-1.363	-.767
	.060	.128	.046	.175	-.894	-1.334	-.758
	.070	.131	.049	.178	-.882	-1.309	-.750
	.080	.134	.052	.181	-.872	-1.286	-.743
	.090	.137	.054	.183	-.862	-1.265	-.736
	.100	.140	.057	.186	-.854	-1.246	-.730
	.150	.152	.068	.197	-.818	-1.167	-.705
	.200	.163	.079	.206	-.789	-1.105	-.685
	.250	.172	.089	.215	-.765	-1.052	-.668
	.300	.181	.099	.223	-.743	-1.005	-.651
	.350	.190	.109	.231	-.722	-.961	-.636
	.400	.198	.120	.239	-.703	-.920	-.621
	.450	.207	.131	.248	-.684	-.881	-.606
	.500	.216	.143	.257	-.665	-.844	-.591
	.550	.225	.156	.266	-.647	-.807	-.575
	.600	.235	.170	.277	-.628	-.770	-.557
	.650	.246	.185	.290	-.609	-.734	-.538
	.700	.258	.201	.305	-.588	-.697	-.515
	.750	.271	.218	.325	-.566	-.661	-.488
	.800	.287	.238	.351	-.542	-.624	-.454
	.850	.307	.259	.390	-.513	-.587	-.409
	.900	.333	.283	.453	-.477	-.548	-.344
	.910	.340	.289	.470	-.469	-.539	-.328
	.920	.347	.295	.490	-.459	-.531	-.310
	.930	.356	.301	.514	-.449	-.521	-.289
	.940	.366	.308	.542	-.437	-.511	-.266
	.950	.377	.317	.577	-.424	-.500	-.239
	.960	.391	.326	.621	-.408	-.487	-.207
	.970	.408	.338	.680	-.389	-.472	-.167
	.980	.433	.353	.770	-.364	-.452	-.114
	.990	.475	.378	.938	-.324	-.423	-.028

a. A heterogeneity factor is used. b. Logarithm base = 10

Confidence Limits							
		95% Confidence Limits for Conc_femGGxWT_Cyr			95% Confidence Limits for log(Conc_femGGxWT_Cyr)[b]		
	Probability	Estimate	Lower Bound	Upper Bound	Estimate	Lower Bound	Upper Bound
PROBIT[a]	.010	.421	.234	.528	-.376	-.630	-.277
	.020	.448	.262	.552	-.349	-.581	-.258
	.030	.466	.282	.567	-.332	-.550	-.246
	.040	.480	.297	.579	-.319	-.527	-.237
	.050	.492	.311	.589	-.308	-.508	-.230
	.060	.502	.322	.598	-.299	-.492	-.223
	.070	.511	.333	.606	-.291	-.478	-.218
	.080	.520	.343	.613	-.284	-.465	-.213
	.090	.527	.352	.619	-.278	-.454	-.208
	.100	.534	.361	.625	-.272	-.443	-.204
	.150	.565	.399	.651	-.248	-.399	-.186
	.200	.591	.432	.672	-.228	-.365	-.172
	.250	.614	.462	.691	-.212	-.335	-.160
	.300	.636	.491	.709	-.197	-.309	-.149
	.350	.657	.519	.726	-.183	-.285	-.139
	.400	.677	.547	.743	-.170	-.262	-.129
	.450	.697	.576	.760	-.157	-.240	-.119
	.500	.717	.605	.778	-.144	-.218	-.109
	.550	.738	.635	.796	-.132	-.197	-.099
	.600	.760	.666	.816	-.119	-.176	-.088
	.650	.784	.699	.839	-.106	-.155	-.076
	.700	.809	.734	.866	-.092	-.134	-.063
	.750	.837	.771	.899	-.077	-.113	-.046
	.800	.870	.809	.942	-.060	-.092	-.026
	.850	.910	.850	1.003	-.041	-.070	.001
	.900	.963	.897	1.094	-.017	-.047	.039
	.910	.976	.908	1.119	-.011	-.042	.049
	.920	.990	.919	1.146	-.004	-.037	.059
	.930	1.006	.932	1.177	.003	-.031	.071
	.940	1.025	.946	1.214	.011	-.024	.084
	.950	1.046	.961	1.258	.020	-.017	.100
	.960	1.072	.980	1.311	.030	-.009	.118
	.970	1.104	1.002	1.381	.043	.001	.140
	.980	1.149	1.033	1.480	.060	.014	.170
	.990	1.223	1.081	1.653	.088	.034	.218

a. A heterogeneity factor is used. b. Logarithm base = 10

Appendix 4 – Overwintering survival

Strain	Replicates	Weeks (4 d.C.)	Total pupae assessed	Total flies survived
WT	4	0	418	367
GG	4	0	225	176
HxGG	5	0	264	205
WT	3	3	200	75
GG	4	3	218	82
HxGG	3	3	162	46
WT	3	5	250	13
GG	3	5	172	37
HxGG	3	5	154	53
WT	3	9	208	0
GG	3	9	163	10
HxGG	3	9	161	25

Appendix 5 – Competitive mating

Paternity of offspring from competitive mating assays were determined based on progeny eye colour. Progeny with wild type (normal) eye colour were sired by the first male, while progeny with white or wild type (normal) eye colour were sired by the second male. Where white eye flies were present in a cross, ratios of 3 normal : 1 white eye were expected. Deviation from this expectation are highlighted in yellow and represent p-values below the Bonferroni corrected threshold and support the occurrence of multiple matings.

Bonferroni correction for Cross E (0.05/21 = $p < 0.0024$), Cross F (0.05/26 = $p < 0.0019$), Cross G (0.05/15 = $p < 0.0033$).

Cross Type	Cross Number	Normal Eyes	White Eyes	Chi-squared p-value
E	101	34	5	0.079
E	102	41	5	0.027
E	103	62	16	0.360
E	104	5	2	0.827
E	107	6	0	NA
E	109	33	4	0.046
E	111	31	4	0.064
E	115	80	17	0.089
E	116	35	13	0.739
E	117	39	7	0.125
E	120	41	10	0.374
E	121	18	4	0.460
E	123	43	10	0.303
E	124	60	10	0.038
E	124	75	20	0.374
E	125	23	6	0.592
E	127	18	3	0.257
E	128	28	7	0.495
E	129	54	10	0.083
E	130	3	0	NA
E	131	37	10	0.556
E	133	25	6	0.468
E	134	73	24	0.953
F	1	177	41	0.035
F	2	119	26	0.049
F	3	30	0	NA
F	4	51	6	0.012
F	5	209	46	0.010
F	6	196	58	0.425
F	7	45	14	0.822
F	8	38	0	NA
F	10	117	21	0.008
F	12	49	0	NA
F	13	122	29	0.100

F	14	138	4	0.000
F	15	67	15	0.161
F	16	30	0	NA
F	17	89	20	0.109
F	18	35	8	0.333
F	19	177	41	0.035
F	20	1	1	0.414
F	25	56	14	0.334
F	26	80	15	0.038
F	27	60	0	NA
F	28	230	57	0.044
F	31	19	6	0.908
F	32	132	40	0.597
F	33	126	44	0.790
F	35	37	3	0.011
F	36	109	24	0.064
F	37	69	0	NA
F	38	80	18	0.129
F	40	70	8	0.003
F	42	95	14	0.003
F	43	74	24	0.907
G	44	64	20	0.801
G	45	152	48	0.744
G	46	99	20	0.039
G	47	2	1	0.739
G	48	31	8	0.518
G	49	147	27	0.004
G	50	29	7	0.441
G	51	43	6	0.039
G	52	117	23	0.019
G	53	101	13	0.001
G	54	12	5	0.674
G	55	53	0	NA
G	56	114	24	0.039
G	57	135	31	0.060
G	58	89	16	0.021
G	60	87	21	0.182

Appendix 6 – PCR primers

PCR primers developed in insecticide resistance screening (objective 2c)

Forward Primer Name	Forward Primer Sequence	Reverse Primer Name	Reverse Primer Sequence	Product Size
Chr6E196F	GAGCTTGCTCTGTTTTGCTGT	Chr6E196R	ACAAATATTAACGGCGGCGG	196bp
Chr6B306F	TGGATGTTCTTCCCTGGGGA	Chr6B306R	TCTGGATGCGACTGTAAAATGGA	306bp
Chr6A285F	ACTTGGGCTAATCGAAATTTTGGA	Chr6A285R	TCGCCAAATCTTACTGAAACAAA	285bp
Chr6CCYP108F	TGGACTAGTCAATAGACCTGACAG	Chr6CCYP108R	TGCACTATTCTGTTAACCAATCTGTG	108bp
Chr6DCYP163F	CGGGTTCAGTTGTATGGGGA	Chr6DCYP163R	CCAGGGACGAAGCCTATTGA	163bp
ChrXA238F	TAACGCCACACTCTTCGAGG	ChrXA238R	TCATCATTGTTGTGGCCCGT	238bp
ChrXB100F	TGAAAATTGCGGCCTGTACC	ChrXB100R	ACCAATCGGCTTAGAATCATTTTCT	100bp
ChrXC338F	GCCAGTCGACTTCGGATCTAA	ChrXC338R	GTTGTTGCTTCTTTTGTTTTGCT	338bp
Chr2C117F	AGAACCGAACATTGTCCAATTAGT	Chr2C117R	TCGGACGAGCACTCCTTTTC	117bp
Chr2A220F	GTGTGTTAACGATACTCTTTCCTTT	Chr2A220R	TTCCATAGAAGAATCTCCAAATGATAC	220bp
Chr2B350F	TCTTAGGGCTGTTCATGCGC	Chr2B350R	GGGAAAAACGGTCCTATAGGCA	350bp
Chr3B128F	TGGAAGCTATGTTTAAAATTGCGA	Chr3B128R	AGATGTTATGCATTTGTACTGGTTC	128bp
Chr3C279F	TGAGGTATTTGGAAAATTTCAAAAGCA	Chr3C279R	AGGATTCGATGCAAGAGACTT	279bp
Chr3A225F	ACCTGTAGTGGGTGCTACA	Chr3A225R	ACGATCCTGTTTTGCAAAAGAAA	225bp
Chr4A110F	GAGCTTGGTTATGAATTTTACCTACTT	Chr4A110F	GCGTTAAAAACATTAGTAAATCAGCTT	110bp
Chr4C317F	CGACCATAGCAAGGGAATACGA	Chr4C317R	GGTATCCTGTGATCTGAAGCGT	317bp
Chr4B208F	ATGTTTTTGAGTGAAAAGTTGGCA	Chr4B208R	TGGTCGAGTTTTGAGTTCCGT	208bp
Chr5B110F	CTGCTGATTTAACTAGGGAGTAGTC	Chr5B110R	CCGCAGTGTAATTGTTGAACAAG	110bp
Chr5A337F	GGAGAGTTGTCAATTCTCTGCCT	Chr5A337R	CGCATTCGGTTCGGAAACTAATT	337bp
Chr5C224F	GCTCTACTGTGGCCGATAGT	Chr5C224R	AGGGATCACGTCATTTTCACCA	224bp

Appendix 7 – Research Capacity Building

Please include the total number of Masters & PhD students and post-doctoral fellowships attached to this project.

Number of research personnel attached to the project	Total
Number of research Masters students	1 (This project supported Mr. Greg Wittert)
Number of PhD students	0
Number of post-doctoral fellowships	1 (This project supported Dr. Trent Perry)