

**AWI Breech Strike R&D Technical Update
Maritime Museum, Sydney
20th August 2014**

Philip Batterham
University of Melbourne

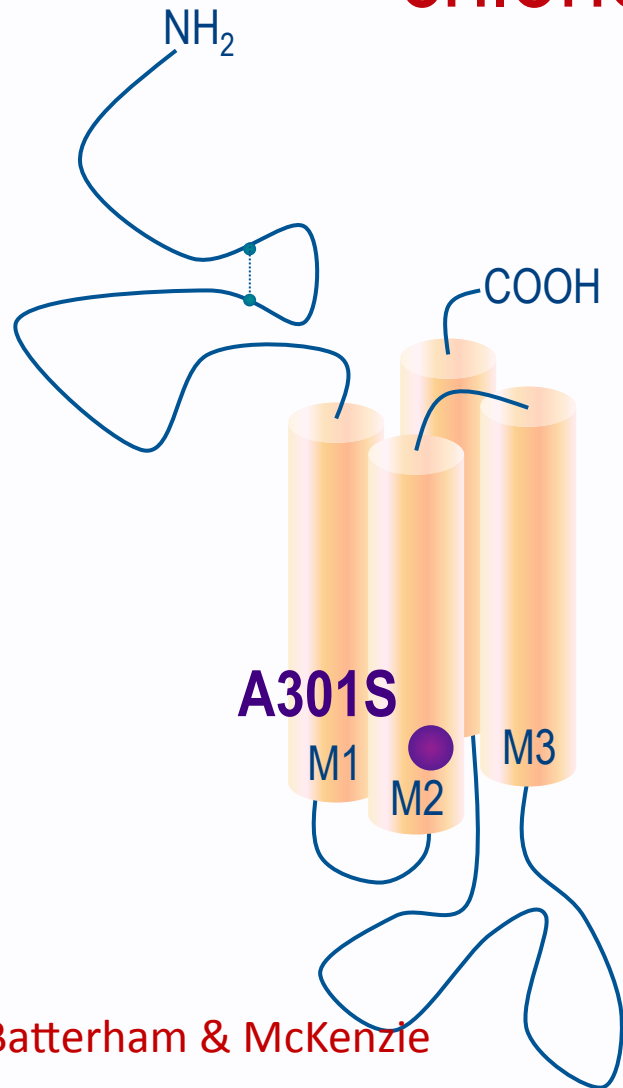


Road to the Genome

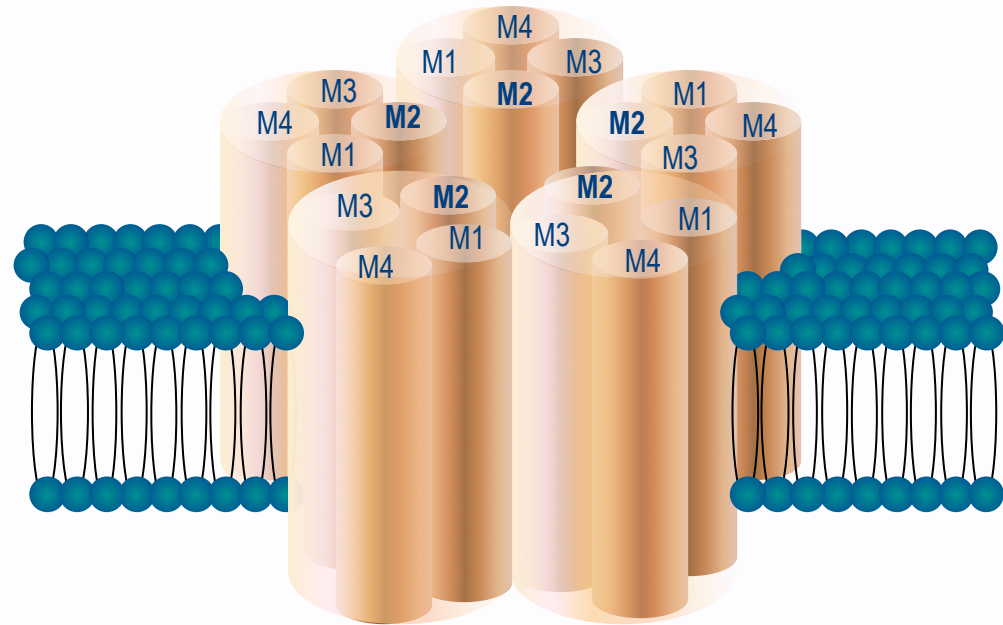


- Blowfly control with Insecticides
 - Insecticide resistance
 - Understanding resistance, new targets?
- Blowfly control with Vaccines??
 - limited knowledge of potential target proteins
- Need for basic biological knowledge – genome sequence as a starting point
- Small scale project – Batterham & Scott
- Current Project (Batterham, Gasser, i5K, James, Kotze)

Dieldrin targets a GABA receptor/ chloride channel subunit



Batterham & McKenzie

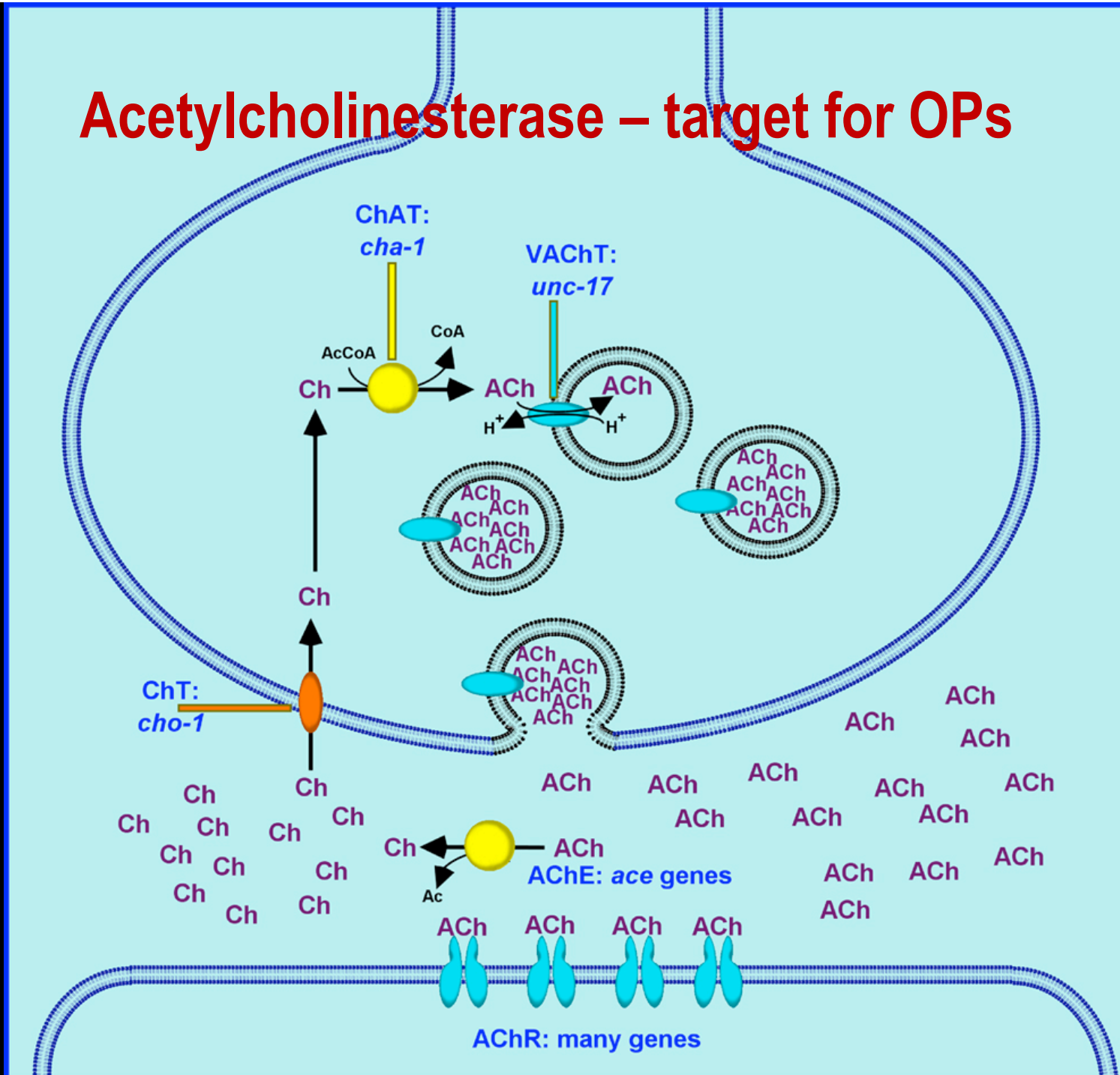


The mutation detected

Rdl^S VTTVGLAVRAPTR

Rdl^R VTTVGLSVRAPTR

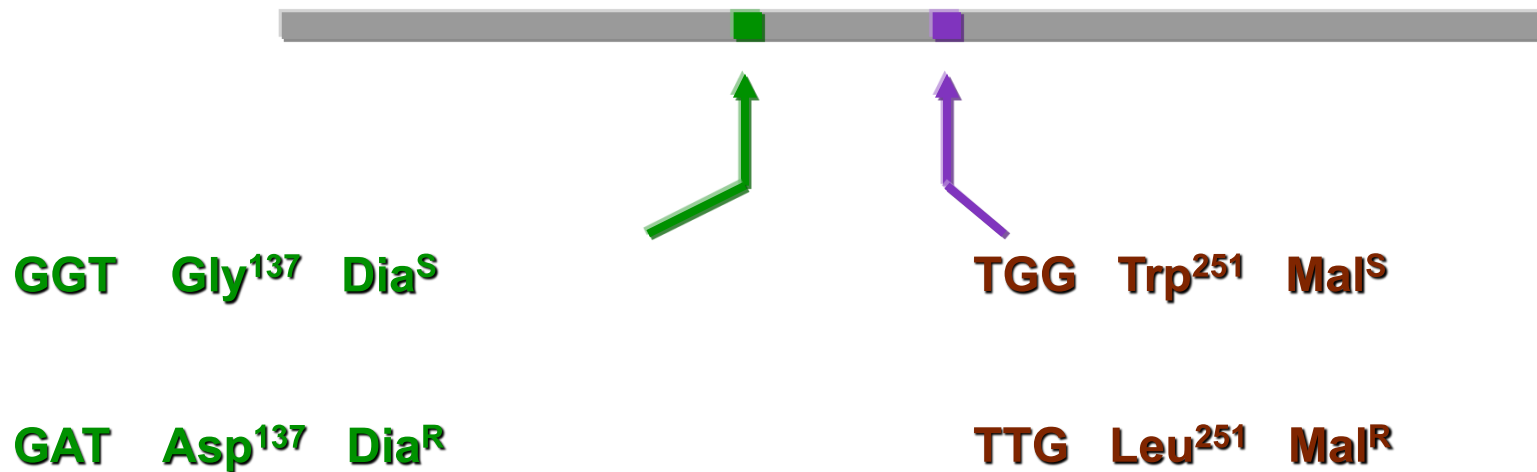
Acetylcholinesterase – target for OPs



Diazinon (OP) Resistance conferred by a mutant carboxylesterase

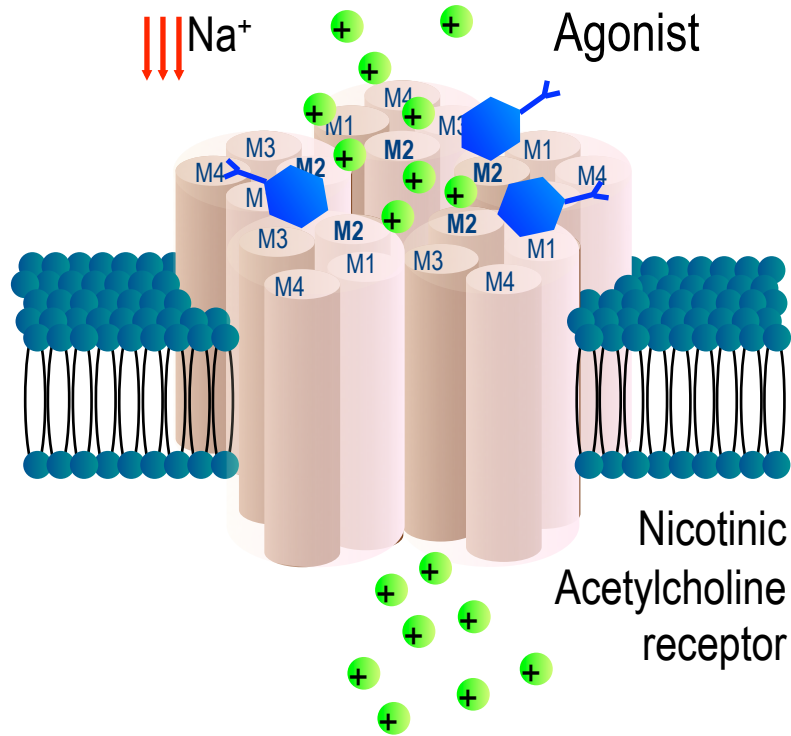
Field Resistance (Newcomb & Oakeshott)

Rop-1 gene



Laboratory Mutants (McKenzie & Batterham)

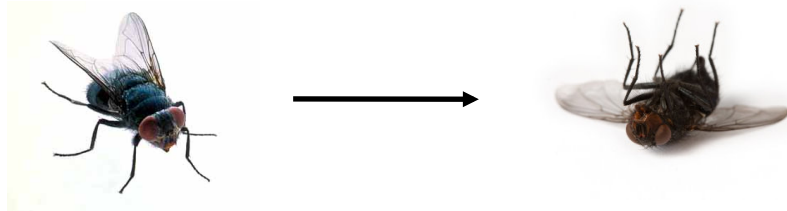
Insecticides targeting Nicotinic Acetylcholine Receptors (nAChRs)



Agonists bind to receptor keeping channel open

Agonists

- Nicotine
- Neonicotinoids
- **Spinosyns**



Agonists not degraded as acetylcholine is

EST Sequencing Project (Batterham)



Lucilia Cuprina EST Database

Home

Sequence Quality

Assembly
Information

Search BLAST

Search InterProScan

Search Gene
Ontologies

Edit and Search
Annotations

Run BLAST Search

Downloads

Read the EST
User Manual

Lucilia Cuprina



Project Statistics	
Total ESTs	29186
Total EST bases	25281113 (866.2 bases per EST)
Total EST q20 bases	17594130 (602.8 q20 bases per EST)
Total EST trimmed bases	15450522 (529.4 trimmed bases per EST)
Total Contigs	2797
Total Contig Bases	2303015 (823.4 bases per contig)
Sequences assembled	24519 (8.8 sequences per contig)
	Lc 880 out of 1663 (53% passed)
	Lce 12990 out of 27006 (48% passed)
	Luc 676 out of 1444 (47% passed)
Total Libraries (4)	Luce 14640 out of 32208 (45% passed) (library statistics include 3' sequences)

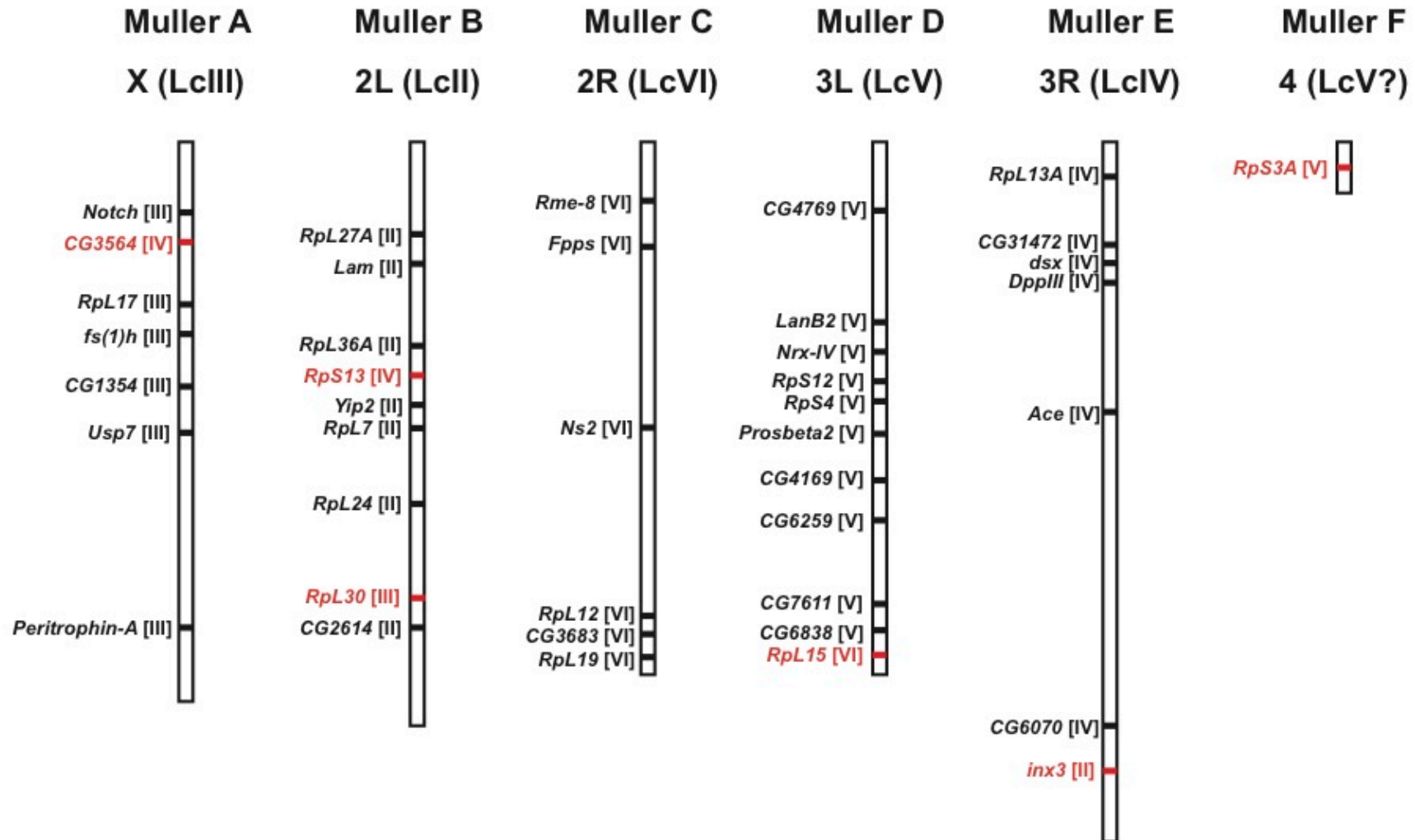
29,186 sequences
(embryo, 1st instar)



7,464 genes

*Assembled using TIGR Gene Indices clustering tools by Annette McGrath AGRF

Conservation of Genetic Maps





awi

Australian Wool
Innovation Limited

Lee et al. *BMC Genomics* 2011, **12**:406
<http://www.biomedcentral.com/1471-2164/12/406>



RESEARCH ARTICLE

Open Access

Identification, analysis, and linkage mapping of expressed sequence tags from the Australian sheep blowfly

Siu F Lee^{1*}†, Zhenzhong Chen^{1†}, Annette McGrath², Robert T Good¹ and Philip Batterham¹



Contents lists available at ScienceDirect

Insect Biochemistry and Molecular Biology

journal homepage: www.elsevier.com/locate/ibmb



Short Communication

Efficient germ-line transformation of the economically important pest species *Lucilia cuprina* and *Lucilia sericata* (Diptera, Calliphoridae)

Carolina Concha ¹, Esther J. Belikoff, Brandi-lee Carey, Fang Li, Anja H. Schiemann, Maxwell J. Scott*

Institute of Molecular BioSciences, Massey University, Private Bag 11222, Palmerston North, New Zealand

Current Project - Partners and Roles

Peter James – inbreeding flies

Andrew Kotze – preparation of nucleic acids (DNA – male and female adults; RNA – adult female, adult male, mixed pre-adult samples)

Stephen Richards et al (Baylor, i5K Pilot) – Sequencing and initial genome assembly

Robin Gasser and Phil Batterham – Assembly and annotation, gene finding

Ultra high-throughput sequencing & supercomputing

De novo genome sequencing and assembly from short sequence read data



nature genetics LETTERS

Whole genome sequence of *Schistosoma haematobium*

10.1038/ng.2500

Abstract

Schistosoma haematobium is a parasitic flatworm that causes schistosomiasis, a major human disease. We have sequenced the genome of *S. haematobium* to a depth of 100x, revealing a genome of 225.2 Mb with 18,100 genes. The genome is highly AT-rich and contains a large number of repetitive elements. We have identified several genes that are unique to *S. haematobium* and may be involved in its parasitic lifestyle. The genome also contains a large number of genes that are shared with other flatworms, suggesting a common ancestor.

Supplementary Information

Supplementary Figure S1: Genomic tracks for the *S. haematobium* genome. The tracks show the genome assembly, gene models, and various annotations. The genome is highly AT-rich and contains a large number of repetitive elements.

LETTER

Ascaris suum draft genome

10.1038/ng.2501

Abstract

Ascaris suum is a parasitic nematode that causes ascaridosis in pigs. We have sequenced the draft genome of *A. suum* to a depth of 100x, revealing a genome of 180 Mb with 12,000 genes. The genome is highly AT-rich and contains a large number of repetitive elements. We have identified several genes that are unique to *A. suum* and may be involved in its parasitic lifestyle. The genome also contains a large number of genes that are shared with other nematodes, suggesting a common ancestor.

Supplementary Information

Supplementary Figure S1: Genomic tracks for the *A. suum* genome. The tracks show the genome assembly, gene models, and various annotations. The genome is highly AT-rich and contains a large number of repetitive elements.

nature genetics ARTICLES

Genome of the human hookworm *Necator americanus*

10.1038/ng.2502

Abstract

Necator americanus is a parasitic nematode that causes hookworm disease in humans. We have sequenced the genome of *N. americanus* to a depth of 100x, revealing a genome of 180 Mb with 12,000 genes. The genome is highly AT-rich and contains a large number of repetitive elements. We have identified several genes that are unique to *N. americanus* and may be involved in its parasitic lifestyle. The genome also contains a large number of genes that are shared with other nematodes, suggesting a common ancestor.

Supplementary Information

Supplementary Figure S1: Genomic tracks for the *N. americanus* genome. The tracks show the genome assembly, gene models, and various annotations. The genome is highly AT-rich and contains a large number of repetitive elements.

Genome Biology RESEARCH

The genome and developmental transcriptome of the strongyloid nematode *Haemonchus contortus*

10.1038/ng.2503

Abstract

Haemonchus contortus is a parasitic nematode that causes anemia in livestock. We have sequenced the genome and developmental transcriptome of *H. contortus* to a depth of 100x, revealing a genome of 180 Mb with 12,000 genes. The genome is highly AT-rich and contains a large number of repetitive elements. We have identified several genes that are unique to *H. contortus* and may be involved in its parasitic lifestyle. The transcriptome data provide insights into the developmental processes of this parasite.

Supplementary Information

Supplementary Figure S1: Genomic tracks for the *H. contortus* genome. The tracks show the genome assembly, gene models, and various annotations. The genome is highly AT-rich and contains a large number of repetitive elements.

NCBI

FlyBase

Abstract

FlyBase is a comprehensive database of the genome and transcriptome of the fruit fly *Drosophila melanogaster*. It provides access to a wealth of genomic data, including the genome assembly, gene models, and transcriptome data. FlyBase is a valuable resource for researchers studying the genetics and development of *Drosophila*.

Supplementary Information

Supplementary Figure S1: Genomic tracks for the *Drosophila melanogaster* genome. The tracks show the genome assembly, gene models, and various annotations. The genome is highly AT-rich and contains a large number of repetitive elements.

Genome comparison – By the numbers

	<i>Lucilia cuprina</i>	<i>Drosophila melanogaster</i>	<i>Musca domestica</i>
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	14,544	15,771	17,508
Repetitive seq. (%)	33.0	36.0	Not yet known
GC content	30	42	35

Genome annotation

Key protein groups^a	Number predicted
Secretome	2,257
Channels and transporters	552
Peptidases	402
Kinases	291
Phosphatases	250
G protein-coupled receptors (GPCRs)	216
GTPases	97
Ligand-gated ion channels (LGICs)	61
Major sperm proteins (MSPs)	34
Peptidase inhibitors	45
Vitellogenins	20
Excretory/secretory (ES) proteins	15

^a Based on first “freeze” of the genome;
some predicted proteins belong to multiple categories

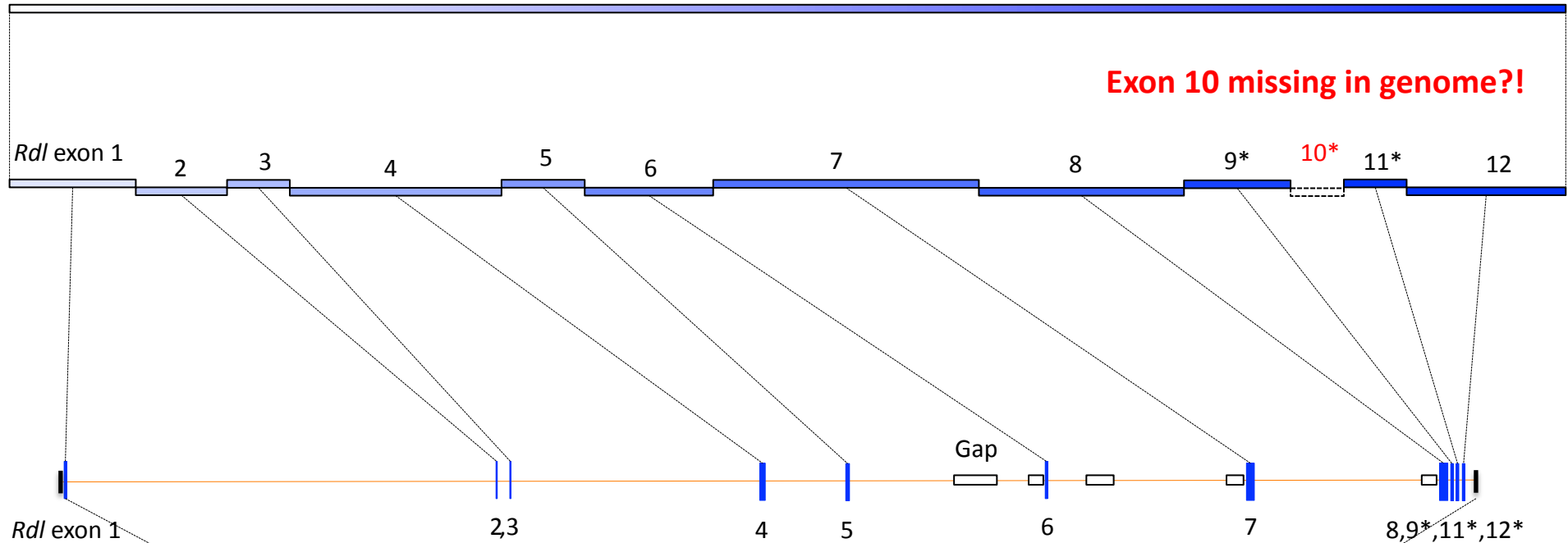
Blowfly Genome



- 14,466 genes identified
- 11,962 genes have a counterpart in housefly
- **2,950 of these unique to the blowfly!!**
 - Tsetse fly has 2,803 unique genes

Annotation of the *Resistance to dieldrin (Rdl)* locus

Rdl coding sequence [GI:2565319; 1653 bp]



Exon 10 missing in genome?!

Rdl exon 1

2

3

4

5

6

7

Gap

8

9*

10*

11*

12

8,9*,11*,12*

Rdl region 56535 bp

Ronald Lee

Scaffold568

1

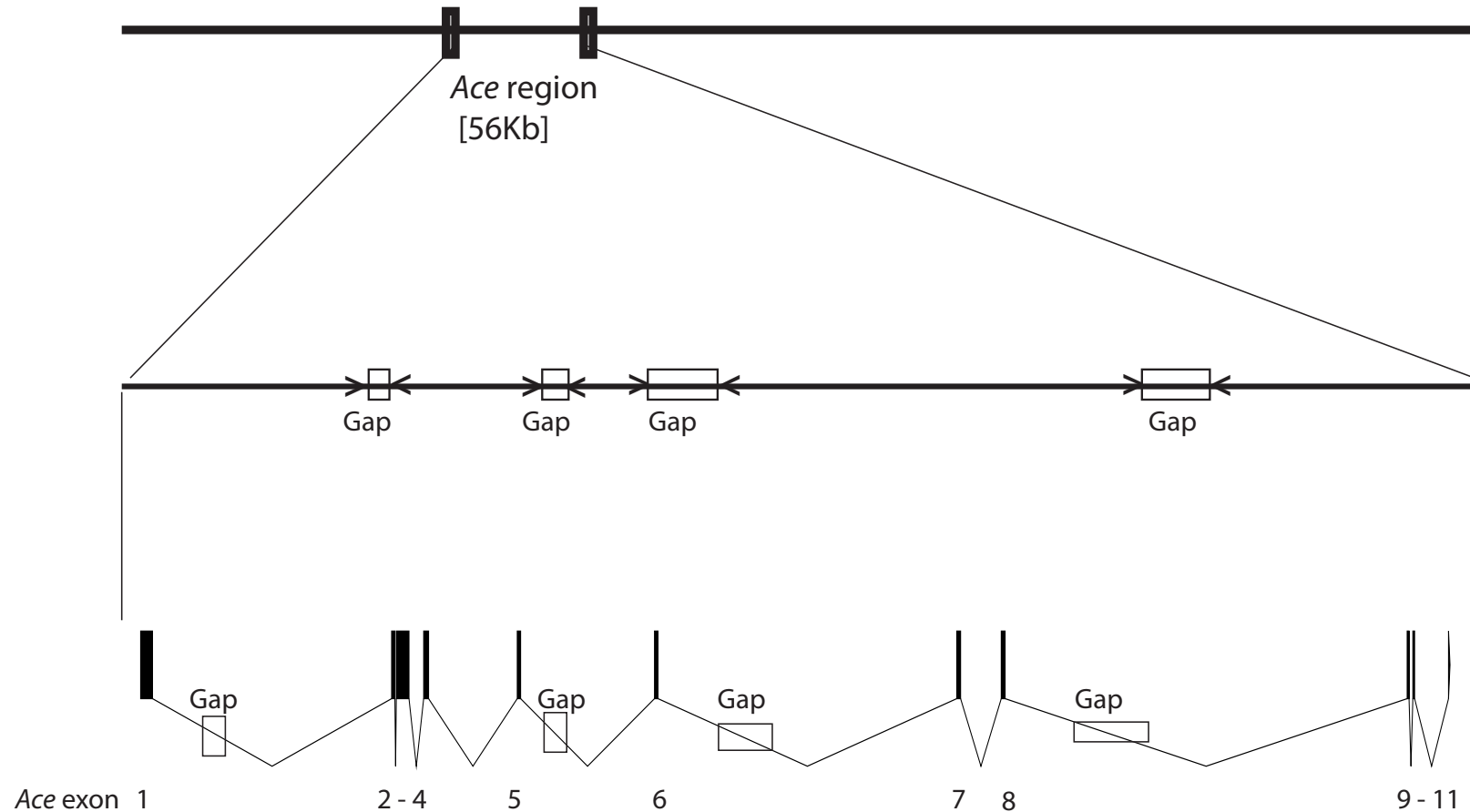
131,720

188,254

345,065

Annotation of the *AChE* gene

Lucilia cuprina genome scaffold105 [1.4Mb]



ACE CDS

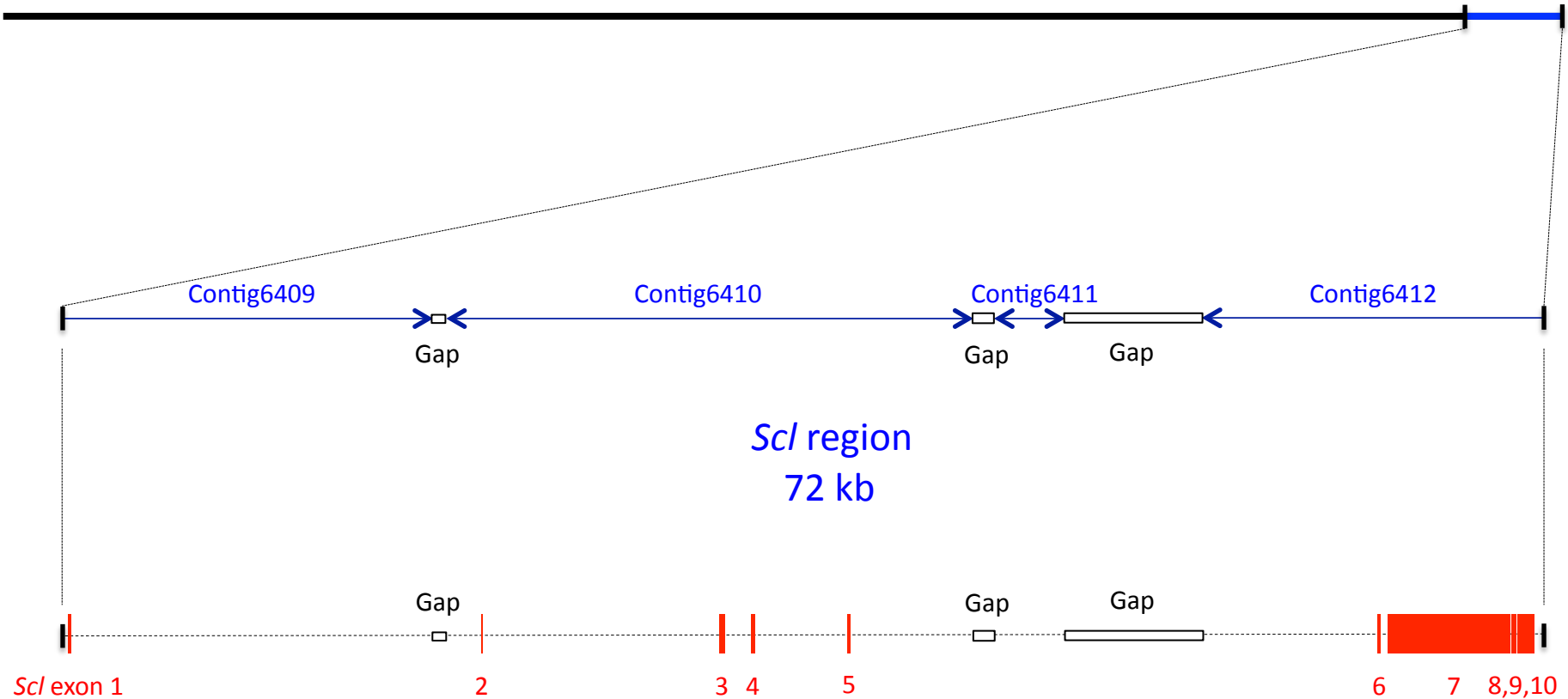


CDS: 2,247bp; 11 exons
Protein: 749 AA

Bert Breugelmans

Annotation of the *Scalloped wings (Scl)* gene

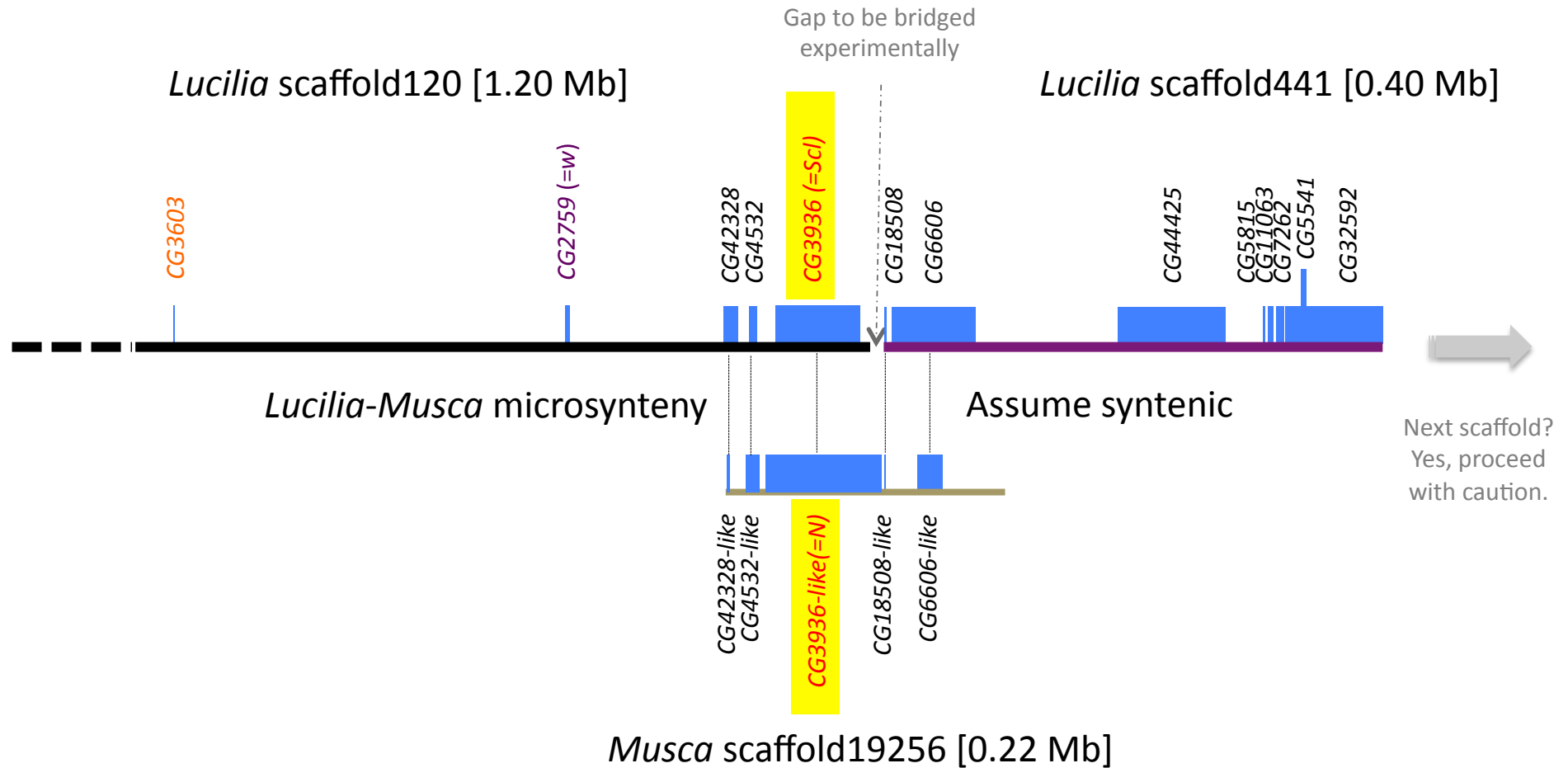
Lucilia cuprina genome scaffold 120 [1.2 Mb]



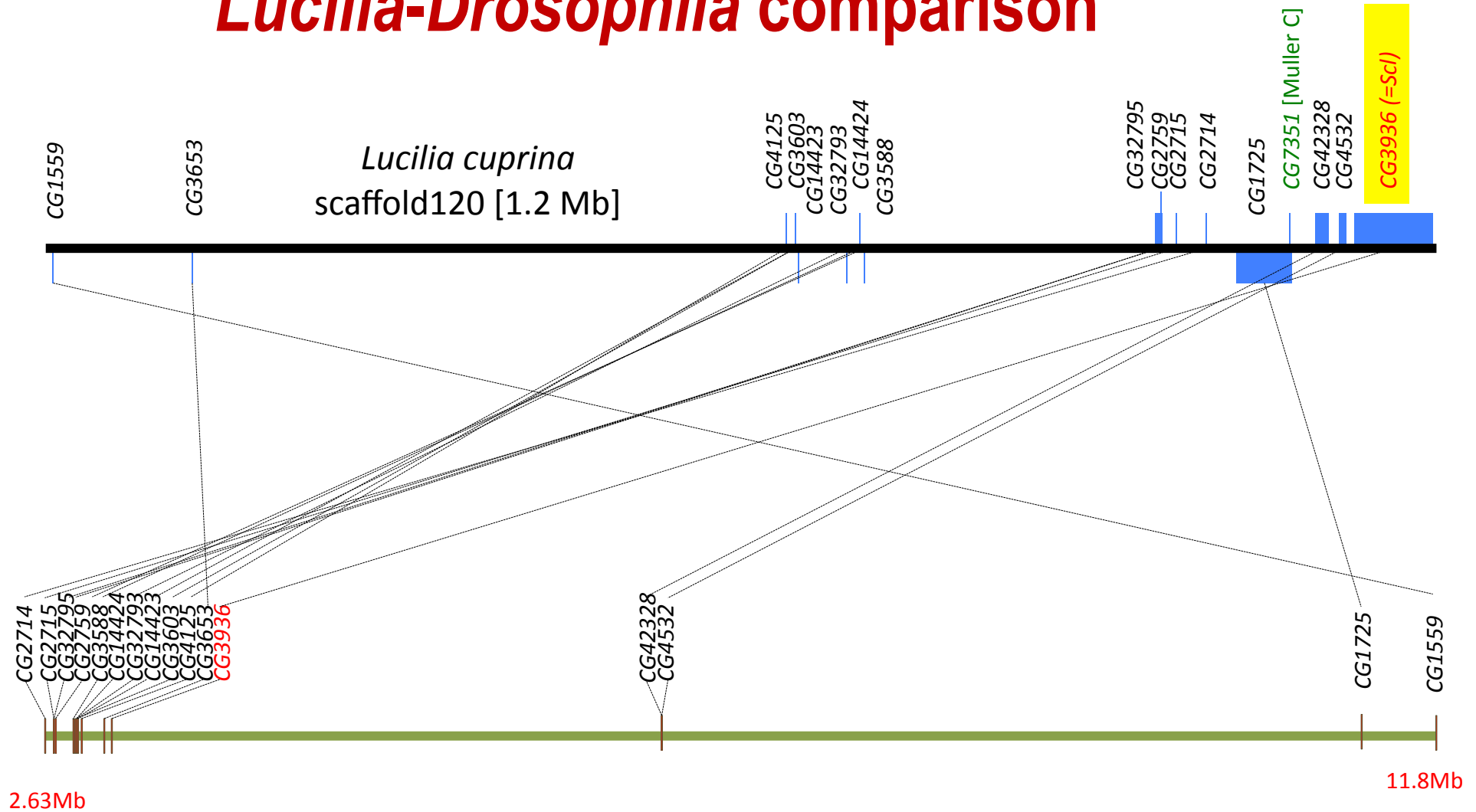
Scl complete coding sequence: 7962 bp; exons 1-10
(Chen et al 1998; GI: 1389670)

Ronald Lee

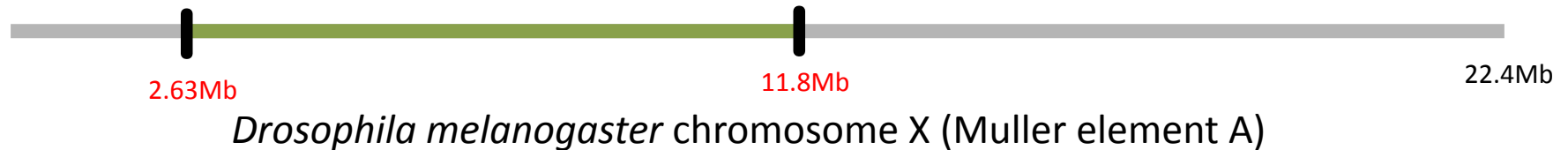
Genome – Up close



Lucilia-Drosophila comparison



Ronald Lee





Available online at www.sciencedirect.com



Insect Biochemistry and Molecular Biology 37 (2007) 184–188

*Insect
Biochemistry
and
Molecular
Biology*

www.elsevier.com/locate/ibmb

Short communication

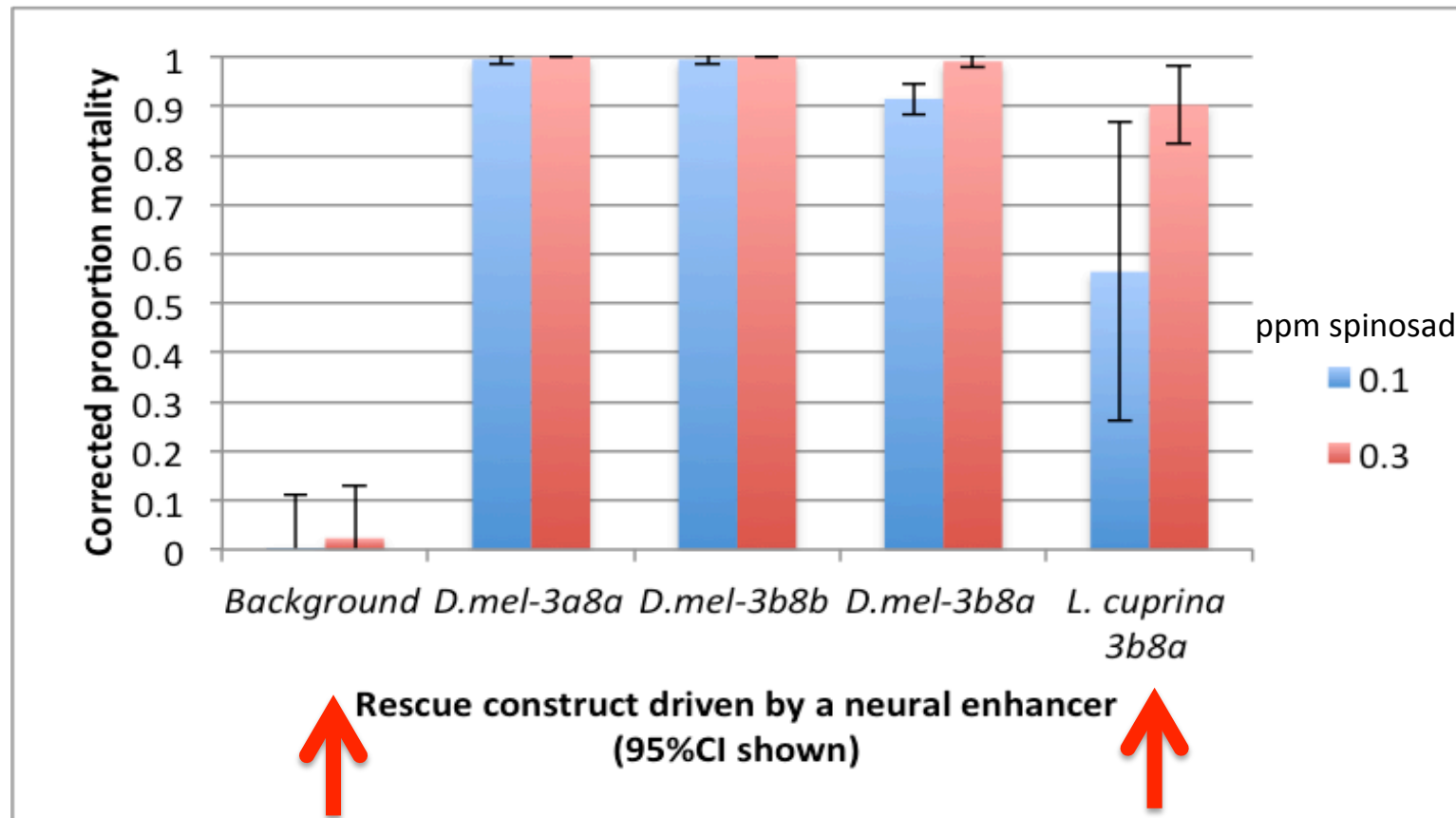
A *Dα6* knockout strain of *Drosophila melanogaster* confers
a high level of resistance to spinosad

Trent Perry*, John A. McKenzie, Philip Batterham

Department of Genetics, Centre for Environmental Stress and Adaptation Research, Bio21 Institute, University of Melbourne, Parkville, Victoria, Australia

Received 24 October 2006; received in revised form 21 November 2006; accepted 21 November 2006

Testing for potential for resistance to evolve via the target identified in *Drosophila*



Rescue construct driven by a neural enhancer
(95%CI shown)
↑
Dα6 gene
deleted

Rescue construct driven by a neural enhancer
(95%CI shown)
↑
Lucilia cuprina ortholog
Perry & Batterham, unpubl

Important questions that can be addressed

- Novel insecticide/vaccine targets?
 - *Lucilia* specific genes??
 - Genes expressed in 1st instar larvae on sheep
 - Essential genes for blowfly survival?
 - CRISPR in *Lucilia*
- Role of smell in strike? (CRISPR)
- Interaction of sheep and blowfly in strike initiation?
- Describing population structure & movement
- Are particular blowfly genotype more successful in striking sheep?

Engineering mutations at will

Highly Efficient Targeted Mutagenesis of *Drosophila* with the CRISPR/Cas9 System

Andrew R. Bassett,^{1,*} Charlotte Tibbit,¹ Chris P. Ponting,¹ and Ji-Long Liu^{1,*}

Cell Reports 4, 1–9, July 25, 2013

Identity	[Green bar]																																																			
1. Line59_Chr2L	825,650	825,660	825,670	825,680	825,690	825,700	825,710	825,720	825,730	825,740																																										
Frame 1	A	A	C	C	T	C	A	G	G	G	G	C	T	C	C	G	A	T	T	C	G	T	T	G	G	C	C	A	G	G	G	T	C	C	A	G	C	G	T	A	T	T	C	A	T	G	A	G	G	T	C	C
	N	L	Q	G	L	R	F	V	G	H	G	S	L	Q	R	I	V	G	Q	M	V	Q	A	L	F	M	R	S																								
2. Da6 Exon 2 CRISPR Deletion	6	16	26	36	46	51	61	71	81																																											
Frame 1	A	A	C	C	T	C	A	G	G	G	G	C	T	C	C	G	A	T	T	C	G	T	T	G	G	C	C	A	G	G	G	T	C	C	A	G	C	G	T	A	T	T	C	A	T	G	A	G	G	T	C	C
	N	L	Q	G	L	R	F	V	G	H	G	S	L	Q	R	R	W	T	A	D	G	S	A	G	A	F	H	E	V																							

Any gene,
Any mutation,
Blowflies??

A

