AWI Breech Strike R&D Technical Update
Maritime Museum, Sydney
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Odour and Bacteria

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11. French and Chinese students

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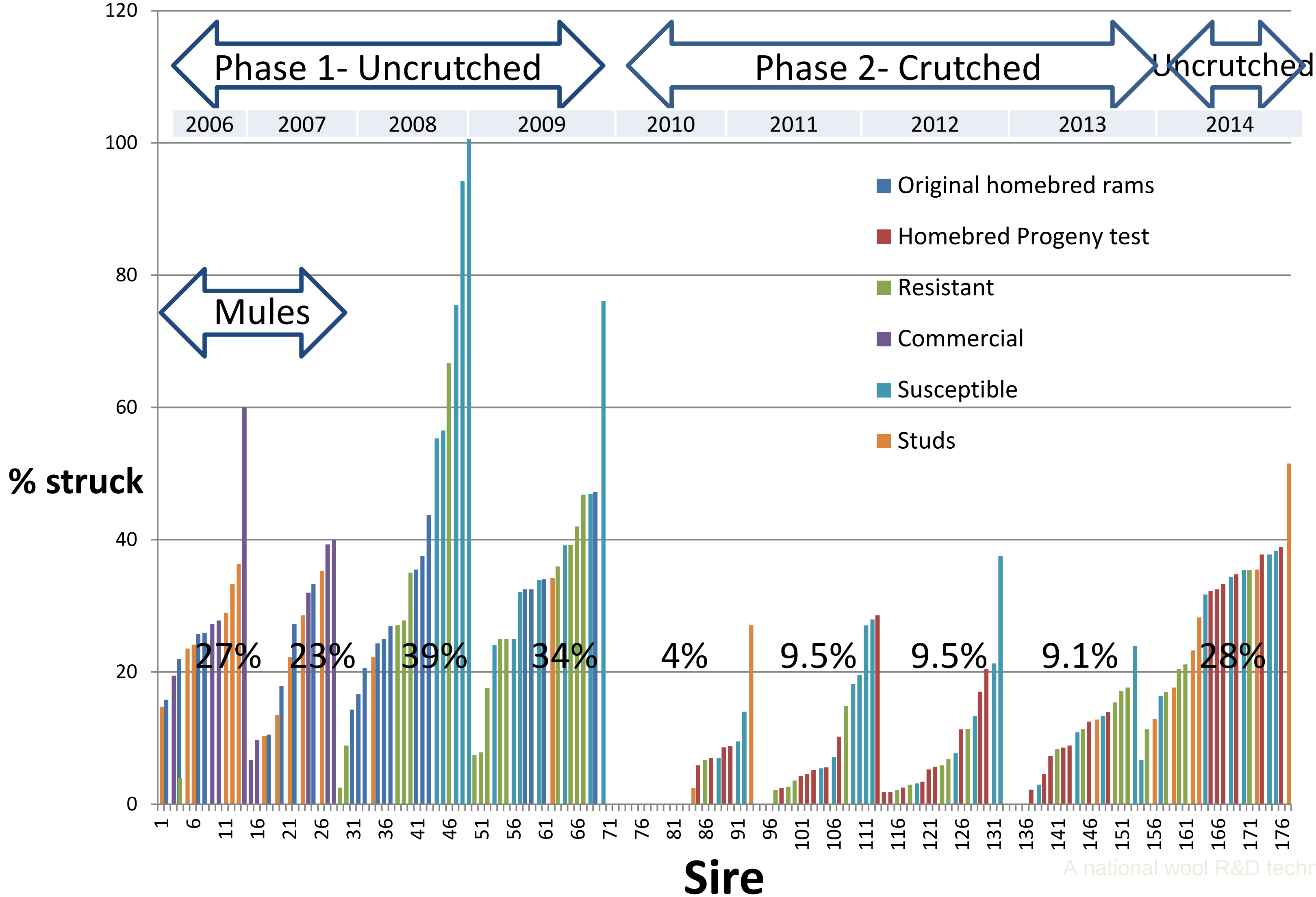
The problem

- *Lucilia Cuprina*, the "Australian" Sheep Blowfly.

Introduced to Australia in the early 1900's.

Large differences in breech strike between sire progeny groups



Heritability of Breech flystrike in a winter and in a summer rainfall region in crutched and uncrutched sheep

Trait	V_p	Crutched	r_g	V_p	Uncrutched	r_g
Weaner (Winter)	0.03	***0.10 (0.02)**** ***0.21(0.03)*****	0.26	0.55	***0.57 (0.13)***	0.44
Hogget (Winter)	0.07	***0.11 (0.02)***		0.58	***0.57 (0.16)***	
Weaner (Summer)	0.21	***0.18 (0.03)***	0.92			
Yearling (Summer)	0.09	***0.16 (0.03)***				

** 2006-2014: *2010-2014

Heritability of Breech Strike with normal crutching is low

Direct selection is not an option

- Animals have to be challenged.
- A reasonable proportion (>25%) must be struck
- It is painful
- Phenotyping is very labour intensive and therefore expensive
- Challenge to commercial animals – economic loss

Important to find indirect selection criteria

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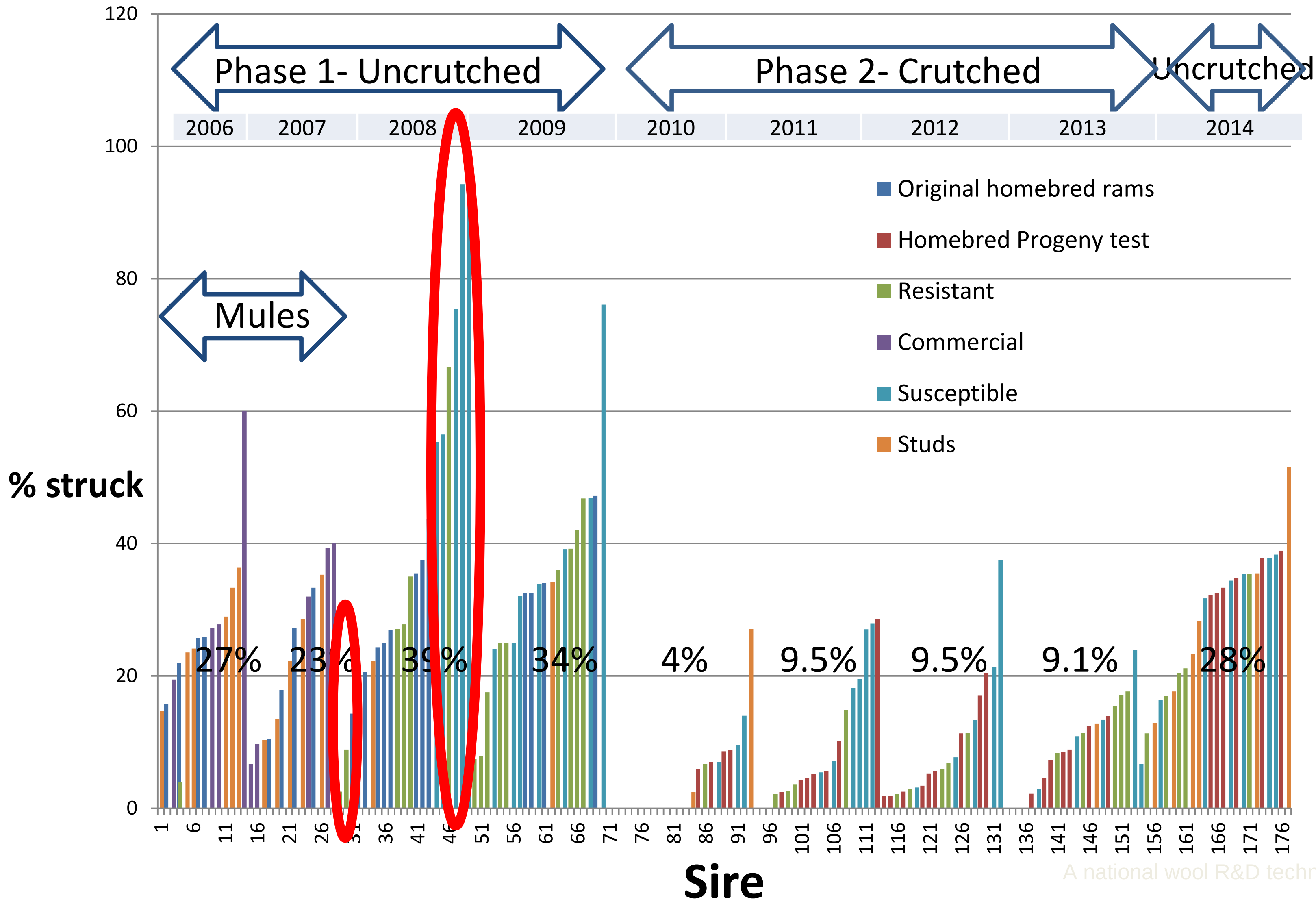
Objectives - Scientific

- **Identify and quantify importance of indicator traits for breech strike in unmulesed sheep in summer and winter rainfall regions**
  - Identify potential management solutions
- To estimate genetic parameters to design effective breeding programs
  - Heritability
  - Phenotypic and genetic correlation between traits
- To provide industry with ASBVs of indicator traits
- Incorporate in breeding programs

Key indicator traits

1. Skin wrinkle
2. Dags
3. Urine stain
4. Face and Breech cover
5. Breech strike (early)

Large differences in breech strike between sire progeny groups



A national wool R&D technical update
June 2010

103% of this sire's progeny were struck!

94% of this sire's progeny were struck!

9% of this sire's progeny were struck!

3% of this sire's progeny were struck!

Averages of indicator traits to weaning of extreme sire progeny groups for breech strike

	Resistant		Susceptible		P-value
	Sire 1	Sire 2	Sire 3	Sire 4	
Incidence of breech strike (%)	**2.5**	**8.9**	**102.9**	**94.3**	<0.001
Number of progeny	41	44	35	31	
Weaning weight (kg)	28.8	25.2	23.3	24.3	<0.001
Dag score	1.3	1.3	1.7	1.6	<0.001
Breech wrinkle	1	1	1	1.1	0.35
Tail wrinkle pre shearing	1.2	1.1	1.1	1.2	0.12
Tail wrinkle post shearing	1.2	1.5	1.7	1.6	<0.001
Breech cover pre shearing	3.6	3.3	3.6	3.5	0.15
Breech cover post shearing	2.8	2.7	3.4	3.1	<0.001
Urine stain	1.2	1	1.3	1.3	0.02
Wool colour	2.6	2.5	2.6	2.5	0.10

Little differences in the indicator traits between the sires

Average of indicator traits to hogget age of extreme sire progeny groups for breech strike

Traits	Resistant		Susceptible		P- value
	Sire 1	Sire 2	Sire 3	Sire 4	
Breech strike%	2.5	8.9	102.9	94.3	<0.001
Progeny No's	41	44	35	32	
Dag score	2.1	2.4	3.3	3.3	0.22
Breech wrinkle	1.0	1.0	1.0	1.0	0.90
Breech cover	2.7	2.6	2.8	2.7	0.20
Urine stain	1.2	1.3	1.5	1.4	<0.01
Wool colour	2.5	2.7	2.8	2.7	0.03

Little differences in the indicator traits between the sires

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Breech strike is repeatable

Progeny of 4 Extreme Sires

Trait	n	Resistant %	n	Susceptible %
Hogget*	85	5.7	66	98.6
3 year	32	0.0	37	54.2
4 year	31	0.0	33	10.7
5 year	27	0.0	30	16.5

* As hoggets they were not crutched before fly season, as mature ewes they were crutched

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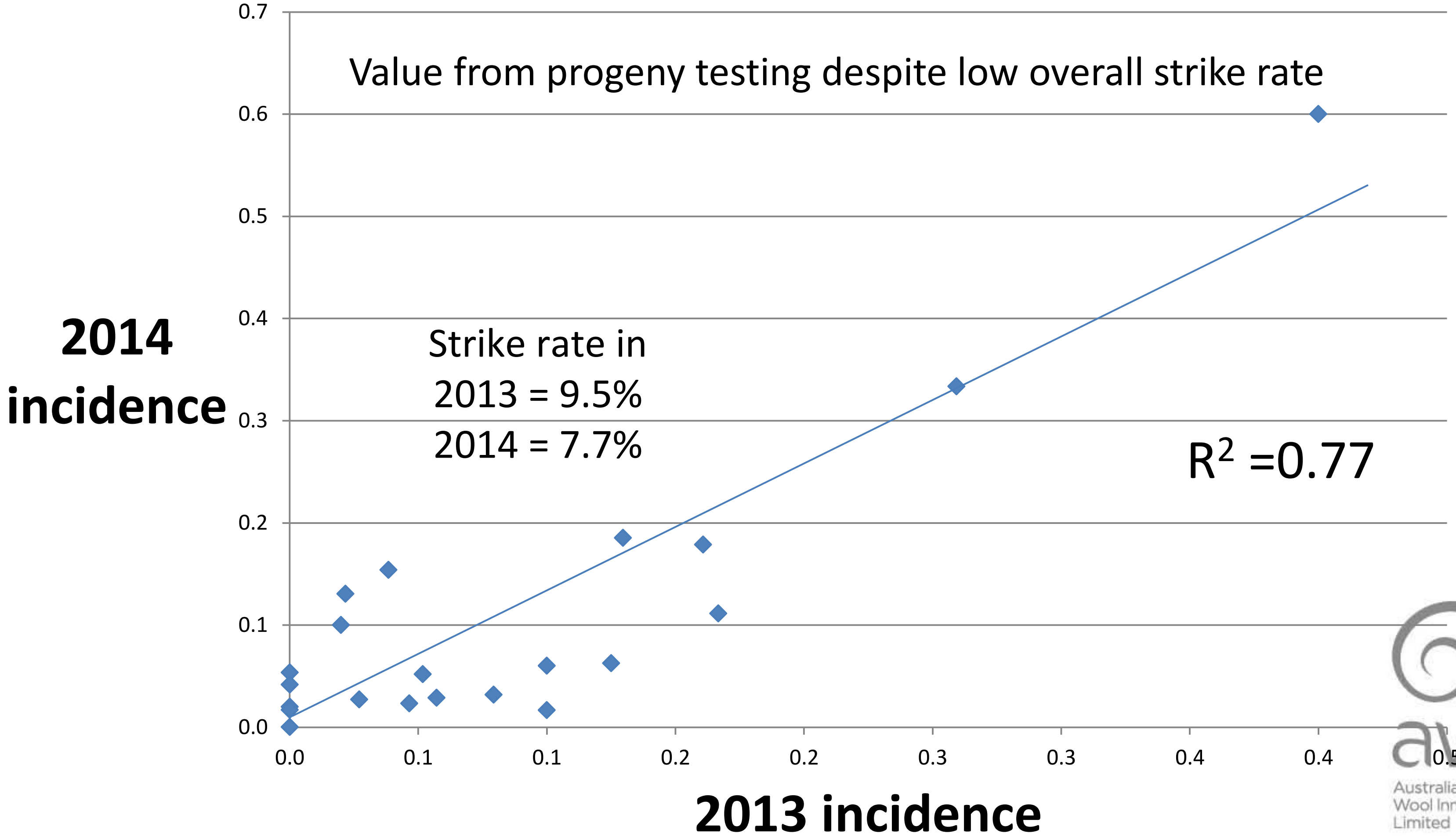
Average breech strike of the 2012 sire progeny groups in 2014 regressed against their average in their 2013 season



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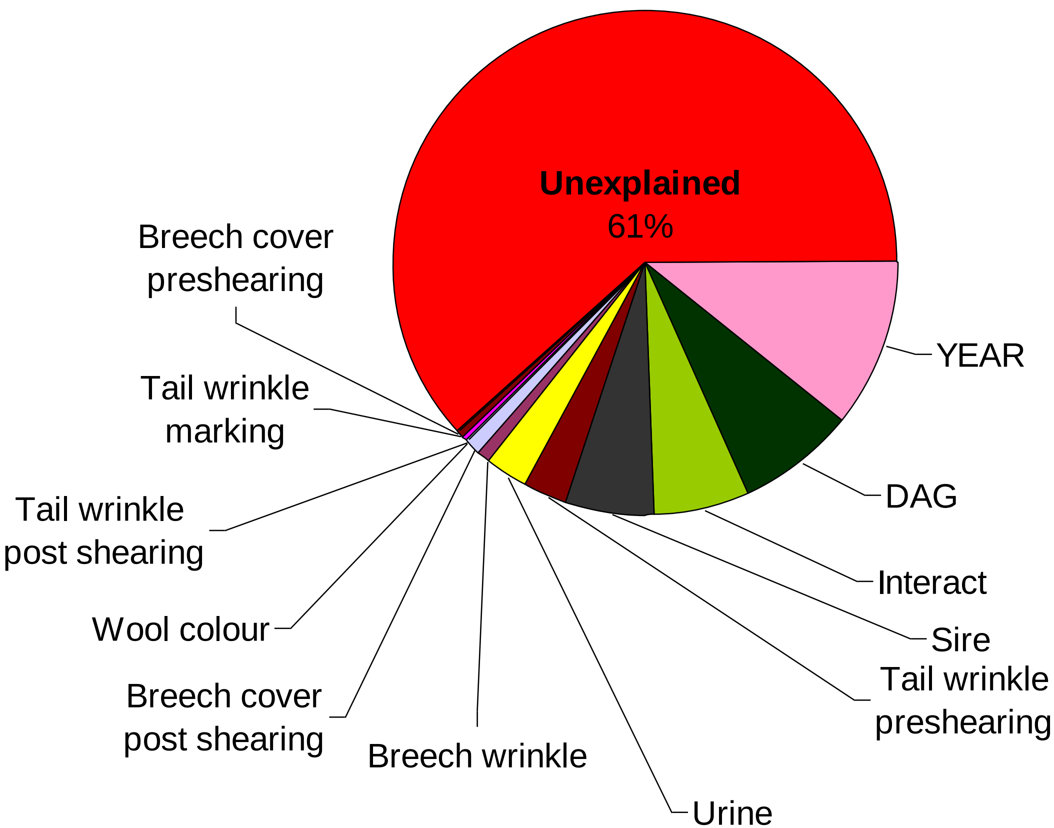
Sources of variation of breech strike at weaning and at hogget age in uncrutched sheep in a winter rainfall region

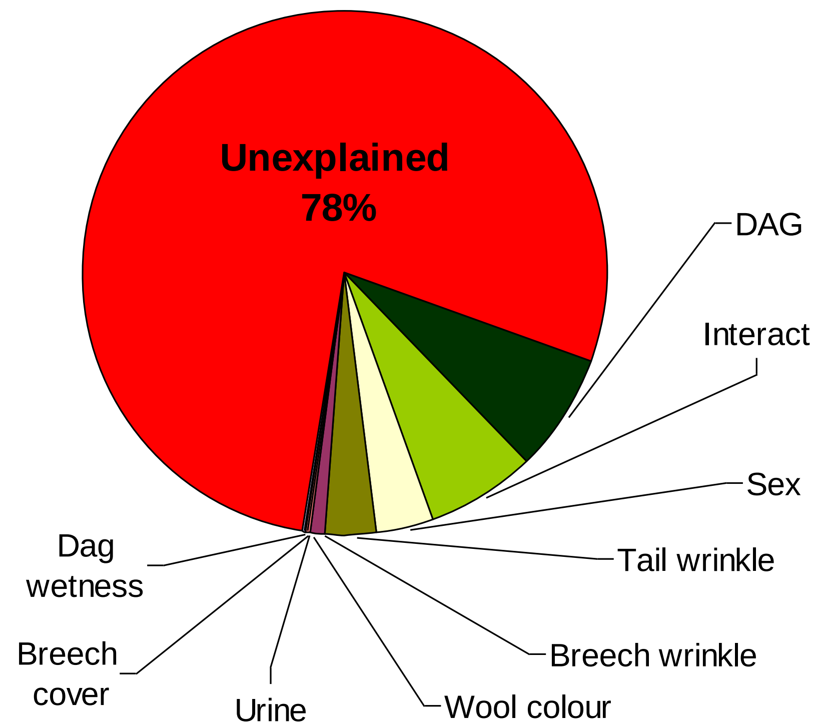
Weaning

2008 and 2009

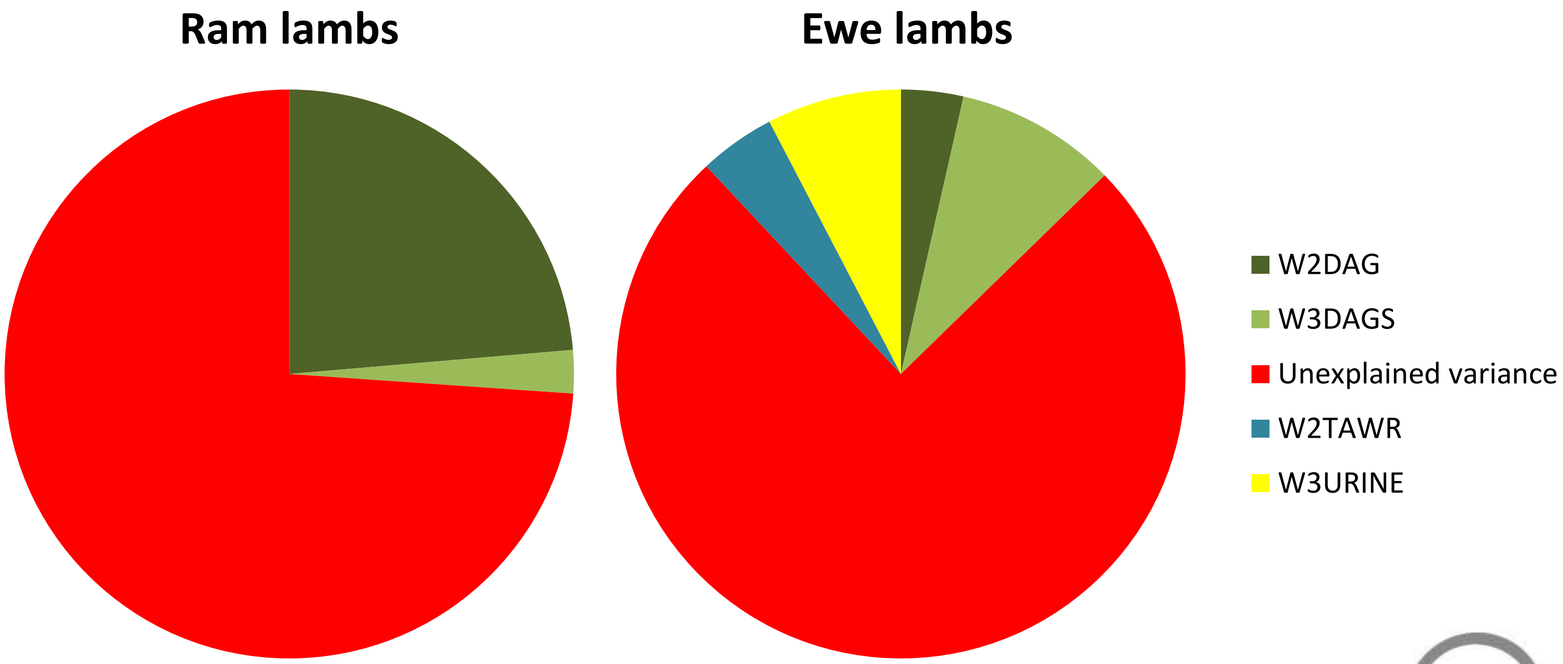


Hogget

2008

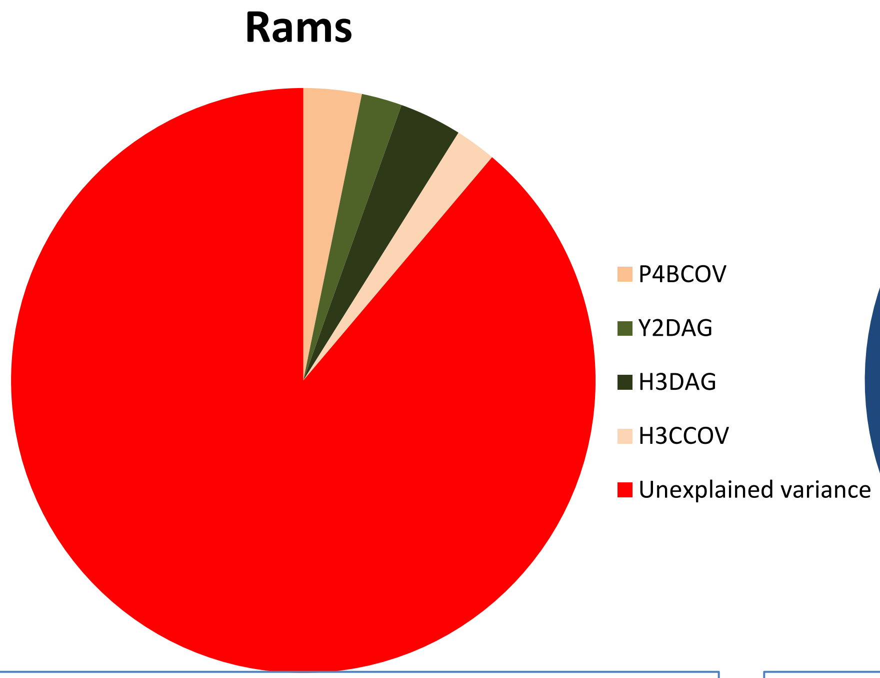


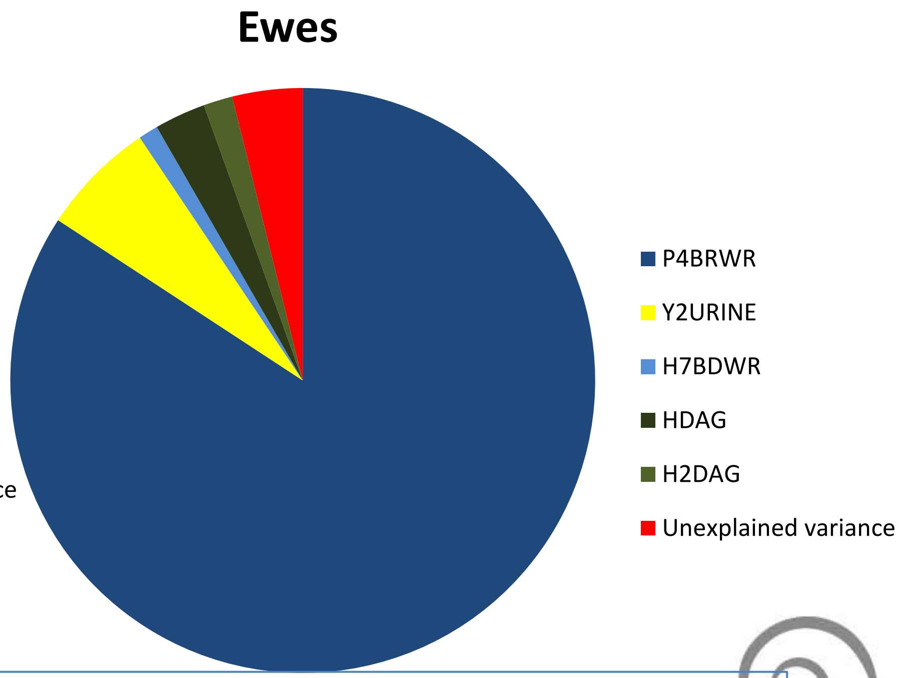
Sources of variation in breech strike at weaning (2010-2013)



Large amount of unexplained strike in ram and ewe lambs from birth to weaning

Factors explaining the variation in breech strike on individual sheep from weaning to hogget age in crutched sheep (2010-2013)





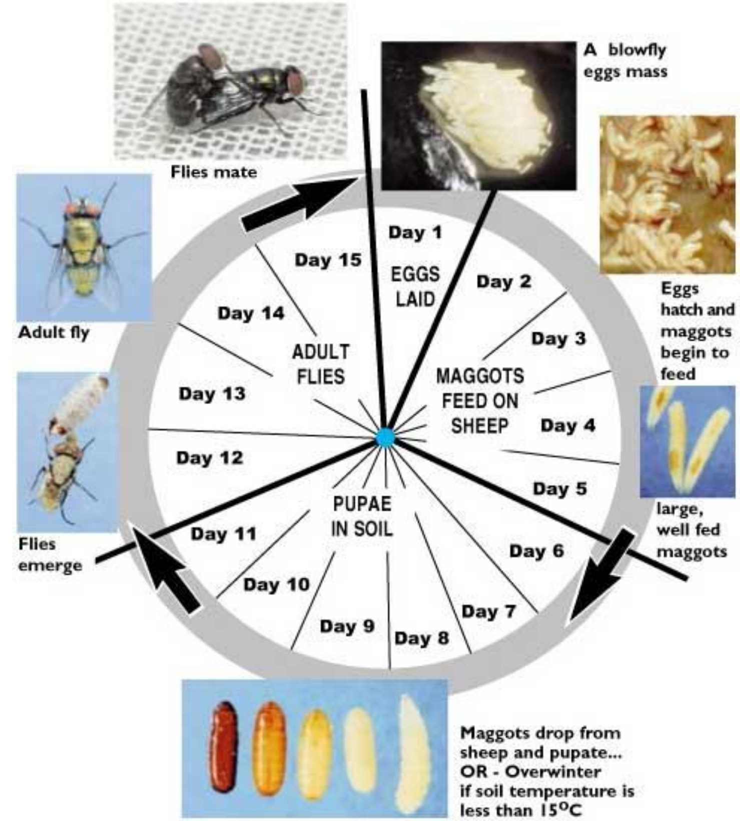
Most variation unexplained

Wrinkle explains most variation

Large amount of unexplained strike in rams from weaning to hogget but not in ewes

The issue

- What attract blowflies to specific sheep??



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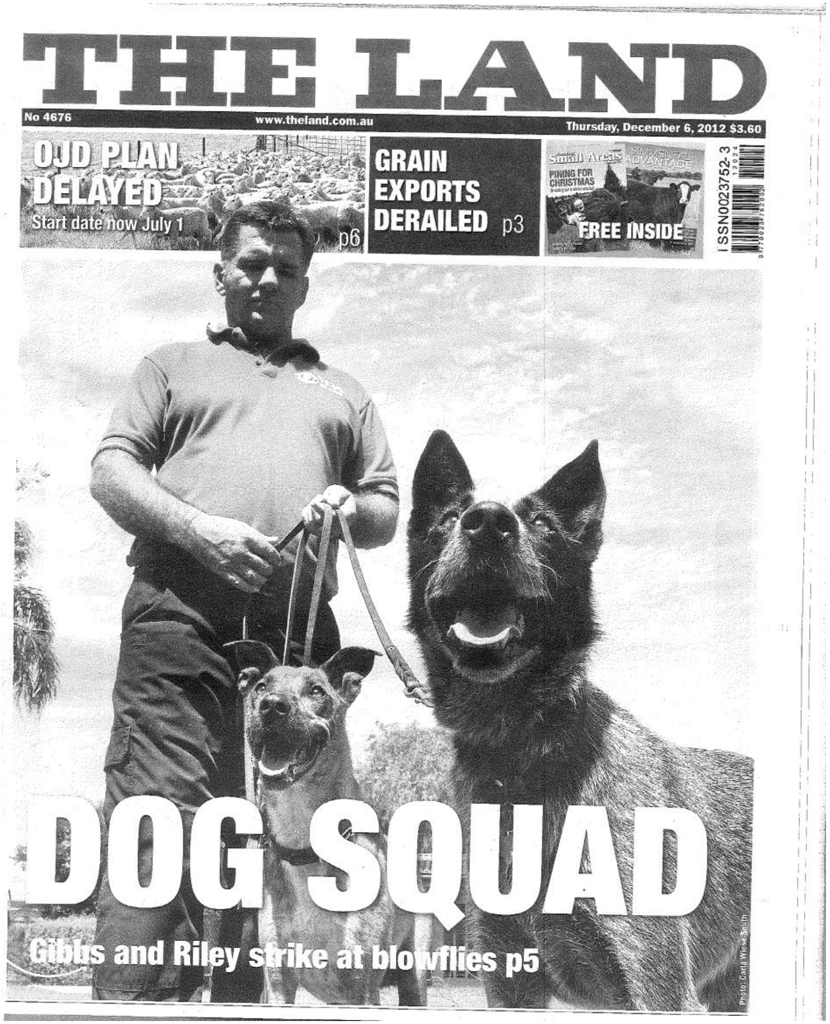
Potential trait?

Odour



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Accuracy of dogs to differentiate between resistant and susceptible wool samples

Test samples	Accuracy	
	Resistant	Susceptible
Trained (Mt Barker samples)	100%	100%
Blind test (CSIRO samples)	82%	92%

Results look encouraging but we are still not sure what the dogs were really smelling

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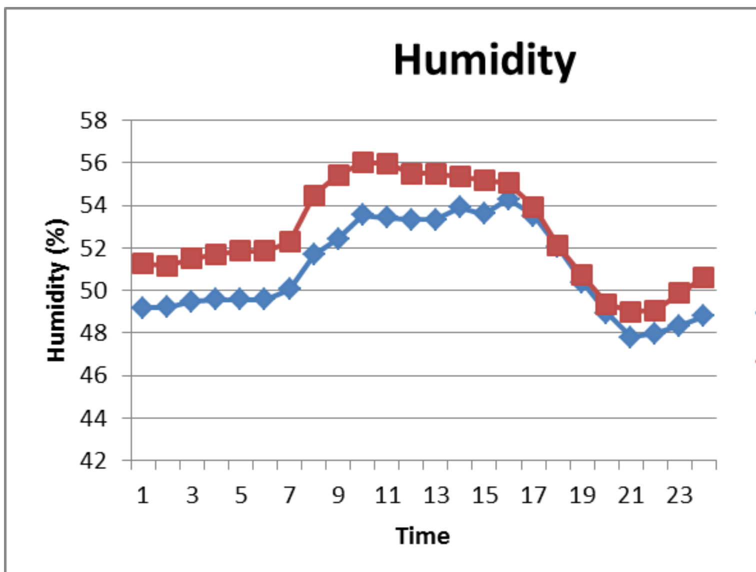
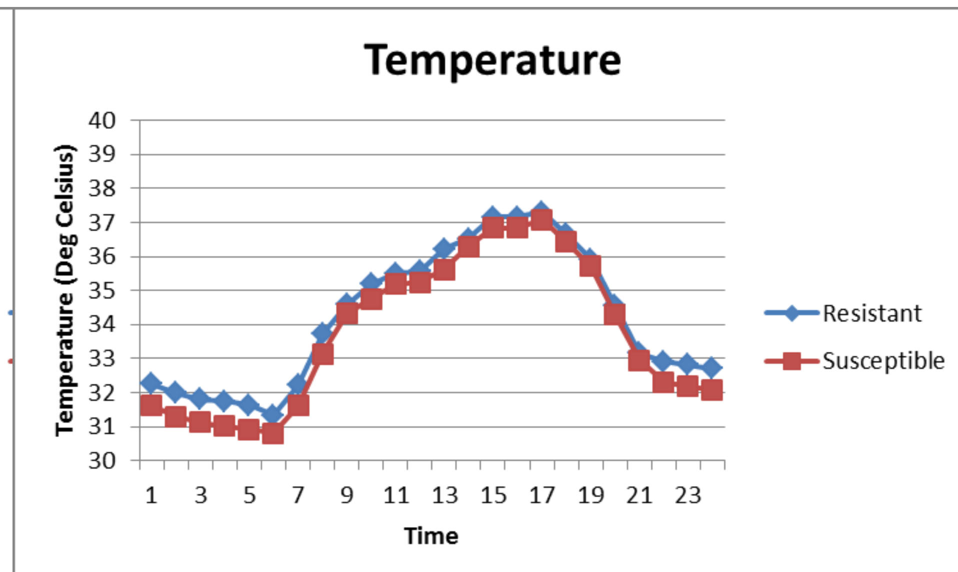
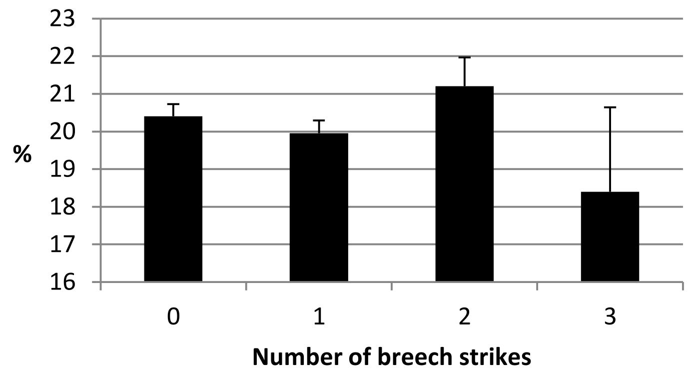
Differences in micro-environment in the breech between extreme resistant and susceptible sires



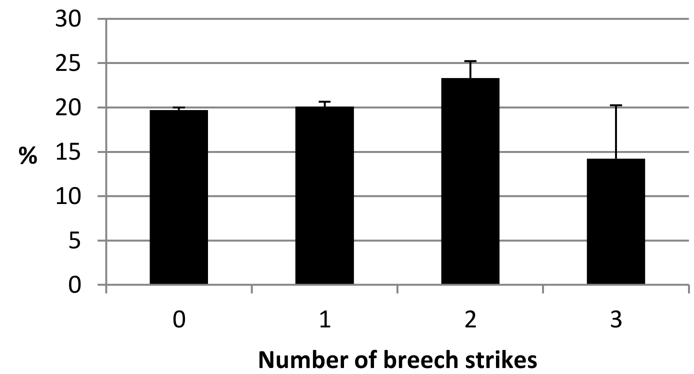


Effect of wax, suint, dust and moisture on breech strike in midside wool

Moisture

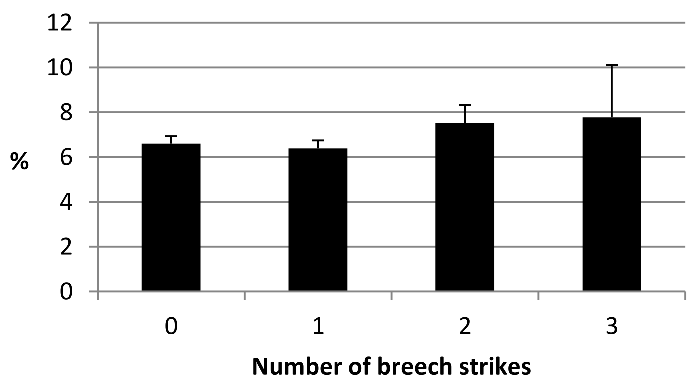


Wax

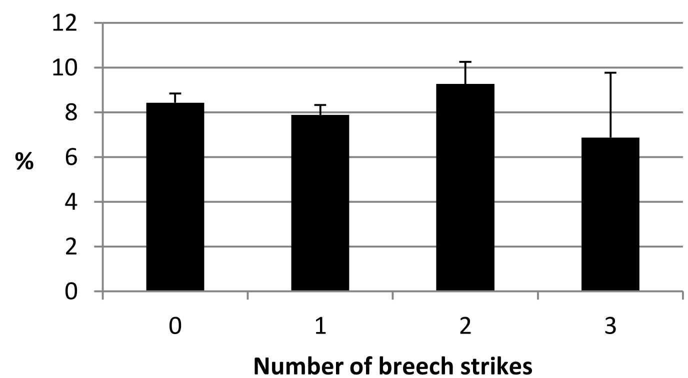


Differences but no clear pattern

Dust



Suint



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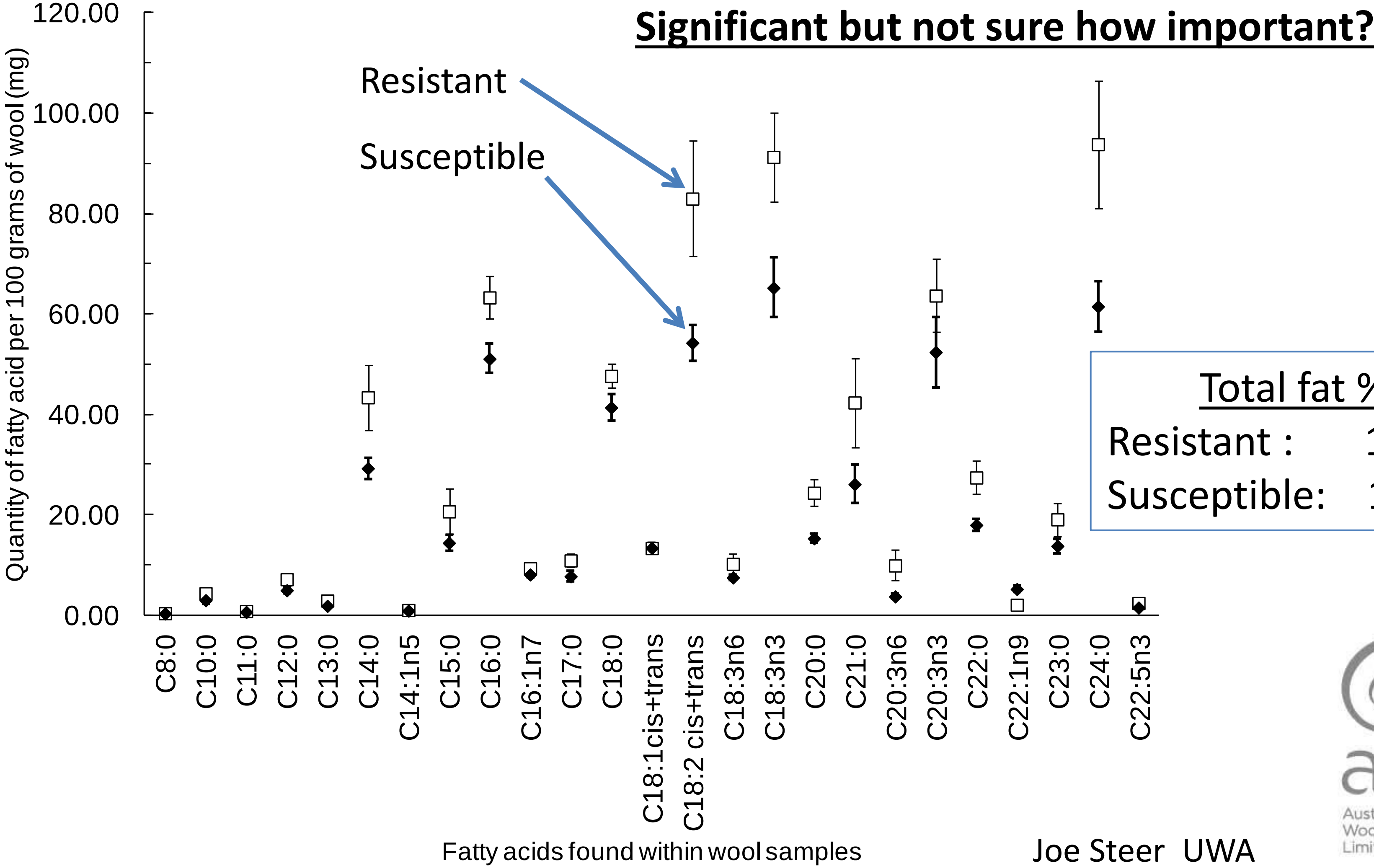
Differences in fatty acids from breech wool samples of extreme resistant (open squares) and extreme susceptible sheep (closed diamonds)



Total fat %

Resistant :	16.2%
Susceptible:	12.4%

Joe Steer UWA

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MICRO – ORGANISMS in 2012 drop progeny

Only 5% bacteria can normally be cultivated in lab

But DNA can test for existence of >5000 bacterial, fungus and yeast species

Identified micro-organisms in and on skin of

30 resistant ewes

30 susceptible rams

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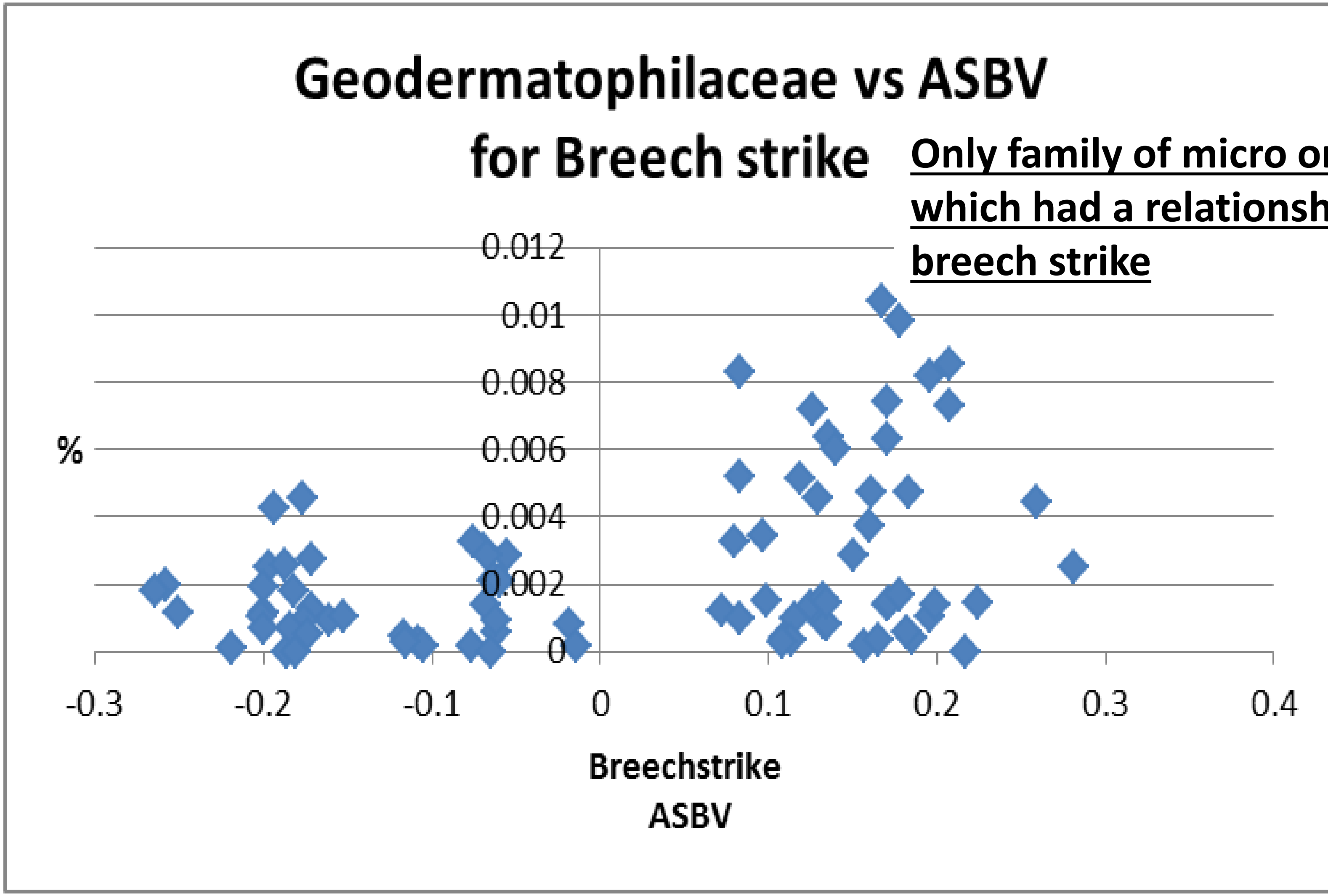
Microbiome differences between 30 resistant and 30 susceptible sheep



Only family of micro organisms which had a relationship with breech strike

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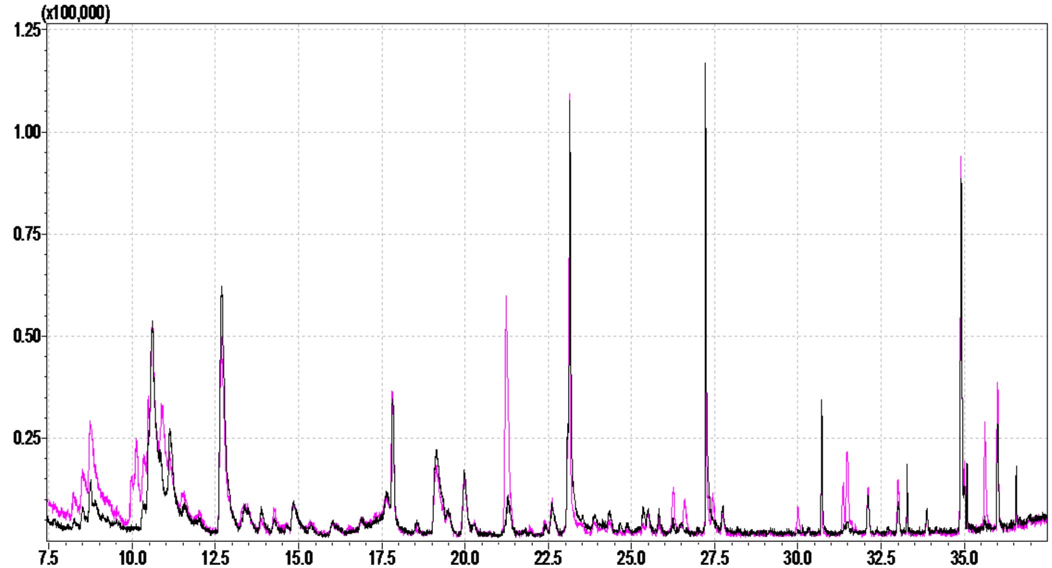
Gaschromatograph profile of odour components of breech wool

>2200 Sheep tested to date

- **Resistant and Susceptible sheep:**
  2008 drop extreme ewes
  (Measured over 4 years on stored wool samples)
- **Mt Barker 2012 drop**
- **Mt Barker 2013 drop**
- **CSIRO 2013 drop**



Identified > 1500 volatile chemical components so far

More work to see if there are differences between R and S sheep

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Chemical components that differ between resistant and susceptible sheep

Metabolite	P-Value
Heptanal	0.002
Dimethyl Sulfone	0.032
Nonanal	0.014

Work continues with evaluation of the 1500 compounds with attractiveness to flies

Annika Karlsson UWA

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Repeatability of odour compounds of wool across years in 2008 drop extreme ewes

Impact and role of these remain unknown

Volatile compound	Repeatability	SE
2(3H)-Furanone, 5-heptyldihydro	0.23	0.10
Unknown part 3-Pentanol	0.20	0.10
Benzene, 1-ethyl-2-methyl- or similar	0.14	0.08
1,1'-Bicyclohexyl-1,1'-diol	0.13	0.07
Unknown	0.13	0.05
Unknown	0.12	0.06
2(3H)-Furanone, dihydro-5-propyl-	0.12	0.06
Unknown	0.11	0.08
Heptanoic acid	0.11	0.06
Octane, 2,2,6-trimethyl- or similar	0.10	0.08

Which factors affect fly behaviour??

1. Sex
2. Gravid vs non-gravid flies
3. Bait (wool vs liver)
4. Age of wool sample
5. Age of the fly
6. Feeding regime

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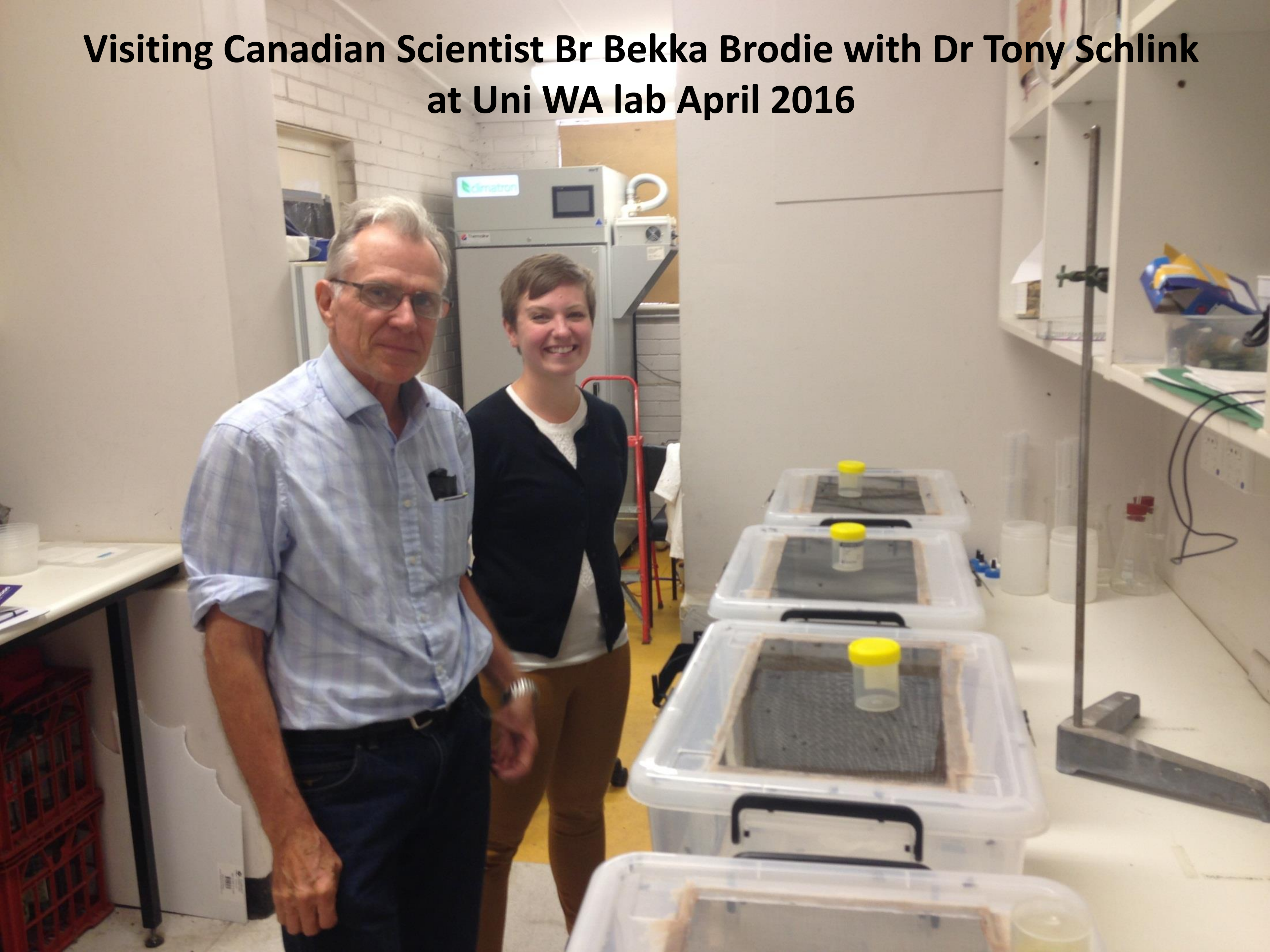
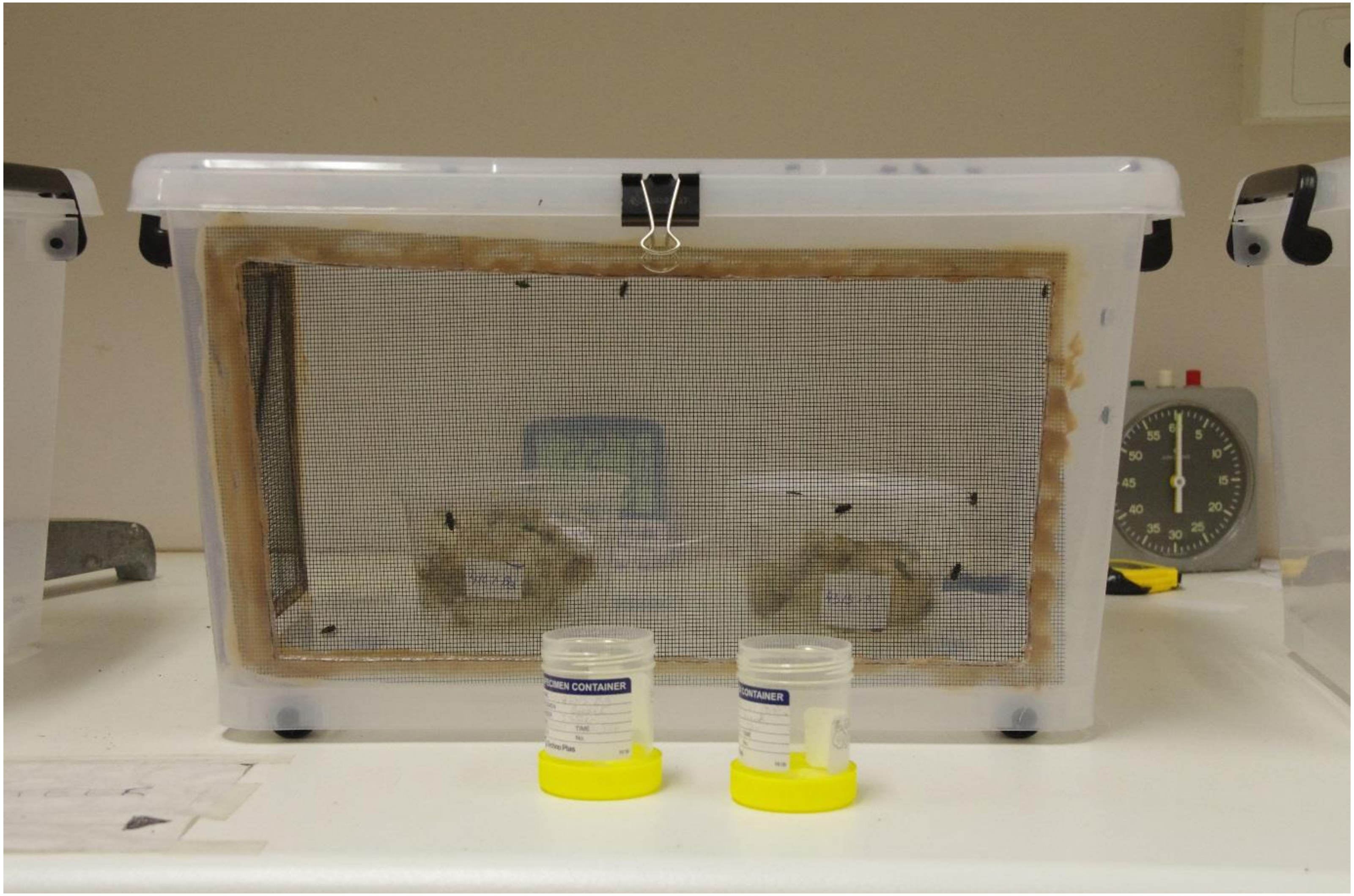


Visiting Canadian Scientist Br Bekka Brodie with Dr Tony Schlink at Uni WA lab April 2016

Brodie test

<u>50 flies per cage with the number of times flies settle on R of S wool recorded</u>



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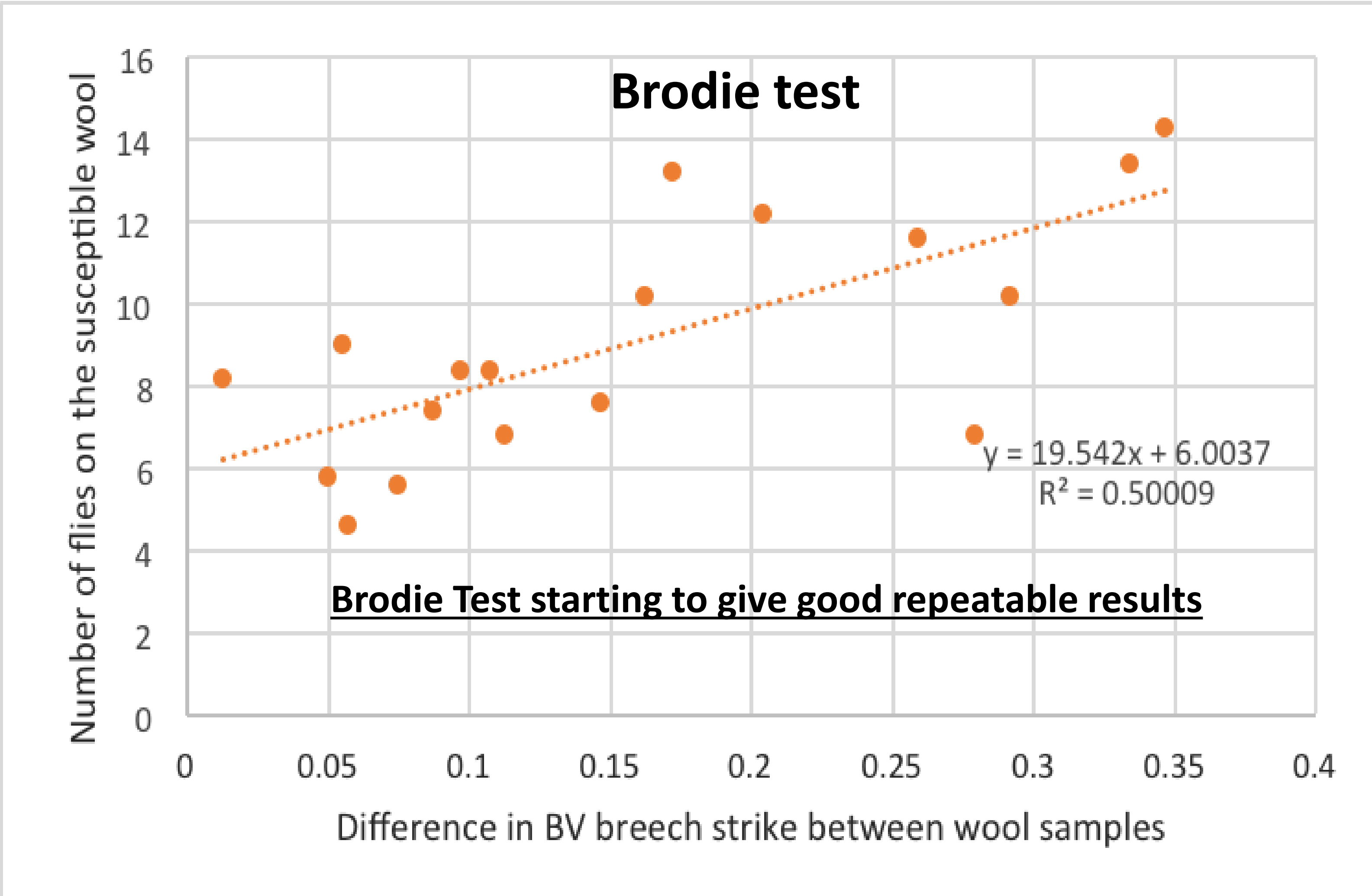
Attractiveness of flies to breech wool from resistant and susceptible sheep



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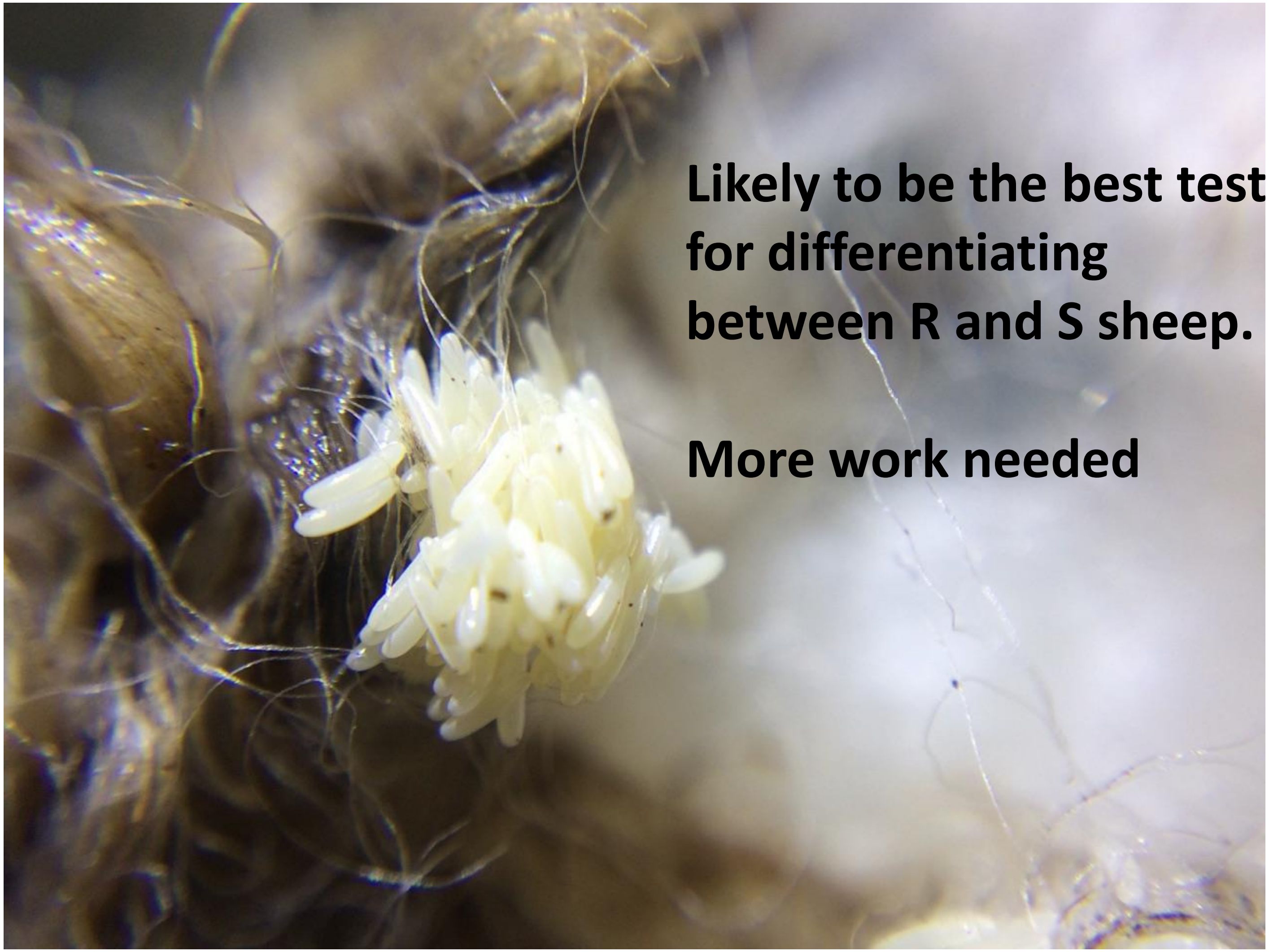
Getting the flies to lay eggs on wool



Likely to be the best test for differentiating between R and S sheep.

More work needed

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Electro-Antennagram AEG

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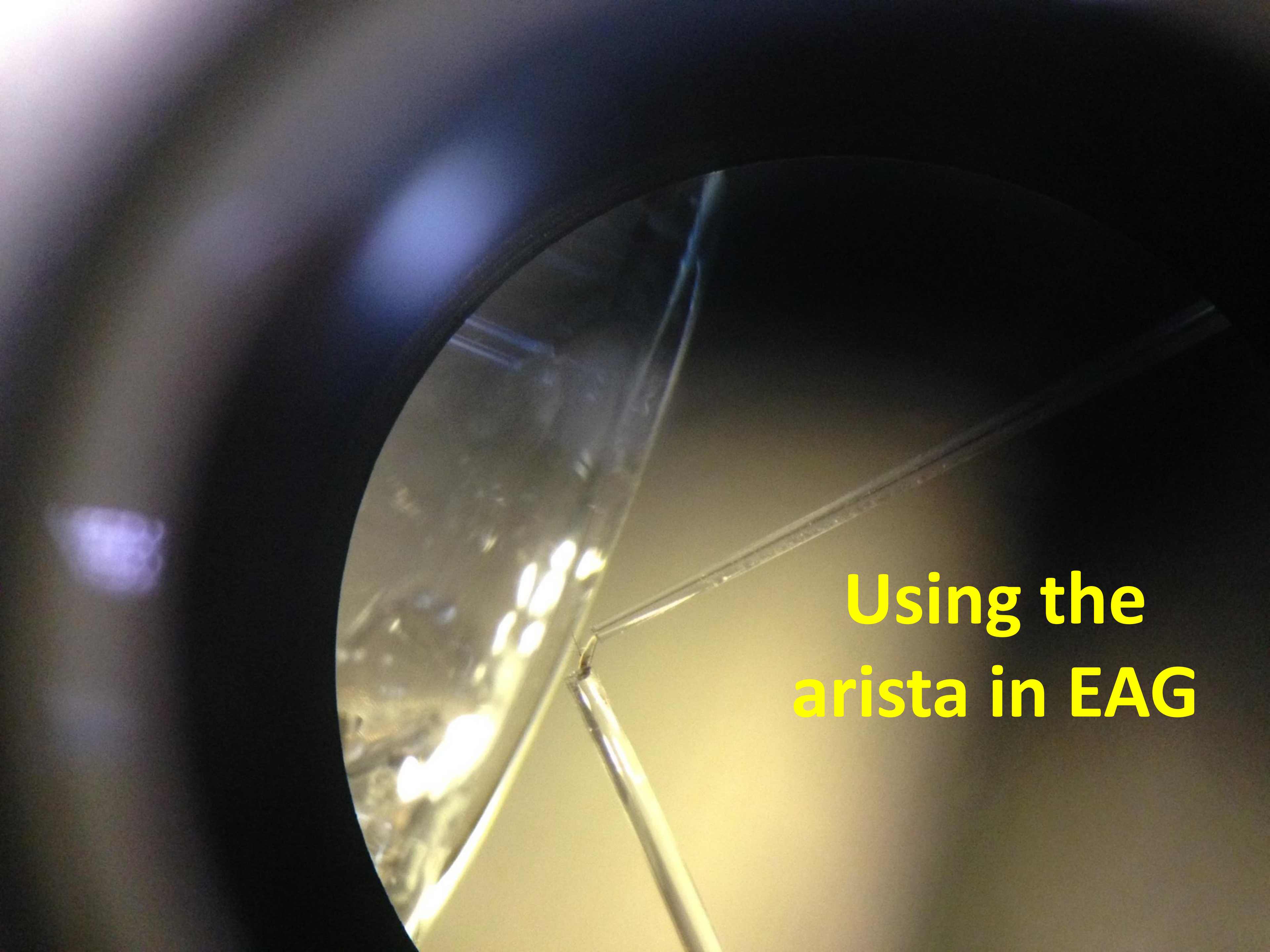
DIAMOND VIEW
Sample
Chromatography
Detectors
0.00
Total Ion
TOSHIBA

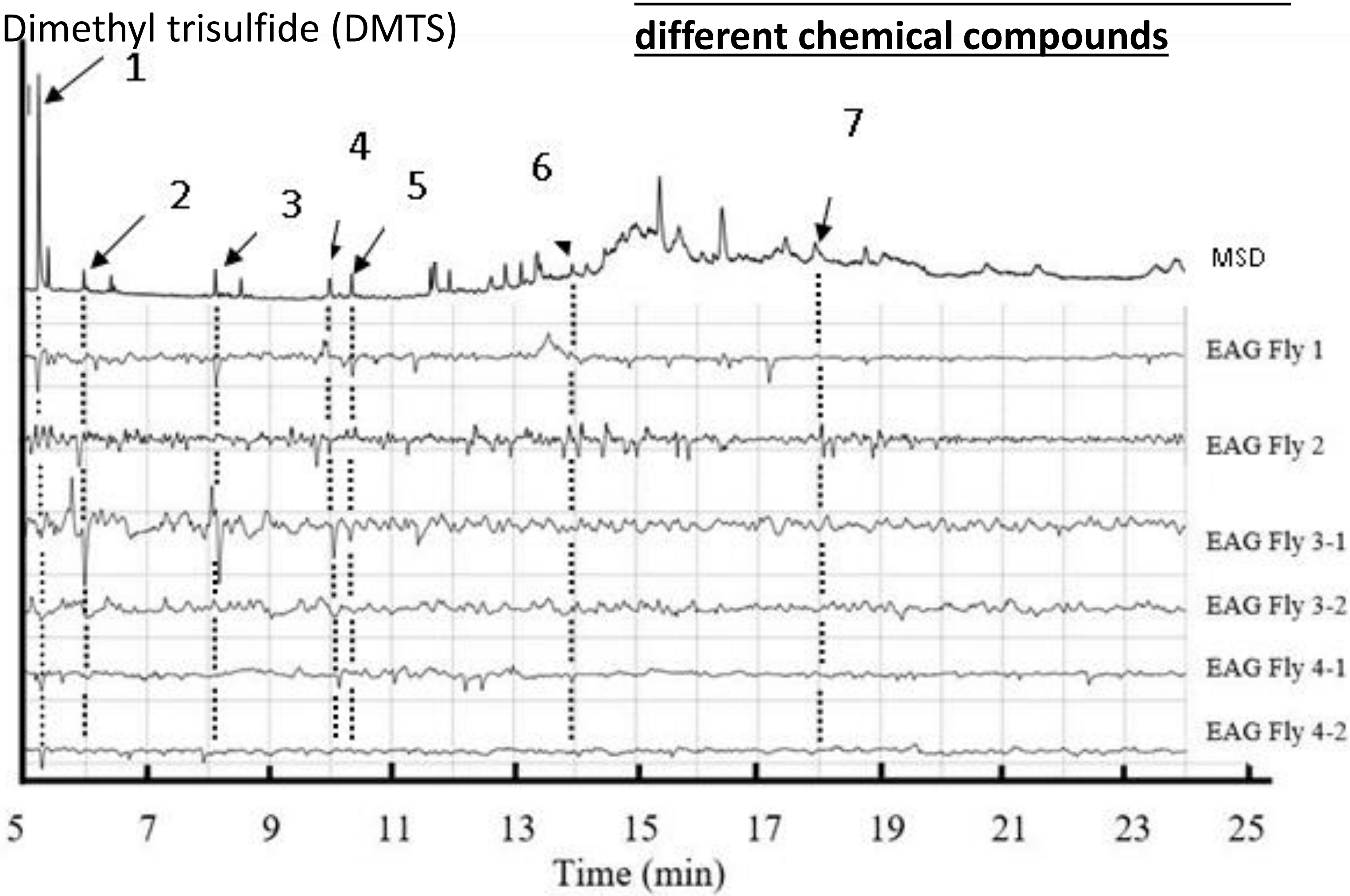
Electro-Antennagram (EAG)

- EAG equipment and technology modified for flies
- Found the best body part to use (arista only)
- Identified the best extraction method of the volatile components


Arista
Bekka Brodie
BLOW FLIES
(FAMILY CALLIPHORIDAE)
Lucilia sericata


Using the
arista in EAG

Mass Spectrometer output against the EAG patterns of the antennae from four different flies, two tested twice.

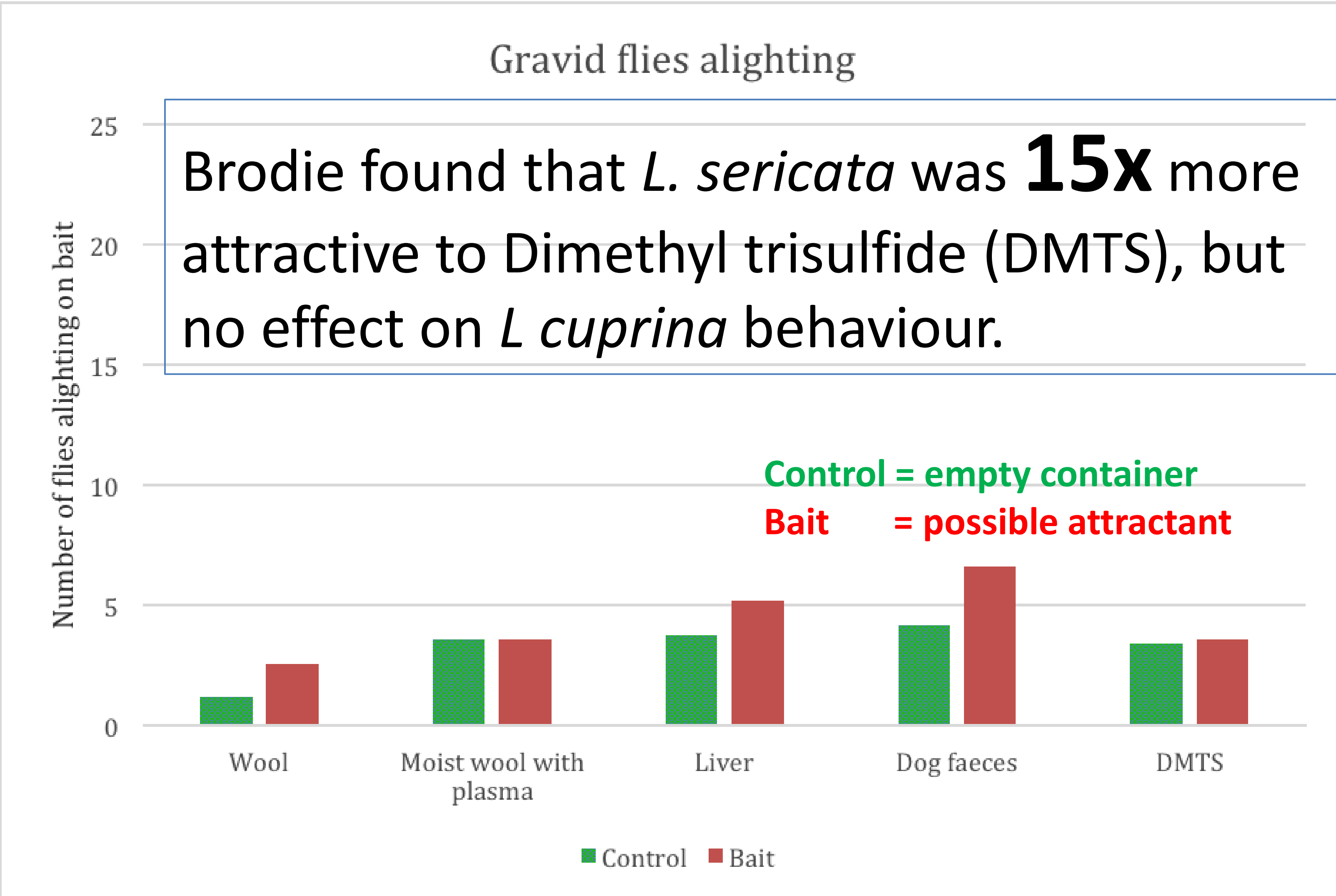


<u>Shows differences between flies for 7 different chemical compounds</u>

Comparing different attractants

Brodie found that *L. sericata* was **15x** more attractive to Dimethyl trisulfide (DMTS), but no effect on *L cuprina* behaviour.

Control = empty container
Bait = possible attractant



Conclusions

1. Differences exist between resistant and susceptible sheep in;
    1. Odour (Dogs and flies with Brodie test)
    2. Micro-environment in the breech
    3. Microbial species
    4. Fatty acid content of breech wool wax
2. Different odour recognition systems exist between very highly related fly species *L. sericata* and *L. cuprina*. Attractants for *L cuprina* have been proving difficult to identify

Where to from here?

- Sheep factor (resistant vs susceptible sheep)
  - Differences in semiochemicals from sheep
  - Validate Brodie test with fresh samples
  - Test olfactory responses with EAG
  - Tracking the fly's searching patterns
- Putrid factor
  - Understanding attractiveness of dags
- Wool moisture factor
  - Differences in sweating rates
  - Differences in drying rates of wool

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Take home message

1. Slow but good progress
2. We solved many basic problems
    1. Fly behaviour tests - identify factors impacting on fly behaviour
    2. Adapted electro-antennagram methodology to flies
3. Technology is now working
4. Different odour recognition systems between *L. sericata* and *L. cuprina*
5. Good experimental material & resources for ongoing work

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AWI gratefully acknowledges the funds provided by the Australian government to support research, development and marketing of Australian wool.