A REVIEW OF PREDISPOSING FACTORS FOR BREECH FLYSTRIKE

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Executive Summary

Australian Wool Innovation has supported research, development and extension towards better flystrike control methods and reducing the welfare impacts of flystrike on sheep over many years. Most recently this research has focussed on development of better methods of breech strike control and finding alternatives to mulesing. General consensus is that breeding sheep with improved breech strike resistance will be a critical component of future programs. Recent research has identified key factors underlying differences between sheep in susceptibility but a significant portion of variability in strike incidence remains unexplained in some flocks and regions.

This project:

- Reviewed Ausvet report (Hillman and Madin 2018) 'Understanding risk factors for ovine sheep flystrike' to summarise risk factors for flystrike and identify areas of knowledge deficit
- Assisted in the planning, conduct and assessment of outcomes from a Breech Flystrike Review Workshop (4th December 2018, Stamford Plaza Sydney Airport, see appendix 1) to identify areas of research and development priority
- Reviewed AWI research conducted to date towards the identification of sheep risk factors for breech strike
- Reviewed the literature on the role of odour in the development of breech strike and in determining differences in strike susceptibility amongst sheep
- A number of areas of interest were not picked up or considered at the Breech Flystrike Review Workshop, probably reflecting the attendee's specialist areas of interest. These areas have been considered briefly and areas of priority included in the review.

Review of the causal web identified five main areas where knowledge was lacking or where further research or development is required. These areas were:

a) The causes and control of scouring, a major breech strike risk factor;
b) The role of bacteria in susceptibility to breech strike;
c) The mechanisms by which flies find susceptible sheep;
d) Genomic associations with breech strike susceptibility and;
e) Limited availability of ASBVs for breech strike characters, in particular for scouring and urine stain and lack of a breech strike/welfare index(s) in MERINOSELECT.

A list of research areas identified at the Breech Flystrike Review Workshop was compiled and prioritised by attendees. The five top priority areas were:

a) Increased understanding of the fundamental biology of the Australian sheep blowfly *Lucilia cuprina* (*L. cuprina)* leading towards better control methods;
b) Invest in a genomics reference flock to generate genomics breeding values;
c) Increase phenotypic data with a view to putting indicator characteristics into a breech strike index;
d) Understand the genes that operate at different times and in different environments in the life cycle of the sheep blowfly and;
e) Support continuation of the breech strike resource flocks.

A list of the top 11 areas and more detailed comment on each is provided in section 3 of the report.

A further list of areas not considered at the Breech Flystrike Review Workshop but which we briefly assessed later are also listed and discussed in section 3. These included:

a) Insecticide resistance in sheep blowflies;
b) Development of new flystrike insecticides;
c) Flystrike vaccines;
d) Biological control of sheep blowflies;
e) Area wide genetic controls for *L. cuprina; and*
f) The need for research to address scouring.

A number of these areas are being addressed by AWI funded research currently underway.

The role of odour in breech flystrike susceptibility and attraction of other livestock ectoparasites was reviewed and the potential for future research was addressed by the review. It appears that with blood feeding flies, there is a core group of compounds found in breath, urine and skin secretions, and often associated with bacterial growth, that are involved in attraction. In the case of blowflies and other strike flies, attraction to livestock is mainly associated with bacteria-derived odours. There are few convincing reports linking intraspecies host differences in susceptibility to attraction in either blood feeding or myiasis flies and even in the, much studied, area of mosquito attraction the reasons for differences between individuals have not been fully elucidated.

In sheep, odours associated with bacterial growth, particularly when in association with urine staining, scouring and diseases such as fleece rot and dermatophilosis, are critical in determining susceptibility to strike. However, there is little evidence to suggest that differences in attraction of flies to sheep, or innate odour differences between sheep, are key factors in breech strike susceptibility, other than when associated with differences in known predisposing conditions. In addition, any innate differences in sheep odour are likely to be overwhelmed by the effects of bacterial odours during strike waves.

It seems unlikely that innate odour traits will be useful criteria for selecting for breech strike resistance. However, odour cues are critical at a number of stages in the development of flystrike. Clarification of olfactory mechanisms in *L. cuprina* and the genetic basis underlying these may lead to novel approaches targeting the genes that operate in host detection, the location of suitable sites for oviposition and at different stages in the establishment of strikes. This may assist in the development of improved bait or deterrent options and identify targets for new families of blowfly strike insecticides and vaccines. Overall recommendations from the different activities undertaken are provided below, not in order of priority.

Recommendations

1. *Invest in a genomics reference flock towards the creation of genomic markers/indexes/ breeding values for flystrike resistance*
   We consider this a high priority area (also considered high priority with high cost, high potential reward by Breech Flystrike Review Workshop participants). Genomic methods have major potential benefits for selecting flystrike resistance because sheep do not need to be exposed to strike, or subject to the predisposing conditions for flystrike and detailed and difficult phenotyping is not required to assess an animal's genetic merit. Rather, the genotype is estimated from a small blood sample. Furthermore, a genetic value can be attributed to all animals in all years and all environments, regardless of level of flystrike challenge.

   We recommend the establishment of a Genomics Implementation Working Group to determine the best path forward with regard to available resources/resource constraints. This panel should include high-level specialist expertise in sheep/animal genomics, sound industry reference and representation from Sheep Genetics to facilitate implementation. The potential value of maintenance of the flystrike selection flocks, which are already phenotyped for a wide range of flystrike and production traits, as part of this reference flock, is emphasised.

2. *Increase the collection of phenotypic data from industry flocks (and other research flocks where relevant) with a view to the development of a breech strike index(es)*
   Encouragement of much more widespread phenotyping for flystrike traits is required to provide more robust and widely applicable estimates in Merino genetics. This is particularly so for urine stain, which currently does not have a breeding value available in MERINOSELECT, and for scouring/dags. To this end there is a need to facilitate easier methods of measurement of 'difficult' traits such as urine stain and scouring/dags. There could be easier methods of assessing them, or perhaps indirect methods of estimating urine stain/risk of urine stain. The recording of alternative more readily measured estimates for the main flystrike traits e.g. faecal consistency for scouring, face cover for bare area, neck and body wrinkle for breech wrinkle for recording in MERINOSELECT and presentation of ASBVs for these traits should also be considered.

   Progeny testing of elite sires directly for breech strike incidence would provide an avenue for increased accuracy and maximising industry genetic gain in flystrike resistance.

3. *Development of breech strike / welfare indexes*
   There is a need to facilitate practical 'useability' of breech strike traits in MERINOSELECT for sheep breeders. Breeding indices incorporating breech strike resistance while maximising genetic gains for other traits are needed for a range of different environments and sheep types. Optimal incorporation of breech strike resistance will require the derivation of an economic value(s) for breech strike resistance.

4. *Better understand the unexplained variation in the occurrence of strike in resistant and susceptible sheep and the effect of management regime on this*
   The amount of variation in breech strike susceptibility not explained by the major indicator characters will be key to a consideration of the need for new or better indirect selection criteria. There is little unexplained variation in some data sets (e.g. crutched ewes in WA where only 9.38% of the variation remains unexplained) and dags and skin wrinkles explain most of the phenotypic variation, as opposed to the NSW flocks and unmulesed, uncrutched flocks in WA where approximately 50% of the variation remains unexplained. There is a need for a 'harmonised analysis' of the WA and NSW data followed by careful consideration of what

percent of the unexplained phenotypic variation is environmental in origin, what percent is likely to be genetic, what fixed effects have been taken into account and likelihood of finding new indicator characters that can markedly increase the accuracy of selection for flystrike resistance.

5. *Support the continuation of the flystrike resource flocks*
   The two flocks provide a source of very accurately pedigreed and phenotyped animals and are in completely different environments with different flystrike profiles. The depth of phenotyping for flystrike incidence in the flystrike selection lines in WA (now at Katanning) and NSW (Chiswick) makes these flocks an important core resource for genomic studies, a prime resource for identifying and testing new indicator characters and valuable for obtaining more precise genetic parameters for the development of more accurate selection and breeding programs.

   The flocks will also be an important resource for research in other areas, for example investigating the role of microbiome profiles in strike etiology and susceptibility, testing the efficacy of new vaccine technologies and resistant phenotypes, and the future development of welfare indices (that incorporate resistance to breech strike) and breeding values.

6. *Increase understanding of the fundamental biology of L. cuprina* (leading to opportunities for control)
   This was considered high priority at the Breech Flystrike Review Workshop because knowledge in this area underpins a large number of potential approaches. These studies need to be carefully targeted to provide knowledge with specific endpoints towards improving control efficacy and will be facilitated by recent advances in molecular technology. Some specific areas of interest are suggested in the body of the review and in other recommendations. We emphasise the importance of a careful review of the abundant work already undertaken in this area and, in particular, the work conducted as part of the CSIRO genetic control program in the 1970s, before new research is commenced.

7. *Explore the expression patterns of L. cuprina genes to understand the molecular basis of establishment of strikes (attraction, oviposition, larval invasion) and regulation of key developmental processes of L. cuprina.*
   This work will facilitate optimal usage of the *L. cuprina* genome to develop new vaccines, new flystrike insecticides and potentially area-wide approaches to control of *L. cuprina*. This work needs to be targeted to specific outcomes in order to ensure efficiency and value of the investment.

   (Work in this area supported by AWI is underway, searching for genes involved in the location of susceptible sheep by *L. cuprina*, dermal invasion by blowfly maggots, the initiation of strike and developmental processes of the blowfly larvae. This work is strongly supported).

8. *Understand the fleece / dag microbiome, and its role in breech strike susceptibility*
   It is well established that bacterial growth is important at various stages in the development of bodystrike; for example in providing odour cues for attraction and oviposition, causing skin scalding and extravasation which provides protein for the development of 1st instar larvae and by providing a focus for skin invasion by newly hatched blowfly maggots. Microbial odours, particularly in association with urine or other decomposing organic matter have also been shown to be important in the attraction of other livestock ectoparasites to their hosts and bacteria often also provide critical nutritive factors for larval development of some livestock-associated flies.

There has been much less study of the importance of the breech fleece microbiome and interactions with urine stain and scouring and the importance of bacteria in the development of breech strike. However, there is indication that bacterial growth could be similarly important in determining breech strike susceptibility. This was identified as an area of knowledge deficit in construction of the causal web, and in our subsequent review of odour and predisposing causes for breech strike and was listed amongst priorities for research at the Breech Flystrike Review Workshop. The microbiome could also influence skin proteomic/metabolomic profiles and associated studies of the fleece/skin proteomics and metabolomics may yield additional important information towards the development of new approaches to control, for example vaccination against key bacteria, blocking bacterial odours, the use of bactericides or biological methods to control critical bacteria.

9. *Development of a detailed business case for investing in genetic improvement of sheep resistant to breech strike*
   To understand if further investment into breeding programs focussed on reducing breech flystrike is worthwhile, and to underpin promotion to woolgrowers about the application of genetic technologies or other approaches, an understanding of the size and scale of potential benefits is required – i.e. a value proposition/business case. A component of this work, for example, would be a benefit cost analysis for establishing genomic evaluation of flystrike. This would also inform the feasibility/attractiveness of different approaches by quantifying the size of trade-offs that growers are willing to make.

10. *Better understand the role of attractants/odour in sheep susceptibility and the genesis of strike*
    Odour is involved at a number of stages during development of strike. In particular location of sheep, the identification of susceptible sites on sheep for oviposition by flies and stimulating egg laying. Many of the main odours involved at different stages appear to be bacterially and environmentally mediated and there is little evidence that innate (genetically controlled) odour differences between sheep influence fly attraction or are related to susceptibility. There appears to be little evidence to support further studies of odour differences with a view to the identification of new selection criteria.

    However, bacterial odours and other volatiles associated with predisposing causes of flystrike, such as urine and faecal staining, are critical to the initiation of strike and methods that interfere with the perception of odour by the flies, for example by targeting critical olfactory genes or processes, or the identification of strongly repellent molecules may lead to novel control approaches. Studies in this area should take into account that odour could be operating at a number of stages in strike development in addition to attraction (for example acting as an arrestant or oviposition stimulant) and design experimental tests accordingly.

11. *Manage insecticide resistance and maintain the efficacy of available flystrike control products*
    The availability of effective flystrike protection and treatment chemicals remains critical to effective management of flystrike in Australian flocks, particularly in non-mulesed flocks. There is a long history of resistance development to flystrike control chemicals and the recent emergence of resistance to keystone control products, dicyclanil and cyromazine is a major threat to sustainability of wool production. This will be particularly important in unmulesed flocks, highly susceptible flocks and flocks in high flystrike risk regions. The characterisation and monitoring of resistance and promotion of resistance management strategies should continue to be an important element of flystrike control programs. Australian Wool Innovation is investing in this area. There has been limited detailed consideration of the best resistance management approaches to prolong the effectiveness of flystrike control compounds. A project to model resistance management programs, towards the development of optimal

recommendations for woolgrowers, informing an integrated pest management (IPM) plan, is required. A detailed IPM plan should be supported by delivery of a well-integrated extension program for woolgrowers.

12. *Develop new insecticidal actives for flystrike control*
With increasing costs of development and registration, the rate of new production animal parasiticide active compounds coming onto the market has "slowed to a trickle". The wool sheep parasiticide market is relatively small in the world context and this is particularly relevant as all of the major pharmaceutical companies that conduct research in this area have a multinational focus. Research in this area will assist the continued availability of effective flystrike preventatives for use by Australian woolgrowers. The availability of the *L. cuprina* genome will provide the possibility of new insecticidal targets (as well as oviposition suppressants) and AWI is currently funding a project in this area. AWI may need to increase their involvement with commercial veterinary pharmaceutical companies to assist new product development. The possibility of developing products based on chemical mixtures, a strategy currently used for ectoparasites, but used widely as a tool for combating resistance for gastrointestinal parasites should also be considered. There may be an opportunity to revisit some previously suggested chemicals. (The case of GH74 and like compounds is noted in the body of the review).

13. *Development of flystrike vaccines*
AWI funded projects are underway towards the development of a flystrike vaccine. This will be facilitated by the recent availability of the *L. cuprina* genome and current AWI projects to identify critical genes in the genesis of flystrike, which offer the possibility of new gene targets for a vaccine. This is a high risk, but potentially very high reward project. A vaccine directed against fleece rot bacteria, critical in susceptibility to bodystrike was previously developed and patented, but never commercialised (Burrell 1985). This vaccine gave extended protection against fleece rot and bodystrike. As preliminary evidence suggests that many of the same bacteria may be important in susceptibility to breech strike, investigation of the potential of this vaccine for use in reducing susceptibility to breech strike may be worthwhile.

14. *Biological control of sheep blowflies*
Biological control could include the release of specialist natural enemies that are expected to persist in blowfly populations keeping fly populations low (classical biological control) or biopesticides (innundative biological controls) where large numbers of pathogenic organisms (fungi, bacteria, viruses,) parasites or predators are released as 'biological pesticides'). *L. cuprina* occurs at low population density at most times and flystrike is episodic with fly populations building rapidly when conditions become suitable. The rate of spread of pathogens and parasites is almost invariably density-dependent. This factor and the lag time generally experienced between a pest outbreak and a corresponding increase in numbers of biocontrol agents would seem to present difficulties for classical biocontrol agents to persist and impact on *L. cuprina* populations, or more particularly, to reduce strike incidence. Biopesticides such as *Bacillus thuringiensis* and some entomopathogenic fungi have shown short term protection when applied to sheep in experimental studies and suitable agents may have application as part of an integrated approach or in organic flocks. However, they are unlikely to provide a level or persistence of protection comparable with chemical pesticides which limits their practicality in many situations. Pathogens that persist in the soil, such as some fungi or entomopathogenic nematodes, may have effect against soil stages of *L. cuprina* (prepupal larvae and pupae) particularly during the overwintering phase. However, better knowledge of the spatial and temporal ecology of the soil phases of *L. cuprina* will be required to assess whether sufficient mortality could be induced to significantly affect flystrike incidence. The potential of biological

control of *Lucilia spp*. using sheep blowfly pathogens is currently being reviewed in more detail as part of AWI Project ON-00620.

15. *Area wide genetic controls for Lucilia cuprina*
    These methods seek to bring about suppression or eradication of the pest population by the release of flies of the same species that have been modified to confer sterility or cause genetic death in pest populations. This approach is also known as autocidal control and is usually used in area wide strategies focussed on eradicating pest populations from an area or reducing pest abundance through ongoing release programs. The most well-known method, the sterile insect technique (SIT) was successfully used to eradicate screwworm flies from north and central America and has also been used for eradicating regional incursions of insects, such as fruit flies in uninfested areas of Australia and an incursion of screwworm flies in Libya.

    In the 1970s, CSIRO investigated the use of compound chromosome strains, sex-linked translocation strains and female killing systems in an attempt to suppress or eliminate *L. cuprina* populations and to address the cost barriers to use of SIT in Australia. In spite of some initial success this was eventually not pursued because of operational difficulties and funding constraints. The availability of gene editing technologies (such as CAS CRISPR) provide the potential for more elegant systems of genetic control such as RIDL (Release of Insects with Dominant Lethality) or potentially using gene drives to spread deleterious (often sex-linked or stage specific genes) through fly populations. Research is currently underway, funded by AWI, to identify critical genes in *L. cuprina* and may facilitate the design of genetically modified strains suitable for use in area wide autocidal approaches. Transgenic sexing “male only” strains have been developed in North American *L. cuprina* strains and consideration should be given to the feasibility of the future use of these strains in the design of area wide strategies in Australia.

16. *Project to address scouring*
    Scouring (diarrhoea) and resultant dags in the breech wool of sheep are major predisposing causes for breech strike in the southern sheep production areas of Australia. Dags are also a major management issue in their own right in these areas. Methods to reduce the incidence of scouring and dags would have a major impact in reducing breech strike incidence.

    Recommendations towards the reduction of dags have been provided to AWI in a previous project (AWI Project WP520 - Minimising dags in sheep) and are currently being updated (AWI Project ON-00610).

1. Introduction

Australian Wool Innovation (AWI) has supported significant research, development and extension towards better flystrike control methods and reducing the welfare impacts of flystrike over many years. Most recently this research has focussed on better methods of breech strike control and finding alternatives to mulesing. General opinion is that breeding sheep with increased resistance will be a key component in breech strike control programs for non-mulesed flocks. This research has identified key factors underlying differences between sheep in susceptibility to breech strike. However, a significant portion of the overall variability between sheep in the incidence of strike remains unexplained. Projects to clarify the contribution of odour to blowfly attraction to the sheep and the part that odour-related characters may play in variation amongst sheep in susceptibility have been underway for several years and the recent mapping of the sheep blowfly genome has provided information to underpin new insecticides, vaccines and area-wide approaches to control. In addition, a recent AWI workshop developed a causal web for flystrike to assist the identification of key risk factors for breech strike and areas of knowledge deficit. We reviewed the outcomes from this research, as well as other odour-related parasite research, towards the development of new selection methods for breeding increased resistance and other new control methods. The project has developed recommendations for future research and development in this area. A workshop of researchers and other stakeholders working in the area of flystrike research (the Breech Flystrike Review Workshop) was conducted in parallel with this review to provide a wider view of opinions on the most promising directions for future research.

2. Review of causal web and risk factors for flystrike

A summarised version and the full version of the causal web developed as part of AWI Project ON-00510 are shown in Figures 1 and 2. Summaries of the results from our review of the causal web are given below in Tables 1 and 2. A number of major areas where there appears to be information lacking, or where further development could provide improved control practice were identified.

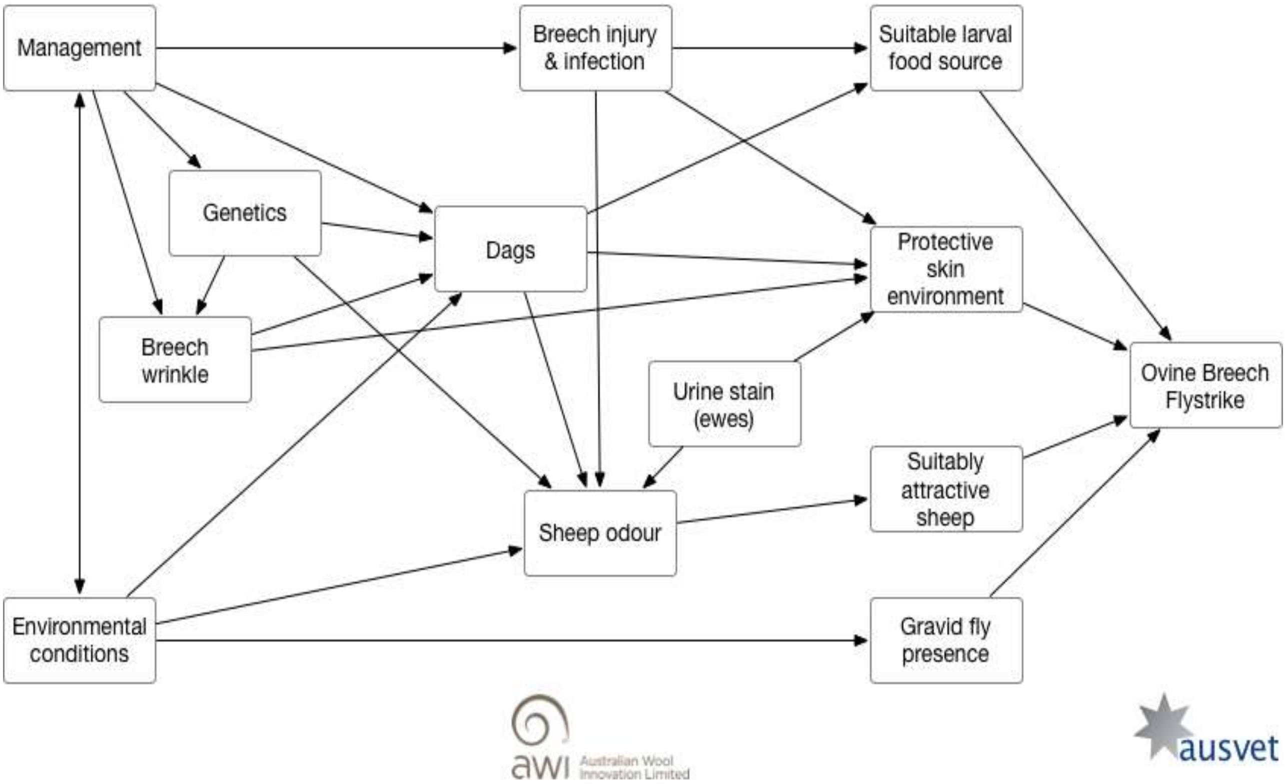

Figure 1. Preliminary causal web of ovine breech flystrike in Australia (AWI Project ON-00510)

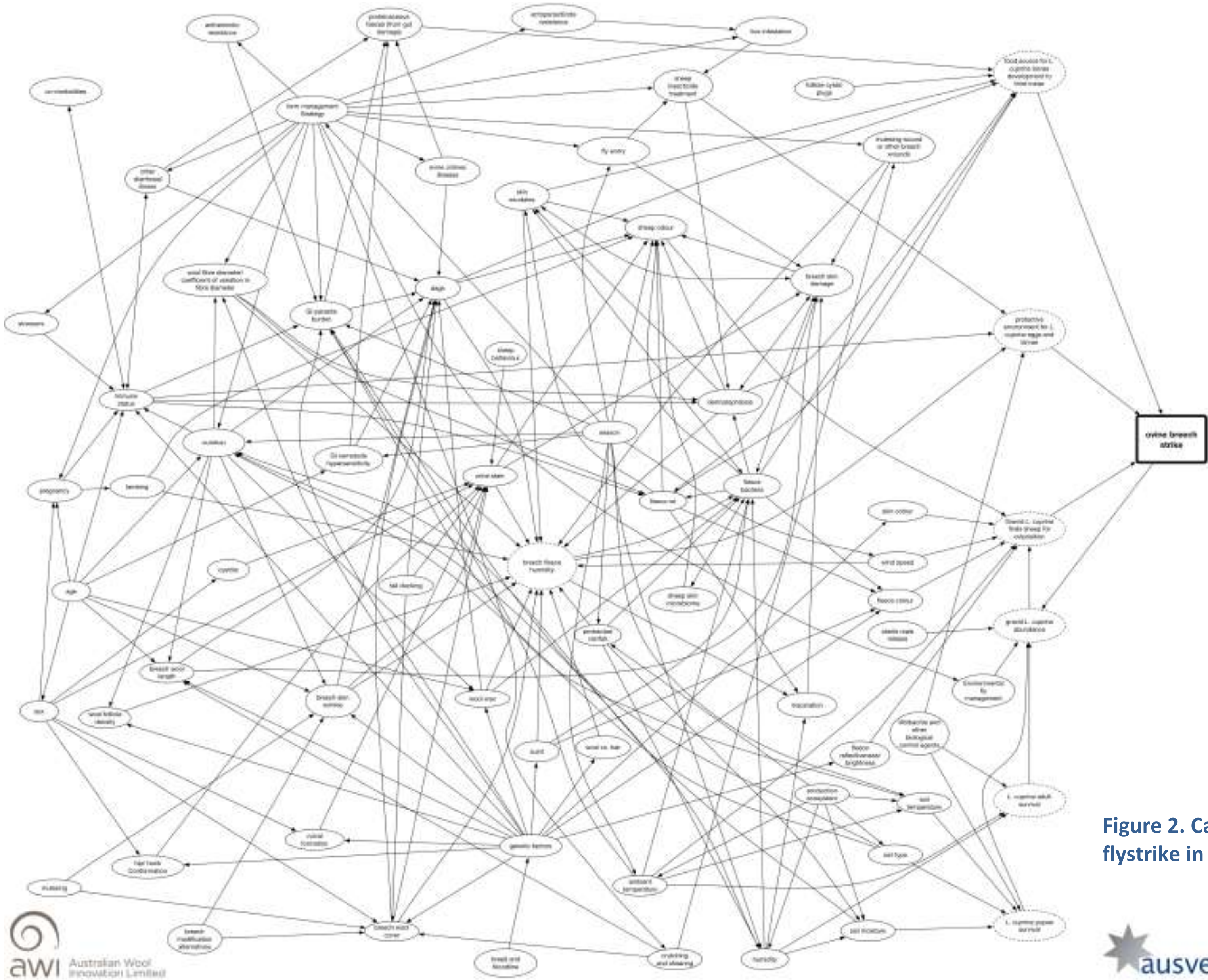

Figure 2. Causal web of ovine breech flystrike in Australia (AWI Project ON-00510)

These areas for further development are:

1. **Scouring:** Its causes, factors relating to sheep susceptibility, means of control, and the exact mechanisms by which it impacts on strike, susceptibility, particularly in later steps in the initiation of a strike.

2. **The role of bacteria in the initiation of breech strike**: This includes the role of odour in the attraction of flies to sheep, but also in later stages of strike initiation including oviposition, the nutrition and survival of first instar larvae, skin invasion, skin inflammation, serous exudate for larval nutrition and a skin focus for initial larval invasion. This has been carefully investigated with respect to *L. cuprina* attraction and oviposition stimulation in body strike, but the role and dynamics of bacteria in the initiation of breech strike and in later stages of strike is less certain. Clarifying this is important as it may provide ideas for other approaches of control. For example, vaccination against *Pseudomonas* bacteria has previously been suggested as a possible control for fleece rot and bodystrike (Burrell 1990).

3. **Sheep location by flies:** The means by which flies locate sheep from a distance in the extensive sheep production systems is also an area of uncertainty and has been explored in this review. Our preliminary assessment was that the presence or absence of well-known attractive predisposing causes such as other strikes, scouring, urine stain and active flystrike as attractants to the sheep is likely to 'drown out' any between sheep variation in odour attraction per se. To be clear, this is not to say that odour per se or the sources of differences in odour may not be associated with flystrike susceptibility, but just that variability in the attraction of flies because of sheep odours, as distinct from odours from known predisposing conditions, is unlikely to explain this.

4. **Genomic factors**: Being able to identify the actual genes or gene combinations that code for traits leading to enhanced breech strike resistance seems to be the 'holy grail' of identification of sheep factors within the causal web, and for selecting breech strike resistance. Currently there is little information available in this area. An initial limited attempt at finding useful genomic associations for variation in breech strike resistance, based on limited data, only found SNPs of small effects, (Dominik, unpublished data). Additional work, utilising all of the available data from the breech strike selection lines has recently been completed (AWI Project ON-00515), with similar results, but indicated that even though no SNPs of large effect were found, the aggregation of the small effects of many SNPs could be effective in the creation of Genomic Enhanced Breeding Values. Although longer term in outlook, further work in this area could significantly improve the efficiency of selection and rates of improvement in breech strike resistance.

5. **ASBVs**: There are still relatively few industry records contributing to the estimation of ASBVs for a number of the major flystrike traits, most particularly for difficult to assess traits such as urine stain, dag score, and breech wool cover. Collection of more industry data will greatly increase the accuracy of ASBVs for these traits and applicability to different industry breeding objectives and management regimes.

Table 1. Assessment of risk factors from the causal web
(*S=sheep; M= management; E=Environmental; F=Fly factors)

Causal web factor	Factor*		Comment	Action
Sheep factors				
Lice infestation	S	M	Increases susceptibility by sheep biting - moist fleece, skin rupture	Not of sufficient import to justify further investigation
Sheep insecticide treatment	S	M	Clear effect depending on chemical, application efficiency and resistance. Application now mainly 'spray on' chemicals	Research towards new chemicals underway. Opportunity for spray-on or other low labour application methods for flystrike control
Proteinaceous faeces from gut damage	S		Not studied; elevated protein in faeces may increase *L. cuprina* L1 survival in dags/stained wool	Consider as factor in future research on dags
OJD	S		Scouring of proteinaceous faeces from OJD could possibly increase the risk of breech strike	Significant incentives to reduce OJD per se and any flystrike risk as a result
Fly worry	S		Unclear what is meant here; non-strike flies around mulesing or docking wounds can delay healing and increase strike susceptibility; season of mulesing important; old work shows repellents can reduce fly worry per se; mulesing wound treatments can reduce likelihood of strikes in broken scabs	Low priority
Follicle cysts plugs	S		Have been related to fleece rot/bodystrike development but importance minor/unclear; don't seem to be documented for breech strike unclear	No sufficient evidence to warrant further action
Mulesing and other breech wounds	S	M	Can become struck; Manage to avoid mustering until wounds healed, avoid dog bites, wound protectants available	Research towards phase-out of surgical mulesing underway
Sheep odour	S		Dogs can smell differences between resistant and susceptible sheep. Odour associated with dags, urine fleece rot dermo an important strike cue.	Addressed in section 4 of this report.

			Importance of innate sheep odours in susceptibility yet to be confirmed.	
Skin exudates	S		Appear to be important for strike (L1) establishment in body strike (fleece rot, dematophilosis). Importance of skin scalding and exudate in breech strike unclear. Importance of protein content of dags (to support L1 larval survival) and development of strike unclear	Clarify importance of inflammation/skin factors in breech strike establishment
Dags	S		Well documented as important in breech strike susceptibility in southern flocks	Research into dag development/ susceptibility required; high priority
GI parasite burden	S	M	Direct effects of scour worms important/ effect of health impacts on immune response? Genetic correlation with strike appears low	As above
Fibre diameter/CV	S		CV is important in susceptibility to fleece rot/ bodystrike (More resistant sheep have lower CV) Conflicting direction of significant genetic associations in WA (-ve) and Armidale (+ve) flocks Results from AWI Project ON-00524 on 'Predictions of genetic gain in reducing breech flystrike – update' indicate that CV of fibre diameter increases (unfavourable direction) as a correlated response to selection for reduced breech flystrike	Investigate as opportunity arises, and with existing data sets. Specific effort low priority
Sheep behaviour	S		Not well studied; tail length and ability to disturb flies suggested, but unlikely to be key factor; Sheep seldom 'groom' (c.f. cattle) or exhibit protective behavioural responses Effect of other sheep behaviours, e.g. sheep camping behaviour, on susceptibility/strike incidence unclear but may be hard to influence	Possibly a review of sheep camp behaviour with a view to targeting soil stages of *L. cuprina* (most pre-pupal larvae leave sheep; emerge from pupae at night when sheep likely to be in camps); low priority

Immune status	S		No evidence that sheep develop functional protective immunity to strike. Research to date on role of immune response in susceptibility to strike has failed to show a strong association (Preliminary review of effect of sheep immune response on susceptibility available in James 2006). Vaccinating against fleece rot to reduce bodystrike susceptibility showed some promise, but for a number of reasons not pursued Importance of bacterial proliferation on breech strike occurrence uncertain. Opportunity?	Blowfly vaccine projects underway (AWI Projects ON-00619 and ON-00624). Role of bacteria in breech strike requires clarification Potential for vaccination against key bacteria in breech strike?
Nutrition	S	M	• Clear effects via scouring • Some forages shown to reduce strike incidence (NZ work) (Was this just by anthelminthic effects/ reducing scouring or were other factors involved? Follow up)	Research on scouring needed; high priority
GI nematode sensitivity	S		Effect due to hypersensitivity scouring well documented, selecting for low hypersensitivity not currently an option	Research on scouring needed; high priority
Urine stain	S	M	A key factor in susceptibility, particularly on unimproved pastures; Relative contribution in breech strike in daggy sheep, i.e. interactions, unclear Difficult to score in presence of dags	Role of urine stain well established Better method of assessment needed ASBVs for urine stain needed Ways to encourage industry collection of data?
Other diarrhoeal incidence	S	M	A couple of reviews of dags and factors causing them available	See comments on dag/scouring research
Fleece rot	S		Importance of fleece rot-like or other bacterial conditions in determining the occurrence of breech strike unclear	Clarification of role of bacteria in breech strike needed, could present new control opportunities

Fleece bacteria	S		No differences could be found in microbial diversity amongst hogget ewes and rams from the Armidale or Mt Barker flocks No differences in Fungi and Yeast species were found between resistant and susceptible animals in either the Armidale and Mt Barker flocks However, member of Geodermatophilacea positively associated with breech strike in WA flock	May not be useful as selection character per se, but role of bacteria in strike susceptibility needs clarification with a view to both breeding and novel means of control Dynamics of bacterial populations likely to be important. What bacteria thrive on wet/urine stained/daggy sheep? What is the role of these bacteria – attraction, skin inflammation, oviposition stimulus, F1 nutrition? Literature review the first step
Skin colour	S		Anecdotally black sheep are more susceptible, evidence doesn't really support, but may be observation artefact, visual attraction of flies?	Of little practical significance
Food source for larval development to L3	S		Development to L2 stage with mouthparts that can actively invade skin may be important, skin exudate/dags/ bacteria per se/other food sources? Bacteria are important in diet of many other young fly larvae. Fleece bacteria effects on survival of *L. cuprina* L1 don't appear to have been investigated	Role of bacteria in breech strike development requires clarification. Discussed briefly in this review
Protective environment for fly eggs and larvae	S	E	Fleece architecture and fleece yolk may have effect on this, but likely to be most determined by management effects on breech wool length	Data available for body strike and breech strike. Given environmental and management effects on this considered low importance
Co-morbidities	S	M	Well documented that few *L. cuprina* breed in the carcases	Low priority
Sheep stressors	S		Potential effects on sheep immune response, but effects of immune response and more particularly variation between sheep, are unclear. No evidence that sheep develop functional immunity to strike	General stress/stressors considered unlikely to be important. Specific stressors different e.g. effects of parasites, digestive upsets

Pregnancy	S		Unlikely per se (See comments on sheep immunity). Post-natal staining and afterbirth adherence could be important if lambing during fly season	Limited impact
Age	S		Young sheep are more susceptible to strike	Management implications are well known
Breech fleece humidity	S	E	Significant work has been conducted on fleece humidity and fleece rot susceptibility, breech strike, see WA, potential effects on bacterial growth, egg and L1 survival	Significant investigations in body strike susceptibility showed positive association Association also evident in WA breech strike susceptibility flocks
Cystitis	S		Could be a factor in pizzle strike, of minor importance in overall strike incidence	Minor importance
Dermatophilosis	S		Body strike precursor in some areas/years/flocks; no effects on breech strike reported	Unlikely to be breech strike issue
Breech wool length	S	M	Crutching/shearing effects well known; effect of any differences in genetically determined wool length likely to be masked by management	Easily manipulated by crutching and shearing
Breech skin wrinkle	S		Clear key factor; affects dags and urine stain; possibly bacterial proliferation in wrinkles	Effects well known
Sex	S		Females more susceptible than males, breech urine staining key factor	Effects well known
Vulval formation	S		Malformations often management induced (e.g. crutching/shearing damage, dog bites etc. can be important; Some genetic differences in tail formation noted	Sheep owners usually cull sheep with abnormalities that result in urine staining or increased dag but opportunity for structured breeding approaches, other than selection against wrinkle, appears small.
Hip/hock conformation	S		Effect of hocks on staining in scouring sheep, but effect on breech strike? 'Hocky' heritable and sheep usually culled anyway. Hip formation effects not reported	Probably minor importance

Breech wool cover	S		Recent Australian and NZ work has demonstrated the importance of this trait and genetic parameters collected	More records required for better ASBVs etc in MERINOSELECT
Breed and bloodline	S		Known to be significant differences between Merino types and bloodlines	Largely accounted for by differences in known susceptibility factors
Genetic factors	S		Heritability estimates and other genetic parameters available and becoming refined as data collected	Collected from resource flock and industry collection continuing in Sheep Genetics. Further/ongoing recording required, particularly for characters such as dags, urine score and breech cover
Wool wax	S		Associated with fleece rot resistance, In WA breech strike selection lines, there was no relationship between moisture, wax, suint and wax content of midside wool and strike. However resistant line had higher wax	Maybe some association with susceptibility; Relatively low level of association suggests limited likely benefit from further investigation
Suint	S		Dubious whether associated with strike and obtaining repeatable measures problematic because of effect of rainfall and management (dipping & jetting)	Not a useful selection character
Wool v hair	S		Any effects confounded with breed; effects within Merinos unlikely to be useful because of wool quality issues, appears to be no data for association with breech strike	Little practical import
Wool follicle density	S		Preliminary investigation of follicle characters in CSIRO flock. Higher density (associated with high wrinkle) on midside, but no association with breech strike	Expensive character to measure, little evidence to suggest importance, further investigation probably not warranted
Maceration	S		See comments on exudation	See above
Humidity	S	E	Wool moisture/humidity differences associated with fleece rot and body strike susceptibility Some association with strike in ewes in WA	Given importance of environmental effects (rainfall, urine, scouring), impact of inherent sheep differences in fleece humidity on breech strike likely to be small

			Obviously important in development of strike. Largely environmental but see comments on breech fleece humidity	Dessicant compounds previously tested for fleece rot and bodystrike susceptibility Effect on bacterial populations?
Fleece colour	S		Key factor with body strike susceptibility; Association with breech strike susceptibility in WA data was not significant	Not thought to be significant in breech strike susceptibility
Fleece reflectiveness/brightness	S		White high reflectance wools suggested to increase resistance to fleece rot and body strike; see comments on wool colour above	WA data suggest no strong association
Management factors				
Anthelminthic resistance	M	E	Compromised control of scour worms from resistance may affect flystrike incidence, most acutely in southern areas	New chemicals, better methods of diagnosis
Tail docking	M		Length at docking important; well documented association with susceptibility	Effects well known
Mulesing	M	S	Known	
Anti flystrike clips	M	S	Known	No longer any significant use
Crutching and shearing	M	M	Reduces susceptibility; sometimes in bad flystrike can lead to strike in shearing cuts	Well known
Sterile male release	M		Proposed (experimental) area wide intervention; not used at moment	Currently under consideration
Environmental fly management eg trapping, insecticides	M		Relatively abundant information available; research to clarify situation with dicyclanil resistance is considered critical	Well researched and effects understood. Long distance attractants/attraction to traps unlikely With emergence of resistance to dicyclanil and growing desire to phase out mulesing, development of new flystrike insecticides is high priority

Fly factors				
Ectoparasiticide resistance	F		Research to clarify resistant situation with dicyclanil and cyromazine is critical	Research underway
L. cuprina adult survival	F		Poor relationship between fly numbers and strike except at low fly density; i.e. sheep susceptibility appears to be a more important determinant of strike incidence	Adult flies best targeted using area wide approaches. Sterile male being considered, but unlikely to be economic using traditional approach except (possibly) for localised suppression. Availability of the *Lucilia* genome provides the opportunity for novel area wide approaches
L. cuprina pupal survival	F		Reducing pupal survival could influence fly populations, particularly early in the fly season or at low fly densities	Opportunities for targeting pre pupal/pupal stages is unclear. Review of information and opportunities in this area may be worthwhile
Gravid *L. cuprina* abundance	F		See above; possible to influence fertility/fecundity by targeting fly genetic mechanisms?	Fly genome will facilitate significant opportunities in this area; currently under consideration
Gravid *L. cuprina* sheep location	F		Information is lacking on the mechanisms by which flies find sheep (at distance) in extensive grazing systems	Area of information deficit
Environmental factors				
Season	E		Clear seasonal patterns in flystrike risk	Design of fly programs with regard to seasonal patterns available in FlyBoss
Ambient temperature	E		Clear effect on fly activity, cease flight at <17^{o}C	Well known
Protracted rainfall	E		Environmental risk factor; interacts with wetting to skin and rate of drying, which in turn influenced by sheep factors	Well known

Wind speed	E		Environmental factor; wind influences *L. cuprina* activity/flight, no flight when wind >30kph	Effects known
Production eco system	E		Clearly effects from many perspectives but realistically best currently explored through effects on key influential factors	Individual farm fly programs vs opportunity for area wide approaches?
Soil type	E		Environmental factor that could influence fly pupation and fly populations	Some information available on survival of pre-pupae/pupae
Soil moisture	E		As above	Effects have been investigated
Soil temperature	E		Environmental factor influencing rate of development and +/- diapause during winter	Effects have been investigated
Wolbachia and other bio-control agents	E		Proposed as research area; +/- *Wolbachi*a between different *L. cuprina* strains? Biological effects? Previous importation of parasitoid wasps was unsuccessful in influencing fly numbers	*Wolbachia* under investigation Survival of *L. cuprina* and ability to cause strike outbreaks at low densities makes classical biological control difficult

Table 2. Correlations with Potential Selection and Economic Criteria, based on data from crutched but unmulesed sheep

Sheep Factors	Site	Age of Measurement	Correlations with Breech Strike (from birth to hogget shearing) – WA Site (Greeff *et al.* 2016)		Correlations with Breech Strike – Armidale, NSW Site (Smith *et al.* 2016)		Comments	
			Genetic	Phenotypic	Genetic	Phenotypic	Confirmed as useful indicator	Comment
Wrinkles	Breech	Early -	0.42 to0.47	0.10 to 0.24	0.26 to 0.62	0.08 to 0.20	Yes	ASBV since 2009
	Tail	Early -	0.44 to 0.49	0.10 to 0.20	-	-	No	WA results suggest further investigation
	Neck	Early -	0.33 to 0.46	0.10 to 0.11	0.13 to 0.42	0.05 to 0.11	Yes	Seen as alternative to breech wrinkle scoring
	Body	Early -	0.45 to 0.53	0.12 to 0.17	0.23 to 0.41	0.07 to 0.11	Yes	Seen as alternative to breech wrinkle scoring
Dags (Score)		Early	0.48 to 0.82	0.02 to 0.12	0.81	0.24	Yes	ASBV since 2009
		Late	0.59 to0.67	0.08 to 0.14				
Faecal moisture of dags		Late	0.34 to 0.92	0.06 to 0.10			Difficult to record	Potential similar to faecal consistency?
Faecal worm egg count		Hogget	0.08	0.01			No or very weak correlation	
Faecal consistency	Breech	Post-Weaning	0.6	0.01			Yes, strong correlation Dag Moisture	ASBV recommended, but not implemented

		Yearling	0.29 to 0.58	0.08 to 0.11				
		Hogget	0.13 to 0.92	0.05 to 0.08				
Wool coverage	Breech	Early	0.34 to 0.61	0.08 to 0.11	0.09 to 0.59	0.03 to 0.04	Yes	ASBV since 2009
	Crutch	Early	0.27 to 0.36	0.05 to 0.07	0.20 to 0.32	0.01 to 0.09	No	Potential useful indicator
	Belly	Early	0.15 to 0.34	0.03 to 0.06			?No	No results for NSW
	Face	Early	0.23 to 0.43	0.11	0.23	0.07	No	Potentially useful indicator - score before 1st shearing
Pluck factor	Belly	Before hogget shearing	0.09	-0.04			No - very low heritability	No results for NSW
Bare area around Anus and Vulva	Length / Depth	Marking	-0.28	-0.02	-0.25 to -0.34	-0.01 to -0.03	No	
	Width	Marking	-0.26	-0.03	-0.08 to -0.35	0.00 to -0.06	No - Not particularly useful	Sheep with bare breeches can still form dags, unless bare area is large
Tail	Length	Marking	0	0	0.12	0.03	No	
		Hogget	-0.28	0			No – but very high heritability	Docked tails were measured, so unclear why heritability is so high
	Width	Marking	0.04	0.03	-	-	No	

		Hogget	0.66	0.02	-	-	No – but re-examine	Greeff *et al.* (2016) argue that the strong genetic correlation may be related to excess skin around the docked tail, but can this adequately explain a genetic correlation?
Tail - bare	Length	Marking	-0.16	0.03				
	Width	Marking	-0.02	0.03				
Tail score		Marking	-0.03	0.08*[1]			No – little evidence of a relationship – WA only	
Urine stain	Breech	Early	0.52 to 0.58	0.10 to 0.14	0.06	0.18	Yes – WA only	ASBV recommended, but not implemented
Wool colour	Midside	Yearling to Hogget	-0.38 to 0.12	-0.02 to 0.02	0.01	0	No	Inconsistent or no correlation with breech strike, unlike body strike
Birth coat		Birth	-0.1	-0.03			No	Uncorrelated with breech strike in uncrutched animals
Other wool traits								
CVFD	Midside	Hogget	-0.05	0.03	w0.31 a0.24	w0.11 a0.13	Yes, but inconsistent	Low correlation with breech strike in NSW data, but weak to uncorrelated in WA data. However, correlated with body strike

FD	Midside	Hogget	0.02	-0.01	-0.25	-0.08	No	Low genetic correlation in NSW data, favouring finer wool
FDSD	Midside	Hogget	-0.05	0.03	0.12	0.05	No	Weak and not significant correlations with breech strike
SS	Midside	Hogget	0.05	-0.01	w-0.17 a-0.22	-0.13	Small	Different sign R_g b/w WA and NSW sites
CE	Midside	Hogget	-0.09	0.01			No	Negligible correlations with breech strike
GFW			0.06	0.02	w0.08 a0.20	w0.04 a0.16	No	Negligible. Adult in NSW slightly unfavourable with higher FW
CFW			0.05*[2]	0.01*[2]	w0.03 a0.21	w0.03 a0.15	No	Negligible. Adult in NSW slightly unfavourable with higher FW

*[1] Genetic correlation was -0.31 in uncrutched sheep but virtually zero in crutched sheep
*[2] Estimated from uncrutched sheep.

A range of other characters have been noted in the causal web and assessed in AWI's current breech strike research program and elsewhere as potential indirect characters for breech strike. For a range of reasons, these have been excluded from appearing in Table 2 as they are unlikely to be practically useful and genetic parameters for their association with breech strike susceptibility have not been estimated. These factors include wool reflectiveness/brightness, fleece humidity, vulval conformation, hip/hock conformation, follicle density, suint, the presence of follicle 'plugs', skin colour, and a number of other skin factors.

However, factors that may warrant further investigation are a number of tail-associated characteristics. Table 2 above, indicates a number of these that may be implicated - tail width (see below for further discussion), tail bare area length (r_g =-0.16), as well as tail wrinkle, which is presumably correlated with other wrinkle scores. These correlations also seem to occur in other data sets including that from uncrutched sheep in Western Australia, reported by Greeff *et al.* (2014). The width of the tail (measured after hogget shearing) had a strong genetic correlation of +0.66 ± 0.23 with breech strike from birth to hogget shearing, with a moderate heritability of 0.22 ± 0.09. Researchers have argued that this may be related to the excess skin around the docked tail, but it is difficult to see how this could have a major impact on a genetic correlation. Interestingly, the Joint Blowfly Committee (1933) describe the breech conformation of the Vermont as follows: "the skin of the rump is so arranged that the tail is wide and flappy with a marked central depression" so it is also possible that a wide tail is a remnant from the Vermont genotype introduced many years ago. Another trait that stood out was tail length (also measured after hogget shearing). It was highly heritable (0.80 ± 0.13), although it only had a genetic correlation with breech strike of -0.15 ± 0.11. It is unclear as to why this is the case because the animals' tails were docked to a standard industry protocol (Greeff *et al.* 2016).

In addition a range of carcase attributes including condition score, fat depth and eye muscle depth, not included in the causal web, were measured at Mt Barker, WA (Greeff *et al.* 2016). These traits are of economic import and thus their associations with flystrike susceptibility are of consequence in breeding programs. All have moderate heritability and their genetic correlations with breech strike are favourable and generally in the weak to low range, with the phenotypic correlations also favourable.

3. Breech flystrike review workshop and other research areas

3.1 Breech flystrike review workshop

3.1.1 Background

A workshop was held with leading researchers in the area of sheep flystrike biology and control and sheep breeding and genetics.

The objectives of the workshop were:

- Identify research gaps and opportunities towards
  - Improved methods of breeding for breech strike resistance; and
  - Development of novel sheep blowfly strike controls
- Recommend research directions and priority areas
- Add these outputs to the Breech Flystrike Risk Review
- Workshop participants were also requested to address
  - The value of/need for the WA and CSIRO breech strike research flocks to future breech strike breeding research and how should these flocks be used?

3.1.2 Methodology

The activities of the workshop are summarised below and detailed agenda for the day is given in Appendix 1. A list of the attendees is given in Appendix 2.

A number of keynote presentations were delivered to provide a background for the day's discussions (Appendix 1). The presentations were followed by the formation of four breakout groups. The first two groups (A1 and A2) addressed the area of sheep genetics, selection and breeding for flystrike resistance. The second two groups (B1 and B2) addressed the area of molecular biology of sheep blowflies and opportunities for novel control approaches presented by the availability of the sheep blowfly genome and recent advances in the area of molecular technology (e.g. CAS/CRISPR).

Members of Groups A1 and A2 were asked to address the following questions.

- What further work needs to be done to improve rates of genetic gain for increasing breeding for breech strike resistance with currently available breeding technologies?
- What is the priority for the development of sheep genomic breeding technologies in comparison to improvement of existing methods?
- What are the researchable issues?

Those in Groups B1 and B2 were asked to address:

- What new opportunities do availability of the *L. cuprina* genome and advances in molecular technology provide for the development of new or improved flystrike controls (e.g. flystrike vaccines, new insecticides, fly population-based or area-wide approaches)?
- What are the researchable issues?

Both groups were also asked to identify other ideas or potential areas for work relating to flystrike control, across research, development or extension/adoption, likely to yield benefits for industry in terms of reduced flystrike incidence or cost.

Outcomes from the discussions of the four groups were presented by group representatives and collated and summarised by the workshop facilitator. Areas of research were assessed collectively by the meeting participants in terms of risk, potential benefit, expected cost and timeframe for the realisation of outcomes. The key research areas were then ranked in order of priority by the allocation of 10 points per participant to the different key areas.

All participants were also asked to consider:

- The value of/need for the WA and CSIRO breech strike research flocks in future breech strike breeding research
- How these flocks should be used
- The value/need for future flystrike researcher forums.

These aspects were discussed later in the day (Appendix 1).

3.1.3 Rankings from workshop

Ideas generated from groups were summarised into the following 12 'opportunities' summarised in Table 3.

Table 3. Final priority and level of risk, magnitude of likely reward, cost and likely timeframe for each area of Research/Development/Extension as assessed by workshop participants

Idea/Area	Priority Rank	Points	Risk (H/M/L)	Reward (H/M/L)	(Cost H/M/L)	Time-frame
Fly biology / population dynamics	1	45	L	L	H	2-4
Genomics reference flock	2	38	L	H	H	>5
↑Data → breech strike index	3	24	L	M/H	L	2-4
Fly genetics and timing	4	23	L	M/H	M	2-4
Support resource flock	5	19	L	M	H	>5
Extension – value proposition	6	15	L	L?	L	1-2
Role of attractants / odour	6	15	H	M/H	H	>5
Fly microbiome - vulnerability	6	15	L	L	L/M	2 - 4
Fleece / dag microbiome	7	11	L	M/H	L	2-4
Unexplained variation	8	10	H	H	H	>5
Sex-determination factors	9	6	M	M	L	2-4
Integrate sheep/fly genome	10	3	M	M	M/H	>5

Further elaboration from the workshop and notes about each of these areas from the review panel are given below

1. *Increase understanding of the fundamental biology of Lucilia leading to opportunities for control*
   This was considered high priority because of the large number of potential approaches which additional knowledge in this area underpins. Some areas of particular interest are:

- Knowledge of the genetic structure and interconnectedness of different *L. cuprina* populations is important to identifying genes that have wide applicability as vaccine or insecticide targets and to designing area wide approaches to population suppression or eradication
- Better knowledge of dispersal and degree of migration and interconnectedness between populations to formulating optimal resistance management programs and to inform recommendations on early season treatments for blowfly strike control
- Microbial involvement in breech strike of sheep, what affects it and how it is involved in flystrike genesis. While quite a lot of work has been done in microbial involvement in the development of body strike, much less is known of the importance of bacteria and other microbes in susceptibility to breech strike
- Better understanding of the spatial ecology of the post feeding (off host) larval stages and pupae. (Where are they, how are they distributed, can they be targeted?).

This is a broad area, that will be facilitated by the use of new molecular techniques. These studies need to be well targeted to provide knowledge with specific endpoints relating to improving control efficacy or specific research outcomes. There is already a substantial literature in this area, and we emphasise the importance of a careful review of (in particular) the work that was done for the CSIRO genetic control program in the 1970s and other relevant studies in this area to establish what is already known before research is commenced.

2. *Invest in a genomics reference flock to generate genomics breeding values*
Genomic approaches to selection, whereby the presence of major genes, groups of genes or genomic indexes are directly measured to predict genetic merit of breeding stock are being used with increasing frequency in selecting programs for many livestock species. These approaches provide major potential benefits to animal breeders because detailed phenotyping is not required. Rather a small sample of genetic material, such as blood or plucked hair is collected and can be used to provide direct assessment of genetic merit for a range of traits.

Genomic methods could have particular advantages for selecting flystrike resistance as animals would not need to be exposed to strike, or predisposing conditions such as scouring or urine stain for a genetic evaluation to be made. A genetic value could be attributed to all animals, regardless of whether they are bred in high or low flystrike environments or whether it is a high or low flystrike year.

This was considered a 2nd priority in the workshop rankings but had an overall point score well above the 3rd ranked and lower priorities. This was considered likely to provide significant gains in the longer term (>5 y) but was considered a high value, but high-risk priority.

The value of maintenance of the flystrike selection flocks in this context was noted.

3. *Increase phenotypic data with a view to putting indicator characteristics into a breech strike index*
There is a need to encourage much more widespread phenotyping for flystrike traits in industry as well as research flocks. There are still relatively few industry records contributing to the estimation of ASBVs for a number of the major flystrike traits, most particularly for difficult to assess traits such as urine stain, dag score, and breech wool cover. Collection of more industry data will greatly increase the accuracy of ASBVs for these traits and applicability to different industry breeding objectives and management regimes. There is a need to facilitate practical 'useability' of breech strike traits for sheep breeders. The development of breeding indices incorporating breech strike resistance while maximising genetic gains for other traits are

needed in MERINOSELECT. Optimal incorporation of breech strike resistance will require the derivation of an economic value(s) for breech strike resistance.

4. *Understand the life cycle of the fly and the genes that operate at different times and in different environments*
   Better understanding of the genes that operate at different stages in strike establishment (host finding, oviposition, egg hatch and larval invasion), critical developmental genes and conditional survival genes (for example for overwintering) will aid the identification of new vaccine and insecticide targets and facilitate development of other novel approaches such as area-wide genetic controls.

5. *Support continuation of the breech strike resource flocks*
   The two flocks provide a source of very accurately pedigreed and phenotyped animals and are in completely different environments with different flystrike profiles. The depth of phenotyping for flystrike incidence in the flystrike selection lines in WA (now at Katanning) and NSW (Chiswick) makes these flocks an important core resource for genomic studies, a prime resource for identifying and testing new indicator characters and valuable for obtaining more precise genetic parameters towards more accurate selection and breeding programs. They will also be key resources such as the development of welfare indexes and ASBVs. In addition to breeding related activities the flocks provide a valuable resource for research in other areas, for example investigating the role of microbiome profiles in strike etiology and susceptibility, testing the efficacy of new vaccine technologies across susceptible and resistant phenotypes, and the future development of welfare indices and breeding values.

6. *Refine the value proposition related to the adoption of breech strike resistance in selective breeding*
   To understand if further investment into breeding programs focussed onto reducing breech flystrike is worthwhile, and to underpin promotion to woolgrowers from applying genetic technologies or other approaches, an understanding of the size and scale of potential benefits is required. A component of this work, for example, would be a benefit cost analysis for establishing genomic evaluation of flystrike. This would also inform the feasibility/attractiveness of different approaches by quantifying the size of trade-offs that growers are willing to make. This was ranked equally in priority with the following three recommendations with points well below the top three recommendations. It was suggested that this may have been ranked higher by people with a more applied focus.

7. *Better understand the role of attractants*
   Odour is involved at a number of stages and represents a key factor in the stages in the development of strike. In particular (probably) distance location of sheep, the identification of susceptible sites on sheep for oviposition of flies and stimulating egg laying. Availability of the fly genome has already enabled the identification of some genes involved in *L. cuprina* odour perception (AWI Project ON-00373). The role of odour at different stages of flystrike and potential differences in susceptibility is addressed elsewhere in this review.

8. *Role of the fly microbiome and its role in relation to vulnerabilities*
   Metagenomic assessment of the bacteria associated with *Lucilia sericata* and *L. cuprina* has indicated that most are acquired from the environment with little evidence to support transgenerational transfer except for *Wolbachia* and possibly *Proteus mirabilis*. It seems that any work in this area will be largely speculative and in the absence of a well-developed hypothesis as to how this work would lead to new controls, we consider it relatively low priority. Characterisation of sheep breech skin/fleece microbiome, the effects of predisposing

factors such as urine staining, scouring and fly interactions is likely to be an avenue of much greater potential benefit.

9. *Understand the fleece / dag microbiome*
   It is well known that bacteria are important at various stages of body strike development, for example in providing odour cues for attraction and oviposition, causing skin scalding and extravasation which provides protein for 1st instar larvae and a focus for skin invasion by early stage larvae. It is also known that in other fly species microbes can provide nutritive elements for the larval stages or adults. It is well known microbial odours are extremely important in the attraction of a range of other livestock ectoparasites to their hosts, particularly when associated with urine contamination. Much less is known about the importance of microbial communities and interactions with urine staining and scouring and roles in the development of breech strike and we consider this an area of research of some priority. In addition to their importance in determining breech strike susceptibility. Dags are an important issue in their own right, and research towards understanding the development of, and susceptibility to, dags could yield substantial labour saving and flystrike reduction benefits. Recommendations from AWI Projects WP520 and ON-00610 'Minimising Dag in Sheep' should be considered.

10. *Better understand the unexplained variation in resistant and susceptible sheep*
    The amount of variation in breech strike susceptibility not explained by the major indicator characters will be key to a consideration of the need for new or better indirect selection criteria. A significant proportion of the variability in susceptibility to breech strike remains unexplained in some data sets but the amount appears to vary with different locations and different management regimes. For example, in crutched ewes in WA only 9.4% of the variation remains unexplained and the presence of dags and skin wrinkles appear to explain most of the phenotypic variation. This is different to NSW flocks and unmulesed, uncrutched flocks in WA where approximately 50% of the variation remains unexplained. There is a need for a 'harmonised analysis' of the WA and NSW data to explain what portion of this is variation is environmental, followed by careful consideration of the likelihood/value of finding selection criteria that can markedly improve the accuracy of selection.

11. *Understand sex determination factors in sheep blowflies*
    The development of 'male only' or female killing strains can enable novel new approaches to genetic/area-wide control and markedly improve the economics of area-wide release techniques (such as the sterile insect technique). Female killing strains of *L. cuprina* have been developed in the US (with US strains of *L. cuprina*). These could be intercrossed with Australian field strains of *L. cuprina* and then tested in field cage studies to evaluate the feasibility of their use in area wide programs for the eradication or suppression of sheep blowflies.

12. *Integrate sheep and fly genomics*
    Considerable work remains to elucidate and annotate genes for both the sheep blowfly and sheep. The opportunities presented by integration of the two genomes should be kept in mind but at this stage we consider that effort should be directed towards further identification of critical genes in the individual fly and sheep genomes. This approach was considered lowest priority by the participants at the Breech Flystrike Review Workshop.

3.2 Other major flystrike research and development areas not discussed at the workshop

Although the objective of the workshop was to identify research gaps and opportunities towards the development of novel sheep blowfly strike controls and to recommend research directions and priority areas, some major potential areas were not considered, probably reflecting the interests of the invited audience. However, a number of these areas were identified in information and ideas submitted by invitees and a number are also the topic of AWI funded projects underway. There were also a number of major areas that we considered were missed and these are also captured and briefly discussed below.

13. *Manage insecticide resistance and maintain the efficacy of available flystrike control products*
    The availability of effective flystrike protection and treatment chemicals remains critical to effective management of flystrike in Australian flocks, particularly in non mulesed flocks. There is a long history of resistance development to flystrike control chemicals and the recent emergence of resistance to keystone control products, dicyclanil and cyromazine is a major threat to sustainability of wool production. This will be particularly critical in unmulesed and highly susceptible flocks. The characterisation and monitoring of resistance and promotion of resistance management strategies should continue to be an important element of flystrike control programs. AWI is currently investing in a project to determine the extent of this resistance and to characterise the effects of resistance on product performance (AWI Project ON-00491). This is considered very high priority research because of the current high level of industry dependence on products based on these active ingredients for effective and labour-efficient flystrike control. There has been limited science-based consideration of the best management approaches to manage this resistance and prolong the effectiveness of flystrike control compounds and a project to model optimal resistance management programs is required.

14. *Develop new insecticidal actives or formulations for flystrike control*
    With increasing costs of development and registration, the rate of new production animal parasiticide active compounds coming onto the market has “slowed to a trickle”. The wool sheep parasiticide market is relatively small in the world context and this is particularly relevant as all of the major pharmaceutical companies that conduct research in this area have a multinational focus. Research in this area will assist the continued availability of effective flystrike preventatives for use by Australian wool growers. The availability of the *L. cuprina* genome will provide the possibility of new insecticidal targets (as well as oviposition suppressants) and AWI is currently funding a project in this area. AWI may need to increase their involvement with commercial veterinary pharmaceutical companies to assist new product development. There may be an opportunity to revisit some previously suggested chemicals. (For example; the case of the oviposition deterrent GH74 and like compounds is mentioned elsewhere in this review) and it is likely that veterinary pharmaceutical companies may have other potential compounds that have not been developed to date as sheep flystrike protectants). In addition, the cardinal rule of toxicology has often been modified to: “Dose makes the poison; but formulation is everything”. Advances in formulation technology, particularly in the areas of controlled release technology (e.g. capsules, slow release flea collars and insecticidal ear tags for cattle), nanotechnology and ‘smart drug delivery systems’ with stimuli-responsive characteristics can greatly increase the efficiency of parasiticides. The development of nanoparticle and stimuli responsive formulations is currently under investigation in an AWI project (ON-00549).

15. *Development of flystrike vaccines*
    AWI project(s) towards the development of a flystrike vaccine is underway. This will be facilitated by the recent availability of the *L. cuprina* genome and current projects to identify critical genes in the genesis of flystrike, which offer the possibility of new gene targets for a vaccine (This is a high risk, but potentially very high reward project).

16. *Biological control of sheep blowflies*

    Classical biological control - *The release of specialist natural enemies*. These may be pathogens (e.g. bacteria and fungi), parasites (e.g. parasitic wasps) or predators of the pest. The objective is to establish persisting populations of these natural enemies that attack the pest and continue to suppress its population. However, *L. cuprina* occurs at low population density at most times and flystrike is episodic with fly populations building rapidly when conditions become suitable. The rate of spread of pathogens and parasites is almost invariably density-dependant. This factor and the lag time generally experienced for a corresponding increase in numbers of biocontrol agents during pest outbreaks (for example flystrike waves) would seem to present difficulties for any classical biocontrol agent to persist and impact on *L. cuprina* populations, or more particularly to reduce strike incidence.

    Biopesticides (innundative biological controls applied to sheep): *Bacillus thuringiensis* and some entomopathogenic fungi have shown short term protection when applied to sheep in experimental studies and suitable agents may have application as part of an integrated approach or in organic flocks. However, they are unlikely to provide a level or persistence of protection comparable with chemical pesticides.

    Pathogens that persist in the soil, such as some fungi (e.g. *Tolypocladium spp*.) or entomopathogenic nematodes (e.g. *Steinernema spp*. *Heterorhabditis* spp. may be able to have effect against soil stages of *L. cuprina*, particularly prepupal larvae and particularly during the overwintering phase. However, better knowledge of the spatial and temporal ecology of the soil phases of *L. cuprina* (see 1 above) will be required to assess whether sufficient mortality could be induced to significantly affect flystrike incidence.

    The potential of biological control of *Lucilia spp*. using sheep blowfly pathogens is currently being reviewed in more detail as part of AWI Project ON-00620.

17. *Area wide genetic controls for Lucilia cuprina*

    These methods seek to bring about suppression or eradication of the pest population by the release of flies of the same species that have been modified to confer sterility or cause genetic death in the target pest population. These methods are also known as autocidal control and are usually used in area wide strategies focussed on eradicating pest populations or reducing pest abundance. The most well-known method, the sterile insect technique (SIT) uses mass releases of male insects that have been irradiated using gamma radiation to cause damage to insect chromosomes or sperm, effectively rendering them sterile. With many species of flies, including *L. cuprina*, the females only mate once. Therefore, if a female mates with a sterile male she is effectively sterilised for life. With serial mass releases of sterilised males, the chance of a fertile female finding a fertile mate is functionally reduced to zero and a population can be eradicated from the release area. In its most well-known use, the SIT method has been successfully used to eradicate screwworm flies from north and central America. This method has also been used for eradicating regional incursions of insects, such as fruit flies in fruit fly-free areas of Australia and an incursion of screwworm flies in Libya. However, because of the widespread areas in which *L. cuprina* is found in Australia this approach has generally been considered uneconomic for widespread use. Regional use of sterile male may be viable for *L. cuprina* control in some situations but would require a significant research and development effort to establish.

    In the 1970s, in an effort to address the cost barriers to use of sterile male in Australia, CSIRO investigated the use of compound chromosome strains, sex-linked translocation strains and female killing systems in an attempt to suppress or eliminate *L. cuprina* populations (Foster

1980; Foster *et al.* 1985). In this case the flies carried recessive eye colour mutations that caused functional blindness and were lethal to females in the field. However, because the males carried the normal eye colour genes on the male sex chromosome they survived and continued to spread the lethal genes to females in later generations. It was considered that this approach could be more cost effective than the sterile insect technique for control of *L. cuprina* in Australia because of the need for lower release ratios than necessary for the SIT technique. However, in spite of some initial success, because of operational difficulties and funding constraints, this was eventually not pursued.

The availability of gene editing technologies (such as CAS-CRISPR) has provided the potential of more elegant systems of genetic control such as RIDL (Release of Insects with Dominant Lethality), RNAi based approaches, homing endonuclease genes (HEGs) (McGraw and O'Neill 2013) and potentially using gene drives to spread deleterious (often sex-linked or stage specific genes) through fly populations. The RIDL approach is currently under investigation for use in other pest species, most particularly mosquitoes (Alphey 2014). It is expected that research funded by AWI currently underway will identify critical genes in *L. cuprina*. This may facilitate the design of suitable genetically modified strains that could spread through the population and compromise survival or fertility of flies, or perhaps their ability to initiate strikes.

The efficiency and economic feasibility of the sterile insect technique and many other genetic control techniques can be significantly improved if only genetically modified males are released (Scott 2014; Scott *et al.* 2014). Transgenic sexing "male only" strains have been developed in North American blowfly strains (*L. cuprina cuprina*) (Li *et al.* 2014). These strains carry a tetracycline repressible female lethality gene whereby the females can be used in the rearing system, but then eliminated in the later stages so that only sterile males or males carrying the female killing chromosome are present for release. There is potential to use these "male only" strains as the basis for a future genetic control of *L. cuprina dorsalis* sheep blowfly populations in Australia. This would require interbreeding of the two strains to introduce the trait into the Australian *dorsalis* strain. Cage studies could then be conducted to test the ability of the resultant strains to suppress Australian populations of sheep blowflies (Li *et al.* 2014).

18. *Project to address scouring*

Scouring (diarrhoea) and resulting faecal accumulations in the breech wool of sheep (dags) are major predisposing conditions for breech strike in southern sheep production areas. Controlling dags to minimise wool staining and avoid faecal contamination of carcases is also a major management issue in its own right. Research to reduce the occurrence of dags could have a major effect in reducing breech strike incidence. Recommendations for research and development to reduce the impact of dags were made in AWI Projects WP520 and ON-00610 – 'Minimising dags in sheep' and are not considered further here.

General points of discussion around priorities from workshop

- Very important to invest in underpinning science
- Need to operate and prioritise within existing resources
- Should consider at what stage resistance may become problematic?
- Silver bullet unlikely so continued focus on multiple solutions (IPM approach)
- Consider how any solutions / technologies fit within 'normal' farm management practices
- For each idea, ensure you have a clear 'route to market'
- Ideas generated by this forum need to be 'ground-truthed' with farmers
- Take account of social issues and research them (marriage of social and technical)
- Review what's been done before, especially on fly biology.

3.4 Continuation of the flystrike resource flocks

- There was strong support for the Armidale and Mt Barker resource flocks. The establishment of genomics reference flock was priority 2 and support for the resource flock priority 5
- The two flocks are in completely different environments and it would be useful to work with both flocks
- Dags are a very big issue in Mt Barker and there is a need for a good faecal consistency score and a breeding value for low dags using summer rainfall data
- Significant advantages of using previously phenotyped and pedigreed flocks as basis for on-going genomic analysis
- It was noted that the Armidale flock was now quite old and alternatives (such as MLP flocks) could be utilised
- Opportunities to use resource flocks included:
  - Uni of Melb – implants into resistant and susceptible sheep
  - Genomics resource flocks – need to capture data on flocks
  - Attractant work with WA flock
  - Develop ASBV for Faecal consistency score
  - Testing prototype vaccines (need approx. 200 animals)
  - Examine whether the resistant or susceptible is the same trait or might be pre-disposition rather than resistance?

3.5 Follow-up forums

There was support for follow up forums:

- They could consider results of current research as well as identifying new ideas. Synergies across projects may also be identified
- Examine the potential for inter-disciplinary teams focussing on a major program of work - focus on a particular problem / opportunity (for example integrated management)
- Involvement of commercial companies (e.g. pharma) in forums should be considered
- Try to align the timing of forums for when overseas researchers may be able to attend (e.g. when attending a conference – parasitology, AAABG)
- Early adopters of research outcomes (producers) should be involved to ensure RD&E relevance
- The option of more 'informal' forums over a couple of days (like Yarra Valley fly forums) should be considered
- When establishing future dates, check timing of other domestic and overseas conferences.

4. Odour review

4.1 Unexplained variation in flystrike susceptibility

The percent of variation in breech strike susceptibility not explained by the major indicator characters will be a key consideration in the likelihood that rates of gain can be improved by the identification of new criteria. The only suitable datasets to determine this are the AWI breech strike selection flocks maintained by DPIRD at Mt Barker and now Katanning in WA and by CSIRO at Chiswick, near Armidale. Data on breech strike incidence is available from 2006 to 2014. Reports on unexplained variation are available from both flocks, following analyses of the impact of risk factors on breech strike incidence (Greeff *et al.* 2016; Smith 2016).

Mt Barker site, WA

The breech strike research flock was established in 2006; progeny born from 2006 to 2009 were not crutched as yearlings, but progeny born from 2010 to 2013 were crutched 4 months prior to the main flystrike season. Greeff *et al.* (2018) report the results of variance partitioning of the incidence of breech flystrike data for animals born from 2006 to 2011, whereas Greeff *et al.* (2016), in the final AWI Project report (ON-00169), present results on progeny born between 2006 and 2014.

Uncrutched and unmulesed animals, born from 2005 to 2009. Table 4, from Greeff *et al.* (2018), presents information on the percentage of variation explained by significant indicator traits of breech strike incidence from birth to hogget shearing. Approximately half the variation in breech flystrike incidence in this data remains unexplained (in either sex). More details on the separate traits of breech flystrike from birth to weaning and weaner to hogget shearing are available in Greeff *et al.* (2018), noting that for the latter period, around 64% for ewes and 61% for males of the variance for breech flystrike remains unexplained.

Table 4. (originally Table 9 in the paper). The percentage of variation explained by the significant indicator traits of the log-transformed breech strike counts from birth to hogget shearing in unmulesed and uncrutched ewes and rams born from 2005 to 2009 (Greeff *et al.* 2018)

Trait[A]	Ewes[B]	Rams
Number of sheep	1433	1148
Body wrinkle at marking	1.5	-
Tail wrinkle post-weaning	21.5	21.0
Body wrinkle at yearling age	-	2.9
Urine stain at marking	1.3	-
Urine stain at weaning	3.8	-
Dag at weaning	2.8	1.3
Dag at post-weaning	2.0	2.1
Dag at yearling age	1.7	16.3
Dag at hogget age	10.6	3.4
Dag moisture in spring	1.5	-
Clean Fleece Weight	-	1.7
Residual (unexplained) variance (%)	***53.2***	***51.5***

Crutched and unmulesed animals, born from 2010 to 2013. When progeny were crutched 4 months prior to the flystrike season, a very different result emerges in terms of unexplained variation in breech flystrike incidence. Table 5 presents information on factors explaining the variation in breech strike from birth to hogget shearing in crutched animals. In ewes, only approximately 10% of the variance remains unexplained, whereas the majority of the variance (about 71%) remains unexplained in male progeny.

Table 5. Factors explaining the variation in breech strike from birth to hogget shearing in crutched animals (originally Table 6b on page 33 of the final report) (Greeff *et al.*, 2016)
Note that columns have been reversed so that they align with Table 4.

Source	Females	Males
Dag at weaning	-	14.40
Breech wrinkle post-weaning	85.56	
Face cover post-weaning	-	5.32
Breech wrinkle at marking	-	6.61
Dag at hogget age	4.64	0.73
Dag moisture at hogget age	-	1.53
Unexplained variance (%)	***9.81***	***71.41***

CSIRO flock, Armidale
Figure 3 a) and b). summarise the relative contribution to variation in weaner and yearling-adult breech strike respectively, of fixed factors, indicator traits and genetic effects.

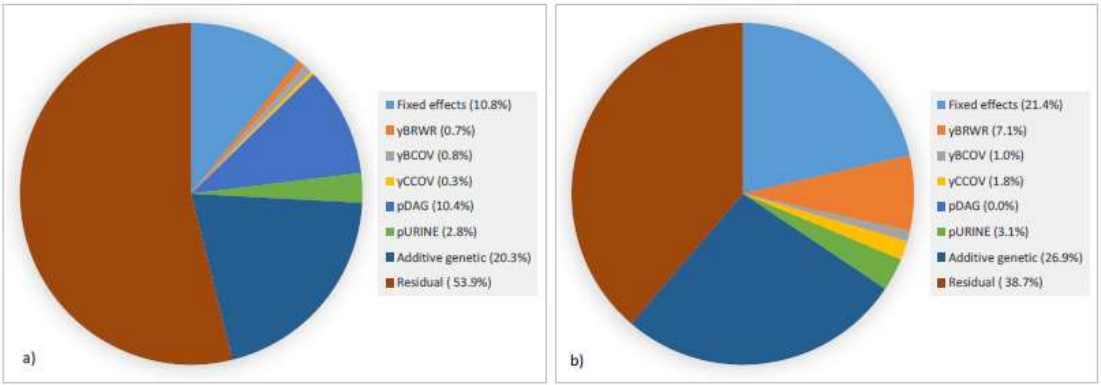

Figure 3. Relative contribution to variance in a) weaner breech strike (males and females), and b) yearling-adult (females only) for fixed effects, key indirect indicators of breech strike, genetics, and residual variation. For weaners, fixed effects included selection line, mulesing, sex, birth-rearing type, age-of-dam, management group (which incorporates birth year), and body weight. For yearling-adult females, fixed effects included selection line, mulesing, contemporary group (which incorporates birth year, number of years retained in the flock, and exactly which years they were), and yearling bodyweight (Smith 2016)

The management of the Armidale flock has been consistent since it was established in 2006, with animals crutched before the onset of the fly season.

In summary, in the Armidale flock the unexplained variance for breech strike is 54% for weaners (females and males) and <40% for yearling/adults (females only). Fixed effects accounted for approximately 11% of the variance for breech flystrike in weaners, but over 21% of the variance in breech flystrike in yearling/adult ewes. The combination of the phenotypic effects of breech indicator traits (breech wrinkle, breech cover, crutch cover, dag and urine stain) and the additive genetic variation accounted for 35% of the variation in breech flystrike in weaner sheep. For yearling-adult breech strike, the phenotypic component of breech wrinkle is more important than it was for weaners, and the importance of dag was lower. The additive genetic component was almost 27% for yearling-adult breech strike. Thus, wrinkle is even more important for breech strike risk in adults than weaners, at both the phenotypic and genetic levels.

Suggested Improvements: It would be useful to have males and female weaner data separated, akin to the WA analysis. However, as the proportion of variance explained by all fixed effects is only 11%, of which sex is only one of 7 effects, the differences between the sexes is unlikely to be a large figure.

In summary, a significant proportion of the variability in susceptibility remains unexplained but the amount varies with different locations and different management regimes. Notably, crutching markedly reduces the amount of variation unexplained in ewes, but not in males. This may suggest that factors associated with the fleece or wool characters are a key source of unexplained variation in ewes and may indicate the importance of urine stain, or potentially fleece wetting or resultant bacterial growth in explaining variability.

4.2 Detection dog studies

This critical study indicates that dogs could smell the difference between susceptible and resistant sheep and was the main stimulus for the work on odour and attractiveness reviewed in this report (Greeff *et al.* 2013). Detection dogs were trained on wool from resistant & susceptible ewes from one flock (WA Mt Barker resource flock) using a positive reward system and tested on a second flock to which they were naïve (CSIRO Armidale resistant flock). Both of these flocks had known flystrike history and accurate pedigree records. In testing an exact result for both dogs of 76.9% (82% in ewes from paper) to identify target (resistant) and 92% to ignore non-targets (susceptible) was achieved when tested on the Armidale flock. These are positive results showing dogs can detect differences in wool samples between groups that are known to be resistant or susceptible to breech strike.

While the wool was collected from resistant and susceptible ewes, the animals had very different histories of strike and treatment. All of the susceptible ewes had been previously struck and treated in previous years (2008, 2009 & possibly 2010 depending on when the wool was collected), whereas the resistant ewes had never been struck or treated. Although no wool grown since the previous shearing was used to train the dogs, and wool samples were collected outside of the fly season, it is not possible to distinguish if the dogs are detecting a heritable difference between animals that are resistant and susceptible, or if they are detecting an odour difference resulting from, or due to, the previous occurrence of strike and treatment per se. This was true of both the training and the test sheep.

It is not possible to tell what differences the dogs are detecting between the groups and what is the physiological basis of this difference. Additionally, the dogs were trained on wool that had been stored in a dry cool place for up to 6 weeks so it is likely that any highly volatile odours present would have dissipated, and that some odorants have degraded and therefore the odour signature may not be indicative of what is experienced by flies when finding hosts. Nevertheless, the dogs did seem to identify a difference between resistant and susceptible animals with a high degree of accuracy and clarification of what the dogs were detecting may assist the identification of indirect selection characters or the development of other novel means of control. However, that dogs were able to detect differences between resistant and susceptible sheep should not imply a mechanistic explanation or that flies detect the same odours. That is the things that dogs can smell may not be responsible for the difference, simply associated with it. As an analogy, fish oil and cod liver oil smell strongly and are known to have significant health benefits, but there is no suggestion that the health benefits are due to their particular smell.

Dogs have exquisite sense of smell and detect intricate odour signatures formed by complex odour mixtures. There is currently no commercially available equipment that can meet the performance of a dog's nose. An 'E-nose-like' technology that seeks to mimic the particular odour signatures detected by dogs, linked to machine learning or artificial intelligence algorithms, may provide the best method of detecting the same odours detected by the dogs, however this type of technology is in its infancy.

4.3 Insect perception of odour

In insects, odour compounds are detected with the antennae or maxillary palps, whereby a variety of receptors, expressed on odorant sensory neurons (OSNs) located in multiple types of odorant sensilla, fire signals to the brain when odours are present (Figure 4). In addition, there are often gustatory receptors and potentially odorant receptors on the tarsi and ovipositors of insects that can also be important in the sequence of events leading to behaviours such as mating, feeding or oviposition. The olfactory organs have three main classes of sensilla; basiconic, trichoid and coeloconic, which can detect different classes of compounds depending on the insect species. In Diptera and Lepidoptera species, basiconic sensilla generally detect fruit or plant odours (Hallem and Carlson 2004), while trichoid sensilla detect pheromones (Hallem *et al*. 2004; Kaissling *et al.* 1978) and coeloconic sensilla detect organic acids and amines (Pophof 1997). These sensilla contain olfactory pores which allow the entry of odorants into the aqueous lymph of the sensilla (Steinbrecht 1997). The lymph contains a range of soluble olfactory proteins; odorant binding proteins (OBPs), chemosensory binding proteins (CSPs), and odorant degrading enzymes (ODE's) which function to bind, transport and degrade odorants in the lymph (Leal 2013).

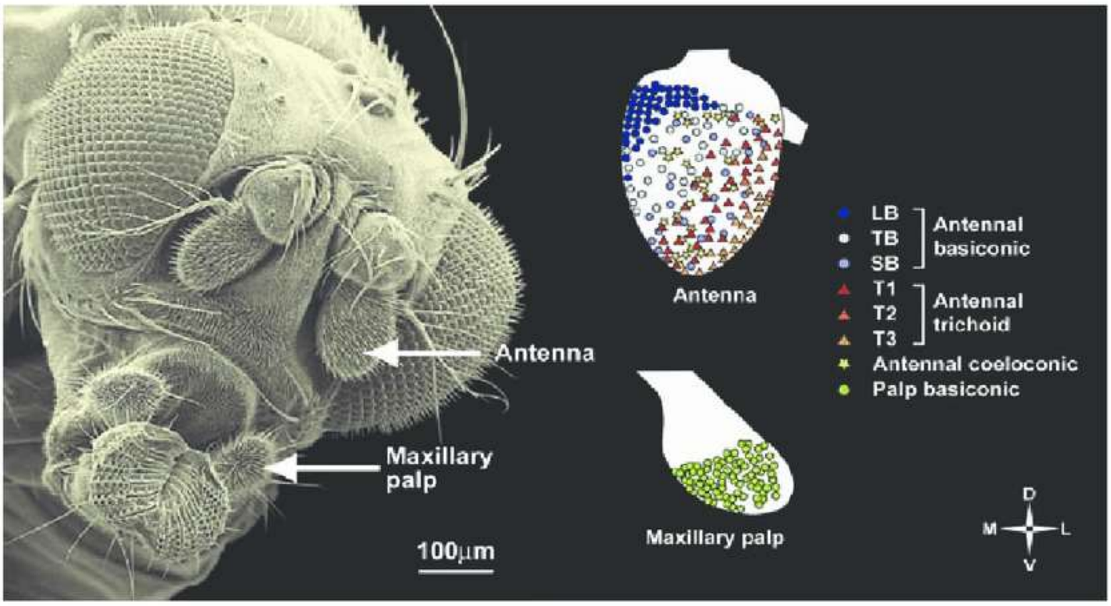

Figure 4. From Laissue and Vosshall, 2008; highlights the main olfactory organs and sensilla types in dipteran insects

The sensilla also contain two to four OSNs which express receptors that detect odorants. There are three families of receptors present in OSNs on the antennae and maxillary palps (Figure 5), the odorant receptors (ORs) (Clyne *et al.* 1999), the gustatory receptors (GRs) (Clyne *et al.* 2000) and the Ionotopic receptors (IRs) (Benton *et al.* 2009). The ORs are tuned to detecting volatile organic compounds (Hallem *et al.* 2004), the GRs located in these OSNs detect CO2 (Kwon *et al.* 2007), and the IRs located in the coeloconic sensilla are tuned to amines and organic acids (Yao *et al.* 2005; Silbering *et al.* 2011).

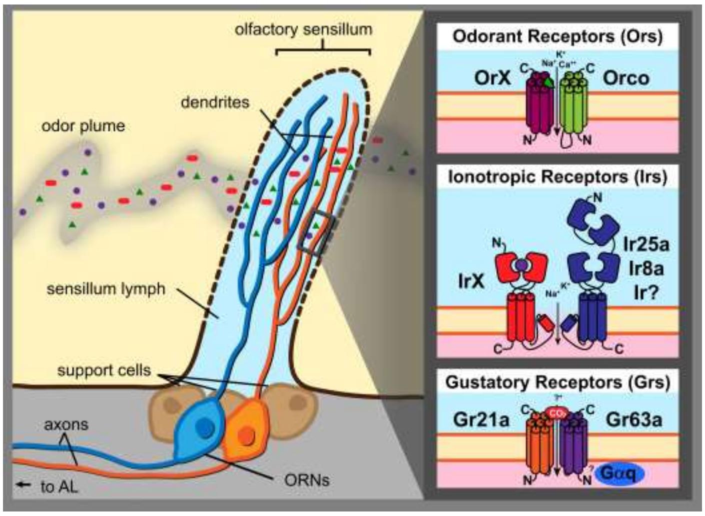

Figure 5. From Pask and Ray, 2016; depiction of an olfactory sensilla, highlighting the architecture of the sensilla and the receptor types expressed in chemosensory neurons

The most deeply studied of the receptor families are the ORs. Almost all OSNs express a co-receptor, *Orco,* which is highly conserved across insect taxa and is required for the detection of odorants (Dobritsa *et al.* 2003; Larsson *et al.* 2004). *Orco* forms a heterodimer in the OSNs with a variable ligand-binding OR and this complex is believed to function as an ion channel (Sato *et al.* 2008; Wicher *et al.* 2008). These ligand-binding ORs are highly divergent both within and between insect species. The size of the OR family ranges from 10 in some insect species up to 400 in Hymenoptera characterised by species-specific expansions of single genes or gene families likely driven by host shifts or specialisations (Hansson and Stensmyr 2011).

ORs work in a combinatorial system to allow the insect to detect and distinguish thousands of odorants using a limited number of receptors (Hallem *et al.* 2004). Some receptors are broadly tuned responding to a range of odorants and some or more narrowly tuned to important ecological odours for the insect, for example pheromone receptors in Lepidoptera. Both *Orco* and ORs with narrow affinity for ecologically important cues may be potential targets for olfactory disruption. More broadly tuned receptors are less attractive for disruption due to the redundancy of the combinatorial system.

Insect GRs are critical for insect detection of gustatory cues (e.g. sweet, bitter, salt), however GRs also have a specialised role in insect odour perception as they detect the universal insect volatile cue CO2 through GRs expressed on the maxillary palps (Robertson and Kent 2009; Lu *et al*. 2007). Surprisingly, GRs located on the palps of mosquitoes have also been shown to respond to an array of human derived odours indicating they may have a significant role in locating and differentiating human hosts (Tauxe *et al.* 2013; McMeniman *et al.* 2014). The GRs expressed in the maxillary palp are important for host seeking may also be potential targets for olfactory disruption.

IRs are a family of receptors related to ionotropic glutamate receptors that are thought to have diverse function in insects (Benton *et al.* 2009). Across insect orders, a subgroup of these receptors, antennal IRs, are specifically expressed in the antennae and are absent in other tissues (Benton *et al.* 2009; Croset *et al*. 2010). Analogous to the heteromeric assembly of Orco with ligand specific ORs, two receptors have been shown to co-express with the antennal IRs in coeloconic OSNs and appear to be required for odorant responses from these sensilla (Abuin *et al.* 2011). The finding that there are orthologous IRs across insect orders suggests IRs are tuned to general and essential chemosensory cues for insects (Rytz *et al*. 2013), which also makes them potential targets for olfactory disruption.

4.5 Definition and terminology/concepts for odour-related insect behaviour

Although the observation of whether insects aggregate near a target or perhaps avoid it is often described in terms of insect movement (attraction or repellency) in reality this is not the only explanation. Dethier *et al.* (1960) note that the commonly used terms attractants, relevant in a consideration of flies finding sheep, and repellents do not accurately define possible responses of insects to animals and defined six categories for designating chemicals in terms of the responses that they elicit in insects. This consideration is particularly relevant in a consideration of the role of odour in, the development of strike and the interpretation of differences in susceptibility to strike between sheep.

The definitions that they use, and which will be adopted in this review are:

Attractant: A compound or feature that causes insects to make oriented movement towards its source and draws insects from a distance. That is, there is a directed locomotory response, either by flight or walking in terrestrial insects, towards the site of attraction. In the case of flystrike this could be causing blowflies to fly towards sheep, or for flies that have already landed on sheep, that might cause them to walk or fly towards the susceptible sites, such as dags or fleece rot lesions.

Arrestant: This is a compound that causes insects to aggregate in contact with it. This may give the appearance of attraction, but the compound is distinct from an attractant in that it does not actively draw insects from a distance. Arrestants are often mistakenly described as attractants as the outcome often appears similar in that insects are seen to aggregate in contact with the compound. The example often used is sugar, which has negligible vapour pressure, and has very little effect in attracting flies from a distance. Flies aggregate near sugar not because they were actively attracted there, but rather because they find the site favourable and stay there longer to feed. In the case of flystrike, it is possible that flies could find sheep randomly in sheep camps or near watering points, and preferentially accumulate on sheep with the necessary arrestant chemicals or fleece attributes.

Feeding, mating or oviposition stimulant: This is a compound which elicits a feeding, mating or oviposition response in exposed insects. In the case of flystrike this is mainly of interest in relation to oviposition. Whether or not the deposition of eggs ultimately occurs depends on a number of cues including moisture, fleece chemicals and proximity to other ovipositing females.

Other of Dethier's definitions, relating to attraction include:
Repellent: A chemical that causes flies to make oriented movements away from its source.

Deterrent: A compound that inhibits feeding or oviposition in a place where insects would, in its absence feed.

Locomotor stimulant: a compound that causes insects to disperse from a region more rapidly than if the area did not contain it (eg pyrethroid chemicals).

Anti-Attractant: This is a compound defined by Wright *et al.* (1971) as a substance with no intrinsic repellent action of its own, but which acts by diminishing the attractiveness of an attractant.

4.6 Effects of odour in the development of strike

4.6.1 Approach to the attractant: Location of sheep by Lucilia cuprina

There have been few studies of the attraction of flies to sheep from a distance. In the extensive production systems of Australia, where sheep may be spread over large areas at low stocking rates, *L. cuprina* is found at relatively low density in the field (Gilmour *et al.* 1946) and a key element in the establishment of strikes will be locating susceptible sheep in this area. The most substantial early consideration of this was by Mackerras and Mackerras (1944). They made several substantial observations on this phase of host finding. Firstly, they noted that virtually every specimen of *L. cuprina* found on sheep is a gravid female (Mackerras and Mackerras 1944). This was later confirmed by the study of Woodburn and Vogt (1982) suggesting that there are particular cues for host finding that are specifically perceived by female *L. cuprina* searching for oviposition sites.

It is not known whether the female fly recognises a sheep's susceptibility status from a distance or whether susceptible areas are recognised only after the fly has landed. Browne (1979) noted that the points at which flies alighted on sheep had no obvious spatial relationship to the susceptible site at which oviposition eventually occurred. He noted that the egg laying site is only located after exploration by walking and by making short flights over the surface of the sheep. Information is almost completely lacking on the sensory cues used by the gravid female in finding sheep, but it is probable that vision and olfaction, and possibly temperature, are involved. Browne (1979) indicated that the olfactory stimuli are likely provided by normal sheep odours and/or the odours arising from conditions which predispose sheep to fly attack. Ashworth and Wall (1994) concluded that upwind orientation and landing of flies is probably in response to putrefactive sulphur rich volatiles originating in large part from the cysteine component of wool. This seems to be supported by the findings of Eisemann (1988). He assessed the response of *L. cuprina* to potential cues from struck sheep, unstruck dry sheep and unstruck wet sheep placed either upwind or downwind of fly cages. The controls were an empty pen or a pen containing a human subject. Struck sheep placed 0-5m upwind of the fly cage elicited the most upwind movement, followed in decreasing order by sound wet sheep, sound dry sheep and the controls. Significant upwind movement of the flies with struck sheep was seen at distances of up to 20m but only up to 10m with dry sound sheep. It should be noted, however, that his study used individual penned sheep. With a mob of similarly affected sheep responsible volatiles may be perceived over greater differences. Wet sheep were significantly more attractive than dry sheep and struck sheep significantly more attractive than wet sheep. This reinforces the findings of Mackerras and Mackerras (1944) that *L. cuprina* only become attracted to sheep when fleece humidity is high and that under laboratory conditions *L. cuprina* would oviposit on wet sheep even in the absence of extrinsic putrefactive odour sources such as faeces or urine staining. This was also shown by Cragg and Cole (1956) who found that *L. cuprina* exhibited no significant response to wool unless moisture was present. Interestingly, this was different to the response of *L. sericata* where reaction to wool was not dependent on high humidity or tarsal contact with moisture. They also showed that at least some of the receptors important in this response were located on the flies' antennae. They related this to difference in the habitat of the two species, with *L. cuprina* occurring in semi-arid environments whereas *L. sericata* is usually found in areas of higher humidity. Mackerras and Mackerras (1944) also noted that *L. cuprina* was never seen to be attracted to sheep with clean dry breech wool, although they might alight on them at random.

Differences in fleece humidity fleece moisture, wettability and drying of the fleece and some associated characters (Lipson 1976; Hall *et al.* 1980) have been associated with susceptibility to flystrike (mainly bodystrike) in a number of instances and there was some association between fleece moisture and breech strike susceptibility in the study of Greeff *et al.* (2016). It is possible that fleece humidity and the wetting and drying behaviour of the fleece could be important in influencing differences in attraction of *L. cuprina*, as well as in the later stages of strike development such as stimulating oviposition by the flies, hatching of the eggs and survival of early instar larvae.

Taken together, these results suggest that at relatively close quarters, odour is very important in the attraction of flies to sheep, but the means of attraction to sheep over greater distances is less certain. There appears to be no study of differences between sheep in the attraction of flies from a distance, other than due to the effects of predisposing conditions such as strike, faeces and urine stain or wet fleece and no studies suggesting differences between sheep in attraction or landing in the presence of predisposing characters or artificially produced putrefactive characters. If long distance attraction of *L. cuprina* does occur, it appears that this is more likely to be due to flock cues than to emanations associated with individual sheep.

That there was some suggestion of attraction to a human subject in the study of Eisemann (1988) suggests the possibility of more generic cues, such as, for example carbon dioxide, warm moist air plumes, visual cues, or in the case of sheep, rumen volatiles. When large numbers of sheep are present in a mob it is conceivable that these odours could attract flies from greater distances than suggested for individual sheep in the study of Eisemann (1988). Alternatively, large numbers of sheep with wet fleece or other predisposing factors could be expected to produce much stronger odour plumes and draw flies from much greater distances than the 20m from individual sheep suggested in the study of Eisemann (1988). It seems likely that flies are not initially drawn specifically to susceptible sheep but determine suitable egg laying sites after later searching activities. Mackerras and Mackerras (1944) conclude that for sheep to become struck there is a definite element of chance. They note that during a fly wave some sheep will be struck one time, some will be struck the next time and some not at all. They suggest that apparently the distance over which they can be perceived by *L. cuprina* is so small that a very high population of flies is necessary to ensure that all susceptible sheep will be found.

It may be that flies 'find' sheep relatively by chance in areas where their habitats coincide. Most 3rd instar *L. cuprina* larvae that have completed their feeding drop off of sheep to pupate between midnight and 9.00am. In addition, *L. cuprina* exhibits strong water-oriented behaviour (Browne 1962; Browne and Dudzinski 1968). This would act to concentrate *L. cuprina* larvae around sheep camp sites or sometimes watering points. Similarly, most adult flies emerge from pupae between midnight and dawn, again when sheep will often be near sheep camps. Rice (1986) describes what he refers to as a 'peridomestic' behaviour whereby *L. cuprina* tends to "loiter" in aggregations in areas near potential resources. This includes in proximity to sheep flocks and near permanent water. These behaviours may act to facilitate contact with sheep and reduce the importance of long-distance attraction mechanisms in host location.

Cragg and Cole (1956) found that there was a difference in the attraction profiles of *L. sericata* from different countries. Flies from Britain, where it is known to be the major strike fly, were much more attracted to wool than *L. sericata* from Australia, where it is seldom found in strikes or *L. sericata* from Denmark. In addition, country strains of *L. sericata* from Denmark were more attracted to wool than flies collected from the city. Cragg and Cole (1956) concluded that sensitivity to a 'wool factor' was a distinguishing attribute of wool myiasis strains of *L. sericata* and *L. cuprina*. Given known differences in *L. cuprina* strains in Australia (Norris 1990), similar differences in attraction of different strains may be present in Australia. However, one small study found little difference in response to different odour cues between city and rural strains of *L. cuprina* (Callander 2007) and Rice (1986) also indicated that urban *L. cuprina* were competent sheep strike flies.

In limited studies working with *L. sericata*, Cragg and Cole (1956) found that there was little difference between wools from different breeds including Merino, Crossbred or Oxford Downs wool.

They also found that storage had little effect on attractiveness and even washing with soapy water did not completely remove the factors responsible for attraction. *L. cuprina* only showed a marked reaction to wool when the floor of the choice chamber was moist, suggesting the involvement of tarsal receptors. They also noted the presence of humidity sensors on the antennae however and suggested that they may be involved. The attraction testing method used by Cragg and Cole (1956) was very short range and detection of moisture on the floor of the chamber may actually be acting in the next phase, the searching and settling phase in identifying a suitable site for oviposition (see below).

The limited studies conducted to date all suggest that sheep with predisposing conditions are considerably more attractive than those without and can be perceived at greater distance than clean dry sheep, albeit a relatively short distance. Although no studies have been conducted to look at the relative attraction of flies to 'resistant' and 'susceptible' sheep from a distance, it seems unlikely that differences in distance attraction can explain a significant portion of the variation in susceptibility. All of the available evidence seems to suggest that the presence of 'putrefactive' or bacterial odours associated with faecal and urine staining, fleece rot or mycotic dermatitis will overwhelm any differences between sheep in dry sheep odour. In addition, the importance of moisture in increasing the attractiveness of flies to sheep suggests that if there is a susceptibility related difference in the attraction, this difference is most likely to be manifest after the wool becomes wet. This doesn't eliminate the possibility that there may be some interaction between 'intrinsic' sheep odours (potentially determined by genetics) and susceptibility, but if this is the case, it seems more likely that this effect will be exerted at the later searching, settling and oviposition phases of flystrike initiation, not in the early attraction stages. This has implications for the design of studies to determine the effects of odour differences on variation between sheep in strike susceptibility.

4.6.2 Searching and settling phase: location of suitable oviposition sites on the sheep

Once flies have found a potential host sheep, the second stage in the initiation of a flystrike where odour is likely to play a part is in the searching and settling stage. After alighting on sheep *L. cuprina* begin searching for potential egg laying sites by walking over the fleece surface and undertaking a series of short flights. During this phase the fly 'tastes' the fleece surface with the sensors on her tarsi, proboscis and ovipositor. When a suitable area is found, the fly continues to explore in a restricted area by walking at a reduced rate. This may continue for some minutes even in the presence of suitable oviposition stimulants. Browne (1979) suggests that this might serve to prime the oviposition behaviour as well as to allow the fly to locate the most favourable egg laying site. It has been suggested that fleece chemicals may be acting as an arrestant at this time, causing the fly to stay on the fleece surface and complete its searching phase. Rogoff and Browne (1958) demonstrated the importance of fleece chemicals by showing that there was little oviposition on plugs of scoured wool, human hair or horse hair made attractive with the putrefactive odours of indole and ammonium carbonate, but oviposition occurred on greasy wool and interestingly on cattle hair tied into staple like bundles with cavities between the artificial staples. It is notable that strike by *L. cuprina* has occasionally been reported on cattle (Wilkinson and Norris 1961). Mackerras and Mackerras (1944) propose the need for a sheep factor, associated with living sheep, as well as the presence of a putrefactive factor, for egg laying to take place. However, Browne and Rogoff (1958), in insectory studies found no difference in the amount of oviposition on putrefactive plugs on a large area of fleece arranged in a 'sheep configuration' from that near plugs on living sheep, bringing this hypothesis into question. Cragg and Ramage (1945) also showed that this factor could be supplied by a component of the fleece, and the presence of a live sheep was not necessary. They concluded that this factor was probably more correctly described as a wool factor than a sheep factor. Furthermore, Browne (1965) found that *L. cuprina* would oviposit in plastic containers in response to indole and CO2 vapours. He hypothesised that the stimuli provided by fleece chemicals might play their largest part during the pre-oviposition phases of behaviour, acting largely as arrestants. There appears to have been no assessment of variation amongst sheep in the time for which gravid flies stay after landing to search for a suitable oviposition site, or of the factors that influence this. It is possible that variations of odour or gustatory stimuli affect this phase of strike induction, rather than attraction to the sheep, and that these differences contribute to variation amongst sheep in the likelihood of them becoming struck.

4.6.3 Oviposition

Once a potential egg laying site is found, the fly begins to search for cavities in the fleece, between the wool staples. These are favoured places for egg laying because of high humidity and lower light intensity (Browne 1958). At this stage the fly begins to extend her ovipositor and test the fleece or surrounding surface to determine suitability for egg laying. Often the fly backs into fleece cavities and extends her ovipositor towards the skin, presumably aiming to bury her eggs as deep in the fleece as possible and away from the drying effects of solar radiation and wind. It is known that sensing of fleece chemicals and moisture by the fly tarsi are important in stimulating oviposition, but there appears to have been no study to correlate potential sheep differences with flystrike susceptibility. It would appear that fleece architecture and the presence of cavities in the fleece could affect this, particularly in the case of very dense, compact tips and may also be a factor influencing whether eggs are ultimately laid.

It is likely that odour could also play a role whether or not *L. cuprina* oviposits at this stage. The observation that flies could be induced to lay in plastic containers when stimulated by odour alone led Browne (1965) to postulate that odour reception by sensors on the ovipositor was also important in determining whether or not eggs were ultimately laid. Rice (1976) identified the presence of olfactory peg sensilla on the *L. cuprina* ovipositor and Merritt (1987; 1989) suggested that the cerci at the tip of the ovipositor of sheep blowflies have olfactory, gustatory and tactile functions.

If more than one fly is in the vicinity, they often aggregate and the existence of a short range aggregation pheromone has been suggested (Rogoff 1958). Laboratory observations suggest that contact between flies reduces locomotory activity to a degree that nearby flies are stimulated to start preparation for egg laying and begin testing the substrate with their ovipositor. Once egg laying starts the fly appears to become almost oblivious to external stimuli and continues to lay eggs until the complete egg mass is deposited. Collective oviposition tends to lead to larger masses of eggs which reduces the likelihood of egg dehydration and death. The collective activities of larger groups of first instar larvae probably also improves the chance of skin invasion and the successful strike establishment. Group oviposition may lead to a more aggregated distribution of egg masses in a sheep mob and increase the chance that a sheep does not become struck even though predisposing factors are present.

A compound that acts at this stage of flystrike initiation as a strike deterrent (alpha-cypermethrin applied at a concentration of 50g/L) is currently registered for protection against body strike in sheep. Another oviposition deterrent from a different chemical group, 1,1-bis (p-ethoxyphenyl)-2-nitropropane (GH74), was claimed to give reliable protection for at least six months against body strike when applied at 0.5 per cent (Virgona *et al.* 1976; Van Gerwen and Browne 1983). These compounds work by suppressing egg laying or by changing the pattern of egg laying from egg masses to scattered single eggs generally laid off-target that don't result in strike initiation. GH74 was stable and very persistent on the fleece and appeared to act as a contact oviposition suppressant, so not strictly against odour. It was suggested that GH74 would probably also give control against strike resulting from urine staining in ewes but may give less reliable protection against breech strike resulting from heavy scouring. However, it was noted that in this case most of the eggs laid on scouring sheep were around the edges of the bare area of mulesed sheep where flies could stand on the bare skin to lay, rather in contact with the wool, suggesting that oviposition deterrents may be more effective on non mulesed sheep. Revisiting this compound or other non-volatile compounds that provide long-term protection by acting as oviposition deterrents may be worthwhile, particularly as they target adult flies, probably affect a different set of genetic mechanisms to current larvicides and may provide an additional tool for use in resistance management.

4.6.4 Egg hatch

Under optimal conditions of high humidity and 37.5^{o}C temperature, egg hatching occurs in as little as 7h, whereas at low temperature (10^{o}C) hatching may take as long as 100h and the likelihood of eggs hatching is low. Dehydration is a major cause of egg mortality and as the eggs desiccate the outer layers of the egg becomes tougher and more difficult for the larvae to penetrate. Although the female fly generally attempts to locate the eggs as close as possible to the skin surface, if there is no external source of wetting, it is unlikely that the eggs will hatch without the presence of extraneous moisture from rainfall or predisposing conditions such as urine staining, scouring or an active strike. Variability in moisture content of the fleece and characters that influence the ease of wetting and the rate of drying have been previously related to body strike susceptibility (Lipson 1976; Hall *et al.* 1980). High moisture content no doubt favours bacterial growth, the attraction of flies and the development of predisposing conditions such as fleece rot and mycotic dermatitis, but increased egg hatching is likely to be partially responsible. Sometimes fly eggs are seen deposited on the surface of dags or urine stained wool, obviously as a result of the presence of powerful oviposition stimulants. These eggs are exposed to rapid rates of dehydration and often fail to hatch, providing a further reason for the failure of strikes to develop in the presence of predisposing factors.

4.6.5 Larval survival and strike establishment

Moisture is also critical to the survival of larvae and first instar larvae are extremely vulnerable to the effects of desiccation until a strike lesion is established. First instar larvae do not have the large mouth-hooks present in later stage larvae and conventional wisdom has been that as a result, first instar larvae were not able to invade intact sheep skin. However, Sandeman *et al.* (1987) indicated that this was not necessarily the case and with electron micrographs showed a line of ridges within the oval cavity with which young larvae appeared to be able to abrade the skin. Their study was conducted with larvae placed under an artificially moistened cotton pad. Experience has shown that the success rates of strike establishment in artificial strikes is markedly enhanced if the skin is lightly abraded. In the field, strikes are often associated with bacterial growth causing conditions such as fleece rot (Merritt and Watts 1978a,b), skin scalding or dermatitis in the case of breech strike (Bull 1931). These conditions provide moisture, a focus for skin invasion and protein via exudate from inflamed skin that act to increase the likelihood of larval invasion and successful strike establishment.

L. cuprina antigens invoke a range of inflammatory and immune responses in sheep. Although multiple repeated infections elicit a protective response, manifest by slight reductions in larval growth and survival, this response is weak and poorly sustained and probably of little practical consequence (Sandeman *et al.* 1986). Chin and Watts (1991) showed that sheep bred with resistance to fleece rot had stronger skin hypersensitivity and humoral immune responses to *Pseudomonas*, which may also relate to flystrike susceptibility. O'Meara *et al.* (1995) found no difference between resistant and susceptible sheep in the establishment of strikes or the growth of larvae where sheep were challenged with larval implants. Smith *et al.* (2008) estimated heritabilities of 0.21 and 0.29 for mean larval weight and total larval weight respectively in sheep challenged with 50 first instar larvae, but survival of larvae was not heritable (h^2 = 0.01). Larval growth was negatively correlated with peripheral blood eosinophil numbers measured either before or after larval challenge and, larval growth *in vitro* on serum collected from challenged sheep was moderately associated with larval growth *in vivo* (Smith *et al*. 2008). They concluded that there was a level of resistance of Merino sheep to growth of *L. cuprina* larvae that may be mediated in part through actions of anti-larval factors in serum and eosinophils. Interestingly, in this study there was a negative phenotypic correlation with fleece rot score after exposure of sheep to simulated rain.

Differences have been demonstrated between flystrike resistant and susceptible sheep in their responses to direct challenge with blowfly larvae and to *L. cuprina* excretory or secretory products. O'Meara *et al.* (1992) found a greater wheal response to blowfly excretory or secretory antigens in sheep selected for bodystrike resistance whereas Colditz *et al.* (1994) found that plasma leakage in response to general inflammatory mediators, in particular activated complement, was greater in susceptible animals. Histological studies indicated higher numbers of mast cells in the skin of more resistant sheep (Colditz *et al.* 1994), consistent with a stronger inflammatory response. Inflammatory response to excretory or secretory antigens has been suggested as the basis for a test to select for resistance to fleece rot and bodystrike (O'Meara *et al.* 1992). However, there was no significant difference between resistant and susceptible sheep in the establishment and growth of larvae in either the study of O'Meara *et al.* (1992) or Colditz *et al.* (1996).

A search for quantitative trait loci (QTL) for larval survival and growth was conducted in data from 94 half-sib progeny of a Merino × Romney sire backcrossed to Merino ewes (Smith *et al.* 2008). Potential QTL for larval growth were identified on chromosome 11 and for larval survival on chromosome 18, although phenotyping greater numbers of sheep and a higher marker density on these chromosomes is necessary to confirm the result. Although there appears to be heritable variation in the rate at which larvae develop, the implications of this variability on flystrike incidence and impact, and more particularly on breech strike impact, requires clarification.

4.6.6 Bacterial involvement in flystrike development

The growth of bacteria is well known to be important in attraction of flies to the sheep but is also involved in the larval phase of strike. *Pseudomonas aeruginosa*, *Bacillus subtilis* and a number of other bacterial species have been implicated. Merritt and Watts (1978a, b) showed that *P. aeruginosa*, which grew rapidly when wool was wetted, had two effects. It produced odours which attracted flies and stimulated oviposition, and it caused production of serous exudate that provided nutrition for the growth of first instar larvae. Interestingly Sandeman *et al.* (1987) noted that one of the first obvious changes in skin structure when larvae were added to intact skin without predisposing causes, was the gradual loss of skin debris, largely keratinized squamous epithelial cells, from the wool adjacent to the skin. This may suggest that when first instar larvae are artificially applied to intact skin without any predisposing condition that they use this debris as an early source of protein. In more natural conditions this is usually obtained from sources such as fleece rot or other skin exudates. Furthermore, bacteria are a critical source of nutrients in the larval stages of other flystrike associated flies (Perotti *et al.* 2001; Romero *et al*. 2006) and it is interesting to hypothesise that the same may be so of newly hatched *L. cuprina* larvae on sheep. Emmens and Murray (1983) found that whereas bacterial species including *Proteus mirabilis, Enterobacter cloacae* and *Bacillus subtilis* were very important in stimulating oviposition, *Pseudomonas* alone was the least attractive of the four species examined. However, *Pseudomonas* greatly increased attraction of the other bacterial species when present in combination with them. Emmens and Murray (1982) also showed that the attractiveness of the different bacterial species varied significantly on different wool substrates. Merritt and Watts (1978a) found that there was much greater growth of these bacteria on wool from susceptible sheep than resistant sheep. In this study the wool was collected from adult sheep previously exposed flystrike and found to be susceptible as weaners. Eisemann *et al.* (1995) discusses the rapid increase in attractiveness of wool that occurs after wetting and suggests that this may be caused by the rapid activation of metabolic activity of many previously desiccated bacteria. A similar mechanism may have been responsible for the differences observed by Merritt and Watts (1978a).

Burrell (1985) demonstrated that immunisation could control the growth of *P. aeruginosa* on the surface of the skin and/or the pathogenic effects of its soluble products on the skin to the extent that fleece-rot lesions were both less attractive for oviposition and less nutritionally supportive for first instar larvae. In a field trial with the vaccine, none of 26 vaccinated sheep developed severe exudative, fleece-rot lesions nor were any fly-struck, whereas 61 of 115 control sheep developed severe, exudative, fleece-rot lesions and 21 of these were struck by *L. cuprina.* Notably immunisation with diffusible antigens of one strain of *P. aeruginosa* protected sheep against experimental dermatitis induced by the homologous and 3 heterologous strains, and against the natural dermatitis of fleece-rot caused by a heterologous strain. This also suggests the possibility that differences in immune response to fleece rot bacteria could be a contributing factor in differences in susceptibility to flystrike amongst sheep. While the aforementioned studies related primarily to susceptibility to body strike, it is known the *Pseudomonas* can also proliferated on urine-wetted skin in the breech (Bull 1931) and similar factors could be contributing to breech strike susceptibility.

4.7 Insect attraction in other mammalian systems

Variability in susceptibility to ectoparasitic insects between host species, between breeds and amongst individual animals within breeds is well recognised across most livestock species.

Differences in odour have been suggested to contribute to this variability. However many other factors including physical factors such as coat colour and hair density (Steelman *et al.* 1997), immunological factors (Nelson *et al.* 1977) and behavioural responses (Schofield and Torr 2002), as well as differences in susceptibility to predisposing conditions in the case of myiasis flies, are also known to play key roles. Although the effect of differences in odour are usually discussed in relation to attraction to suitable hosts, odour can also affect susceptibility in many other ways. For example, odour cues may be important in stimulating an insect to land on a host after it has been attracted to the host vicinity (tsetse), guiding an insect to suitable oviposition or feeding sites on a host, stimulating an insect to bite and begin feeding in the case of blood feeding parasites (Torr and Mangwiro 2000), or inducing egg laying in the case of myiasis flies such as *L. cuprina*. Determining the stage at which odour cues are operating is critical as it determines the appropriate methodology for testing effects of odour on susceptibility. For example, if odour is acting as an arrestant, or acting by stimulating oviposition, methods testing attraction such as olfactometer testing may fail to identify significant effects.

Most studies of odour responses in livestock insects have been conducted with a view to the development of traps, or in some instances with a view to the use of repellents as a more benign method of insect control. This has been so with *L. cuprina* where large numbers of volatile compounds have been investigated in an effort to identify attractive compounds that can improve trapping efficiency. There have been very few studies to determine whether differences in odour could underlie differences in host susceptibility to parasites. The most detailed examination of effects of odour on host susceptibility to date has been in relation to the attraction of mosquitoes to humans (see section 4.8.2.1).

There appears to be a high level of commonality in the volatiles that are attractive to insect parasites across different host-ectoparasite-associations and it has been suggested that the olfactory systems of blood feeding insects have evolved convergently to respond to a number of generic host volatiles, and even blends of these (Isberg *et al.* 2016). Compounds that attract insects include volatiles from host breath (particularly in the case of ruminant hosts), compounds from urine, dung and faeces, particularly in association with bacterial activity, odours from skin and skin microbiota, and glandular secretions in some particular insect-animal associations (Tommeräs *et al.* 1996; Russell and Hunter 2005). Logan and Birkett (2007) hypothesise that there is a basic 'core' suite of olfactory signals that, when present, convey information to an insect that a vertebrate is nearby. Some of these compounds, found ubiquitously in humans and other vertebrates that frequently cause positive behavioural responses in blood feeding insects include carbon dioxide, 1-octen-3-ol, lactic acid, ammonia, acetone and various fatty acids. Logan and Birkett (2007) also note that the addition or increase of certain other chemicals may repel insects or 'mask' the activity of these core attractants and could be the mechanism causing avoidance of non-host vertebrates by host-seeking insects. Most of the evidence for this hypothesis appears to come from differences between species in attractiveness, rather than from differences amongst individuals within species.

Odours associated with bacteria have also been commonly linked to attraction of parasitic flies (Tsetse flies, New World screwworm, *Lucilia spp.* And other strike flies, mosquitoes) and volatiles produced by putrefaction of host substrates such as urine, faeces, body tissues or wool, mediated by bacteria, are also commonly involved in ectoparasite attraction. This is particularly the case with facultative myiasis flies, such as *L. cuprina* that can parasitise live animals, but which are also able to breed in carcases or other decomposing biological substrates. In this case there appears to be significant commonality in the odours that attract these flies to carrion and to live host animals (Tomberlin *et al.* 2017a; Ashworth and Wall 1994).

4.7.1 Volatiles in animal breath

Carbon dioxide is a universal host emission in mammalian breath and has been related to attraction of many of parasitic insect and tick species. Carbon dioxide has often been used to improve the effectiveness of insect traps, most notably in the attraction of biting flies such as mosquitoes, biting midges and tabanid flies (deer flies, horse flies and March flies) and sheep head flies, *Hydrotaea irritans* (Ball and Luff 1981). Although it seems that CO2 is not strongly attractive to *L. cuprina*, (Ashworth and Wall 1994) it appears to act synergistically when in combination with indole, a common putrefactive odour, in stimulating ovipositon by *Lucilia spp*. (Hobson 1936; Cragg 1956; Browne 1965).

Interestingly, Torr *et al*. (2006) found that attraction of stable flies to cattle increased with body size, and that CO2 production was strongly related to weight. Artificially adjusting amounts of CO2 produced by individual cattle to make the emissions equivalent removed the differences between animals in attraction of stable flies, but not tsetse flies. This suggests a possible causal effect for different levels of CO2 production in differential attraction of stable flies. Acetone and butanone, present in ruminant breath have also been shown to contribute to the attraction of tsetse flies and stable flies (Torr *et al.* 1995). 1-Octen-3-ol, also a frequent component of ruminant breath, has been shown to be attractive to zoophilic species including mosquitoes (Kline *et al.* 1990), biting midges, (Isberg *et al.* 2017), tsetse flies, stable flies and tabanids (horse and deer flies) (Krcmar *et al.* 2005). In addition, when used in combination with CO2, 1-Octen-3-ol has been shown to increase catches of *Stomoxys* in traps (Mihok *et al.* 1995).

4.7.2 Compounds in animal urine

Various phenols and phenol derivatives in volatiles associated with cattle urine are attractive to parasitic flies including tsetse flies (Bursell *et al.* 1988; Vale *et al.* 1988), stable flies and biting midges (*Culicoides* spp) (Torr *et al.* 1995; Kline *et al.* 1990; Isberg *et al.* 2017). For the tsetse fly species *Glossina pallidipes* and *G. morsitans morsitans* the most attractive compounds found in urine were 4-methylphenol and 3-n-propylphenol (Owaga *et al.* 1988) and Cilek (1999) showed that a mixture of these compounds, significantly increased collection of stable flies in Alsinite traps. Aged horse or cattle urine is much more attractive to tsetse flies than fresh urine, almost certainly as a result of bacterial activity, and phenolic compounds present in aged cattle urine have played a significant role in control strategies for tsetse flies in Africa (Krcmar and Lajos 2011; Baldacchino *et al.* 2014). Urine odours appear to be particularly attractive to livestock pests in which the larval stages grow in excrement or contaminated bedding material, such as stable flies and *Culicoides* midges. While these compounds may be primarily acting to guide flies to suitable larval substrates for oviposition, they also act to guide parasites to the vicinity of suitable hosts for blood feeding. This suggests, at least in these instances, a general concurrence of odours that act to attract flies to feeding and oviposition sites. Of course, odours from urine stained sheep are well known to be strongly attractive to sheep blowflies and a number of compounds found in urine (for example phenols indole and ammonium carbonate) have been included in baits for L. *cuprina* and *L. sericata* (Cragg and Ramage 1945; Urech *et al.* 2004). A number of these compounds are also powerful oviposition stimulants.

Sheep with wool stained by scouring (diarrhoea) or faecal accumulation in the wool (dags) are also known to be highly susceptible to breech strike, buts as noted by Ashworth and Wall (1994) little work has been done to quantify the precise nature of the attractive volatiles released by scouring or faeces-soiled sheep. However, *L. cuprina* is known to be highly attracted to indole (Urech *et al.* 2004) which frequently occurs in high concentrations in faeces and is known to be used as a cue by other flies to locate this resource (Tomberlin *et al.* 2017b). Interestingly Morris *et al.* (1997) showed that gut mucus, produced in greater quantities by sheep with a high gastrointestinal parasite count, attracts and induces probing behaviour by *L. cuprina.*

4.7.3 Odours derived from animal skin or hair

A wide range of volatiles in skin secretions or isolated from the hair coats of different animals have been shown to be variously attractive or repellent parasitic flies. Often attraction is concentration dependent and compounds which are attractive at one concentration may be repellent at others (Birkett *et al.* 2004). This may suggest that odours from different sources act at different levels in host location, some acting to attract parasites from a distance and others, where concentration is important, acting to stimulate landing or feeding (Warnes 1995).

1-Octen-3-ol, heptanal, octanal, nonanal, decanal, E-2- nonenal, and 6-methyl-5-hepten-2-one, identified in the headspace of cattle hair (Birkett *et al.* 2004; Gikonyo *et al*. 2002), are detected by the olfactory system of *Culicoides* biting midges (Bhasin *et al.* 2000), as well as of other haematophagous insects, including mosquitoes (Ghaninia *et al.* 2008; Logan *et al*. 2008; Syed and Leal 2009), tsetse flies (Gikonyo *et al.* 2002), bed bugs (Harraca *et al.* 2012), and triatomine bugs (Guerenstein and Guerin 2001) and a range of other livestock-associated flies (Birkett *et al.* 2004). However, Birkett *et al.* 2004 who tested 23 volatiles isolated from cattle against face flies (*Musca autumnalis*) horn flies head flies, stable flies and Palearctic screwworm showed that only some of the compounds were active across the range of flies tested. These compounds included 1-octen-3-ol, 6-methyl-5-hepten-2-one, (Z)-3-hexen-1-ol, naphthalene, and all of the electroantennagram-active compounds they identified from urine.

A number of studies have shown that biting midges are differentially attracted to host volatiles when they are presented together with CO2 (Mands *et al.* 2004). Isberg *et al.* (2016) isolated 9 constituents in cattle hair that were bioactive against midges (as well as 14 from urine). They found that decanal (derived from both cattle hair and urine) and 1-octen-3-ol and which is a common volatile present in body odour of humans and animals (also found in bovine breath), were attractive but heptanal and octanal were repellent in comparison to CO2 alone. Of the urine derived volatiles 2-phenylethanal, 2-ethylhexanol, 3-methylindole, phenol, and 3-ethylphenol were attractive whereas nonanal, 3-propylphenol, and 4-propylphenol inhibited midge attraction when compared to

CO_2 alone. 6-Methyl-5-hepten-2-one, 3-methylphenol, 4-methylphenol, and 4-ethylphenol elicited either attraction or inhibition, dependent on the concentrations tested

In the case of sheep flystrike, wool has a high sulphur content, largely present as sulphur amino acids and sulphurous compounds are known to be highly attractive to sheep blowflies (Ashworth and Wall 1994). The action of *P. mirabilis* a bacterial species commonly associated with flystrike degrades wool into a range of sulphurous compounds (Emmens and Murray 1982). Notably sulphurous compounds are also produced by the action of bacteria in the decomposition of other animal proteins (Pittard *et al.* 1982; Miller *et al.* 1973) which may suggest that this is generic, rather than a sheep specific attraction cue.

4.7.4 Bacterial odours

Bacterial odours have been widely implicated in the attraction of livestock ectoparasites to their animal and human hosts. This is not surprising as bacterial nutrients are often important components of the larval diet of manure or refuse breeding flies associated with livestock, such as house flies, stable flies and horn flies. Larval growth is seriously reduced where bacterial growth is experimentally inhibited in these media (Romero *et al.* 2006; Perotti *et al.* 2001). Odours produced by bacterial decomposition have also been shown to be attractive for blood feeding flies (Krcmar and Lajos 2011) and for mosquitoes where differences in attraction to human hosts have been associated with differences in the skin microbiome (Verhulst *et al.* 2011).

Odours associated with bacterial decomposition of a range of biological substrates are also key attractants for blowflies that may breed in carcases and other decomposing biological materials (Tomberlin *et al.* 2017a). A number of the key bacterial species involved in the production of attractant or arrestant odours and in stimulating oviposition on carcases have also been implicated in strike initiation by *Lucilia spp*. (Tomberlin *et al.* 2017b). Bacteria also appear to be important in obligate parasites of live animals such as New World screwworms, and the Palearctic screwworm *Wohlfahrtia magnifica*, that have lost the ability to breed in carcases (Devaney *et al.* 1973; Khoga *et al.* 2002). Notably *W. magnifica*, which also most commonly strikes the genital area of sheep and goats (Sotiraki *et al.* 2005), is in the family Sarcophagidae, a different family to blowflies and New World and Old World screwworm flies.

Eisemann and Rice (1987) showed that larval cultures that were maintained microbe-free did not strongly attract gravid female *L. cuprina* whereas larvae cultured in media where bacteria were allowed to grow were highly attractive. This strongly suggests that the odours produced by struck sheep or non-sterile cultures of *L. cuprina* larvae, which cause them to be extremely attractive to gravid *L. cuprina,* are primarily bacteria mediated.

One species of particular interest is *Proteus mirabilis* which has been prominently identified in studies with *L. cuprina* and *L. sericata* (Burrell 1990) as well as with New World screwworms (Chaudhury *et al.* 2010) and the secondary screwworm fly (*Cochliomyia macellaria*) (Chaudhury *et al.* 2015). Emmens and Murray (1982) found that odours produced by *P. mirabilis* provided the strongest oviposition stimulation of four bacterial species isolated from the fleeces of sheep (*P. mirabilis*, *B. subtilis*, *E. cloacae* and *Ps aeruginosa*) when examined as pure cultures. Interestingly, *Ps. aeruginosa* was not highly attractive when present alone in their tests but resulted in significantly increased egg laying when present in combination with the other bacterial species (Emmens and Murray 1983). Morris *et al.* (1997) tested the responses of gravid *L. cuprina* to odours from varying sources in laboratory studies and found that *P. mirabilis* odours stimulated significant movement towards their source and elicited an increased probing response, associated with preparation for oviposition. *P. mirabilis* was also effective in attracting *Lucilia spp*. When presented in traps in New Zealand field studies (Morris *et al.* 1998).

Interestingly, *P. mirabilis* has also been shown to persist in the salivary glands of *L. sericata* that are otherwise relatively free of vertically transmitted resident microbes *(*Singh *et al.* 2015*). P. mirabilis* has been shown to persist in the digestive tract of *L. sericata* through immature development to the adult stage (Wei *et al.* 2014) and it has been suggested that *L. sericata* transports *P. mirabilis* between feeding and oviposition sites. Ma *et al.* (2012) suggest that *L. sericata* and *P. mirabilis* have a mutualistic relationship. Bacterial signals that promote 'swarming' behaviour of *P. mirabilis* (whereby a motile form of the bacteria moves to cover a food surface) also results in strong attraction of blowflies to contaminated sites and promotes further dispersal of the bacteria by the flies (Tomberlin *et al.* 2017b).

It is well established that bacterial growth is important at various stages in the development of body strike; for example, in providing odour cues for attraction, searching and oviposition, as well as causing skin scalding and serous exudation that provides protein for the development of 1st instar larvae. Although the role of bacteria in susceptibility to breech strike is less certain, it has been hypothesized that sulphur-rich volatiles resulting from microbial decomposition of wool are important in attraction of flies and oviposition is known to frequently occur in response to ammonia rich compounds resulting from urine. Based on studies in other species it seems that the effects of bacteria on urine, and probably faeces-fouled wool could act to enhance attraction of sheep blowflies to the breeches of flystrike susceptible sheep. Bacteria often also provide critical dietary nutritive factors for larval development in other livestock-associated flies and may also facilitate larval survival and strike establishment in myiasis flies by this means.

4.7.5 Oviposition pheromones and the fly factor

Although with myiasis flies there are no known long-distance pheromones, similar to those identified for a number of other insect groups, sheep blowflies commonly aggregate in groups during oviposition, producing large composite egg masses that reduce the chance of eggs dehydrating and increasing the likelihood of successful strike initiation. This behaviour is likely to be mediated by a pheromone that attracts other gravid flies, reduces their activity and stimulates them to oviposit (Browne *et al.* 1969; Emmens 1981). These actions were suggested to rely on an olfactory component but also a strong contact component (Browne *et al.* 1969).

However, the existence of this pheromone has been controversial, and no compound has to date been characterised. Brodie *et al.* (1915), from studies with *Lucilia sericata* and black blowflies (*Phormia regina*), suggested that aggregation may be due to more general feeding signals, which affect both gravid and non-gravid flies, and which also act across blowfly species. They suggest that these cues relate to more general signalling of the presence of food resources but could potentially also indicate the suitability of a site for oviposition.

These signals may also be due to a long recognised "fly factor" (Holl and Gries 2018) whereby areas where flies have previously fed, with the presence of fly faeces and regurgitate, attract flies in greater numbers than to similar areas not previously visited by flies. These authors suggest that the attractive odours produced may be microbial in origin. Aggregation of flies during oviposition, whether fly or microbially mediated, would affect the spatial distribution of oviposition and potentially strike amongst sheep in a flock, especially when fly densities are low, and may help to explain why some sheep are not struck even when predisposing conditions and favourable environmental conditions for flystrike appear to be present.

4.8 Effect of odour differences in livestock hosts

4.8.1 Between host species

Although some species, such as stable flies will feed on blood from a wide range of species and are broadly considered generalists, others are more restricted or specialised in their host range. Differences in odour are often suggested to play a key role in in determining differences in host attraction in host-specialist species (Logan and Birkett 2007). Blood meal analysis showed that tsetse flies (*G. morsitans*) readily landed and fed on cattle, bush pigs and warthogs, but avoided impala in Zimbabwe whereas in Kenya a different species of tsetse (*G. pallidipes* fed on cattle and buffalos, but avoided waterbuck, even though the animals were kept in adjacent field enclosures (Gikonyo *et al.* 2003). In this case the difference in attraction appears to be due to repellent compounds in the waterbuck (*Kobus defassa*) odour. Tsetse flies were shown to be attracted to a host-specific volatile blend from cattle or buffalos, but they actively avoided the blend of odours from waterbuck and moved preferentially towards the clean air control (Gikonyo *et al.* 2003). This was linked to fewer aldehydes and more phenolic components, octalactone and a series of methyl ketones (C8–C13) in the odours from waterbuck. Testing in the field by Saini *et al.* (2017) showed that application of the repellent waterbuck compounds to susceptible cattle conferred substantial protection from attack by tsetse flies. The preference of different mosquito species for different host species is also widely recognised and at least in some instances, has been linked to odour cues (Raji and DeGennaro 2017). However, the evidence for host volatiles determining differences between individuals within species is more tenuous.

4.8.2 Within host species

4.8.2.1 Variation in attractiveness of human hosts to mosquitoes

Insect host-seeking behaviours have been most studied in mosquito species because of their importance as deadly disease vectors. However, even with these species where there is strong anecdotal evidence for difference in susceptibility amongst individuals. Logan and Birkett (2007) concluded that so far investigations have merely scratched the surface in their attempts to explain the chemical basis for these differences.

Most mosquito species are opportunistic, biting vertebrate animals that are readily available and restricting to broad taxonomic groups rather than narrow species ranges (McBride 2016). *Aedes* and *Anopheles* mosquitoes have however evolved to selectively target humans, and now display strong preference for human odour over that of other animals. For these mosquitos, olfaction is considered to be the most important sense utilised to detect hosts, however thermal and visual cues are also important. There is also evidence that mosquitos have intraspecies host preferences however the reasons some individuals are more attractive than others has not been fully elucidated and it appears to be complex (Qiu *et al.* 2006).

It is well established that anthropophilic mosquito species use two main cues to select and navigate to human hosts, CO2 and host specific odours (reviewed in Webster and Card 2017). These stimulants can be categorised by their behavioural effect. CO2 is described as an activator, as it promotes flight and increases mosquito sensitivity to other host stimuli. Carbon dioxide may a similarly in the stimulation of oviposition by *Lucilia spp*. Browne (1965) notes that indole was a strong oviposition simulant except in the complete absence of CO2 and that the effect of Indole and CO2 together was more than additive. A similar effect was seen with *L. sericata* where ammonia did not stimulate oviposition in the absence of CO2 (Cragg 1950). Host specific attractant odours can also elicit upwind flight and attraction. Mosquitoes tend not to land on the source unless it is heated and CO2 is also present, suggesting the combination of CO2, host specific odours and body heat are all required for host selection by mosquitos. The attractiveness of odours differs for the different mosquito species.

Very early studies show *Anopheles gambiae* are more attracted to individuals with blood group O than other blood types, however the mode of attraction is unknown (Wood *et al.* 1972). A similar observation was made with another mosquito species, *Aedes albopictus,* however O blood groups were only significantly more attractive than A blood groups (Shirai *et al.* 2004). This study also showed the source of the attraction could not be attributed to the ABH antigens on the skin as they did not influence the landing preference of mosquitoes among ABO blood groups (Shirai *et al.* 2004). This difference between the species may be due to the evolutionary history of the mosquitos with *A. gambiae* evolving in Africa where the O blood type is more prevalent, while *A. albopictus* evolved in Asia where the O blood group is less prevalent (Shirai *et al.* 2004).

It has also been shown that pregnant women (Lindsay *et al.* 2000) or people with larger body mass (Port *et al.* 1980) are more attractive than non-pregnant or smaller body mass respectively. These observations may relate to the relatively larger surface, increased CO2 production, relatively higher body temperature and higher humidity (De Jong and Knols 1995). One study has tested the role of CO2 in differential attraction by standardising the amount of CO2. However this did not lead to equal attraction (Costantini *et al*. 1996). All of these factors can also lead to increased odour production which may alter attractiveness (Olanga *et al*. 2010).

It is known that chemical cues alone can result in differential attractiveness of human subjects to mosquitoes (Qiu *et al.* 2006). In this study, variations in attractiveness between individuals were tested without confounding factors such as body size, hue, heat and moisture and clearly show odour differences alone can influence attraction. Further studies have attempted to identify the physiologically relevant chemicals that drive differential attraction. Attraction can be increased either because there is an increase in specific odours that are attractive, a decrease in repellent odours, or a 'masking' effect of attractive odours. There are well known mosquito attractants such as lactic acid and ammonia and reducing or increasing the amounts of these odours can result in increased attraction (Steib *et al.* 2001; Geier *et al.* 2007). Conversely, secondary metabolites such as 7-octenoic acid, (*E*) and (*Z*)-3-methyl-2-hexenoic acid, have been shown to reduce attraction of *Anopheles gambiae* at some concentrations in odour baited entry traps (Costantini *et al.* 2001). Logan and Birkett (2007) hypothesise that as many of the attractive odours are produced by primary metabolism, they may indicate the presence of humans to mosquitos, whereas differential attraction may due to differential secondary metabolism that produces repellent type chemicals.

The mechanisms underlying the production of volatiles in hosts has been attributed to production by skin cells (which could have a genetic basis) or by the microbiota *(*Fernández-Grandon *et al.* 2015). There is limited work on the genetic basis of mosquito attraction to humans, however one study demonstrated an underlying genetic component detectable by mosquitoes through olfaction by comparing the attractiveness of identical and non-identical twins body odour to mosquitos (Fernández-Grandon *et al.* 2015). While there were limited sample numbers in this study the authors show a strong narrow-sense heritability of 0.62 (SE 0.12) for relative attraction.

Skin microbiota is an important determinant of odour (Rennie *et al.* 1991; Ara *et al.* 2006; Shelley *et al.* 1953) with an absence of bacteria resulting in odourless sweat (Shelley *et al*. 1953), and evidence that microbiota composition affects mosquito attractiveness (Verhulst et al., 2011b). It has been shown that humans that are more attractive to mosquitos have an overall higher abundance of total skin bacteria, but a decreased diversity of bacteria (Verhulst et al., 2011b). Studies have also shown specific bacterial species can increase attractiveness (e.g. *Staphylococcus epidermidis)* or decrease attractiveness (e.g. *Pseudomonas aeruginosa*) of humans to mosquitos (Verhulst et al. 2009; Verhulst et al., 2011a; Verhulst et al., 2011b), however the specific odour compounds resulting from the skin and bacteria interaction that directly affect the attraction have not been elucidated.

4.8.2.2 Differences in attraction to animal hosts

In animal hosts, the evidence for qualitative differences in host volatiles determining differences in intraspecific susceptibility to ectoparasite attack is similarly somewhat equivocal. With horn flies, buffalo flies, stable flies and black flies there is a relatively strong positive correlation between body size and attraction with older and larger animals bitten more than smaller and younger ones. Although CO2 is generically produced by mammalian hosts it has also been suggested as an as an explanation for differences in attraction of stable flies to different sized cattle (Torr *et al*. 2006). For tsetse and stable flies, the order of attraction to cattle was ox > cow > heifer > calf and oxen were twice as attractive as calves of less than 12 months old. These authors found that smaller animals produced lower levels of carbon dioxide, acetone, octenol and phenols than oxen, but for older calves and cows, levels of production of other known kairomones and repellents were similar to those of an ox. Artificially adjusting the doses of carbon dioxide produced by individual cattle to make them equivalent did not alter differences in attractiveness for tsetse flies but it did for stable flies. These authors concluded that in the case of stable flies at least, differential attractiveness is related to the quantity of CO2 emitted. A similar effect of level of CO2 emissions was seen in a study of attraction of black flies to human hosts (Scholfield and Sutcliffe 1996). Removing CO2 from breath emissions reduced attraction by 85% and removed differences between individuals. This study also suggested that, even after the removal of CO2 from exhaled breath, the remaining components of breath odours were more important in attraction than body odours.

Thomas *et al.* (1987) examined factors responsible for differential attraction of cattle headflies (*Hydrotaea irritans*), which have been implicated in the transmission of mastitis in dairy cattle in Europe. Tests of these cattle in wind tunnels showed that there was a significant correlation between attraction to numbers of headflies seen on the animals, and numbers attracted to water washes from the cattle, but no association with either odours from ether washes or urine. There was also a strong correlation between the numbers of headflies and the total counts of all fly species on cattle, suggesting that the level of attraction was a more general fly phenomenon. Whether this was due to qualitative or quantitative differences in the odours from the different cattle was not determined but the work did suggest that volatile compounds were involved.

Although preferential attraction of ectoparasites to different components of host odours can often be demonstrated in laboratory choice studies or trapping studies, the case for these odours playing a large part in determining differences in susceptibility between individuals within a species on live animals is less compelling. Birkett *et al.* (2004) identified 23 host compounds, collected by suspending collection vessels above the backs of penned cattle or above cattle urine, that caused electroantennagram responses in face fly or horn fly antennae. When tested against five other fly species only some of these compounds showed electroantennagram (EAG) responses in all species. These compounds included 1-octen-3-ol, 6-methyl-5-hepten-2-one, (Z)-3-hexen-1-ol, naphthalene, and all compounds identified from urine. Unique compounds identified from a low carrier animal included propylbenzene, styrene, camphene, 2-heptanone and propyl butanoate. In wind tunnel studies with *M. autumnalis*, 1-Octen-3-ol, 6-methyl-5-hepten-2-one and 3-octanol showed significant attraction at certain concentrations and naphthalene, propyl butanoate and linalool showed strong repellence at low concentrations, but no response at higher concentrations.

The case for a role of qualitative differences in host odour in attraction of biting flies in the field is less compelling, however. In a field study using small, separate herds of cattle in Denmark, slow-release formulations of two compounds selected on the basis of laboratory testing, 1-octen-3-ol and 6-methyl-5- hepten-2-one were applied to low and high horn fly-carrier cattle (Birkett *et al.* 2004). When 6-methyl-5-hepten-2-one was applied to two heifers with low fly numbers, there was no significant change in fly loads on either of the two days measured. When applied to heifers with high fly loads, the numbers of flies were reduced significantly on one of the two measurement days for one of the heifers, but no effect was observed for the other. For 1-octen-3-ol, a repellent response was seen on one of the four animals on one of the two days tested. That is, although both compounds showed an attractive effect in the olfactometer studies, both had a repellent effect in the field studies. The effect of these compounds has been shown to vary with concentration in other studies and concentration differences may also have been important for the variable effects in the aforementioned study. In similar work in Chile, Oyarzun *et al.* (2009) found that extracts collected from high and low fly carrier cattle showed no behavioural activity against horn flies when tested for activity in Y-tube olfactometer tests. Horn fly attraction to 1-octen-3-ol was confirmed, as in the previous studies, and both m-cresol and p-cresol, which are breakdown products of cattle urine, were also found to be attractive. Of the other compounds tested in olfactometer studies 2-decanone was shown to be repellent as similarly found with tsetse flies (Vale 1980), and 2-undecanone was attractive, similar to previously found with *Aedes aegypti* and *Anopheles stephensi* mosquitoes (Haas *et al.* 2006). 6-methyl-5-hepten-2-one showed no behavioural activity for *H. irritans* in contrast to the results of Birkett *et al*. (2004). Oyarzun *et al.* (2009) suggest that lack of similarity in the effects seen in their work and that of Birkett *et al.* (2004) could be due to the use of inappropriate concentrations or perhaps the need for complementary volatiles, such as CO2 for an effect to be manifest.

4.8.2.3 Variation in attraction to sheep

There is limited information to suggest that innate odour differences play a significant role in determining susceptibility of sheep under field conditions. Cragg and Cole (1956) showed that the factor or factors in wool that attracted *L. cuprina* and *L. sericata* were strongly persistent, were not confined to either the suint or wool grease fractions and were in all probability intimately bound to the wool fibre. However, in their work there was no marked difference in attraction of flies between Crossbred, Merino and Oxford Downs wool or to different fleeces within each sheep group. Mackerras and Mackerras (1944) note that if there is some factor involved in the attraction which is not perceptible to humans, it is not constantly present from year to year or even day to day. In their work, frequently the first sheep laid on by flies got most of the eggs, no doubt because of the aggregation factor associated with ovipositing *L. cuprina* (Browne *et al.* 1969; Emmens 1981). They noted that in all of their studies they had not encountered an animal which was consistently unattractive to flies. This seems to match the observations of Eisemann (1995) who showed that that there was a high degree of variability in the attractiveness of freshly clipped, wetted fleece samples, including in those taken from the same sheep on different dates. He suggests that this may be related to the large fluctuations that are known to occur in fleece microorganisms under field conditions. Eisemann (1995) also suggests that the rapid increase in attractiveness of wool following wetting may be due to the activation of the metabolic activity of desiccated bacteria already present in the fleece. These observations infer strong environmental effects, and by implication relatively low heritability of fleece odours. Mackerras and Mackerras (1944) conclude that there is a high degree of chance involved in whether not sheep become struck and the distance over which sheep can be perceived by flies is so small that a very high population of flies is necessary to ensure that all susceptible sheep will be found. Both Emmens and Murray (1983) and Merritt and Watts (1978a) suggested the possibility that differences in wool composition could influence attractiveness following bacterial growth and that this could be due to differences in the chemical composition of the fleece or wool yolk. However, the presence of resident microflora was not tested or controlled in either of these studies and the multiplication of the resident species following wetting of the wool could have also contributed to the observed differences. Both mechanisms imply a high environmental component in the production of odours suggesting that heritability of odour production is likely to be low.

More recently, extensive investigations have been undertaken in WA to clarify the potential importance of odour and attraction of *L. cuprina* in explaining differences in breech strike susceptibility. Despite an exhaustive investigation using a range of different assays, the results remain equivocal. Where an association was found the level of correlation was generally low and the results often inconsistent. Gas Chromatography (GC) did not identify any consistent differences between resistant and susceptible sheep in either the Mount Barker or Armidale flocks. This is likely due to the high variation of odour signature observed between individual sheep and between the flocks, also the low levels of odour repeatability across time points for individuals.

In behavioural studies with flies, Y-tube olfactometer and arena tests gave inconsistent results although in one study using the arena test there was a significant low association between breeding value and the time the flies spent on the most susceptible wool samples. In studies with samples collected from resistant and susceptible sheep, the results were counterintuitive with the wool from flystrike resistant sheep determined to be more attractive to gravid *L. cuprina* females than wool from flystrike susceptible sheep (Yan *et al.* 2019). In this same study octanal and nonanal induced electro-antennogram? (EEG) responses and were shown to be attractive to gravid flies in behavioural choice tests. However, as noted, the more attractive wool samples came from sheep determined as resistant in the field.

Taken together, these results suggest it is unlikely that any differences in innate sheep attraction to flies that are not related to known predisposing causes are large enough, consistent enough or easily enough measure to be practically useful in the selection of resistant sheep. Although odours cues are critical at a number of stages in the development of strike, the most important odours seem to be associated with predisposing conditions such as urine staining, scouring or bacterial growth and it seems likely that these odours would overwhelm the effects of any innate sheep differences in odour.

4.8.2.4 Opportunities for using odour control approaches other than breeding

It is well documented that odour approaches can be successfully used to reduce flystrike incidence in controlled tests, with most effort concentrated on attract and kill trapping (Hall 1995; Urech *et al*. 2004; Urech *et al.* 2009). In order for attract and kill methods to efficiently reduce fly populations it requires economical and efficient capture of large proportions of female flies. In Australia the LuciTrap® is commercially available and has been shown it can suppress 60% of the *L. cuprina* population compared to matched controls (Urech *et al.* 2009). However, these traps are not routinely used as they are not cost-effective, due to the large number of traps needed, the trap maintenance required, also due to the low level of flies present before fly strike occurs.

Trapping systems can be useful as a monitoring tool to alert to the presence of flies and the need for intervention, however such systems currently require labour intensive monitoring. Autonomous monitoring services that provide real-time information about the presence of pests have been developed for Queensland fruit fly to aid in pest management and will be available commercially from Australian start-up RapidAIM. A similar approach for monitoring of *L. cuprina* could be considered.

An alternative approach to attract and kill or localised monitoring, is the 'push-pull' strategy. This strategy uses odorants in order to repel insects from normally attractive hosts (push) while simultaneously attracting insects to an alternate area (pull) where they can be removed. This approach has been widely used for agricultural pests, first developed for the non-toxic control of *Helicoverpa armigera*, a generalist pest feeding on more than 200 host species (Pyke *et al.* 1987). The best example of the push pull strategy is for the control of Maize stem borers in sub-Saharan Africa, where farmers use intercropping with some plant species that emit repellent volatiles and other plants that emit highly attractive odours but do not support larval growth (Khan *et al.* 2000). Similar push pull strategies for livestock pests in Africa have also been considered (Hassanali *et al.* 2008).

For a push-pull strategy to be effective for sheep blowflies there are two requirements; firstly, an effective area wide repellent that interferes with natural host attractions and secondly an effective and efficient trapping system that can substitute the host. The mobility of flocks and the low numbers of sheep blowflies required for flystrike may present some challenges for the development of effective push-pull strategies.

Another opportunity to manipulate an insect's host seeking behaviour is through disruption of the olfactory system. This disruption could be achieved in multiple ways. Repellent chemicals such as DEET (*N*,*N*-diethyl-3-methylbenzamide) have been extensively studied to understand the mode of action that elicits avoidance behaviour by many insects. Modes of action include; stimulating repellence related receptors mediating avoidance (Davis 1985), sequestering volatiles making them unavailable for detection by the olfactory system (Syed and Leal 2009), acting as an agonist to olfactory components and confusing the olfactory system (Bohbot and Dickens 2010), or acting as an antagonist to the receptors rendering them non-functional (Ditzen *et al.* 2008). An understanding of the molecular basis of the insect olfactory system is crucial to the selection of disruptive targets.

5. Conclusions

Accumulating evidence thus suggests that the olfactory systems of haematophagous insects have evolved convergently to respond to generic host volatiles, and even blends of these and it is suggested that there is a basic 'core' suite of olfactory signals that, when present, convey information to an insect that a vertebrate is nearby (Isberg *et al.* 2016). Volatiles from a number of different sources can be involved in this process and compounds associated with host breath, animal urine and its decomposition products, dermal secretions and skin microbiota are prominent amongst these.

With myiasis flies, and particularly with facultative myiasis flies such as *L. cuprina* there is a significant similarity between those that breed on live animals and those that breed in carrion in the odour cues important to the location and utilisation of breeding resources (Tomberlin *et al.* 2017a). In the early 1900's Froggatt (1915) suggested that in Australia it was the occurrence of large numbers of dead sheep and the associated smell of rotting wool that led to carrion flies developing the habit of ovipositing on living sheep. Sheep blowflies are attracted to and will oviposit as readily on carcases as on live sheep and in many instances the odour cues have been shown to be common. With myiasis flies, both facultative myiasis flies such as blowflies, and obligate parasites such as screwworms, bacterial odours have been shown have a strong involvement in the sequence of events leading to oviposition. *L. cuprina* readily oviposits on carcases and urban refuse and has been reported to strike cattle and lay eggs on horse blankets, even though specific attractive sheep odours are presumably not present.

It is rarely the case that an individual compound is essential for attraction and most parasites will respond to generic host cues and feed or oviposit on non-host species or low susceptibility animals under the right conditions (Isberg *et al.* 2016). With most blood feeding and myiasis flies, mixtures of odours are generally more attractive than single compounds. Where kairomones have been defined the concentrations and ratios of compounds in these mixtures can be critical and some compounds that are attractive at low concentrations may be non- attractive or even repellent at higher concentrations. In addition, the attractiveness of some is even context specific (Hansson 2011). It is also notable that these odours are not necessarily constant even within individual hosts and can vary according to health status and environmental factors. This high level of variability would present difficulties for use of odour as a selection criterion in breeding programs, even if an association was demonstrated.

In the area of livestock parasite control there are few previous suggestions that odour differences can be used as a basis for breeding more resistant animals and even in the most often cited reference suggesting this may be possible (Birkett *et al.* 2004), the evidence presented is tenuous.

There have been suggestions that species such as *L. cuprina* and *L. sericata* are attracted to wool more strongly than most other flies and this difference has been suggested to be due to attraction to sulphurous compounds associated with the decomposition of wool proteins. It has also been shown that the attractiveness of bacterial species may vary on different wool types. However, it appears that this attraction is also often variable, even on the same sheep over time. Indeed Mackerras and Mackerras (1944) noted from their extensive observations on attraction of *L. cuprina* to sheep that they had not encountered an animal which was consistently unattractive to flies and there was a strong element of chance, dependent to some degree on fly density, in the likelihood that sheep would or would not become struck.

Although odour cues are no doubt key in host finding and feeding or strike initiation, visual, and gustatory stimuli are often also involved, physical factors such as integument type and temperature and humidity gradients can have an effect. Variations in susceptibility may also be a result of differences in host behavioural response to the presence of parasites. In addition, usually the successful initiation of feeding or oviposition is dependent on a sequence of responses to host stimuli. Different odours or mixtures of odours, together with the effects of other stimuli will determine if a fly finds a sheep, identifies a suitable site for oviposition and ultimately deposits an egg mass on a sheep and this potential multifactorial effect of odour adds a further level of variability to sheep odour - fly interactions.

However, odour cues are critically important. It seems that the key odours are mainly bacterial in origin and associated with predisposing conditions such as urine and faeces stain, bacterial conditions such as fleece rot and dermatophilosis and possibly bacterial growth associated with wet fleece. It seems that these odours would likely overwhelm any innate sheep variations in odour.

However, given the importance of olfaction at many stages in host finding and feeding or strike initiation by *L. cuprina*, clarification of olfactory mechanisms and the genetic basis underlying these may lead to novel approaches targeting the genes that operate in host detection, the location of suitable sites for oviposition and at different stages in the establishment of strikes. This may assist the development of improved bait or deterrent options and identify targets for new families of blowfly strike insecticides and vaccines.

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7. Appendices

Appendix 1: Breech Flystrike Review Workshop documents

a. Workshop objectives and agenda

BREECH FLYSTRIKE REVIEW WORKSHOP
4 December 2018, Stamford Plaza Sydney Airport
9.00 am – 4.30 pm
Introduction
Peter James, Forbes Brien and Alisha Anderson are currently undertaking an AWI funded review of breech flystrike risk factors to identify potential R&D opportunities to reduce these risks. As part of that review, this workshop has been initiated by AWI to gather input (gaps, R&D ideas) from leading breech flystrike researchers and consultants.

Objectives of Workshop:

- Identify research gaps and opportunities towards:
  - improved methods of breeding for breech strike resistance; and
  - development of novel sheep blowfly strike controls
- Recommend research directions and priority areas
- Add these outputs to the Breech Flystrike Risk Factors Review.

AGENDA

Time	Topic	Presenter
8.00 – 9.00	**Coffee and Tea available**	
9.00 – 9.10	**Welcome/Background**	**Bridget Peachey**
9.10 – 9.15	**Introduction to Day**	**Russell Pattinson - Facilitator**
Summary of Projects		
9.15 – 9.40	**Review of Breech Flystrike Risk Factors**	**Peter James** (15 mins + 10 mins)
9.40 – 10.10	**Overview of Breeding for Breech Strike Resistance - where to now?**	**Geoff Lindon** (20 mins + 10 mins)
10.10 – 10.40	**Overview of Mapping the *Lucilia cuprina* genome – where to now?**	**Trent Perry** (20 mins + 10 mins)
BREAK (20 mins)		
Identification of Research Opportunities		
11.00 – 12.00	**Breakout Groups** A. Breeding for Breech Strike Resistance - where to now (2 groups) B. *Lucilia cuprina* genome and the research opportunities it presents (2 groups)	**All in 4 groups**
12.00 – 12.45	**Reporting back & discussion**	**4 presenters and facilitator**
LUNCH (60 mins)		
Prioritisation of Research Opportunities		
1.45 – 2.15	**Criteria for prioritisation of ideas**	
2.20 – 3.10	**Prioritisation of ideas**	
Break (20 minutes)		
Collaboration		

3.30 – 4.00	**Collaboration Opportunities/Access to resources** Availability and access to resources • Sheep flocks (where next?) • Blowfly Genome How can we collaborate better?	
4.00 – 4.20	**Breech Flystrike Researcher Forums?**	
4.20 – 4.30	**Wrap Up/Next Steps**	
4.30 pm	**FINISH**	

b. Attendees

First Name	Last Name	Organisation
Sheep genetics		
Geoff	Lindon	AWI
Forbes	Brien	University of Adelaide
Daniel	Brown	AGBU
Johan	Greeff	DPIRD
Mike	Goddard	University of Melbourne
Raul	Ponzoni	Consultant
Jen	Smith	CSIRO
Sonja	Dominik	CSIRO
Joan	Lloyd	Consultant
Herman	Raadsma	University of Sydney
Bruno	Fernandes Sales Santos	AbacusBio (replacing Peter Amer)
Fly genome		
Peter	James	University of Queensland
Vern	Bowles	University of Melbourne
Phil	Batterham	University of Melbourne
Alisha	Anderson	CSIRO
Clare	Anstead	University of Melbourne
Coral	Warr	University of Tasmania
Dave	Leathwick	AgResearch
Tony	Vuocolo	CSIRO
Trent	Perry	University of Melbourne
Schroder	Johann	MLA
Owain	Edwards	CSIRO
Peter	Hunt	CSIRO
Other		
Russell	Pattinson	Facilitator
Vidette	Moore	AWI
Bridget	Peachey	AWI
Jane	Littlejohn	AWI
Apologies/No response/TBC		
Ben	Hayes	University of Queensland
Andrew	Kotze	CSIRO
John	Oakeshott	CSIRO

Peter	Amer	Consultant
Richard	Newcomb	Plant & Food Research NZ
Mark	Sandeman	Federation University
Tim	Mahoney	University of Queensland

c. Notes from presentations

1. Review of Breech Flystrike Risk Factors - Peter James

a) Breeding - opportunities

Where we should go next could include but not limited to:

- Use of data already collected
- Further data/ development needed in this area
- New/better indicator characters
- Facilitating adoption of what is already available

Genomic methods

b) Sheep blowfly genome - opportunities

- New chemicals
- Managing resistance (preventing, monitoring, other?)
- Vaccines
- Area wide controls/ Genetic (blowfly) controls
    - Sterile male, RIDL, New approaches Gene drives?

c) Other

- Resistance
- Biocontrols – classical biocontrols, biopesticides,
    - 'Natural compounds'
- Predisposing conditions
    - Scouring, Fleece rot/ dermatophilosis, Bacterial roles
- 'Physical'/management controls
    - Mulesing alternatives, Crutching/shearing, 'Environmental' management,
    - Fly traps/ population
- Blowfly/ strike ecology
- Integrated /systems approaches
- The science is good but some essential science needs to be considered

2. Overview of Breeding for Breech Strike Resistance – where to now? – Geoff Lindon

<u>Planned but not activated R&D</u>

- Data Quality "Index" a game changer, increased focus on precision (and volume)
- Increase number of animals assessed for breech wrinkle, dags and cover
- Add neck and body wrinkle to breech wrinkle ASBV (NM and Dohne Studs)
- Create Urine Stain (H2 low) and Faecal Consistency ASBVs
- Create welfare enhanced indexes (Promote high fleece weight, low breech trait sires, with high survival)
- Publish stud average ASBVs for breech traits, similar to wether trials
- Improve worm egg count monitoring, more accuracy at lower burdens

<u>As yet unplanned R&D</u>

- Work with today's elite sheep that will be relevant commercially in 10 to 15 years
- Find unexplained factors using 2 resources
    - Progeny test elite fleece weight and breech traits genetics, 2 sites

    - Retain 200 ewe R (and perhaps not S line) at Mt Barker and Armidale   Is this decided?
    - (AMSEA site and or MLP sheep?)
    - Can artificial breech wetting assist? Rate of drying?
- What causes dags? Issue is we don’t have enough animals in high dag breeding areas
- What causes urine stain? (males and females)
- Is odour and bacteria an issue?
- Keep investigating genomics
- Indexes for welfare traits.
- It was also mentioned that there is no breeding value for strike – Breeding strike index?

3. Blowfly genome – Trent Perry

ON-00373 Genetics of Blowfly Parasitism – Completed

- Improved the *L. cuprina* genome
- Examined genes that might be critical for development
- Assessed whether they impact larval survival

ON-00570 Development of gene knockout technology – Continuing

- Develop a robust genetic manipulation technique for *L. cuprina*
- CRISPR = highly transferrable between organisms
- There was also discussion around the scare mongering going on, at the moment, around CRISPR and what are the regulatory issues and what would the public perception be of releasing genetically modified organisms
- Adapting and developing tools from *Drosophila melanogaster* to study *L. cuprina*

ON-00624 Informed development of a flystrike vaccine - Future

- *L. cuprina* population genetics study
- Using proteomics to examine the interactions between larvae and sheep during myiasis
- Collaborating with CSIRO on vaccine development

d. Notes from discussion groups

Sheep Genetics Groups

Group A
Opportunities

i. Increase phenotypic data of indicator traits so as to develop a breech strike index
ii. Facilitate a flock(s) to establish genomics Breeding Values (could be AMSEA flocks, MLP flocks, industry flocks)
iii. Clarify the value proposition for use of breeding values and communicate / extend to industry
iv. Better understand ‘attractiveness factors’ (e.g. odour)
v. Support for resource flocks

Notes:

Need Increased phenotyping for current factors
Need ASBV for faecal consistency
Index for breech strike resistance?
Genomics reference flock – need 10,000+ sheep
Structure, combine breech strike flock and industries (case, control protocol)
Focus on breech strike
AMSEA & MLP flocks?

Extension/ Adoption
- More phenotyping
- Prepare for when there is a significant value proposition to adopt technology

Research
- Odour and attractiveness
- How does the fly identify targets

Resource Flocks
- Keep and link with industry flocks
- Diversity

Group B
Opportunities

i. Clarify the aim of your breeding objective – reduce breech strike or stop mulesing?
ii. Clarify the value proposition for use of breeding values for breech strike – reduce strike, impact on fleece weight but increase in reproductive performance
iii. Develop integrated package of solutions – not just selective breeding
iv. Increase genomic data
v. Investigate the unexplained variation (e.g. odour, microbiome)
vi. Support for resource flocks

Notes:

What is the breeding objective?
- Reducing breech strike or stopping mulesing?

Adoption & Value
- > Design gains or $ value?

Need an integrated plan
- Maybe package better to accommodate GFW
- Repro benefits lost in translation?

Need more phenotypes
- Encourage phenotypes from industry
- Investigate value of different quality of flystrike phenotypes

Unexplained variation
- Selection lines
- Other populations
- Joint analyses of 2 flocks?

Other flocks
- Progeny testing – desirably where incidence allows direct measure of breech strike

Fly Genomics Groups

Group C
Opportunities

i. Research the fundamental biology of the fly (e.g. life cycle), if they are not on the sheep, where are they? Review past studies.
ii. Investigate the dynamics of the fly population
    a. Apply to genome to look for new control options
iii. Better understand attractants including the potential role of nanoparticles (sacrificial lamb – e.g., that can pull flies away from the other sheep)
iv. Investigate microbes on sheep
v. Examine fly microbiome and identify areas of potential vulnerability
vi. Support for resource flocks

vii. Good benefit in keeping the genetic resource flock, use to inform fly biology and to look further into the resistant and susceptible sheep and what is different in the fly?

Gaps

More extensive transcriptomes
Life stage
Tissues
Environmental effects – microbial effect
Diet (carrion/rubbish versus "on sheep"
Attractants / Baits; Push – Pull (traps on sheep)

Group D

Opportunities

i. Fly population studies – location, hybrids, genetic variation etc (build on other work such as Q fly)
ii. Examine attractants (olfactory)
iii. Understand sex determination factors
iv. Support for resource flocks

Gaps

Dispersal Distances
Hybridisation
Population variation
Value Add (Analytical values, other insect data, transport routes – Sheep)

Note

Representative sampling:
Trapping (Luci Traps?)
Sheep Collections
Rural / Non-Rural.

Appendix 2: AWI response to the Recommendations presented in the Review of Predisposing Factors for Breech Flystrike Report

AWI welcomes the recommendations presented in the Review of Predisposing Factors for Breech Flystrike, with work already underway on many under AWI'S flystrike RD&E program.

AWI's responses to the recommendations in the Review are below**.** Where AWI agrees with recommendations, it remains under budgetary constraints and may not proceed with investment.

RECOMMENDATION 1. Invest in a genomics reference flock towards the creation of genomic markers/indexes/ breeding values for flystrike resistance
We consider this a high priority area (also considered high priority with high cost, high potential reward by Breech Flystrike Review Workshop participants). Genomic methods have major potential benefits for selecting flystrike resistance because sheep do not need to be exposed to strike, or subject to the predisposing conditions for flystrike and detailed and difficult phenotyping is not required to assess an animal's genetic merit. Rather, the genotype is estimated from a small blood sample. Furthermore, a genetic value can be attributed to all animals in all years and all environments, regardless of level of flystrike challenge.

We recommend the establishment of a Genomics Implementation Working Group to determine the best path forward with regard to available resources/resource constraints. This panel should include high-level specialist expertise in sheep/animal genomics, sound industry reference and representation from Sheep Genetics to facilitate implementation. The potential value of maintenance of the flystrike selection flocks, which are already phenotyped for a wide range of flystrike and production traits, as part of this reference flock, is emphasised.

AWI RESPONSE:
AWI supports the recommendation to form an expert group to assist AWI plan the flystrike genomics path for sheep. A recent AWI funded genome wide association study by CSIRO using genomic information from over 1,500 sheep from the Mt Barker WA and Armidale NSW flystrike resource flocks concluded that there are no major sheep genes associated with breech flystrike and therefore genetic marker assisted selection is unlikely to be effective. However, as SNP gene polymorphism may assist, AWI will investigate the formation of a virtual reference flock, with oversight from an industry Flystrike Genomics Working Group, if funding permits. The group would facilitate the collection of further genomic data to inform the creation of genomic breeding values and/or indexes for flystrike resistance.

RECOMMENDATION 2. Increase the collection of phenotypic data from industry flocks (and other research flocks where relevant) with a view to the development of a breech strike index(es)
Encouragement of much more widespread phenotyping for flystrike traits is required to provide more robust and widely applicable estimates in Merino genetics. This is particularly so for urine stain, which currently does not have a breeding value available in MERINOSELECT, and for scouring/dags. To this end there is a need to facilitate easier methods of measurement of 'difficult' traits such as urine stain and scouring/dags. There could be easier methods of assessing them, or perhaps indirect methods of estimating urine stain/risk of urine stain. The recording of alternative more readily measured estimates for the main flystrike traits e.g. faecal consistency for scouring, face cover for bare area, neck and body wrinkle for breech wrinkle for recording in MERINOSELECT and presentation of ASBVs for these traits should also be considered.

Progeny testing of elite sires directly for breech strike incidence would provide an avenue for increased accuracy and maximising industry genetic gain in flystrike resistance.

AWI RESPONSE:
AWI agrees with the need for more data recording to boost the MERINOSELECT dataset of flystrike traits, hence the robustness of genetic parameters and predictions of breech flystrike resistance. AWI will continue to encourage industry projects and individual wool growers to submit their data.

Additionally, the Merino Lifetime Productivity (MLP) project is collecting genotypes on 5,500 ewes and annual phenotypes for the breech flystrike indicator traits of urine stain, faecal consistency, dags, worm egg count, body and breech wrinkle breech cover and face cover. Industry can now collect body wrinkle as a highly correlated trait to breech wrinkle, allowing all mulesed animals to be phenotyped for breech wrinkle. The MLP project is essential fundamental investment for the success of breeding for breech flystrike resistance. It will inform woolgrowers of the benefits and disadvantages of a wide range of breeding tools so they can make an informed decision on using new tools such as genomics for difficult to measure traits such as flystrike resistance.

RECOMMENDATION 3. Development of breech strike / welfare indexes
There is a need to facilitate practical 'useability' of breech strike traits in MERINOSELECT for sheep breeders. Breeding indices incorporating breech strike resistance while maximising genetic gains for other traits are needed for a range of different environments and sheep types. Optimal incorporation of breech strike resistance will require the derivation of an economic value(s) for breech strike resistance.

AWI RESPONSE:
AWI supports this recommendation to develop selection indexes. Information collected under the MLP project is intended to contribute towards the development of flystrike/welfare index(es).

RECOMMENDATION 4. Better understand the unexplained variation in the occurrence of strike in resistant and susceptible sheep and the effect of management regime on this

The amount of variation in breech strike susceptibility not explained by the major indicator characters will be key to a consideration of the need for new or better indirect selection criteria. There is little unexplained variation in some data sets (e.g. crutched ewes in WA where only 9.38% of the variation remains unexplained) and dags and skin wrinkles explain most of the phenotypic variation, as opposed to the NSW flocks and unmulesed, uncrutched flocks in WA where approximately 50% of the variation remains unexplained. There is a need for a 'harmonised analysis' of the WA and NSW data followed by careful consideration of what percent of the unexplained phenotypic variation is environmental in origin, what percent is likely to be genetic, what fixed effects have been taken into account and likelihood of finding new indicator characters that can markedly increase the accuracy of selection for flystrike resistance.

AWI RESPONSE:

AWI supports this recommendation to explain the variation in the incidence of flystrike. A project, using the data sets from the WA and NSW flystrike resource flocks, has been scoped to improve the understanding of the causal role of key factors contributing to breech flystrike occurrence and to measure the extent to which these factors influence the risk of breech flystrike. Funding for this project does not exist at present.

RECOMMENDATION 5. Support the continuation of the flystrike resource flocks

The two flocks provide a source of very accurately pedigreed and phenotyped animals that are in completely different environments with different flystrike profiles. The depth of phenotyping for flystrike incidence in the flystrike selection lines in WA (now at Katanning) and NSW (Chiswick) makes these flocks an important core resource for genomic studies, a prime resource for identifying and testing new indicator characters and valuable for obtaining more precise genetic parameters for the development of more accurate selection and breeding programs.

The flocks will also be an important resource for research in other areas, for example investigating the role of microbiome profiles in strike etiology and susceptibility, testing the efficacy of new vaccine technologies and resistant phenotypes, and the future development of welfare indices (that incorporate resistance to breech strike) and breeding values.

AWI RESPONSE:

AWI does not support this recommendation to continue investment in the flystrike resource flocks. AWI could no longer justify investment in flocks that were losing genetic relevance to the national flock and when compared to other, more contemporary industry flocks such as the MLP flock. Instead, resources have been directed into priority blowfly research areas including chemical resistance and investigation of a blowfly vaccine. However, AWI agrees that the data and genetic samples collected from these flocks continue to offer useful insights into breech flystrike resistance, and current and recently completed projects are providing valuable analysis of the information generated from these flocks. AWI is looking to contribute over 1,500 genotypes from the flystrike resource flocks to MLA's Genomic Reference Flock. Furthermore, whilst no flystrike incidence data is being collected there is a significant amount of phenotyping of breech flystrike indicator traits and genotyping data being collected at the 5 MLP sites and available for research.

Full pedigree and phenotype information has been collected from the flystrike resource flocks, as well as a bank of genetic samples, and individuals from the Armidale NSW flystrike resource flock are being used in other industry projects (including the MLP and flystrike vaccine projects). Should there be a requirement for sheep with diverse flystrike resistant phenotypes for future projects these are expected to be sourced from other industry flocks including the MLP and AMSEA flocks and possibly a virtual flock using existing ram breeding flocks.

RECOMMENDATION 6. Increase understanding of the fundamental biology of *L. cuprina* (leading to opportunities for control)

This was considered high priority at the Breech Flystrike Review Workshop because knowledge in this area underpins a large number of potential approaches. These studies need to be carefully targeted to provide knowledge with specific endpoints towards improving control efficacy and will be facilitated by recent advances in molecular technology. Some specific areas of interest are suggested in the body of the review and in other recommendations. We emphasise the importance of a careful review of the abundant work already undertaken in this area and, in particular, the work conducted as part of the CSIRO genetic control program in the 1970s, before new research is commenced.

AWI RESPONSE:

AWI supports this recommendation to increase fundamental knowledge in fly biology. There is a knowledge gap on the national genetic variation of fly genes. This work is needed to inform research targets to understand the fundamental biology of the fly as it may assist identifying opportunities for control. A 3-year-long population genomics study, currently underway, is examining the population structure of *L. cuprina* from both rural and urban areas. DNA samples across three collection seasons will be analysed for levels of variation and sequenced to estimate the size of the blowfly populations and levels of migration/gene flow between them. A proteomic analysis of early blowfly parasitism of sheep will complement recent work that generated a detailed profile of the gene expression changes of *L. cuprina* during larval development on sheep.

These projects are expected to provide critical information required for future planning and flystrike control strategies such as sterile insect release, biological control, gene drive or new chemicals or vaccines. It will also provide valuable information in managing and containing the spread of insecticide resistance outbreaks.

Historical fundamental research to explore the potential to develop a flystrike vaccine did not realise a commercial vaccine at the time. However, the significant advances in foundation knowledge at the time are now helping underpin new approaches in flystrike vaccine development. New age technologies, including Next Generation Sequencing, the availability of the sheep blowfly genome, lifestage and tissue-based transcriptomics, enhanced methodologies for production of recombinant antigens and synthetic biology, are now being applied in an advanced approach to developing a flystrike vaccine. This is allowing new questions to be asked, and processes undertaken, to produce answers and leads in much quicker timeframes. This has already resulted in the discovery of novel candidate antigens that are under investigation for potential use in a flystrike vaccine.

RECOMMENDATION 7. Explore the expression patterns of *L. cuprina* genes to understand the molecular basis of establishment of strikes (attraction, oviposition, larval invasion) and regulation of key developmental processes of *L. cuprina*

This work will facilitate optimal usage of the *L. cuprina* genome to develop new vaccines, new flystrike insecticides and potentially area-wide approaches to control of *L. cuprina*. This work needs to be targeted to specific outcomes in order to ensure efficiency and value of the investment.

(Work in this area supported by AWI is underway, searching for genes involved in the location of susceptible sheep by *L. cuprina,* dermal invasion by blowfly maggots, the initiation of strike and developmental processes of the blowfly larvae. This work is strongly supported).

AWI RESPONSE:

AWI supports this recommendation to explore the expression of fly genes. A proteomic/ metabolomic analysis of early blowfly parasitism of sheep, that is currently underway, will complement recent work that generated a detailed profile of the gene expression changes of *L. cuprina* during larval development on sheep. This, and related projects, are expected to provide critical information required for future planning and implementation of flystrike control strategies such as sterile insect release, biological control, gene drive or the development and use of new chemicals or vaccines. It will also provide valuable information in managing and containing the spread of insecticide resistance outbreaks.

RECOMMENDATION 8. Understand the fleece/dag microbiome, and its role in breech strike susceptibility

It is well established that bacterial growth is important at various stages in the development of bodystrike; for example in providing odour cues for attraction and oviposition, causing skin scalding and extravasation which provides protein for the development of 1st instar larvae and by providing a focus for skin invasion by newly hatched blowfly maggots. Microbial odours, particularly in association with urine or other decomposing organic matter have also been shown to be important in the attraction of other livestock ectoparasites to their hosts and bacteria often also provide critical nutritive factors for larval development of some livestock-associated flies.

There has been much less study of the importance of the breech fleece microbiome and interactions with urine stain and scouring and the importance of bacteria in the development of breech strike. However, there is indication that bacterial growth could be similarly important in determining breech strike susceptibility. This was identified as an area of knowledge deficit in construction of the causal web, and in our subsequent review of odour and predisposing causes for breech strike and was listed amongst priorities for research at the Breech Flystrike Review Workshop. The microbiome could also influence skin proteomic/metabolomic profiles and associated studies of the fleece/skin proteomics and metabolomics may yield additional important information towards the development of new approaches to control, for example vaccination against key bacteria, blocking bacterial odours, the use of bactericides or biological methods to control critical bacteria.

AWI RESPONSE:

AWI agrees that the fleece/dag microbiome, particularly with respect to its interactions with urine stain and scouring is a key area of knowledge deficit in our understanding of breech flystrike susceptibility. However, any further investment needs to consider the outcomes from existing projects and existing funding priorities. The population genomics study will provide information on the blowfly microbiome present in samples collected from different regions of the country and this is expected to provide some insights and directions for any potential further R&D into this important area.

RECOMMENDATION 9. Development of a detailed business case for investing in genetic improvement of sheep resistant to breech strike
To understand if further investment into breeding programs focused on reducing breech flystrike is worthwhile, and to underpin promotion to woolgrowers about the application of genetic technologies or other approaches, an understanding of the size and scale of potential benefits is required – i.e. a value proposition/business case, A component of this work, for example, would be a benefit cost analysis for establishing genomic evaluation of flystrike. This would also inform the feasibility/attractiveness of different approaches by quantifying the size of trade-offs that growers are willing to make.

AWI RESPONSE:
AWI sees this work on development of a business case for breeding resistant sheep as a lower priority to other recommendations and anticipates it will, in part, be covered by recommendations regarding creation of a flystrike reference flock and flystrike/welfare index(es). Several constraints for genetic improvement exist including the use of analgesia and anaesthesia for mulesing and the commercialisation trials of the liquid nitrogen process for breech modification. Furthermore, a key constraint is the level of knowledge and trust in advanced breeding tools such as ASBVs and genomics, hence the priority of the MLP project to generate knowledge.

RECOMMENDATION 10. Better understand the role of attractants/odour in sheep susceptibility and the genesis of strike
Odour is involved at a number of stages during development of strike. In particular location of sheep, the identification of susceptible sites on sheep for oviposition by flies and stimulating egg laying. Many of the main odours involved at different stages appear to be bacterially and environmentally mediated and there is little evidence that innate (genetically controlled) odour differences between sheep influence fly attraction or are related to susceptibility. There appears to be little evidence to support further studies of odour differences with a view to the identification of new selection criteria.

However, bacterial odours and other volatiles associated with predisposing causes of flystrike, such as urine and faecal staining, are critical to the initiation of strike and methods that interfere with the perception of odour by the flies, for example by targeting critical olfactory genes or processes, or the identification of strongly repellent molecules may lead to novel control approaches. Studies in this area should take into account that odour could be operating at a number of stages in strike development in addition to attraction (for example acting as an arrestant or oviposition stimulant) and design experimental tests accordingly.

AWI RESPONSE:
AWI supports this recommendation for exploring fly perception of odour and resultant fly behaviours. A recently completed project to breed an Orco knockout blowfly that cannot detect odour, using CRISPR, provides a tool that will be valuable in understanding the role of odour in blowfly attraction to the sheep or the extent to which additional cues are involved, such as CO_2 or heat. However, any further investment needs to consider the outcomes from existing projects and existing funding priorities.

RECOMMENDATION 11. Manage insecticide resistance and maintain the efficacy of available flystrike control products

The availability of effective flystrike protection and treatment chemicals remains critical to effective management of flystrike in Australian flocks, particularly in non-mulesed flocks. There is a long history of resistance development to flystrike control chemicals and the recent emergence of resistance to keystone control products, dicyclanil and cyromazine is a major threat to sustainability of wool production. This will be particularly important in unmulesed flocks, highly susceptible flocks and flocks in high flystrike risk regions. The characterisation and monitoring of resistance and promotion of resistance management strategies should continue to be an important element of flystrike control programs. Australian Wool Innovation is investing in this area. There has been limited detailed consideration of the best resistance management approaches to prolong the effectiveness of flystrike control compounds. A project to model resistance management programs, towards the development of optimal recommendations for woolgrowers, informing an integrated pest management (IPM) plan, is required. A detailed IPM plan should be supported by delivery of a well-integrated extension program for woolgrowers.

AWI RESPONSE:

AWI supports this recommendation to manage insecticide resistance. A project to monitor the development of blowfly resistance to commonly used chemical treatments in all the major wool producing regions is currently underway. The results from this work have already informed the development of an Australian sheep blowfly resistance management strategy that incorporates integrated pest management practices. Information on best practice flystrike control for growers that already have evidence of resistance on their properties is under development. Further research to better understand blowfly resistance mechanisms is currently under consideration.

RECOMMENDATION 12. Develop new insecticidal actives for flystrike control

With increasing costs of development and registration, the rate of new production animal parasiticide active compounds coming onto the market has "slowed to a trickle". The wool sheep parasiticide market is relatively small in the world context and this is particularly relevant as all of the major pharmaceutical companies that conduct research in this area have a multinational focus. Research in this area will assist the continued availability of effective flystrike preventatives for use by Australian woolgrowers. The availability of the *L. cuprina* genome will provide the possibility of new insecticidal targets (as well as oviposition suppressants) and AWI is currently funding a project in this area. AWI may need to increase their involvement with commercial veterinary pharmaceutical companies to assist new product development. The possibility of developing products based on chemical mixtures, a strategy currently used for ectoparasites, but used widely as a tool for combating resistance for gastrointestinal parasites should also be considered. There may be an opportunity to revisit some previously suggested chemicals. (The case of GH74 and like compounds is noted in the body of the review).

AWI RESPONSE:

AWI supports this recommendation to develop new insecticidal actives and improve existing actives. Phase II of a project to prove the viability of a new class of insecticidal target proteins is nearing completion. It is intended that the outcomes from this study will put industry in a good position to attract investment from a pharmaceutical company for further development and commercialisation of a novel insecticide for blowfly control. A current project, funded in partnership with a pharmaceutical company, to investigate the development of nano-encapsulated formulations of available blowfly chemical treatments may offer improved options for managing flystrike with minimal residues and off-target effects, whilst countering the development of resistance to chemical treatments.

RECOMMENDATION 13. Development of flystrike vaccines

AWI funded projects are underway towards the development of a flystrike vaccine. This will be facilitated by the recent availability of the *L. cuprina* genome and current AWI projects to identify critical genes in the genesis of flystrike, which offer the possibility of new gene targets for a vaccine. This is a high risk, but potentially very high reward project. A vaccine directed against fleece rot bacteria, critical in susceptibility to bodystrike was previously developed and patented, but never commercialised (Burrell 1985). This vaccine gave extended protection against fleece rot and bodystrike. As preliminary evidence suggests that many of the same bacteria may be important in susceptibility to breech strike, investigation of the potential of this vaccine for use in reducing susceptibility to breech strike may be worthwhile.

AWI RESPONSE:

AWI supports this recommendation, and preliminary work to investigate the development of a flystrike vaccine commenced in early 2019. The information garnered from using new research approaches and the highly informative sheep blowfly genome, coupled with pre-existing knowledge, is helping to rapidly progress AWI's flystrike vaccine initiative. The development of a flystrike vaccine presents the industry with a potential new paradigm in flystrike control. A vaccine offers a "clean and green", whole animal protection control measure for flystrike, complementing an integrated pest management approach to best practice flystrike management. Vaccines are broadly accepted by livestock producers meaning the adoption of a vaccine for flystrike control should be well taken-up by the industry. Pharmaceutical companies have already shown a high degree of interest in this research and have endorsed the approach that is being taken. Candidate vaccine antigens for inclusion in experimental flystrike vaccines, that target the establishment of larvae on sheep and their growth, have been identified, representing several interesting classes of insect proteins. Research scale manufacture of the antigens for trialling in sheep is in process. This approach is high-risk, however success will result in high impact outcomes that will potentially transform the industry.

RECOMMENDATION 14. Biological control of sheep blowflies

Biological control could include the release of specialist natural enemies that are expected to persist in blowfly populations keeping fly populations low (classical biological control) or biopesticides (innundative biological controls) where large numbers of pathogenic organisms (fungi, bacteria, viruses,) parasites or predators are released as 'biological pesticides'). *L. cuprina* occurs at low population density at most times and flystrike is episodic with fly populations building rapidly when conditions become suitable. The rate of spread of pathogens and parasites is almost invariably density-dependent. This factor and the lag time generally experienced between a pest outbreak and a corresponding increase in numbers of biocontrol agents would seem to present difficulties for classical biocontrol agents to persist and impact on *L. cuprina* populations, or more particularly, to reduce strike incidence. Biopesticides such as *Bacillus thuringiensis* and some entomopathogenic fungi have shown short term protection when applied to sheep in experimental studies and suitable agents may have application as part of an integrated approach or in organic flocks. However, they are unlikely to provide a level or persistence of protection comparable with chemical pesticides which limits their practicality in many situations. Pathogens that persist in the soil, such as some fungi or entomopathogenic nematodes, may have effect against soil stages of *L. cuprina* (prepupal larvae and pupae) particularly during the overwintering phase. However, better knowledge of the spatial and temporal ecology of the soil phases of *L. cuprina* will be required to assess whether sufficient mortality could be induced to significantly affect flystrike incidence. The potential of biological control of *Lucilia spp*. using sheep blowfly pathogens is currently being reviewed in more detail as part of AWI Project ON-00620.

AWI RESPONSE:

AWI supports this recommendation for biological control of blowflies. Recommendations from a recent review of the biological control of Lucilia spp. using sheep blowfly pathogens in soil and the fly microbiome are under consideration. *Wolbachia* are a bacteria that infect the majority of insect species on the planet and have already been harnessed to prevent the spread of viral infections by mosquitos. Many strains are being studied in other pest insects for use as biological control agents for population suppression of elimination. Prior AWI funded research has identified the presence of naturally occurring *Wolbachia* bacterial infections in the Australian sheep blowfly samples, and current work as part of the population genomics study will provide a detailed analysis of how many different *Wolbachia* (and other bacteria) species are present in *L. cuprina* and profile their distribution across Australia.

RECOMMENDATION 15. Area wide genetic controls for *Lucilia cuprina*
These methods seek to bring about suppression or eradication of the pest population by the release of flies of the same species that have been modified to confer sterility or cause genetic death in pest populations. This approach is also known as autocidal control and is usually used in area wide strategies focussed on eradicating pest populations from an area or reducing pest abundance through ongoing release programs. The most well-known method, the sterile insect technique (SIT) was successfully used to eradicate screwworm flies from north and central America and has also been used for eradicating regional incursions of insects, such as fruit flies in uninfested areas of Australia and an incursion of screwworm flies in Libya.

In the 1970s, CSIRO investigated the use of compound chromosome strains, sex-linked translocation strains and female killing systems in an attempt to suppress or eliminate *L. cuprina* populations and to address the cost barriers to use of SIT in Australia. In spite of some initial success this was eventually not pursued because of operational difficulties and funding constraints. The availability of gene editing technologies (such as CAS CRISPR) provide the potential for more elegant systems of genetic control such as RIDL (Release of Insects with Dominant Lethality) or potentially using gene drives to spread deleterious (often sex-linked or stage specific genes) through fly populations. Research is currently underway, funded by AWI, to identify critical genes in *L. cuprina* and may facilitate the design of genetically modified strains suitable for use in area wide autocidal approaches. Transgenic sexing "male only" strains have been developed in North American *L. cuprina* strains and consideration should be given to the feasibility of the future use of these strains in the design of area wide strategies in Australia.

AWI RESPONSE:
Further information on the potential success of area wide genetic controls for *L. cuprina,* under Australian conditions, would be required before AWI could support this recommendation. AWI is in consultation with MLA during their review of SIT use for *L. cuprina.*

RECOMMENDATION 16. Project to address scouring
Scouring (diarrhoea) and resultant dags in the breech wool of sheep are major predisposing causes for breech strike in the southern sheep production areas of Australia. Dags are also a major management issue in their own right in these areas. Methods to reduce the incidence of scouring and dags would have a major impact in reducing breech strike incidence. Recommendations towards the reduction of dags have been provided to AWI in a previous project (AWI Project WP520 - Minimising Dags in Sheep) and are currently being updated (AWI Project ON-00610).

AWI RESPONSE:
AWI supports this recommendation. A 2019 review of recent research outcomes on minimising dags in sheep included R&D recommendations for the prevention of dags, which are currently under consideration. AWI has updated its suite of extension resources for managing dag, including the complex relationships between larval hypersensitivity scouring and worm egg count.

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