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Summary

Fleece rot and body strike form a disease complex. The major components of resistance to body strike so far identified are wool and body conformation traits affecting susceptibility to fleece rot. The role of the host response in contributing to resistance to fleece rot and body strike is not clear, although the enhanced antibody responses to Pseudomonas aeruginosa *seen in sheep selected for resistance to the disease complex may be important. The sporadic occurrence of body strike due to climatic variability necessitates indirect selection. Fleece rot has a high genetic correlation with body strike and is therefore a candidate for indirect selection; however, culling of sheep with fleece rot is already the industry norm. Thus indicator traits with higher prevalence or higher genetic correlation with body strike than is the case for fleece rot are needed to enhance rates of genetic improvement. Direct challenge with internal parasites has been a successful strategy for selecting sheep for resistance to internal parasites. It is unknown whether genetic variation occurs between sheep in survival and growth of larvae after eggs are deposited on the sheep. This question should be investigated to establish whether there is scope for selection for resistance to body strike independently of fleece rot. Applying contemporary molecular genetic techniques such as gene expression arrays to sheep with variability in resistance to fleece rot or larval survival and growth may lead to identification of gene markers for resistance.*

Keywords

Resistance, fly strike, fleece rot, selection, direct challenge

Introduction

Studies on resistance of sheep to blowfly strike commenced early in the 20th Century when fly strike emerged as an economically important disease. Blowfly strike was uncommon until 1903 when it became widespread in New South Wales and Victoria, extending throughout the range of sheep over the next few years (Gilruth *et al*., 1933). Emergence of the disease coincided with introductions of American Merinos from Vermont, which had a high grease content in the fleece and pronounced skin wrinkle (Belschner, 1966). With the realisation that body conformation was a predisposing factor for susceptibility to blowfly strike (Seddon *et al.*, 1931a) and that these predisposing traits were heritable (Seddon *et al.*, 1931b) interest turned to breeding resistant sheep. Wetting of fleece had been found to induce bacterial growth (Stuart, 1894), which in turn induced green or red discoloration of wool (Seddon and McGrath, 1929) and dermatitis (Bull, 1931). These studies led to the conclusion that predisposing conditions for body strike are wool characteristics and body conformations that favour, principally through susceptibility to wetting and resultant dermatitis, attraction of flies, oviposition and subsequent nutrition of larvae (Gilruth *et al.*, 1933). It was realised that fleece rot and blowfly strike occur as a disease complex, with the strongest interdependence when blowfly strike occurs over the shoulders, back and flanks (body strike). This early work provided the basis for the next 70 years of research into resistance to blowfly strike.

Pathogenesis of fleece rot

In a review of fleece rot and body strike, McGuirk and Watts (1983) concluded that there appear to be three barriers providing resistance to blowfly strike: wool, skin and the immune system. Much attention has been focused in the last three decades on skin changes following wetting. With artificial wetting, the free sterol content of waxes increases and the hydrophobic properties of remaining wax decrease (Hay and Mills, 1982). *Pseudomonas aeruginosa* is thought to contribute to the breakdown of the skin wax layer, which appears to be an important step in progression to fleece rot (James *et al*., 1984). This conclusion is supported by the observation that chemical disruption of the sebaceous layer on skin

greatly enhances development of fleece rot (James and Warren, 1979). A positive correlation between suint content of wool before wetting and fleece rot susceptibility has been seen in some studies (discussed by James *et al*., 1984). Rates of change in the water content of fleece during wetting and drying phases, but not the water content of fleece prior to wetting, were associated with fleece rot incidence (Raadsma, 1989). The variability of fibre diameter and birth coat score have also been identified as potential traits for indirect selection (Raadsma, 1993).

Artificial wetting can induce local dermatitis and plasma exudation within six hours (Chapman *et al*., 1984), which can progress to disruption of normal follicular structures, with fibre breakages and development of plugs within follicles (Nay and Watts, 1977; Hollis *et al*., 1982). These features appeared to be more frequent in sheep susceptible to fleece rot (Nay and Watts, 1977). A predominance of *P. aeruginosa* is seen amongst bacteria proliferating on wetted skin (Merritt and Watts, 1978). During fleece rot, bacteria remain superficially on skin and do not penetrate the dermis (Chin *et al.*, 1995). Perhaps reflecting the predominance of *P. aeruginosa* is the observation that much higher antibody titres develop to this organism during fleece rot than to other skin bacteria (Chin and Watts, 1992). Skin wetting is necessary for systemic sensitisation to antigens from *P. aeruginosa* (Chin *et al.*, 1995). Further characterisation of skin and wool traits may be necessary before they can be successfully used as indicator traits for resistance to fleece rot and fly strike.

Direct and indirect selection for resistance to fly strike

Hayman (1953) confirmed Seddon's observations on heritability of resistance to fleece rot. Quantitatively, McGuirk and Atkins (1984) estimated a heritability of susceptibility to fleece rot of 0.40 on a continuous, underlying scale of liability in unselected medium-wool Peppin Merinos from 15 years of field data at Trangie Agricultural Research Centre in NSW. Heritability of susceptibility to blowfly strike in the same environment was between 0.26 and 0.37 (Gilmour and Raadsma, 1986; Raadsma, 1991). In practice, though, information is usually only available on presence or absence (binomial scale) of these conditions which, under typical incidences, results in an effective heritability of 0.1-0.25 (McGuirk and Atkins 1984). Experimental conditions for inducing fleece rot and fly strike were developed at Trangie using artificial wetting by overhead sprinklers of sheep temporarily housed indoors (McGuirk *et al.*, 1978). Fleece rot and subsequent body strike resulting from artificial wetting provided the basis for establishing selection lines with resistance or susceptibility to the disease complex (McGuirk *et al.*, 1978). These selection lines have been the subjects of intensive study of both wool characteristics and physiological responses associated with resistance. Heritability of susceptibility to fleece rot under artificial wetting (Raadsma *et al.*, 1989) was comparable to that seen under field conditions (Raadsma and Rogan, 1987). Following 17 years of selection, the lines had diverged at an annual rate of 2.8% for natural fleece rot and 0.4% for natural body strike (Mortimer *et al.*, 1998). With the absence of a random bred control line in this study it is unclear whether there has been a greater impact of selection on resistance or susceptibility to the disease complex. The sporadic occurrence in many of the grazing zones of Australia of weather conditions that induce fleece rot and that accompany fly waves, stimulated research on correlated wool traits that could provide the basis for indirect selection for resistance. Wettability of the fleece (Raadsma, 1989), objectively measured wool colour (Raadsma and Wilkinson, 1990) and certain fibre characteristics (Raadsma, 1993) are genetically correlated with resistance to the disease complex.

Mechanisms of resistance in the Trangie selection flock

An understanding of host defence mechanisms that underlie resistance may aid the identification of strategies for indirect selection for resistance. Freshly hatched larvae feed on proteins in exudates associated with bacterial dermatitis (Gilruth *et al.*, 1933; Sandeman *et al.*, 1987). Skin inflammatory responses have therefore been examined to determine whether underlying differences in plasma leakage could account for differences between the selection lines in susceptibility to blowfly strike. Leakage of radiolabelled albumin from the vascular compartment into skin was measured following intradermal injection of the permeability agents histamine, bradykinin, activated complement, platelet-activating

factor or serotonin. Plasma leakage induced by activated complement was greater in susceptible sheep, and for all mediators there was a tendency for plasma leakage to be greater in susceptible sheep (Colditz *et al.*, 1992). This finding suggested that a smaller plasma leakage response following release of endogenous permeability mediators might contribute to resistance to blowfly strike and is in accord with the nutritive role of blood proteins for larvae. Serum complement C3 levels, however, did not differ between lines (O'Meara and Raadsma, 1995), suggesting that differences might lie at the level of receptor number or function controlling vascular permeability rather than in supply of mediator. Contrary to this finding is the observation that when excretory and secretory products from larvae were injected intradermally, there was a greater wheal response in resistant animals (O'Meara *et al.*, 1992). The wheal response to excretory and secretory products has also been correlated with resistance to fleece rot and blowfly strike in unselected sheep (Broadmeadow, 1988) and has been proposed as a selection marker for resistance to the disease complex (Raadsma *et al.*, 1992). Co-heritability of the skin test response with resistance to fleece rot is estimated to be 0.25 (Raadsma, 2000).

Cellular components of the inflammatory response in skin and resident leucocytes in skin have also been examined in the resistant and susceptible lines. Intense neutrophil accumulation is a feature of the dermatitis that accompanies fleece rot and fly strike (Bull, 1931; Burrell *et al.*, 1982; Bowles *et al.*, 1992) and there is extensive production of inflammatory cytokines interleukin (IL)-1α, IL-1β, IL-6 and IL-8 (Elhay *et al.*, 1994) at the wound site. Neutrophil accumulation in response to intradermal injection of the inflammatory mediators ovine IL-1β, human IL-8, human IL-1α, human tumour necrosis factor (TNF)α activated complement, leukotriene B_4 and endotoxin from *Pseudomonas aeruginosa* did not differ between lines (Colditz *et al.*, 1994). Leucocyte populations in untreated skin and in skin sites collected six hours following intradermal injection of TNFα were examined by immunohistology. WC1+ lymphocytes and eosinophils were more prevalent in skin of susceptible sheep, and IgE+ cells (probably mast cells) were more prevalent in skin of resistant animals (Colditz *et al.*, 1994). In an independent study (Nesa, 1994), mast cells were also found to be more prevalent in skin from animals of the resistant genotype. This difference in the prevalence of mast cells may underlie the greater wheal response to intradermal injection of larval antigens in resistant sheep noted above. Furthermore, in an examination of the IgE gene, Engwerda *et al.* (1998) found a restriction fragment length polymorphism between resistant and susceptible lines. Thus the role of mast cells in resistance to blowfly strike deserves further attention.

The response of resistant and susceptible sheep to artificial infestation with larvae has been examined in two experiments. O'Meara *et al*. (1995) found greater exudation of serum proteins onto the skin surface during the first 12 hours of infestation in resistant animals, which is in accord with greater skin wheal responses seen in these animals. In the second experiment, the effects of lymphocyte and (IFN)-γ depletion on larval establishment and growth have been studied by treatment of sheep with monoclonal antibodies. In contrast to results seen with the internal parasite, *Trichostrongylus colubriformis,* where depletion of CD8+, WC1+ lymphocytes or IFNγ enhanced resistance to infection (McClure *et al.*, 1996), depletion of CD4+, CD8+, WC1+ lymphocytes or interferon IFNγ had no effect on establishment or growth of blowfly larvae (Colditz *et al.*, 1996). Importantly in both artificial infestations, there were no differences between the resistant and susceptible lines in the establishment or growth of larvae.

Lymphocyte subsets in blood do not differ between resistant and susceptible lines (Colditz *et al.*, 1996; McColl *et al.*, 1997). Following intravenous challenge with endotoxin from *Pseudomonas aeruginosa* there are higher neutrophil counts and monocyte counts in blood from the resistant line (Colditz, *unpublished*). Antibody responses to intradermal injection of *P. aeruginosa* antigens are generally greater in resistant sheep (Chin and Watts, 1991; Gogolewski *et al.*, 1996), however, no significant differences between the lines were found in the antibody response to *L. cuprina* antigens (O'Meara *et al.*, 1997). Sheep from the resistant line develop higher titres of antibody to *P. aeruginosa* antigens during simulated fleece rot conditions when live cultures of *P. aeruginosa* are applied epicutaneously to wetted skin (Chin and Watts, 1991). This may result from differences in skin characteristics between the lines resulting in greater uptake of antigens through skin in the resistant animals, or alternatively to differences between lines in immunological recognition and response to *P. aeruginosa* antigens. Systemically administered *P. aeruginosa* vaccines can confer resistance to fleece rot (Burrell, 1985) so the differences in antibody titres noted by Chin and Watts (1991) and Gogolewski *et al*. (1996) may well contribute to the

differences in prevalence and severity of fleece rot seen between the selection lines. Taken together, these extensive studies on the Trangie resistant and susceptible lines suggest that reactivity of skin may contribute to resistance but probably plays a subsidiary role to wool characteristics that predispose to fleece rot.

Innate immunity in unselected sheep

The host response to fleece rot and fly strike results in production of high levels of inflammatory cytokines in skin (Elhay *et al.*, 1994). We therefore examined the direct toxic effects of these endogenous mediators on larvae in an *in vitro* feeding assay (Colditz and Eisemann, 1994). At concentrations that stimulate inflammatory activity in skin (Colditz, 1991; Colditz and Watson, 1992; Mulder and Colditz, 1993), histamine, bradykinin, platelet-activating factor, serotonin, activated complement, leukotriene B_4, IL-8, IL-1β, TNFα and IFNγ all failed to affect larval growth. Antibody to peritrophic membrane antigens (PM) was an exception, inducing dose dependent inhibition of larval growth. Protease inhibitors in plasma inhibit growth of larvae *in vitro* (Bowles *et al.*, 1990), and anti-inflammatory drugs retard larval growth (O'Sullivan *et al.*, 1984). These findings suggest that, except in the presence of specific anti-PM antibody, the promotion of larval growth by nutrients is greater than the concomitant inhibition induced by protease inhibitors present in the inflammatory exudate (O'Meara *et al.*, 1995). In accord with the finding in the Trangie selection lines noted above, IgE+ cells are more prevalent in skin of sheep with no history of fleece rot than in sheep with a record of fleece rot (Colditz *et al.*, 1994).

We recently examined factors contributing to the break in wool fibres that accompanies strike. Blowfly strike was induced in a group of five 18 month old Merino wethers by application of approximately 500 freshly hatched larvae per day for 8 days to an abraded skin site on the flank of each sheep. Daily feed intakes were recorded and pair-fed sheep were offered the quantity of feed consumed by their pair on the preceding day. A third group of uninfested controls was fed *ad libitum* for the duration of the experiment. Severe strike was induced in four of the infested sheep with one sheep exhibiting only mild strike. Average daily gain was significantly lower in infested sheep than control sheep during week one of infestation. Rectal temperature was elevated in infested sheep from the first day of infestation until three days after the last application of infective larvae, which coincided with the treatment of all sheep with the organophosphate Diazinon to terminate infestations. Plasma cortisol levels were elevated in infested sheep from day 2 to day 6 of infestation. The cytokines IL-1β, IL-6, IL-8 and TNFα were assayed in plasma. IL-6 was elevated from day 2 to day 6 of infestation, whereas no significant differences were observed between treatment groups for concentrations of the other cytokines. Longitudinal growth of wool fibres did not differ between groups, though there was a trend towards less growth in struck and pair-fed sheep than in controls. Staple strength was significantly lower in struck sheep than in control and pair-fed sheep, but did not differ between sites near to and distant from the strike lesion. Taken together these findings suggest that the host response to infestation has systemic consequences that lead to reduced fibre strength throughout the fleece, and that the reduced feed intake accompanying blowfly strike is not primarily responsible for reduced fibre strength. Cortisol and IL-6 are implicated as systemic mediators that contribute to reduced fibre strength, however, the interdependence, pleiotropism and redundancy of mediators associated with stress and inflammatory responses make it unlikely that a single mediator will be identified as accountable for the effect of blowfly strike on fibre strength. Rather a temporary resetting by pro-inflammatory mediators of the priorities for nutrient utilisation is likely to occur during flystrike.

The findings of this study illustrate the potential importance of the acute phase response in the fibre break that accompanies fly strike. The acute phase response is a host defence response that mobilises nutrients for enhanced activity of innate and adapted immune systems in the face of tissue damage and disease challenge (reviewed by Colditz, 2001). The major endogenous messengers activating the acute phase response are the pro inflammatory cytokines, IL-1β, IL-6, TNFα and IFNα/β. These messengers induce synthesis by the liver of acute phase proteins (e.g. serum amyloid A, C-reactive protein). These cytokines also provide afferent inputs to the central nervous system where they provoke changes in behaviour, thermoregulation, cognition and endocrine regulation.

Important changes in utilisation of amino acids accompany leucocyte proliferation and the acute phase response. Leucocytes have a particularly high requirement for glutamine (Wilmore and Shabert, 1998), cysteine (Malmezat *et al.*, 1998) and polyamines (Grimble and Grimble, 1998). Glutamine consumption by leucocytes exceeds requirements for energy production and is also increased two to three fold in lymphocytes during the intense cellular activity that follows stimulation with antigens (Pond and Newsholme, 1999). It has been postulated that the high requirement for glutamine may be to provide substrate for purine and pyrimidine nucleotides during periods of leucocyte proliferation (Pond and Newsholme, 1999). Enterocytes, as do leucocytes, utilise glutamine as an energy source, and the gut mucosa, which is heavily populated with leucocytes, utilises much of the glutamine provided by the diet (Pond and Newsholme, 1999). Almost all cells possess the enzyme glutamine synthetase, however, few tissues export glutamine (Wilmore and Shabert, 1998). Interestingly, muscle has a high capacity for synthesis of glutamine and may provide a reservoir, which is drawn upon by the pro-inflammatory cytokines, particularly TNFα for fuelling leucocyte proliferation during periods of intense antigenic challenge (Wilmore and Shabert, 1998; Pond and Newsholme, 1999). Glucocorticoids (e.g. cortisol during fly strike) also stimulate production and release of glutamine from muscle, and around 25% of the amino acid efflux from muscle in a catabolic state is glutamine (Wilmore and Shabert, 1998).

In common with leucocytes and enterocytes, the germinal layer of the wool follicle has a high requirement for glutamine and cysteine (Hynd, 2000). The changes in energy metabolism and glutamine and cysteine availability, and the decreases in insulin-like growth factor-1 and zinc that accompany the acute phase response may contribute to fibre weakness (Hynd, 2000) seen following internal parasitism and blow fly strike. This suggestion is supported by the observation that sheep selected for high fleece weight have increased susceptibility to internal parasitism (Morris *et al.*, 2000). Competition between the wool follicle and the immune system for sulphur amino acids and polyamines may contribute to susceptibility to parasites as cysteine supplementation of the diet reduces such susceptibility in the high fleece weight selection line (Miller *et al.*, 1998). The binding of copper to ceruloplasmin which increases in serum during the acute phase response may limit the availability of copper for tyrosinase activity in the wool follicle and thus also contribute to impaired wool fibre strength due to poor keratinization (Hynd, 2000).

Acquired immunity in unselected sheep

There have been several studies on the acquisition of immunity following repeated experimental infestation of sheep. Sheep rapidly produce antibodies to larval antigens (O'Donnell *et al.*, 1980; Sandeman *et al.*, 1985; Eisemann *et al*., 1990; Seaton *et al.*, 1992) and there are dramatic changes in leucocyte populations at the site of infestation (Bowles *et al.*, 1992) and in draining lymph (Bowles *et al.*, 1994). There is, however, very limited effect of such sensitisation from experimental infestations on survival and growth of larvae during subsequent challenge. It is noteworthy that larvae exert an immunosuppressive effect on the host, which has been attributed, in part, to excretion of ammonia (Guerrini, 1998). While lymphocyte depletion in the Trangie selection lines failed to affect larval establishment and growth, the effect of immunosuppressive treatments on growth of larvae in unselected sheep has not been examined. An infiltrate of CD4+, WC1+ and CD1+ leucocytes is seen at sites of infestation following immunisation with larval antigens (Bowles *et al.*, 1996). However, leucocyte depletion studies to determine the contribution of this infiltrate to reduced larval survival in immunised sheep have not been performed. Thus the role of cellular responses in resistance to blowfly strike remains an open question.

Lessons from breeding for resistance to other diseases

Analysis of results from 12 sheep and three cattle experiments on responses to selection for disease resistance yielded an average heritability of disease resistance of 0.28 (Morris, 1998). This figure is of similar magnitude to heritability of production traits such as milk yield, body weight or fleece weight. Currently, breeding for resistance to disease requires that candidates for selection, or their relatives, are exposed to the disease-causing agent so that levels of resistance can be compared (Woolaston and Baker,

1996). Natural or artificial challenge of sheep with internal parasites has been highly successful in revealing variation in faecal egg counts. Experiments on selection for this trait in several countries have led to the establishment of sheep with resistance or susceptibility to the economically important species of internal parasites (Windon *et al*., 1987; Woolaston and Piper, 1996; Bisset *et al*., 1996). Physiological traits that can predict resistance while animals are free from internal parasitism have proved to be elusive, while genetic markers of resistance have yet to be identified. Nonetheless, molecular genetic techniques offer promise that genetic markers will be found.

To date, sheep have not been examined in a suitably designed experiment for genetic variation in survival and growth of blow fly larvae following artificial challenge. This question deserves attention and has been proposed as a project within the Australian Sheep Industry CRC. Applying contemporary molecular genetic techniques such as gene expression arrays to sheep with variability in resistance to fleece rot or larval survival and growth may lead to identification of gene markers for resistance.

The transgenic approach to resistance

As an alternative to selection for resistance from within the natural gene pool of a population, the transfer of genes encoding a protein with inherent disease resistance properties has been explored by Ward *et al*. (1993) as a path to establishment of sheep with resistance to fly strike. Tobacco chitinases that are larvicidal have been investigated *in vitro* in the search for a form that is sufficiently potent to be considered a candidate for insertion into transgenic animals. A suitably potent chitinase has not been identified (Ward, 2000), however, the principle of conferring disease resistance by transgenesis has recently been demonstrated in transgenic mice that express the bactericidal product lysostaphin in milk. Resistance to experimental mastitis caused by *Staphylococcus aureus* occurred in mice expressing the transgene (Kerr *et al*., 2001). As biotechnologies mature, methods such as gene therapy (introduction of foreign DNA into differentiated somatic cells) or genetic modification of skin commensal bacteria (Lyness *et al*., 1994; Moore, *these proceedings*) will be devised for conferring resistance by inducing expression on, or by, skin of environmentally-safe, biologically-active molecules with selective toxicity for blow fly larvae. Many molecules with larvicidal activity are known and could be candidates for such approaches.

Conclusions: Implications for selection strategies

Salient features of the research described above are:

- High heritability of resistance to fleece rot and flystrike implies potential for genetic progress but the lack of gradation in resistance limit responses that can be achieved through direct selection.
- Sporadic prevalence of fly strike due to climatic variability necessitates indirect selection.
- High genetic correlation of fleece rot with bodystrike (>0.9; Raadsma, 2000) means selection on basis of fleece rot is feasible.
- However, climatic variability also leads to a sporadic occurrence of fleece rot so identification of further indicator traits is desirable to aid selection when fleece rot disease prevalence is low.
- Skin test response to intradermal injection of larval products, which has a co-heritability with fleece rot of 0.25 (Raadsma 2000), provides one option for indirect selection.
- Mechanistic studies suggest resistance in Trangie selection lines is due to characteristics affecting fleece rot susceptibility rather than survival and growth of larvae.
- Variation may occur in magnitude of the acute phase response once sheep are struck and this variability may manifest as resilience, however seeking animals with resilience to the disease complex would seem to be an unrealistic goal. Identifying up-stream mechanisms of resistance either pragmatically by molecular genetic technologies or intuitively by proteomic analyses would seem more likely to yield tools for selection.
- Whether genetic variation occurs in survival and growth of blow fly larvae remains an unanswered question. Research within the Australian Sheep Industry CRC will investigate this issue with the potential to lead, via analysis of gene expression arrays, to genetic markers for this trait.

- The ethical implications of strategies for controlling body strike need to be considered. Selecting sheep for resistance is likely to have few ethical implications although inducing disease expression, say via wetting sheds, which could be considered for seed stock, would raise some ethical issues. From both an ethical and a practical viewpoint, molecular genetic markers of resistance that can be identified while animals are free from disease are highly desirable. Chemical and transgenic strategies raise many ethical issues.

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