Acid red 1 dye absorption by wool found to not be heritable for wool pre-treated with a chlorination process

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Declaration

This thesis has been composed by myself and has not been accepted in any previous application for a degree. The work, of which this is a record, has been done by myself and all sources of information have been cited.

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Introduction

The major determinants of wool value are generally related to the physical properties of wool such as fibre diameter, clean fleece weight, staple strength and staple length. However, the chemical properties and their subsequent effects for processing have not been considered to any great extent other than wool colour (Schlink *et al.* 2006). Dyeing of wool is considered to be one of the most important practices in the wool processing sector. As wool is a heterogeneous fibre, uptake of dye and rate of dye diffusion across the fibre surface occurs at an uneven rate. The ability for a fleece to absorb dye evenly is of importance as wool with a lower propensity to be evenly dyed has limited use in the wool industry, especially for the worsted wool system. This narrows the range of colours that such wool could potentially be dyed. A dye which is useful in determining fibre damage and is frequently used to dye textiles is the anionic acid red 1 dye. It is likely that there is variation in composition with regards to the components which hinder dye absorption. Previous studies carried out by (Dowling *et al.* 2006; Schlink *et al.* 2006) have found that wool dyeability of acid red 1 dye was heritable suggesting that there was genetic variation linking biological phenotypic variation of the wool to potential of the wool of absorbing dye. The genetic variation suggested was based on the 'F' cuticle layer which is known to confer hydrophobicity as well as a barrier to absorption of dye molecules (Medley 1959; Leeder and Rippon 1985; Molina *et al.* 2005; Schlink *et al.* 2006). However, these previous studies focused on the absorption of dye prior to a common commercial process carried out prior to dyeing; shrink proofing. As shrink proofing partially removes and modifies the surface properties of wool fibres, it is expected that the evenness and rate of dye uptake will increase due to the disruption or removal of barriers to dye absorption.

In this review, we will first explore the general overview of the wool industry in Australia. The next section will be regarding structure of wool and how wool structure varies genetically, therefore having differential effects on dyeing. Following will be with regards to shrink proofing and in particular, how the chlorine Hercosett process achieves anti-felting and possible effects on dyeability of wool. Finally, the review will examine previous similar studies which were carried out based on the heritability of dye absorption, and then concluding with the proposed hypothesis of the experiment.

The Wool Industry

The Australian climate and environmental conditions is well suited for sheep production, thus wool production is one of Australia's most important forms of land use. Although the Australian wool industry is only a fraction of what it used to be, Australia is still the largest wool producing country in the world, producing an estimate of 341 million kilograms of greasy wool in the 2013/14 season of which 90% is exported to other countries, primarily to China and Italy (AWI 2014). The estimated gross value for this amount was valued at $2.5 billion (ABS 2015). However, wool production has been declining in Australia as well as the rest of the world over the past couple of decades due to the decreasing demand for wool, changes in lifestyle, and increased competition from other quality synthetic fibres over the decades. Sheep flock numbers in Australia have declined from an opening number of 170 million in 1990 to 73.1 million at the start of 2011 (AWI 2013). On top of being a natural, renewable, biodegradable and sustainable fibre, wool serves as an extra source of income for farmers and it is therefore important to ensure its competitiveness to increase the demand and thus profitability of a sheep enterprise.

Structure of Wool

Wool is a heterogeneous fibre, and the composition of the fibre is highly variable within as well as between sheep. Wool is estimated to contain 170 different types of polypeptide molecules which are not uniformly distributed throughout the fibre (Lewis and Rippon 2013). The structure of a wool fibre is composed of an aggregation of external cuticle and internal cortical cells held collectively by the cell membrane complex (Meritxell *et al.* 2011). A third kind of cell may be present in coarse fibres called the medulla.

Figure 1: Schematic diagram of a wool cuticle (Feldtman *et al.* 1983)

The external overlapping cuticle cells are comprised of two different layers; the exo and endocuticle layers (Fig. 1). Every single cuticle cell is surrounded by a thin membrane known as the epicuticle; part of a cuticle cell resistant membrane that is located on the fibre surface approximately 3-6nm thick representing 0.1% of the total fibre mass (Rippon 1992, Meritxell *et al.* 2011; Lewis and Rippon 2013). The hydrophobic nature of clean untreated wool is due to a lipid layer that is covalently bound to the surface of the epicuticle. This lipid component was dubbed as the 'F-layer' by Leeder and Rippon (1985). This layer will therefore affect the uptake of any water based molecules such as dyes with hydrophilic properties.

The cortical cells make up the remaining 90% of the fibre, consisting of two main types of cortical cells; para and ortho-cortical cells. The ortho and para-cortical cells differ in chemical proportion and composition of the intermediate filament/matrix system within each macrofibril (Lewis and Rippon 2013). The different proportion and composition of cortical cells were initially thought to be responsible for crimp in wool, however recent studies have found that this was not the case and curvature is the result of differential cell division in the follicle bulb (Hynd, *et al*. 2009). The cell membrane complex accounts for approximately 3.5% of the fibre at around 25nm wide (Bradbury 1973, Meritxell *et al.* 2011). The cell membrane complex serve to provide adhesion between cells. The cell membrane complex is comprised of three major components; an intercellular cement, a lipid component and a chemical resistant proteinaceous membrane (Meritxell *et al.* 2011). These components of the cell membrane complex serve as the dye diffusion pathway throughout the cell.

Genetic variation in wool traits

The price received for a sale lot of wool is based on the characteristics of wool present in the specific sale lot. These wool characteristics are the outcome of both genetics and environment influence of the sheep. The genetic parameters for any given trait, includes the heritability; proportion of the variation of a trait which is passed onto the offspring, as well as genetic correlations; how one trait is genetically related to another (MLA, 2009). These parameters are used to estimate Australian Sheep Breeding Values (ASBVs) that is made available

through Lambplan and Merinoselect . ASBVs give the best indication of an animal's true breeding value based on pedigree and performance (MLA, 2009). ASBV's are used to predict how an individual's progeny will perform for a range of traits and this can be used to shift the genetic pool of a given flock towards a farmer's breeding objective.

Genetic variation in fibre composition

Wool is a heterogeneous fibre, where variation in fibre composition not only occurs between fibres from different sheep, but also between fibres within an individual sheep as well. Protein composition is affected by various factors including genetics, nutritional status as well as physiological state of the animal. The two major proteins which the wool fibre is composed of are the keratin intermediate filament proteins (KRTs) and keratin intermediate filament associated proteins (KAPs) (Itenge *et al.* 2011). KRTs form the microfibrils which provide a skeletal framework, and are enclosed within a KAP matrix (Itenge *et al.* 2011). Rogers *et al.* (1994); Beh *et al.* (2001) and Flanagan *et al.* (2002) all described variation within both KAP and KRT genes, therefore affecting expression of the protein and thus final composition of the wool fibre. Millington *et al*. (2011) states that trace elements strongly bind to keratin proteins and in turn may influence wool colour and dyeability. It is likely that differences in fibre composition due to genetic variance affects the physical and chemical routes of dye absorption and diffusion, suggesting dye absorption can be influenced on a genetic level.

Effect of fibre structure in wool dyeing

Dyeing of wool is often carried out by one of the most popular dyeing techniques; exhaust dyeing. This process is carried out with the wool material in contact with dye liquor, and the fibres absorb the dyes such that the concentration of dye in the dye bath decreases over time. As a result, the dyeing operation proceeds in several stages. Firstly, the diffusion of dye through the aqueous dyebath to the fibre surface, followed by the transfer of dye across the fibre surface and finally diffusion of dye from the surface throughout the whole fibre (Holmes

et al. 1956; Rippon 1992). To produce an acceptable even shade and degree of colour fastness, complete penetration of dye into the fibre is required. For fibres that are assumed to be cylindrically uniform, Holmes *et al.* (1956) as well as Medley and Andrews (1959) suggested that Fick's laws of diffusion dictates that graphing dye uptake against square root of time should be linear over most of the dyeing curve. However, for wool, linearity of the dyeing curve is achieved after an initial concave of the dyeing curve (Holmes *et al.* 1956; Medley and Andrews 1959; Musnickas *et al*. 2005). Medley and Andrews (1959) suggested that there is a barrier with small capacity for dye existing at the fibre surface. The concept of a surface barrier to the entry of dye into the fibre is supported by studies showing that treatment with an alkaline reagent; potassium tert-butoxide, removes or modifies the surface properties such that rate of dye uptake by the fibre dramatically increases (Medley and Andrews 1959; Leeder *et al*. 1985; Rippon and Leeder 1986). Consequently, a process such as shrink proofing which partially removes lipid layers off the surface of the fibre will logically lead to an increase in rate of dye uptake.

Shrink proofing

Mechanism of Felting Shrinkage in wool

Shrink proofing processes are the most common chemical treatments aside from dyeing that is carried out on wool in order to prevent felting or shrinkage of wool fabrics. Under warm alkaline conditions, the cuticle cells rise and thus increase exposure to their neighbouring fibres and these cuticle cells are the primary cause of felting through entanglement of individual fibres in untreated wool fabrics causing shrinkage. Without any preventative treatment, woven and knitted wool products under conditions of warm, moist conditions with mechanical disturbance will shrink due to the interlocking of fibres through a directional frictional effect (Holt 1975; Udakhe *et al.* 2011). Directional frictional effects are achieved according to the direction in which a fibre is pulled over another surface or in this case, other fibres (Udakhe *et al.* 2011). Given that two separate fibres have the same root and tip orientation, there is little chance of interlocking, as friction is at a minimum when there is movement in either direction as in wool on the sheep (Fig. 2). On the other hand, if the fibres are lying in opposite orientations, directional friction between fibres will be at a maximum resulting in fibres wedging with each other after processing resulting in shrinkage and the tendency to felt (Fig. 2). During machine washing of untreated wool fabrics, the mechanical

agitation in warm, alkaline and moist conditions is inevitable, resulting in fibre interlocking and thus felt shrinkage of wool, which is irreversible (Dobozy 1958). As a result, there is demand for easy care, machine washable wool that will not shrink or felt.

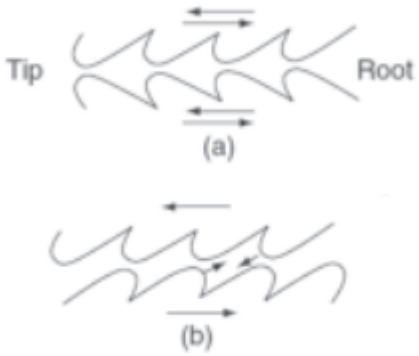

Figure 2: Differential friction in wool: (a) between fibres lying in same direction; (b) between fibres lying in opposite direction (Udakhe *et al.* 2011)

Processes of Shrink Proofing

There are a diverse range of methods available to produce anti-felting and shrink resistance in wool. Shrink resistance processes which are commercially practiced can be classified into oxidative process or resin process. Oxidative processes achieve anti-felting by reducing the rough outer surface layer, either by partial or complete removal of the cuticle scales through chemical treatment (Udakhe *et al.* 2011). To the contrary, resin processes prevent shrinkage by coating the cuticle scales with a polymer, thus preventing the entanglement and wedging of the scales (Udakhe *et al.* 2011). The most commonly used method for shrink proofing is the chlorine Hercosett process, which utilises both the oxidative and resin process to provide a cost efficient practice for conferring anti-felt properties to wool fabrics.

Chlorine Hercosett Process

The chlorine Hercosett process is well recognised as the first commercially viable polymer process for the treatment of wool tops. It has had the most success for producing a soft handle, easy care, machine-washable non-shrinking wool. Although the chlorine Hercosett process produces a very successful commercial product, the environmental impact of this process is increasingly of concern to regulators and consumers. The use of high levels of chlorine leads to sizeable concentrations of organic halogen compounds (AOX) produced in

the chlorination step, and is found in waste effluent ranging from 80 – 100 mg/l Cl (Bechtold *et al.* 2012). These levels are particularly of concern to European processing companies as a European Union has a legislation which restricts the acceptable concentration of AOX in discharge effluents to 0.5mg/l Cl (El-Sayed *et al.* 2001). Despite the various alternatives under research and development for an AOX free shrink resistant process, the chlorine Hercosett process is still currently the most cost beneficial and commercially viable shrink proofing process for wool (El-Sayed *et al.* 2001, Udakhe *et al.* 2011).

The continuous chlorine Hercosett process has several stages applied sequentially to wool tops and slivers which seeks to modify wool fibre surfaces to allow polymer adhesion as well as oxidising cuticle cells to achieve anti-felting (Udakhe *et al.* 2011). The first stage involves acid chlorination, typically utilising a solution of sodium hypochlorite (NaClO) at a temperature of 15-20°C at a pH of 1.5-2.0 (AmtexYarns 2015). This chlorination pre-treatment should be uniform through the cross section, width and length of wool, to ensure conformity of treatment (AmtexYarns 2015). The acid chlorination is followed by neutralisation and anti-chlorination which involves sodium carbonate and sodium sulphite. The function of the sodium carbonate and sodium sulphite combination considerably increases the absorption of resin in later stages, suggesting that the affinity of the resin is greatest when sodium bisulphate is utilised on the chlorinated wool (Brooks 1985). Rinsing after neutralisation is required in order to avoid the contamination of sulphite in the next phase of the application of resin (AmtexYarns 2015). Contamination of sulphite impedes the rate and extent of exhaustion of resin onto the surface of wool fibres associated with the conversion of cationic sites in the resin to anionic sites (Cockett *et al.* 1978, Benisek and Craven 1980). This might not only result in insufficient shrink proofing of wool, but results in differential dyeing effects as well (Cockett *et al.* 1978). These first three stages are collectively known as the “pre-treatment”, prior to the application of the resin. The objective of the pre-treatment is to establish charged sites on the surface of fibres in which oppositely charged resin molecules are attracted to, as well as assist the spreading of resin by raising the surface tension of the fibre surface to a value higher than that of the resin (Benisek and Craven 1980; AmtexYarns 2015).

One of the most important stages with regards to conferring shrink resistant properties to wool is the resin application phase. The most widespread used resin in the chlorine Hercosett process is usually Hercosett 57, a water soluble, cationic polyamide epichlorhydrin resin (De

la Maza, Parra *et al.* 1989). Although this resin application stage is critical to conferring shrink proofing properties, without the pre-treatments, shrink resistance is not imparted as the resin doesn't spread to form a film over the fibre surface, but instead polymerises into individual globules (Tester and Makinson 1982). Following resin application, is the application of softener. This phase is to transmit a softening effect as well as to remove an excess unbonded resin which might result in fibre to fibre bonding in the final drying phase. The wool then reaches its final stage of the chlorine Hercosett process which involves drying of the treated wool.

Genetic parameters for dyeability

There have been few studies which have quantified heratibility of dyeability. Schlink *et al.* (2006) carried out a study looking at the inheritance of acid red 1 dye absorption of non-shrink proofed wool and its genetic relation to other merino wool traits. This experiment proposed that there is extensive natural variation between sheep in the cuticle 'F' layer as measured by acid red 1 absorption, and sought to estimate the heritability of acid red 1 absorption and determine its phenotypic and genetic correlations to other wool traits (Schlink *et al.* 2006). The experiment was carried out on fully pedigreed Merino Resource flocks where mid side wool samples from 1824 progeny from ninety sires of different merino strains were collected at hogget shearing and used for analysis (Schlink *et al.* 2006). Absorption was determined by a spectrophotometer and phenotypic variances, heritability estimates, phenotypic and genetic correlations were determined using ASREML, where age of dam, type of birth and group were fitted as fixed effects (Schlink *et al.* 2006). Absorption of the anionic acid red 1 dye was shown to be a heritable trait in merino wool with a heritability estimate of 0.47±0.07 (Schlink *et al.* 2006). This heritability estimate is comparable to that of staple strength, staple length, and fibre diameter. The value of 0.47±0.07 is considered high and therefore given that there is large enough variation in a flock, it will respond to selection pressure, allowing the breeding of fleeces on the basis of dye absorption. This ensures uniformity in dyeing performance of the dye (Schlink *et al.* 2006). The mean of acid red 1 absorption of 82.0% in this experiment was vastly different to another experiment by Schlink *et al*. (2005) that demonstrated a mean absorption of 36.5%. The difference between the two experiments was that the former utilised a solvent extraction procedure that removes residual wool wax, as opposed to a simple hand carded, aqueous scoured wool. The solvent extraction method removes residual wool wax which may hinder the capability of wool to absorb dye

molecules. However, it should be noted that this heritability study was carried out on wool that had yet to undergo any shrink resistant processes prior to the testing.

It is well acknowledged that the 'F' layer confers the hydrophobic property to the wool fibre that have considerable impact on the shrinkage properties as well as dyeability of wool fabrics (Medley 1959; Leeder and Rippon 1985; Molina *et al.* 2005; Schlink *et al.* 2006). The thin layer of fatty acids was shown to contribute and act as a barrier to entry of dye into the fibre in experiments that resulted in a dramatic increase of dye uptake when a severe potassium tert-butoxide treatment was used to fully remove the 'F' layer (Medley 1959; Leeder *et al.* 1985; Rippon and Leeder 1986; Lewis and Rippon 2013). These results are also supported by a more recent study by Naebe, *et al.* (2010) where the increase in dye uptake was attributed to the increase of effectiveness of ionic interactions between the proteins of the epicuticle with the dye molecules after removal of the hydrophobic 'F' layer by plasma treatment. As shrink proofing through the chlorine-Hercosett process partially oxidises the 'F' layer as well as allowing the adhesion of a polymer coat around the surface of the fibre, it is important to consider wool that have been subjected to the chlorine-Hercosett process when looking at heritability of dyeability.

Conclusion

With synthetics occupying the vast majority of the market, it is important to improve the competitiveness of wool, especially for coloured fabrics as dyeing is one of the most crucial finishing stages in the wool processing sector. Although there are no apparent studies looking at the variation in F-layer composition between sheep, genetic variation in structure and composition of the wool fibre is clearly evident and well known. Schlink *et al.* (2006) found absorption of acid red 1 dye to be heritable in pre-shrinkproofing wool, which implies that one would be able to capitalise on this genetic variation within a flock. The hypothesis is that there is significant genotypic variation in the absorption of acid red 1 dye between wool from progeny of different sires after undergoing a chlorination shrink proofing process. This would suggest that the potential for dye uptake is heritable even after shrink proofing.

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Abstract

Genetic variation in chemical composition of wool fibres and their correlating absorption of dye by different sheep is the basis of determining the potential for heritability of dye absorption. Optimising the ability for a fleece to absorb dye is of economic importance as wool with a lower propensity to be dyed has limited use in the wool industry. While the effect of a chlorination process on both the fatty acid layer as well as epicuticle is well documented,

little is known about how it affects the heritability of dye absorption. This study tested the hypothesis that there is significant genotypic variation in the absorption of acid red 1 dye between wool from progeny of different sires after undergoing a chlorination process, and as such, potential for dye uptake is heritable after chlorination. 1441 mid side wool samples from progeny of 84 different sires were tested for absorption of acid red 1 dye using a spectrophotometer read at 520nm following a chlorination process with dichloroisocyanurate. There appeared to be no significant differences in acid red 1 absorption after chlorination between progeny of different sires. The results rejected our hypothesis, suggesting that potential for dye absorption by wool after chlorination is unlikely to be heritable (0.035 ± 0.0387). These results imply that the genetic variation in dyeability can be found in a component of the wool, most likely lipids found at intercellular junctions of cuticles which act as a barrier to dye entry, which is removed or disrupted after chlorination.

Introduction

Dyeing of wool represents an economically important practice along the wool processing industry due to the costs associated with materials, capital, labour and time. As wool is a heterogeneous fibre, the uptake of dye and rate of dye diffusion across the fibre surface occurs at an uneven rate. Therefore optimising and ensuring the reproducibility of wool dyeing is of prominence to wool processors as wool with lower propensity to be dyed has a more narrow range of colours that it could be dyed along with requiring an increase in duration of dyeing (Schlink *et al*. 2006). Conventionally, physical properties of a fleece such as fibre diameter, clean fleece weight, staple strength and staple length are primarily the major determinants of wool value. However, the chemical composition and properties along with their subsequent effects on inhibiting dye penetration has not been considered to any great extent (Schlink *et al*. 2006). By producing a fibre more susceptible to dye uptake, we will be able to decrease costs associated with the dyeing process and increase profitability.

Anti-felting of woollen products is of importance to the wool industry with regards to demand for washability in comparison to other fibres. Without any treatment, wool products have a tendency to felt and shrink due to directional friction caused entanglement of cuticle cells between fibres in opposite orientations. As a result, shrink proofing processes are amongst most common chemical treatments aside from dyeing of wool that is carried out on wool in order to prevent felting or shrinkage of wool fabrics and to ensure wool to compete against other fibres. This importance is

more prominent with wool in the worsted process where wool ends up in woven apparel such as clothing where anti-felting is absolutely required. Currently, the most common and commercially viable process for the treatment of wool tops is the chlorine hercosett process. In addition to partial or complete removal of cuticle scales responsible for felting, chlorination is also known to chemically modify the surface of fibres.

According to Fick's laws of diffusion, the plot of uptake versus square root of time should be initially linear and remain so for most of the dyeing curve (Medley 1959). While many fibres follow this diffusion pattern, wool in contrast, does not. The diffusion curve for wool has an initial concave pattern, with the curve only becoming linear after some time (Zhao and Pailthorpe 1987). This resulted to an assumption that there is a barrier with a small capacity for dye is present on wool fibres.

The layer of fatty acids covalently bound to the surface of the epicuticle is known as the F-layer (Molina *et al.* 2005). Negri *et al.* (1992) and Jones and Rivett (1997) both established that the F-layer of the cuticle contains 18-methyl eicosanoic acid (18-MEA) as the major lipid component ranging from 58% to 65% of total fatty acids (Jocic *et al.* 2005). The 18-MEA fatty acid chains are oriented away from the fibre such that it produces a polyethylene like layer on the outer surface which makes the epicuticle hydrophobic and resistant to chemical agents. (Negri *et al*. 1993a, 1993b). It is this F-layer which confers hydrophobicity to keratin fibres.

Previous studies by (Dowling *et al.* 2006; Schlink *et al.* 2006) reported that without any pre-treatment, acid red 1 absorption to be heritable. Schlink *et al*. (2006) proposed that the genetic basis for differences between wools in dyeing performance derives from biological variation in the cuticle 'F' layer. The heritability estimate obtained for acid red 1 absorption without pre-treatment was similar to that obtained for staple strength, fibre diameter and staple length which are all traits that are known to be heritable and have been bred for in the past few decades with high success rates in increasing profitability. The heritability of this trait will therefore allow breeders to utilise genetic variation within the flock to breed for wool that is more susceptible to dye absorption, thus decreasing costs associated with the dyeing process while increasing profitability.

This study therefore tested the hypothesis that there is significant genetic variation in the absorption of acid red 1 dye between wool from progeny of different sires after undergoing a chlorination shrink proofing process. Given that there is significant genotypic variation, it would

suggest that the potential for dye uptake is heritable even after shrink proofing, and that the basis for differences between wools in acid red 1 absorption is attributable to more than just chemical composition differences that is not affected by chlorination.

Materials and Methods

Research site & experimental design

The research was performed on stored wool samples from fully pedigreed, Merino Resource flocks of the Department of Agriculture of Western Australia at Katanning (latitude 33° 41', longitude 117° 35'). Wool samples were derived from 1103, 184 and 154 progeny born between June and July of 2001, 2002, and 2003 respectively. The total 1441 progeny were produced from eighty four sires of different Merino strains. All progeny were reared under normal commercial conditions. Progeny were shorn in September as lambs and again at 15 months of age after 12 months of wool growth. Mid-side samples were collected at hogget shearing and were the samples stored and utilised for analysis.

Chlorination of wool samples

Chlorination prior to dyeing was carried out using a modification on the method used in Leeder and Rippon (1985) and Ito *et al.* (1994) on the clean wool samples after wax, suint and dust determinations. In brief, 0.15g of conditioned, clean scoured wool remaining after wax, suint and dust extraction was placed into a 25ml vial with 15ml of 5% weight on volume (w/v) of 99.925% pure sodium dichloroisocyanurate (DCCA). The sample was then vigorously shaken and left on a dry rotary shaker for 60 minutes.

Anti-chlorination

DCCA was removed from the sample after 60 mins. 10ml of 3% w/v of 98.5% pure sodium thiosulphate was added to the vial, vigorously shaken and removed after 30 minutes. The wool sample and vial was then rinsed with deionised water and excess water was blotted out. 10ml of 20g/L of 100% pure disodium tetraborate decahydrate (Borax) was added to each sample and left to soak overnight (Leeder and Rippon 1985). The following day, the excess borax was poured off and the sample was washed in deionised water afterwhich excess water in the wool was blotted out again.

Absorption of acid red 1 dye

To each sample was measured on a modification of the method of Leeder and Rippon (1985), 10ml of 0.15g/L acid red (60% dye content) in 0.005M hydrochloric acid was added, then vigorously shaken such that the wool was completely saturated. Samples were then left on a rotary shaker to be mixed for 1hr. Each wool sample was removed from the vial, and 0.25ml of the dye was mixed with deionised water at a 1:4 ratio in a 3 ml cuvette. This mixed solution was utilised to determine absorption of acid red 1 by measuring absorbance of light on the UVmini-1240 UV-VIS spectrophotometer at 520nm. A control of 10ml of 0.15g/L acid red (60% dye content) in 0.005M HCl without any wool and mixed with deionised water at a 1:4 ratio was used. Percentage of absorption was calculated using the following equation

$$\text{Acid Red 1 ABS (\%)} = \frac{\text{Avg Control ABS} - \text{Sample ABS}}{\text{Control ABS}} \text{ x } 100$$

Statistical analysis

All statistical analysis determining phenotypic variances, and heritability estimates were performed using ASREML (Gilmour *et al.* 2002). A linear mixed animal model was used to estimate the genetic variation between animals. Dam age, type of birth (single, double or triple), birth year and sex of the progeny were fitted as fixed effects, while animal was fitted as a random effect. As males and females were managed separately, sex was confounded with group. For this analysis, interaction terms between fixed effects were only included if they were statistically significant ($p<0.05$). Interaction terms included all possible two way combinations of dam age, birth type (single, twin or triplets), sex, birth year (Table 1). The data was log-transformed in order to normalise the distribution of the data. 159 data entries were removed due to obtaining invalid negative absorption percentages after calculation that would have otherwise affected the statistical analysis. Any obvious outliers were removed from the analysis as well.

Results

For the animal model used in the analysis, the non-significant interactions, followed by the non-significant fixed effects were removed one at a time until only the remaining fixed effects were all statistically significant (P<0.05) (Table 1). Table 1 shows that sex is the only main effect that was found to be statistically significant (p value = 0.016). The one significant

Table 1. Statistical significance of various fixed effects on our linear mixed animal model (left- including non-significant effects, right – only significant effects remaining)

Type III Tests of Fixed Effects[a]

Source	Numerator df	Denominator df	F	Sig.
Intercept	1	1182.000	401.378	.000
Sex	1	1182.000	1.938	.164
Byr	2	1182.000	1.083	.339
DamAge	7	1182.000	.744	.635
BirthType	1	1182	1.103	.294
Sex * Byr	2	1182	6.220	.002
Sex * DamAge	7	1182	.171	.991
Sex * BirthType	1	1182	2.887	.090
Byr * DamAge	14	1182	1.078	.374
Byr * BirthType	2	1182	.228	.796
DamAge * BirthType	6	1182.000	.728	.627

a. Dependent Variable: LogABS.

Type III Tests of Fixed Effects[a]

Source	Numerator df	Denominator df	F	Sig.
Intercept	1	1220.000	1462.481	.000
Sex	1	1220.000	5.873	.016
Sex * Byr	4	1220	4.199	.002

a. Dependent Variable: LogABS.

interaction was between sex and birth year (p value = 0.002).

Total phenotypic variance (σ_p^2) was calculated by using the equation

$$\sigma_p^2 = \sigma_g^2 + \sigma_e^2$$

where σ_g^2 is the genotypic variance and $\sigma_e^2$is the environmental variance and heritability estimates were obtained using the equation

$$h^2 = \frac{\sigma_g^2}{\sigma_p^2}$$

where $\sigma_g^2$is the genotypic variance and σ_p^2 is the total phenotypic variance for each respective wool trait.

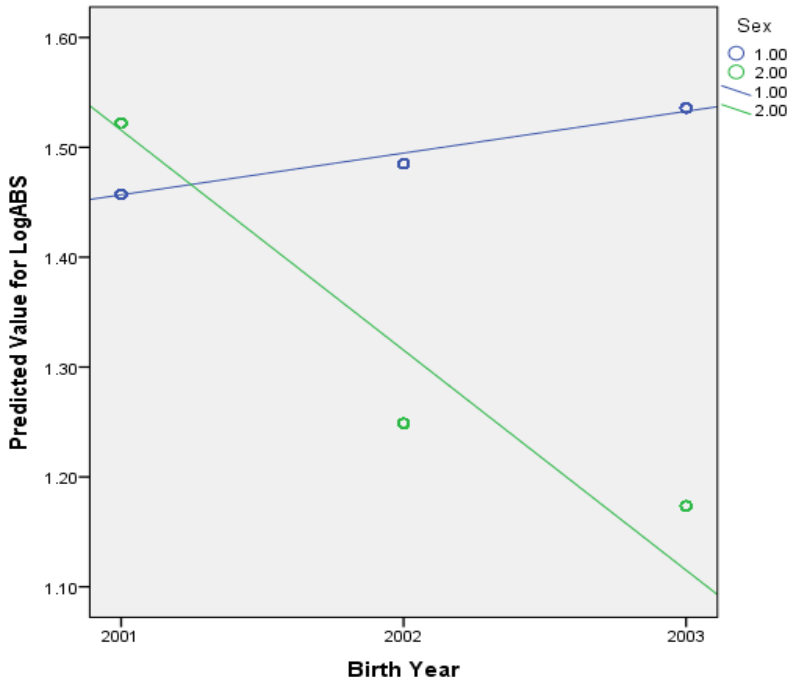

Figure 1: Scatterplot of predicted logABS against birth year for different sex (males, females)

Figure 1 displays the significant interaction term as indicated by the p-value of 0.041 for birth year and sex. The scatterplot was constructed using predicted values obtained from the mixed linear model and the data was back transformed from logABS. The corresponding coefficient for gradient for males (sex = 1) is 0.04 while the gradient for females (sex = 2) is -0.2 for figure 1. Absorption slightly increases for males as birth year ascends, while absorption appears to decrease as birth year ascends. Figure 2 shows the significant fixed effect, sex, and its relationship to absorption of acid red 1 dye. It appears that the distribution for log absorption for both sexes is similar.

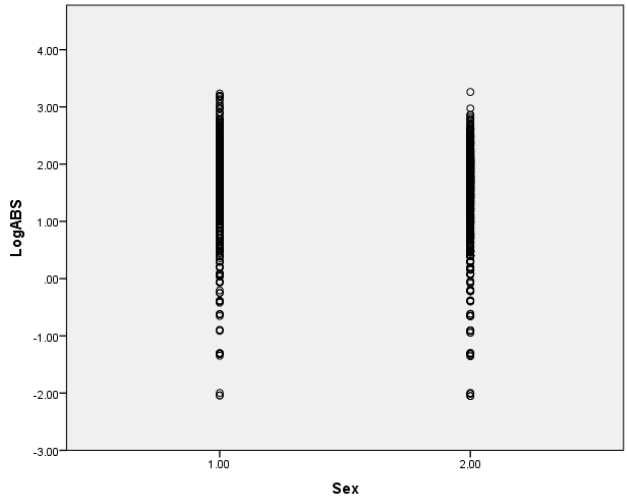

Figure 2: Boxplot showing relationship between LogABS and sex (1=male, 2=female)

Table 2. Mean values, coefficient of variation (CV), phenotypic variance (σ_p^2 ± s.e.) and heritability (h^2 ± s.e.) for Acid red 1 absorption after chlorination, and other wool traits

Wool Traits	Mean	CV	σ_p^2	h^2
Acid Red 1 absorption (%)	5.5304	69.9	1.3 ± 0.05	0.035 ± 0.04
Fibre diameter (µm)	19	7.8	2.2 ± 0.06	0.49 ± 0.04
Staple strength (N/ktex)	27.5	32.5	80.1 ± 2.04	0.38 ± 0.04
Staple length (mm)	98.4	9.2	82.5 ± 2.27	0.51 ± 0.04
Yield (%)	70	6.7	21.8 ± 0.61	0.63± 0.04
CVFD (%)	23.7	12.4	8.6 ± 0.24	0.62 ± 0.04

[A]CVFD, coefficient of variation of fibre diameter

The mean, CV, phenotypic variance and heritability estimations for the wool traits are shown in Table 2. Acid red 1 absorption after chlorination had a low heritability estimate of 0.035 ± 0.0387, highly dissimilar to heritability estimates obtained for acid 1 absorption of 0.45 ± 0.07 in Schlink *et al*. (2006). The coefficient of variation (CV) obtained for acid red 1 absorption after chlorination was 69.9%. Total phenotypic variance for acid red 1 absorption was 1.3 ± 0.05. By reversing the equation used for h^2, we obtain an estimate of 0.04696 for genotypic variance.

Discussion

Looking at the linear mixed animal model utilised in our statistical analysis, no main effects from our list of fixed factors were found to be significant in determining acid red 1 absorption after chlorination. Table 1 shows statistical output for our log transformed linear mixed model highlighting that the interaction between sex and birth year, and the main effect sex to be significant in determining the dependent variable; absorption of acid red 1 dye after chlorination. The corresponding p-values are 0.005 and 0.013 respectively (Table 1).

The significant interaction term between birth year and sex with a corresponding p-value of 0.002 appears to have a strong relationship in determining absorption. Table 3 shows log absorption to be statistically different for 4 out of 5 of the other possible parameter combinations to the reference parameter of females*birth year of 2003. The change in birth year may be due to the increase in length of storage, or may have been due to environmental effects which are unique to each specific year that the progeny were born in, and the differential effects the environmental effect such as nutrition has on separate genders with

regards to barrier to dye entry. It should be noted that storing and handling conditions may have been different for both sexes as sex as management/storage was confounded, thus it is difficult to determine the actual cause of the significance for this interaction variable. This may also be the reason for the significance in main effects of sex.

Table 3. Regression analysis output for significant interaction between sex*birth year

Parameter Estimates

Dependent Variable: LogABS

Parameter	B	Std. Error	t	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
Intercept	1.174	.110	10.707	.000	.959	1.389
[Sex=1.00] * [Byr=2001.00]	.284	.117	2.425	.015	.054	.513
[Sex=1.00] * [Byr=2002.00]	.311	.169	1.841	.066	-.020	.643
[Sex=1.00] * [Byr=2003.00]	.362	.149	2.435	.015	.070	.654
[Sex=2.00] * [Byr=2001.00]	.348	.115	3.018	.003	.122	.575
[Sex=2.00] * [Byr=2002.00]	.075	.137	.547	.585	-.194	.344
[Sex=2.00] * [Byr=2003.00]	0[a]	.	.	.	.	.

a. This parameter is set to zero because it is redundant.

The heritability estimate obtained for absorption of acid red 1 after chlorination was low (0.035 ± 0.0387) and not significantly different from zero and therefore unlikely to respond to selection pressure. In addition, the low phenotypic variance of 1.3 ± 0.05 suggests that high selection differentials can't be achieved in breeding programs, and that genetic progress for selecting this particular trait will be extremely slow. This result is vastly different to those wool traits which are known to be heritable such as fibre diameter (0.49 ± 0.04), staple strength (0.38 ± 0.04), staple length (0.51 ± 0.04), etc. Our heritability estimate for acid red 1 absorption is closer to heritability estimates obtained for reproductive traits such as fertility (number of ewes lambing per ewe joined) with average estimates 0.08 ± 0.01 from a study carried out by Safari *et al.* (2005), and 0.045 ± 0.005 in a similar study by Safari *et al.* (2007). Although fertility has a low and similar heritability estimate to acid red red 1 absorption, the benefits in profitability for reproductive performance in sheep far outweighs the benefits obtained from increasing dyeability.

However, in contrast, Schlink *et al.* (2006) reported that acid red 1 absorption was heritable without any pre-treatment effects, obtaining a heritability estimate of 0.45 ± 0.07 and a phenotypic variance of 130.6 ± 4.94. These estimates are highly dissimilar to those obtained for dyeing after chlorination. It is therefore plausible to suggest that the effects of chlorination dramatically lowers heritability of acid red 1 dye absorption by lowering genotypic variation.

This is likely due to the removal of a component which acts as a barrier for dye absorption, in which has varying effects on dye penetration in respect to genetic variation. It was initially proposed in Schlink *et al.* (2006) that there might be a biological basis for the observed variation in dye performance was attributable to the variation in chemical composition of the external fatty acid monolayer; the F-layer, found on the epicuticle of the wool fibres.

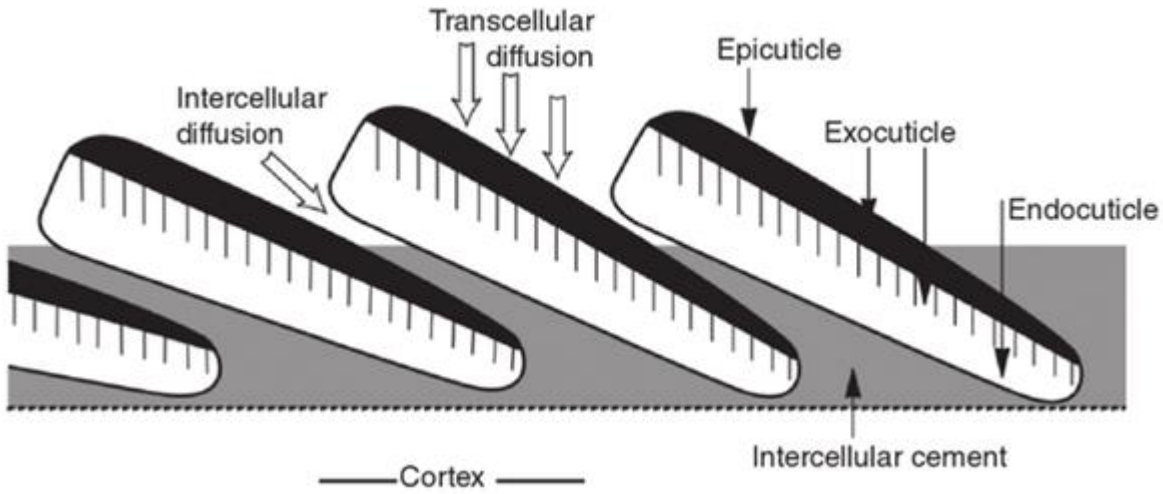

Figure 3: Diffusion pathways of dyes into wool (Simmonds 1955)

While acid red 1 absorption has been previously associated as a barrier to dye penetration, Leeder, Rippon and Rivett (1985) found that for undamaged wool fibres, transcellular diffusion of dyes did not occur in long liquor dyeing when the covalently bound F-layer was removed without any further modification of cuticle or cortical cells. This finding supports the hypothesis proposed in Leeder and Rippon (1985); Leeder, Rippon and Rivett (1985) that it is not the lipids of the F-layer, but rather the highly cross linked A layer which lies immediately beneath the epicuticle cell membrane, that acts as the barrier to transcellular dye diffusion instead. Therefore, the focus for barriers to transcellular diffusion should be ascribed to the A layer instead of the fatty acid F-layer.

The A-layer is not a continuous membrane, but instead surrounds individual cuticle cells of the wool fibre (Lewis and Rippon 2013). In unmodified wool, dyes find an easier route into the wool fibre. Figure 3 shows a schematic diagram of the gaps between cuticle cells where the intercellular material extends to the exterior of the fibre (Lewis and Rippon 2013). These

gaps between cuticle cells allow dyes to penetrate into the cortex of a wool fibre without diffusing through the cuticle cells (Fig. 3). Figure 4 shows the initial stages of dye diffusion, where fluorescent dye is shown to diffuse between cuticle cells where the main pathway of diffusion is intercellular rather than transcellularly for undamaged wool. This finding supports the observations of Millson and Turl (1950) where the rate of uptake of dye at edges of cuticle cells could be manipulated and increased by distorting the wool fibre as well as when wool fibres are extended (Koga *et al.* 1985). Hence, we should consider the barriers to dye penetration present at these positions on the fibre surface instead of solely the F-layer.

Figure 4: Light micrograph showing diffusion of a fluorescent dye at scale junctions (Lewis and Rippon 2013)

In addition, Leeder, Rippon and Rivett (1985) used specially synthesised nuclear dense heavy metal dyes to demonstrate the location of dye in the fibre throughout the dyeing process to be between cuticle cells. Consequently, it was widely recognised that lipids which are concentrated at the intercellular junctions between cuticle cells are the main forces which restrict dye penetration and intercellular diffusion of dyes into the non-keratinous regions of the cell membrane cortex for non-damaged wool (Joko *et al.* 1985; Leeder, Rippon, Rothery and Stapleton 1985; Forslind *et al.* 2005*).* Wools with surface damage will respond differently when exposed to the same dye conditions (Simpson and Crawshaw 2002). Depending on the type and intensity of treatment, modified wools may also show transcellular diffusion of dyes across the cuticle cells (Rippon and Evans 2012). Consequently, it is important to understand how the chlorination process affects both barriers to dye diffusion, the A-layer as well as lipids at intercellular junctions.

While both the A-layer and lipids found at intercellular junctions are both recognised as barriers to dye entry through different diffusion pathways, the chlorination process affects both barriers. The process of chlorination cleaves the thioester linkages between the A-layer of the exocuticle and the F-layer by oxidising cysteine (Leeder, Rippon and Rivett 1985; Thomas, 2007) resulting in the removal of around 60% of the surface lipids from the epicuticle F-layer (Negri *et al.* 1992). These effects increase the hydrophilicity of the fibre, which was found to increase the dyeing rate, but not affect the equilibrium dye uptake (Baritt and Elswor 1948). It was found that this result was due to the fact that chlorination increases uptake rate by promoting intercellular diffusion following removal of lipids at cell junctions rather than removal due to the removal of the F-layer and A-layer and initiating transcellular diffusion (Lewis and Rippon 2013). Transcellular diffusion was only shown to occur when the fibre went through a more severe chemical treatment or through the complete removal of the cuticle layer (Kopke *et al.* 1960; Hampton and Rattee 1979). This suggests that the variation in acid red 1 dye absorption prior to chlorination lies in the lipids found at cell junctions rather than an A layer barrier.

This study found that absorption of acid red 1 dye after chlorination has low genetic and phenotypic variances as well as a heritability estimate that is not significantly different from zero. This implies that this trait is likely to not respond to genetic selection. The outcome is thought to be due to the removal of a component which acts as a barrier to acid red 1 dye entry by chlorination. It is within this component that previous studies have identified variations in dye absorption. Further research is therefore required to identify this component which is likely to be lipids found at intercellular junctions, and how the variation in composition of this component affects dye penetration. Alternatively, new research could look at more modern methods of shrink proofing such as plasma treatments and whether there is genetic variation for increased absorption of dye after such treatments, thus being able to respond to selection pressure by being a heritable trait.

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