THE INHERITANCE AND CONTROL OF ISOLATED PIGMENTED WOOL FIBRES IN MERINO SHEEP

by

Malcolm Russell Fleet

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in

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ABSTRACT

THE INHERITANCE AND CONTROL OF ISOLATED PIGMENTED WOOL FIBRES IN MERINO SHEEP

This thesis provides an account of research on the occurrence and inheritance of isolated melanin pigmented wool fibres and macroscopic pigmentation in Merino sheep. Dark fibres in greasy wool cannot be reliably measured prior to sale and can limit the flexibility of end-use and result in financial loss by wool processors. In view of this limitation of wool, Merino breeders have traditionally selected against most types of pigmentation.

Following the literature review (Chapter 1), three experiments are presented in detail. The first experiment (Chapter 2) involved a private Merino flock in which pigmented leg fibres had increased. Hogget ewes without pigmented leg fibres had a distinctly lower incidence of isolated pigmented fibres (22 vs 1136 per kg of scoured staples) relative to sheep with this macroscopic pigmentation. Leg fibre pigmentation also involved greater amounts of other pigmentation and was highly repeatable (0.9) during adult life.

The second experiment (Chapter 3) involved a Merino resource flock at the Agricultural Centre, Trangie (NSW) and provided estimates of heritabilities and correlations for pigmentation traits and some production traits (Chapter 3). Most types of pigmentation had moderate or high heritabilities and were positively correlated with each other. Exclusion of sheep with macroscopic fibre pigmentation reduced the concentration of isolated pigmented fibres in the hogget wool clip from a mean of 231 per kg to as low as 15 per kg.

Pigmented halo-hair on the birthcoat had the highest correlations (r_p = 0.33 and r_g = 0.66 $\pm$ 0.19) with the concentration of isolated pigmented wool fibres and their heritabilities were 0.61 $\pm$ 0.16 and 0.18 $\pm$ 0.12, respectively. Even though the heritabilities and genetic correlation coefficients mainly had high standard errors, being based on a sample of 24 to 42 sires, the pigmentation parameters are the first values to be generated for Merinos.

The phenotypic correlations between the hogget production characters (clean fleece weight, average fibre diameter and off-shears body weight) were low (-0.7 to 0.13). However, the genetic correlations between pigmentation and clean fleece weight or body weight were generally positive (0.1 to 0.7) while those with average fibre diameter were generally negative (-0.1 to -0.5). The importance of these genetic trends on future generations arising from industry selection practices and in other Merino resource flocks requires further clarification.

The mode of inheritance of key indicators (pigmented leg fibres and pigmented birthcoat halo-hair) of isolated pigmented wool fibres was investigated at Turretfield Research Centre, Rosedale (SA) (Chapter 4). The segregation of phenotypes (presence vs absence) for leg fibre pigmentation was consistent with simple Mendelian inheritance though penetrance of the proposed allele was not complete. The data for pigmented birthcoat halo-hairs was inconsistent with the hypothesis for simple inheritance.

Also conducted at Turretfield, were other experiments (reported briefly) that increased understanding of the occurrence of isolated pigmented wool fibres (foetal development, distribution in the fleece, change with age), changes in macroscopic pigmentation with age and the relationship between measurements of pigmented fibres in raw wool and processed tops.

This thesis confirms that the occurrence of isolated pigmented fibres in hogget Merino fleeces is associated with the presence and degree of types of remnant macroscopic fibre pigmentation. The opportunity to exploit these associations to improve wool quality in relation to dark fibre risk is examined.

DECLARATION

This work contains no material which has been accepted for the award of any other degree or diploma in any university or other tertiary institution and that, to the best of my knowledge and belief, contains no material previously published or written by another person, except where a due reference has been made in the text.

I give consent to this copy of my thesis, when deposited with the University Library, being available for loan and copying.

Date: 5th March 1997

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ACKNOWLEDGMENTS

I express my sincere thanks for the guidance from my main University supervisors, Dr Philip Hynd and Dr Jim Gallagher, and to direction provided by Dr John Brooker. The opportunity to complete this post-graduate study in conjunction with employment by the South Australian Research and Development Institute (SARDI) and with projects financially supported by Australian wool growers, through the International Wool Secretariat, is gratefully acknowledged. Mr Rob Lewis and Dr Don Plowman of SARDI were very supportive of this post-graduate training.

My interest in pigmentation in sheep was initiated and facilitated by Mr C.H.S. (Scott) Dolling. Mr Roger Foulds conducted a parallel project on dark fibre metrology at CSIRO Division of Wool Technology (Ryde, N.S.W.) and provided assistance and guidance in this aspect of the work. For many of the aspects of data analysis I thank Dr Raul Ponzoni for providing direction.

I thank all the sheep breeders who showed an interest in the studies and especially those who provided access to their flocks. I greatly appreciate the access allowed to the Merino resource flocks and data at the Agricultural Research Centre, Trangie. In particular, I thank Dr Don Saville and Ian Rogan for facilitating that access, to Dr Sue Mortimer for provision of data and assistance with the analysis, and other staff who helped in the field. Anne Burbidge, Chris McInnes and John Crowley from CSIRO (Division of Wool Technology) assisted with the preparation and measurement of tops and Professor George Rogers and Dr John Forrest (University of Adelaide) organised the histological preparations.

The wool metrology conducted involved a major labour input and the efforts of Mrs Taherah Pourbeik, in particular, are gratefully acknowledged. Other technicians who assisted this task were Barry Lynch, Annette Mackie, Julie Absolum and Hussein Pourbeik. The field activities involving sheep flocks at SARDI's Turretfield Research Centre were largely assisted with commitment from Darryl Smith. Others involved in the field or laboratory were Greg Mattiske, Lindon Fullwood, Philip Ancell and Bob Lampe.

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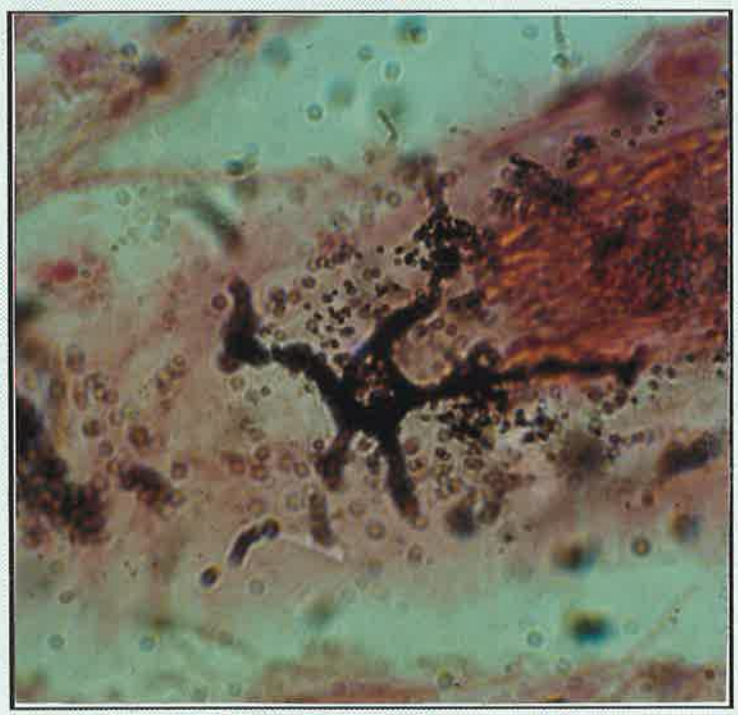
My greatest debt is to my family for their patience and support.

CHAPTER 1

LITERATURE REVIEW

THE BASIS AND CONTROL OF MELANIN PIGMENTATION

- IN SHEEP AND OTHER MAMMALS-



"Select whole flocks with fleeces soft and white. As for the ram, though dazzling white he may be, If but the tongue beneath the slimy palate is black, for fear the fleeces of your lambs Bear dusky spots; reject him: look around, there is ample room for choice".

Publius Verilius Maro (29 BC), from the third book of Vergil's Georgics (386-391), as translated from Latin by L.P. Wilkinson (1982).

CHAPTER 1

THE BASIS AND CONTROL OF MELANIN PIGMENTATION

- IN SHEEP AND OTHER MAMMALS -

1.1. PREAMBLE

This thesis was conceived with the discovery of isolated pigmented fibres in some Merino fleeces and the identification of significant phenotypic correlation coefficients with macroscopic pigmentation. There is a paucity of scientific information relating to pigmentation in white sheep and especially for Merino sheep despite the beliefs of Merino breeders that such pigmentation is undesirable.

Selection against pigmentation outside the fleece has been questioned by some scientists promoting emphasis on selection for traits of direct economic benefit. However, the realisation of the existence of isolated pigmented fibres in some Merino fleeces provided a new area of concern among Merino breeders; especially those breeders using performance recording without attention to macroscopic pigmentation outside the fleece.

Even though there is little literature about isolated pigmented wool fibres in white sheep, a wealth of other information relating to pigmentation in coloured sheep and other mammals is documented. It is expected that the principles involved in the basis and control of pigmentation in other mammals (e.g. mice) will be similar to those existing in sheep. This review provides a foundation for understanding the existing knowledge specific to the research on Merinos as well as general information on pigmentation in mammals.

1.2. EVOLUTION OF THE FLEECE

Through the course of evolution genes have arisen in mammals that produce coat colour variations conferring selective advantages in body temperature regulation or survival through camouflage, warning and sexuality (Owen 1980; Haliday 1980). At the cellular level the natural pigment (melanin) provides protection from the harmful effects of ultra-violet radiation (Pathak 1967).

The evolution of most domesticated sheep has involved artificial selection for changes in pigmentation and fibre structure of the coat. The domestication of sheep started perhaps 11000 years ago with capture and retention of wild sheep being hunted by primitive man. Once captured the destiny of sheep was shaped by human needs. As early as 8000 years ago the fleece of sheep was coloured, annually moulted, and consisted of coloured arrays of kemps and hairy fibres, combined with a fine undercoat. A modern example of such primitive sheep is preserved in a wild breed, named Mouflon, on the islands of Corsica and Sardinia that were settled in about 6000BC (Ryder 1987). Also on the island of St Kilda off North-West Scotland is the feral Soay breed that have either hairy or woolly fleeces and are thought to be similar to sheep that existed in the bronze-age (3000BC to 1500BC). Figure 1 shows present day examples of sheep in relation to the inferred end points of evolution of wool and hair fleeces (Ryder 1987).

The development of white animals among the varicoloured herds is referred to in the Holy Bible (Moses 1445-1405BC). Wool felt found by excavators at Catal Huyuk in Turkey dates to 6500BC and the remains of woven wool cloth found in a Denmark bog is dated to about 1500BC. A new stimulus for breeding sheep, in relation to coat colour,

arose around 1000BC in the Middle East with the development of dyeing. A dye industry for wool implies a demand for white wool and this is supported by textile artefacts from the first millennium BC in the Middle East (Ryder 1987). The problem of pigmented progeny in white flocks was referred to by the Roman poet, Virgil in 29BC (Wilkinson 1982). The translated text advised the selection of entirely white flocks and suggests that pigmentation of the tongue can be used as a guide to sheep that are likely to produce spotted lambs (see quotation on the title page of this Chapter). Such beliefs have persisted until recent times among the breeders of sheep (Graham 1870, Dry 1924, "Old Hand" 1953, Body et al. 1962).

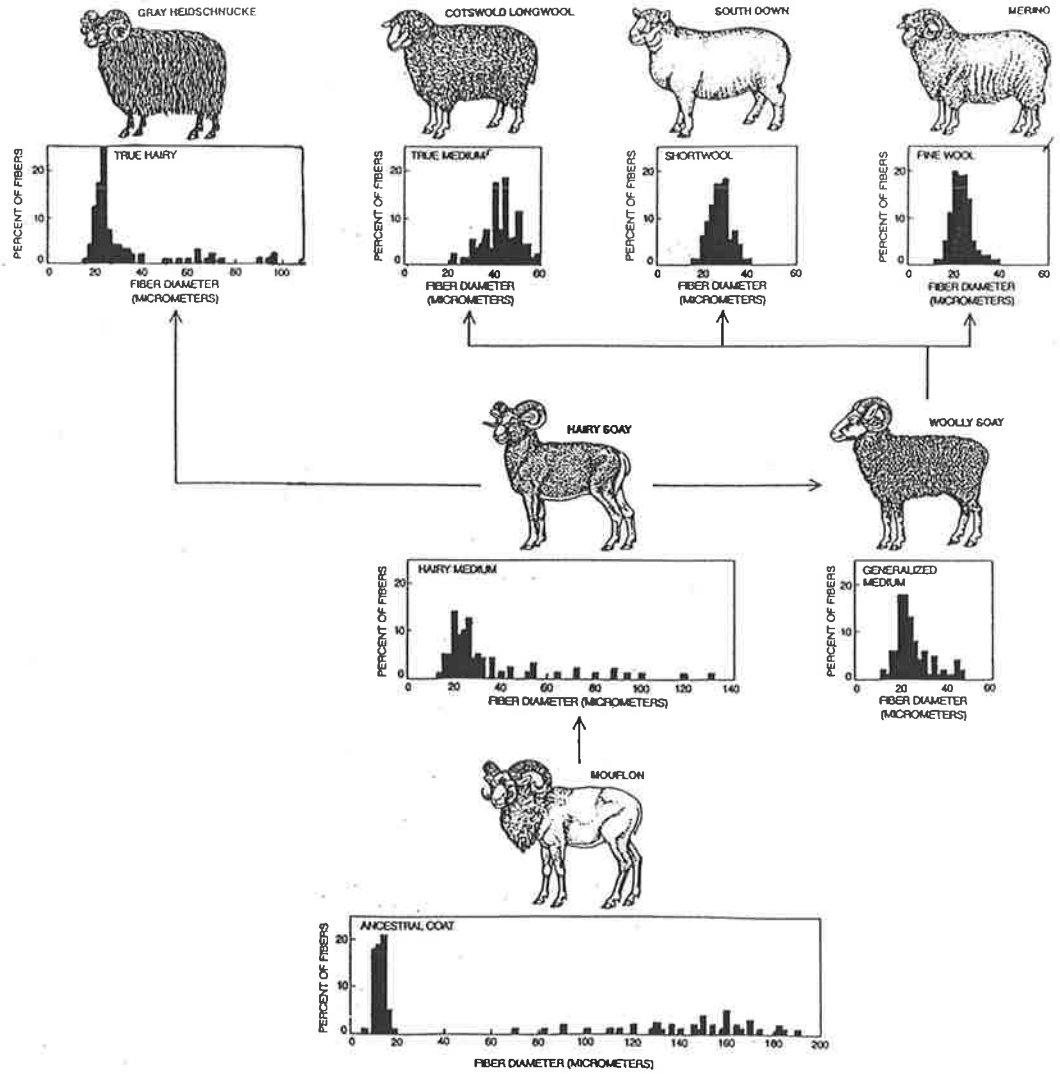


Figure 1: Relationships between present day examples of different types of fleece (Ryder 1987).

The Mouflon and Merino stand at opposite poles of fleece development and colour (Ryder 1987). The hair coat colour of the Mouflon is a reddish fawn to reddish grey, with some banded fibres and a yellowish to white saddle. A black line is present down the backline, and another black stripe separates the body from the pale belly (Sponenberg et al. 1996). In contrast, the Merino coat has been selected for freedom from pigmented fibres and pigmented skin (Graham 1870). However, the development of the Australian Merino, following the sheep imports in 1788, involved some crossing of Afrikaner fat-tail and Bengal sheep with Spanish Merinos (Austin 1987). Such crossing would have introduced pigmentation of the coat that has since been removed or diluted by selection.

The lack of pigmentation arising from albinism or white spotting in mammals is often accompanied by disadvantages including reproduction loss, impaired hearing and vision, or susceptibility to cancer (Robinson 1955, Witkop 1971; Bergsma and Brown 1971; Pulos and Hutt 1969; Silvers 1979; Spradbrow and Hoffman 1980). However, lack of pigment on the coat of sheep is considered "normal" and, if disadvantages exist, these are accepted in many breeds in order to produce wool with few pigmented fibres.

The inheritance of most characters is complicated by involvement of several genes and environmental factors. Variations in pigmentation often involve single genes that are not greatly affected by environmental factors so their effects can be readily distinguishable and provide a basis for models of gene action and interaction. In mice over 50 loci and 130 alleles are involved in the control of pigmentation (Silvers 1979). The model proposed for mice should be a starting point for comparison with other animals. A catalogue of loci and alleles for sheep has been produced by the Committee for Genetic Nomenclature of Sheep and Goats (COGNOSAG)(Sponenberg et al. 1996).

1.3. COMMERCIAL RELEVANCE OF DARK FIBRES IN WHITE WOOL

Buyers and processors of Australian wool consider contamination-free wools, and systems to prevent contamination from any sources (pack and in-bale), as being among the most important of the quality issues to be addressed by the Australian wool industry (Chant Link and Associates 1994). Of the in-bale sources of contamination, dark fibre is seen as an increasing problem by some customers of Australian wools (IWS 1996).

The importance of dark fibres in processed wool involves reduced flexibility for end-use and reduced value that is not entirely predictable. Due to the sporadic nature of the fault (sampling difficulty) and lack of practical measurement techniques it is not possible to measure the dark fibre content of greasy wool. The first time at which dark fibre content can be practically assessed is during early stage manufacture (tops). The procedure involved is labour intensive and provides an indication of the likely risk of exceeding a particular threshold (e.g. 100 dark fibres per kg) rather than an absolute measure of dark fibre content (IWTO 1988). Adoption of this technology is at the innovative stage and not widely conducted at present though less rigorous testing may be practiced. Recently an imaging system developed for measuring fibre diameter distribution has been adapted to measure the colour of scoured fibres and dark fibre content (Lupton et al. 1995).

In terms of commercial relevance, it is likely that black pigment is more readily identified in processed wool than shades of brown, tan or yellow and this inference is reflected in the CSIRO Reference Scale used to assess the darkness of fibres (Foulds et al. 1984).

The CSIRO Reference Scale is based on standard dyed wool fibres with differing light transmittance values, as follows; grade 8 (black) <55% transmittance; grade 7, 55-70%; grade 6, 71-85%; grade 5 or (threshold of visible distinction), 86-93%; and grade 4, (very pale) >92%. Single dyed fibres of similar fibre diameter in a white wool web are mounted between glass plates and compared with the dark fibres identified in wool webs being viewed with the CSIRO Dark Fibre Detector (Foulds 1988; Fleet and Foulds 1988). Isolated fibres of any colour in greasy wool that are dark enough to be visually discernible in processed wool constitute a serious fault. In contrast, fibres of pale colouration (CSIRO Darkness less than level 5) will only be a problem when in large quantities causing bulk discolouration of processed wool (Figure 2).

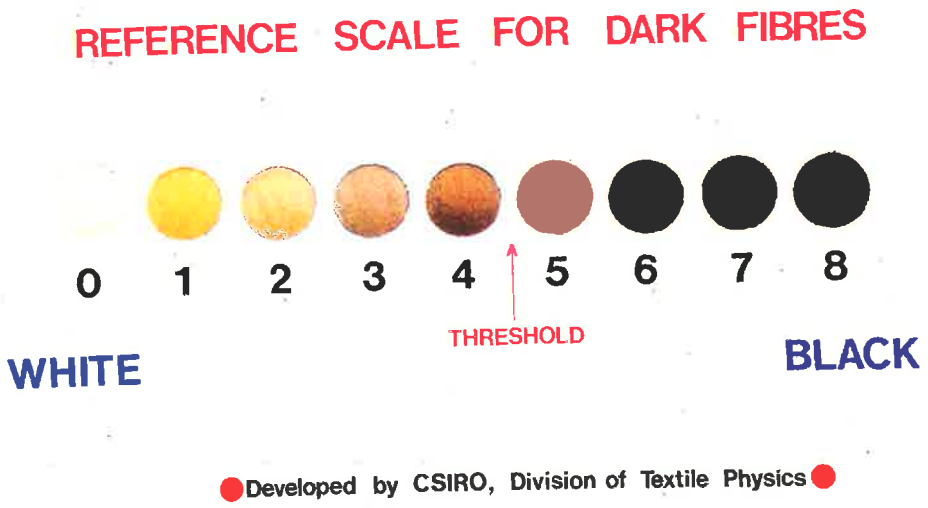


Figure 2: Diagrammatic representation of the CSIRO Reference Scale, giving the bulk appearance of the dyed wool fibres, devised for the purpose of categorising the darkness of fibres identified in wool webs with the CSIRO Dark Fibre Detector (Foulds et al. 1984, Foulds 1988).

When dark fibres are recognised in late stage manufacture of sensitive products (white and pastel coloured fabrics and garments) the offending fibres may be manually "picked" involving costs ranging from nominal to prohibitive and claims passed back to early stage manufacturers (Strydom and Gee 1982; Foulds et al. 1984). Other methods of dealing with dark fibres are selective bleaching (Bereck et al. 1982) or automated removal from yarns using SIROCLEAR (Plate 1992) but they involve limitations and additional costs. These methods by no means relieve the grower of his responsibility to minimise dark fibre content of white wool (Plate 1992).

Extensive surveys have shown that commercial wool tops which are completely free of dark fibres are, for practical purposes, virtually non-existent (Strydom and Gee 1982). Nevertheless, the Australian wool industry has gained a reputation for producing wool with a low incidence of dark fibres as reflected in a survey to wool mills (Cardellino 1978).

Within Australian auction sales the discount for visible pigmentation in wool ranged as high as 50% but averaged 16% for 1992/93 (Pattinson and Hanson 1993). The average discount for visibly pigmented Merino wool declined from about 43% in 1973-74 to 27% in 1977-78; apparently in conjunction with the growth of a coloured wool industry and increased demand for naturally coloured wool (Curtis 1979).

Specifications of dark fibre limits in tops and fabric may be as low as 1 per kg top but are more commonly specified at 100 per kg top (Foulds et al. 1984). Surveys of dark fibres in commercial tops have been reported by Satlow (1963), Henning (1975) and

Cardellino (1992) and tops made from skirted fleece wools of Australian or Merino origin tended to have lower dark fibres contents than other origins. Foulds (1989) concluded that in tops made of Australian Merino wools, with dark fibre concentrations far in excess of 100 per kg, the predominant source of dark fibres was urine stain. However, when around 100 dark fibres per kg both pigmented fibres and urine stained fibres were important.

Burbidge et al. (1991) and Burbidge and McInnes (1994) report the dark fibre concentrations in 161 small lots of Merino wool processed to top. Based on this survey, and a sample of commercial consignments, the levels of pigmented fibres in Merino wool can be excessive, especially in young Merino sheep, once urine stained fibres are minimised. The evidence implicating young Merino sheep with a higher risk of pigmented fibres is consistent with results included in this thesis and reported in Fleet et al. 1991).

The extent of the problem of pigmented fibres in Australia's white wool clip is not accurately determined and the reason for this is the practical problem of measurement and lack of reliable data from wool processors. It is not known whether the problem of pigmented fibres is increasing or decreasing as a result of industry selection programs (e.g. WOOLPLAN) or Wool Quality Management Programs (Brien et al. 1991; Ponzoni 1991; Vandeleur 1993). The research of Burbidge et al. (1991), Burbidge and McInnes (1994) and this thesis provide the most thorough investigations currently available to gauge the extent of the problem.

1.4. MELANIN

Melanin is a generic term for a wide variety of natural pigments responsible for shades of black, brown, tan and yellow found in plants and animals. In mammals there are two classes of melanin, termed eumelanin and phaeomelanin, and their mixture and concentration can lead to a wide variety of shades. Eumelanin is a dark brown to black pigment which is insoluble in acid and alkali, contains nitrogen (6-9%) but no sulphur (0-1%), and consists of monomer units of 5,6-dihydroxyindole and 5,6-dihydroxyindole-2-carboxylic acid. Phaeomelanin is a yellow to reddish-brown pigment which is soluble in alkali, contains nitrogen (8-11%) and sulphur (9-12%), and is made up of benzothiazine units derived from cysteinyldopas forming pigments with a diverse range of physical and chemical properties. One group of phaeomelanins, with a well defined chemical structure of low molecular weight, is called the trichomes which consist of two conjugated 1,4-benzothiazine units (Prota and Searle 1978; Ito and Jimbow 1983; Prota 1993; Ito 1993; Tsukamoto et al. 1992; Furumura et al. 1996). Figure 3 is a scheme of the melanin synthesis pathway.

The distinction between eumelanins and phaeomelanins cannot be accurately determined from visual or light microscope study and there is overlap between pigment types. Distinction of pigment type can be determined from chemical analysis (Ito and Jimbow 1983; Sponenberg et al. 1988; Renieri et al. 1993; Prota et al. 1995; Ozeki et al. 1995), electron spin resonance (Vsevolodov et al. 1987) or electron microscopy (Jimbow et al. 1983; Inazu and Mishima 1993; Renieri et al. 1993). Both eumelanin and phaeomelanin are produced together even in genotypes that favour production of one of these groups of

pigments (Sakura et al. 1975; Quevedo and Fleischmann 1980; Jimbow et al. 1993). For example, even in the hair of yellow mice (A^y) a small amount (0.02%) of the melanin is eumelanin (Ito and Jimbow 1983). Inazu and Mishima (1993) provide evidence of eumelanosomes (mainly eumelanin) and phaeomelanosomes (mainly phaeomelanin) occurring simultaneously in the same melanocyte.

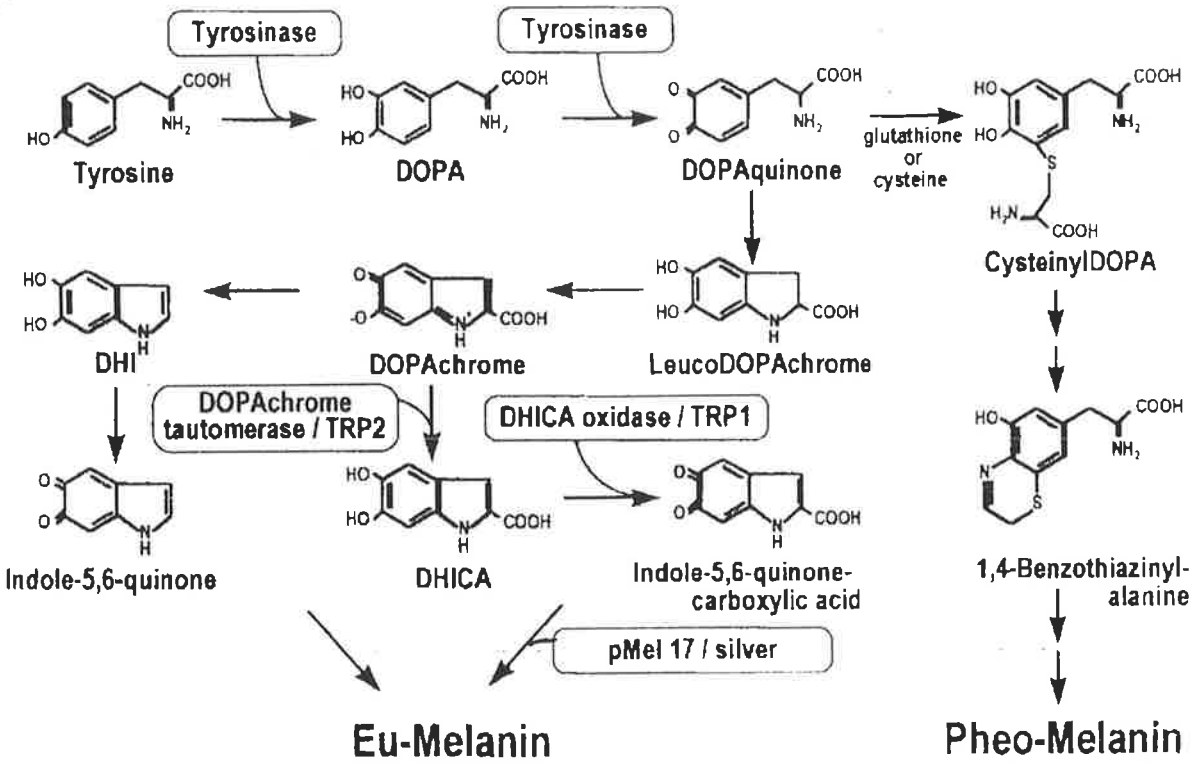


Figure 3: Scheme for the pathways of melanin synthesis (Furumura et al. 1996).

1.5. MELANOCYTES AND MELANOSOMES

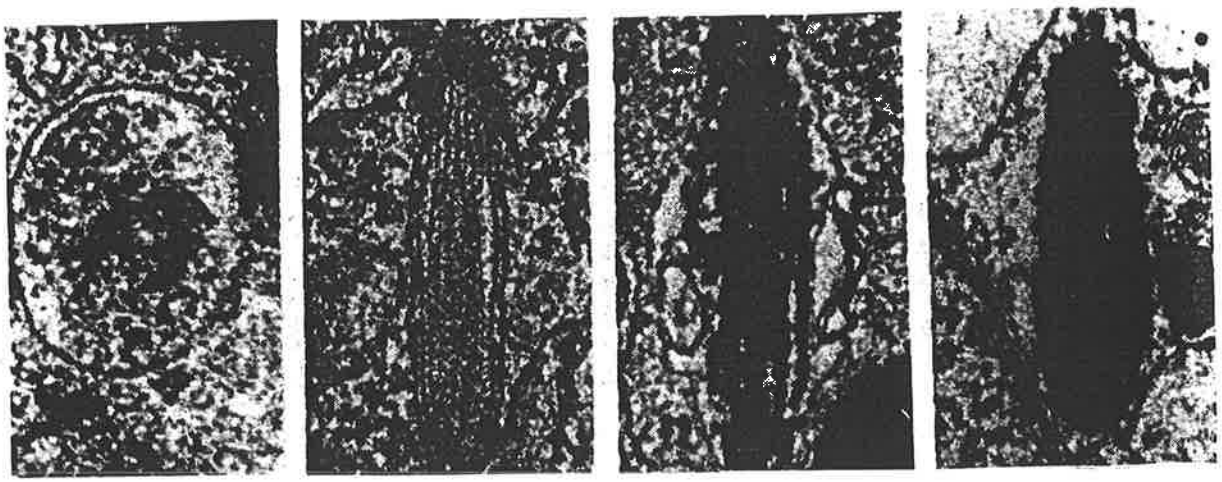
In vertebrates, melanin is synthesised in melanosomes, a cellular organelle, within dendritic cells called melanocytes (Riley 1976). These cells are found in the skin, eyes, ears and brain of mammals (Rawles 1947; Jackson 1994). Normally the pigment visualised in skin, hair and wool, is not within the melanocytes themselves, but in

keratinocytes that were in contact with melanocytes early in their development. A melanocyte and melanosomes in the outer root sheath of a wool follicle is shown on the cover page of this Chapter. Fitzpatrick and Breathnach (1963) reported that in the human epidermis each melanocyte may be in contact with 36 keratinocytes.

The transfer of melanin granules to keratinocytes has been observed by electron microscopy and involves penetration of the tip of a melanocyte dendrite that is phagocytosed by the keratinocyte. After phagocytosis the dendrite tip forms a pouch that has two membranes; one from the dendrite and one from the keratinocyte. The pouch of melanin granules gradually moves toward the cell nucleus and as this occurs the membranes are digested leading to the dispersal of melanosomes (Seiji et al. 1976; Okazaki et al. 1976). These keratinocytes then fill with keratin and flatten forming the protective outer layer of the epidermis (stratum corneum) or the hair and wool fibres (Fitzpatrick and Breathnach 1963).

There is some uncertainty about development of tyrosinase (primary enzyme for melanogenesis) and premelanosomes in the region of the endoplasmic reticulum and golgi complex (Wellings and Siegel 1963; Seiji et al. 1963; Foa and Aubert 1977; Jimbow et al. 1991). Jimbow et al. (1991) suggest that tyrosinase and structural proteins form on ribosomes in the rough endoplasmic reticulum. The tyrosinase becomes glycosylated and is moved to the golgi complex. Structural proteins transfer to the smooth endoplasmic reticulum and are then expelled in a membrane bound vesicle (premelanosome) of about 0.5μm diameter with internal structure. Microvesicles from the golgi apparatus that contain the glycosylated tyrosinase and regulatory factors fuse with the premelanosome membrane and pass into the vesicle to bind with the internal matrix proteins.

Although initially spherical, eumelanosomes develop an elliptical shape and inner membrane structures (Figure 4). The outer membrane involves two dense lines about 70A apart but internal membranes consist of a single structure. At the premelanosome stage (Stage I), tyrosinase synthesis and arrangement is complete but melanisation has not commenced.



Stage I Stage II Stage III Stage IV

Figure 4: Electron micrographs of eumelanosome development

Melanin deposits on both sides of internal and external membranes (stage II and III) until the granule is saturated (stage IV). The mature eumelanosome is about 0.7μm long with a diameter of 0.3μm (Birbeck 1963; Seiji et al. 1963). The specific proteins and lipids incorporated into the melanosome, as well as the nature of the tyrosinase, and several loci (e.g. *B* locus) will influence the melanosome type ultimately formed (Moyer 1966). The melanosomes of hair bulbs are larger (about 2x) than those of epidermal melanocytes (Ortonne and Prota 1993). Eady et al. (1983) found that tyrosinase-negative albinism in human foetal skin can be determined at 20 weeks gestation as melanocytes will contain only stage I and stage II melanosomes while tyrosinase-positive albinos will also show stage III melanosomes.

1.6. TYROSINASE

The enzyme tyrosinase was first identified in mushrooms and shown to catalyse the conversion of tyrosine to melanin (Bourquelot and Bertrand 1895). Bloch (1927) produced melanin with dopa as the substrate in human skin epidermis but the reaction was negative for tyrosine. In plants and insects, dopa was found to be the first product formed from tyrosine and that tyrosinase could use either substrate (Raper 1928).

Tyrosinase and dopa oxidase activities were both later identified in murine melanoma, skin from horses and in human skin after irradiation by ultraviolet light. The lack of tyrosinase activity in human epidermis (Bloch 1927) was attributed to tyrosinase being in an inactive or partially inhibited state in unexposed human skin (Fitzpatrick and Becker 1950). Dopamine, noradrenaline and adrenalin were once considered precursors of melanin but *in vivo* experiments have shown these substrates are not normally used in the melanin synthesis pathway (Goodall 1976).

Tyrosinase is a copper containing enzyme that catalyses the hydroxylation of tyrosine to dopa and the oxidation of dopa to dopaquinone at separate binding sites. As well as being a substrate, dopa acts as an initial co-factor to tyrosinase and stimulates melanin synthesis (Lerner 1949, Fitzpatric and Becker 1950; Hearing and Ekel 1976: Jimbow et al. 1991). The stimulating effect of dopa is not just a function of the first reaction since 5μM dopa increased tyrosinase activity (measured by dopa production) by 6-fold but melanin synthesis increased 15-fold (Husain et al. 1982). Tyrosinase catalyses a third eumelanin reaction; the oxidation of 5,6-dihydroxyindole (DHI) to indole-5,6-quinone (Korner and Pawelek 1982; Tsukamoto et al. 1992).

Several forms of tyrosinase have been demonstrated to occur in melanocytes including three soluble forms T1, T2 and T3 and the membrane-bound T4 form located in melanosomes that plays the essential role in melanogenesis. T1 and T3 are thought to be precursors of T4 and can convert dopa to dopaquinone in a test tube but cannot produce melanin within the melanocyte. T3 contains indole blocking factor and inhibits conversion of 5,6-dihydroxyindole to melanin. T2 is thought to be an artefact of electrophoresis isolation of T3 (Hearing et al. 1982).

Three tyrosinase related proteins (TRP-1, TRP-2 and TRP-3) are now recognised that have 35-45% amino acid identity with each other (Jackson 1994). The *Albino* locus encodes tyrosinase, the enzyme with three melanogenic functions (Figure 3), the *Slaty* locus encodes TRP-2 (tyrosinase-related protein-2), that functions as a dopachrome tautomerase, and the *Brown* locus encodes TRP-1, that is able to oxidise the DHICA produced by TRP-2 (Tsukamoto et al. 1992; Hearing et al. 1992; Jimenezcervantes et al. 1994; Kobayashi et al. 1994; Jackson 1994; Cassady and Sturm 1994; Yokoyama et al. 1994; Furumura et al. 1996). Winder et al. (1994a) suggests that tyrosinase, TRP-1 and TRP-2 may stably interact within a melanogenic complex in the melanosome to regulate the quantity and quality of melanin synthesised.

Phaeomelanogenesis requires a lower level of tyrosinase activity than does eumelanogenesis and it has been suggested that regulation of tyrosinase activity may play a part in the switch between the two pigment types (references cited in Furumura et al. 1996). Human TRP-1 has tyrosine hydroxylase activity, in common with tyrosine, but no dopa oxidase activity. It is proposed that one function of TRP-1 is to modulate tyrosinase activity by making DOPA available as a cofactor (Zhao et al. 1994).

Another gene identified in the mouse and thought to affect a late step in the melanin pathway is known as *Silver*. A recessive allele at this locus, the *silver* (*si*) mutation, induces hypopigmentation by the premature loss of melanocytes and greying of some hair follicles (Silvers 1979; Kwon 1993). The Pmel17 gene expresses specifically in melanocytes and encodes a melanogenic enzyme (Kwon et al. 1995). Pmel17 maps to human chromosome 12 and mouse chromosome 10 in a region known to contain the locus *Silver* (Kwon 1993; Kwon et al. 1995). The *si* mutation has recently been explained by a single base insertion in the domain of the Pmel17 gene (Kwon et al. 1995). The *Pmel17/silver* protein is an internal melanosomal matrix protein that plays a role in the polymerisation of melanogenic intermediates to form eumelanin (Donatien and Orlow 1995; Chakraborty et al. 1996; Furumura et al. 1996).

1.7. HORMONES

The main hormone involved in melanogenesis is melanocyte stimulating hormone (MSH). Two forms of MSH (α and β) are produced in the pituitary (intermediate lobe) of mammals and both induce darkening of human skin (Lerner and McGuire 1961). The main regulator of MSH is MSH-Release Inhibiting Factor (MIF) which is excreted by the hypothalamus and acts on the pituitary to restrain release of MSH. A second pituitary factor is proposed that has the opposite effect (Kastin and Schally 1972). Melatonin produced by the pineal gland during periods of darkness reduces MSH levels possibly by interaction with the mechanism controlling MIF release or function (Kastin et al. 1967; Snell 1972). Melatonin has been found to have a small inhibitory effect on tyrosinase activity, dramatically inhibits α-MSH induced melanogenesis, and slightly stimulates dopachrome tautomerase activity in cultured melanoma cells (Valverde et al. 1995a).

Change in colour, from dark to light in the Carmaque horse, or from eumelanin to phaeomelanin in C3H-HeA^{vy} and A^{y}/a mice, has been attributed to a reduction in the level of circulating MSH (Geschwind and Huseby 1972; Altmeyer et al. 1984; Levine et al. 1987). Seasonal and racial differences in pigmentation, and interactions with ultra violet light, have also been associated with differences in circulating MSH levels in humans (Holtzmann et al. 1983; Altmeyer et al. 1986).

MSH is a large polypeptide that cannot enter the cell and it was predicted that receptors on the cell membrane, possibly overlying the golgi region, provide the signal for eumelanogenesis (Fritsch and Varga 1976). The signal from MSH appears to involve an increase in cAMP, though for human melanocytes this relationship was less certain until recently, and in turn increased tyrosinase activity and melanin (Pawelek 1976; Hirobe and Takeuchi 1977; Kwon et al. 1988; Ranson et al. 1988; Pawelek and Gilchrest 1990; Furumura et al. 1996).

α-MSH is a post-translational cleavage product of the precursor proopiomelanocortin (POMC) which can also give rise to ACTH and β-endorphin. There is evidence that α-MSH may also be produced in skin and influence pigmentation via paracrine mechanism. The action of α-MSH on melanogenesis is medicated through the melanocortin-1 receptor (MC1R), one of five closely related G-protein-coupled receptors that react to α-MSH, ACTH and, or, γ-MSH. All of the melanocortin receptors are coupled to adenylate cyclase *in vitro*, and nearly all of the melanogenic effects of MSH can be reproduced by treatment with cAMP analogs. ACTH also appears to bind with the MC1R receptor (or related receptor) inducing cAMP formation and stimulating melanocyte proliferation and melanogenesis (Cone and Mountjoy 1993; Furumura et al. 1996).

The enzyme dopachrome oxioreductase (DCOR) is active in the conversion of dopachrome to dihydroxyindole (DHI). DCOR is absent in recessive yellow mice (*e/e*) and dominant yellow (A^y/a) and low in Sienna yellow (A^{sy}/a) mice. MSH had no effect on DCOR in *e/e* mice but increased DCOR production in the other yellow genotypes (Barber et al. 1985). In the adult C3H-HeA^{vy} mouse, the phaeomelanin production is associated with less glycosylation of tyrosinase and less membrane bound active tyrosinase than in the pubertal eumelanic mice (Burchill et al. 1989; Burchill 1991).

The *Agouti* locus encodes a small protein (131 residue) that appears to be an antagonist to MSH that may compete with or prevent MSH binding or signalling with the melanocyte receptor. Expression of *TRP-1*, *TRP-2* and *Pmel17/silver* are extinguished during phaeomelanogeneis, while expression of tyrosinase is reduced but not eliminated. *Agouti* mRNA is produced by the cells of the dermal papilla and its protein product acts on the cells of the overlying hair follicle, but not on adjacent follicles, demonstrating the paracrine action (references cited in Furumura et al. 1996). In contrast, the product of the *Extension* locus is the MSH receptor. Alleles at this locus can affect the MSH receptor so that signalling for eumelanin is prevented or occurs completely or partially independent of MSH binding (Robbins et al. 1993; Jackson 1993 1994). Other hormones implicated in melanogenesis are adrenocorticotropic (ACTH) hormone (Lerner and McGuire 1964; Maeda et al. 1996), oestradiol (Ranson et al. 1988; Maeda et al. 1996), estriol, progesterone, follicle stimulating hormone and luteinising hormone (Maeda et al. 1996), calcitonin (Mason et al. 1988) and prostaglandin E1 and E2 (Nordlund et al. 1986).

1.8. ULTRAVIOLET LIGHT (UV)

1.8.1. Tanning responses

The absorption spectrum of melanin extends into the ultraviolet (UV) region and protects the skin from UV damage. White skin from normally unexposed regions can transmit 5-15% of the UV and those wavelengths of less than 300nm may reach the dermal papillae. Sunburn is primarily caused by wavelengths less than 320nm (Pathak 1967) and the major protective factors are skin thickening and melanisation (Anderson and Parish 1981).

An initial response to increased visible light (wavelength >400nm) and the long wave ultraviolet light (UVA 320-400nm) involves immediate pigment darkening (IPD). An IPD plateau occurs within an hour of irradiation and then the pigment fades within a few hours after ceasing exposure (Jimbow et al. 1973).

Repeated exposure of previously shielded white skin of humans or mice led to activation of precursor melanocytes and formation of unmelanised melanosomes (0-2 days). During 3-5 days of UV exposure, tyrosinase synthesis within these cells resulted in darkened melanosomes. Between 6-7 days (human) or 6-12 days (mouse), mitosis resulted in an exponential increase in dopa-positive melanocytes followed by melanosome transfer with melanocyte differentiation, arborisation of dendrites, and plateau of melanocyte numbers (Pathak 1967; Quevedo and Smith 1963; Jimbow and Uesugi 1982). After cessation of UV there is a tendency for the melanocyte density to decline toward levels existing prior to irradiation (Pathak 1967; Quevedo et al. 1965).

Stimulation of melanogenesis by UV light involved a change in tyrosinase activity, without any change in the amount of tyrosinase or TRP-1 and decreased TRP-2 in cultured human melanocytes. These results are different from mechanisms by which other melanogenic agents (cholera toxin and isobutyl methylxanthine) stimulated melanogenesis where the amounts of tyrosinase, TRP-1 and TRP-2 were increased (Abdelmalek et al. 1994).

1.8.2. Lack of tanning

Tanning in the skin usually, but not always, follows exposure from sunlight or UV. For example, Quevedo and Smith (1963) found that the feet and de-haired trunk skin of yellow mice (A^y/A^a), with or without white spotting (S^s/S^s), and the white spotted feet of their black litter mates (A^a/A^a // S^s/S^s), failed to tan in response to UV. The white spotted areas and yellow skin had fewer epidermal melanocytes than unspotted skin from black litter mates. This factor or the reduced ability of the melanocytes present to respond to UV (transfer melanosomes and proliferate) may explain the lack of tanning in A^y/A mouse epidermis (Quevedo and Mc Tague 1963).

All *pink-eyed dilute* (*p/p*) mice also failed to tan and few melanin granules were found in epidermal cells (Quevedo and Smith 1963). Despite an increase in the number of active melanocytes after UV exposure they retained the characteristic fine, spherical to ovoid, yellow-brown characteristic melanosomes. The *pink-eyed dilute* with *dilute* (*p/p // d/d*) showed reduced dendrite formation. Whereas, in the black-eyed white and albino strains no pigmented melanocytes were observed after irradiation (Quevedo and Smith 1963).

Another two loci in the mouse named *Leaden* and *Ashen* can have restrictive effects on dendrite extension like *Dilute* (Silvers 1979; Jackson 1994). The *Dilute (d)* locus encodes a myosin-related protein (myosin 12) which is speculated to be necessary for the elaboration, maintenance, and/or function of melanocyte dendrites and melanosome transport (Mercer et al. 1991; Moore et al. 1994).

The murine *Pink-eyed Dilute (p)* gene encodes a melanosomal membrane protein (Rosemblat et al. 1994) that was suggested to be involved in the transport of tyrosine in the melanocyte (Jackson 1994; Lee et al. 1995). The murine *p* gene has been mapped close to the locus for tyrosinase-positive albinism (Nakatsu et al. 1992; Kedda et al. 1994; Brilliant et al. 1994) and molecular characterisation has confirmed that these mutations are homologous (Lee et al. 1995). Colman et al. (1993) for tyrosinase-positive albinism gene and Gahl et al. (1995) for the murine pink-eyed dilution gene provide evidence contrary to the involvement of a tyrosine transport system.

Gahl et al. (1995) report that pink-eyed dilution melanosomes are immature by virtue of their low density, high hexosaminidase activity and lack of pigment. Although the *p* protein is thought to function as a transport protein through the melanosomal membrane, influence levels of members of the TRP family in the melanosome, and may also be associated with anchoring and/or stabilising of the melanogenic complex (tyrosinase, TRP-1 and TRP-2) within the melanosome (Winder et al. 1994a; Orlow et al. 1994; Donatien and Orlow 1995; Lamoreux et al. 1995; Furumura et al. 1996).

In white Merino wool-bearing skin the sparsely distributed presumptive epidermal melanocytes are described as being rounded, weakly dopa-positive but darkened by ammoniacal silver nitrate, lacking dendrites, and with little evidence of melanin transfer to adjoining keratinocytes. Ammoniacal silver nitrate identified tyrosinase sites or premelanin in sheep melanocytes that were induced to an amelanotic state by copper deficiency (Forrest et al. 1985).

In sheep the majority of skin is shielded from UV by wool. Kemp-bearing or bare skin areas on white sheep often show no evidence of pigmentation despite continuous exposure to UV. On shearing the fleece, the wool-bearing skin is transiently exposed to the affects of sunlight (Forrest and Fleet 1985 1986). Chapman et al. (1984) report on the erythema responses of the skin of biochemically denuded sheep when exposed to sunlight. Forrest and Fleet (1985) found that application of UV to clipped wool-bearing skin on Merino sheep did not induce a tanning response in most of the sheep tested but epidermis thickness increased 5-fold after 28 days of exposure. The thickening of epidermis appears to be the only visible mechanism for protection against UV in such Merino sheep.

Some Merino sheep developed tan freckles and black-grey skin spots after exposure of wool-bearing skin to UV (Forrest and Fleet 1985 1986). Daniels and Johnson (1987) implicate the Merino breed with a high incidence of squamous cell carcinoma that is most likely associated with old age and reduced pigmentation as in cattle (Spradbrow and Hoffman 1980; Daniels and Johnson 1987). The lack of pigment can also contribute to susceptibility to photosensitisation (Daniels and Johnson 1987).

1.9. FOETAL DEVELOPMENT

1.9.1. Histological characterisation

A melanoblast (precursor or inactive melanocyte) is a round or ovoid premelanin-positive (ammoniacal silver nitrate-reacting) but dopa-negative cell (Zimmermann and Becker 1959; Mishima 1960; Mishima and Loud 1963; Mishima and Wildlan 1966 1967). The embryonic neural crest was demonstrated (by transplanting portions of mouse embryos to the embryonic coelom of the White Leghorn fowl) to be the source of the migrating melanoblasts that differentiate to melanocytes in the skin (Rawles 1947). The clonal origin of melanocytes, from the neuroectodermal cells of the neural crest, is crucial to understanding the development of the numerous coat colour patterns that occur between and within various mammals.

Melanoblasts usually migrate from the neural crest to the skin and spread in a dorsoventral direction until the skin is saturated (i.e. when these migratory cells meet ventrally). Having completed migration, a melanoblast may proliferate and colonise, establishing a spot of pigment cells, or differentiate to become active and dendritic, or remain in a precursor state until conditions are suitable for proliferation and, or, melanogenic activity. The major increase in skin melanocyte numbers will normally arise from proliferation of the original migratory melanoblasts. While the capacity for melanoblasts to differentiate into active cells may be determined by genotype, the morphology and activity of mature cells can also be influenced by factors in the surrounding tissue (Reams 1963). Unlike other melanocytes, those of the pigmented retinal epithelium develop from neuroectoderm *in situ* (Jackson 1991).

The colonisation of differentiating human foetal skin by melanoblasts (or inactive melanocytes) and melanocytes has been characterised by use of the dopa-reaction and premelanin reactions (Figure 5). At the pregerm stage and hair-germ stage there is a mixture of active (dopa-positive) and inactive (dopa-negative) melanocytes randomly distributed through the epidermis. At the hair-peg stage the melanocytes locate along the epidermal-dermal border and are distributed randomly in the developing hair follicles. By the bulbous peg stage (before 6 months gestation) the melanocytes are concentrated in the epidermis and outer root-sheath and developing follicle bulb. Later in gestation the outer root-sheath becomes deficient in melanocytes with only occasional melanoblasts evident after a 6 month term (Mishima and Wildlan 1966).

Holbrook et al. (1989) used a monoclonal melanoma antibody (HMB-45) to detect melanoblasts and melanocytes in human foetal skin. At 50 days gestation labelled cells are in the epidermis (1050 per mm ^{2}), peak in number (2300 per mm^2) by 12-14 weeks gestation, and then decline to levels evident in neonatal skin (800 per mm^2).

Cable et al. (1995) followed the development of melanocytes in mouse embryos by in situ hybridisation to TRP-2 (Dopachrome tautomerase) mRNA, which labels migratory melanoblasts from 10 days post-coitum. Numerous melanocytes migrate to the inner day around 11 days. In contrast, few melanocytes are associated with the eye or skin at this stage and melanoblast distribution within the trunk and tail is patchy.

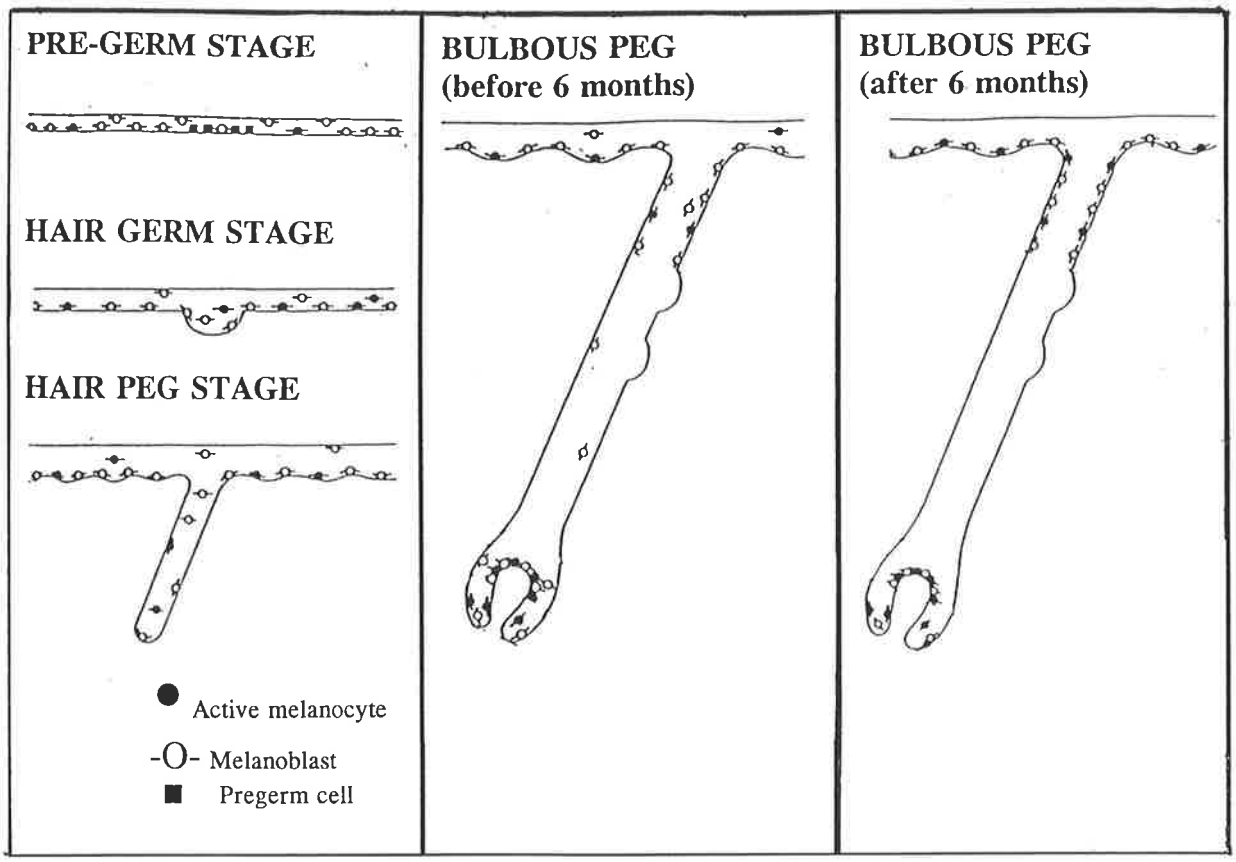


Figure 5: Development of melanocyte populations in foetal human skin (Mishima and Wildlan 1966).

1.9.2. Factors influencing melanocyte migration and colonisation

The factors involved in directing melanocyte migration to the epidermis, the crossing of the basement membrane, adhesion and localisation in the basal layer of the epidermis are starting to be discovered. Cell adhesion receptors and the composition of the extracellular matrix in the migration pathway are likely to be involved in initiation and termination of migration. Melanocytes are known to attach and migrate on fibronectin while integrin is a cellular receptor that connects extracellular matrix proteins and is involved in melanocyte adhesion (Scott et al. 1992; Etoh et al. 1993). Slominski and Paus (1993) propose that melanocytes may migrate along pathways marked and modified by the mesenchyme which includes the dermal papilla.

Deficiencies of stem cell growth factors (soluble and membrane-bound forms), encoded at the *Steel* locus of the mouse, are identified as involved in melanoblast survival and migration or melanocyte adhesion and proliferation in skin (Fleischman 1993). Lahav et al. (1994) provide evidence in support of the notion that stem cell growth factor (steel factor) sustains the survival of neural crest derived cells and stimulates the rate of melanogenic differentiation. Yoshida et al. (1996) has demonstrated that a Steel factor regulated transgene (*lacZ*) is expressed in the enteric ganglion cells of the intestine and in the dermal papillae of the hair follicle where it has a role in supporting *c-kit* dependent growth and development of melanocytes. Cable et al. (1995) studied development of melanocytes in normally pigmented and dominant spotting mouse embryos. They suggest that mutations of the c-kit receptor tyrosine kinase encoded at the *Dominant White* (*W*) locus do not alter early migration or differentiation of melanoblasts but severely affect melanoblast survival.

Cell surface glycoprotein CD44 may be involved in the migration and invasion by melanoma cells (Thomas et al. 1993) and nerve growth factor is important for melanocyte migration and dendricity and may be required for survival (Yarr and Gilcrest 1991). Morelli et al. (1992a) report that leukotriene C4 and transforming growth factor (TGF)-α, which can be elevated in skin after trauma, are stimulators of melanocytes migration.

1.9.3. Melanocyte proliferation

In the process of developing culture techniques for normal human melanocytes, the essential factors and stimulating agents for melanocyte mitosis and survival *in vitro* have been determined. Substances promoting melanocyte mitosis (Yarr and Gilcrest 1991) are:

- Cyclic AMP;
- dibutyryl cyclic AMP (dbcAMP);
- cholera toxin and isomethyl xanthine (IBMX) which increase cAMP;
- basic fibroblast growth factor (βFGF), keratinocyte conditioned media and other sources of βFGF; and
- 12-O-tetradecanoyl-phorbol-13-acetate or 12-myristate-13-acetate.

The early successful culture, and autonomous growth of melanoma cells, has been attributed to autocrine production of βFGF but other factors are involved *in vitro* (Dotto et al. 1989). Endogenous production of growth factors by melanocytes, nevus melanocyte cells, primary melanoma cells and metastatic melanoma cells reflect levels of increased autonomy toward the metastatic state (Shih and Heryln 1993). Morelli and Norris (1993) predict that inflammatory mediators, cytokines and growth factors that affect keratinocyte growth will also alter melanocyte function. For example, the leukotrienes C4 and B4 are potent mitogens and leukotriene B4 increases melanogenesis. These leukotrienes are present in elevated amounts and associated with hyperpigmentation in inflammatory dermatosis (Morelli et al. 1992b). Cholera toxin has been shown to induce stem cell factor (steel factor) production in murine intestinal tract (Klimpel et al. 1996).

Active melanogenesis and presence of melanocytes in the murine hair bulb is closely coupled to anagen (the period of active keratinocyte proliferation). Accompanying anagen is expression of the *proopiomelanocortin* (*POMC*) gene that encodes the POMC protein required for production of ACTH and MSH. The demonstration of *in vitro* production of MSH and ACTH by cultured human keratinocytes, raises the possibility of local tissue production of these potent stimulators of melanogenesis; in addition to supply from the

pituitary. During anagen, of the hair follicle cycle, keratinocytes will be actively producing cytokines and growth factors that also stimulate melanocyte proliferation and melanogenesis (e.g. βFGF, interleukins (IL)-1α and 6, endothelins, nerve growth factor, α β and γ interferons, TGF α and β, and tumor necrosis factor α (Slominski and Paus 1993). Where hair bulb melanocytes locate during catagen (regression) and their replacement after telogen (rest) is clarified by Ortonne and Prota (1993). The *c-kit* gene may play a critical role in replacement of hair bulb melanocytes after telogen as injection of an anti-*c-kit* monoclonal antibody resulted in non-pigmented hair.

1.10. PIGMENTATION CHANGES AFTER BIRTH

1.10.1. Juvenile changes

Extension of pigmentation from primordial centres of migration, is often transitory, occurring in the foetus and during neo-natal life, leading to relatively stable adult patterns of clearly defined spots or irregular patterns (Schumann 1960; Wendt-Waegner 1961; Schaible 1972; Schaible and Brumbaugh 1976; Petters and Markert 1979). In spotted or patterned animals there can be pigment spread in early life (Billingham and Silvers 1963). Where melanocytes of differing genotype exist in diffuse mixtures or discrete spots (e.g. allophenic and mosaic mice) such changes may relate to competition between melanocyte genotypes (Gordon 1977; Gearhart and Oster-Granite 1981) or preprogrammed early mortality (Mintz 1971; Gordon 1977).

In the neonatal mouse, extrafollicular melanocytes disappear from the trunk epidermis but they persist in the epidermis of the relatively hairless skin of the extremities (Quevedo et al. 1966; Hirobe and Takeuchi 1977). However, dopa-positive melanocyte numbers on

the trunk at 16 days postpartum (approx 50 per mm^2) increase 10-fold after exposure with ultraviolet light (UV) for six days (Quevedo et al. 1966). Fleet et al. (1993a) found melanocyte populations in white wool-bearing skin declined between birth and lamb shearing (5 months age).

1.10.2. Change in adult life

The melanocyte population of white human hair from "grey" individuals is greatly reduced if not completely absent. The surviving melanocytes are often vacuolated, have very little endoplasmic reticulum and golgi zone, contain few melanosomes and some are partly melanised with an affinity for dopa (Fitzpatrick 1965). In "greyed" human hair follicles that lacked melanin, as indicated by negative silver nitrate stain, there was evidence of tyrosinase mRNA or tyrosinase in the outer-root sheaths and between the follicle bulb and the outer root sheath (Takada et al. 1992).

Greying usually involves an increase with age in the number of follicles without melanocytes. However, in the murine *silver (si)* mutant there may be a decrease of greying with age on some colour backgrounds (Silvers 1979). In sheep, greying of a pigmented coat is an effect of some of the alleles at the *Agouti*, *Roan* and *Extension* loci (Sponenberg et al. 1996). Dilution effects also occur for some loci and alleles (e.g. recessive brown) or result from sunlight (Lundie 1989; Lauvergne et al. 1981b).

Brooker (1968) detailed changes in various types of non-wool pigmentation throughout life. In general, the degree and incidence of skin pigmentation increased during life, tan hairs on the ears and hoof pigmentation declined in early life, black hairs on the ears

increased, and pigmented hairs on the legs was similar in adult life. Some Merino sheep in later adult life develop black-grey pigmented spots in wool-bearing areas (Figure 6), which appear to be associated with a sensitivity of the skin to freckle (Figure 7) when exposed to sunlight. The freckles arise in response to sunlight and fade in its absence (between annual shearings) but the black-grey spots usually persist and may give rise to pigmented fibres (Kelly and Shaw 1942; Fleet and Forrest 1984; Forrest and Fleet 1986).

Freckles arise from a mosaic variation in the activity of epidermal melanocytes and not from an increase in numbers (Breathnach 1957). The histology of black-grey skin spots (Figures 8 and 9) showed varying stages of migration of altered epidermal melanocytes (active, extremely prolific which produce black pigment instead of tan) down the outer root sheaths to the follicle bulbs and eventually leading to production of pigmented wool fibres (Fleet and Forrest 1984; Forrest and Fleet 1986).

The "age-related" development of pigmented wool fibres (Forrest and Fleet 1986) provides evidence for pigmentation of follicle bulbs by migration of melanocytes from different compartments (epidermis or outer root-sheaths). These local increases in melanocyte density are junctional, with similarity to Senile Lentigo (Mishima 1967), unlike malignant melanoma that involves disruption of the epidermis basement membranes and invasion of the dermis (Schmoeckel et al. 1989). Nevertheless, Lentigo Senilis is one step closer than a "normal" melanocyte to development of a melanocytic condition and malignant melanoma (Mishima 1967). The reversion of cultured albino melanocytes to an active form through mutation has been observed (Jackson and Bennett 1990). A similar event may initiate dormant melanocytes or active tan "freckle" melanocytes to a prolific eumelanin producing form in "age-related" pigmented spots of sheep.

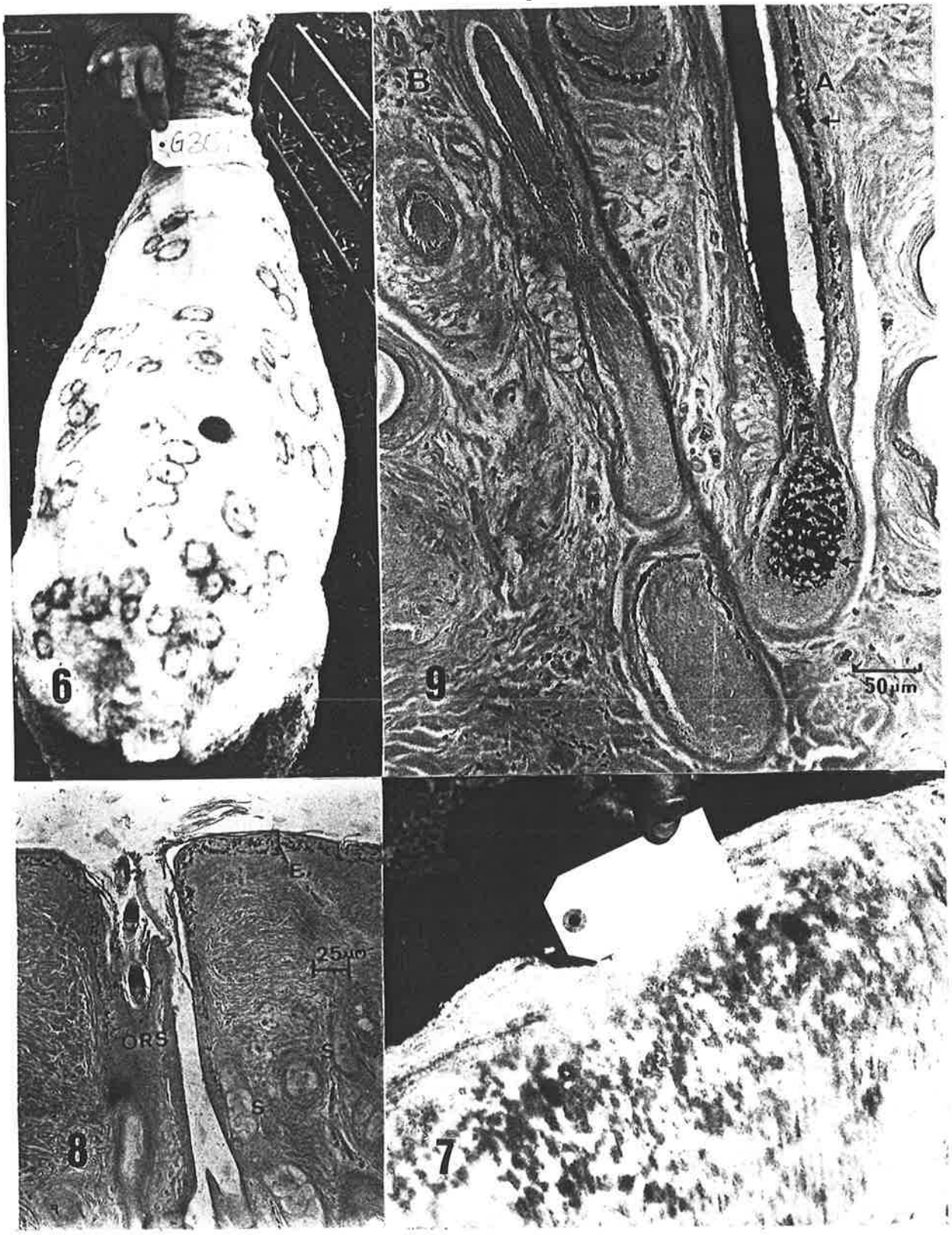


Figure 6: This Merino sheep (8.5 years old) had 75 black-grey pigmented skin spots (circled) most with pigmented wool fibres.

Figure 7: This 8.5 year old ewe was re-shorn 3 months later and showed gross freckling of the skin with the black-grey spots.

Figure 8: A vertical section through a black-grey skin spot showing pronounced pigmentation in the epidermis (E) and outer root sheath of a wool follicle to the level of sebaceous glands (S).

Figure 9: A black-grey skin spot showing a white wool follicle with pigment cells in the outer root sheath at B. The pigmented follicle alongside had melanocytes in the outer root sheath (A) and follicle bulb (arrow).

1.10.3. Trauma changes

Silvers and Russell (1955) with adult mouse skin and Rawles (1955) with foetal rat skin found that white spotted areas can support melanocyte invasion when transplanted to pigmented regions. Also, transplant of cultured melanocytes to white areas in the recessive spotted mouse led to pigmented skin and some pigmented fibres (Klaus 1979). Grafts of black wool-bearing skin to a white lamb (2 month old) showed pigment spread after 15 days from the graft into the adjoining margin of the white host skin (epidermis and outer root sheaths). After 25 days some pigmented wool fibres had developed and after 40 days many previously white host follicles on the margin of the graft had become pigmented (Hardy et al. 1952). Other grafting experiments of wool-bearing skin in adult sheep also demonstrated slow pigment spread to the host epidermis and then to host wool follicles after a long period (Ryder 1979; Lyne and Hollis 1968 1980).

Freezing the wool-bearing and hair-bearing areas on black Merino sheep led to permanent reduction in pigmented fibres (Lyne and Hollis 1968) as does freeze branding of horses (Gallagher - personal communication). However, in a white spot on a black Merino and in the white wool-bearing skin of Suffolk sheep, trauma by freezing, plucking or incision, led to generation of pigmented wool fibres. In both cases, the melanocytes nearest the follicle bulbs prior to the trauma were in the outer root-sheaths (Lyne and Hollis 1968).

In white Merino sheep, melanocytes are usually absent from the outer root sheaths and of low concentration in the epidermis (Forrest et al. 1985). When wool-bearing skin from normal white Merino sheep was frozen there was no induced pigmentation (Lynne and Hollis 1968). Staricco (1963) found that amelanotic melanoblasts in the middle to lower outer root-sheath of human hair can move to the epidermis, to become dopa-positive,

melanogenic and which proliferate, in response to trauma of plucking and dermabrasion or UV exposure. Grichnik et al. (1996) showed kit-reactive cells, distinctly different from Langerhans and Merkel cells, were present near the basement layers of the epidermis and in deeper follicular regions and constitute a precursor melanocyte reservoir of human skin. Horikawa et al. (1996) using immuno-techniques and premelanosome-related antigens reported that the majority of precursor melanocytes can be found in the mid to upper portion of the outer root-sheaths of human hair follicles. These precursor cells could not be identified with antibodies to tyrosinase, TRP-1, TRP-2 or the HMB-45 antibody that recognises a melanosome-associated cytoplasmic protein.

Hyperpigmentation of human skin after being grafted on to athymic nude mice was accompanied by a marked increase in the quantity of melanogenic enzymes and melanogenic peptides. Tyrosinase, TRP-1, TRP-2, were markedly enhanced one week after grafting and persisted until 4 weeks post-graft. Alpha-MSH and ACTH were detected in the epidermis soon after grafting and also in the dermis 2-4 weeks post-graft (Matsumoto et al. 1996).

1.11. COAT COLOUR

More than 60 distinct genes have been identified, largely as a result of basic studies of mice, that influence mammalian pigmentation. At this time, approximately 25% of those 60 genes have been cloned and characterised, and their general, often their specific, function(s) in affecting melanogenesis in mammals have been identified (Furumura et al. 1996). Coat colour is discussed in relation to the most widely recognised genes (*Agouti*, *Brown*, *Extension*, *Albino*, and Spotting genes) that determine pattern, colour or absence of pigmentation.

1.11.1. The *Agouti (A)*-locus

1.11.1.1 A locus alleles

The *Agouti (A)* locus controls the distribution (pattern) and amount of eumelanin and phaeomelanin over the coat and within individual hairs of mammals. The *A* locus has been highly conserved throughout evolution with variants of this locus being recognised in most mammalia (Siracusa 1991). In mice over 25 alleles are reported at the *A* locus (Siracusa 1991; Siracusa et al. 1995) and in sheep 20 alleles are proposed (Sponenberg et al. 1996).

The extremes of expression and dominance of the *A*-locus alleles in the mouse are dominant lethal yellow (A^y), which survives when heterozygous to give full extension of yellow over the coat, and recessive extreme non-agouti (A^{ae}) which in the homozygous state generates a completely black coat. A switch exists that determines whether eumelanin or phaeomelanin will be produced at any given time by hair bulb melanocytes. Apart from regional variation in the amount of tan or white and black over the coat, characteristic of different pattern types, the effect of the agouti switch (white or tan to black) can be visible within individual hairs (Cleffman 1963; Silvers 1979).

In sheep, the alleles of highest and lowest dominance designated at the *Agouti* locus are, respectively, *white or tan* (A^{Wt}), for full tan, white and tan, or full white (in the absence of tan), and *nonagouti* (A^a), for uniformly eumelanic or black coat without tan. Dominance of the intermediate alleles, that also give rise to recessive black lambs, increases with degree of white or tan (phaeomelanin) in the coat pattern (Brooker and Dolling 1969a; Adalsteinsson 1970; Sponenberg et al. 1996).

1.11.1.2. Basis of A locus function

Skin transplant experiments have provided evidence that action of *A*-locus alleles is from outside of the melanocyte within the microenvironment of the hair follicle. Furthermore, this action is independent of the *Agouti* genotype of the melanocyte (Silvers 1958 a,b; Silvers 1979). The *Agouti* locus in the mouse is on chromosome 2 (Silvers 1979) and was originally mapped using linkage homology nearby within an ectotrophic virus (Siracusa 1991).

Recent molecular genetic studies have found that the *A* locus makes a protein of 131 amino acids that prevents MSH signalling and switches the melanocytes from eumelanin to phaeomelanin synthesis (Bultman et al. 1992; Miller et al. 1993; Jackson 1993; Lu et al. 1994a; Willard et al. 1995; Furumura et al. 1996). Lack of production of the gene product results in black pigment only, over production in yellow pigment only, and wild-type expression results in a pulse of production midway through the hair growth giving the typical agouti banding (Jackson 1993). Mutations that up-regulate agouti expression, resulting in yellow coat, are dominant to those causing reduced agouti expression (Hustad et al. 1995).

The light-bellied agouti mutant (A^W) produces banded hairs on the dorsal surface and yellow or cream coloured hairs on the ventral surface. Animals that carry A^W express two sets of *agouti* mRNA isoforms. One of these isoforms is expressed only in the mid-portion of the hair growth cycle and accounts for the banded phenotype and the other isoform is expressed throughout the entire growth cycle but only in the ventrum (Vrieling et al. 1994; Millar et al. 1995; Furumura et al. 1996).

The most dominant allele at the murine *Agouti* locus, named *lethal yellow* (A^y), involves a chromosomal rearrangement resulting in over production of an abnormal-sized agouti protein (from a chimeric mRNA) that is expressed in all tissues. The A^y mutant is caused by a large deletion that involves an adjoining locus (Merc/Raly5) that plays a role in preimplantation development of the embryo (Bultman et al. 1992; Miller et al. 1993; Duhl et al. 1994a).

Another mutation producing an identical coat colour phenotype but without lethal effects, named *viable yellow* (A^{vy}), involves over expression of a normal-sized mRNA (Bultman et al. 1992). Four other dominant agouti mutants (A^{vy}, A^{iapy}, A^{iy}, and A^{sy}), causing yellow coat, have been shown to involve insertions (Duhl et al. 1994b; Perry et al. 1994). Molecular characterisation of some of the recessive alleles of the murine agouti locus are also reported (Bultman et al. 1994; Hustad et al. 1995).

The human homologue of the agouti gene, that has been associated with a form of maturity-onset diabetes of the young, has recently been mapped to human chromosome 20q11.2 and is 85% identical to the dominant yellow agouti mutations of the mouse (Kwon et al. 1994). However, Wilson et al. (1995) exclude the *Agouti* locus as a candidate for the locus for maturity-onset diabetes of the young (MODY1). While the human agouti gene product will produce yellow mice when expressed as a transgene the function of this protein in human coat colouration is not yet clear (references cited in Furumura et al. 1996).

1.11.1.3. Detection of recessive black

A requirement for a diagnostic test for identification of sheep that carry a gene that allows expression of recessive black coat patterns remains unsatisfied; leaving progeny testing as the only practical method available. Radiation studies with mice have induced mutations involving the *Agouti* locus that have caused lack of function and a *nonagouti* pattern (full black phenotype) when homozygous. For example, the *nonagouti lethal* mutation (A^{al}) involves a 75 Kb deletion relative to the wild type allele (A) of the *Agouti* locus (Siracusa 1991).

The ability to discriminate white or tan sheep that are carriers of a recessive gene for black lambs has been proposed as being possible in some other breeds. For example, in white Wensleydale sheep and white Blueface Maine sheep, a blue colouration of skin was proposed to be a characteristic of heterozygotes (Dry 1924; Lauvergne 1962). However, each comparison of known heterozygous sheep with control sheep (unlikely carriers) did not support the effective discrimination of carriers based on visible pigmentation. Lauvergne et al. (1979) have subsequently attributed the partial expression of an allele at the (Bl^B) at the *Blue* locus that allows production of eumelanin (bluish skin colour) on a white or tan (A^{Wt}) background, but this explanation is hypothetical (Sponenberg et al. 1996).

Vsevolodov et al. (1981) found a difference in the electron spin resonance of the wool from white ewes that produced a black lamb (heterozygotes) and control sheep that produced white lambs. This difference is reported to allow 80% effective discrimination of carriers from non-carriers in the Russian sheep breeds tested (i.e. Kazakh finewool

ewes crossed with Lincoln, Romney Marsh, Border Leicester and Tien Shen rams). However, this method used on wool from South Australian Merino sheep, known to be carriers of the gene for recessive black or control sheep (unlikely carriers), showed no significant difference between genotypes (Fleet and Lincoln 1984; Fleet et al. 1988). Furthermore, it was demonstrated that management differences and sex of sheep had significant effects on the electron spin resonance of white wool.

In Peppin Merinos (Brooker 1968) and South Australian Merinos (Fleet et al. 1989), some forms of black-grey skin (i.e. on the nose and lips, around the eyes and under the tail) and black-grey in the hooves showed significant differences ($P < 0.05$) between carriers and non-carriers of the gene for recessive black. However, the small magnitude of the differences and large degree of overlap between genotypes prevents practical distinction. This evidence was based on sheep generated in controlled matings to produce known heterozygotes (A^{Wt}/A^{a}) and other sheep with a high probability of being homozygous (A^{Wt}/A^{Wt}). Also there were no differences in the content of isolated pigmented fibres in fleeces of homozygotes and heterozygotes (Fleet et al. 1989) or the distribution of melanocytes in white wool-bearing skin (Forrest et al. 1985).

Molecular genetic studies are beginning to characterise the variation of DNA at the *Agouti* locus in the mouse (Miller et al. 1993) and human genome (Kwon et al. 1994) and the allelic differences at that locus (Siracusa 1991; Michaud et al. 1994). Following this knowledge and technology is experimentation with Merino sheep to locate the ovine *Agouti* locus and to identify differences in the DNA that are anticipated to be responsible for white or tan and nonagouti phenotypes in sheep (Fleet et al. 1995b). The gene responsible for recessive black (nonagouti phenotype) has shown linkage with markers on

ovine chromosome 13. DNA fragments from the *ovine Agouti* gene show a relatively high degree of homology at the protein and DNA level with those from the murine, human and bovine genomes (Parsons et al. 1997).

1.11.1.4. Pleiotropic effects related to the A locus

The murine A^y allele is lethal when homozygous and causes abnormalities like obesity (involving high insulin levels), reduced fertility (implantation failure and embryo mortality) and susceptibility to neoplasms, when heterozygous (Wolff and Bartke 1966; Silvers 1979; Wolff et al. 1986). Klebig et al. (1995) concluded that most, if not all, of the pleiotropic traits associated with the dominant yellow mutant of mice can be reproduced in an ectopic manner through transgenesis. Perry et al. (1996) concluded that the same functional domains of gene producing the mouse agouti protein that affect coat colour are also important for inducing obesity. This finding is consistent with the hypothesis that the agouti protein induces obesity by antagonising melanocortin binding to other melanocortin receptors.

Alleles for yellow hair (A^y, A^{vy} and *e*) in the mouse, interact with factors causing white spotting (e.g. *S*, *Bt*, *W*, and *Mi* loci) to reduce the unpigmented area relative to non-yellow litter mates (Dunn et al. 1937; Hauschka et al. 1968; Lamoreux and Russell 1971; Lamoreux and Russell 1979). The reduction of white spotting on a phaeomelanin background is contrary to the situation proposed for sheep (Lauvergne 1975).

Adalsteinsson (1970) concluded that white or tan (A^{Wt}/-) ewes have reduced fertility relative to those with eumelanin coats. The proportion of white or tan lambs was higher

for singles than twins (86.5% vs 80.2%) and there were fewer (-7.2%) white or tan lambs born than expected relative to black or brown lambs. He inferred that the reduced fertility involved embryo mortality or selective fertilisation that disadvantaged production of foetuses with the A^{Wt} allele. Drymundsson and Adalsteinsson (1980) also report that out of season breeding activity is less in white sheep than eumelanin coloured sheep. It is not clear whether these effects of reduced fertility are solely dependent on the A^{Wt} gene or involve the affects of other loci that produce extensive white spotting in its presence.

1.11.2. The *Brown (B)* locus

1.11.2.1. B locus alleles

The *B*-locus alleles other than *wildtype* (B^+) change eumelanin production, both epidermal and follicular, from black to brown when homozygous but there are no effects on phaeomelanin. In the mouse there are five *B*-locus alleles, namely B^{Lt} and B^W (*light* and *white-based* brown), B^+ (black or *standard*), (b^c) *cordovan* or dark brown, and (*b*) *brown*, in order of dominance (Silvers 1979). The *B* locus determines changes in the melanocyte as can be inferred from skin transplant experiments. Host skin of different genotypes had no effect on the colour of melanin production within *b/b* melanocytes that had migrated from the grafted (*b/b*) skin (Reed and Henderson 1940). The *b/b* genotype has greater tyrosinase activity than *B/B*, associated with high concentrations of T1 isozyme, but melanin production is reduced as a result of considerably less membrane bound T4 tyrosinase, and melanosomes appear pale, granular and spherical (Foster 1963; Moyer 1966; Holstein et al. 1967; Quevedo 1971). Tamate et al. (1989) found that melanocytes from brown (*b/b*) mice have 20% to 30% higher levels of mRNA for tyrosinase than black agouti mice.

Sponenberg et al. (1996) detail the various reports of brown fleece in sheep, including Nell (1967) that had been attributed to the *E* locus. In this report of the Committee for Genetic Nomenclature of Sheep and Goats (COGNOSAG), the accepted *B*-locus alleles for sheep are B^+ (for *wild*) and B^b for *brown*. Sponenberg (1990) suggested that recessive brown may have a selective advantage in white sheep flocks through paling of isolated pigmented fibres. However, Gregor et al. (1984) mated 62 black or grey Merinos originating from white Merino flocks to moorit rams and, from the progeny generated, the predicted gene frequency of the B^b allele in those flocks was 0.1.

1.11.2.2. Basis of B locus function

The product of the brown locus is a Tyrosinase-Related Protein (TRP-1) produced by the wild allele B^+ (Bennett et al. 1990; Tomita et al. 1991). When originally isolated the DNA for *TRP-1* was thought to encode tyrosinase (Shibahara et al. 1986) but was later found not to confer tyrosinase activity (Muller et al. 1988) and map to the *B* locus on mouse chromosome 4 and to human chromosome 9 (Jackson 1988; Abbot et al. 1991). A brown phenotype in humans involving a mutation and lack of expression of the TRP-1 gene is reported (Boissy et al. 1996).

Gregor et al. (1984) and Lundie (1989) report that brown lambs are dark at birth but, as they age, the wool of many of these sheep fades to almost white after 1-2 years. The TRP-1 protein shows catalase B activity that was proposed to convert hydrogen peroxide, produced during melanogenesis, to water and prevent the degradation of melanin and melanin precursors (Halaban and Moellmann 1990). More recently, TRP-1 has been

proposed as being involved in stabilising a melanogenic complex with tyrosine that prevents premature melanocyte death due to tyrosinase-related toxicity (Lu et al. 1994b).

The *Brown* locus is believed to have 5,6 dihydroxyindol-2-carboxylic acid (DHICA) oxidase activity (Tsukamoto et al. 1992; Jackson 1994; Jimenezcervantes et al. 1994; Kobayashi et al. 1994; Cassady and Sturm 1994; Furumura et al. 1996) though it has also been ascribed a dopachrome tautomerase activity (Winder et al. 1994b). Zaho et al. (1996) reports that TRP-1 stimulates the activity of tyrosinase and promotes melanogenesis.

Two alleles for dark brown, *cordovan* and *cordovan-Harwell*, make detectable low levels of TRP-1 mRNA (1% of wild allele) allowing some catalase production and protection of melanin. The dominant mutations at the *B* locus called *light* or *white-based* brown have hairs with a dark tip and white base that arise due to melanocytes in the hair bulb dying prematurely during the growth cycle. It has been proposed that the melanocyte death in the *light* mutation is due to a defect in the melanosomal membrane that normally allows the escape of toxic products of melanogensis. In the *white-based* mutant it is proposed that expression of a linked gene is triggered that causes a lethal affect on the melanocytes (Jackson 1991; Halaban and Moellmann 1993; Jackson 1994).

1.11.3. The *Extension (E* locus)

1.11.3.1. E-locus alleles

The *E*-locus alleles involve changes in phaeomelanin and eumelanin synthesis that arise from within the melanocyte. The highest allele at the *E* locus is *dominant black* (E^D)

which results in a black coat indistinguishable from the *nonagouti* (A^a/A^a) pattern. The other alleles are *sombre* (E^{so}) and *tobacco* (E^{to}), which do not completely mask agouti expression, E^+ for *wildtype* (i.e. no colour modification) and *recessive yellow* (*e*) which when homozygous suppresses dark pigment production. Unlike dominant lethal yellow A^y and viable yellow A^{vy}, the *e* allele does not confer undesirable pleiotropic effects (Hauschka et al. 1968; Silvers 1979).

Dominant black (E^D) is well documented in sheep (Nell 1967; Sponenberg et al. 1996). Other alleles proposed in sheep are *blackish* (E^{bl}), *brownish* (E^{br}) and *yellow* E^y identified in Tajik sheep (Aliev and Rachkovosky 1987) and E^j (*japanese brindling*) proposed for the Mouten Vendeen breed and other Downs breeds (Denis and Malher 1990; Malher and Denis 1990; Malher 1991).

The proposed alleles involve pronounced effects on the birthcoat with the fleece fading to white soon after birth. The American Tunis, Tajik, Degeres, Mouten Vendeen, Grivette and Suffolk are examples of breeds born with dark coats of dominant pigmentation where the fleece or wool-bearing areas fade to grey or white soon after birth (Nichols 1927; Bokenbaev 1964; Adalsteinsson 1983; Aliev and Rachkovosky 1987 1989; Denis and Malher 1990; Malher 1991).

1.11.3.2. Basis of E locus function

The murine extension locus is known to be located on chromosome 8 (Silvers 1979). In the horse, the *e/e* genotype gives rise to the Chestnut pattern. The *E* locus in horses is linked to loci for *Tobiano (To)*, *Roan* (*Rn*), *Serum Esterase* (*Es*), *Serum Albumin* (*Al*), and

Vitamin D binding protein (*Gc*). The horse group is thought to be homologous to the *e-Es* cluster of loci in the mouse (chromosome 8) and linkage group IV in the rabbit (Andersson and Sandberg 1982).

The fact that α-MSH induced eumelanin production in A^y/- mice but not *e/e* mice (Quevedo et al. 1981) led to the hypothesis that the *E* locus encodes the α-MSH receptor (Jackson 1991). The melanocyte α-MSH receptor gene (MC1R) has since been cloned (Mountjoy et al. 1992) and the pigmentation phenotypes of variant extension locus alleles result from point mutations within this gene that alter MSH receptor function (Robbins et al. 1993). The *recessive yellow* allele results in a non-functional MSH receptor so the melanocyte defaults to a yellow pigment. In the *sombre-3J* mutation, the receptor is constitutively 60% active whereas the *tobacco* mutation has a hyperactive receptor. The dominant mutations at the *E* locus signal eumelanin production even in the absence of MSH (Robbins et al. 1993; Jackson 1993 1994).

The presence of variants for the MC1R gene in humans and an association of these variants with red hair and fair skin has recently been reported (Valverde et al. 1995b). In horses the molecular characterisation of the *e* mutation giving rise to the Chesnut pattern involves a single missense mutation (83Ser to Phe)(Marklund et al. 1996). Variations in the MC1R gene in chickens are associated with proposed *E*-locus alleles producing either uniformly yellow-red or black pigmentation (Takeuchi et al. 1996). The *E* locus has also recently been mapped in the pig genome to the short arm of chromosome 6 (Mariani et al. 1996).

1.11.4. The *Albino* (*C* locus)

1.11.4.1. C-locus alleles

The term albino was used by a Portuguese explorer in 1660 to describe white negroes in Africa and comes from the Latin *albus* or white (Fitzpatrick and Quevedo 1966; Witkop 1971; Hamori 1983). In this context, the term has been used to include a wide variety of phenotypes which show a deficiency of pigmentation (e.g. Hamori 1983; Fitzpatrick and Quevedo 1966; Witkop 1971).

Silvers (1979) made the distinction between lack of pigment due to the effects of white spotting genes, that act by causing a deficiency of melanocytes, and true albinism where a full complement of melanocytes is present but there is a defect in melanin synthesis involving tyrosinase. The classic albino (*c/c*) shows no visible pigmentation at any site resulting in pink eyes due to the translucent iris revealing the capillary bed of the choroid and retina. Amelanotic melanocytes are both tyrosine and dopa-negative but can be identified by light microscope and electron microscope (Silvers 1979; Eady et al. 1983).

Adalsteinsson (1977 1978) reported the occurrence of complete albinism in Icelandic sheep. These animals showed no pigment of the coat though genetically coloured, the eyes were bluish-pink and photophobic to the extent that vision was obviously impaired in bright light, and the mode of inheritance was recessive. This mutant allele has been given the symbol C^c (Sponenberg et al. 1996).

Recently another albino sheep has been documented within South Australia in a Suffolk flock. The proposed allele has been named *albino marrabel* (C^{cmar}) and has been allocated to the *C* locus. This allocation of the proposed allele to the *C* locus is based on the

phenotype, apparent mode of inheritance (consistent with being an autosomal recessive) and characteristic histochemistry (Witkop et al. 1972). The *albino marrabel* has an almost completely white coat and bluish-white eyes, with a red reflex in reduced light, though some diffuse tan pigmentation may be evident or develop in the hooves or around the edge of the iris at the pupil opening and the leg hairs are a pale yellow (Rowett and Fleet 1993). This phenotype is similar to an allele for blue-eyed albino (c^a) in cats, that is allelic to the heat sensitive *siamese albino* mutant (c^s) (Turner et al. 1981).

1.11.4.2. Pseudo-albino phenotypes

A distinction between recessive complete albinism (causing white coat and white-pale blue irides) and other forms of hypopigmentation in cattle was made by Greene et al. (1973). The dominant forms in cattle (Leipold and Huston 1966 1968a,b) had occasional pigmented body spots, irides that varied from grey to blue and occasionally with a brown fragment, and pigment in the posterior layer of the iris; although reduced and abnormally distributed. The external colour of the iris, general white coat, and similar ocular fundus reflections can cause mistaken identity to tyrosinase related albinism.

Dysfunction of a recessive gene other than for tyrosinase was realised when normal progeny were born to albino parents. It was found that a tyrosinase positive form of albinism existed that produced a similar albino effect (Witkop 1971). The gene for tyrosinase-positive albinism (oculocutaneous albinism type II) on human chromosome 15 (Ramsay et al. 1992) is homologous with the murine *pink-eyed dilute* (*p*) gene (Nakatsu et al. 1992; Gardner et al. 1992; Kedda et al. 1994; Brilliant et al. 1994; Lee et al. 1995). The *p* locus encodes a melanosomal membrane protein (Rosemblat et al. 1994).

1.11.4.3. Basis of C locus

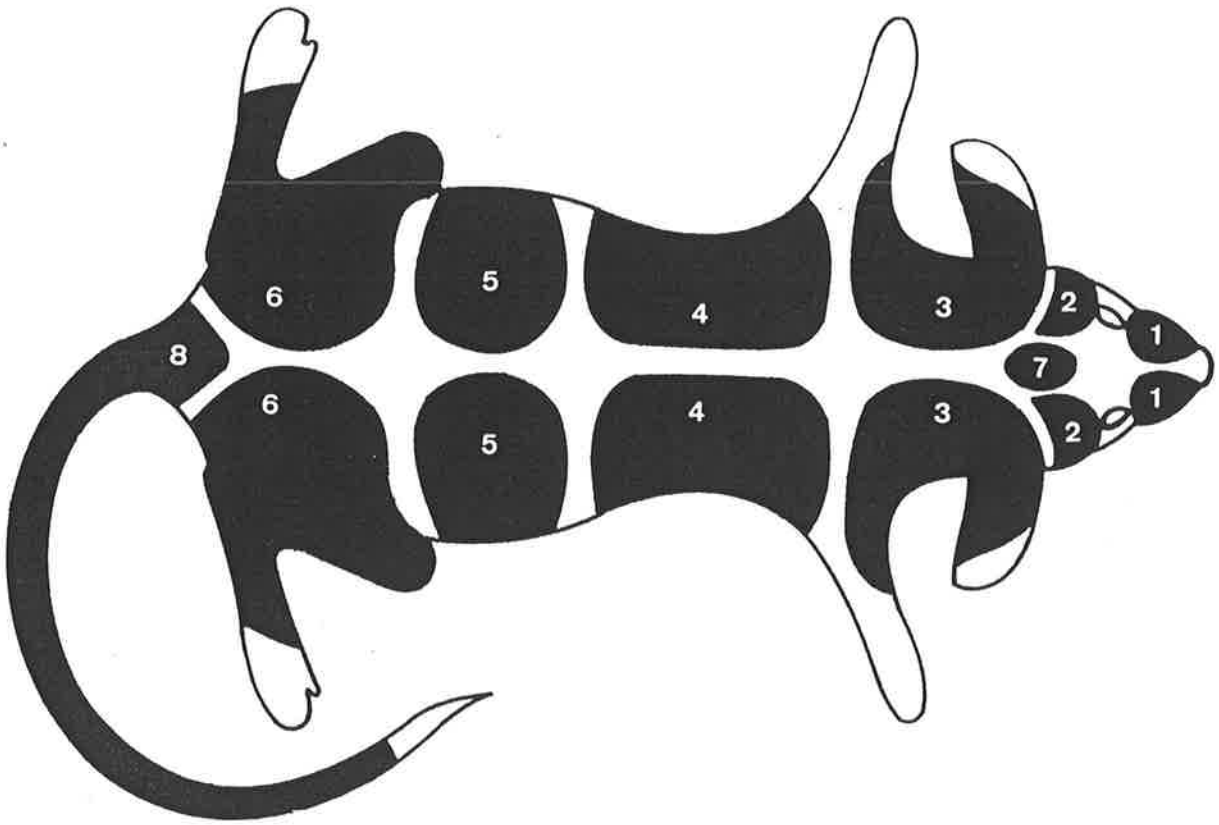
The gene encoding tyrosinase maps to mouse chromosome 7 (Kwon et al. 1987) and human chromosome 11 (Barton et al. 1988). Mouse tyrosinase is predicted to consist of 533 amino acids and have a molecular weight of 60,536 (Muller et al. 1988). Linkage between the albino *C* locus and the *Haemoglobin beta* locus (7.5 to 9 cM apart) exists in mice, rats, cats and rabbits (Sandberg and Anderson 1987; O'Brien et al. 1986). However, in humans *Haemoglobin beta* is on the short arm of chromosome 11, close to a tyrosinase-related DNA segment, while the *C* locus is on the long arm of chromosome 11 (Chaganti et al. 1985; Giebel et al. 1991). In cattle, a probe for human tyrosinase was not syntenic with *Haemoglobin beta* but did hybridize to a fragment that was syntenic with lactate dehydrogenase A (Foreman et al. 1994). The *Brown* locus is linked to the *C* locus in the Syrian hampster (28.4cM to 32.3cM) and rabbit (42.8cM) whereas in rodents they are on different chromosomes (Robinson 1973; Sandberg and Andersson 1987).

Many of the human *C*-locus mutations (Oetting and King 1992; Tomita 1993) and those in the mouse (Muller et al. 1988; Beermann et al. 1990; Kwon et al. 1989a; Shibahara et al. 1990; Halaban and Moellmann 1993; Schmidt and Beermann 1994; Juriloff et al. 1994) have been found by gene sequencing to arise from point mutations. In humans, twenty seven *C*-locus mutations (involving oculocutaneous albinism type I) have been characterised. They involve no melanin production (24), small to moderate amounts of melanin (2), or unusual pigment (1) patterns (Tomita 1993). Type I albinism that involve a partially active or unstable tyrosinase exist in other animals and include the *chinchilla* and *platinum* mutants in the mouse (Silvers 1979; Orlow et al. 1993; Beermann et al. 1995) and heat sensitive forms, including the murine *himalayan* mutant (Kwon et al. 1989b), the *siamese albino* in cats and a human mutant (Giebel et al. 1991).

1.12. WHITE SPOTTING

1.12.1. Pattern formation

Pigment deficient areas in the skin of mice involve over 20 distinct loci. As a result of studying crosses between mice with different spotting mutants a hypothesis developed that involved 14 pigment centres from which clonal expansion and migration occurs to fill in the void areas of skin (Figure 10). It is argued that the clearly defined white areas in some spotting mutants separate clones of melanocytes of different primordial origins and are deficient of pigment cells due to restricted proliferation (Schaible 1963 1969).



Bilateral centres: 1=Nasal, 2=Temporal, 3=Aural, 4=Costal, 5=Lumbar, 6=Sacral
Medial centres: 7=Coronal, 8=Caudal

Figure 10: A diagram of the 14 primary sites of melanoblast colonisation in the mouse (Schaible 1963 1969)

Each of the pigment centres can be traced back to a primordial melanoblast in the neural crest that is preprogrammed for destination and differentiation (Schaible 1963 1969). Further to interpretations by Schaible (1963 1969), other studies of allophenic mice involving the artificial fusion of two eight-cell embryos with differences in spotting genes, albinism or other pigment markers (Mintz 1967), provided evidence consistent with there being 34 primordial centres of melanoblasts (head, 3 pairs; body, six pairs; and tail, eight pairs). However, Schaible (1972) suggested that a doubling of the number of pigment areas could be an effect of fusing embryos, through increased cell numbers, involved in producing the allophenic animals.

Schaible (1972) suggested that, in general, small animals have fewer pigment centres than large animals. He cited the cow as an example, which is proposed to have 36 primary pigment centres including 2 sites on each lower leg (Figure 11). Searle (1968) suggested that the tail tip, forehead, feet and mid-ventral region are especially prone to exhibit white spotting due to distance from pigment centres (Searle 1968).

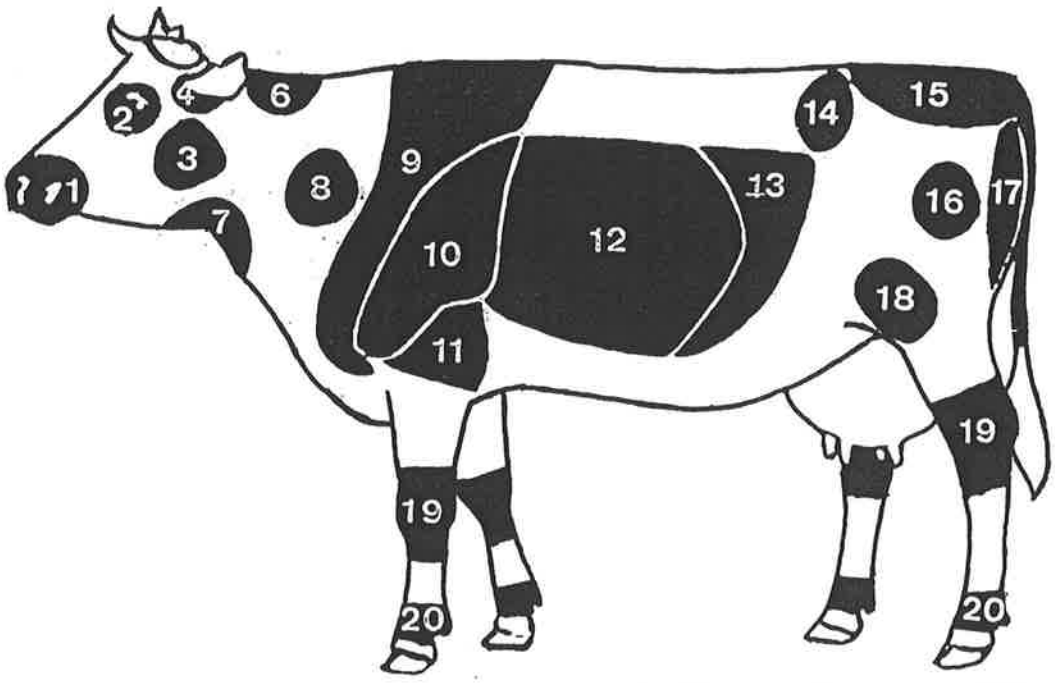


Figure 11: Pigment centres of cattle (Schaible 1972 from Lauprecht 1931)

1.12.2. White spotting in mice

1.12.2.1. Recessive spotting (S locus) alleles

Two of the recessive alleles causing white spotting at the Piebald or *Spotting* (*S*) locus of the mouse are *piebald* (*s*) and *piebald lethal* (s^l) (Silvers 1979). Other induced mutants involving the *S* locus are described by Metallinos et al. (1994). Spotting is extremely variable and ranges from an entirely or almost entirely depigmented coat, or white collars, to a few white spots on the belly, feet or forehead. There is clear demarcation between pigmented and non-pigmented areas.

Doolittle et al. (1975) provided a historic account of selection for enhancement of white spotting. Heritability for spot score for the first 18 years of selection was 0.19 with spots confined to the head. During the next 10 year period, spotting also developed on the body and the heritability of spot score was higher (0.51). Brooker (1968) showed a similar enhancement of white spotted areas arising from selection in recessive black Merinos.

The wide variation in spotting has been previously attributed to a group of modifiers termed the *k-complex* (Silvers 1979). Recent molecular genetic studies have characterised nine induced mutations involving the murine *S* locus. These variants involved specific deletions around this locus that reflect varying degrees of effect on the coat (Metallinos et al. 1994). However, variation in extent of recessive spotting in the mouse has also been identified as effects of six other modifier genes on chromosomes 2, 5, 7, 8, 10 and 13. The loci differed in their spatial contribution to spotting on the dorsal versus ventral surfaces on mice. The loci on chromosomes 2 and 5 had non-additive effects (Pavan et al. 1995).

1.12.2.2. Basis of S locus function

In the recessive spotted mouse, melanoblasts migrate to all areas of skin but fail to differentiate in the white-spotted areas due to a limiting factor(s) in the epidermis. This limitation occurs at around 11 days of gestation but is temporary. If migration is delayed to 13 days of gestation then some pigmented hairs develop despite inhibition of epidermal pigmentation and by 16 days gestation conditions are suitable for both epidermal and follicular pigmentation (Mayer 1967 a,b).

The *s/s* genotype involves a low frequency of deaths (10%) due to megacolon while the homozygotes for piebald lethal allele (s^l) invariably die soon after birth due to the same condition. The megacolon is caused by paralysis of the digestive tract resulting from a deficiency of myenteric ganglia which, like melanocytes, are of neural crest origin (Jackson 1991). This affect on the myenteric ganglia of the digestive tract is not unlike the condition described for grey and white Karakul sheep (Nell 1967; Groenewald 1992; Groenewald and Booth 1992) and human Hirschsprung disease (Pavan et al. 1995; Ceccherini et al. 1995).

The murine *S* locus has been mapped to the distal half of chromosome 14 and is 9.1 $\pm$ 5.4cmM from the *Slaty* locus (Metallinos et al. 1994) and shows highly significant linkage with the endothelin-B receptor gene (Ceccherini et al. 1995). Nine induced mutations characterised in the region of the *S*-locus showed the extent of the deletions involved was initially associated with the severity of the homozygous phenotype (Metallinos et al. 1994). Targeted disruption or mutations of the gene producing endothelin-B receptor also produce congenital megacolon and pigment abnormalities in mice, rats and humans

(Hoscoda et al. 1994; Gariepy et al. 1996). Crossbreeding studies show the two genes are not complementary, that *piebald lethal* exhibits a recessive phenotype identical to endothelin-B gene knockout mice while the milder piebald (s) allele, that produces coat colour spotting only, expresses low levels of structurally intact endothelin-B mRNA and its protein (Hosoda et al. 1994).

1.12.2.3. Dominant White (W) and Steel (Sl) mutants

White spotting alleles at the *Dominant White* (*W*) locus in mice involve phenotypes where melanocytes were thought did not migrate to produce pigment. The pigmented regions that occurred on these animals (e.g. skin pigment) were thought of as representing normal melanocytes from an unaffected neural crest clone or secondary wave of migration (Mayer 1973 1979). However, Cable et al. (1995) using more specific labelling for migrating melanoblasts found that *W*-locus mutations do not alter early migration or differentiation of melanoblasts but severely affect melanoblast survival. White spotting alleles at the *Steel* (*Sl*) locus involve a tissue environment that will not support melanocytes (Mayer 1973 1979).

The *W* locus on mouse chromosome 5 is homologous with the *c-kit* oncogene on human chromosome 4 that causes dominant piebald. There is linkage homology with other white spotting loci including: *Tobiano* and *Roan* in horses, *Dominant White*, *Patch* and *Roan* in the pig, and *Patch* and *Rump-white* in the mouse (Johannson et al. 1992).

Germ cells and haematopoietic stem cells are other types of migratory cells affected by the *W* and *Sl* gene products and their deficiency leads to anaemia and sterility. The

effects of *W* alleles vary from no pigment, severe anaemia and infertility when heterozygous and lethal when homozygous for the highest allele, to white patches with mild anaemia and normal fertility of the lowest allele when homozygous. This trend of reduced severity with alleles of lower dominance is referred to as "dominant-negative" (Jackson 1994). Molecular characterisation of several mutations at the *W*-locus are reported (Nagel et al. 1995; Paquette et al. 1996; Fleischman et al. 1996).

The *W* locus encodes the tyrosine kinase membrane receptor protein *c-kit* which is signalled by the *Steel*-locus (SF) protein. The SF protein is also known as mast-cell growth factor, stem-cell factor and *c-kit* ligand. The *kit* signalling by the SF protein is clearly essential and a deficiency of either results in a failure of embryonic development of melanoblasts, haematopoietic stem cells and primordial germ cells (Fleischmann 1993).

The *Sl* allele results in complete loss of melanocytes in the white spotted areas and is lethal when homozygous. The *steel dickie* (Sl^d) allele is viable when homozygous or heterozygous with other *Sl* mutants and allows melanocyte migration to the skin (Jackson 1993; Fleischmann 1993; Spitz et al. 1993; Jackson 1994). The *steel panda* is another viable murine mutation but when homozygous it disrupts ovarian follicle development. Expression of RNA for kit-ligand (SF) is reduced in *steel panda* (Besmer et al. 1993).

Steel Factor is required for the early development of melanoblasts after migration. It also suppresses programmed cell death of primordial germ cells in culture and a similar mechanism probably operates in melanoblasts (Jackson 1994). Lahav et al. (1994) provide evidence in support of the notion that stem cell growth factor (steel factor) sustains the survival of neural crest derived cells and stimulates the rate of melanogenic

differentiation. Yoshida et al. (1996) demonstrated expression of a transgene, incorporating the lacZ reporter gene under the control of fragment of the steel factor that is essential for development of melanocytes and other neural crest derived cells, in the enteric ganglion cells of the intestine and in the dermal papillae of the hair follicle. They suggest that the transgene expression in the dermal papilla is suggestive of a role in supporting *c-kit* dependent growth and development of melanocytes.

Two forms of SF are produced; one soluble and one membrane-bound. The *Sl* allele produces neither form of SF but the *steel dickie* allele produces the soluble form and allows mouse survival. SF may act as a proliferative signal, a homing mechanism for migration, or a factor critical for clonal survival. SF levels are highest at the destination site of the migrating cells. While migration of melanoblasts is normal in Sl/Sl^{d} and Sl^{d}/Sl^{d} mice, the survival of melanocytes requires the membrane-bound SF-factor (Fleischmann 1993; Halaban and Moellmann 1993). The membrane-bound SF not only stimulates melanocyte proliferation but also mediates cell-cell adhesion (Flanagan et al. 1991).

1.12.2.4. Other white spotting loci

The *Patch (Ph)* and *Rump-white (Rw)* loci are closely linked to the *W* locus and produce clearly defined bands around the trunk (*Ph*) and a white spotted hind (*Rw*) when heterozygous and are lethal when homozygous. Mice with the allele for *Patch Extended* ($Ph^{e}/+$) are white except for the head and shoulders producing a pattern similar to *Pigmented Head* in sheep. The white spotted areas on *Ph*/+ mice, like dominant white, contain few melanocytes (Silvers 1979). In *Rw*/+ mice, epidermal melanocytes are common and may indicate that delays of migration have caused white spotting or the

melanocytes may arise from a secondary migration (Silvers 1979). Murine *Rump-white* involves a large chromosomal inversion that was thought could disrupt *W*-locus and *Ph*-locus regulatory sequences (Stephenson et al. 1994; Nagle et al. 1994) though recent evidence suggests that Kit and PDFR-R-α continue to express and that homozygotes die around 9.5 days of gestation (Bucan et al. 1995). The *Ph*-locus encodes a receptor tyrosine kinase for platelet derived growth factor (PDFR-R-α). Other receptor tyrosinase kinase genes (*KDR* and *Flt3/Flk1*) from humans, that map to mouse chromosome 5, are candidates for the murine *Rump-white* spotting locus (Halaban and Moellmann 1993).

The *Splotch* locus is on murine chromosome 1 and when homozygous for the *splotch* allele (*Sp/Sp*) is lethal (around 13 days gestation). The *splotch* allele prevents melanoblast clone development and heterozygotes (*Sp/+*) display white spotting on the belly and sometimes the back that results from inviable melanocyte clones (Auerbach 1954; Markert 1960; Mayer and Maltby 1964). *Splotch* encodes the HuP2 protein. This locus is possibly related to the locus for Waardenberg syndrome on human chromosome 2 (Hearing 1993). Details about the molecular lesions that give rise to *splotch* mutants are provided in Jackson (1994) and Fleming et al. (1996).

In the belted mouse (*bt/bt*) there is usually a pigmented spot located dorso-laterally in the white belt region. This spot has been interpreted as the primordial centre for the pigmentation of the belt region. Selection for wide belt leads to the elimination of this spot (Schaible 1969 1972). Epidermal melanocytes are common in the white areas of the belted mouse but melanocytes are absent from the hair follicles. The lack of fibre pigmentation is presumed due to an inability of melanocytes to gain access to the follicle bulb or their inability to persist in that environment (Mayer and Maltby 1964).

White spotting induced by the *Micropthalmia* locus on mouse chromosome 6, with the alleles (Mi^{wh} *or mi*), involves either the prevention of clonal development of melanoblasts or the premature death of melanoblasts during migration. When homozygous these alleles produce a white coat and when heterozygous with the *wildtype* allele ($Mi^{wh}/+$ or $mi/+$) a spotted coat develops (Silvers 1979; Jackson 1994).

Based on the above examples, it is evident that the mechanisms involved in white spotting vary. White spots can occur as a result of inviable melanoblast clones in the neural crest, the premature death of melanoblasts, an inability of melanoblasts to differentiate and colonise the skin, delayed migration and inability to pigment follicles after arrival in the skin. The effects of different white spotting mutants are often additive increasing the degree of spotting. Even though the separate spotting effects of different loci may provide only partial depigmentation of the coat (e.g. $Ph^e/+$ or $W^v/+$) when acting together they can result in complete depigmentation (Silvers 1979).

1.12.3. White spotting in sheep

1.12.3.1. Genetic background of white sheep

The most dominant allele (A^{Wt}) at the *A* locus inhibits the production of black or brown (eumelanin) pigmentation of the coat but allows production of red or tan pigment (phaeomelanin). Sheep carrying the A^{Wt} allele will either be fully tan, tan piebald or have a completely tan coat; the amount of tan being given by genes at other loci (Adalsteinsson 1970; Adalsteinsson and Wardum 1978; Adalsteinsson et al. 1980; Lauvergne et al. 1981a). Adalsteinsson (1970) reported a case of a mutation from black and tan (A^t) to white or tan (A^{Wt}). Adalsteinsson et al. (1980) suggest that a similar mutation could have

occurred during the early domestication of sheep and together with the effects of other loci (e.g. *S* locus) selection for extension of white areas on the tan coat was pursued.

1.12.3.2. S locus interpretations for sheep

Spotting from the *S* locus is recognised as allowing the opportunity for broken colour in sheep of European origin, including Black Welsh Mountain, Jacob, Icelandic, Corsican, Berrichon, Solognot, Bizet and Merino d' Arles sheep Australian Merino, Italian Merino, and Upper Visso sheep (Renieri et al. 1989; Sponenberg et al. 1996). Lauvergne (1969 1975) suggested that the white spotting (blaze, tail-end and socks) in the dominant black Bizet breed was due to the same gene (S^b) that produced white coat in the Berrichion breed (S^b/S^b) and tan piebald (S^b/S^+) when crossed to solid tan coloured Solognot sheep (S^+/S^+). The S^b allele was found to be dominant to S^+ with 50-100% penetrance in the Bizet breed and near complete penetrance in the Solognot x Berrichion sheep. Without data to confirm the separate effects of S^b it has been grouped with other white spotted effects attributed to the *spotted* (S^s) allele (Sponenberg et al. 1996).

In a study of colour patterns in Corsican sheep, the hypothesis that S^s was dominant on a tan background and recessive on recessive black (e.g. A^a/A^a) background was found to be acceptable (Lauvergne and Adalsteinsson 1976). The differential expression of S^s, on backgrounds of different colour, was further supported by examination of coat colour in the Merino d'Arles (Lauvergne et al. 1981a). Acceptance of the *S* locus as the main locus allowing broken colour in European sheep breeds implies that the degree of effect of S^s and relative dominance varies depending on the genetic (Agouti, Extension) and colour (eumelanin and phaeomelanin) background which may be over simplistic in view of the number of loci and alleles producing such white spotting effects in mice.

1.12.3.3. Other white spotting in sheep

For Asiatic sheep and some European breeds there is conflict in the literature about the mode of inheritance and relative dominance of white patterns. Examples of evidence consistent with a simple recessive mode of inheritance of white spotting include 'Anon' (1963) for Ouda ewes mated to Merino rams, Kijatkin (1968) for crosses between Karakul and Afgan Pied sheep, Cooper (1966) for Merino sheep and Perepelicina and Tapiljskii (1967) for matings between Corriedale rams and Jaidira x Merino F1 ewes.

Examples of evidence consistent with a simple dominant mode of inheritance of white spotting include Lauvergne (1969 1975) from crosses between Berrichion and Solognot breeds, Lauvergne and Hoogshagen (1978) for Texel and Zwartbles breeds, Nell (1967) for the Karakul and Persian White breeds, Singh and Singh (1971) and Singh and Chaudhary (1972) for Chokla sheep mated to Merino, Malher (1988 1991) and Denis and Malher (1990) for Downs breeds (Mouten Vendeen, Grivette and Suffolk).

Figure 12 (Lauvergne 1969 1975) shows the range of white spotting evident from crosses between the Berrichion breed that is dominant black with white forehead blaze and the Solognot breed that is solid tan. These patterns are attributed to the *Spotting* locus, where the depigmentation involves the legs and head before other areas. In contrast, Figure 13 shows white spotting patterns evident in crosses between the Downs breed, Mouten Vendeen and the Solognot breed. These patterns of spotting, in which the dorsum is affected before the head and legs, are attributed to a dominant allele at the locus *Depigmentation laterale (Dl)* (Malher 1988; Denis and Malher 1990). However, COGNOSAG recognises that such lateral depigmentation could equally be allocated to the *Spotting* locus (Sponenberg et al. 1996).

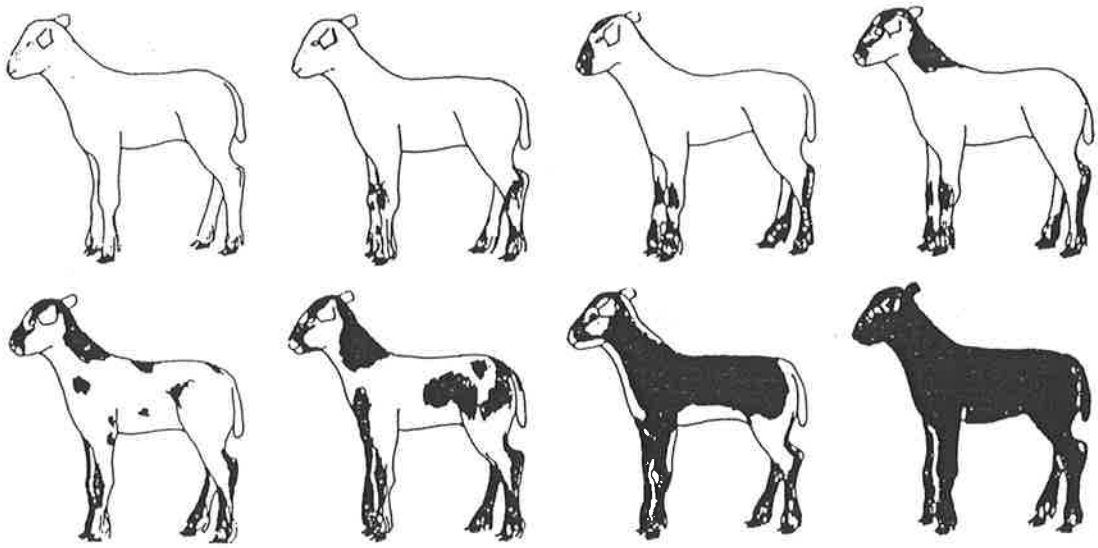


Figure 12. White spotting in Berrichion x Solognot progeny (Lauvergne 1969 1975)

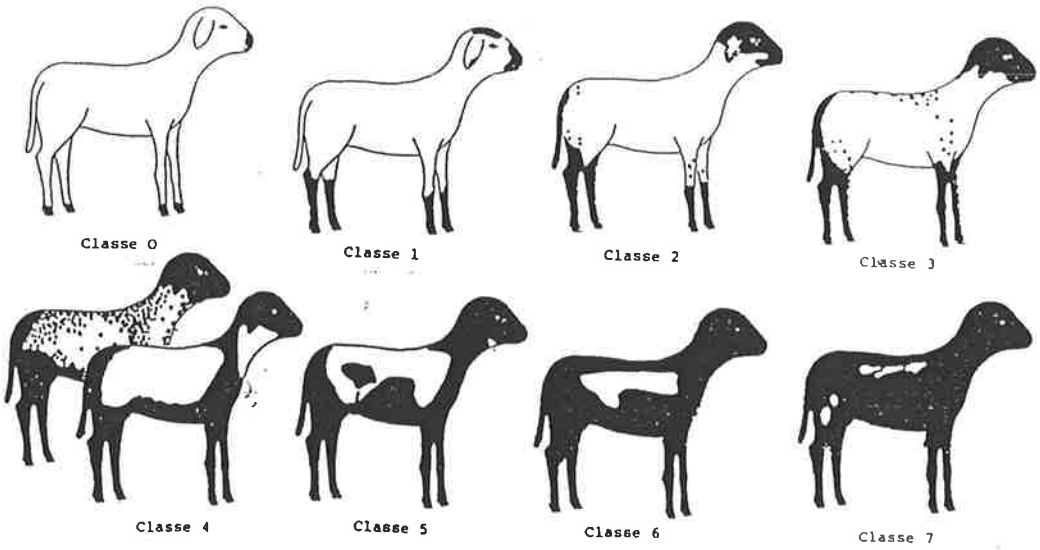


Figure 13. Classes of white spotting in progeny of Mouten Vendeen x Solognot (Malher 1988; Denis and Malher 1990)

Evidence consistent with a multi-genic explanation of white spotting includes Kijatkin (1957) for Fat Rump ewes mated to Lincoln rams, Duzgunnes et al. (1960) and Lauvergne (1976) for white Karaman sheep mated to German Mouten Merinos, 'Anon' (1963) for Yansa ewes mated to a Merino ram and Schmitz (1968) for Karakul sheep.

The dominant genes *roan* and *dominant white* impart sub-vital effects. Nell (1967) presented segregation data that show these two gene effects are allelic rather than epistatic. Sponenberg et al. (1996) suggest that the *afghan lethal* allele, proposed for the *Pigmented Head* locus, may be Nell's *dominant white*. Many homozygotes for these dominant lethal genes can die soon after birth but actual mortality is variable. This variation is perhaps due to a modifying complex or recombination if the lethal effect is due to a closely linked gene (Nell 1967; Sukharkov and Pliev 1992). Baatar (1990) reported that lethal grey (*roan*) also occurs in the Grey Mongolian sheep, Turcana and Sokolki sheep breeds. In a study of foetal weights of Karakul lambs it was found that black were heavier than grey and grey heavier than Sur[A] (Iosunov and Mamatkazin 1990). The lethal effect of *dominant white* and *roan* involves paralysis of the digestive tract (Nell 1967; Groenewald 1992; Groenewald and Booth 1992) resulting from a deficiency of myenteric ganglia which, like melanocytes, are of neural crest origin.

In view of the situation in the mouse (Silvers 1979), the many phenotypes evident within and between various breeds of sheep, it is likely there are many white spotting loci and alleles that can interact and confuse the interpretation of genotypes for pigmentation types; especially on predominantly white sheep. The loci currently accepted by COGNOSAG as involved in white spotting on the sheep coat (Sponenberg et al. 1996) are *Spotting (S)*, *Pigmented Head (PH)* and *Roan (Rn)*, though a number of other loci are proposed.

[A] Sur is an affect of delays in the initiation of pigmentation in the foetus resulting in a birthcoat where the fibre tips are weakly pigmented (white or rarely yellow) and the fibre bases are fully pigmented. Sur effects are attributed to two recessive genes (*Sur Bukhara* and *Sur Surkhandarya*) at different loci; though possible allelism can not be excluded (Sponenberg et al. 1996).

The relationships between types of white spotting and their molecular genetic homology with characterised white spotting genes in other mammals (e.g. Halaban and Moellman 1993) would clarify the origins of white spotting and white fleece in sheep.

1.13. PIGMENTED SPOTS ON WHITE COATS

1.13.1. Somatic change

Somatic mutations of pigment cells of the embryo give rise to random variations in pigment phenotype in the form of spots on the coat. Large numbers of somatic cells forming the coat are available per individual for spontaneous or induced mutations. While such changes, in any zone of proliferation (e.g. neural crest, skin), during mitosis are most likely to involve one or few bases (e.g. a molecular deletion or insertion) within a gene, other spots may arise from reciprocal recombination or chromosomal aberrations. However, only those changes that could survive several mitosis would cause a spot with the expression of the recessive allele. It is possible that many deletions are lethal in germ cells but survive in somatic cells and produce spot changes in the coat (Fahrig and Neuhauser-Klaus 1985).

1.13.2. Australian Piebald (*AsP*)

The Australian piebald locus (*AsP*) is proposed to explain the occurrence of one or more rounded spots of light grey to black fibres in an otherwise white coat of Merino sheep. (Brooker and Dolling 1969b; Sponenberg et al. 1996). The spots vary in size, may be located anywhere on the coat and typically display positional asymmetry if there is more than one spot (Brooker and Dolling 1969b; Fleet et al. 1985; Fleet and Smith 1990).

The penetrance of this proposed recessive allele *piebald* (*AsP*^{p}), responsible for the black-grey spots, is reported to be low and widely variable. Mating of piebald rams to piebald ewes produced between 0 and 45% of affected progeny (Brooker and Dolling 1969a). The low penetrance suggests a multi-genic inheritance and the phenotype resembles somatic changes perhaps with an inherited liability involving melanocytes (migration time, colonisation, numbers). The proposal for an identifiable locus that results in sheep with such random pigmented areas is questionable given the evidence available.

The white fleece wool from Merino sheep with the Australian Piebald phenotype usually does not have a high incidence of pigmented fibres. Nevertheless, such random spots represent a serious fault since they may not be removed completely, due to oversight or not being visible (greyed with age), allowing pigmented fibres into the white wool line (Fleet and Smith 1990). There is no relationship documented between "age-related" pigmented spots occurring in old Merino ewes and piebald or black lambs occurring in their progeny (Fleet et al. 1985).

1.13.3. Other forms of spotting

The Dalmatian dog is born with a white coat and pigmented spots develop after birth. The delayed pigmentation is in the form of small spots distributed as if migration was from the end of nerve fibres or blood vessels (Schaible and Brumbaugh 1976). Adalsteinsson (1983) and Dolling (1989) reported the occurrence of small pigmented spots developing after birth in the white spotted areas of recessive black sheep.

White spotting can also be associated with hypopigmentation or heterochromia of the irides and deafness and, or, reproductive loss. Examples of such reports include the following species; Dalmatian dogs (Greibrokk 1994), other white spotted dogs (Sorsbey and Davey 1954; Gwin et al. 1981), white cats (Bergsma and Brown (1971), white horses (Pulos and Hutt 1969), white spotted cattle (Leipold and Huston 1968 a,b), white foxes (Belyaev et al. 1975), mice (Cable et al. 1994) and some white Merino sheep (Lang 1995). For Merino sheep (Lang 1995) and Dalmatian dogs (Griebrokk 1994) a recessive autosomal allele is proposed as implicated, in conjunction with white spotting factors, to produce heterochromia irides or a blue-eyed effect.

Terrill (1947) studied the occurrence of pigmented fibre spots on the legs of white Columbia and Targhee sheep and reported a heritability of 0.26 $\pm$ 0.05 and 0.34 $\pm$ 0.07, respectively. There was no relationship found between leg colour and the weaner traits of face cover, length of staple, body weight, condition and neck wrinkles.

Terrill (1947) reported that mating parents without leg colour produced 7% of the progeny in the Columbia sheep breed and 12% in the Targhee breed that were affected. The percentages of affected offspring, when one parent was affected were 22% and 24% and when both parents were affected were 44% and 38%, respectively. Mating of ewes with varying degrees of leg colour (scores 2, slight; 3 and score 4, considerable colour) resulted in 16%, 19% and 50% affected, respectively, in Columbias and 16%, 42% and 29% in Targhees. Mating of rams with colour score 2, to ewes without colour, resulted in 23% affected in Columbias and 25% affected in Targhee. In his study of leg colour, Terrill (1947) proposes that:

* The inheritance of leg fibre colour in these breeds is multi-factorial.
* Progress toward elimination of leg hair pigment from these breeds would be slow because of the low incidence (15% in Columbia and 12% in Targhee).
* There is little to be gained from selection against leg colour in ewes.
* Selection against leg colour in rams attaches an economic importance to the trait which is difficult to justify because of the lack of relationship to a market value.

The last two proposals of Terrill represent personal interpretations that are arguably not well supported by the data presented or sufficiently investigated.

Parnell (1950) attributed the amount of black spotting on Scottish Blackface sheep to the additive effects of several genes. Adalsteinsson (1975) reported the heritability of tan areas on the coat of white and tan Icelandic sheep to be 0.46 ± 0.05 and found and association exists between the amount of macroscopic tan fibre in the lamb coat and occurrence of isolated tan fibres in the white or faded adult fleece. The isolated tan fibres detected in the adult fleeces had an average diameter of 97.4μm compared to the fleece average of 30.7μm.

Nichols (1927) found that the incidence of isolated pigmented fibres in the white adult Suffolk fleece could be graded from an assessment of macroscopic fibre pigment in the lower thigh region of the fleece. Labban (1957) found that increased frequency of pigmented halo-hairs on the tail of Suffolk lambs was associated with an increased incidence of isolated pigmented fibres, increased hairiness and reduced quality (higher visual count) of the adult fleece. He proposed that hairiness and isolated pigmented fibres were related.

Bokenbaev (1964) made crosses between Degeres rams and (Precoce x fat-rumped) and studied characteristics of the white and coloured progeny. The relationships between fleece colour and wool fineness or tail thinness were positive for white fleeces, negative for brown or grey fleeces, and no relationship was evident for black fleeces. Wool fibres of black areas, on white spotted black sheep, are reported to be longer and coarser than the wool fibres of the surrounding white areas (Brooker 1968; Ryder and Adalsteinsson 1987). Ryder and Adalsteinsson (1987) proposed that the absence of melanocytes is somehow associated with the production of finer wool.

1.13.4. Pigmented spots in Merinos

The occurrence of black or brown pigmented fibre or skin spots in the non-fleece areas on sheep has been a historic concern of the Merino sheep breeder. It is a traditional belief of Merino breeders that certain types of remnant pigmentation involve an increased risk of black or spotted (Australian piebald) progeny (Graham 1870; Pearse 1945; "Old Hand" 1953; Body et al. 1962; Dun and Eastoe 1970). While intuitively these beliefs seem rational, at least for spotted sheep, there was a paucity of scientific data (Brooker 1968) about this aspect of sheep selection. As Terrill (1947) pointed out, selection against pigmentation reduces the amount of emphasis that can be placed on traits of direct economic importance to commercial producers.

Brooker (1968) and Fleet et al. (1989) have provided objective information for white Merinos about pigmentation differences between carriers and non-carriers of the recessive gene for black lambs. Carriers had slightly more black-grey pigmentation for bare skin areas (nose-lips, around eyes or under tail) and in the hooves than non-carriers.

However, selection against such black-grey pigmentation in white Merinos, as a method of reducing the occurrence of black lambs, is unlikely to be effective for reduction of the frequency of black lambs or carriers in white Merino flocks. Brooker (1968) came to a similar conclusion in relation to reducing the occurrence of Australian Piebald (random spot).

In the absence of supporting scientific evidence, some geneticists have questioned the emphasis placed on non-wool pigmentation by Merino breeders during sheep selection. An example of the extent of knowledge and disagreement with these selection practices is provided within a quotation from Dun and Eastoe (1970), as follows:

> *"Many Merino breeders concentrate a lot of selection effort against pigmentation that is found outside the wool-growing skin. Some breeders and sheep classers are extremely careful in this regard, culling even for small black and brown spots on the muzzle, lips, face, ears and legs. Sheep with prominent pigmentation of this type, such as large areas of brown pigmented hair on the legs are almost universally culled. There is also the worry at times about brown birthcoat fibres on lambs - commonly seen on the back of neck. These fall out and are not subsequently associated with a pigmented fleece. Hayman reports that brown patches of skin, obvious on lambs at birth, disappear quickly without ever being associated with pigmented wool fibres.*
>
> *As such pigmentation has no bearing on the animals production, it is obvious that it can be ignored and selection effort concentrated on the economic measurements of lamb and wool production".*

Dolling (1970) recognised the existing scientific evidence and that support on this basis for these traditional selection practices was lacking. Nevertheless, Dolling also realised that absence of pigmentation can have a cash value, that may be considered by ram breeders and their clients, as expressed in the following quotation:

> *"Considering rams as the sale product, the absence of black streaks in the horns would be considered by most buyers to have a cash value. It is known from experimental work, however, that these black streaks are not associated with production of black lambs. It is not yet known that they are not associated adversely with any other aspect of production. Should the absence of such associations be established and accepted by the ram buyer, however, and should black streaks still affect the price paid for a ram, the streaks would then have a marketing value - they would represent unfortunate packaging."*

In the development of the Selection Demonstration Flocks at the Agricultural Research Centre, Trangie, a high degree of pigmentation outside the fleece was introduced with one of the most productive sire lines. It is suggested by Dun and Eastoe (1970) that:

> *"These sheep showed black hooves, and a lot of brown pigment on face and legs, in combination with heavy wool cuts, good quality wool, large frames, low level of skin fold and good reproductive performance. These productive qualities could not be sacrificed just to eliminate a fancy point, like external pigment, which is of no importance in wool or meat production".*

Steyn (1963) expressed concern about pigmented fibres on horn sites or legs of South African Merinos as these pigmented fibres can directly contaminate the otherwise white fleece. Another aspect of pigmentation in sheep that was apparently not appreciated by breeders and scientists of Merino sheep, as a potential problem, was the occurrence of isolated pigmented wool fibres scattered through the otherwise white fleece. This fault is identified in other breeds in association with macroscopic pigmentation; for example, Suffolk sheep (Nichols 1927; Labban 1957), Icelandic sheep (Adalsteinsson 1975) and Corriedale sheep (Fleet et al. 1984; Fleet and Stafford 1989).

The first documented evidence of isolated pigmented wool fibres in Merino sheep (Fleet et al. 1989; Fleet and Smith 1990) provided the stimulus for further research on the inheritance and control of these dark fibres "like needles in hay stacks" within some white fleeces. The experiments described in this thesis provide an account of that research.

1.14. CONCLUSION

There is a great deal known about the control of pigmentation in the mouse. The knowledge for the mouse has developed as a result of the availability of various patterns, maintained or induced by fanciers and scientists, and the suitability of mice for basic laboratory studies. Analogous to well documented and studied white spotting patterns of the mouse are variations in pigmentation that characterise the many breeds of white or white spotted sheep (e.g. Merino, Corriedale, Suffolk). These breeds have developed over thousands of years to improve the flexibility of end-use of wool and meat products or satisfy the interests of their fanciers. In mice there are over 20 loci that cause pigment deficient areas and similar genetic systems are likely to exist in sheep.

Control of pigmentation faults has a been a priority of sheep breeders since the development of dyeing technologies and has increased in importance with recent fashion and competition from alternative fibres (cotton and synthetic) without this fault. Surveys of wool sale lots and commercial consignments, sampled after production of wool top, have shown that pigmented fibres in Merino wool are an important source of dark fibres when urine stained fibres are controlled and, especially, in wool from young sheep.

The recent development of Quality Management systems for the Wool Industry (e.g. Vandeleur 1993) has a priority of minimising non-wool fibre contaminants (e.g. polypropylene), urine stained wool and visible pigmented wool. The occurrence of isolated pigmented fibres is a threat to the integrity of these Wool Quality Management Systems and this thesis provides an account of this potential problem and its control.

There is considerable reliance on phenotype similarities, with well documented pigment loci in humans, mice and other animals, to explain pigmentation phenotypes in sheep. Gene mapping investigations (e.g. Broad et al. 1996; Parsons et al. 1997) are beginning to establish the homologies between characterised genes of humans and other animals with those in the genome of sheep. In view of the rate of progress with such endeavours, the confident explanation of pigment phenotypes in sheep (e.g. Sponenberg et al. 1996) may be revised or validated in the near future by genetics studies at the molecular level. There is considerable potential to study variations in pigmentation of sheep. This thesis involves the important and little studied aspects of pigmented fibres in white wool and relationships with macroscopic pigmentation and the main production characters of Merino sheep.

The work in this thesis was conceived with the discovery of isolated pigmented fibres in some white Merino fleeces and the identification of significant phenotypic correlation coefficients with macroscopic non-wool pigmentation. This discovery involved Strong-wool Merinos, originating in South Australia, and a medium-wool Peppin Merino sire from a selection line in the multiple-bloodline flock at Trangie. Further to these discoveries was concern among some Merino breeders that had been selecting for economic traits alone and had noted increased non-wool pigmentation in their flocks.

The first experiment conducted involved sampling a private Merino flock in Western Australia for hogget ewes with and without pigmented fibres on the legs. This experiment identified the potential problem of isolated pigmented fibres in fleeces of some of the ewes that could have been resolved by culling those sheep with pigmented leg fibres.

The multiple-bloodline flock at the Agricultural Research Centre, Trangie, New South Wales, provided a resource to study in a quantitative manner the inheritance of pigmentation in white Merinos and the relationships with the main production characters (lamb survival, hogget fleece weight, body weight and average fibre diameter). The genetic parameters (heritabilities and genetic correlations) obtained from the half-sib data from this flock are the first available for Merino sheep. In these flocks, several types of macroscopic fibre pigmentation were associated with isolated pigmented fibres and could have been used in a culling criterion to minimise isolated pigmented fibres in the hogget wool clip. The heritabilities of most types of macroscopic pigmentation were moderate to high (0.2 to 0.8) but the mode of inheritance of each type was unknown.

The investigations at Turretfield Research Centre, Rosedale, South Australia, involved genetic studies of a qualitative manner to study the mode of inheritance of the key indicators (pigmented leg fibres and pigmented birthcoat halo-hair) of isolated pigmented fibres in the fleece. The segregation of phenotypes (absence vs presence) for pigmented leg fibres was supportive of simple Mendelian inheritance. However, the data for pigmented birthcoat halo-hairs was inconsistent with expectations for simple mode of inheritance. Other experiments at Turretfield Research Centre provided greater understanding of the nature of the wool fault (foetal development, change with age, distribution in the fleece), changes in macroscopic pigmentation with age and the relationship between the measurements of pigmented fibres in fleeces and those obtained from processed top (combed sliver).

A flow chart on the following page shows how the experimentation was organised to overcome the gaps in knowledge on the inheritance and control of isolated pigmented

wool fibres in Merino sheep. The main hypothesis being tested in each of the experimental works was as follows:

Chapter 2: Leg fibre pigmentation on Merino sheep has no significant effect on isolated pigmented fibres in the hogget fleece.

Chapter 3: Isolated pigmented fibres and macroscopic pigmentation are not heritable and there are no significant genetic relationships between them or with production traits.

Chapter 4: Pigmented fibres on the legs and pigmented halo-hair on the birthcoat have simple Mendelian inheritance.

Chapter 5:

Section A: The timing of pigment cell (melanoblast) migration during foetal life in not related to the development of isolated pigmented fibres.

Section B: There is no significant regional variation in the concentration of isolated pigmented fibres in affected fleeces.

Section C: There is no significant variation with age of sheep in the concentration of isolated pigmented wool fibres (1.5 - 5.5 years) and macroscopic pigmentation (birth to 1.5 years).

Section D: There is no significant difference between the concentration of isolated pigmented fibres in batches of hogget fleeces and the pigmented fibre concentrations found in tops (combed sliver) processed from the batches.

As an indication of the historic beliefs and the level of understanding among sheep breeders or scientists, about the importance of selection against pigmentation in sheep, quotations are provided on cover pages of Chapters and the General Discussion section.

FLOW CHART FOR THE ORGANISATION OF THIS THESIS

PROBLEM IDENTIFICATION

⇓

Review existing knowledge
(Chapter 1)

INFORMATION GAPS

⇓

Observations in a private flock
(Chapter 2)

INHERITANCE STUDIES

⇓

Quantitative study
- Merino resource flock
(Chapter 3)

Qualitative study
- Mode of inheritance
(Chapter 4)

UNDERSTANDING THE PROBLEM

⇓

Foetal development
Distribution in the fleece
Age-related changes
Processing to top
(Chapter 5)

CONCLUSIONS

⇓

General Discussion

CHAPTER 2

OBSERVATIONS ON PIGMENTATION OF MERINO SHEEP SELECTED FROM A PRIVATE MERINO FLOCK

"All sheep without exception should be free from black, yellow, or tan spots on the legs or face; and in the case of rams, no black stripe on the horns, nor yellow or tan colour on the roof of the mouth can be tolerated".

John Ryrie Graham (1870)

SYMBOL	CHAPTER 2 - DESCRIPTION FOR SYMBOL
P	Pigmented fibres on the legs evident (Present)
A	No pigmented fibres on the legs evident (Absent)
PFC	Pigmented fibre concentration (No. per kg scoured staples).
LPFC	Transformed data (LPFC=[$\log_{10}$(PFC+1)].
SSp	Black-grey skin spots on the sides and backline of the sheep that developed after hogget age and were evident at 3.5 or 5.5 years age.
FSp	Diffuse spots of pigmented wool fibres that developed after hogget age and were evident at 5.5 years age. These spots usually arise in conjunction with SSp but occasionally appear without underlying visible skin pigmentation.
Br, Bl and T	Brown-tan, black-grey and total pigmentation.
Bare skin	
EYs	Pigment on bare skin around each eye.
Ns	Pigment on bare skin on the nose and lips.
Ls	Pigment on bare skin between the legs.
Ms	Pigment inside the mouth.
EVs	Pigment on bare skin inside the ears.
Ts	Pigment on bare skin around tail.
Skin under kemp	
Fs	Pigment of skin on face.
EDs	Pigment of skin on dorsal ears.
Fibres	
Lf1 and Lf2	Pigmented fibres on all legs (Lf1) or posterior rear legs (Lf2). - A = pigmented leg fibres absent on all legs. - P = pigmented leg fibres evident on one or more of the legs.
Hf	Pigmented fibres on horn sites.
Ef	Pigmented fibres on ears.
El	Pigmented eye lashes.
Bf	Pigmented birthcoat halo-hairs.
Other	
Hv and Hn	Pigment in hooves and horns, respectively.

CHAPTER 2

OBSERVATIONS ON PIGMENTATION OF SELECTED MERINO SHEEP FROM A PRIVATE FLOCK

2.1. INTRODUCTION

In 1985 a group of ram breeders in Western Australia who relied heavily on performance recording, with minimal attention to other traits considered to be "fancy points" (Dun and Eastoe 1970), became concerned about increases in pigmentation outside the fleece. This concern was reinforced by reports of associations between non-fleece pigmentation (pigmented fibres on the horn sites and legs) and isolated pigmented fibres in the fleece of Corriedale sheep (Fleet et al. 1984).

In one of the Western Australian flocks there was 17% of the ewe weaners affected by varying amounts of pigmented fibres on the legs. The initial investigation involved sampling this flock to establish whether there was any relationship between the leg fibre pigmentation and isolated pigmented fibres in the hogget fleece.

This chapter describes the assessment of pigmentation in 95 non-Peppin Merino sheep selected on the basis of presence or absence of pigmented leg fibres. The changes in non-fleece pigmentation with age, the associations between the various types of non-fleece pigmentation, and relationships with isolated pigmented fibres in the hogget fleece or pigmented wool fibre spots in the fleece at 5.5 years age were also investigated.

2.2. MATERIALS AND METHODS

2.2.1. Sheep and location

One hundred and ten yearling Merino ewes were selected from a flock in Western Australia on the basis of the macroscopic presence (P) or absence (A) of pigmented fibres on the legs. These ewes were generated in a syndicate mating involving several sires. The selected sheep were transferred to a private property in South Australia and then later to Turretfield Research Centre. The sheep were assessed in 1985 at hogget age (1.5 years) for macroscopic non-fleece pigmentation and the hogget fleeces measured in 1986 for isolated pigmented fibres. They were kept until 5.5 years age with twice yearly shearings introduced after 3.5 years age to promote the development of pigmented skin and pigmented wool fibre spots in the fleece area (Fleet and Forrest 1984). At age 5.5 years there were 95 sheep remaining to re-assess macroscopic pigmentation and to check for pigmented skin (SSp) and wool fibre spots (FSp) that had developed in the dorsal wool-bearing (fleece) regions of the body since 3.5 years age.

2.2.2. Recording macroscopic pigmentation

Each sheep was scored for the amount of black and grey (Bl) or brown and tan (Br) and total (T) pigmentation of skin and fibres visible to the observer without assistance (i.e. macroscopic). The descriptions here include additional types of pigmentation relevant to sheep described in other chapters. The scores for macroscopic pigmentation were based on a system developed by Brooker (1968) as follows:

2.2.2.1. Bare skin areas: The areas observed were the bare skin around each eye (EYs), on the nose-lips (Ns), between the legs (Ls), inside the ears (EVs) and mouth (Ms

- including tongue, internal lips and mouth roof), and under the tail (Ts). A single score was allocated to each area and for the eyes and ears the two scores were summed. (Score 0, no pigment; score 1, 1-10% pigmented; score 2, 11-25%; score 3, 26-50%; score 4, 51-75%; score 5, 76-99%; score 6, 100%). In the case of the sheep discussed in Chapter 2, the score for total pigmentation was obtained using the method of Brooker (1968). In this procedure, if bare skin was not completely pigmented and both colours (Bl and Br) were present, the score for total pigmentation was obtained by combining the median percentage values for each of the colour scores (Brooker 1968). In the subsequent experiments (Chapter 3 and 4) the procedure for scoring total (T) pigmentation was improved by direct assessment like the component colours (Bl and Br).

2.2.2.2. Pigmented fibres: The areas scored were the face (Ff), ears (Ef), legs (Lf), horn sites (Hf) and eye lashes (El). For the legs a single score was allocated to the anterior and posterior of each leg and these were summed to give Lf1 and the scores for the posterior of the rear legs summed to give Lf2 (see Figures 1 to 4). The scores for both ears and both eye lashes were summed. (Score 0, no pigmented fibres; score 1, few spots or pigmented fibres; score 2, speckled or many pigmented fibres; score 3, large patches; score 4, large portion (>50%) of the area). For the sheep discussed in Chapter 2, classification on presence (P) or absence (A) of pigmented leg fibres was based on the Lf1 score at hogget age but at 5.5 years age only the rear legs (Lf2) were reassessed. If pigmented leg fibres are present then, with few exceptions, they will be found and most prominent on the posterior of the rear legs (i.e. Lf2).

2.2.2.3. Pigmented skin under kemp fibre areas: The areas scored were the face (Fs) and ears (EDs) with the separate ear scores summed. Scores (0-4) were the same as for *Pigmented fibres*.

2.2.2.4. Figures 1 to 4: Shows various degrees of leg fibre pigmentation.

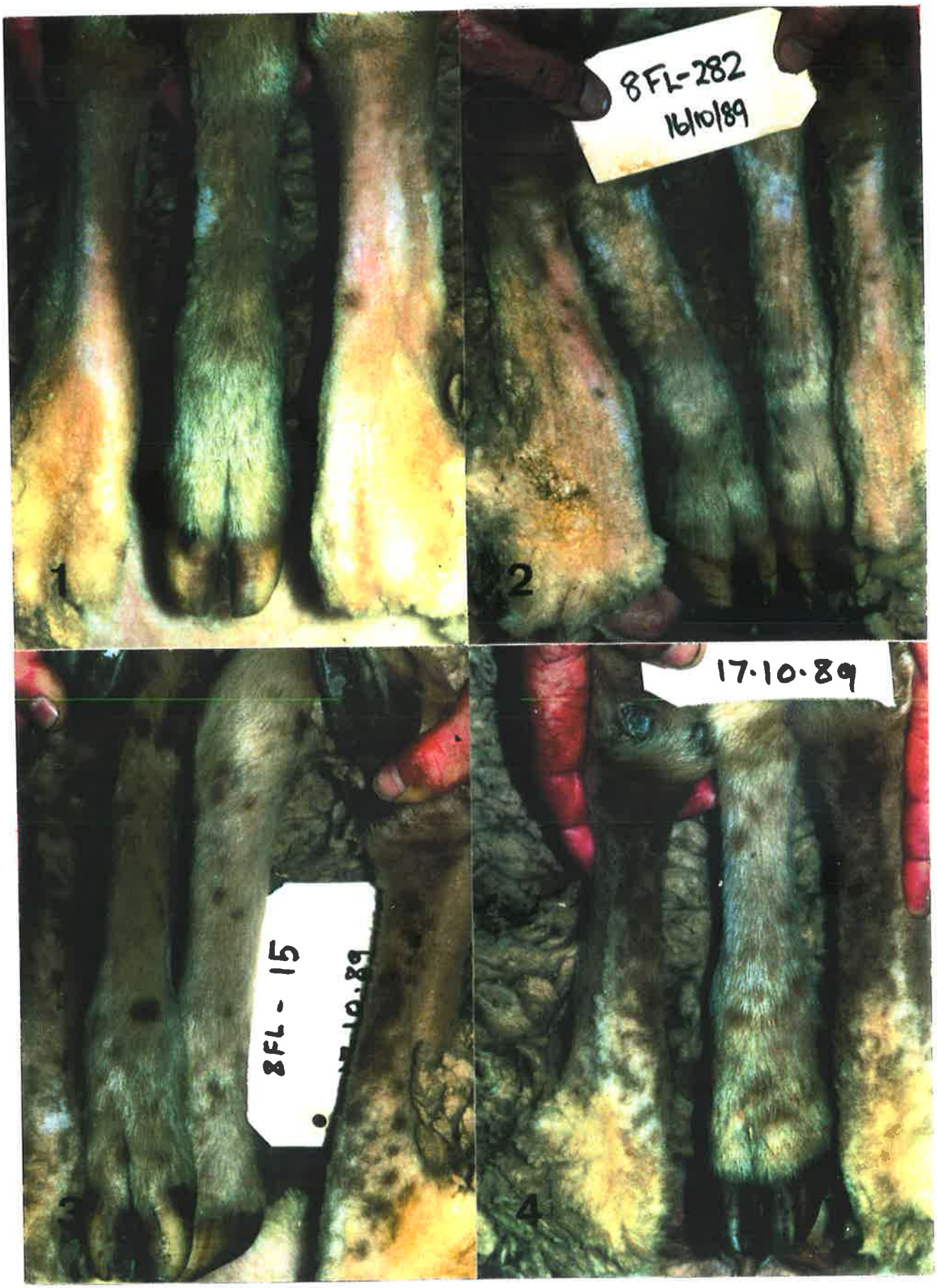


Figure 1 to 4: Show various degrees of leg fibre pigmentation.
Figure 1, few spots.
Figure 2, score 1 rear legs and score 2 front legs.
Figure 3, score 2 front legs and score 3 rear legs.
Figure 4, score 3 on front legs to score 4 on rear legs.

2.2.2.5. Hooves and ram horns: A score was allocated to each hoof (Hv) and horn of rams (Hn) and these were summed. (Score 0, no pigment; score 1, <26%; score 2, 26-50%; score 3, 51-75%; score 4, >75%). Horn scores apply to rams included in other chapters. In Chapter 2, the rear hooves only were scored at 5.5 years age and compared with records for the same hooves at hogget age.

2.2.2.6. Birthcoat halo-hair: This fibre pigmentation occurs mainly on the back-of-neck and is usually tan in colour. (Scores 0, no halo-hair or hairs white; score 1, 1-10 pigmented hairs; score 2, 11-20 hairs; score 3, 21-100 hairs; score 4, 101-200 hairs; score 5, >200 hairs). Birthcoat halo-hair scores apply to sheep described in other chapters.

2.2.2.7. Piebald spots: The occurrence, location on the coat, and colour of any distinct and rounded pigmented fibre spots, with positional asymmetry (random location), in non-wool areas or in the fleece were recorded (See Figures 7, 9 and 10).

2.2.2.8. Pigmented wool and skin spots developing on adult sheep: The wool-bearing areas (i.e. upper sides and backline) of the sheep of Chapter 2 were closely inspected immediately after shearing at 3.5 and 5.5 years age for black-grey skin spots (SSp) and pigmented wool fibre spots (FSp), other than piebald spots, that had apparently developed since hogget age. Location on the coat of each skin/fibre spot was recorded.

2.2.2.9. Figures 5-10: Show lambs with *pigmented birthcoat halo-hair.*

- Figure 5, score 2; Figure 6; score 3.
- Figure 7 and 10; score, 4; and Figures 8 and 9, score 5.
- *Piebald spots:* The lambs in Figures 7 and 9 have black random spots on the shoulder and between the ears and in Figure 10 the lamb has a tan coloured piebald spot on the hips and tan hairs around the anal area.
- *Tan ear tips:* The lambs in Figures 8, 9 and 10 have tan ear tips.

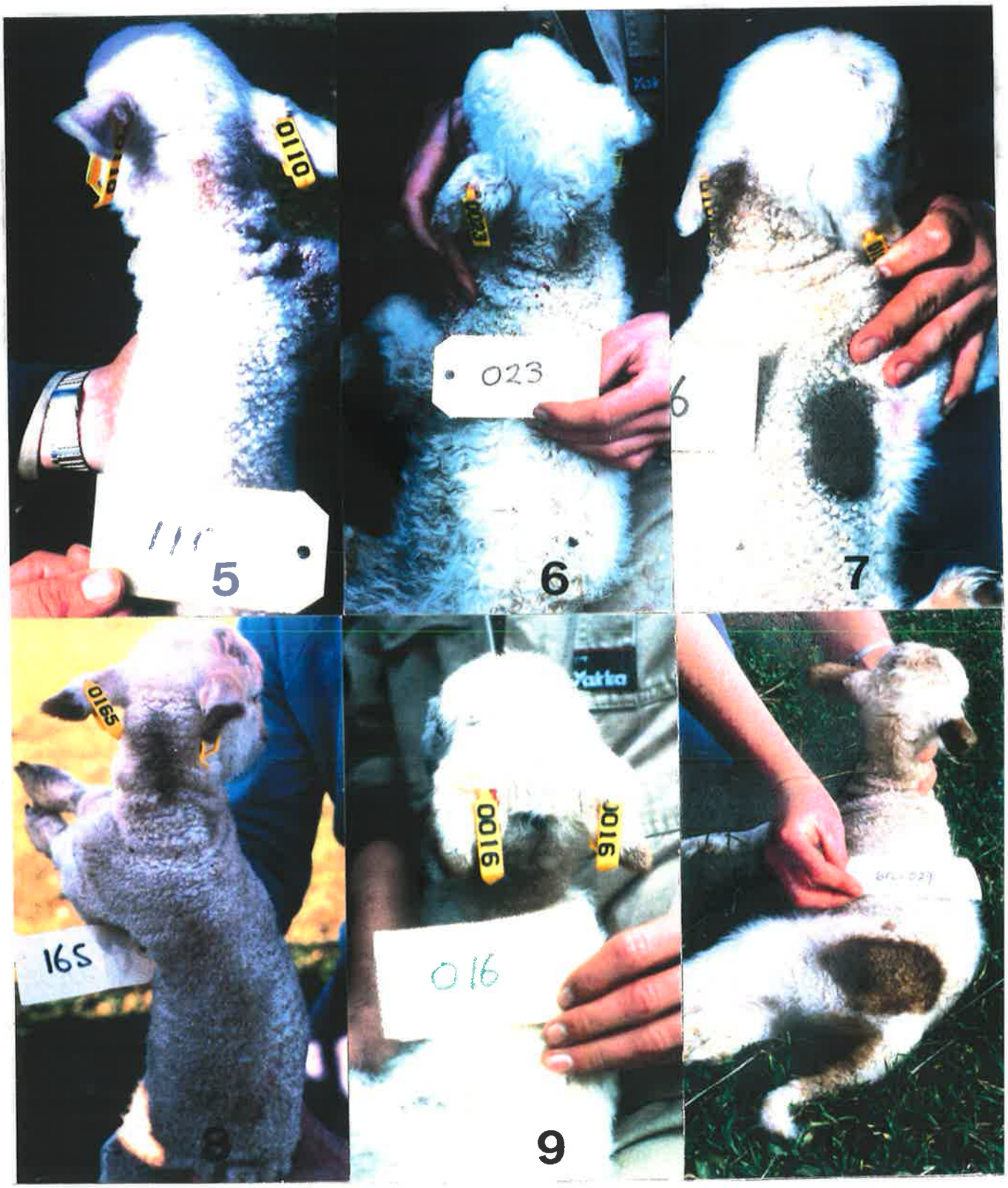


Figure 5 to 10: Show various degrees of pigmented birthcoat halo-hair.
Figure 5: Score 2, 11 to 20 hairs.
Figure 6: Score 3, 21 to 100 hairs.
Figure 7 and 10: Score 4, 101 to 200 hairs.
Figure 8 and 9: Score 5, >200 hairs.

Note in Figures 7, 9 and 10 the lambs also have black or tan Australian Piebald (random spots). Also, in Figures 8, 9 and 10 the lambs have tan fibres on the ears.

2.2.3. Fleece measurement

The fleeces were all skirted and had 12 months wool growth having been previously shorn as lambs at 4-6 months age. Each fleece was spread on a table and sampled using a perspex grid (1.8m x 1.2m) with 104 holes. Two samples of 52 staples were produced from alternate holes in the grid. Staples were trimmed to remove dusty tips, leaving a 70mm length, and solvent scoured with Mobil B1 while held in bundles. The samples were randomised and allocated to three observers and the results for each pair combined. All staples were inspected with a CSIRO Dark Fibre Detector (Foulds et al. 1984; IWTO 1988) and all darkened fibres of more than 20mm length were removed from the wool staple web and permanently mounted on glass slides. Pigmentation was confirmed by microscope inspection (magnification to 400x). The total mean weight of scoured staples inspected for each fleece was 38.2g. The concentration of pigmented fibres found in the fleece samples (PFC) is reported as the number per kg of scoured staples.

2.2.4. Statistical analysis

To quantify the differences between A and P sheep and the changes between 1.5 and 5.5 years age, for each type of non-fleece pigmentation, the data were analysed by least squares analysis of variance (procedure GLM of SAS 1987) using the following model:

$$Y_{ijk} = u + L_i + SN(L)_j + Age_k + (L*Age)_{ik} + e_{ijk}$$

Where: Y_{ijk} = the observed pigment trait on an individual sheep;

u = the mean; L_i = Class for presence (P) or absence (A) of pigmented leg fibres;

$SN(L)_j$ = sheep nested within L was used to test the L mean square;

Age_k (1.5 years and 5.5 years); and e_{ijk} = the random residual effect.

For PFC, SSp and FSp, that only included one of the age levels, the data were analysed by fitting the effect of L class alone. PFC counts were also transformed ($\log_{10}$[PFC+1]) in order to more closely approximate a normal distribution (Falconer 1964).

The data for pigmentation traits tend to have non-normal distributions being skewed one way or the other in the range of counts or scores available. In these circumstances, transformation of the data may help (e.g. reduce coefficients of variation) or non-parametric statistical methods can be used. Spearman's rank method provides the best known non-parametric procedure to obtain ordinary correlation coefficients (Snedecor and Cochran 1967). When records are available from only two intervals, and no other fixed effects are to be fitted, then the ordinary correlation equals the repeatability (Ponzoni - personal communication).

Spearman's correlation coefficients (r_s) were calculated between the various types of macroscopic non-fleece pigmentation, the concentration of pigmented wool fibres (PFC) in the hogget fleece, and the number of skin spots (SSp) or pigmented wool fibre spots (FSp) observed at 5.5 years age. These correlation coefficients were calculated for non-fleece pigmentation scored at 1.5 and 5.5 years age for all ewes (n=95) and for those ewes with (P=57 sheep) and without (A=38 sheep) pigmented fibres on the legs.

2.3. RESULTS

2.3.1. Frequency and mean score of pigmentation at 1.5 and 5.5 years age

Repeatabilities for each macroscopic non-fleece pigmentation type (total score), between the two ages (1.5 and 5.5 years), and the percentage of affected sheep (score>0) are

shown in Table 1. Most of these repeatabilities for the entire sample, and within the A and P subgroups, were significant and higher than 0.5.

All sheep with pigmented leg fibres on the posterior of the rear legs (Lf2) at hogget age also had pigmented fibres evident on the legs at 5.5 years age. Conversely, with one exception, all of the sheep without pigmented fibres on the posterior of the rear legs at hogget age were also unaffected at 5.5 years age. The repeatability for Lf2 (total score) between 1.5 and 5.5 years age was 0.93. However, there were 5 sheep with a zero Lf2 score at hogget age that were classified to the (P) group, based on the Lf1 score at hogget age, due to pigmented fibres being evident on other areas of the legs (front legs or anterior of rear legs). The correlation coefficients between the Lf1 and Lf2 scores at hogget age was 0.94 (Appendix 1).

The proportion of sheep affected by specific types of non-fleece pigmentation increased between 1.5 and 5.5 years age for most types (e.g. ear skin and fibres, face skin, skin between legs and under the tail)(Table 1). For other types of non-fleece pigmentation the proportion affected remained relatively stable (e.g. eye skin, leg fibres in P) or declined slightly (hooves and horn site fibres).

In Table 2 the effect of presence (P) and absence (A) of pigmented leg fibres is quantified. The first conclusion is that A sheep had a minor incidence of isolated pigmented fibres in the fleece (PFC) relative to P sheep (22 vs 1136 per kg scoured staples). This difference became significant ($P<0.001$) when transformed data (LPFC) were used. The percentage of sheep with pigmented fibres in the fleece sample was 18% for A and 65% for P.

Table 1: Repeatability of non-fleece pigmentation between 1.5 and 5.5 years age, and the percentage of sheep affected (pigment evident), for sheep with (P) or without (A) pigmented leg fibres.

Type of pigment		Repeatability			Lf Absent (A)		Lf Present (P)	
Age	Colour	All	A	P	1.5	5.5	1.5	5.5
Leg fibres (Lf2)	Black	0.85*	--	0.86*	0	0	19	23
	Tan	0.93*	--	0.85*	0	0	72	70
	Total	0.93*	--	0.79*	0	0	91	89
Horn site (Hf)	Black	0.73*	--	0.77*	0	3	14	20
	Tan	0.88*	0.72*	0.83*	3	5	75	63
Face fibre (Ff)	Black	0.50*	--	0.47*	0	3	25	32
	Tan	0.79*	--	0.78*	0	0	33	25
Ear fibre (E)	Black	0.34*	0.04	0.38*	8	24	37	46
	Tan	0.49*	--	0.48*	0	0	12	9
Face skin (Fs)	Black	0.62*	0.41*	0.41*	8	42	60	95
	Tan	0.88*	0.62*	0.80*	39	68	88	100
Ear skin (Es)	Black	0.72*	0.33*	0.63*	16	62	68	96
	Tan	0.85*	0.64*	0.72*	66	92	98	100
Eye skin (EYs)	Black	0.55*	0.58*	0.39*	100	100	100	100
	Tan	0.47*	0.58*	0.36*	100	100	98	98
Nose-lips (Ns)	Black	0.76*	0.63*	0.70*	63	71	95	96
	Tan	0.82*	0.83*	0.64*	84	84	98	100
Mouth (Ms)	Black	0.75*	0.50*	0.74*	24	26	82	80
	Tan	0.38*	--	0.44*	11	0	14	18
Bet. Legs (Ls)	Black	0.04	--	-0.12	0	53	7	98
	Tan	--	--	--	0	76	0	100
Tail skin (Ts)	Black	0.71*	0.62*	0.47*	47	74	93	98
	Tan	0.75*	0.78*	0.40*	82	89	96	100
Hooves (Hv)	Black	0.85*	0.38*	0.61*	26	13	93	88
	Tan	0.23*	--	0.29*	5	0	5	5

* $P < 0.05$ otherwise not significant ($P \geq 0.05$)

Table 2: Least square means for pigmentation at 1.5 and 5.5 years age for sheep with (P) or without (A) pigmented leg fibres.

Type of pigment[A]		Model r^2	Signif. effects[B]	Without (A) 1.5 years	5.5	With (P) 1.5 years	5.5
PFC		0.03	None	22 a[C]	--	1136 a	--
LPFC		0.18	L	0.3 a	--	1.5 b	--
FSp		0.09	L	--	0.0 a	--	0.7 b
SSp		0.05	L	--	0.1 a	--	0.6 b
Leg fibre	Bl	0.0	None	--	--	1.4 a	1.2 a
(Lf2)	Br	0.0	None	--	--	4.7 a	4.5 a
Horn site	Bl	0.98	L	0.0 a	0.0 a	0.9 b	0.9 b
(Hf)	Br	0.94	L T L*T	0.2 a	0.2 a	5.1 c	3.9 c
Face fibre	Bl	0.88	L	0.0 a	0.0 a	0.3 b	0.4 b
(Ff)	Br	0.92	L T L*T	0.0 a	0.0 a	0.8 b	0.6 b
Ear Fibre	Bl	0.69	L T	0.1 a	0.4 ab	0.6 b	1.1 c
(Ef)	Br	0.81	L	0.0 a	0.0 a	0.5 b	0.4 b
Face skin	Bl	0.82	L T L*T	0.1 a	0.4 b	0.9 c	1.7 d
(Fs)	Br	0.93	L T	0.7 a	1.1 b	2.9 c	3.5 d
Ear skin	Bl	0.89	L T L*T	0.3 a	1.0 b	1.7 c	3.2 d
(Es)	Br	0.93	L T	1.7 a	3.2 b	6.3 c	7.1 d
Nose-lips	Bl	0.89	L T	0.9 a	1.1 a	2.0 b	2.5 c
(Ns)	Br	0.93	L T	2.1 a	1.9 a	3.9 b	3.6 c
Eye skin	Bl	0.89	L T	2.6 a	5.4 c	3.6 b	6.7 d
(EYs)	Br	0.83	T L*T	8.6 b	7.3 a	9.1 a	7.1 a
Mouth	Bl	0.91	L	0.3 a	0.3 a	1.5 b	1.7 c
skin	Br	0.61	L	0.2 ab	0.0 a	0.4 b	0.4 b
Bet. legs	Bl	0.81	L T L*T	0.0 a	0.6 b	0.1 a	1.6 c
(Ls)	Br	0.89	L T L*T	0.0 a	1.6 b	0.0 a	3.3 c
Tail skin	Bl	0.86	L T L*T	0.5 a	1.0 b	1.1 b	2.2 c
(Ts)	Br	0.89	L T	1.9 a	2.3 b	4.1 c	4.3 c
Rear	Bl	0.89	L T L*T	0.7 a	0.2 a	6.3 b	3.3 c
hoof (Hv)	Br	0.53	None	0.1 a	0.0 a	0.2 a	0.2 a

A PFC is the concentration of isolated pigmented fibres in the hogget fleece and LPFC is the $\log_{10}$ transformed variable; FSp and SSp are the number of age-related pigmented fibre and skin spots, respectively

B Significant effects ($P<0.05$): Including class L for presence (P) or absence (A) of pigmented leg fibres and age (T) for 1.5 and 5.5 year observations.

C Means within rows and colours (Bl and Br) with a common letter (a,b,c,d) do not differ significantly ($P \geq 0.05$).

At the hogget shearing, seven of the sheep had a piebald spot and only one of these came from the A group. Two sheep with a pigmented skin spot in the dorsal wool-bearing regions (SSp) at 3.5 years age, that apparently had developed since hogget age, subsequently produced the pigmented wool fibres (FSp) evident at 5.5 years. Significant differences between A and P sheep were also obtained for SSp ($P<0.01$) and FSp ($P<0.001$) at 5.5 years age. The incidence of 5.5 year old sheep with one or more skin spot (SSp) or wool fibre spot (FSp) for A sheep was 13% and 3%, respectively, and for P sheep was 28% and 32%, respectively.

The significance of main effects for class L for presence or absence of pigmented leg fibres, class T for age 1.5 or 5.5 years, and the interaction (L*T) fitted in the least squares analysis of variance are shown in Table 2. Except for brown-tan skin around the eyes and brown-tan in the hoof, all the other types of macroscopic pigmentation had significantly different A and P means. Age (T) had a significant effect except for leg fibres, black horn site and face fibres, tan ear fibres, mouth skin and brown hoof pigment. The significant interactions between age and leg fibre pigment class (T*L) occur because sheep (P) with leg fibre pigment showed a greater change (mainly increases between 1.5 and 5.5 years age) than sheep (A) without leg fibre pigmentation.

2.3.2. Correlations between pigmentation types at 1.5 and 5.5 years age

The correlation coefficients (Spearman's) between the various types of macroscopic non-fleece pigmentation and the concentration of isolated pigmented fibres in the hogget fleece (PFC) are shown in Table 3 (black-grey or brown tan) and Appendix 1 (total score).

Also in Table 3, the correlation coefficients between the Bl and Br colour classes are shown. At hogget age, the highest correlation coefficients with PFC (≥ 0.39) were obtained with brown-tan pigmented fibres on the legs, horn sites and face, brown-tan skin on the ears and black-grey of the nose-lips skin and in the hooves. Both pigment colours (black-grey and brown-tan) also had significant positive correlation coefficients with PFC for most of the other types of non-fleece pigmentation. Appendix 1 shows the correlation coefficients between the total score of each type of non-fleece pigmentation and PFC in the entire sample (ALL), at both ages, were positive (0.22 to 0.54) and significant ($P < 0.05$); except for between leg skin at hogget age (-0.11; n.s.).

Table 3: Correlation coefficients (Spearman's) between the concentration of isolated pigmented fibres (PFC) and scores for black-grey (Bl) and for brown-tan (Br) visible pigmentation, and between the two colours (Bl and Br) of pigmentation scored on the 95 sheep at 1.5 and 5.5 years age (brackets).

Non-fleece pigmentation		PFC with Black-grey Bl		PFC with Brown-tan Br		Bl vs Br	
Leg fibres	Lf2	0.11	(0.10)	0.39***	(0.47)***	-0.30**	(-0.23)*
Horn site	Hf	0.10	(0.11)	0.44***	(0.36)***	-0.27**	(-0.17)
Face fibres	Ff	0.14	(0.24)*	0.43***	(0.40)***	0.08	(0.22)*
Ear fibres	Ef	0.17	(0.27)**	0.22*	(0.26)*	0.26*	(0.29)**
Face skin	Fs	0.30**	(0.42)***	0.28**	(0.41)***	0.64***	(0.74)***
Ear D skin	Es	0.29**	(0.37)***	0.41***	(0.50)***	0.59***	(0.75)***
Eye skin	EYs	0.23*	(0.25)*	0.13	(-0.06)	-0.39***	(-0.79)***
Nose-lips	Ns	0.39***	(0.39)***	0.24*	(0.23)*	0.51***	(0.46)***
Mouth skin	Ms	0.34***	(0.22)*	-0.04	(0.12)	0.16	(0.15)
Between legs	Ls	-0.11	(0.36)***	-	(0.40)***	-	(0.66)***
Under tail	Ts	0.36***	(0.43)***	0.32**	(0.34)**	0.49***	(0.45)***
Rear Hooves	Hv	0.45***	(0.47)***	-0.14	(0.14)	-0.07	(0.09)

*** $P < 0.001$; ** $P < 0.01$; $P < 0.05$; otherwise not significant ($P \geq 0.05$). significant.

The non-fleece pigmentation types, within colour classes, having non-significant correlations with PFC at hogget age were black-grey fibres on the legs, horn sites, face and ears, black-grey skin between the legs and brown-tan in the skin around the eyes, in the mouth and between the legs, and brown-tan in the hooves (Table 3). Brown-tan skin around the eyes was prominent on most sheep, while the other non-significant types were infrequent (Table 1 and 2). At 5.5 years age, the types with non-significant correlations with PFC were black-grey fibres on the legs and horn sites, brown-tan of the mouth skin, around the eyes, and in the hooves. Brown-tan skin around the eyes was prominent on most sheep, while the other non-significant types were infrequent. The significant correlations between the Bl and Br colour classes were positive except for leg fibres (both ages), horn site fibres (hogget age) and eye skin (both ages).

The number of pigmented wool fibre spots (FSp) at 5.5 years age also had significant positive correlation coefficients with most types of non-fleece pigmentation and PFC (Table 4). The number of pigmented skin spots (SSp) was not significantly correlated with PFC but had significant positive correlation coefficients with several types of non-fleece pigmentation and with FSp (0.54).

The correlation coefficients among the total scores for the various types of non-fleece pigmentation for all sheep were mainly higher than 0.5 and positive at both ages (Appendix 1). The main difference between ages occurred for pigmentation of bare skin between the legs (Ls) which was infrequent and not correlated with PFC at hogget age but subsequently developed and showed the same trend as other sites by 5.5 years age. Between leg skin developed pigment after hogget age apparently as a result of exposure of the mature and lactating udder to sunlight.

Table 4: Correlation coefficients between the number of black-grey pigmented skin spots (SSp) in the fleece area and wool fibre spots (FSp), recorded for the 3.3 and 5.5 year old sheep, and the non-fleece pigmentation types (total score) scored at both 1.5 and 5.5 years age.

Pigmentation types	Skin spots (SSp)						Fibre spots (FSp)					
Age	1.5 years			5.5 years			1.5			5.5		
Colour	T	Bl	Br	T	Bl	Br	T	Bl	Br	T	Bl	Br
PFC	0.18			--			0.30*			--		
Leg fibres (Lf1)	0.22*			--			0.35*			--		
Leg fibres (Lf2)	0.25*	0.24*	0.10	0.30*	0.12	0.18	0.36*	0.14	0.30*	0.39*	0.04	0.36*
Horn sites (Hf)	0.31*	-0.05	0.33*	0.22*	-0.11	0.25	0.39*	0.05	0.35*	0.33*	0.06	0.27*
Face fibres (Ff)	0.17	0.15	0.13	0.23*	0.15	0.16	0.29*	0.12	0.24	0.34*	0.17	0.35*
Ear fibre (Ef)	0.01	-0.04	0.19	0.35*	0.36*	0.10	0.18	0.15	0.30*	0.33*	0.34	0.27*
Face skin (Fs)	0.06	0.17	0.05	0.19	0.22*	0.19	0.17	0.24*	0.17	0.27*	0.35*	0.29*
Ear D skin (EDs)	0.17	0.34*	0.20	0.17	0.28*	0.17	0.29*	0.19	0.29*	0.33*	0.26*	0.32*
Eye skin (EYs)	0.15	0.06	0.10	0.13	0.18	-0.06	0.10	0.16	-0.07	0.19	0.18	-0.10
Nose-lips (Nls)	0.31*	0.32*	0.18	0.33*	0.24*	0.15	0.35*	0.38*	0.22*	0.41*	0.43*	0.19
Mouth skin (Ms)	0.23*	0.23*	0.09	0.19	0.17	0.16	0.31*	0.36*	-0.03	0.39*	0.37*	0.25*
Bet. legs (Bls)	0.0	0.0	--	0.14	0.11	0.14	0.17	0.17	--	0.19	0.20*	0.17
Under tail (UTs)	0.20*	0.21*	0.20	0.24*	0.29*	0.06	0.29*	0.20	0.27*	0.37*	0.34*	0.28*
Rear Hoof (Hv)	0.30*	0.31*	-0.12	0.30*	0.29*	0.06	0.44*	0.45*	-0.01	0.35*	0.37*	-0.09

* $P < 0.05$, otherwise not significant.

Also shown in Appendix 1, are correlation coefficients obtained within the A and P groups between PFC and total score for each non-fleece pigmentation type. Correlation coefficients for the component colours (Bl or Br) within the A and P subgroups are not presented. In each of these subgroups the correlation coefficients between each type of non-fleece pigmentation and PFC, or among the non-fleece pigmentation types, were mainly lower than 0.3 and non-significant ($P \geq 0.05$). This result reflects the large impact

in this flock of the sampling method, based on presence and absence of pigmented leg fibres, on the concentration of isolated pigmented fibres in the hogget fleeces and associations with other types of macroscopic pigmentation.

The significant correlation coefficients at hogget age for PFC within A sheep were total score for pigmented skin around the eyes (0.39) and for P sheep were brown-tan and total scores for pigmented fibres on the horn sites (0.26 and 0.30) and face (0.32 and 0.33). The A sheep at 5.5 years age had significant correlation coefficients between PFC and brown-tan and total score for pigmented skin on the ears (both 0.38). For P sheep at 5.5 years age the significant correlations were between PFC and brown-tan and total score for fibres on the face (0.31 and 0.32) and legs (0.33 and 0.38) and total score for nose-lips skin (0.28).

SSp and FSp had correlation coefficients with non-fleece pigmentation and PFC that were mainly lower than 0.2 and non-significant within A and P sub-groups (data not presented). Only one A sheep had a positive FSp record (single spot). For P sheep the significant correlation coefficients for FSp were obtained with black-grey and total score for hoof pigment (0.34 and 0.31) and black-grey mouth skin (0.27) at hogget age and, at both ages, with black-grey and total score for nose-lips skin (1.5 = 0.33 and 0.25; 5.5 = 0.31 and 0.34), and at 5.5 years age with brown-tan and total score for face fibres (0.28 and 0.27) and black-grey and total score for ear fibres (0.39 and 0.36).

For SSp there were no significant correlation coefficients among A sheep at hogget age. However, for P sheep the significant correlations at hogget age were with black-grey and total score for hoof pigment (0.32 and 0.29), black-grey and total score for skin of the

nose-lips (0.36 and 0.26), brown-tan and total score for horn site fibres (0.34 and 0.32) and, at both ages, were black-grey ear skin (1.5 = 0.26; 5.5 = 0.27), and at 5.5 years age were black-grey and total ear fibres (0.42 and 0.39), total score for leg fibres (0.27), and black-grey tail skin (0.35) and FSp (0.61).

2.4. DISCUSSION

2.4.1 Differences between Merinos with and without pigmented leg fibres

It should be recognised that the sheep were not sampled in a random manner but, rather, were selected on the basis of presence or absence of pigmented fibres on the legs. Nevertheless, this experiment needs to be viewed as a preliminary investigation to determine if a potential problem (related to pigmented leg fibres) existed in a Merino breeder's flock and to look for possible useful trends. It is 'usual' for commercial Merino flocks to contain few sheep with pigmented fibres on the legs. Nevertheless, this type of pigmentation became a concern in some breeders' flocks. Therefore, a comparison that considers trends both across all sheep sampled (All) and within the subclasses for presence (P) or absence (A) of pigmented leg fibres is very relevant for such a qualitative trait.

The most important finding in this Chapter is the general large difference between A and P sheep for the various types of pigmentation (Table 2). It appears that the factor(s) which increase presence of pigmented fibres on the legs simultaneously increase pigmentation in other areas; including isolated pigmented fibres in the hogget fleece. The mean concentration of isolated pigmented fibres (PFC) among the sheep sample (A), without pigmented fibres on the legs, is well below the commonly accepted upper limit (i.e. 100 per kg) for sensitive end-uses (Foulds et al. 1984). In contrast, the sample of

sheep (P), with pigmented leg fibres, had a PFC over 11-fold greater than the commonly tolerated upper limit. More of the P sheep were also found to be affected (18%) by isolated spots of pigmented wool fibres (FSp), developing between 3.5 and 5.5 years age, than were the A sheep (3%). However, these wool fibre spots are expected to have been encouraged by twice yearly shearing between these ages.

2.4.2 Correlations between the pigmentation traits

The proportion of sheep with pigmented leg fibres in this sample was greater (3.5 fold) than in the original flock. The magnitude of correlations between the different types of pigmentation will depend on the frequency of leg fibre pigmentation in the flock for those types associated with this trait. Therefore, it could be expected that correlations between traits associated with pigmented leg fibres will be higher than would have been evident with a random sample of the original flock or within the A and P subgroups. The correlations within A and P subgroups are relevant when leg fibre pigmentation is considered as a qualitative trait and the affected and unaffected sheep as different strains.

The various types of pigmentation were generally positively correlated among themselves for all sheep but were mainly of lower magnitude within A and P subgroups. Most types of non-fleece pigmentation within the entire sample had significant positive correlation coefficients with PFC and FSp. However, within the A and P subgroups the correlations were mainly lower and not significant. Variability of pigmentation types would be reduced and the effect of leg fibre pigment removed, within the A and P subgroups, so it is not surprising that the correlations are lower. The correlation coefficients and the least square means obtained with all sheep, indicate that presence of leg fibre pigmentation was

having a large effect on isolated pigmented fibres in the hogget fleece (PFC), isolated wool fibre spots (FSp) in late adult life, and all of the other pigmentation traits to some degree or at some stage (1.5 and, or, 5.5 years age).

2.4.3. Repeatability of macroscopic pigment and control of isolated pigmented fibres

With few exceptions (e.g. skin area between the legs) the various types of non-fleece pigmentation had repeatabilities higher than 0.5 (Table 1). Most non-fleece pigmentation traits increased during adult life; especially for sheep with pigmented leg fibres.

Brooker (1968) reported that hoof pigment, tan fibres on the ears and brown skin around the eyes tend to decline, while black fibres on the ears, black and brown skin on the nose-lips, and black skin around the eyes, tend to increase between birth and adult life. The results documented here add to that evidence of changes in non-fleece pigmentation with age in Merinos. In other sheep (e.g. Suffolk) and other mammals (e.g. mice) there are often changes of pigmentation in early life followed by relatively stable adult patterns (Nichols 1927, Schumann 1960, Wendt-Waegner 1961, Billingham and Silvers 1963, Mintz 1971, Schaible 1972, Gordon 1977, Schaible and Brumbaugh 1976, Petters and Markert 1979, Gearhardt and Oster-Granite 1981, Adalsteinsson 1975 and 1983).

The type of pigmentation which had the highest correlation with isolated pigmented fibres varied between the groups of sheep (All, A and P) and age (1.5 and 5.5) considered. For culling decisions of the practising Merino breeder, the associations at hogget age for all sheep (mixture of A and P) or those sheep (A) without pigmented leg fibres, are most relevant. In the case of all of the hogget sheep, the total score for pigmented fibres on

the legs, horn sites and face, and pigmented hooves had the highest correlations (0.44 to 0.50) with isolated pigmented wool fibres. The scores for pigmented fibres on the legs were highly correlated (r_s = 0.75 to 0.85) to these other types of pigmentation. There were relatively few ewes (18%) within the A group with detectable levels of isolated pigmented fibres. Nevertheless, in this case, the only type of non-fleece pigmentation significantly correlated with PFC was pigmented skin (total score) around the eyes (0.39).

It is clear that presence of pigmented leg fibres, in this sample of sheep, had a large impact on the levels of isolated pigmented wool fibres in hogget fleeces and isolated spots of pigmented wool fibres that developed later in adult life. Pigmented leg fibres had a high repeatability between 1.5 and 5.5 years age (0.85 to 0.92). Therefore, Merino breeders could substantially reduce (possibly eliminate) this fault in the current generation, and simultaneously reduce other associated pigmentation including isolated pigmented wool fibres, through culling of affected hogget sheep and any remaining affected sheep identified later. Information is now required about the inheritance of leg fibre pigmentation, isolated pigmented fibres in the hogget fleece and other related macroscopic pigmentation.

2.5. CONCLUSION

The positive associations between the various types of macroscopic non-fleece pigmentation and isolated pigmented fibres in the hogget fleece, or wool fibre spots in old age (5.5 years), depended greatly on the presence of leg fibre pigmentation. Therefore, the magnitude of the correlations obtained would have also depended on the frequency of sheep in the sample with pigmented leg fibres.

The high positive correlation coefficients between leg fibre pigmentation and other types of macroscopic pigmentation imply that traditional Merino breeders who selected against pigmented skin on the nose and lips or ears could also have been indirectly selecting, to some extent, against the occurrence of animals with leg fibre pigmentation. However, this experiment has shown that direct selection against pigmented leg fibres can lead to a dramatic decline in pigmented fibres in the hogget wool and is expected to be more efficient (especially when affected sheep represent a minority) than indirect culling of other more common types of associated pigmentation.

Most types of macroscopic non-fleece pigmentation had high repeatabilities between hogget age and normal culling age (e.g. pigmented leg fibres 0.93). Careful examination of Merinos for pigmented fibres on the legs at hogget age and culling of affected individuals would be expected to substantially reduce (possibly eliminate) this fault within that generation of sheep for the remainder of adult life.

A potential problem (isolated pigmented wool fibres) and a potential solution (cull sheep with pigmented leg fibres), was identified in this experiment but the information relates only to one sample and one generation of hogget Merino ewes. In the following chapters, other samples of sheep are examined for the inheritance of isolated pigmented wool fibres and associated macroscopic pigmentation and their relationships with production traits.

CHAPTER 3

HERITABILITIES AND CORRELATIONS BETWEEN PIGMENTATION AND PRODUCTION TRAITS IN A PEPPIN MERINO FLOCK

"Many Merino breeders concentrate a lot of selection effort against pigmentation that is found outside the wool-growing skin. Some breeders and sheep classers are extremely careful in this regard, culling even for small black and brown spots on the muzzle, lips, face, ears and legs. Sheep with prominent pigmentation of this type, such as large areas of brown pigmented hair on the legs are almost universally culled. There is also the worry at times about brown birthcoat fibres on lambs - commonly seen on the back of neck. These fall out and are not subsequently associated with a pigmented fleece. Hayman reports that brown patches of skin, obvious on lambs at birth, disappear quickly without ever being associated with pigmented wool fibres.

As such pigmentation has no bearing on the animals production, it is obvious that it can be ignored and selection effort concentrated on the economic measurements of lamb and wool production".

_____ *Dun and Eastoe (1970).*

SYMBOL	CHAPTER 3 - DESCRIPTION FOR SYMBOLS
Pigmentation	
PFC	Pigmented fibre concentration (No. per Kg scoured staples).
LPFC	Transformed data (LPFC=[$\log_{10}$(PFC+1)].
Br, Bl and T	Brown-tan, black-grey and total pigmentation.
Bare skin	
EYs	Pigment on bare skin around each eye.
Ns	Pigment on bare skin on the nose and lips.
Ls	Pigment on bare skin between the legs.
Ms	Pigment inside the mouth.
EVs	Pigment on bare skin inside the ears.
Ts	Pigment on bare skin around tail.
Skin under kemp	
Fs	Pigment on skin on face.
EDs	Pigment on skin on dorsal ears.
Fibres	
Lf1 and Lf2	Pigmented fibres on all legs (Lf1) or posterior rear legs (Lf2). A=pigmented leg fibres absent and P=Present.
Hf	Pigmented fibres on horn sites.
Ef	Pigmented fibres on ears.
El	Pigmented eye lashes.
Bf	Pigmented birthcoat halo-hairs.
Other	
Hv and Hn	Pigment in hooves and horns.
Production traits	
BWt	Birth weight of lambs.
Surv	Lamb survival (5 levels; birth to 10 months of age).
CFW	Clean hogget fleece weight.
Fd	Mean fibre diameter.
OBW	Off-shears body weight.
Other	
BRT	Birth and rearing type for hogget records or
BT	Birth type (singles and multiples) for lamb records.
DA and FK	Dam age (maiden or adult) and Flock (4 groups)
SIR and BD	Sire and birth date within the year.

CHAPTER 3

HERITABILITIES AND CORRELATIONS BETWEEN PIGMENTATION TRAITS AND PRODUCTION TRAITS IN A PEPPIN MERINO FLOCK

3.1. INTRODUCTION

Buyers and processors of Australian wool consider contamination-free wools, and systems to prevent contamination from any sources (pack and in-bale), as being among the most important of the quality issues to be addressed by the Australian wool industry (Chant Link and Associates 1994). Of the in-bale sources of contamination, dark fibre is seen as an increasing problem by some customers of Australian wools (IWS 1996).

Any dark fibres in wool can result in reduced flexibility of end-use, mending operations and financial losses during wool processing (Foulds et al. 1984). A reliable method of presale measurement of dark fibres in greasy wool is not yet available and even during early stage manufacture the measurement procedure (IWTO 1988) is labour intensive. It remains the responsibility of wool growers to minimise occurrence of dark fibres in white wool, especially wool that is Quality Assured for this aspect (e.g. Vandeleur 1993), or identify the wools at risk of containing dark fibres (AWC 1993).

The minimisation of pigmented wool has been a traditional goal of the Merino breeder (Graham 1870, "Old Hand 1953", Body et al. 1962), involving selection against macroscopic pigmentation, but these methods have been subject to little scientific

examination to determine their effectiveness and efficiency. Such traditional selection in the Merino breed has been questioned by some scientists (e.g. Dun and Eastoe 1970) who could not justify such sheep culling based on the evidence available at the time (note quote on the cover page of this chapter).

Positive phenotypic correlation coefficients between macroscopic pigmentation and isolated pigmented fibres in the fleece of Merino sheep were identified in Chapter 2 and in earlier reports (Fleet et al. 1989; Fleet and Smith 1990). These correlation coefficients provide information that is useful for current flock performances. The impact on future generations requires knowledge of the heritabilities, genetic correlation coefficients, or mode of inheritance in the case of an identifiable gene.

While remnant or remaining pigmentation on white sheep is likely to be heritable there is no information on genetic parameters for the Merino breed and little evidence available for other breeds. Terrill (1947) reported the heritability of pigmented fibre spots on the legs of Columbia and Targhee sheep breeds to be 0.26 $\pm$ 0.05 and 0.34 $\pm$ 0.07, respectively. Adalsteinsson (1975) reported that score for areas of tan fibres on the coat of weaner Icelandic sheep has a heritability of 0.46 $\pm$ 0.05 and found that such macroscopic tan areas were related to the occurrence of isolated coarse tan fibres in the adult fleece. Fleet et al. (1990) in a progeny test of 20 Corriedale sires found significant differences between sires in the concentration of isolated pigmented wool fibres in their ewe hogget progeny and the estimated heritability was 0.45 $\pm$ 0.22.

The Multiple-bloodline flock at the Agricultural Centre, Trangie, provided a resource to record pigmentation and obtain genetic parameter estimates for these traits and the major

production characters. It was from one of these bloodline flocks (MP10) that a sire had been identified that produced a high proportion of progeny with high concentrations of isolated pigmented wool fibres (Fleet 1985; Chapter 4).

In this Chapter, estimates of heritabilities are reported for isolated pigmented wool fibres and macroscopic pigmentation traits in white Merinos. Estimates of the phenotypic and genetic correlations among isolated pigmented wool fibres, various types of macroscopic pigmentation and the major hogget production traits (clean fleece weight, average fibre diameter and off-shears body weight) are presented. Also evaluated, was the likely phenotypic impact on isolated pigmented fibres of the existing flock from culling those sheep with selected types of macroscopic pigmentation. This information is required to evaluate the scope for selection and genetic improvement of wool quality through reduction of pigmented fibres. As the measurement procedure for isolated pigmented fibres concentration is not practical, the potential use of related macroscopic pigmentation on sheep as indicators of this fault, either alone or in a selection index, is clearly important for investigation and industry breeding programmes. Simultaneous fast and cumulative gains against pigmented fibres to improve wool quality are desired.

3.2. MATERIALS AND METHODS

3.2.1. Sheep and location

The sheep were located at the Agricultural Research Centre, Trangie NSW, and were the unculled ewe progeny (1984, 1985, 1986 and 1987 drops) of four bloodline flocks of medium-wool Peppin Merinos in a multiple-bloodline flock. The bloodline flocks involved are referred to as MP2, MP4, MP6 and MP10 and had been maintained since 1974-75 for genetic parameter estimates (Mortimer and Atkins 1989). Coinciding with

the commencement of this project, the flocks were maintained with 80 breeding ewes for MP2, MP4 and MP6 and 180 breeding ewes for MP10. The only selection of ewes imposed in these flocks was for obvious black wool on lambs (recessive black or piebald spot) and extreme physical disabilities. Flock MP10 was based on the Fertility Selection flock founded in 1959 and involved the use of rams selected from highly fertile ewes (Dun and Eastoe 1970). Whereas, for flocks MP2, MP4 and MP6 the rams used prior to 1984 were purchased as average replacements from industry and, therefore, pigmentation other than macroscopic wool pigmentation may have been considered by stud of origin. The rams used after 1983 (generating the progeny used in this study) were bred from within the flocks and the only selection imposed was the same as for the ewes.

The experiment commenced in July 1985, with the scoring of live-born ewe lambs for pigmentation, and in October 1985 the first fleeces and scores of pigmentation from hogget ewes of the 1984 drop were collected. These activities were repeated for three successive years giving rise to a sample of 515 hogget ewes (1984 to 1986 drop) and 681 ewe lambs (1985 to 1987 drop). The 1985 and 1986 drops were assessed at birth and at hogget age and involved 330 ewes. The 1984 drop was only scored as hoggets and the 1987 drop only as lambs. For the estimation of genetic parameters, sheep from sires with less than 5 offspring were deleted, to provide a "reasonable" number of progeny for each sire, leaving the total number of sires and progeny shown in Table 1.

3.2.2. Measurements

3.2.2.1. Macroscopic pigmentation

The scores for macroscopic pigmentation (i.e. visible to observer without assistance) on the hogget sheep were as described in Chapter 2. The score system for lambs was similar

(Appendix 2) and provided a score of total pigmentation of birthcoat halo-hair, leg fibres, face fibres, ear fibres, horn site fibres, nose-lips skin, hooves and piebald spot (Table 1).

3.2.2.2. Microscopic pigmentation

Each skirted fleece (12 months wool growth) was enclosed in a labelled plastic bag and pressed into bales for transport to Adelaide for fleece measurement. The basic method of fleece sample preparation and measurement is described in Chapter 2. In this experiment there were three observers initially employed to inspect the samples. Two of the observers left for alternative employment after the second year and were replaced with a single observer for the third year of measurements. Each set of 2 samples of 52 staples from each of the skirted fleeces was randomly allocated to the three (first two years) or two (final year) observers. Each sample of 52 staples was inspected in a step-wise manner and, depending on the proportion found to contain pigmented fibres, 50% (all staples affected) or 75% (50% or more of the staples affected) or all of the staples were inspected. The mean number of staples inspected per sample was 50, the mean number of staples containing pigmented fibres was 1.3, and the mean weight of staples inspected was 15.3g.

Darkened fibres of length greater than 20mm were scored for CSIRO Darkness grade (grade 4, pale to grade 8, black) and darkened fibre length (grade 1, $<$20mm; 2, 20-40mm and 3, $>$40mm darkened) and then permanently mounted on glass slides to confirm melanin pigmentation with a microscope (magnification to 400x). The concentration of pigmented fibres found in the fleece samples is reported as the number per kg of scoured staples.

Table 1: Number of sires and their ewe progeny assessed in 2-3 drops at Trangie between 1985 and 1987 for each of the traits considered.

Trait	Age of sheep	Number of sires	No. of ewe progeny
LPFC	Hogget	38	499
Birthcoat halo-hair	Lamb 1985-86 Lamb 1985-87	24 42	307 679
Piebald spot	Lamb	42	679
Hooves	Lamb Hogget	42 38	679 498
Leg fibres	Lamb Hogget	42 38	679 499
Face fibres	Lamb Hogget	42 38	679 499
Ear fibres	Lamb Hogget	42 38	679 499
Horn site fibres	Lamb Hogget	42 38	678 499
Eye lashes	Hogget	24	311
Face skin	Hogget	38	499
Ear dorsal skin	Hogget	38	499
Ear ventral skin	Hogget	38	499
Eye skin	Hogget	38	499
Nose-lip skin	Lamb Hogget	42 38	679 499
Inside mouth skin	Hogget	38	496
Under tail skin	Hogget	38	495
Birth weight	Lamb 1985-87	42	679
Lamb survival	Lamb 1985-87	42	679
Clean fleece wt. (kg)	Hogget	38	485
Fibre diameter (μm)	Hogget	38	492
Body weight (kg)	Hogget	38	494

3.2.2.3. Other records and measures.

A large number of records and measures were routinely assessed in the Multiple-bloodline flocks and the other characters considered were:

- Birth weight
- Score for white halo-hairs on the birthcoat. Range 1-7 according to degree of birthcoat coverage by the white hairs.
- Survival score for lambs born alive. Score 1, dead within 7 days of birth; 2, dead at marking; 3, dead at weaning; 4, dead or 5 alive at 10 month weighing.
- Clean fleece weight (hogget age).
- Mean fibre diameter (midside hogget fleece sample).
- Off-shears body weight (hogget age).

3.2.3. Statistical analyses

3.2.3.1. Analysis of variance

The data were analysed by least squares analysis of variance using the procedure GLM of SAS (1987). The $\log_{10}$ transformed concentration of isolated pigmented fibre in fleece samples (LPFC) was tested for differences between observers, as a single effect in a least squares analysis of variance, within each year of measurement. Significant differences ($P<0.05$) between observers were identified so the data were adjusted (using the difference between least square means) to match the observer that stayed for the entire period of the experiment.

The model fitted, in the least squares analysis of variance, to the data on each trait was as follows:

$$Y_{ijklmn} = u + BRT_i/BT_i + DA_j + b\,(X_{ijklmn} - X) + FK_k + YR_l + (YR*FK)_{kl} + (BRT*DA)_{ij} + (BRT*FK)_{ik} + (BRT*YR)_{il} + (DA*FK)_{jk} + (DA*YR)_{jl} + SIR_m(FK\ YR) + e_{ijklmn}$$

Where Y_{ijklmn} = the observed trait on an individual sheep.

u = the overall mean.

BRT_i = the effect of the *i*th birth-rearing type for traits recorded on ewes as hoggets (i = 1, 2, 3; 1 = single born and reared, 2 = multiple born and single reared, 3 = multiple born and reared).

BT_i = the effect of the *i*th birth type for traits recorded on ewes as lambs (i = 1, 2; 1 = single born, 2 = multiple born).

DA_j = the effect of the *j*th dam age (j = 1, 2; 1 = age 2 years at first, 2 = age 3-6 years at lambing).

X_{ijklmn} = the birthbate for that individual sheep.

X = the mean birthdate.

b = the linear regression coefficient on birthdate.

FK_k = the effect of the *k*th flock (k = 1, 2, 3, 4; 1 = MP2, 2 = MP4, 3 = MP6, 4 = MP10).

YR_l = the effect of the *l*th year (l = 1, 2, 3; 1 = 1984, 2 = 1985, 3 = 1986 for hoggets or l = 1, 2, 3; 1 = 1985, 2 = 1986, 3 = 1987 for lambs).

$SIR_m(FK\ YR)$ = the random effect of the *m*th sire nested within the *k*th flock and *l*th year.

e_{ijklmn} = the random residual error.

The FK, YR and FK*YR effects were tested with the SIR (FK YR) mean square, while the birth-rearing type and dam age main effects, and the other first order interactions were tested against the error mean square.

The pigmentation traits and reproduction trait (lamb-weaner survival) had skewed distributions and were transformed [e.g. LPFC = $\log_{10}$(PFC+1)] prior to analysis. The $\log_{10}$ transformation makes the data more symmetrical and approximates to a normal distribution (Falconer 1964). However, the heritabilities of pigmentation traits (total scores) and most of the correlation coefficients (between LPFC and non-fleece pigmentation traits) obtained with untransformed data were similar to those obtained from transformed data.

Table 2 shows significance of the main effects for most of the variables analysed. Day of birth was significant ($P<0.05$) for ear fibres on lambs and black skin on the face of hoggets, birth weight, clean fleece weight, mean fibre diameter and off-shears body weight. The effect of day of birth was removed from the data with an adjustment obtained from the option SOLUTION in the procedure GLM (SAS 1987) prior to determination of heritabilities and correlation coefficients.

3.2.3.2. Heritabilities, phenotypic and genetic correlations

The heritabilities were estimated using variance components obtained from the Restricted Maximum Likelihood (REML) method of the SAS procedure VARCOMP (SAS 1987). This method involves the separation of variance components in two parts: one that contains the fixed effects and one that does not. Initial estimates are obtained that are

invariant with respect to the fixed effects in the model. These are locally the best quadratic unbiased estimates given that the true ratio of each component to the residual error component is zero. The procedure iterates until convergence is reached for the log-likelihood objective function of the portion of the likelihood that does not contain the fixed effects (SAS 1987). The model included flock, year, other significant main effects and interactions treated as fixed effects and sire (nested within flock x year) treated as a random effect.

The formulae for calculating heritabilities from half-sib data (A) is found in Falconer (1964) while the standard errors of estimates (B) were calculated as indicated by Becker (1984). Phenotypic, genetic and environmental correlations (C) among all traits compared were estimated using variance and covariance components obtained with equal design matrices from the same procedure used for the heritabilities. The formula for estimating phenotypic or genetic correlations from half-sib data, together with an approximation of the standard error of the genetic correlation, were as described by Falconer (1964) and calculated as detailed below (C and D). The phenotypic and genetic covariance between two traits was calculated in a manner described by Raul Ponzoni (personal communication).

A. Heritability (h^2) $= 4 \times [\sigma^2 S / (\sigma^2 S + \sigma^2 E)]$

Where: σ^2 S = Variance component SIR (FK YR)

σ^2 E = Variance component Error

B. Standard Error (SE) of h^2 = $\sqrt{[16 \times V(\sigma^2S / \sigma^2S + \sigma^2E)}$

Where: $V(\sigma^2S / \sigma^2S + \sigma^2E) =$

$[((\sigma^2S + \sigma^2E)^2 \times V(\sigma^2S) + (\sigma^2S)^2) \times (V(\sigma^2S) + V(\sigma^2E) + 2COV\sigma^2SE)) -$

$(2\sigma^2S \times (\sigma^2S + \sigma^2E) \times V(\sigma^2S) + COV\sigma^2SE)] / (\sigma^2S + \sigma^2E)$

and: $V\sigma^2S$ = Variance of Sire;

$V\sigma^2E$ = Variance of Error

$2COV\sigma^2SE$ = 2 x Covariance σ^2S,E

C. Phenotypic (r_p), genetic (r_g) and environmental correlation coefficients (r_e) - Variance and covariance components are determined for each trait (e.g. A, B) and a variable equal to their sum (i.e. A+B) using the same animals and same model (equal adjustment for fixed effects found significant for one or both traits) for each of the three calculations (i.e. for A, B and A+B). Flock and Year were always fitted as fixed effects and SIR(FK YR) was fitted as the Random Effect. Correlation coefficients were determined with the following equations:

Phenotypic variance of A (V_{pA}) = $(\sigma^2S_A + \sigma^2E_A)$

Phenotypic variance of B (V_{pB}) = $(\sigma^2S_B + \sigma^2E_B)$

Phenotypic variance of A+B (V_{pA+B}) = $(\sigma^2S_{A+B} + \sigma^2E_{A+B})$

Phenotypic covariance A+B (COV_{pA+B}) = $(V_{pA+B} - V_{pA} - V_{pB}) / 2$

Genetic covariance A+B (COV_{gA+B}) = $(\sigma^2S_{A+B} - \sigma^2S_B - \sigma^2S_A) / 2$

Phenotypic correlation $r_p = COV_{pA+B} / \sqrt{V_{pA} \times V_{pB}}$

Genetic correlation $r_g = COV_{gA+B} / \sqrt{\sigma^2S_A \times \sigma^2S_B}$

Environmental correlation $r_e = r_p - (\sqrt{h^2_A} \times \sqrt{h^2_B} \times r_g) / (\sqrt{1-h^2_A} \times \sqrt{1-h^2_B})$

D. Standard error (SE) of genetic correlation coefficient:

$$SE\ r_g = ((1-r_g^2) / \sqrt{2}) / \sqrt{(\sigma h^2_A \times \sigma h^2_B) / (h^2_A \times h^2_B)}$$

Standard errors for phenotypic and environmental correlations can be estimated with formula provided in Becker (1984) but were not calculated. Standard errors for phenotypic correlations with the number of observations reported here are expected to be low ($\pm$ 0.05 to $\pm$ 0.07 e.g. Wade et al. 1992) whereas the standard errors for the genetic correlations were expected to be relatively large in view of the limited number of sires (24 - 48). As this is a small sample of sheep, the estimated genetic parameters are considered as not necessarily indicative of general parameters of the wider sheep population. In particular, the genetic correlation coefficients should be viewed with caution.

3.3. RESULTS

3.3.1. Analysis of variance

Table 2 shows the significance of the main fixed and random effects fitted to the least square analysis of variance. For many of the pigmentation traits the only significant effect was from the sires. In some cases, sire or other effect was significant in only one colour (black-grey Bl or brown-tan Br) or only the total score (T). The significance of sire (within flock and year) for LPFC was $P<0.06$. Other main effects (birth and rearing type, age of dam, flock, year and day of birth) were mainly not significant for the pigmentation traits. In contrast, for most of the production traits, year and, or, flock were significant and other effects such as birth type or birth and rearing type and age of dam age were sometimes significant.

Table 2: Significance of the main fixed and random effects fitted in the least squares analysis of variance on pigmentation traits (hogget records other than birthcoat halo-hair on newborn lambs) and production traits.

Source [A]	LPFC	Birth coat (Bf)	Pie-bald spot	Hoof (Hv)	Leg fibre (Lf1)	Face fibre (Ff)	Face skin (Fs)	Ear fibre (Ef)	EarD skin (EDs)	EarV skin EVs)	Horn fibre (Hf)
BRT	ns	ns	ns	* Br	ns	ns	ns	ns	ns	ns	ns
DA	ns	ns	ns	* Br	ns	ns	ns	ns	ns	ns	ns
FK	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
YR	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
SIR FK YR	ns	***	ns	*** * Br	*** ** Bl	** T * Bl	***	** T * Br	***	*** T Br	*** T Br
r^2 (CV)	0.23 (125)	0.35 (149)	0.09 (588)	0.29 (88)	0.34 (153)	0.16 (338)	0.28 (95)	0.17 (243)	0.24 (62)	0.25 (68)	0.28 (149)

Source [A]	Eye skin EYs)	Eye lash (El)	Nose skin (Ns)	Mth skin (Ms)	Tail skin (Ts)	Birth Wt. BWt	Survi -val Surv	Cln flc. CFW	Fib diam. Fd	Off-Shr OSW
BRT/BT	ns	ns	ns	ns	ns	***	**	***	ns	ns
DA	ns	ns	ns	ns	ns	**	ns	*	ns	ns
FK	ns	ns	ns	ns	* T ***Bl	*	*	***	***	***
YR	ns	ns	ns	ns	ns	*	ns	**	*	**
SIR FK YR	*** Bl	*** T Br	***	*** T Bl	***	ns	ns	***	***	**
r^2 (CV)	0.15 (10)	0.25 (35)	0.35 (48)	0.22 (188)	0.30 (35)	0.32 (14)	0.13 (23)	0.53 (14)	0.58 (6)	0.40 (10)

[A] Source of Variation: BRT = birth and rearing type; BT = Birth type; DA = age of dam; FK = flock; YR = year and SIR(FK YR) = sire nested within flock and year (see *Analysis of Variance 3.2.3.1.*).

Significance: * $P<0.05$; ** $P<0.01$; *** $P<0.001$; ns $P\geq0.05$.

Model r^2 and Coefficient of Variation are from the analysis of total score in the case of macroscopic pigmentation.

Pigment colour: T, total score; Bl, score for black-grey; Br, score for brown-tan. Where none of these letters are shown then the significance level applies to all three scores.

Significant interactions occurred for birth type or birth-rearing type and age of dam (BT/BRT*DA) for face and horn site fibres of lambs, brown pigment in the hoof, black skin inside the ear, and pigmented fibres at the horn sites (Br and T scores) of hoggets.

The year and age of dam (YR*DA) interaction was significant for black nose-lips skin, and the flock and birth-rearing type (FK*BRT) interaction was significant for LPFC, pigmented eye lashes (Br and T scores) and nose-lips skin (Br and T scores) of hoggets ($P<0.05$). Birth type and year (BT*YR) interactions were significant for horn site fibres on lambs and the flock and age of dam (FK*DA) interaction was significant for face fibres on lambs.

The significant effect of FK*BRT for LPFC occurred as a result of a higher LPFC for BRT=3 (multiple born and reared) in one flock alone (LPFC=1.67 vs 0.12 to 0.94). However, when the data for that flock were analysed alone the differences were not significant ($P=0.06$) and the only large difference was between BRT=2 (-0.4) and the other two alternatives (1 = 1.1; 3 = 1.4).

3.3.2. Heritabilities and incidence of pigmentation traits

Of the 515 hogget fleece samples measured, 217 (42%) had pigmented fibres detected. Based on the observer adjusted values, 20.4% of samples had a PFC greater than or equal to 100 per kg. The mean PFC was 169 per kg and the darkened fibres had a mean darkness grade of 5.1 (around the threshold of importance) and mean score for darkened fibre length of 2.3 (i.e. 20-40mm).

Appendix 3 shows the heritabilities with standard errors, the percentage of sheep having some visible pigmentation, and the mean score of the untransformed variable of the macroscopic pigmentation traits, within colour classes (Bl and Br) and the total score. There was considerable difference in occurrence of various types of macroscopic

pigmentation (e.g. 1% for horn site fibres on lambs to 100% eyes skin pigmentation on hoggets). Pigmentation types scored on lambs had a greater incidence among the hogget sheep and, in some cases, the change was substantial (e.g. leg fibre pigment increased from 3.5% affected at birth to 29% at hogget age).

Table 3 provides the heritabilities of LPFC and total scores for macroscopic pigmentation traits together with percentage of sheep affected and the mean score and phenotypic variance of the $\log_{10}$ transformed trait. LPFC had a low heritability (0.18 $\pm$ 0.12). Macroscopic fibre pigmentation traits that had high heritabilities at hogget age were leg fibres (0.82 $\pm$ 0.21), horn site fibres (0.71 $\pm$ 0.20) and eye lashes (0.45 $\pm$ 0.22). Heritabilities were moderate for ear fibres (0.25 $\pm$ 0.12) and low for face fibres (0.18 $\pm$ 0.11).

Bare skin pigmentation traits for hoggets were highly inherited (0.36 $\pm$ 0.14 to 0.69 $\pm$ 0.20), except for pigmentation around the eyes (0.14 $\pm$ 0.10). Pigmented skin under kemp fibre on the face and ears of hoggets were both highly heritable (0.65 $\pm$ 0.19 and 0.51 $\pm$ 0.17, respectively), as was pigmentation in the hooves (0.60 $\pm$ 0.18). The heritabilities for pigmentation traits scored on lambs were high for hooves, leg fibres and nose-lips skin (estimates all greater than 0.4). Pigmented birthcoat halo-hair in lambs had an estimated heritability of 0.61 $\pm$ 0.16. Ear fibres scored on lambs had a moderate heritability (0.23 $\pm$ 0.12). Horn site fibres (0.03 $\pm$ 0.05) and face fibres (0.06 $\pm$ 0.06) and piebald spot (0.08 $\pm$ 0.07) had low heritabilites for lambs. Except for pigmented leg fibres, those types of macroscopic pigmentation with low incidences of affected or unaffected individuals also had low heritabilities (Table 3).

Table 3: For each type of pigment the % sheep affected (value > 0), mean value, heritability and standard error ($h^2 \pm SE$), and the phenotypic (r_p) and genetic correlation ($r_g \pm SE$) coefficients between macroscopic pigment and isolated pigmented wool fibre concentration (LPFC), are shown.

Pigment type	Age[A]	Affected %[B]	Mean (σ^2_P)[C]	$h^2 \pm SE$	r_p	$r_g \pm SE$
LPFC	H	42	0.77 (0.97)	0.18 ± 0.12		
Birth hair	L	23	0.08 (0.02)	0.61 ± 0.16	0.33	0.66 ± 0.19
Piebald spot	L	3	0.008 (0.002)	0.08 ± 0.07	0.08	0.77 ± 0.28
Hooves	L	35	0.15 (0.04)	0.48 ± 0.15	0.27	0.35 ± 0.32
	H	59	0.49 (0.22)	0.60 ± 0.18	0.27	0.15 ± 0.31
Leg fibres	L	3	0.01 (0.01)	0.44 ± 0.14	0.11	0.04 ± 0.34
	H	29	0.27 (0.21)	0.82 ± 0.21	0.26	0.18 ± 0.28
Face fibres	L	1	0.005 (0.002)	0.06 ± 0.06		
	H	8	0.03 (0.01)	0.18 ± 0.11	0.12	0.08 ± 0.43
Ear fibres	L	8	0.04 (0.02)	0.23 ± 0.12	0.13	0.55 ± 0.77
	H	16	0.06 (0.03)	0.25 ± 0.12	0.21	0.43 ± 0.31
Horn site	L	1	0.005 (0.002)	0.03 ± 0.05		
fibres	H	29	0.21 (0.12)	0.71 ± 0.20	0.27	0.18 ± 0.29
Eye lashes	H	91	0.76 (0.08)	0.45 ± 0.22	0.29	0.39 ± 0.36
Face skin	H	53	0.25 (0.07)	0.65 ± 0.19	0.22	0.31 ± 0.28
EarD skin	H	76	0.51 (0.12)	0.51 ± 0.17	0.22	-0.10 ± 0.32
EarV skin	H	73	0.47 (0.12)	0.65 ± 0.20	0.16	-0.22 ± 0.30
Eye skin	H	100	1.06 (0.01)	0.14 ± 0.10	0.16	-0.16 ± 0.48
Nose skin	L	54	0.21 (0.04)	0.48 ± 0.15	0.23	0.28 ± 0.38
	H	83	0.49 (0.07)	0.69 ± 0.20	0.21	0.08 ± 0.30
Mouth skin	H	22	0.08 (0.03)	0.36 ± 0.14	0.29	0.14 ± 0.34
Tail skin	H	93	0.55 (0.04)	0.49 ± 0.18	0.18	0.06 ± 0.34

[A] Ages represented are newborn lambs (L) and hoggets (H).

[B] Percentage of sheep with a count or score >0 (pigment present) based on the non-transformed and unadjusted count\score data.

[C] Mean and phenotypic variance for the transformed (log10[trait+1]) variable.

3.3.3. Heritabilities of production traits

Clean fleece weight, fibre diameter and off-shears body weight had moderate to high heritabilities of 0.44 $\pm$ 0.17, 0.47 $\pm$ 0.16 and 0.31 $\pm$ 0.14, respectively (Appendix 5). These heritability estimates were of similar magnitude to those reported for a larger sample of sheep from this flock (Mortimer and Atkins 1989). The heritability of birth weight and lamb survival were low (0.14 $\pm$ 0.08 and 0.09 $\pm$ 0.08, respectively).

3.3.4. Phenotypic and genetic correlations

Appendix 4 provides a matrix of correlation coefficients between LPFC and the total scores for macroscopic pigmentation. Heritabilities and standard errors are provided along the diagonal, phenotypic correlation coefficients (in bold) with environmental correlations above the diagonal and genetic correlation coefficients below the diagonal.

Appendix 4 shows that phenotypic correlation coefficients between the various types of pigmentation were all positive and most being moderate to high. Most of the genetic correlation coefficients between macroscopic pigmentation types were higher than the phenotypic correlation coefficients and in some cases were close to unity (e.g. pigmented fibres on the legs, face and horn sites). Environmental correlation coefficients, in general, had the same sign (positive) as the genetic correlations; the most common exceptions involved birthcoat halo-hair on lambs.

Table 3 provides the phenotypic and genetic correlations between LPFC and the total score of the macroscopic pigmentation traits. All of these pigment traits had positive phenotypic correlation coefficients with LPFC that were low to moderate (0.08 to 0.33).

The genetic correlation coefficients with LPFC were also positive (0.06 to 0.77) except for skin pigment on the ears (dorsal and ventral scores) and around the eyes (-0.10 to -0.22). A strong genetic correlation was found between LPFC and pigmented halo-hair (0.66 $\pm$ 0.19) and piebald spot (0.77 $\pm$ 0.28). The genetic correlations between the other macroscopic pigmentation traits and LPFC were positive, in general, but not significantly different from zero based on the standard errors. The phenotypic and genetic correlation coefficients between pigmented birthcoat halo-hair score and score for white halo-hairs on the birthcoat were 0.24 and 0.26 $\pm$ 0.17. respectively.

3.3.5. Repeatabilities and correlations with LPFC

In Table 4 are correlation coefficients or repeatabilities for scores of pigmented fibres visible at birth and hogget age (1985 and 1986 drops) for the same site on the coat as well as correlation coefficients of the lamb scores with birth weight and lamb-weaner survival (1985 to 1987 drops). For hoof pigment and nose-lips skin pigment, scored at birth and again at hogget age, the phenotypic and genetic correlation coefficients were both high and of comparable magnitude. For leg fibres the phenotypic correlation coefficient was low, reflecting the large difference in incidence at these ages (Table 3), but the genetic correlation coefficient was close to unity. In the case of ear fibres, the phenotypic and genetic correlations were both low and may reflect the difference in incidence or type of pigmentation being recorded between the two ages. The ear fibres on lambs are usually tan in colour and this disappears in early life while those scored at hogget age are usually of black colour and increase with age (Chapter 5D).

3.3.6. Production characters

In Table 4 the phenotypic and genetic correlations between pigmentation on lambs and birth weight or lamb-weaner survival are provided. The phenotypic correlations were low and most were close to zero while most of the genetic correlations were positive. The genetic correlations significantly different from zero were hoof pigment with birth weight (0.72 $\pm$ 0.15) and nose-lips skin pigment with birth weight (0.84 $\pm$ 0.09) and lamb survival (0.65 $\pm$ 0.22).

Appendix 5 provides correlations coefficients between the pigmentation traits and the hogget production traits. The phenotypic correlations of LPFC with clean fleece weight (-0.02) and body weight (-0.11) were slightly negative and LPFC with fibre diameter was slightly positive (0.08). The corresponding genetic correlations tended to be stronger and were negative between LPFC and clean fleece weight (-0.35 $\pm$ 0.31) and fibre diameter (-0.67 $\pm$ 0.21) and positive between LPFC and off-shears body weight (0.15 $\pm$ 0.35).

The phenotypic correlations between the macroscopic pigmentation traits and production traits were all small in size (between -0.07 and 0.13). The correlations involving clean fleece weight (except the phenotypic correlation with under tail skin) and body weight were all positive. Phenotypic correlations involving fibre diameter tended to be negative.

The genetic correlations between the macroscopic pigmentation traits and clean fleece weight and off-shears body weight were in general positive, while those with fibre diameter tended to be negative. An important exception to the general trend for clean fleece weight was the negative genetic correlation with LPFC.

Table 4: Phenotypic correlations and genetic correlations ± SE between pigment on new-born lambs and hogget sheep (1985-1986; 32 sires and 307 progeny) and correlations with lamb birth weight or survival (1985-1987; 42 sires and 679 progeny).

Lamb vs hogget	Birthcoat halo-hair	Hoof	Leg fibre	Ear fibre	Nose skin
Repeatability (r_p) r_g ± SE (Lamb vs Hogget)		0.62 0.88 ± 0.06 ***	0.39 0.92 ± 0.04 ***	0.06 0.25 ± 0.42 n.s.	0.65 0.78 ± 0.12 ***
Lamb score vs Birth weight	0.01 0.36 ± 0.25 n.s.	0.04 0.72 ± 0.15 ***	0.07 0.34 ± 0.28 n.s.	0.05 -0.03 ± 0.40 n.s.	0.07 0.84 ± 0.09 ***
Lamb score vs Survival	0.04 0.34 ± 0.31 n.s.	0.01 0.50 ± 0.29 n.s.	0.04 0.02 ± 0.39 n.s.	0.05 0.16 ± 0.49 n.s.	0.09 0.65 ± 0.22 **

Significance: * $P<0.05$ ** $P<0.01$; *** $P<0.001$; n.s. not significant $P\geq0.05$

The significant genetic correlations were between: clean fleece weight and ear ventral skin (0.48 $\pm$ 0.19), ear dorsal skin (0.60 $\pm$ 0.16), eye skin (0.66 $\pm$ 0.21) and eye lashes (0.63 $\pm$ 0.22); off-shears body weight and ear fibres (0.55 $\pm$ 0.23), eyes skin (0.57 $\pm$ 0.29) and eye lashes (0.72 $\pm$ 0.16); and fibre diameter and hooves (-0.45 $\pm$ 0.18) or face fibres (-0.62 $\pm$ 0.20) and isolated pigmented fibre concentration (-0.67 $\pm$ 0.18).

3.3.7. Effects of culling based on macroscopic fibre pigmentation

Most types of macroscopic fibre pigmentation were infrequent (3 to 29%) relative to skin or hoof pigmentation (22% inside mouth and 53% to 100% for other types). The exception was pigmented eyelashes in which case 91% of the sheep were affected to some degree. In order to visualise the impact of selecting against macroscopic fibre pigmentation the following culling criteria were defined:

Criterion 1: Only the presence of pigmented halo-hairs on the birthcoat was used.

Criterion 2: Macroscopic fibre pigment present on the hogget (legs, horn sites, face and ears) except for pigmented eye lashes were considered.

Criterion 3: As for the second criterion with the maximum score for pigmented eye lashes added (i.e. > 75% of the eyelashes pigmented).

Criterion 4: As for criterion 3 but scores for pigmented fibres on lambs (birthcoat halo-hair, legs, face, ears, horn sites and piebald spots) were added.

The data from th 1985 and 1986 drops included 330 progeny, for which all types of pigment had been scored, and the average PFC was 231 per kg. Table 5 details the effect on PFC based on separation of these sheep into two groups (absent and present) for the fibre pigmentation in each culling criterion.

Table 5: No. of sheep with and without macroscopic fibre pigmentation within four culling criterion and effect on isolated pigmented wool fibres (PFC).

Criteria for culling of pigmentation	Macroscopic Pigment Absent	Present
(1) Birthcoat-halo hair alone Number of sheep. PFC (No. per kg scoured staples)	231 **147**	89 **460**
(2) Hogget records[1] excluding eyelash records Number of sheep. PFC (No. per kg scoured staples)	201 **115**	129 **413**
(3) Hogget records including eyelash records Number of sheep. PFC (No. per kg scoured staples)	127 **33**	199 **363**
(4) Hogget and lamb[2] records Number of sheep. PFC (No. per kg scoured staples)	104 **15**	214 **345**

1 Hogget records include fibre pigmentation present on the legs, horn sites, face and ears, with the maximum score (>75% pigmented) for eyelashes included/excluded.

2 Lamb records include fibre pigmentation visible on the birthcoat halo-hair, legs, face, ears, horn sites and piebald (random) spots.

Culling on presence of pigmented birthcoat halo-hair (criterion 1) excluded 28% of the sheep leading to an average PFC of 147 per kg in the unaffected group. When other macroscopic fibre pigmentation on hogget sheep (criterion 2 - excluding eyelashes) was used to cull animals the PFC averaged 115 per kg in unaffected hoggets (61% of the total). Culling animals based on criterion 3 (including pigmented eyelashes) reduced PFC to 33 per kg in unaffected hoggets (39% of the total). When all fibre pigmentation types on the lamb and hogget were considered (criterion 4) the unaffected sheep (33% of the total) had an average PFC of 15 per kg.

The effects of the above culling criteria on hogget production traits (greasy and clean fleece weight, average fibre diameter and body weight) and the other types of macroscopic pigmentation (skin and hooves) were considered by Fleet et al. (1997). There was no significant effect on mean productive performance of the existing flock but the incidence and mean score of the other types of macroscopic pigmentation (skin and hooves) were reduced in the selected group (Appendix 8).

3.4. DISCUSSION

3.4.1. General discussion

Any dark fibres in top can lead to costly repairs and financial losses in wool processing and fabric manufacture. The commonly quoted maximum number of dark fibres tolerated for sensitive end-uses is 100 per kg (Foulds et al. 1984). In this sample of 515 Peppin hogget ewes, there was 42% of fleece samples with pigmented fibres detected and the estimated mean concentration was 169 per kg. However, some of the pigmented fibres were pale (around CSIRO darkness grade 5) and may not have been of commercial importance but the mean score for darkened fibre length was high (20 to 40mm).

The wool metrology provided a basis (problem identification) for investigation. Pigmented fibres have been identified as a risk element for young Merino sheep in processing surveys conducted by CSIRO (Burbidge and McInnes 1994). Therefore, the relationships between isolated pigmented fibres and macroscopic pigmentation, as possible indicators of this hidden fault in white wool from young sheep, and the relationships with production traits (number of sheep, body weight and fibre diameter), are clearly relevant.

With few exceptions, the only significant ($P<0.05$) effect in the analysis of variance of pigmentation traits was for sires. In contrast, most of the production characters were also affected by other effects fitted in this analysis (Table 2). The environmental effects considered, in general, did not influence pigmentation.

Most types of macroscopic pigmentation had moderate to high heritabilities, for example - pigmented leg fibres 0.82 $\pm$ 0.21 and nose-lips skin 0.69 $\pm$ 0.20, but the heritability for LPFC was low (0.18 $\pm$ 0.12). Phenotypic correlation coefficients between the various types of macroscopic pigmentation (total score) were all positive (r_p = 0.1 to 0.8) and generally consistent with the trends reported in Chapter 2. The genetic correlation coefficients were also mainly positive and higher in magnitude than the phenotypic correlation coefficients (Appendix 4). The environmental correlation coefficients were usually of the same sign (positive) as the genetic correlation coefficients, and the most common exceptions involved pigmented birthcoat halo-hair scored on lambs with the hogget pigmentation traits. A difference in sign between the two correlation coefficients suggests that the environmental and genetic affects are through different physiological mechanisms (Falconer 1964).

Limitations of the sample (24 to 42 sires) restrict confidence in the genetic correlation coefficients. Isolated pigmented wool fibre concentration had relatively low phenotypic and genetic correlations with the macroscopic pigmentation traits, except the relatively strong phenotypic (0.33) and genetic correlations with pigmented birthcoat halo-hair (0.66 $\pm$ 0.19). A similar phenotypic association between score for pigmented birthcoat halo-hairs and isolated pigmented fibres was reported by Fleet et al. (1989) for a group of South Australian Merino sheep. The heritability of pigmented birthcoat halo-hair was

high (0.61 $\pm$ 0.16) relative to that of LPFC (0.18 $\pm$ 0.12). Therefore, even if measurement of LPFC was practical for routine assessment of fleeces, selection against pigmented birthcoat halo-hairs may be more efficient.

Indirect selection may lead to a correlated response that is higher than direct selection provided the two traits are highly genetically correlated and the heritability of the correlated trait is sufficiently greater than that of the character of primary interest (Turner and Young 1969). The merit of indirect selection (CR_X) via character Y relative to that of direct selection (R_X) of character X can be expressed as a ratio where:

$$\frac{CR_X}{R_X} = r_g \cdot \frac{i_Y}{i_X} \cdot \frac{h_Y}{h_X} \quad \textit{(Falconer 1964)}$$

Where: r_g = the genetic correlation coefficient between character X and Y (0.66)

i = selection intensity (assumed equal)

The h^2 of character X (LPFC) = 0.18 so h_X = 0.42.

The h^2 of character Y (birthcoat halo-hair) = 0.73 so h_Y = 0.85

Based on the above equation and components, the CR_X/R_X ratio is 1.34 so the response of LPFC to selection against pigmented birthcoat halo-hair (assessed at birth) is expected to be more effective than direct selection. However, of greater relevance is the practical application of selection. Measurement of isolated pigmented fibres by the procedure used is difficult and expensive whereas macroscopic pigmentation can be readily and cheaply assessed in the field.

Pigmented birthcoat halo-hair is temporarily visible on the lamb coat. Lamb marking is usually the first occasion that lambs are handled and can be assessed for this character. However, some shedding of fibres can occur between birth and marking (Chapter 4, Chapter 5D and Appendix 6); especially in naturally mated flocks where lambing is spread over 6-8 weeks. Therefore, to gain the full effect of birthcoat halo-hair as an indicator of isolated pigmented wool fibres, inspections should be made around the time of birth. Such inspections may not be practical unless newborn lambs are to be mothered and tagged. Ewes synchronised for oestrous and artificially inseminated would also allow lamb marking to occur closer to the start of lambing (within 3-4 weeks) and overcome some misclassification of affected lambs due to fibre shedding.

3.4.2. Relationships between pigmentation and production traits

The phenotypic correlation coefficients between pigmentation traits and the production traits considered were all low and many were close to zero. Therefore, selection against pigmentation would be expected to have little impact on the average production of the current flock (Fleet et al. 1997) but will reduce the number of sheep available for assessment on production traits.

The majority of the genetic correlations between pigmentation traits and the production traits tend to be unfavourable, with the exception of the negative genetic correlation between concentration of isolated pigmented fibres and clean fleece weight, and not significantly different from zero. Most of the genetic correlations between pigmentation and hogget clean fleece weight (LPFC being the important exception) and off-shears body

weight were positive with some coefficients being significant ($P<0.05$). Whereas, the genetic correlations with average fibre diameter were generally negative. For example, LPFC had a significant genetic correlation with average fibre diameter (-0.67 ± 0.18) indicating a possible adverse effect with selection for finer wool. The negative genetic correlation between LPFC and clean fleece weight (-0.35 ± 0.31) may indicate a favourable genetic relationship in selection for increased clean fleece weight.

It is difficult, given the estimated genetic correlations currently available, to evaluate the responses to selection from industry-relevant strategies aimed at joint improvement of wool production and quality. Some preliminary simulations (Appendix 6), using the genetic parameter estimates for pigmentation from this study and genetic parameter estimates for production traits from Ponzoni (1988), did predict that increases in isolated pigmented fibres and macroscopic pigmentation would result from index selection, as described by Ponzoni (1991), with increasing emphasis on reduced average fibre diameter. However, any predictions based on genetic parameter estimates of this study must be made with extreme caution. Robust prediction of responses to realistic selection strategies requires more precise estimates of the genetic parameters for the pigmentation traits or knowledge of the mode of inheritance in the case of simply inherited traits.

The estimated genetic correlations are not considered necessarily precise in view of the limited sampling in this study. Nevertheless, they provide a possible basis for the experiences of practising breeders who have pursued selection for production traits (Chapter 2), without controlling pigmentation outside the fleece, and have witnessed increases in pigmentation that have caused concern from both the aspect of wool quality (pigmented fibres) and of sheep marketing (buyer preference for absence or minimisation of visible pigmentation). Also, it may not be coincidental that highly productive sheep

used to form the selection lines at the Agricultural Research Centre, Trangie, also had high degrees of macroscopic pigmentation outside the fleece (Dun and Eastoe 1970).

Lamb-weaner survival is an important aspect of reproduction and, together with birth weight, showed positive genetic correlation coefficients (0.34 to 0.84) with scores for pigmented halo-hairs, hoof and nose-lips skin pigmentation on the live born lambs. However, birth weight and survival had low heritabilities (0.14 and 0.09, respectively). White spotting genes in sheep can have undesirable pleiotropic effects or be linked to genes with adverse effects on production and survival. For example, *Roan* and *Pigmented Head* in sheep are examples where a sub-vital effect is associated with the greying or white spotting (Nell 1967; Sponenberg et al. 1996).

The large differences between genetic and phenotypic correlation coefficients between pigmentation and production traits highlights the dual nature (environmental and genetic) of the phenotypic correlation. Therefore, the magnitude or sign of the phenotypic correlation are not necessarily indicative of the genetic correlation (Falconer 1964). Other than the work reported in this chapter, there is no other information published about genetic associations between remnant pigmentation on white Merinos and wool quality and sheep production. Even for other breeds of sheep there is little information on this subject reported. Terrill (1947) found in Targhee and Columbia sheep there was little association between scores for pigmented fibres on the legs and face cover, length of staple, body weight, type, condition or neck folds of weaners.

Adalsteinsson (1970) found that fertility of ewes was lower than expected in white or tan Icelandic sheep relative to those with recessive eumelanin coloured coats. Adalsteinsson (1975) found that score for tan areas on the coat was associated with pigmented fibres in

the white or faded adult fleece. Fleet and Stafford (1988) identified associations between isolated pigmented fibres and visible pigmentation in Corriedale hogget ewes and Fleet et al. (1990) showed such isolated pigmented fibres were heritable and that phenotypic correlation coefficients with the main production characters were low (0 to -0.18).

3.4.3. Effect of culling based on macroscopic fibre pigment to reduce PFC

Culling sheep using various types of macroscopic fibre pigmentation as independent selection criteria can dramatically reduce the average concentration of isolated pigmented fibres (PFC) in hogget Merino fleece wool (Table 5). For example, when all the scored types of macroscopic fibre pigmentation on lambs and hoggets were considered in the culling criterion, the PFC was reduced from 231 per kg to 15 per kg in the selected group.

This culling exercise demonstrated that wool from young Merino sheep need not be a high risk for isolated pigmented fibres if selection practices have aimed to minimise the incidence and degree of expression of macroscopic fibre pigmentation; being consistent with traditional standards for the Merino breed (Graham 1870, "Old Hand 1953", Body et al. 1962). In this flock, such culling involved up to 68% of the sheep which would not normally be practical. In any similar flocks the decision could be made to select against the highest degree and gradually work toward elimination of macroscopic fibre pigmentation.

It may be a decision of the commercial breeder or wool marketer to not be concerned about any pigmentation outside the fleece. However, ram breeders and innovative

marketers need to cater for the movement toward Quality Management Systems and their goal of preventing 'surprises' for processors. In the case of isolated pigmented fibres such surprises can be minimised in two ways:

(a) A general penalty for wool from young sheep as proposed in the CSIRO Dark Fibre Risk scheme. This opportunity arises because isolated pigmented fibres appear to decline with age (Fleet et al. 1991; Chapter 5C; Burbidge and McInnes 1994).

(b) Selection against visible indicators so that the risk of isolated pigmented fibres is also minimised in the wool from young sheep. This option also has the advantage of reducing other associated pigmentation faults (e.g. piebald spots on lambs, other hair pigmentation on the edges of the fleece and pigmented wool fibre spots that can develop in old sheep) that may be overlooked during shearing and contaminate the wool lines.

It is not practical, at present, to reliably measure the concentration of isolated pigmented fibres as part of routine fleece measurement. It is practical and inexpensive for ram breeders and commercial growers to observe macroscopic pigmentation associated with isolated pigmented fibres in the fleece. Knowledge is provided in this thesis to allow option (b) to proceed more efficiently than undertaken in the past by Merino breeders.

3.4.4. Associations between the various types of macroscopic pigmentation

The phenotypic and genetic correlation coefficients (Appendix 4) between the various types of macroscopic pigmentation (total score) were all positive. Even though the

genetic correlations were generally higher than the phenotypic correlations there was still a wide range of values (0.05 to 1.05). Heritabilities also varied widely (0.03 to 0.82) making it difficult to propose a single genetic explanation for the occurrence of the various types of macroscopic pigmentation. However, the genetic correlations between some types of pigmentation were close to unity and with low standard errors (e.g. pigmented fibres on the face, legs and horn sites) and may reflect the effects of the same gene (or linked genes).

The white coat of sheep is usually attributed to the effect of the most dominant allele (*white or tan*) at the *Agouti* locus, that allows a solid tan, tan and white, or solid white coat. In many breeds of European origin, such as the Merino, the white area predominates the coat and the amount of tan remaining is determined by genes at other loci, such as the *Spotting* locus (Adlasteinsson et al. 1980; Sponenberg et al. 1996). In Chapter 4, there is an investigation of the hypothesis of involvement of an identifiable locus in determining the presence of pigmented leg fibres or pigmented birthcoat halo-hairs; which both have high heritabilities and have been associated with the risk of isolated pigmented fibres.

The strong association between pigmented leg fibres or horn site fibres and isolated pigmented fibres, evident in the sample of Merino sheep assessed for Chapter 2, is not as clearly repeated in this group of Peppin sheep. This may be due to the lower frequency of sheep with pigmented leg fibres (29%), other sampling differences or, possibly, to an association that is transient because of recombination between linked genes influencing each character (Falconer 1964). Nevertheless, pigmented fibres on the edges of the fleece (horn sites and legs) are undesirable in Merino sheep as they may directly contribute to dark fibre levels in the oddments or skirted fleece (Steyn 1963).

Based on the present information, it seems unnecessary to cull sheep for non-fibre pigmentation in the pursuit of fleeces with minimal isolated pigmented fibres. However, it may also be unwise to allow such pigmentation to increase to degrees evident in breeds like the Corriedale. Apart from possible problems with pigmented fibres (darker fibres, piebald spots on lambs and pigmented wool fibre spots developing in adult life) there is also the important aspect of satisfying the expectations of clients (ram buyers) and to a lesser degree breed standards. Dolling (1970) recognised that even if pigmentation outside the fleece had no undesirable associations with any aspect of production the absence of such pigmentation may still remain to be valued by Merino ram buyers.

3.5. CONCLUSION

This work has shown that most types of macroscopic pigmentation in hogget Merino sheep have moderate to high heritabilities and most were positively correlated (phenotypic and genetic) with the log concentration of isolated pigmented fibres in the hogget fleece. However, the heritability of the concentration of isolated pigmented fibres was low (0.18 $\pm$ 0.12). Pigmented birthcoat halo-hair had the highest phenotypic (0.33) and genetic (0.66 $\pm$ 0.19) with the concentration of isolated pigmented wool fibres. The various types of visible pigmentation were also generally positively correlated among themselves.

Culling the less common types of macroscopic fibre pigmentation (birthcoat halo-hair, piebald spot, legs, horn sites, face and ears), and the maximum score pigmented eye lashes, in this sample of sheep was sufficient to reduce the concentration of isolated pigmented in hogget fleeces to low levels (e.g. 15 to 33 per kg). Nevertheless, this culling involved up to 68% of the flock which is unlikely to be acceptable in practice and, therefore, a longer term reduction may be required in any similar flocks.

The phenotypic correlation coefficients reflect little or no effect on average production of the existing generation from selection against pigmentation but such culling will reduce the number of animals available for assessment on production traits. However, the pigmentation traits, generally, had positive genetic correlation coefficients with hogget clean fleece weight and off-shears body weight and, generally, had negative genetic correlations with average fibre diameter.

There is evidence of adverse genetic correlations between production traits and pigmentation traits which may affect wool quality (pigmented wool fibres or fibre diameter), clean fleece weight and body weight, or pigmentation of possible importance to breed standards and sheep marketing. However, the genetic correlations presented here are imprecise due to limitations of sampling (24 - 42 sires). To assess with greater certainty the effects of selection strategies on sources of dark fibre contamination or other pigmentation in Merinos, larger data sets than the one currently available are needed to obtain precise estimates of the genetic parameters for isolated pigmented fibre concentration and non-fleece pigmentation traits. Until these estimates are available, control of pigmented fibre contamination through breeding in practice will need to continue to rely on selection against pigmentation independent of strategies designed to improve production and wool quality.

The current estimates indicate that isolated pigmented fibres concentration is less heritable than the majority of macroscopic pigmentation traits, as well as production traits. Pigmented birthcoat halo-hair is a likely indirect selection criterion for reducing isolated pigmented fibres in the fleece wool of future generations and, together with other types of macroscopic fibre pigmentation on the coat, can be used as a culling criteria for reducing isolated pigmented wool fibres in the hogget wool of the current generation.

CHAPTER 4

THE MODE OF INHERITANCE OF PIGMENTED LEG FIBRES AND PIGMENTED BIRTHCOAT HALO-HAIRS

"I will pass through all thy flock today, removing from thence all the speckled and spotted cattle, and all the brown cattle among the sheep, and the spotted and speckled among the goats: and of such shall be my hire. So shall my righteousness answer for me in time to come, when it come for my hire before thy face: every one that is not speckled and spotted among the goats, and brown among the sheep, that shall be counted as stolen with me".

Jacob's covenant to Laban, in 'Genesis' the first the first book of Moses (approx. 1445-1405 BC) translated for the Holy Bible.

"I have had considerable experience in sheep breeding since my early youth and have no hesitation in saying that if one is careful to always use sires with wool of true wool character he will find that the noses of sheep (pigmented skin spots) will gradually clean up. .....It is also true that when sheep have red or heavily-marked red legs the condition is also associated with a dull and rusty coloured wool. So much so is this fact that when I encounter it my eyes always turn to inspect the legs of that particular sheep. In my opinion it would be best to disregard sheep with badly-red legs".

"Old Hand" (1953).

CHAPTER 4

THE MODE OF INHERITANCE OF PIGMENTED LEG FIBRES AND PIGMENTED HALO-HAIRS IN MERINO SHEEP

4.1. INTRODUCTION

The white coat of sheep is usually attributed to the effect of the most dominant allele (*white or tan*) at the *Agouti* locus, that allows a solid tan, tan and white, or solid white coat. In many breeds of European origin, such as the Merino, the white area predominates the coat and the amount of tan remaining is determined by genes at other loci, such as the *Spotting* locus (Adalsteinsson 1970; Lauvergne 1975; Adlasteinsson et al. 1980; Lauvergne et al. 1981a; Sponenberg et al. 1996).

The observations made within a sample of Western Australian Merinos (Chapter 2) clearly implicate the presence of leg fibre pigmentation (mainly brown-tan in colour) with the occurrence of isolated pigmented fibres in the hogget fleece of Merino sheep. This finding is consistent with observations on Suffolk (Nichols 1927), Icelandic (Adalsteinsson 1970) and Corriedale sheep (Fleet and Stafford 1989). Data from South Australian strong-wool Merinos has also implicated pigmented fibres on the horn sites (Fleet et al. 1989; Fleet and Smith 1990) and pigmented halo-hair on the birthcoat of lambs (Fleet et al. 1989) with this wool fault.

The quantitative genetics study described in Chapter 3 provided further evidence of association between the various types of macroscopic pigmentation and isolated pigmented fibres in the hogget fleeces of medium-wool Peppin Merinos. Based on the quantitative

analysis, the score for pigmented birthcoat halo-hair had the highest phenotypic and genetic correlation coefficients with isolated pigmented fibres concentration. However, qualitative analysis of the data showed how other types of macroscopic fibre pigmentation could have been used to reduce, in an additive manner, the incidence of isolated pigmented fibres.

Pigmented leg fibres and pigmented birthcoat halo-hair on Merino sheep have high heritabilities (Chapter 3) but the mode of inheritance of these types of pigmentation is uncertain. Whether there are identifiable loci or a linkage group determining the presence of such macroscopic pigmentation on white Merino sheep remains to be clarified.

A genetic study with Targhee and Columbia sheep (Terrill 1947) did not provide a basis for a simple explanation for the occurrence of pigmented leg fibres. However, the heritability of leg colour in these breeds (0.34 ± 0.07 and 0.26 ± 0.05, respectively) is considerably lower than found in Merinos (0.82 ± 0.21) and may reflect a different genetic determination.

The first quotation on the cover page of this chapter is taken from the Holy Bible (1st book of Moses) and apparently reflects beliefs about the mode of inheritance of some colour patterns in sheep and goats. The second quotation is provided, like others in this thesis, to reflect the level of understanding and lack of knowledge advanced by science even in relatively recent times. In this case, the author ("Old Hand" 1953) refers to pigmentation of the nose and pigmented fibres on the legs in relation to characteristics of the fleece. The thoughts conveyed are similar to many others offered by sheep classers and some sheep breeders who promote indirect selection as a means of improvement.

This chapter presents the results of the analysis of the segregation of phenotypes, classified on the basis of presence and absence of pigmented leg fibres or pigmented halo-hairs on the birthcoat, among the progeny of parents classified for the same phenotypes. The hypothesis tested for each of these macroscopic forms of pigmentation is that their inheritance can be related to an identifiable locus (i.e. show evidence of simple Mendelian inheritance). In addition, the qualitative relationship between pigmented birthcoat halo-hair or pigmented leg fibres and isolated pigmented fibres in the hogget fleece was monitored.

4.2. MATERIALS AND METHODS

4.2.1. Location and sheep

In the first three matings at Turretfield Research Centre between (between 1985 and 1987) all of the rams were affected by pigmented leg fibres. The rams came from the Fertility Flock (MP10) at the Agricultural Research Centre, Trangie (Rams 1 to 4) or were 8 sons of one of the Fertility Flock rams (Rams 5 to 12). It was known (Fleet 1985) that most of the progeny of Ram 1 could be affected and have high concentrations of isolated pigmented fibres. In year 5, some of the sons of Ram 1 were mated again to obtain additional observations.

The rams were mated to South Australian Merino ewes mainly not affected by leg fibre pigmentation from four origins, as follows:

Origin 1: Ewes unlikely to be carriers of recessive black (A^{Wt}/A^{Wt}).

(Generated from white x white sheep matings, Fleet et al. 1989).

Origin 2: Ewes that were carriers of recessive black (A^{Wt}/A^{a}).

(Generated from white x black sheep matings, Fleet et al. 1989).

Origin 3: Ewes with a piebald spot (Fleet and Smith 1990).

Origin 4: Other unselected ewes that were available at the time of mating.

The information available about genotype for recessive black and phenotype for Australian Piebald enabled an opportunity to assess independence of these alternative pigment classes from the inheritance of pigmented leg fibres among their progeny.

The fourth mating involved four origins of rams and ewes and within each origin the sheep were allocated on the basis of being unaffected or affected by leg fibre pigmentation. Two of the origins involved Trangie Fertility cross SA Merino sheep that were either related or not related to Ram 1 and the other sheep were from two private Merino flocks. Six unaffected rams (Rams 13 to 18; 1 to 2 per origin) and 9 affected rams (Rams 20 to 28; 1 to 4 per origin) were single sire mated to equal numbers of ewes randomly allocated from each origin.

For the last two drops, additional progeny of Rams 7, 9 and 11 (Sons of Ram 1) were generated. In addition, two affected rams (Rams 29 and 30), an unaffected ram (Ram 19), and a black Merino ram were mated. The black Merino ram (Ram 31) was the son of Ram 29 and a ewe without pigmented leg fibres. Table 1 summarises the matings conducted to study in a qualitative manner the inheritance of pigmented leg fibres.

Table 1: Matings conducted to study the mode of inheritance of pigmented leg fibres

Ram Number	Year / Drop	Ram Phenotype	Ewe Phenotype	Results
Rams 1-4 (from MP10)	3	Affected	Most Unaffected	Table 3
Rams 5-12 (Sons of Ram 1)	1,2,5	Affected	Most Unaffected	Table 3
Rams 13-18	4	Unaffected	Unaffected & Affected	Table 4
Rams 20-28	4	Affected	Unaffected & Affected	Table 5
Ram 19	5	Unaffected	Unaffected & Affected	Table 4
Ram 29-30	5	Affected	Unaffected & Affected	
Ram 31 (Son of Ram 29)	6	Black	Unaffected	Table 5

As macroscopic leg fibre pigmentation appeared to be inherited in a dominant manner, and is 'usually' infrequent and likely to be culled in commercial Merino flocks, the following was assumed:

(a) Those sheep with no pigmented leg fibres were termed **Unaffected** and assumed to be homozygous for the hypothetical recessive allele responsible for the elimination of this fibre pigmentation from the white coat of Merinos.

(b) Those sheep with any pigmented leg fibres were termed **Affected** and assumed to be heterozygous for the alternative allele(s) that allow the presence of pigmented leg fibres. Any sheep from parents that were known to be both affected were excluded. The reason for excluding these sheep is that at least half of them would be expected to have two alleles allowing the presence of pigmented leg fibres (i.e. homozygous for presence).

As the matings progressed, there were more records available for the parents regarding pigmented birthcoat halo-hair. The assumptions used for leg fibre pigmentation were also applied to dark birthcoat halo-hair. Sheep that had pigmented birthcoat halo-hair were assumed to be heterozygous and the unaffected individuals assumed homozygous for the proposed recessive allele that eliminates pigmented halo-hair. However, due to the higher frequency of this character within the Merino, possibly around 23% (Fleet et al. 1989), there was likely to be a greater proportion of individuals homozygous for presence of pigmented halo-hairs. This meant that the assumption about heterozygous status of affected individuals may have been compromised.

4.2.2. Measurements

4.2.2.1. Fleece measurement

For the first three drops of progeny, each skirted hogget fleeces was grid sampled to provide two samples of 52 staples. The two samples from each fleece were allocated to a different observer for measurement of the concentration of isolated pigmented wool fibres. In the first two drops, all the staples tips were removed (trimmed to 70 mm) and all suspected pigmented fibres (with length > 20 mm) from each staple were collected for microscopic inspection (80x or 400x). The fleeces from progeny of the third drop were measured in a similar manner as described in Chapter 5E.

In the fourth year, the fleece sampling procedures involved clipping wool from three body sites (thigh, dorsal neck, and midside) prior to shearing. Twenty scoured staples (untrimmed) were inspected from each site. Each set of three samples from each sheep was inspected by one of three observers.

In the first three years a single measurement derived from a grid sample of the whole skirted fleece (PFC) was available, while for year 4 the measurements were based on a sample from the neck (PFCN), midside (PFCS) and thigh (PFCL) and a pooled result (PFC).

4.2.2.2. Macroscopic pigmentation

Macroscopic pigmentation was either scored in the same manner described in Chapter 2 or was simplified to a single score for multiple sites (e.g. legs) and recording the greatest degree of expression. Pigmented leg fibres (Lf) were scored at birth, lamb marking (years 1 to 3: age 0 to 2 months), lamb shearing (3 to 5 months) and hogget shearing (1.5 years). Pigmented birthcoat halo-hair (Bf) was observed at birth and lamb marking with any score 0 (none present) at birth being replaced by a score >0 at lamb marking. Classification of sheep for presence and absence of (Lf) involved all records between birth and hogget age.

4.2.3. Records, variable expression and change of classification

All lambs born were scored for pigmented leg fibres and pigmented birthcoat halo-hair except for those severely mutilated by predatory animals and in year 6 only pigmented leg fibres were recorded. All lambs, or at least those lambs without pigmented leg fibres, were inspected again for pigmented leg fibres at lamb shearing (3 to 5 months), some at lamb marking (0 to 2 months age), and at hogget shearing (1.5 years). In the first four drops of lambs, all the surviving offspring were scored at lamb shearing and hogget shearing.

For those sheep classified at birth as having pigmented leg fibres (affected), the proportion apparently unaffected by the time of lamb shearing was 3.1% and hogget shearing was 1.7%. For those classified as unaffected at birth the proportion affected at lamb shearing was 13.5% and hogget shearing 21.3% (Table 2). This cross-over of phenotypes (unaffected to affected) has an effect of increasing the apparent survival of affected hoggets.

Table 2: Proportion of progeny (%) within classes for being affected or unaffected by leg fibre pigmentation at birth and lamb shearing or birth and hogget shearing.

Comparison	Affected → Affected	Affected → Unaffected	Unaffected → Unaffected	Unaffected → Affected
Birth vs Lamb shear	43.3%	3.1%	40.1%	13.5%
Birth vs Hogget	38.5%	1.7%	38.5%	21.3%

The degree of expression of leg fibre pigmentation on Merino lambs varied from single fibres or few spots to fully pigmented legs (Figures 2 to 4). In occasional extreme cases, the spots extended from the legs into the wool bearing areas. The colour of the pigmented fibres on the face, legs and horn sites of Merinos was usually tan-brown and occasionally black-grey. In addition, there were some distinct Piebald spots that occurred without the lateral symmetry that is usually evident with the more diffuse speckling. In Figure 1 and 2 the sheep show black Piebald spots occurring in conjunction with the usually symmetrically distributed diffuse tan fibre pigmentation. In Figures 3 and 4 the Piebald spots are comparable in colour to the many diffuse tan fibre spots.

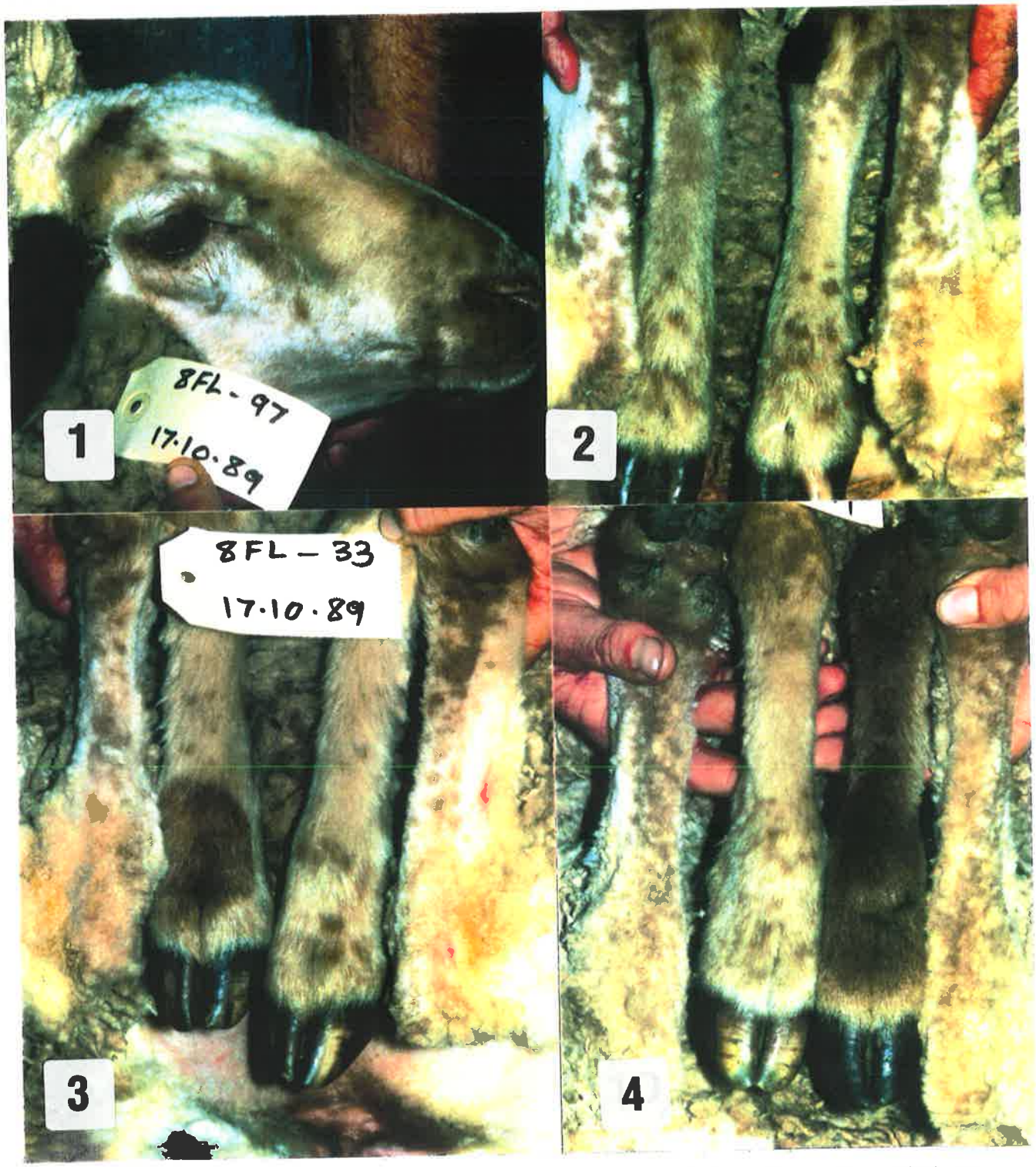


Figure 1: Shows tan fibres on the face and horn sites together with a black ear (Australian Piebald spot) on this sheep.

Figure 2: Tan fibre spots are evident on all legs of this sheep and a black Piebald (random) spot is present on the left hand front knee.

Figure 3: Tan fibre spots are evident on all legs of this sheep and a brown Piebald (random) spot is present on the right hand pastern.

Figure 4: In this case, the brown Piebald spot totally covers the front left hand leg. Also note the black hoof.

Pigmented birthcoat halo-hair is a reddish-tan colour or occasionally black-grey and located on the dorsal neck with other areas of the body occasionally affected. Degree of expression varied from single hairs to a dense patch of variable size (see Chapter 2 for examples). In the first four drops of lambs, pigmented birthcoat halo-hair was scored again at lamb marking (0 to 2 months age). For those lambs originally classified as affected, the proportion apparently unaffected by lamb marking was 20.3%. For those originally classified as unaffected 1.6% were later identified as affected.

4.2.4. Statistical analysis

The association between dam group (origins 1, 2 and 3) or sex of lamb (male and female) and presence or absence of pigmented leg fibres or pigmented birthcoat halo-hairs was examined by contingency analysis. The Chi-square statistic was used to test for departures from independence. The segregation data were tested with the Chi-square statistic by comparison of observed and expected frequencies; provided the expected frequency for each class was at least 1 (Snedecor and Cochran 1967). This simple approach was appropriate because when parental records were available, inference can be made about the likely genotype, and the effects of other factors are minimal (Nicholas 1984).

The associations between visible pigmentation and isolated pigmented fibres were examined by Spearman's correlation coefficients and contingency tables. The contingency tables involved comparisons between level of isolated pigmented fibres in the fleece (PFCG) and grade based on degree of expression of pigmented leg fibres (Lf) or pigmented birthcoat halo-hairs (Bf) or class LB for presence of Lf and Bf.

For the contingency tables the range of scores for pigmented leg fibres and birthcoat halo-hair were reduced to 4 grades: Grade 0, no visible pigment; Grade 1, few pigmented halo-hairs (1-10) or few pigmented leg spots; Grade 2, many halo-hairs (21-100) or legs speckled; and Grade 3, many pigmented fibres (>100) or large pigmented patch/area(s) on legs. In years 1 to 3 the multiple scores on legs provided a range of scores between 0 and 32 for pigmented fibres. These scores were grouped to produce the 4 grades used in the contingency tables: Grade 0, (absent); Grade 1, score 1-8; Grade 2, score 9 to 16; Grade 3, score >16.

4.3. RESULTS

4.3.1. Dam type and sex of lamb

There were no significant differences in the frequencies of progeny affected or unaffected by pigmented leg fibres and the sex of lambs. The same outcome applied to progeny affected and unaffected for pigmented birthcoat halo-hair. Table 3 shows that there was no significant association between presence or absence of pigmented leg fibres of the progeny and dam type (i.e. carrier of recessive black, unlikely carrier of recessive black or Piebald). Based on this outcome, all dams were subsequently classified only for the pigmentation of primary interest.

The piebald spots in the progeny (Table 3) were mainly black-grey (Figure 1 and 2) in colour (83%) and the remainder were recorded as having a brown-tan colour (Figures 3 and 4). Figure 5 shows a lamb with a random brown-tan spot on the shoulder and note the single pigmented hoof.

Table 3: Number of progeny affected or unaffected by leg fibre pigmentation and piebald spots (in brackets) in relation to dam classification for recessive black (carrier or non-carrier) and Australian piebald phenotype.

Dam type	Leg fibre pigment Present	(Piebald spot) Absent	Chi-square
Black carrier (A^{Wt}/A^{a})	40 (8)	43 (75)	0.1 ns
Unlikely carrier (A^{Wt}/A^{Wt})	21 (6)	21 (36)	ns
Piebald (AsP^{p}/AsP^{p})	43 (15)	29 (57)	2.7 ns
	Chi-square for:	Legs spots Piebald	= 2.2 ns = 3.9 ns

Figure 5: A newborn lamb (dead) showing a single pigmented hoof and a Piebald tan fibre spot on the shoulder.

4.3.2. Inheritance of leg fibre pigmentation from Ram 1

Table 4 shows the segregation of leg fibre pigmentation among the progeny of four Trangie Fertility rams and eight sons of Ram 1. Ram 1 produced 7.0% of unaffected progeny when mated to unaffected dams whereas for the other Trangie rams at least half of the progeny were unaffected. The five unaffected lambs from Ram 1 died before the second scheduled inspection for pigmented leg fibres at lamb marking. The absence of pigmented leg fibres in these lambs may be due to their death before the visible on-set of the pigmentation.

Table 4: Incidence of leg fibre pigmentation among the progeny of four Trangie Fertility Rams and eight sons of Ram 1.

Dam Progeny	Unaffected Dam Affected	 Unaffected	Prob. 1 : 1[C]	Affected Dam Affected	 Unaffected	Prob. 3 : 1[C]
Ram 1: 26[A]	66	5	***	17	0	*
Ram 2: 4[A]	19	21	ns	6	0	ns
Ram 3: 2[A]	18	35	*	1	2	
Ram 4: 10[A]	8	8	ns			
Ram 5: 20[A]	15	12	ns	3	0	
Ram 6: 16[A]	17	12	ns	6	0	ns
Ram 7: 10[A]	23	19	ns	30	9	ns
Ram 8: 9[A]	10	10	ns	4	1	ns
Ram 9: 23[A]	31	22	ns	19	5	ns
Ram 10: 16[A]	16	14	ns	7	3	ns
Ram 11: 14[A]	23	26	ns	26	11	ns
Ram 12: 2[A]	8	16	ns	4	0	ns
Total 5 - 12	143	131	ns	99	29	ns

Significance: *** $P<0.001$; ** $P<0.01$; * $P<0.05$; ns not significant ($P \geq 0.05$).
[A] Score for leg fibre pigmentation of the ram (all legs - maximum score = 32).
[C] Probability the ratio of affected to unaffected is different from expectation (e.g. 1:1)

A significant departure from a 1 : 1 ratio for the progeny phenotypes also occurred for Ram 3 while the other Trangie rams had segregation ratios consistent with the ratios being tested. Note also from Table 4, that Ram 3 had the lowest mean score for pigmented leg fibres. All Sons of Ram 1 (Rams 5 to 12) produced segregation ratios that were not significantly different ($P \geq 0.05$) from expectation for the 1 : 1 and 3 : 1 ratios.

4.3.3. Inheritance of leg fibre pigmentation among other sires

4.3.3.1. Unaffected sires

The results for the seven Merino sires that were unaffected by pigmented leg fibres are shown in Table 5. Overall 10.8% of progeny from unaffected ewes were affected. This result is inconsistent with the hypothesis for a single locus determination unless other non-genetic factors can explain the unexpected phenotypes. All rams, when mated to affected ewes, each had a segregation of progeny phenotypes consistent with the expectation.

Table 5: Segregation of leg fibre pigmentation in progeny from unaffected sires

Dam	Unaffected Dams		Affected Dam		Prob.
Progeny	Affected	Unaffected	Affected	Unaffected	1 : 1[C]
Ram 13	1	11	5	8	ns
Ram 14	1	6	11	11	ns
Ram 15	0	12	7	7	ns
Ram 16	1	10	6	5	ns
Ram 17	0	16	5	5	ns
Ram 18	0	5	9	15	ns
Ram 19	8	31	10	4	ns
All sires	11	91	53	55	ns

Significance: ns not significant ($P \geq 0.05$).
[C] Probability that the ratio of affected to unaffected is different from expectation (e.g.1:1)

4.3.3.2. Other affected sires

Table 6 provides the segregation ratios for leg fibre pigmentation on progeny of other affected sires mated to affected and unaffected dams. Ram 29 was a son of Ram 1 and a ewe that had pigmented horn site fibres but no pigmented leg fibres were recognised. The segregation of phenotypes, with one exception, was consistent with expectation for a homozygous status. As with Ram 1, this exception involved a lamb that died before lamb marking and a second inspection for pigmented leg fibres.

Table 6: Segregation of leg fibre pigmentation in progeny from other affected sires

Dam / Ram	Unaffected Affected	Dams Unaffect.	Prob. 1 : 1[C]	Affected Affected	Dams Unaffect.	Prob. 3 : 1[C]
Ram 29: 32[A]	28	0	***	21	1	*
Ram 20: 8[A]	3	11	*	14	6	ns
Ram 21: 23[A]	6	9	ns	12	5	ns
Ram 22: 32[A]	4	1	ns	17	2	ns
Ram 23: 32[A]	6	4	ns	11	7	ns
Ram 24: 26[A]	8	3	ns	25	15	ns
Ram 25: 23[A]	5	7	ns	12	6	ns
Ram 26: 24[A]	7	4	ns	11	2	ns
Ram 27: 28[A]	10	4	ns	9	2	ns
Ram 28: 22[A]	3	9	ns	12	6	ns
Ram 30: 8[B]	11	8	ns	6	3	ns
Ram 31 - NA	25	19	ns			
Rams 20 - 31	88	79	ns	129	54	ns

[A] Score for leg fibre pigmentation of the ram (all legs - maximum score = 32).
[B] Maximum score for posterior of the rear legs only. NA Not applicable - self-colour.
[C] Probability the ratio of affected to unaffected is different from expectation (e.g. 1:1)
Significance: * $P<0.05$; ns not significant ($P \geq 0.05$).

Most of the other rams listed in Table 6 showed a segregation of phenotypes among their progeny that were not significantly different from expectation for 1 : 1 and 3 : 1 ratios. Ram 20 was the only case where the segregation of progeny phenotypes differed significantly from expectation for the 1 : 1 ratio but was not significant for the 3 : 1 ratio.

Ram 20 also had the lowest score for pigmented leg fibres. However, given that there were 21 tests made in Table 6, for proposed heterozygous rams, the one significant difference (Chi-square, 1 D.F. = 4.57) could simply be due to chance alone. Furthermore, the overall results involving 350 progeny did not differ significantly from expectations (1 : 1 and 3 : 1 ratios).

4.3.4. Inheritance of dark birthcoat halo-hair

In Table 7, two sires (Rams 19 and 29) are considered as possibly homozygous for hypothetical alleles for absence (Ram 19) or presence (Ram 29) of pigmented halo-hair. However, in both cases, progeny occurred with unexpected phenotypes. As with leg fibre pigmentation, this result is inconsistent with the hypothesis of involvement of a single locus unless other non-genetic factors can explain the unexpected phenotypes. The proposed heterozygous status of Ram 30 was based on parental records as well as own phenotype and, in this case, the segregation of phenotypes among his progeny was close to that expected. However, other sires (Rams 3, 7, 8 and 10) each had a segregation of phenotypes among their progeny that was inconsistent with the hypothesis.

Table 7: Segregation of pigmented birthcoat halo-hair phenotypes.

Dam	Unaffected Dams		Prob.	Affected Dams		Prob
Progeny	Affected	Unaffected	1 : 1[C]	Affected	Unaffected	3 : 1[C]
Ram 19[B]	5	26		11	10	
Ram 29	10	2	*	29	0	***
Ram 1[A]	20	7	*	10	2	ns
Ram 2[A]	12	9	ns	13	4	ns
Ram 3[A]	3	11	*	0	8	***
Ram 5	8	6	ns	5	0	ns
Ram 6	10	7	ns	5	0	ns
Ram 7	22	10	*	20	5	ns
Ram 8	9	1	*	2	0	
Ram 9	15	12	ns	22	6	ns
Ram 10	11	2	*	8	2	ns
Ram 11	12	22	ns	21	8	ns
Ram 30	8	10	ns	14	4	ns

Significance: *** $P<0.001$; ** $P<0.01$; * $P<0.05$; ns not significant ($P\geq0.05$).
[A] Birthcoat halo-hair phenotype of these rams was not known.
[B] This ram was unaffected while the remaining rams were affected.
[C] Probability that the ratio of affected to unaffected is different from expectation.

4.3.5. Association between leg fibre pigmentation and halo-hair pigmentation

Table 8 shows the frequencies of progeny affected or unaffected by pigmented leg fibres and pigmented birthcoat halo-hairs from Merino sires forming drops 1 to 5 that had leg fibre pigmentation and were mated to ewes without both pigmented leg fibres and pigmented birthcoat halo-hairs. Among these progeny, there was a significant trend ($P<0.001$) for pigmented leg fibres and pigmented halo-hairs to be inherited together.

However, it is clear that the association is not complete (also see correlations in 4.3.7.1) and, therefore, that different loci are likely to be involved in determining the presence of each type of pigmentation.

Table 8: Number of progeny with pigmented leg fibres and, or, pigmented birthcoat halo-hair from dams unaffected by both types of visible pigmentation mated to rams with pigmented leg fibres.

Type of pigmentation	Leg fibre - absent	Leg fibres - present
Birthcoat hairs - absent	50	36
Birthcoat hairs - present	28	75
Chi-square[A]	18.5 ***	

[A] Chi-square with 2 degrees of freedom tests for independence between the two phenotypes (absent vs present) for each character (leg fibre vs birthcoat hairs).

4.3.6. Summary of the segregation for pigmented leg fibres

Table 9 summarises the segregation of progeny affected and unaffected by pigmented leg fibres from parents that were unaffected (Unaff), assumed heterozygous (Aff1) or assumed homozygous (Aff2) for the proposed allele that allows the pigmentation. The occurrence of unexpected progeny phenotypes, from matings involving proposed homozygous individuals, contradicts the hypothesis of a single locus of two alleles with complete dominance and full penetrance. However, there are limitations of classification of phenotypes that will be discussed later. The progeny phenotypes produced from the other matings (Unaff x Aff1; Aff1 x Aff1) provide results that are very close to expectation.

Table 9: Summary of the frequencies of progeny phenotypes observed and expected (brackets). The hypothesis is for a single locus, two allele model, determining presence of pigmented leg fibres and is tested with matings based on parental phenotype and assumed genotype [A].

Progeny Phenotypes	Parental Classification			
	Unaff x Unaff	**Aff1 x Unaff**	**Aff1 x Aff1**	**Aff2 x Unaff**
Leg fibres				
Affected	0.108 (0)	0.5 (0.5)	0.734 (0.75)	0.949 (1.0)
Unaffected	0.892 (1.0)	0.5 (0.5)	0.266 (0.25)	0.051 (0)
Total	102	658	320	99

[A] Parental phenotypes and assumed genotypes:

Unaff = Unaffected and assumed homozygous for the allele that prevents leg fibre pigmentation.

Aff1 = Affected and assumed to be heterozygous for the hypothetical allele allowing pigmented leg fibres.

Aff2 = Affected and assumed to be homozygous for the hypothetical allele allowing pigmented leg fibres.

4.3.7. Associations between pigmentation scores or counts

4.3.7.1. Correlation coefficients

The simple correlation coefficients (Spearman's) between the various types of pigmentation and concentration of isolated pigmented fibres in the hogget fleece (PFC) for the first three drops were all positive and generally significant (Table 10). All types of macroscopic pigmentation were also positively associated among themselves. For example, the correlation coefficient between pigmented leg fibres and hoof pigment or pigmented birthcoat halo-hairs was $r_s = 0.83$ and 0.51, respectively.

Table 10: Spearman's correlation coefficients (r_s) between the concentration of isolated pigmented fibres and macroscopic pigmentation in years 1-3.

Pigment type	Correlation (r_s) with PFC	Pigment type	Correlation (r_s) with PFC
Birthcoat halo-hairs	0.50 ***	**Under tail skin**	0.36 ***
Leg fibres	0.56 ***	**Between leg skin**	0.10 ns
Face fibres	0.41 ***	**Face skin**	0.45 ***
Ear fibres, lambs	0.39 ***	**Ear skin dorsal**	0.43 ***
Ear fibres, hogget	0.34 ***	**Ear skin ventral**	0.30 ***
Horn site fibres	0.54 ***	**Eye skin**	0.41 ***
Eye lashes	0.39 ***	**Nose-lips skin**	0.50 ***
Hooves	0.52 ***	**In-mouth skin**	0.51 ***

Significance: *** $P<0.001$; ns not significant $P\geq0.05$

Table 11 shows correlation coefficients for the fourth lamb drop and includes pigmented fibre concentration at three body sites as well as the combined result. All of these correlation coefficients are positive and significant ($P=0.0001$). The macroscopic pigmentation that was most highly correlated ($r_s > 0.37$) with pigmented fibres in the neck wool (NPFC) was birthcoat halo-hair (0.49) and leg fibres (0.41) while for the midside wool (PFCS) they were leg fibres (0.38) and horn site fibres (0.38). For thigh wool the highest correlation coefficients were with horn site fibres (0.42), hooves (0.40), leg fibres (0.39) and nose-lips skin (0.39). Note that the correlation coefficients between scores for pigmented fibres on the legs, ears (hogget) and horn sites together with nose-lips skin and hooves were higher than other combinations of macroscopic pigmentation.

Table 11: Spearman's correlation coefficients (r_s) between various types of pigment and the concentration of isolated pigmented fibres in each of the three fleece samples (neck PFCN; midside PFCS; thigh PFCL) and pooled PFC.

Pigment type	PF CS	PF CL	PF C	Bf[L]	Ef[L]	Lf	Ef	Ff	Hf	Ns	Hv
PFCN (neck)	0.48	0.51	0.80	0.49	0.20	0.41	0.33	0.34	0.37	0.35	0.37
PFCS (side)		0.63	0.61	0.24	0.25	0.38	0.28	0.29	0.38	0.35	0.36
PFCL (thigh)			0.84	0.33	0.22	0.39	0.33	0.38	0.42	0.39	0.40
PFC (pooled)				0.48	0.27	0.47	0.36	0.38	0.44	0.43	0.44
Halo-hair (Bf[L])					0.30	0.40	0.26	0.33	0.39	0.41	0.35
Ear fibres (Ef[L])						0.32	0.31	0.25	0.31	0.37	0.28
Leg fibres (Lf)							0.55	0.65	0.84	0.72	0.82
Ear fibres (Ef)								0.53	0.56	0.50	0.51
Face fibres (Ff)									0.67	0.52	0.54
Horn site (Hf)										0.71	0.73
Nose skin (Ns)											0.71
Hooves (Hv)											

[L] Pigment scored on lambs. Other types of pigment include the hogget record.

Significance: All coefficients $P < 0.001$.

4.3.7.2. Contingency tables

Table 12 provides contingency tables for years 1 to 3, with the pigmented fibre concentration (PFC) graded according to three levels (grade 1, <100 per kg; grade 2, 100 - 1000 per kg and grade 3, >1000 per kg) and related to grade for degree of pigmented leg fibres (Lf) or pigmented birthcoat halo-hairs (Bf) or class LB for presence of Lf and Bf. The trend for a higher concentration of isolated pigmented fibres (PFC grade) with increasing degree of pigmented birthcoat halo-hairs (Bf) was continuous throughout the full range of grades. For Bf grade 2 and 3, the order of frequencies of

sheep was reversed relative to the lower degrees. For leg fibre pigmentation there was little difference between grades 0 and 1, while for grade 2 and 3 the trend for increased likelihood of isolated pigmented fibres was clearly evident.

Table 12: Numbers of hoggets within grades for degree of pigmentation of the birthcoat halo-hairs (Bf) or leg fibres (Lf) or class for presence of Lf and Bf, and grade for the concentration of isolated pigmented fibres in the hogget fleece (PFC) for years 1-3.

PFC grade	**Unaffected (0)**	**Few Bf fibres (1)**	**Many Bf fibres (2)**	**Distinct Bf (3)**
<100 per kg	94	71	10	8
100-1000	16	35	14	22
>1000 per kg	7	21	17	28
Chi-square	= 93***			
PFC grade	**Unaffected (0)**	**Few Lf fibres (1)**	**Many Lf spots (2)**	**Distinct Lf (3)**
<100 per kg	88	51	19	25
100-1000	15	12	13	47
>1000 per kg	4	9	12	48
Chi-square	= 101***			
PFC grade	**Unaffected LB = (0)**	**Bf only LB = (1)**	**Lf only LB = (2)**	**Lf and Bf LB = (3)**
<100 per kg	54	34	40	55
100-1000	6	9	10	62
>1000 per kg	0	4	7	62
Chi-square	= 86***			

*** Significance ($P<0.001$) for Chi-square with 6 Degrees of Freedom that tests for departure from independence between PFC grade and grade for pigmentation (0 - 3)

The majority of fleeces with pigmented fibre concentrations exceeding 100 per kg occurred within LB class = 3 (pigmented birthcoat halo-hair and leg fibres). Of particular importance is the low incidence of unaffected sheep (LB class = 0) with high concentrations of pigmented fibres (10%) relative to sheep (LB class = 3) affected by pigmented leg fibres and pigmented halo-hairs (69%).

Table 13 provides contingency tables for year 4 in which grades for pigmented fibre concentration (pooled result) are related to the grades of Bf and Lf and classes of LB. The trends were similar to those described for years 1 to 3. Of particular importance is the low incidence of unaffected sheep (LB class = 0) with high concentrations of isolated pigmented fibres (3%) relative to sheep (LB class = 3) affected by presence of pigmented leg fibres and pigmented halo-hairs (52%).

Table 13: Numbers of hogget sheep within grades based on degree of pigmentation of the birthcoat halo-hairs (Bf) or leg fibres (Lf), or class for presence of Lf and Bf, and grade for the concentration of isolated pigmented fibres in the hogget fleece (PFC) for year 4.

PFC grade	Unaffected (0)	Few Bf fibres (1)	Many Bf fibres (2)	Distinct Bf (3)
<100 per kg	135	76	30	4
100-1000	11	19	17	5
>1000 per kg	7	10	10	16
Chi-square	= 97 ***			
PFC grade	**Unaffected (0)**	**Few Lf fibres (1)**	**Many Lf spots (2)**	**Distinct Lf (3)**
<100 per kg	141	22	27	53
100-1000	10	6	9	27
>1000 per kg	3	3	4	33
Chi-square	= 72 ***			
PFC grade	**Unaffected LB = (0)**	**Bf only LB = (1)**	**Lf only LB = (2)**	**Lf and Bf LB = (3)**
<100 per kg	93	50	40	62
100-1000	2	8	9	33
>1000 per kg	1	2	6	34
Chi-square	= 73 ***			

*** Significance ($P < 0.001$) for Chi-square with 6 Degrees of Freedom.

4.4. DISCUSSION

4.4.1. Leg fibre pigmentation

The segregation of the two phenotypes (affected or unaffected for leg fibre pigmentation) is consistent with the proposal that one locus is providing most of the affect responsible for the elimination of pigmented fibres from the legs of white Merino sheep. The occurrence of unexpected progeny in matings intended to involve the same alleles appears inconsistent with the theory of simple Mendelian genetics. Clearly the frequency of unexpected phenotypes is much higher than would be anticipated from gamete mutation (McKusic 1964). Progeny with unexpected phenotypes arose from both proposed homozygotes with pigmentation and proposed homozygotes without pigmentation (Table 4, 5 and 6).

Hanset (1985) faced with similar data for Belgian cattle tested other models (two loci-epistatic; additive - two pairs of genes) but concluded that these other models were incompatible with the segregation data. Despite imperfect data, Hanset (1985) used the information to identify the alleles producing all-White animals with blue ears (R/R), Blue or Roan (R/r^+) and Black or Red (r^+/r^+) and attributed unexpected off-spring to overlap of phenotypes and errors of recording.

A similar occurrence of unexpected progeny is reported for the fowl. In that case, 5.4% of progeny expected to be homozygous for a dominant allele, named *erminette* (*En*) that produces full white, showed some pigmented flecks in feathers. About the same percentage of fowls expected to be heterozygous (speckled) showed no pigment remnants (Hutt 1963).

Possible explanations for the unexpected progeny are as follows:

(a) Loci, other than the one being studied, may interact through overlapping effects to induce apparent non-penetrance in some individuals.

(b) There is the problem of temporary inhibition or misclassification of fibre pigmentation. It was found that among lambs classified unaffected by leg fibre pigmentation at birth, there were 13.5% reclassified as affected at lamb shearing and 21.3% reclassified as affected at the hogget shearing. Similar increases in pigmented spotting on white coats during early life are noted for the Dalmatian dog (Schaible and Brumbaugh 1976) and Leopard pattern of horses (Sponenberg et al. 1990). All of the 6 unaffected lambs from Ram 1 (5) and Ram 29 (1) died before lamb marking so their classification relied on the birth score. Had these lambs survived to a later age they may have been reclassified as affected and total agreement attained for the segregation.

(c) Some individuals identified as affected at birth by pigmented leg fibres were re-classified at lamb shearing (1.7%) and hogget shearing (3.1%) as unaffected. These changes with age allow an opportunity for misclassification of parents not inspected as lambs. In ewes, all degrees of expression (even single hairs) were accepted as positives or affected for breeding purposes. In contrast, most of the affected rams had distinct leg fibre pigmentation. Rams with the lowest scores for pigmented leg fibres had the lowest proportions of affected progeny. Progeny of parents with low degrees of expression may be more likely to show non-penetrance or greater opportunity for misclassification due to oversight of low expressivity.

(d) The depigmentation of the legs may involve a complex locus or linked loci. Recombination within this gene complex or between linked loci may explain the progeny with unexpected phenotypes. Also, the association between pigmented birthcoat halo-hairs and pigmented leg fibres could involve linkage between loci impacting on each type of pigmentation.

Bearing these factors in mind, and the close agreement with expectation for most of the 1:1 and 3:1 ratios, it is concluded that the evidence available is consistent with the hypothesis that a specific locus or complex gene is having the principal affect on the elimination of pigmented leg fibres. There was no significant differences relating to sex; being consistent with an autosomal (not X-linked) inheritance.

The study of leg fibre pigmentation in Columbia and Targhee sheep (Terrill 1947) was supportive of a multigenic inheritance. However, those sheep appear to have been scored as lamb or weaners and, as shown in this thesis, incidence of individuals affected by leg fibre pigmentation can increase in early life. Furthermore, the heritabilities were much lower and it is conceivable that a different genetic determination is involved in Merinos.

4.4.2. Pigmented birthcoat halo-hair

The assumptions for parents are less satisfactory due to a likely higher frequency of affected individuals with two hypothetical alleles allowing presence of pigmented birthcoat halo-hair. Other factors such as overlapping effects of other loci causing non-penetrance, oversight of low expressivity, and absence of birthcoat halo-hairs may have prevented general agreement with identification of a single genetic factor involved in elimination of

pigmented birthcoat halo-hair. For Ram 30 (Table 6), his parental phenotypes added to the basis for a proposed heterozygous status and, in this case, the segregation of progeny phenotypes was close to expectation.

However, the results for individual rams showed several inconsistencies and significant departures from expectation. Therefore, based on the data available for pigmented birthcoat halo-hairs the hypothesis, for involvement of a single locus determination of absence of this type of pigmentation, is rejected. Huston and Leipold (1993) demonstrate how more complex models can provide segregation frequencies approaching that expected for a single locus.

The trend for pigmented leg fibres and pigmented birthcoat halo-hairs to be inherited together (Table 8) may reflect linkage or pleiotropy (overlapping effects). The locus proposed to determine presence of pigmented leg fibres appears also to impact on the presence of pigmented birthcoat halo-hairs. It is conceivable, that the legs being more distant from the body and most of the primordial pigmentation centres (Schaible 1963 1969) are less likely to be affected by other loci affecting pigmented birthcoat halo-hair.

4.4.3. Gene nomenclature.

There is no certainty about the homology of the proposed locus, involved in the elimination of pigmented fibres on the legs of Merino sheep, with loci mapped in other animals. Such assurance about homology requires molecular genetic studies (e.g. Johannson et al. 1992). Nevertheless, it is conceivable that the locus involved is homologous with one of the white spotting loci documented in mice (Silvers 1979) or other animals (Johannson et al. 1992).

Other investigators of sheep have attributed the opportunity for development of white fleece in the European sheep breeds to the *Spotting* (*S*) locus and other modifier loci (Adalsteinsson 1970; Lauvergne 1975; Adalsteinsson et al. 1980; Lauvergne et al. 1981a; Sponenberg et al. 1996). Recently, the murine *S* locus has been mapped to chromosome 14 and 9 induced deletions involving white spotting from this locus have been characterised. The degree of extension of white spotting is related to the extent of deletion around the *S* locus (Metallinos et al. 1994) and the effects of identified modifier genes on six other chromosomes (Pavan et al. 1995). Some of the alleles at the murine *S* locus impart lethal effects involving a deficiency of myenteric ganglion and resulting megacolon (Jackson 1991). These affects are not identified in European sheep breeds for which the *S* locus is thought to be a key gene in production of white coat. However, such lethal effects are identified in some African/Asian sheep breeds (e.g. Karakul) where white or grey coat is thought to involve the *Roan*, *Dominant White* or *Pigmented Head* locus (Sponenberg et al. 1996). The murine locus for *Dominant White* (*W*) is closely linked to other spotting loci (*Patch* and *Rump-white*) on chromosome 5 (Silvers 1979).

Even though the data provided for pigmented leg fibres are not unequivocal the evidence and discussion provided are considered sufficiently supportive to warrant maintenance of the hypothesis for a locus and alleles having a major effect on the occurrence of leg fibre pigmentation. However, molecular genetic studies are required to ascertain how much of the unexplained variation is associated with other than a single locus or linkage group.

To accommodate the recognition of this locus and alleles, outputs of the Committee for Genetic Nomenclature of Sheep and Goats (COGNOSAG)(Andresen et al. 1995; Dolling 1996; Sponenberg et al. 1996) were considered. Without the benefit of segregation or molecular genetic studies that establish allelic relationships or allele homology, the

proposed locus that affects leg fibre pigmentation in Merinos is provisionally allocated to the *Spotting* (*S*) locus and the allele named here *white legs turretfield* and given the allele symbol S^{wlt}. This nomenclature is allocated in view of past classifications for sheep and similarities of effect on phenotype of the *spotted* (S^s) allele, and the allele name indicates more specifically the effect (*white legs*) and provides a geographic reference (*turretfield*) since tests of allelism are not established.

4.4.4. Association with isolated pigmented fibres

Further evidence of positive associations between macroscopic pigment and scattered isolated pigmented fibres in the hogget fleece (PFC) is provided in two samples of Merino sheep generated for the purpose of studying the inheritance of leg fibre pigmentation. The various types of pigmentation were all positively correlated with each other. Pigmented birthcoat halo-hairs and pigmented leg fibres had correlation coefficients with PFC (0.47 to 0.56) that were among the highest of all macroscopic pigmentation traits. The qualitative analysis (Table 12 and 13) reflects how selection against these types of macroscopic pigmentation on white sheep can dramatically reduce the risk of isolated pigmented fibres in the hogget fleece.

Among sheep with neither pigmented birthcoat halo-hair nor pigmented leg fibres, only 10% (years 1 to 3) or 3% (year 4) had concentrations of isolated pigmented fibres (PFC) exceeding the often quoted threshold of 100 per kg. Among the sheep with pigmented birthcoat halo-hair or pigmented leg fibres there was a trend for an increased level of isolated pigmented fibres in the hogget fleece samples with increased degree of expression. Furthermore, the risk of isolated pigmented fibres was greated when both

pigmented leg fibres and pigmented birthcoat halo-hairs were present. These two pigment types tended to be inherited together with the isolated pigmented fibres (Tables 8, 12 and 13).

4.5. CONCLUSION

Pigmented leg fibres and pigmented birthcoat halo-hair on white Merino sheep can influence the quality of wool through the presence of associated isolated pigmented fibres in the fleece. Explanation of the occurrence of pigmented birthcoat halo-hairs, based on parental phenotypes, was less predictable than for pigmented leg fibres. The data are consistent with the involvement of a locus that is responsible for the elimination of pigmented fibres from the legs of Merino sheep. This effect on phenotype is provisionally allocated to the *Spotting* (*S*) locus and the allele is named *white legs turretfield* and given the symbol (S^{wlt}).

The proposal for simple mode of inheritance is not entirely supported by the data due to the occurrence of unexpected phenotypes (5-10%) from parents expected to be homozygotes. The unexpected phenotypes may be explained by misclassification due to change with age or over-sight (low expression) or non-penetrance. Alternatively, the inheritance may be more complex where the effects of other loci, or recombination in the case of a gene complex or linkage group, are involved. One or more of these explanations may also apply to the inheritance of pigmented birthcoat halo-hair.

Merino sheep breeders should be able to quickly reduce and virtually eliminate pigmented leg fibres from their flocks, where this type of pigment is present or develops, by culling

all affected sheep. The elimination of pigmented halo-hair from the flock may not be as rapid due to a less predictable inheritance, reduced opportunity for phenotype classification (lambs only), and greater opportunity for misclassification of phenotypes (fibre shedding). Inspection of newborn lambs and well as at lamb marking is required to maximise the detection of affected individuals.

There was a trend for pigmented leg fibres and pigmented birthcoat halo-hairs to be inherited together and this may reflect linkage or overlapping effects of the loci involved. Isolated pigmented fibres were clearly related to the degree of expression and co-expression of both types of macroscopic fibre pigmentation. In flocks with a high frequency of these types of macroscopic pigmentation, then selection against individuals with the most pronounced expression should reduce the risk of isolated pigmented fibres. To minimise or eliminate pigmented leg fibres will require careful inspection of lambs and adult sheep whenever convenient opportunities arise (tagging, marking, shearing, crutching and classing) before selected stock are used for breeding.

CHAPTER 5

SHORT REPORTS RELATING TO ISOLATED PIGMENTED FIBRES IN MERINO FLEECES

"The majority of sheep classers, ram buyers and ram judges are most conscious of black or brown spots or patches on Merinos. One or two small spots would probably not make a great deal of difference to a sheep's chances at a show or at a sale but if the sheep were to have numerous spots its chances of winning or selling for much money would be greatly lessened. Most sheep breeders agree that rams with black spots on their faces are likely to have them on other parts of their body as well, and if these spots are allowed to go unchecked eventually the flock would end up with an increasing number of lambs being born with patches of black wool on them. It would be fair to claim that flocks which have been classed with particular attention paid to spots have evolved a sheep on which it is rare to find a black patch of wool, and rarer to find a black patch of wool, and rarer still to find a black lamb".

E.M. Body, Bundemar Stud, Trangie, NSW

(Body et al. (1965)

CHAPTER 5

SHORT REPORTS RELATING TO ISOLATED PIGMENTED FIBRES IN MERINO FLEECES

PREAMBLE

In the previous Chapters the potential problem of isolated pigmented fibres in Merino wools has been identified. The associations of this wool fault with macroscopic fibre pigmentation offer a potential mechanism of control by the practising sheep breeder. However, it is not clear from these experiments how the isolated pigmented wool fibres develop in some sheep and not in others nor is it clear whether the problem persists into adult life. Furthermore, the measurements described so far have been conducted on raw wool samples. How these raw wool measurements relate to processed wool requires clarification.

Fleet et al. (1993) proposed that both the concentration of melanoblasts and their time of arrival at the skin epidermis during foetal development is likely to determine the likelihood that isolated wool fibres will become pigmented. In order to clarify this hypothesis an investigation of the foetal development of isolated pigmented wool fibres was conducted. How isolated pigmented wool fibres are distributed in the fleece relative to the location of the visible fibre pigmentation is not clear from grid sample measurement. Therefore, the density of isolated pigmented fibres at various locations in the fleece was assessed on Merinos with leg fibre pigmentation. It is relevant to determine whether the isolated pigmented wool fibres are in greatest concentration near the edges of the fleece.

Changes in pigmentation with age have an effect on the extent of the problem in the wool clip and on the use of indicators that are only temporarily evident. Some surveys of commercial consignments of Merino wool processed to top have shown that pigmented wool fibres are a "borderline" problem (Foulds 1989). However, in this thesis the occurrence of isolated pigmented fibres is identified as exceeding industry tolerances and some individuals have extremely high concentrations of pigmented fibres in their fleeces. This conflict over the importance of pigmented fibres in Merino wools may involve sampling differences (including age of sheep) or the method of measurement. To clarify these questions, changes in pigmentation with sheep age were investigated and some fleeces were processed to wool top (combed sliver) and comparisons made between the raw wool and processed wool.

The investigations reported in this Chapter may not each be fully comprehensive but they provided further knowledge to better understand the problem of isolated pigmented wool fibres and how they relate to the wool industry. This Chapter has five parts as follows:

* **Part 5A:** Foetal development of melanocyte populations.
* **Part 5B:** Distribution of isolated pigmented fibres in the fleece.
* **Part 5C:** Change in isolated pigmented fibres with age.
* **Part 5D:** Changes in macroscopic pigmentation in early life.
* **Part 5E:** A metrological comparison of pigmented fibres in fleeces and top.

CHAPTER 5: SHORT PAPER A

FOETAL DEVELOPMENT OF MELANOCYTE POPULATIONS

5A.1. INTRODUCTION

Melanocyte populations that give rise to fibre pigmentation in mice and humans develop during foetal life (Mishima and Wildlan 1966; Holbrook et al. 1989). Schaible (1963) suggested that delays in melanoblast (precursor melanocyte) migration until after development of hair follicles was the main effect of white spotting. Mayer (1967 a,b) found that timing of migration of melanoblasts was important in the recessive white spotted mouse but, in addition, there is a period when melanocytes cannot differentiate or proliferate in the epidermis.

Adalsteinsson (1970) proposed that delays in migration of melanoblasts was the usual mechanism causing lack of pigmentation in sheep together with a deficiency of melanocytes in white spotted areas. The evidence of Forrest et al. (1985) indicates that in addition to reduced melanocyte numbers the epidermal melanocytes in white wool-bearing skin were relatively inactive. Fleet et al. (1993a) found that Merino lambs with pigmented leg fibres and isolated pigmented fibres had high concentrations of wool-bearing skin melanocytes at birth. It was proposed that both the concentration of melanoblasts and their time of arrival in the wool-bearing skin determine the likelihood that isolated pigmented wool fibres become pigmented.

In order to clarify the aetiology of development of isolated pigmented fibres in the fleece the development of recessive black and white foetal skin was examined.

5A.2. MATERIALS AND METHODS

Foetal and neonatal lambs were derived from either recessive black parents, "usual" white parents, or from white rams with pigmented leg fibres and the associated isolated pigmented fibres in the fleece mated to "usual" white dams. The rams expected to produce progeny with isolated pigmented fibres were also considered likely to be heterozygous for the proposed allele allowing expression of pigmented leg fibres. The ewes were synchronised for oestrous and artificially inseminated (AI) then slaughtered at various intervals after AI. Only a small number of foetal sheep were available within periods of foetal development. Therefore, the results presented are not definitive in relation to the timing of developments but reflect differences between the genotypes.

Skin samples from the loin (between sites 2 and 3 defined by Chapman and Young 1957) were prepared for vertical sections and microscope inspection by the method described in Forrest et al. (1985). The procedure utilised ammoniacal silver nitrate staining (Mishima 1967) to identify melanoblasts and highlight melanocytes. Ten alternate sections were inspected with a light microscope and the number of melanin\premelanin stained cells counted that were located in the epidermis, upper dermis and lower dermis (below the midline of the dermal tissue). The melanocyte\melanoblast counts were divided by the length of the epidermis (i.e. number per mm of epidermis) determined by tracing an enlarged outline of the epidermis using a micro-fiche and measuring it with a planimeter. These counts were analysed by least squares analysis of variance (Procedure GLM of SAS 1987) and the model included the effects of genotype, age and age*genotype interaction.

5A.3. RESULTS AND DISCUSSION

Examples of the macroscopic appearance of pigmentation in black foetal sheep are shown in Figures 1 to 4. Figure 1 shows a genetically black foetus at 50 days gestation in which there is no macroscopic pigmentation evident. Figures 2 and 3 each show a black foetus phenotype at 70 days gestation and in Figure 3 there is a white foetus for comparison. Figure 4 shows a black foetus at 100 days gestation that has a white-spotted phenotype with clear demarcation between the black and white areas. Such white spots usually persist during life though there can be an encroachment of pigmentation (Billingham and Silvers 1963).

Table 1 provides least square means for melanocyte (or melanoblast) concentration within periods of foetal development. The first weakly staining melanoblasts (M) became apparent in recessive black foetal skin at day 50 of gestation (Figure 5). By day 58, melanoblasts were present in the epidermis and they were darkened and becoming dendritic (Figure 6). The budding of primary follicles commences around 64 to 70 days gestation (Hardy and Lyne 1956) and at that time melanocytes were prolific in the epidermis and concentrated along the basement layer where the budding occurs. The process of incorporation of melanocytes into the budding follicles could be clearly visualised (Figure 7). Around day 100 of gestation the initiation of follicles that bud off the epidermis (primaries and original secondaries) is completed and at that stage melanocytes were concentrated in the epidermis, upper reaches of outer root sheaths, and in follicle bulbs of differentiating follicles (Figure 8). After 100 days gestation the secondary derived follicles begin branching from the upper outer root sheaths of the original secondaries (Hardy and Lyne 1956) where melanocytes may have also colonised.

Figure 1: Black Merino foetus at 50 days gestation.

Figure 2 and 3: Black Merino foetus (above) and a white foetus (Figure 3) at 70 days gestation.

Figure 4: Black Merino foetus that is white spotted at 100 days gestation.

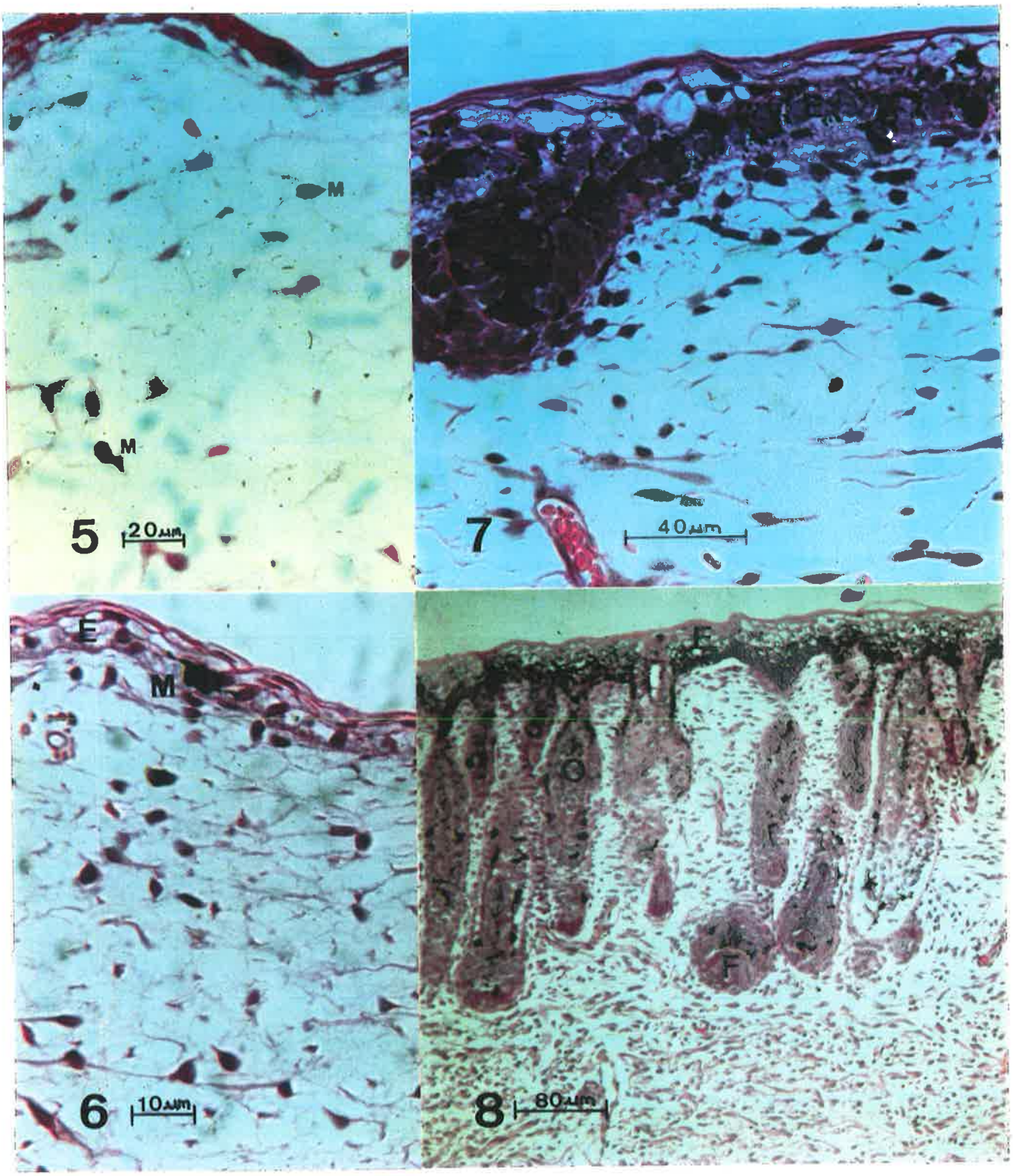


Figure 5: Melanoblasts (M) in the dermis of a black foetus at 53 days gestation.

Figure 6: Melanoblast (M) in the epidermis of a black foetus at day 58.

Figure 7: Melanocytes (M) are prolific in the epidermis (E) of a black foetus at the time of commencement of wool follicle budding (70 days gestation).

Figure 8: Original follicles are well formed at 100 days gestation in this black foetus and the melanocytes are prolific in the epidermis (E), upper reaches of the outer root sheaths (O), and in the follicle bulbs (F).

In foetal progeny of "usual" white Merino parents, the first sporadic melanoblasts were evident in the dermis at 100 days gestation and then in the epidermis 10 days post-partum together with increased numbers of dermal melanoblasts (Figures 9, 10, 11 and 12). Among the progeny of rams with isolated pigmented fibres the first melanoblasts were evident in the dermis at 57 days gestation and in the epidermis at 70 days. Around 70 days gestation the dermal melanoblasts appeared more frequently than in "usual" white and were often associated with capillaries. It appeared as if these blood vessels were being used by the melanoblasts as pathways for migration toward the skin epidermis or developing wool follicles (Figures 13 to 16). The occurrence of melanoblasts in the epidermis remained sporadic throughout the foetal (after day 57) and neonatal periods monitored.

The observations of foetal development are consistent with the hypothesis (Adalsteinsson 1970) that delays in melanoblast migration play a major role in determining the absence of pigmented fibres in white sheep. However, it is also possible that the timing of the primary melanoblast migration is not altered but these precursor cells are not detectable with the stain (ammoniacal silver nitrate) procedure used. Mayer (1973 1979) concluded that in *dominant white* mutants there was no apparent primary migration. The pigmentation that developed on these mutants was thought as representing normal melanocytes from unaffected neural crest clones or a secondary wave of migration. However, Cable et al. (1995), using specific labelling methods for migrating melanoblasts, found the timing of primary migration of these precursor cells was not altered but their survival was severely affected. If this was also the case, producing white coat in Merinos, then the primary migrating melanoblasts would not have survived to differentiate to a stage when detection with ammoniacal silver nitrate stain was possible.

In white Merino sheep likely to develop isolated pigmented fibres, the peak of migration coincided with the initiation of primary follicles (Table 1). The majority of follicles that eventually develop would remain non-pigmented due to the inability of melanoblasts to localise and proliferate in the epidermis. The number of isolated pigmented fibres that develop is likely to be very sensitive to time of the peak and magnitude of the apparent melanoblast migration.

In the "usual" white Merino sheep sampled, it appears likely that the peak of the apparent melanoblast migration would have occurred after birth (Figure 9 to 12). Fleet et al. (1993a) proposed that differences in melanocyte populations of white sheep (with and without) pigmented leg fibres, at birth and lamb marking (2 months age), were attributed to varying times and intensities of melanoblast migration during foetal life.

Figures showing white foetal and neonatal skin

Figure 9: White neonatal (10 days) wool-bearing skin showing a melanocyte (M) in the epidermis that is possibly in a state of lysis.

Figure 10: White neonatal (30 days) wool-bearing skin showing a melanocyte (M) in the dermis and possibly the remains (R) of melanocytes in the epidermis (E).

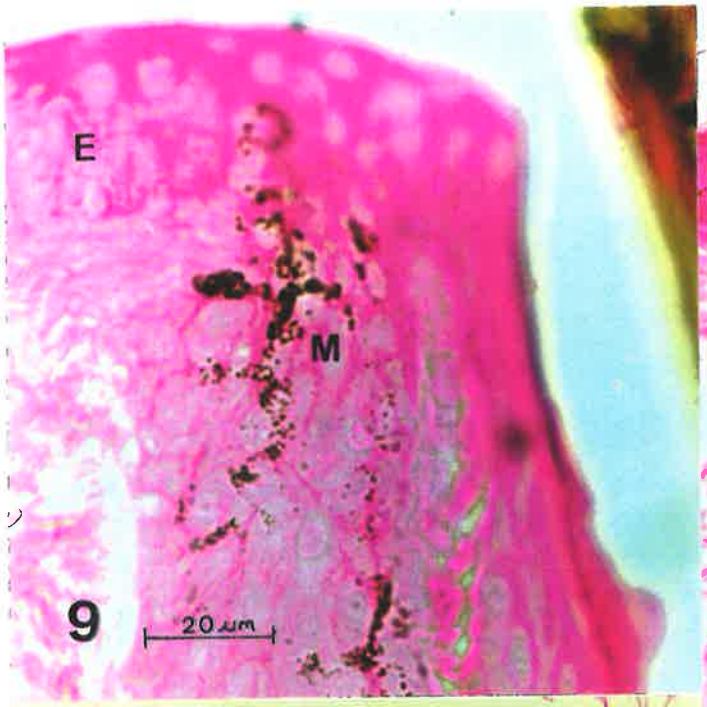
Figure 11: White neonatal wool-bearing skin (40 days) with melanocytes (M) around the sebaceous glands (S) and near the epidermis (E). Note the absence of epidermal melanocytes.

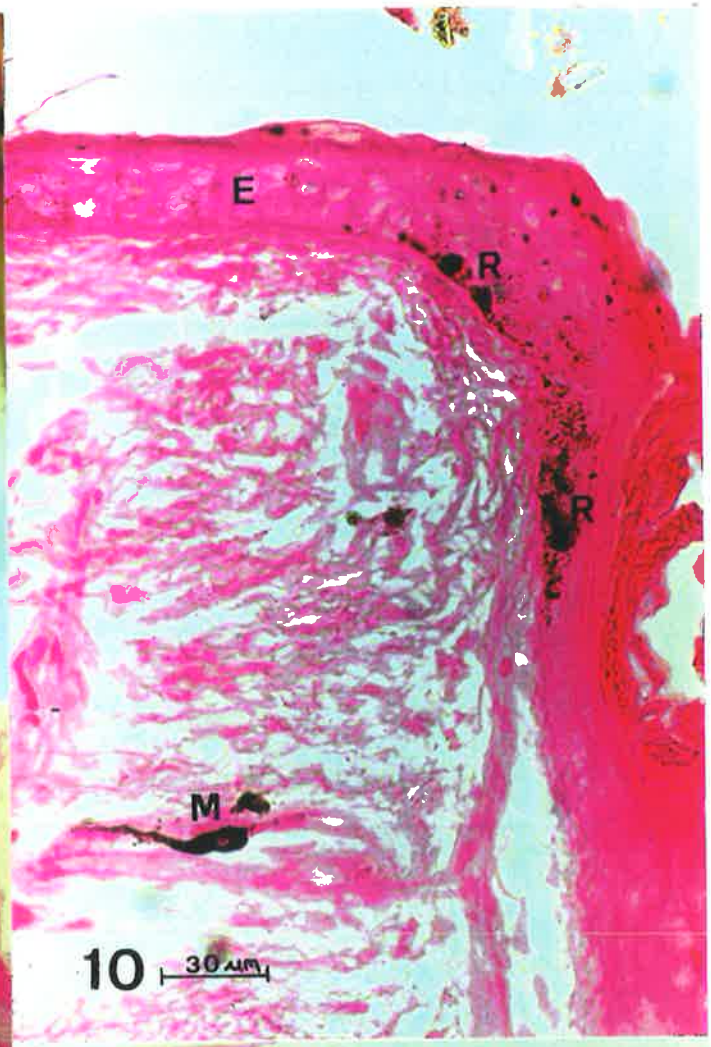
Figure 12: White neonatal wool-bearing skin (40 days) showing a melanoblast (M) in the lower dermis with characteristic specific staining of premelanin in melanosomes (pigment granules).

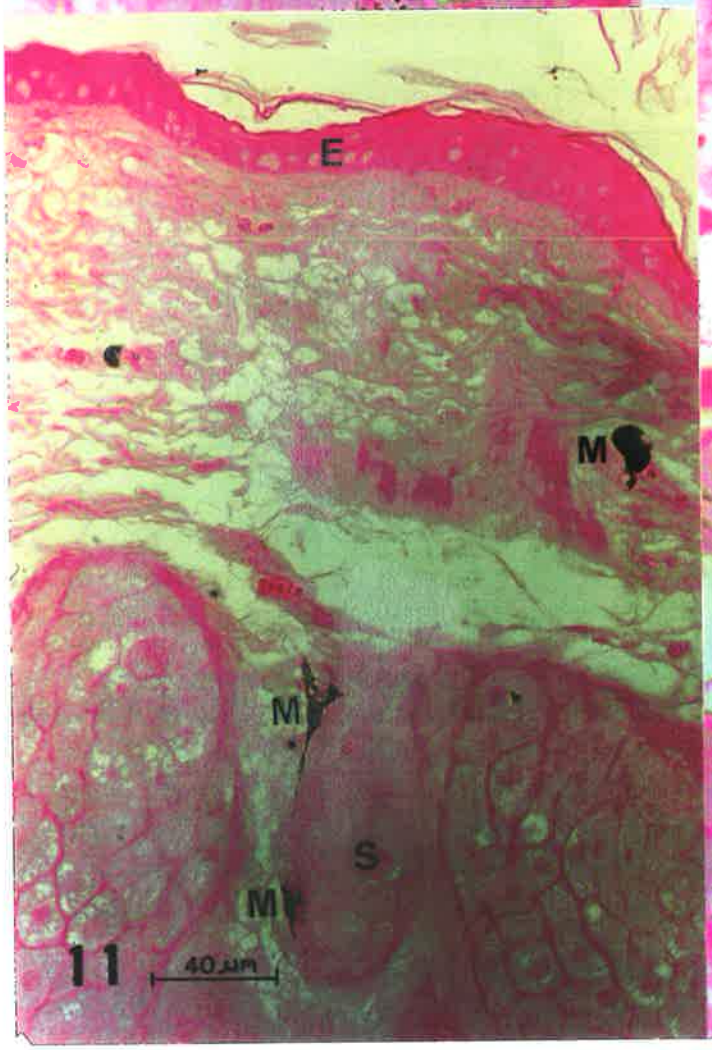
Figure 13: White foetus (IPF) at 70 days gestation with melanoblasts (M) on the walls of a capillary (C). Note the absence of epidermal melanocytes and the budding follicle (F).

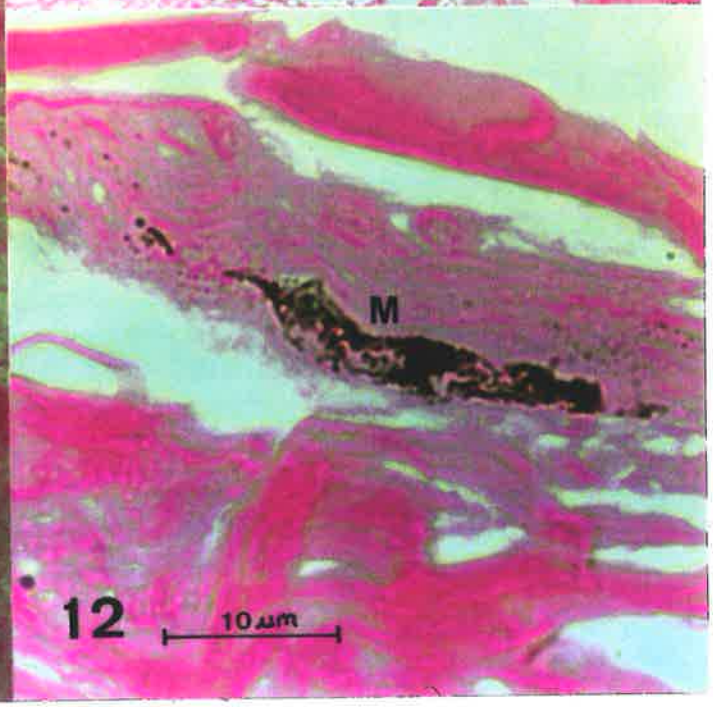
Figure 14: and 15: White foetus (IPF) with melanoblasts near the epidermis (E) and budding wool follicles (F) and on the walls of capillaries (C) in the dermis.

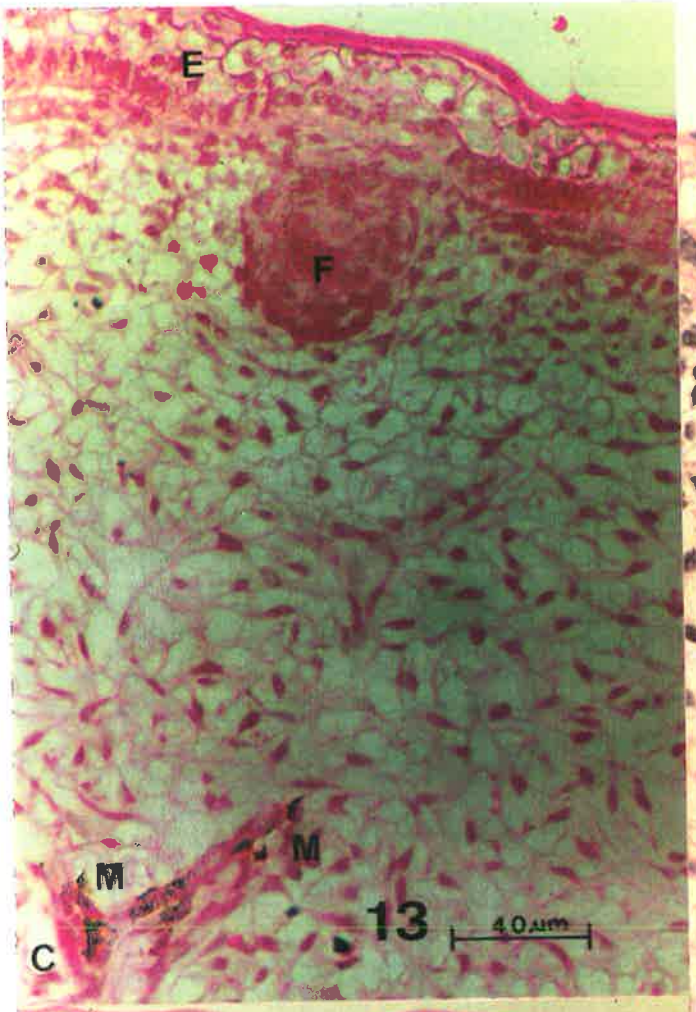
Figure 16: White foetus (IPF) at 85 days gestation showing melanoblasts (M) on the walls of capillaries (C). Note the absence of melanocytes from the epidermis.

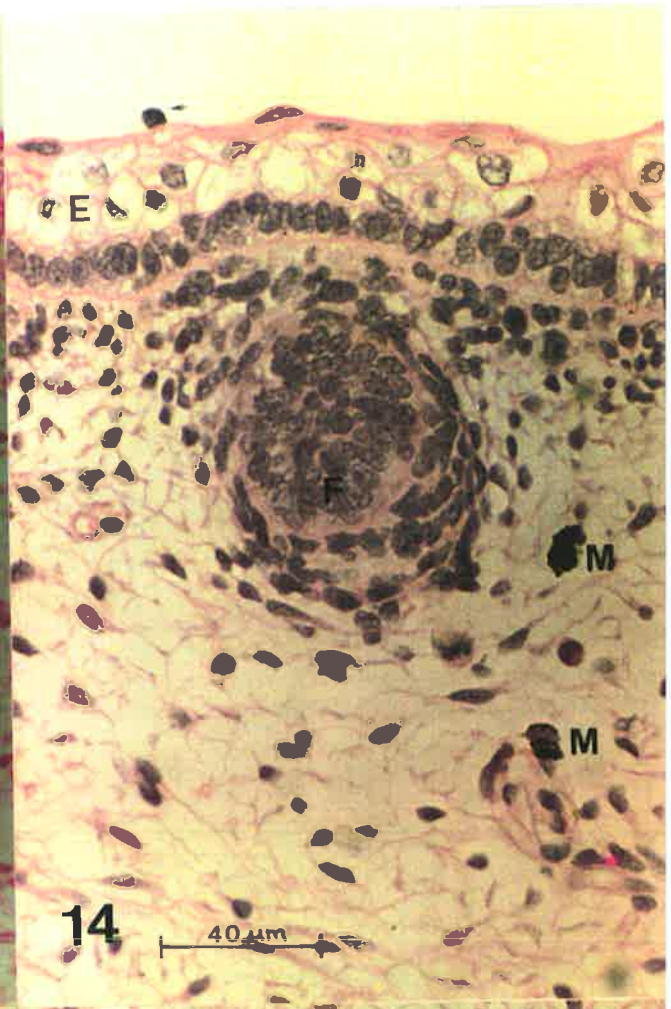

E
M
9
20 µm

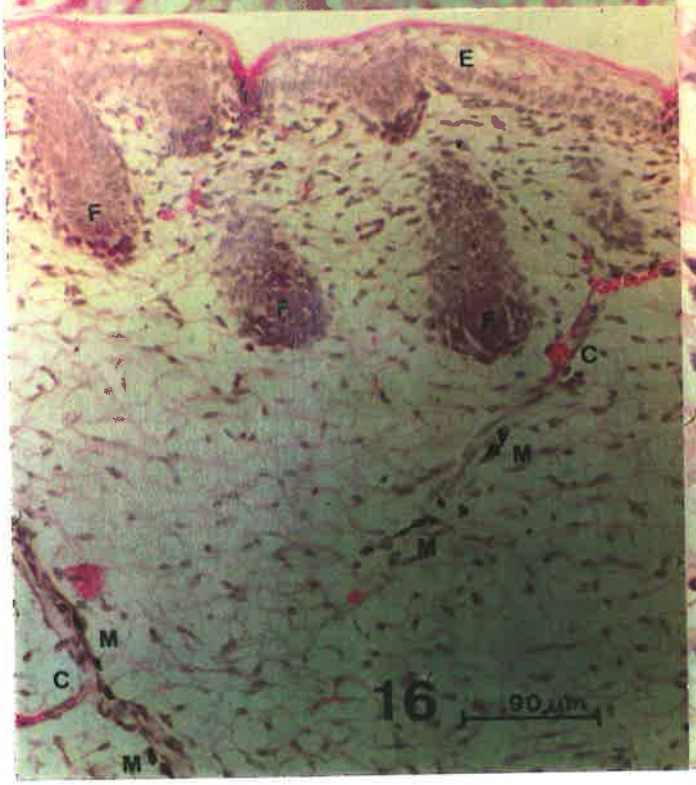

E
R
R
M
10
30 µm


E
M
M
S
M
11
40 µm


M
12
10 µm


E
F
M
M
C
F
13
40 µm


E
F
M
M
14
40 µm


E
F
F
F
C
M
M
M
C
M
16
90 µm

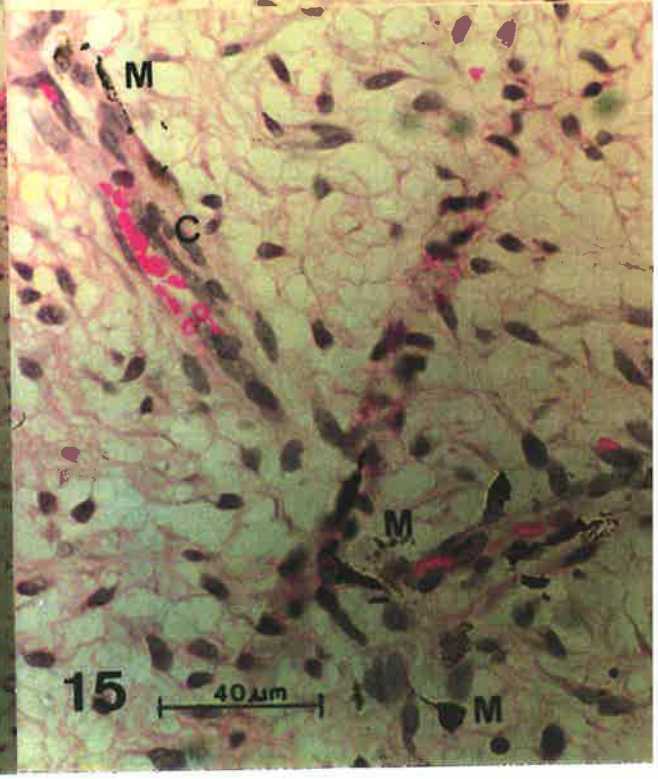

M
C
M
15
40 µm
M

Table 1: Development of wool-bearing skin melanoblast or melanocyte populations in foetal Merino sheep that are either recessive Black, "usual" White alone or "usual" White and isolated pigmented wool fibres (IPF).

SITE \ GENOTYPE	Days After Artificial Insemination 40	50 - 55	57 - 62	64 - 75	77 - 86	90 - 100	Birth
EPIDERMIS							
Black (A^a/A^a)	0.0 a (2)	0.007 a (4)	2.527 a (3)	18.727 b (3)	42.197 b (1)	21.710 b (3)	49.741 a (2)
White ($A^{Wt}/-$)	0.0 a (2)	0.0 a (1)	0.0 a (2)	0.0 a (3)	0.0 a (4)	0.0 a (3)	0.0 a (2)
White + IPF^A	- (0)	0.0 a (3)	0.0 a (3)	0.006 a (5)	0.014 a (5)	0.005 a (3)	0.007 a (2)
UPPER DERMIS							
Black	0.0 a	0.148 a	0.473 a	1.431 b	1.492 b	-	
White	0.0 a	0.0 a	0.0 a	0.0 a	0.0 a	0.004 a	0.097 a
White + IPF^A	-	0.0 a	0.026 a	0.588 a	1.118 b	0.197 a	0.178 a
LOWER DERMIS							
Black	0.0 a	0.040 a	0.138 a	0.091 a	0.0 a	-	-
White	0.0 a	0.0 a	0.0 a	0.0 a	0.0 a	0.004 a	0.012 a
White + IPF^A	-	0.0 a	0.007 a	0.346 a	0.413 a	0.266 a	0.043 a

Letters: Means within columns with a common letter do not differ significantly ($P > 0.05$).
Brackets: The brackets enclose the foetal or new-born sheep within that period and type.

The inability of melanoblasts to colonise the epidermis during early foetal life is similar to the mechanism occurring in the recessive white spotted mouse (Mayer 1967 a,b) and dominant white spotted mutants (Cable et al. 1995). The detection of dermal melanoblasts and the development of isolated pigmented wool follicle bulbs means that melanoblasts do migrate, survive and differentiate in the dermis and colonise to produced pigmented wool fibres in isolated follicle bulbs. However, the epidermis of white Merino sheep apparently will not support melanocytes, at least during foetal life, and may reflect the absence of a necessary factor(s) necessary for their survival in that tissue environment. This situation has similarities with the effects from the murine *S* locus where the *spotted* allele causes a temporary period in which the foetal epidermis will not support melanoblast differentiation or proliferation (Mayer 1967 a,b).

There is also a similarity with effects of the murine *steel dickie* allele of the *Steel* locus where the product allows migration (i.e. to dermis) but will not support the colonisation and activity required to produce visible skin pigmentation (Fleischmann 1993). Yoshida et al. (1996) found expression of a transgene, incorporating the lacZ reporter gene and a fragment of the steel factor that is essential for development of melanocytes, in the dermal papilla of the mouse hair follicle. They suggest strongly that dermal papilla cells provide steel factor to support the c-kit dependent growth and development of hair follicle melanocytes.

Slominski and Plaus (1993) propose that melanocytes may migrate along pathways marked and modified by mesenchyme which includes the dermal papilla. Melanocytes are known to attach and migrate on fibronectin while integrin is involved in melanocyte adhesion (Scott et al. 1992; Etoh et al. 1993).

Cable et al. (1995) reported that many melanoblasts which migrate deep within the mesenchyme lie close to the posterior and anterior cardinal vein. This association supports that structural heterogeneities, such as blood vessels and the neural tube, may act to confine the direction of migrating crest cells. Apart from achieving a blockade effect, these structures may also serve as a contact guide for migrating cells. However, it is unclear whether the melanoblasts associated with the cardinal veins are destined for the skin or are targeting some internal site. Schaible and Brumbaugh (1976) proposed that a requirement for melanoblast movements along circulatory pathways, rather than direct from the neural crest to the skin, may be a reason for the delayed development (after birth) of pigmented spots in the Dalmatian dog. The striking association of melanoblasts with capillary walls (Figures 14, 16 and 17) of foetal sheep likely to develop isolated pigmented wool fibres may involve a substance on that tissue that is required for survival or migration.

The absence of melanocytes from the epidermis may indicate the deficiency of a necessary factor(s) for survival or colonisation. The presence of melanocytes in the dermis, and isolated pigmented follicle bulbs that include the dermal papilla, indicate those tissue environments can allow survival (dermis) and effective melanogenic processes (follicle bulb) at least during early life. The fact that there is clear demarcation between pigmented and white-spotted areas (Figures 4), without any variegation of pigmentation, supports the hypothesis that differences in the tissue environment are restricting pigment spread from centres of location of primordial melanocytes. However, the results of skin grafting experiments, using a 2 month old lamb or adult sheep, are consistent with the notion that such white skin can support melanocytes that move from the adjoining pigmented skin (Hardy et al. 1952; Ryder 1979; Lyn and Hollis 1968 1980).

It seems likely that only the primary follicles and original follicles incorporate isolated melanoblasts that reach the epidermis at the time of follicle budding. The question arises as to whether the dermal papilla can provide an alternate route into wool follicles. Secondary derived follicles bud off the upper outer-root sheaths which are most unlikely to contain melanocytes in white Merino sheep skin. Therefore, the numerous secondary follicles would dilute the concentration of isolated primary or early secondary follicles that have incorporated a melanocyte in the follicle bulb. This mechanism could explain the occurrence of a pigmented outer coat (primary fibres) and a non-pigmented undercoat (secondary follicles) present in some coloured sheep noted by Adalsteinsson (1970). The higher fibre diameters of isolated pigmented fibres relative to the bulk of white fibres (Adalsteinsson 1975; Fleet et al. 1993b) is consistent with the idea of association with different follicles types.

5A.4. CONCLUSION

In white sheep likely to develop isolated pigmented wool fibres, the apparent timing of migration of melanoblasts (ammoniacal silver nitrate staining cells) coincided with initiation of primary and original secondary follicles. However, the melanoblasts did not colonise the foetal epidermis and the deficiency of a cell growth or adhesion factor in that tissue environment, at that critical time of development, is proposed. It is concluded that the number of isolated pigmented fibres that develop in white sheep will be very sensitive to the timing and magnitude of melanoblast migration during the period of development of primary and original secondary follicles in foetal life.

CHAPTER 5: SHORT PAPER B

DISTRIBUTION OF ISOLATED PIGMENTED FIBRES IN THE FLEECE

5B.1. INTRODUCTION

In the previous chapters, the wool measurements have been confined to a representative grid sample taken from the skirted fleece. There was no information available about the distribution of isolated pigmented fibres in the fleece. Such knowledge could provide a basis for simple sampling procedures and understanding the relationship between macroscopic pigmentation and isolated pigmented fibres.

This short paper reports the results of investigations into the distribution of isolated pigmented fibres in the fleece of sheep with pigmented leg fibres and associated isolated pigmented wool fibres.

5B.2. MATERIALS AND METHODS

The sheep had pigmented fibres on the legs and associated high concentrations of isolated pigmented fibres in the fleece. They were sampled from three groups of sheep at Turretfield Research Centre for which these records were available. It is recognised that the isolated pigmented fibres associated with other types of macroscopic pigmentation may show different trends. The sources (groups) of sheep were as follows:

Group 1: 15 ewes aged 1.5 that were progeny of four rams (Rams 5, 6, 7 and 8) described in Chapter 4.

Group 2: 9 rams aged 2.5 years that were sons of Ram 1 described in Chapter 4.

Group 3: 12 ewes, aged 2.5 or 3.5 years from origins unrelated to group 1 and 2.

The sheep sampled were not intended to be representative of Merinos in general but rather those with pigmented leg fibres and associated isolated pigmented fibres. The fleece sampling and measurement procedures are described in detail by Fleet and Pourbeik (1990). Briefly, seven sites in the fleece were sampled (S1 to S7 of Figure 1) together with a grid sample of the skirted fleece.

Darkened fibres of length 20mm or longer, isolated from the inspected scoured wool samples, were checked with a microscope (80 to 400 x). In total 23,878 fibres were confirmed as melanin pigmented. The counts of pigmented fibre concentration were transformed [$\log_{10}$(value+1)] and analysed by least squares analysis of variance using the following model:

$$Y_{ijk} = u + G_i + SN(G)_j + S_k + (G*S)_{ik} + e_{ijk}$$

Where: Y_{ijk} = the pigmented fibre concentration trait of an individual sheep;

and u = the mean; G_i = Group (1 - 3)

$SN(G)_j$ = Sheep nested within group used to test the G mean square; and

S_k = Sample (GS and S1-S7); and

e_{ijk} = the random residual effect.

The analysis included group (G), representing different sources of sheep, and the interaction with wool Sample (G*S) allows a means of assessing whether different trends in the Sample means existed between Groups. The degree of fit of the model was high ($r^2 = 0.81$).

5B.3. RESULTS AND DISCUSSION

The least squares analysis of variance for LPFC found the effects of Group and Sample as significant ($P<0.001$) and the interaction not significant ($P\geq0.05$). The differences between the group means are not important in the context of this study and reflect sampling differences (including source, age and sex). Since the interaction was not significant, the trends between wool sample means within each of the groups of sheep are taken to be similar. Figure 1 shows the least square means [(antilog$_{10}$ LPFC)-1) for pigmented fibre concentration for each wool sample with an indication of significant differences ($P<0.05$).

The thigh (S5) and foreleg (S6) samples had the highest pigmented fibre concentrations, the mid-back (S1) had the lowest concentration, while the wool samples from the neck (S7) and horizontal mid-line of the side (S2, S3 and S4) had the intermediate values. All of the GS and S5 sites contained pigmented fibres while the other sites only had one or two zero values indicating the pigmented fibres were widespread through the fleece.

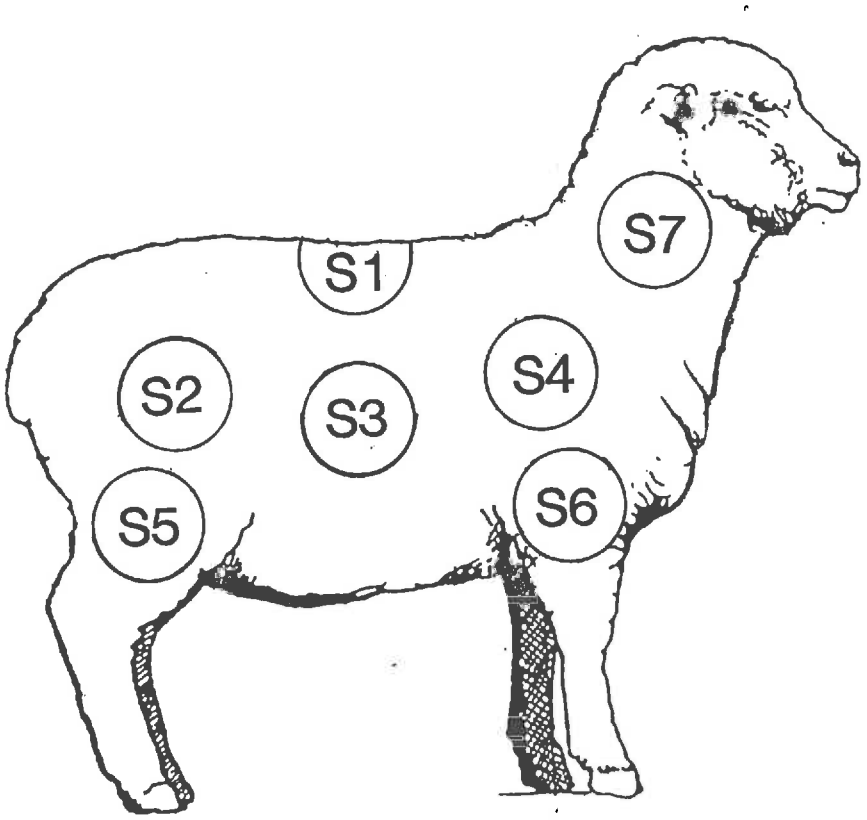
The results are consistent with the legs being the primary site of colonisation of melanoblasts (producing the macroscopic fibre pigment) but with some dispersal of these

cells to wool-bearing skin producing the isolated pigmented fibres. However, this proposal would require primary pigment centres in addition to those identified in the normal mouse (Schaible 1963 1969 1972). Schaible (1972) suggested that, in general, small animals have fewer pigment centres than large animals. He cited the cow, as an example, which is proposed to have 36 primary pigment centres including 2 sites on each lower leg.

5B.4. CONCLUSION

The white Merinos with pigmented leg fibres had the highest concentrations of isolated pigmented wool fibres near the legs, intermediate concentrations at the neck and along the horizontal mid-line of the body, and the lowest concentrations at the mid-back. This distribution of isolated pigmented fibres in the fleece of Merino sheep follows a pattern consistent with the legs being the primary destination for colonisation of melanoblasts producing the macroscopic fibre pigmentation. The declining concentrations of isolated pigmented fibres with distance from the macroscopic fibre pigmentation appear to reflect a dispersal effect on melanoblasts programmed for destination to the legs.

Distribution in the fleece



Sample	Pigmented fibres per kg
GS Grid	1675 c
S1 Midback	443 a
S2 Hip	1248 bc
S3 Midside	1020 bc
S4 Shoulder	1396 bc
S5 Thigh	5682 d
S6 Foreleg	3722 d
S7 Neck	809 ab

Figure 1: The concentrations of isolated pigmented fibres (no per kg scoured staples) at different locations in the fleece (S1 to S7) and in the grid sample (GS). Means with a common letter do not differ significantly ($P \geq 0.05$)

CHAPTER 5: SHORT PAPER C

AGE-RELATED CHANGE IN ISOLATED PIGMENTED FIBRES

5C.1. INTRODUCTION

In previous chapters, the measurements of isolated pigmented fibres have involved hogget fleeces (sheep age 1.5 years). As sheep are usually retained for a period of 5.5 years it is necessary to determine whether there are changes in concentration of isolated pigmented fibres in the fleece as sheep age.

The Suffolk is a breed where there are obvious changes in pigmentation of the fleece (from black to white) in early life. Nichols (1927) noted that pigmented fibres in the white ("greyed") fleece of Suffolk sheep often had a pigmented tip and white base. Aliev and Rachkovosky (1989) reported that pigmented fibres from the "greyed" fleece of Tajik sheep often contained melanocytes apparently shed from the follicle bulb region.

This short paper reports results of investigation of the change with age of isolated pigmented fibres in the fleeces of Merino sheep with leg fibre pigmentation.

5C.2. MATERIALS AND METHODS

The sheep all had leg fibre pigment and associated high concentrations of isolated pigmented fibres in the hogget fleece. They were sampled from groups of sheep at

Turretfield Research Centre for which these records were available. It is recognised that isolated pigmented fibres associated with other types of macroscopic pigmentation may show different trends. The sources (groups) of sheep were as follows:

Group 1: 14 ewe progeny of Ram 1 described in Chapter 5, plus one other ewe from a different origin, that were sampled between 1.5 and 5.5 years age.

Group 2: 5 ewes selected from the sheep described in Chapter 2 and sampled between 1.5 and 3.5 years.

Group 3: 6 grand daughters of Ram 1 at 1.5 and 2.5 years age.

The sample was not intended to be representative of Merinos in general but rather those with pigmented leg fibres and associated isolated pigmented fibres. The skirted fleeces were sampled by grid method, as described in Chapter 2, except that the staple tips were left intact. A sample of 52 solvent scoured staples from each fleece was stored until all samples could be measured by a single observer with the method described in Chapter 2. Only sheep with measurements available for all ages (within groups) were included in the data set for each analysis (group 1 to 3, group 1 and 2 and group 1).

The $\log_{10}$ transformed pigmented fibre concentrations (LPFC), within age groups 1 to 2 years and 1 to 3 years, were each analysed by least squares analysis of variance (Procedure GLM of SAS 1987) using the following model:

$$Y_{ijk} = u + G_i + SN(G)_j + A_k + (G*A)_{ik} + e_{ijk}$$

Where: Y_{ijk} = the observed pigmented fibre concentration of an individual sheep;

u = the mean; $_{Gi}$ = Group (1 - 3);

$SN(G)_j$ = Sheep nested within group used to test the G mean square;

A_k = Age of sheep; and e_{ijk} = the random residual effect.

The analysis including group (G) and the interaction with Age (G*A) allows assessment for different trends between the Age means existing between Groups. The model r^2 for these analysis were 0.81 and 0.88. For sheep group 1, only the effect of age was fitted within the analysis and the model r^2 was 0.40.

5C.3 RESULTS AND DISCUSSION

The least squares analysis of variance for LPFC found the effects of Group and Age were significant ($P<0.05$) and the interaction not significant. The differences in the means between groups are not important in the context of this study and reflect sampling differences that cannot be related to the general Merino population. Since the interaction was not significant, the trends between ages within each of the group of sheep are taken to be similar.

Table 1 shows the least square means [(antilog$_{10}$ LPFC)-1)] for pigmented fibre concentration for ages from each analysis (1 to 2 years, 1 to 3 years and 1 to 5 years). There was a dramatic and significant ($P<0.01$) decline in isolated pigmented fibres between 1.5 years and 2.5 years age. By 2.5 years age and in subsequent ages the concentrations of isolated pigmented fibres were near or well below the recognised industry threshold of 100 per kg (Foulds et al. 1984) despite the very high initial concentrations.

The substantial decline in the concentration of isolated pigmented fibres after 1.5 years age (also reported in Fleet et al. 1991) could have important implications for buyers of wool destined for end-uses which are sensitive to dark fibres; if similar declines occur for

the general Merino population. This possibility has since been supported by data within processing surveys conducted by CSIRO (Burbidge et al. 1991; Burbidge and McInnes 1994). The wool of young sheep appears to be most at risk of containing isolated pigmented fibres.

Table 1. Age-related changes in the concentration of isolated pigmented wool fibres (No. per kg)

Age range - Number of sheep	Age (years) 1.5	2.5	3.5	4.5	5.5
1 - 2 years - 26 (Group 1, 2 and 3)	2005 a	104 b	—	—	—
1 - 3 years - 20 (Group 1 and 2)	1016 a	30 b	7 c	—	—
1 - 5 years - 12 (Group 1)	1181 a	52 b	10 c	6 c	8 bc

Letters: Means in the same row with a common letter (a,b,c) do not differ significantly ($P > 0.05$)

Microscope inspection of the pigmented fibres revealed that many had pigmented tips or were banded. At the point of cessation of pigmentation there were often what appear to be melanocytes in the wool fibres, apparently flushed out from the follicle bulb region, and then a complete cessation of pigment granules (Figure 2). These observations are consistent with those of Nichols (1927) and Aliev and Rachkovosky (1987) for the "greyed" fleece of Suffolk and Tajik sheep, respectively.

It is possible that the cessation of pigmentation may be related to a remnant effect of the growth cycle characteristic of moulted hair fibres or another mechanism. It is usual for

melanocytes to be shed or move from the follicle bulb during catagen (regression) and be replaced after telogen (rest) for active melanogenesis during anagen (growth period). Precursor melanocytes or surviving melanocytes in the outer root-sheaths are proposed to replace melanocytes shed from the bulb during catagen and telogen (Ortonne and Prota 1993). In Merino sheep there may be no reserve melanoblasts or surviving melanocytes in the outer root sheaths (Lyne and Hollis 1968; Forrest et al. 1985) to allow replacement so the fibres permanently "grey". The epithelial tissue environment (including the outer root sheath) may be unfavourable for melanocyte retreat. However, there were no obvious declines in fibre diameter, as expected during catagen, at the point of cessation of pigmentation.

Figure 2: Melanocyte like structures and melanin granules in the wool fibre at the point of cessation of pigmentation.

5C.4. CONCLUSION

There was a dramatic decline in isolated pigmented fibres in the fleece between 1.5 and 2.5 years age to mean levels close to or well below the 100 per kg threshold. This level being the often quoted maximum concentration of dark fibres tolerated by processors for end-uses sensitive to dark fibres. The finding of a substantial decline in pigmented fibre concentration within fleeces before adult life (2.5 years) has since been shown to have wider relevance in Merino clips and, therefore, clearly has important implications for wool buyers in terms of minimising risk of problems associated with dark fibres in wool sale lots.

CHAPTER 5: SHORT PAPER D

CHANGES IN MACROSCOPIC PIGMENTATION DURING EARLY LIFE

5D.1. INTRODUCTION

Brooker (1968) studied age-related changes in macroscopic pigmentation in white Peppin Merinos by a qualitative and graphical examination of data. He found the incidence of tan hairs on the legs (20%) was similar at birth and in adult life though less adult sheep had hoof pigmentation and tan fibres on the ears. Adults were more often affected by black fibres on the ears, black and brown skin on the nose, and black skin around the eyes than lambs.

In Chapter 2, the changes in macroscopic pigmentation, of Merino ewes with (P) and without (A) pigmented leg fibres, between 1.5 and 5.5 years age were reported. Pronounced differences existed between these two classes of sheep (A and P) for the degree of expression of most types of macroscopic pigmentation and change with age. While this information is relevant, most decisions about selection based on pigmentation occur in early life prior to breeding.

This short paper reports on the assessment of changes in macroscopic pigmentation between birth and hogget age among sheep with and without pigmented leg fibres. These results supplement information presented in Chapter 2 for adult sheep by producing similar observations for young Merino sheep.

5D.2. MATERIALS AND METHODS

The 95 hogget sheep were located at Turretfield Research Centre and the progeny of four Trangie Fertility (Dun and Eastoe 1970) rams, with varying amounts of pigmented fibres on the legs, mated to South Australian Merino ewes mainly without this character.

The various types of macroscopic pigmentation described in Chapter 2 (section 2.2.2) and Appendix 7 were scored at birth (BS), lamb shearing - age 6 months (LS) and hogget shearing - 18 months (HS). At lamb marking - age 2 months (LM), birthcoat halo-hair pigmentation was scored again as later ages are unlikely to show this pigmentation (removal by fibre shedding and shearing).

The differences in various types of macroscopic pigmentation and change with age was assessed by least squares analysis of variance using procedure GLM and the Repeated measures option (SAS 1987) for Age and including the effects of class (L) for presence (P) or absence (A) of leg fibre pigment, Sex (S) and L*S. This analysis does not provide least square means and significant differences for comparison of Ages or the interactions between Age and S or L class. To obtain this information the data was first analysed in a univariate form by procedure GLM using model (i). The least square means for significant effects ($P < 0.05$) were then obtained using a simplified model in which non-significant effects had been removed. In most cases, the effect of Sex was non-significant leading to model (ii) for derivation of L*Age least square means. The model r^2 for the different types of pigmentation were high (0.71 to 0.91).

(i) $Y_{ijklm} = u + L_i + S_j + L*S_k + SN(L\ S)_l + Age_m + L*Age_{im} + (S*Age)_{ij} + e_{ijklm}$

(ii) $Y_{ijk} = u + L_i + SN(L)_j + Age_k + L*Age_{ik} + e_{ijk}$

Where : Y = the dependent variable and u = the mean

L = Leg fibre pigment class (present or absent)

S = Sex (ewe or ram)

Age = Age at inspection (BS LS HS)

SN(L S) = sheep number nested within L and S

e_{ijklm} or e_{ijk} = the random residual error.

Appendix 7 contains the publication (Fleet et al. 1995a), in which the results for Chapter 5D are presented and discussed in detail. Table 1 of Appendix 7 shows the significance of effects of Age and Age*L determined from the Repeated Measures analysis of variance, the Spearman's correlation coefficients (repeatabilities) between hogget shearing records (HS) and birth (BS) or lamb shearing (LS), the number of individuals affected by each type of macroscopic pigmentation (i.e. score>0), and the least square means and significant differences for each age (BS, LS and HS) for sheep within L class for presence (P) or absence (A) of leg fibre pigmentation.

5D.3. RESULTS AND DISCUSSION

For each type of macroscopic pigmentation assessed there were significant differences between sheep with (P) and without (A) pigmented fibres on the legs at one of more of the inspection times during early life. The types of pigmentation where there was no

difference between L classes at birth (BS) were face fibres, eye lashes, ear dorsal skin, eye skin and under tail skin. For LS and HS, except for between leg skin pigmentation at LS, all of the types of pigmentation had significant differences between L classes. At HS the difference for between leg skin pigment was also significantly different ($P < 0.05$).

There were differences in the change in pigmentation with age. Sheep (A) without pigmented fibres on the legs either showed no change or a reduced change with age relative to sheep (P) with pigmented leg fibres (Appendix 7, Table 1). The Age*L interactions were significant for face fibres, ear fibres, eye lashes, birthcoat halo-hair, face skin, ear dorsal skin, eye skin, mouth skin, between leg skin and tail skin (Appendix 7, Table 1). The interaction for birthcoat halo-hair involved a reduced mean at lamb marking (LM), apparently as a result of fibre shedding, and a reduced number affected among sheep (P) with leg fibre pigment. The other interactions arise because of a larger change (mainly increases) between BS and LS or HS among those sheep with leg fibre pigmentation (Appendix 7).

Sex of sheep was not significant ($P > 0.05$) except for horn site fibres ($P < 0.05$), between leg skin ($P < 0.001$) and under tail skin ($P < 0.01$). The significant effects of Sex and Age*Sex interaction for pigmented fibres at the horn sites related to the development of horns on rams and exclusion of some fibre pigmentation. The Age*Sex interaction for between leg skin pigmentation arises because of higher scores for rams with leg fibre pigment at HS (age 1.5 years) and is associated with development of skin pigment on the scrotum. A Sex*L interaction for between leg skin pigment occurred because at HS the females of each L class had significantly higher scores than males.

Most types of macroscopic pigmentation increased between BS and LS or HS in at least the P group (Table 1). The exceptions were hoof and horn pigment, ear fibres, ear ventral skin, and birthcoat halo-hair pigment which declined with increasing age in at least the P group. In the case of face and ear fibres, birthcoat halo-hair, face and ear ventral skin, mouth and between leg skin, there was no significant change in pigmentation (from zero or a low value) with age among those sheep (A) without pigmented leg fibres.

Correlation coefficients (Spearman's) or repeatabilities between LS and HS were often higher than between BS and HS. However, nose-lips skin for both A and P groups had high repeatabilities for both intervals.

The frequencies of affected sheep in Table 1 reveal qualitative changes in addition to quantitative change. For example, the incidence of pigmented skin on the ears and under the tail increased from virtually few sheep affected at BS to most sheep affected at LS or HS.

It appears that pigmented leg and horn site fibres will be most readily discernible at lamb shearing and hogget shearing or when sheep are crutched and wigged. Other convenient times to check for pigmented horn site fibres and obvious leg fibre pigment are lamb marking and sheep classing. The changes in macroscopic pigmentation with age and the large effects associated with presence of leg fibre pigment, add to data presented by Brooker (1968) and in Chapter 2.

Differences in change with age between sites on the body may relate to differences in the type of tissue (i.e. skin ⇑; kemp ⇑; fleece ⇓; cornified ⇓), physical differences between sexes or differential exposure of skin to sunlight (e.g. scrotal skin of rams and under tail skin of ewes), or differences in the melanocyte (pigment producing cells) populations. In sheep with pigmented leg fibres there was generally more of other types of pigmentation. Fleet et al. (1993a) found melanocyte numbers in white wool-bearing skin were higher for sheep with leg fibre pigmentation.

5D.4. CONCLUSION

The sheep with pigmented leg fibres had higher amounts of other types of macroscopic pigmentation, than sheep without pigmented leg fibres, at one or more stages in early life. Several types of macroscopic pigmentation increased in frequency or degree of expression with age (between birth to lamb shearing or hogget shearing); especially among sheep with pigmented leg fibres. Types of pigmentation that showed a decline with age were pigmented halo-hairs on the birthcoat, tan ear fibres, hoof and horn pigment, and ear ventral skin pigmentation. Hence assessment of these types of pigmentation would be best undertaken in early life whereas other types of pigmentation that increase in early life (e.g. leg and horn site fibres) should also be checked at hogget age or during adult life.

CHAPTER 5: SHORT PAPER E

A METROLOGICAL COMPARISON OF PIGMENTED FIBRES IN FLEECES AND TOP

5E.1. INTRODUCTION

Top or combed wool sliver is a product of early stage processing of wools other than those very short or heavily contaminated with vegetable matter. To produce top the process involves scouring of greasy wool, drying, carding, gilling and combing. The process removes impurities, including dirt and vegetable matter, removes short fibres, aligns fibres parallel, and produces thorough blending (Harrowfield 1987). The blending allows the opportunity for reliable measurement of dark fibres in top (IWTO 1988).

The relationship between measurements of fleece samples and processed top, the first stage that dark fibres are measured in industry, is important for interpretation of the industry relevance of results presented in the previous chapters. Two experiments are reported elsewhere that involve investigation of this aspect of research on pigmented fibres in white wool. The first experiment involved processing eight batches of two Corriedale fleeces with varying concentrations of pigmented fibres. A high correlation coefficient ($r=0.97$) and higher concentration of pigmented fibres (1.7-fold) in top relative to wool staple measurements was found (Fleet and Foulds 1988). The other experiment involved three groups of Merino fleeces (9 per group) that were processed to top and fabric in a trial assessing transfer of pigmented fibres from coloured sheep (black Merino or Awassi). The concentration of pigmented fibres in the processed top and fabric was lower than found in staple wool (Lightfoot 1993).

In this short paper, the relationship between results based on staple measurement and those based on measurement of processed tops are reported together with objective assessments of the industry relevance of the pigmented fibres present. In this regard, the results add to the findings of Fleet and Foulds (1988).

5E.2 MATERIALS and METHODS

5E.2.1. Fleece preparation

This experiment utilised the fleeces from the 95 hogget sheep described in Appendix 7 and Chapter 4[A]. The hogget progeny of these rams were crutched a week before shearing, to remove urine stain, and after shearing each skirted fleece was placed in a labelled plastic bag.

From each skirted hogget fleece 4 samples of 26 staples were generated using a grid of 104 holes (i.e. a staple from every fourth hole). Two of the samples had the staple tips removed leaving 70 mm (sample A) and the others were left intact (sample B). The practice of removing the dusty staple tips facilitates scouring and had been adopted for other experiments (Chapter 2, 3 and 4) but the effect of this treatment on pigmented fibre concentration was unknown. The samples were scoured in Mobil B1 solvent and the replicates of each sample type (A and B) allocated to 2 observers.

[A]**Note:** Ram 1 in Chapter 4 is referred to as Ram 4 in Chapter 5E and Appendix 7; Ram 2 is Ram 3; Ram 3 is Ram 1; and Ram 4 in Chapter 4 is Ram 2 in Chapter 5E and Appendix 7.

Each staple was inspected using the CSIRO Dark Fibre Detector and fibres were counted that had a dark section of more than 10 mm and of darkness grade 5 (threshold of importance) or greater as determined by comparison with the CSIRO Dark Fibre Reference Scale (Foulds 1988). For sample A, the observers counted the dark fibres (excluding those obviously not pigmented) in each affected staple. For sample B, in addition to counting the dark fibres, up to 3 were taken from each staple for microscopic inspection. The concentration of pigmented fibres per kg of scoured staples was determined for each sample. There were no significant differences ($P \geq 0.05$) between observers or samples (A,B) so the results for pigmented fibre concentration from all their samples for each fleece were pooled to give a single estimate (PFC).

The main indicators of isolated pigmented fibres were identified using a stepwise regression analysis (SAS 1987). In this analysis the various types of macroscopic pigmentation (Chapter 2, section 2.2.2 and Appendix 7) were included as the independent variables and the log transformed values of pigmented fibre concentration (PFC) [$Log_{10}(PFC+1)$] was the dependent variable. In this case, the birth records for pigmented halo-hair were used and for the other types of pigmentation the lamb shearing and hogget shearing records were summed. This analysis selected score for pigmented leg fibres ($P < 0.0001$) and pigmented birthcoat halo-hair ($P < 0.012$) as the best indicators of isolated pigmented fibres in these hogget fleeces. Pigmented ear fibres and piebald spot score were next in order for explaining variation in the concentration of isolated pigmented fibres but were not significant ($P > 0.05$).

5E.2.2. Preparation and measurement of tops

The greasy fleeces were weighed and allocated to a processing batch based on paternity (four sires) and Class (1-6) for indicators of isolated pigmented fibres (Appendix 7) in anticipation that a wide range of pigmented fibre concentrations would be generated. The 13 batches formed from these 95 hogget fleeces were processed through to top by CSIRO (Division of Wool Technology, Ryde), in the order of the lowest anticipated risk of pigmented fibres to highest risk, with thorough cleaning of machinery between batches. Six 20 g samples of top from throughout each batch were measured by a single observer. The procedure involved either inspecting all of the sample or that weight sufficient to generate 50 dark fibres with a coloured length of greater than 10 mm and of darkness level 5 (threshold) or greater.

The dark fibres isolated from the tops were permanently mounted on slides for examination with a microscope at magnification 400x. A random sample or all of the pigmented fibres collected (up to 109 per batch) were measured for diameter, light transmittance and darkened length of fibre.

The percentage of light transmitted through the fibre (Tr) was measured using a microphotometer (Foulds 1988) at a point in the darkest region of the fibre, and the fibre diameter was measured at the fibre base and in the darkest region of the fibre. The mean fibre diameters for both sites on the fibre were similar (0.3 μm difference) so the 2 values were averaged for each fibre. The percentage of fibres with a Tr measurement equivalent or greater than CSIRO Dark Fibre Reference Scale level 5 (%Tr$<$86) was also calculated. Fibre length was measured using a microscope (magnification up to 400x),

micro-fiche image tracing and planimeter, to give the length of fibre visibly dark (DFL) when the slide was viewed with a CSIRO Dark Fibre Detector.

The results for pigmented fibre concentration of individual fleeces were weighted according to the relative contribution to the batch (based on greasy fleece weight) and a pooled result (PFC) obtained. The pigmented fibre concentration for the top (TPFC) was then compared with the pooled raw wool estimate (PFC) by regression analysis (SAS 1987). TPFC values of the 13 tops were 25(2), 33, 50(3), 58, 192, 425, 733, 2514, 3340 and 8566 per kg. The slope of the regression line was found to be very sensitive to the batch with the highest TPFC. As this high value (8566) was solitary and widely separated from other points the results of the regression analysis with and without the highest value are reported.

The hypothesis proposed was that the concentration of isolated pigmented fibres in top equalled the weighted raw wool values. Therefore the regression line was tested for significant difference from a slope of one ($\beta = 1$) an intercept of zero ($\alpha = 0$).

5E.3. RESULTS AND DISCUSSION

Of the 1541 dark fibres removed from the tops, 93.6% were classed as melanin pigmented, 4.6% urine stained, 1.1% non-sheep and non-animal, and 0.8% were medullated white sheep fibres that appeared dark with transmitted light. Table 1 shows the characteristics of the pigmented fibres from the tops within a grouping based on the sire of the progeny. Most of the pigmented fibres were from the progeny of Ram 4.

The percentage of dark fibres with Tr<86 varied from 66% to 89% between the ram groups. Therefore, most of the fibres had a dark area above the threshold for classification of fibres with potential to cause problems in end-uses sensitive to this fault. The mean coloured length visible in fibres (DFL) using the CSIRO Dark Fibre Detector varied from 33mm to 50mm between the ram groups. These lengths are well in excess of the most generous criterion (>25mm) sometimes used to classify dark fibres (Fleet and Foulds 1988). The diameter of the pigmented fibres exceeded that of the wool tops by 6.4μm to 14.7μm between the ram groups. The high fibre diameter of pigmented fibres would increase the likelihood of visible distinction in processed wool.

Table 1. Characteristics of the pigmented fibres removed from the tops.

	Ram 1	Ram 2	Ram 3	Ram 4
No. of fibres	17	59	32	960[A]
Transmittance (%Tr)	76	76	82	78
% with Tr<86	71	85	66	89
Dark length (DFL mm)	50	48	33	47
Diameter (μm)				
Fibres (PFD)	34.8	31.7	37.2	34.1
Top (TFD)	23.7	25.3	22.5	23.8

[A] For Tr a DFL in ram group 4 a random sample of 411 pigmented fibres was measured.

Table 2 shows results of simple linear regression analysis between the pooled and weighted raw wool measurements with those obtained from top and indicate that the raw fleece grid sample provided a reliable measure (r^2 = 0.95) of isolated pigmented fibres in fleece wool processed to top. The tops had higher concentrations of pigmented fibres

than the fleece grid sample (1.52 times greater). However, the extreme solitary point (8566 per kg) influenced the slopes obtained. When this point was excluded from the data set the r^2 remained high (0.91) but the slope became 1.12; being not significantly different ($P \geq 0.05$) from a slope = 1.

Table 2. Regression analysis between the concentration of pigmented fibres in tops (TPFC No. per kg) and the fleece sample measure of pigmented fibre concentration (PFC No. per kg) for 13 or 12 (extreme point deleted) batches.

Raw Wool Measure	Number of batches	Model r^2	Model Signif.	Slope $\beta \pm$ SE $P(\beta = 1)$	Intercept $\alpha \pm$ SE $P(\alpha = 0)$
PFC	13	0.95	***	1.52 ± 0.11 ***	34 ± 179 ns
	(12)[A]	(0.91)	***	(1.12 ± 0.11 ns)	(140 ± 112 ns)

[A] Regression excluding the batch with the highest PFC value (8566 per kg)
Significance *** $P < 0.001$; ns not significant.
$\beta \pm$ SE is the regression coefficient ± the standard error.
$\alpha \pm$ SE is the regression intercept ± the standard error.

A smaller processing trial with Corriedale fleeces (Fleet and Foulds 1988) showed higher concentrations of pigmented fibres in the tops (1.7 fold) relative to the fleece grid sample. Factors which could contribute to a higher concentration of pigmented fibres in top are fibre breakage during processing and easier detection of dark fibres in top relative to solvent scoured wool staples. Harrowfield (1987) reported that carding and gilling processes result in a 41% increase in fibre frequency though this increase will be reduced

by combing. In contrast, Lightfoot (1993) reported that pigmented fibres transferred from coloured sheep to white sheep were reduced in frequency between greasy wool, top and fabric. These reductions presumably reflect the low fibre length of the transferred pigmented fibres (Fleet et al. 1986) leading to their biased removal to the card wastes and noils, compared to the proportion left in tops, and being hidden in the yarn and fabric

5E.4. CONCLUSION

The measurements of isolated pigmented fibres in fleeces showed a high correlation ($r \geq 0.95$) with the pigmented fibres of commercial importance found in tops processed from groups of these fleeces. Pigmented fibre concentrations found in staples collected by grid sampling the hogget fleece were at least as high (1.5 or 1.1 fold) as those found in tops. These results provide confidence for conclusions, relating to sheep culling to minimise occurrence of isolated pigmented fibres in white wool, derived in several other experiments that involved measurement of raw wool alone.

GENERAL DISCUSSION

"The objectionable black is usually heavily, indistinct, or streaky whether on the nose or about the eyes. When really bad around the eyes it appears as if the colour is apparent under the skin as well as on it, and often is of a streaky nature. On the male it is bad to see dark colouration in the horn or horns while another very bad black is sometimes found as deep smudges or many spots on the roof of the mouth, or a collection of black spots in a close group (but not smudgy) on the nose higher up than the nostrils. Sheep with these types of black will most assuredly give black or spotted lambs among their progeny".

"Old Hand" (1953).

"Considering rams as the sale product, the absence of black streaks in the horns would be considered by most buyers to have a cash value. It is known from experimental work, however, that these black streaks are not associated with the production of black lambs. It is not yet known that they are not associated adversely with any other aspect of production. Should the absence of such associations be established and accepted by the ram buyer, however, and should black streaks still affect the price paid for the ram, the streaks would then have a marketing value - they would represent unfortunate packaging."

C.H.S. (Scott) Dolling (1970).

GENERAL DISCUSSION

The occurrence of black or brown pigmented fibre or skin spots in the non-wool areas on sheep has been a historic concern of the Merino sheep breeder (Graham 1870; Pearse 1945; "Old Hand" 1953; Body et al. 1962). However, there was a paucity of scientific investigation of these beliefs and the evidence available did not substantiate selection against pigmentation outside the fleece (Brooker 1968; Dun and Eastoe 1970). The two quotations on the cover page of this section provide examples of the beliefs held by a sheep breeder ("Old Hand" 1953) and discussion advanced by scientific investigation (Brooker 1968) about the importance of pigmentation outside the fleece and possible perceptions of ram buyers in terms value placed on absence of such pigmentation (Dolling 1970).

Dark fibres cannot be reliably measured prior to sale of greasy wool and the first stage that industry may assess this wool fault is during early stage manufacture of wool top (combed sliver). The maximum threshold of 100 dark fibres per kg of top is often quoted for wools destined for end-uses sensitive to dark fibres but even one dark fibre per kg may be unacceptable in some cases (Foulds et al. 1984). The unpredicted occurrence of dark wool fibres, like other unwanted contaminants (synthetic fibres and chemical residues), can result in financial loss by wool processors and lost markets for all involved in the wool pipeline.

To obtain a reliable measurement of dark fibres in top requires labour intensive procedures (Foulds et al. 1988) and adoption of such testing is in a development phase and may not be routinely conducted. Therefore, problems may not be identified until late stage processing when expensive mending operations are required. Mending alternatives to manual "picking" of dark fibres from fabrics have been developed (bleach procedures and SIROCLEAR^{R}) but effectiveness is limited and additional costs and delays are involved (Bereck et al 1982; Plate 1992; Plate, Foulds and Turk - personal communications). **It remains the responsibility of wool growers, wool classers and wool marketers to minimise occurrence of dark fibres and identify wool at risk (or low risk) of containing dark fibres.**

The experiments described in this thesis provide an account of research on the occurrence and inheritance of isolated melanin pigmented fibres in the fleece and associated macroscopic pigmentation on Merino sheep. There is little published information on this subject despite the concerns about dark fibres in white wools.

In the Merino sheep samples tested there was a pronounced **effect of one or more types of macroscopic fibre pigmentation on occurrence of isolated pigmented fibres in the hogget fleeces.** Among the sheep from Western Australia and South Australia the presence and degree of leg fibre pigmentation was closely associated with the occurrence of isolated pigmented fibres. For the Merino resource flock sampled in New South Wales a wider range of types of fibre pigmentation were implicated. In this case, the type of pigmentation with the highest correlation coefficients with isolated pigmented fibres was pigmented halo-hair on the birthcoat ($r_p = 0.33$; $r_g = 0.66 \pm 0.19$). Most macroscopic

fibre pigmentation was generally infrequent (3 to 29%), had moderate to high heritabilities (0.2 to 0.8) and had positive phenotypic correlation coefficients with isolated pigmented fibres.

Culling of sheep based on macroscopic fibre pigmentation involved between 28% (pigmented birth halo-hair alone) to 68% (all types of macroscopic fibre pigmentation) of the sheep. These options reduced the concentration of isolated pigmented fibres in the hogget fleeces from 231 per kg to between 147 and 15 per kg. Such heavy culling may normally not be practical and, therefore, in any similar flocks individuals the distinct pigmentation (highest risk) could be culled first and gradually work toward elimination of the unwanted fibre pigmentation.

Phenotypic correlation coefficients between the various types of pigment and hogget production characters (clean fleece weight, average fibre diameter and off-shears body weight) were all low (-0.07 to 0.13) in the Peppin flock sampled. Therefore, selection against pigmentation should have little effect on the average production of the flock; though the number of sheep available for assessment on production traits will be reduced.

Several of the genetic correlations between pigmentation and production traits were considered adverse or antagonistic relative to expectations from industry selection programs. The genetic correlations were positive, in general, between pigmentation traits and clean fleece weight (except isolated pigmented fibres) or off-shears body weight, and generally negative with average fibre diameter. **The importance of genetic relationships between pigmentation and production traits, in terms of effects on future generations within industry selection programmes requires further investigation.**

The possibility that an identifiable locus was involved in determining the absence of pigmented leg fibres and pigmented birthcoat halo-hairs was investigated in matings of selected parents at Turretfield Research Centre (SA). These matings provided data that were consistent with the hypothesis for the existence of **a gene responsible for removal of pigmented fibres from the legs of Merino sheep.** However, the data did not support the proposal for simple Mendelian inheritance for presence and absence of pigmented birthcoat halo-hair.

Pigmented fibres on the legs and pigmented halo-hairs on the birthcoat were both useful indicators of isolated pigmented fibres in the hogget fleece for the sheep produced at Turretfield Research Centre. As found in other samples of Merino sheep the various types of pigmentation were all positively correlated.

Investigation of melanoblast populations in foetal Merino sheep indicated that **white fleece involves a deficiency of melanocytes and any apparent migration of melanoblasts was delayed relative to black foetal sheep and was unable to colonise the epidermis** during prenatal development. In "usual" white Merino sheep, any melanoblast migration observed in the dermis was delayed until well after the initiation of primary and original secondary follicles. **In white sheep likely to develop isolated pigmented wool fibres the timing of migration of the observed melanoblasts coincided with initiation of primary follicles** (around 70 days gestation). It is concluded that the number of isolated pigmented fibres that develop in white sheep will be very sensitive to the timing and magnitude of melanoblast migration.

The concentrations of isolated pigmented fibres in the fleece of Merino sheep with pigmented fibres on the legs were highest concentrations near this macroscopic pigmentation, had intermediate concentrations on the neck and along the horizontal mid-line of the side, and lowest concentrations at the mid-back. This pattern is consistent with the legs being the primary destination of colonisation of melanoblasts but some of these precursor pigment cells appear to disperse to other areas of the coat allowing the opportunity for development of isolated pigmented wool fibres.

The **isolated pigmented fibres in the fleece** of Merino sheep with pigmented leg fibres **declined dramatically after hogget age** to low levels. This finding, if relevant to a the general population of Merino sheep, has **important implications for wool buyers** in terms of minimising risk of problems from dark fibres in wool sale lots. In recent surveys of tops, wool sale lots and commercial consignments, the CSIRO has provided evidence that is in support of higher risk of pigmented fibres in wool from young Merino sheep and a low risk from older sheep. Nevertheless, this thesis has shown that wool from young sheep (hoggets) can also have a low risk of pigmented fibres if those affected by macroscopic fibre pigmentation have been culled from the flock.

The changes in macroscopic pigmentation with age varied between body sites and with presence or absence of pigmented fibres on the legs. In several cases, pigmentation increased between birth and hogget age and the degree of increase was greater among sheep with pigmented leg fibres. The latter increase may be explained by a higher incidence of melanocytes (Fleet et al. 1993a). Most types of macroscopic pigmentation showed high repeatabilities between 1.5 and 5.5 years age. In the case of pigmented leg

fibres, the careful inspection at shearing and crutching together with culling of the affected individuals in early life (<2 years age) would be expected to almost eliminate this feature from that flock of sheep during adult life. Furthermore, in view of the apparent simple mode of inheritance (absence vs presence) for pigmented leg fibres, the occurrence in future generations should also be minimised.

Measurements of isolated pigmented fibres in fleeces showed high correlation coefficients with the pigmented fibres of commercial importance found in tops processed from groups of these fleeces. The concentration of pigmented fibres in top was at least equal to that found in the raw wool grid sample of the skirted fleece. This outcome provided confidence in the conclusions, relating to sheep culling to minimise occurrence of isolated pigmented fibres in white wool, that were based on measurement of fleece samples.

This thesis provides substantial evidence of associations between macroscopic pigmentation on white Merino sheep and the occurrence of hidden isolated pigmented fibres in the hogget fleece. Macroscopic fibre pigmentation seems likely be more efficient at indicating a potential for isolated pigmented fibres in the hogget fleece than the more common types of non-fibre pigmentation (e.g. nose-lips). Therefore, Merino breeders have the option of relaxing selection against minor skin and hoof pigmentation and focus on reduction or elimination of fibre pigmentation. In view of the high degree of association between the various types of macroscopic pigmentation, selection against fibre pigmentation should also reduce to some degree pigment of skin and hoof.

The following unresolved issues or opportunities in regard to control of pigmentation in white Merino sheep require further consideration:

(a) At the population genetics level, there is need for further estimates of the genetic relationships between pigmentation, production and reproduction traits. Some of the adverse genetic correlations identified may be antagonistic to expectations of industry selection programmes. Selection that removes pigmentation unnecessarily may, possibly, reduce aspects of production. Alternatively, selection emphasis on production traits may, possibly, lead to increases in pigmentation that has undesirable effects on wool quality or sheep marketing.

(b) Development of a practical test for identification of recessive black. The knowledge and molecular tools now available from murine studies has provided the opportunity to apply genetic engineering to practical sheep breeding.

(c) The assessment of albinism as a novel alternative method of controlling pigmentation in Merino sheep.

(d) Determine the homology between genes characterised or mapped in other mammals with other genes that affect pigmentation including white spotting and albinism.

(e) Determine the aetiology at the biochemical level of white spotting effects in sheep. The striking migration of melanoblasts along capillaries of white foetal Merino sheep likely to develop isolated pigmented fibres is suggestive of a substance on that tissue that is allowing survival and guiding migration. The failure of these melanoblasts to colonise the epidermis is suggestive of a deficiency (at least temporarily) of a factor(s) required by melanoblasts to locate and colonise in that tissue environment.

(f) The differential changes in melanogenesis between body sites may involve tissue specific differences that affect melanocyte viability and activity. The rapid decline (greying) of isolated pigmented wool fibres, while pigmentation in exposed skin or kemp persist or increase, requires clarification. Tissue specific differences must be involved if the melanocytes that disperse to the fleece arise from the same neural crest clones that colonise the face and legs.

APPENDICES

APPENDIX 1: Correlation coefficients (Spearman's) between the types of visible pigmentation (total scores) at 1.5 years age (above the diagonal) and 5.5 years age (below the diagonal) and with isolated pigmented fibre concentration in the fleece (PFC) at 1.5 years age for all sheep (ALL) and sheep with (P) and without (A) pigmented leg fibres[A].

Pig-ment	Leg Fibre[A]	PFC	Hv	Lf2	Hf	Ff	Ef	Fs	Es	Ns	EYs	Ms	Ls	Ts
Lf1[A]	ALL	0.52*	0.81*	0.94*	0.88*	0.75*	0.44*	0.73*	0.82*	0.73*	0.60*	0.64*	0.06	0.74*
	A													
	P	0.30*	0.51*	0.81*	0.62*	0.72*	0.27*	0.40*	0.54*	0.60*	0.34*	0.45*	-0.19	0.44*
PFC: 1.5 years	ALL		0.45*	0.44*	0.50*	0.45*	0.22*	0.28*	0.39*	0.38*	0.31*	0.31*	-0.11	0.34*
	A		0.02		-0.08		-0.14	0.11	0.04	0.12	0.39*	0.14		0.28
	P		0.16	0.07	0.30*	0.33*	0.11	-0.08	0.06	0.20	-0.09	0.14	-0.25	-0.06
Hoof (Hv)	ALL	0.85*	0.48*		0.82*	0.77*	0.54*	0.40*	0.66*	0.76*	0.73*	0.56*	0.63*	0.11	0.68*
	A		0.17			0.24		-0.17	0.17	0.27	0.05	0.27	0.26		0.46*
	P	0.66*	0.25		0.53*	0.43*	0.29*	0.33*	0.22	0.39*	0.63*	0.20	0.36*	0.01	0.28*
Leg fibre (Lf2)	ALL	0.91*	0.54*	0.84*		0.85*	0.75*	0.46*	0.75*	0.84*	0.78*	0.60*	0.61*	0.05	0.76*
	A														
	P	0.76*	0.38*	0.57*		0.55*	0.68*	0.27*	0.51*	0.67*	0.71*	0.42*	0.36*	-0.16	0.54*
Horn fibre (Hf)	ALL	0.77*	0.44*	0.78*	0.82*		0.66*	0.41*	0.68*	0.76*	0.68*	0.58*	0.60*	0.12	0.73*
	A		0.11	0.16				-0.05	0.13	0.08	0.16	0.22	-0.09		0.25
	P	0.56*	0.24	0.58*	0.64*		0.52*	0.20	0.30*	0.40*	0.44*	0.29*	0.36*	-0.07	0.37*
Face fibre (Ff)	ALL	0.65*	0.45*	0.67*	0.71*	0.64*		0.50*	0.53*	0.64*	0.65*	0.43*	0.50*	-0.05	0.55*
	A		0.30	0.40*		-0.05									
	P	0.58*	0.32*	0.59*	0.69*	0.58*		0.44*	0.26	0.50*	0.60*	0.25	0.38*	-0.17	0.32*
Ear fibre (Ef)	ALL	0.33*	0.29*	0.46*	0.37*	0.32*	0.43*		0.34*	0.43*	0.45*	0.29*	0.25*	0.00	0.39*
	A		0.32	0.36*		0.09	-0.09		-0.01	0.08	-0.18	-0.08	-0.16		-0.22
	P	0.28*	0.15	0.45*	0.35*	0.24	0.48*		0.15	0.25	0.48*	0.17	0.10	-0.08	0.28*
Face skin (Fs)	ALL	0.81*	0.39*	0.75*	0.80*	0.65*	0.56*	0.26*		0.80*	0.67*	0.70*	0.56*	0.12	0.73*
	A		0.11	0.33*		0.25	0.25	-0.18		0.35*	0.29	0.35*	0.02		0.45*
	P	0.51*	0.06	0.41*	0.55*	0.28*	0.34*	0.18		0.63*	0.47*	0.59*	0.34*	-0.01	0.53*
EarD skin (Es)	ALL	0.80*	0.50*	0.77*	0.83*	0.71*	0.58*	0.43*	0.77*		0.74*	0.66*	0.63*	0.07	0.76*
	A		0.38*	0.25		0.32	0.25	0.43*	0.26		0.33*	0.29	0.38*		0.31
	P	0.58*	0.24	0.53*	0.67*	0.45*	0.48*	0.32*	0.56*		0.58*	0.52*	0.35*	-0.10	0.52*
Nose skin (Ns)	ALL	0.76*	0.42*	0.80*	0.80*	0.69*	0.59*	0.50*	0.73*	0.81*		0.63*	0.68*	0.06	0.72*
	A		0.05	0.35*		0.29	0.15	0.30*	0.31	0.53*		0.41*	0.36*		0.47*
	P	0.60*	0.28*	0.66*	0.69*	0.47*	0.55*	0.55*	0.44*	0.65*		0.33*	0.50*	-0.10	0.53*
Eye skin (EYs)	ALL	0.59*	0.27*	0.58*	0.57*	0.54*	0.42*	0.24*	0.68*	0.67*	0.64*		0.52*	0.16	0.72*
	A		0.21	0.34*		0.35*	0.20	0.27	0.49*	0.52*	0.49*		0.29		0.49*
	P	0.31*	-0.11	0.23	0.37*	0.25	0.28*	-0.02	0.50*	0.53*	0.32*		0.30*	0.13	0.57*
Mouth skin (Ms)	ALL	0.65*	0.25*	0.65*	0.66*	0.61*	0.52*	0.27*	0.57*	0.62*	0.61*	0.52*		0.11	0.57*
	A		0.14	0.09		0.27	0.26	0.08	-0.04	0.20	0.11	0.13			0.31
	P	0.39*	-0.03	0.44*	0.43*	0.38*	0.39*	0.19	0.32*	0.44*	0.48*	0.41*		0.05	0.21
Leg skin (Ls)	ALL	0.68*	0.44*	0.62*	0.62*	0.58*	0.44*	0.22*	0.61*	0.63*	0.57*	0.61*	0.54*		0.16
	A		0.29	0.42*		0.07	0.28	0.34*	0.34*	0.39*	0.26	0.56*	0.21		
	P	0.37*	0.13	0.15	0.29*	0.34*	0.18	-0.09	0.08	0.16	0.11	0.06	0.22		0.05
Tail skin (Ts)	ALL	0.82*	0.45*	0.77*	0.81*	0.68*	0.53*	0.42*	0.77*	0.77*	0.78*	0.73*	0.67*	0.68*	
	A		0.25	0.48*		0.35*	0.25	0.36*	0.36*	0.51*	0.44*	0.79*	0.48*	0.68*	
	P	0.67*	0.21	0.46*	0.70*	0.39*	0.37*	0.37*	0.54*	0.56*	0.67*	0.39*	0.38*	0.22	

* P < 0.05 otherwise not significant

[A] Class for presence (P) or absence (A) of pigmented leg fibres was based on the Lf1 score (all legs) at hogget age. Lf1 was not scored at 5.5 years age.

APPENDIX 2

Field sheet for recording pigmentation on new-born lambs.

LAMBING RECORD SHEET FOR MAJOR PIGMENTATION, ASYMMETRICAL SPOTTING AND PATCHES OF PIGMENTED BIRTHCOAT HAIRS

LOCATION: _____ DATE: / /

LAMB NO.: _____ EXPERIMENT: _____

DAM NO.: _____

*CLASSIFICATION (tick appropriate box)

	Black/Grey	Brown/Fawn ‡‡	Red/Tan ‡
WHITE			
AGOUTI			
Badger face			
Rev. Badger face			
Self colour			
Spotted self colour			
PIEBALD			
HALO HAIR			

*see description sheet

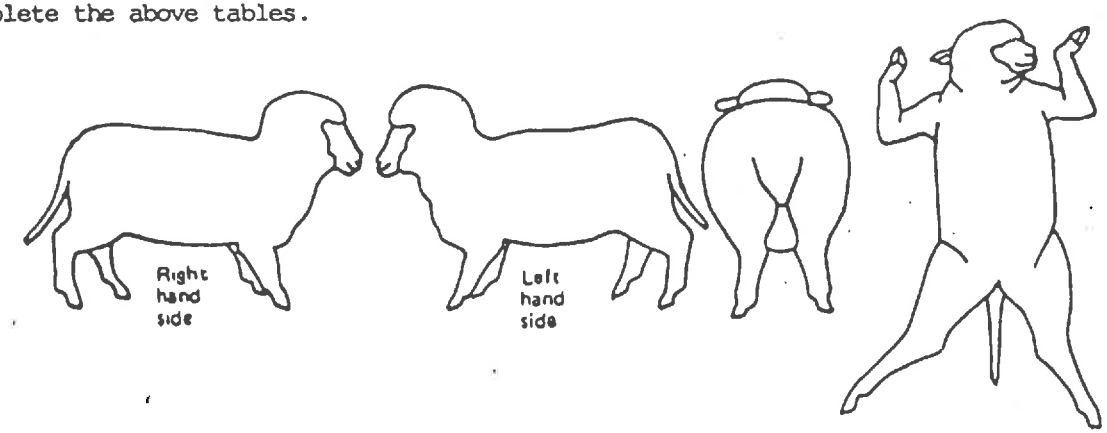
‡‡Fawn = yellowish brown ‡Tan = brownish red

DESCRIPTION \ NUMBER	DESCRIPTION OF SPOTS OR PATCHES							
	1	2	3	4	5	6	7	8
COLOUR								
FIBRE TYPE								
SKIN								
DIMENSIONS cm x cm								

Colour: Black (B), Grey (G), Brown (A), Fawn (F), Red (R), Tan (T)
Fibre type: Wool (W), Kemp (K), Halo Hair (H)
Skin: Pigmented (P) or Not Pigmented (N)

Pigmented areas:

Outline areas of pigmented wool, halo hair or kemp approximately to scale on the diagrams below. Number these pigmented areas (spots/patches) and complete the above tables.



General comments

RECORDING NON-WOOL PIGMENTATION (LAMBS)

(tick appropriate description).

1. BIRTHCOAT

Inspect back-of-neck closely and make a more general inspection of the remainder of the birthcoat. Record colour and extent of birthcoat hairs. Lamb age not to exceed 7 days.

Colour		Scores	
Black/Grey	( )	No halo hairs	( )
Brown/Fawn*	( )	Halo hairs white	( )
Red/Tan*	( )	Few coloured hairs	( )
		Coloured hairs readily discernible	( )
		Patch(es) of coloured hairs	( )
		Large portion (> 50%) of birthcoat covered by coloured hairs.	( )

2. LEGS, FACE AND EARS

Inspect front, sides and back of kemp areas on each leg and record main colour and score for pigmented fibres - usually present as spots or patch(es). Also inspect kemp areas on face and ears.

Colour	Legs	Face	Ears	Scores	Legs	Face	Ears
Black/Grey	( )	( )	( )	No spots or pigmented fibres	( )	( )	( )
Brown/Fawn*	( )	( )	( )	Few spots or pigmented fibres	( )	( )	( )
Red/Tan*	( )	( )	( )	Speckled	( )	( )	( )
				Large patch(es)	( )	( )	( )
				Large portion (> 50%) of area being scored.	( )	( )	( )

3. HORN SITES

Inspect the area surrounding each horn site for pigmented fibres. Record main colour and average score for pigmented fibres at horn sites.

Colour		Scores	
Black/Grey	( )	No pigmented fibres	( )
Brown/Fawn*	( )	< 11 pigmented fibres	( )
Red/Tan*	( )	11-100 pigmented fibres	( )
		> 100 pigmented fibres	( )

4. BARE SKIN/HOOF AREAS

Inspect the bare skin/hoof areas (nose/lips, between legs and hooves) and record colour and average score.

Colour	Nose/lips	Between legs	Hooves
Black/Grey	( )	( )	( )
Brown/Fawn*	( )	( )	( )

Score			
No pigment	( )	( )	( )
< 26% pigmented	( )	( )	( )
26-50% pigmented	( )	( )	( )
> 50% pigmented	( )	( )	( )

N.B.: Fawn = yellowish brown Tan = brownish red

APPENDIX 3: Heritabilities (h^2) $\pm$ standard error (SE) for pigment traits of hogget sheep and lambs [square brackets[A]], phenotypic correlation coefficients between hoggets scores and LPFC, the percent affected[B] and mean score of the untransformed variables.

Pigment type	Black and Grey $h^2 \pm SE$ (r_p - LPFC)	% Aff. (Mean)	Brown and Tan $h^2 \pm SE$ (r_p - LPFC)	% Aff. (Mean)	Total $h^2 \pm SE$ (r_p - LPFC)	% Aff[B] (Mean)
Birth coat hair	–	–	–	–	0.73 $\pm$ 0.26 [0.61 $\pm$ 0.16]	28 (0.4) [23]
Hooves	0.52 $\pm$ 0.17 (0.25)	56 (3.9)	0.26 $\pm$ 0.12 (0.12)	15 (1.0)	0.60 $\pm$ 0.18 [0.48 $\pm$ 0.15]	59 (4.7) [35]
Leg fibres	0.25 $\pm$ 0.13 (0.15)	4 (0.1)	0.86 $\pm$ 0.21 (0.24)	28 (3.4)	0.82 $\pm$ 0.21 [0.44 $\pm$ 0.14]	29 (3.5) [3]
Face fibres	0.20 $\pm$ 0.32 (0.04)	6 (0.1)	0.05 $\pm$ 0.08 (0.12)	3 (0.1)	0.18 $\pm$ 0.11 [0.06 $\pm$ 0.06]	8 (0.1) [1]
Face skin	0.52 $\pm$ 0.17 (0.24)	21 (0.3)	0.64 $\pm$ 0.19 (0.22)	52 (1.1)	0.65 $\pm$ 0.19	53 (1.2)
Ear fibres	0.17 $\pm$ 0.11 (0.23)	13 (0.2)	0.13 $\pm$ 0.10 (0.06)	3 (0.1)	0.25 $\pm$ 0.12 [0.23 $\pm$ 0.12]	16 (0.3) [8]
Ear dorsal	0.53 $\pm$ 0.17 (0.18)	33 (0.6)	0.50 $\pm$ 0.17 (0.22)	74 (3.3)	0.51 $\pm$ 0.17	76 (3.3)
Ear ventral	0.10 $\pm$ 0.09 (0.11)	10 (0.14)	0.68 $\pm$ 0.20 (0.16)	72 (2.9)	0.65 $\pm$ 0.20	73 (2.9)
Horn site fibres	0.08 $\pm$ 0.09 (0.22)	5 (0.14)	0.81 $\pm$ 0.22 (0.23)	26 (1.3)	0.71 $\pm$ 0.20 [0.03 $\pm$ 0.05]	29 (1.4) [1]
Eye skin	0.28 $\pm$ 0.15 (0.13)	91 (2.1)	0.07 $\pm$ 0.09 (0.09)	100 (9.4)	0.14 $\pm$ 0.10	100 (10.7)
Eye lashes	0.02 $\pm$ 0.10 (0.13)	5 (0.1)	0.42 $\pm$ 0.21 (0.28)	91 (5.6)	0.45 $\pm$ 0.22	91 (5.7)
Nose skin	0.49 $\pm$ 0.16 (0.24)	58 (0.8)	0.74 $\pm$ 0.21 (0.21)	82 (2.5)	0.69 $\pm$ 0.20 [0.48 $\pm$ 0.15]	83 (2.7) [54]
Mouth skin	0.32 $\pm$ 0.13 (0.29)	22 (0.3)	0.01 $\pm$ 0.08 (0.04)	1 (0.0)	0.36 $\pm$ 0.14	22 (0.3)
Tail skin	0.25 $\pm$ 0.12 (0.12)	40 (0.4)	0.47 $\pm$ 0.17 (0.17)	93 (2.8)	0.49 $\pm$ 0.18	93 (2.9)

[A] The heritability $\pm$ standard error ($h^2 \pm SE$) and % Affected in [square brackets] relate to records collected at birth (all lambs born alive) in years 2 to 4 (No. Obs. = 679) instead of records collected at hogget (1.5 year) shearing.

[B] % Aff. = The percentage of sheep with a pigmentation score > 0 (i.e. some pigmentation visible).

APPENDIX 4: Heritabilities (along the diagonal), phenotypic correlation coefficients (in bold) with environmental correlation coefficients (above the diagonal), and genetic correlation coefficients ± standard error (below the diagonal) for pigmentation traits (total scores) in a Peppin Merino flock (Based on progeny born in 1984, 1985 and 1986).

Pigment type	LPFC	Birth coat (Bl)	Hooves (Hv)	Leg fibres (Lf1)	Face fibres (Ff)	Face Skin (Fs)	Ear Fibres (Ef)	Ear D Skin (EDs)	Ear V Skin (EVs)	Horn Site (Hf)	Eye Skin (EYs)	Eye lashes (El)	Nose Skin (Ns)	Mouth Skin (Ms)	Tail Skin (Ts)
LPFC	**0.18 ± 0.12**	**0.33** 0.11	0.27 0.38	**0.26** 0.49	**0.12** 0.13	**0.22** 0.21	**0.21** 0.14	**0.22** 0.40	**0.16** 0.43	**0.27** 0.42	**0.16** 0.22	**0.29** 0.26	**0.21** 0.36	**0.29** 0.35	**0.18** 0.26
Bl	0.66 ± 0.19	**0.73 ± 0.26**	**0.35** -0.64	**0.27** -2.02	**0.07** -0.44	**0.26** -0.48	**0.17** -0.48	**0.21** -0.04	**0.10** 0.09	**0.24** -0.75	**0.24** -0.14	**0.25** -0.55	**0.26** -0.81	**0.34** 0.04	**0.13** 0.09
Hv	0.15 ± 0.31	0.69 ± 0.13	**0.60 ± 0.18**	**0.64** 0.39	**0.31** 0.27	**0.57** 0.40	**0.25** 0.10	**0.52** 0.44	**0.29** 0.10	**0.60** 0.45	**0.31** 0.36	**0.24** 0.11	**0.51** 0.21	**0.55** 0.40	**0.56** 0.26
Lf1	0.18 ± 0.28	0.63 ± 0.14	0.76 ± 0.08	0.82 ± 0.21	**0.48** 0.34	**0.62** 0.10	**0.35** 0.06	**0.55** 0.17	**0.40** -0.27	**0.79** 0.42	**0.30** 0.38	**0.30** 0.21	**0.53** -0.15	**0.59** 0.33	**0.44** 0.16
Ff	0.08 ± 0.43	0.92 ± 0.07	0.46 ± 0.24	0.91 ± 0.05	**0.18 ± 0.11**	**0.34** 0.23	**0.34** 0.21	**0.32** 0.13	**0.23** -0.02	**0.40** 0.03	**0.16** 0.07	**0.19** 0.05	**0.29** 0.05	**0.24** 0.14	**0.20** 0.05
Fs	0.31 ± 0.28	0.52 ± 0.19	0.68 ± 0.11	0.82 ± 0.06	0.65 ± 0.18	**0.65 ± 0.19**	**0.22** 0.10	**0.67** 0.46	**0.39** 0.10	**0.61** 0.15	**0.31** 0.28	**0.26** -0.07	**0.59** -0.06	**0.56** 0.34	**0.47** 0.32
Ef	0.43 ± 0.31	0.76 ± 0.13	0.52 ± 0.20	0.73 ± 0.12	0.86 ± 0.10	0.41 ± 0.23	**0.25 ± 0.12**	**0.21** 0.14	**0.20** -0.12	**0.37** -0.08	**0.07** -0.04	**0.19** 0.01	**0.23** 0.24	**0.28** 0.15	**0.17** 0.02
EDs	-0.10 ± 0.32	0.39 ± 0.25	0.60 ± 0.15	0.77 ± 0.08	0.81 ± 0.11	0.84 ± 0.07	0.36 ± 0.25	**0.51 ± 0.17**	**0.48** 0.07	**0.53** 0.11	**0.34** 0.25	**0.30** 0.36	**0.55** 0.20	**0.47** 0.25	**0.44** 0.22
EVs	-0.22 ± 0.30	0.11 ± 0.29	0.40 ± 0.18	0.64 ± 0.12	0.72 ± 0.15	0.54 ± 0.15	0.65 ± 0.16	0.79 ± 0.09	**0.65 ± 0.20**	**0.43** -0.24	**0.31** 0.01	**0.27** 0.31	**0.34** 0.18	**0.31** 0.25	**0.38** 0.14
Hf	0.18 ± 0.29	0.59 ± 0.17	0.68 ± 0.11	0.91 ± 0.03	1.05 ± 0.03[A]	0.84 ± 0.06	0.98 ± 0.02	0.81 ± 0.08	0.75 ± 0.09	**0.71 ± 0.20**	**0.25** 0.09	**0.28** 0.36	**0.50** -0.18	**0.54** 0.32	**0.44** 0.07
EYs	-0.16 ± 0.48	0.63 ± 0.19	0.35 ± 0.30	0.44 ± 0.25	0.63 ± 0.29	0.53 ± 0.24	0.56 ± 0.29	0.68 ± 0.19	1.01 ± 0.01[A]	0.67 ± 0.19	**0.14 ± 0.10**	**0.55** 0.36	**0.38** 0.31	**0.24** 0.31	**0.30** 0.28
El	0.39 ± 0.36	0.76 ± 0.12	0.33 ± 0.26	0.39 ± 0.24	0.60 ± 0.30	0.52 ± 0.23	0.47 ± 0.28	0.24 ± 0.33	0.22 ± 0.33	0.25 ± 0.29	0.94 ± 0.04	**0.45 ± 0.22**	**0.34** 0.47	**0.27** 0.30	**0.21** 0.37
Ns	0.08 ± 0.30	0.64 ± 0.15	0.68 ± 0.11	0.76 ± 0.08	0.74 ± 0.13	0.91 ± 0.04	0.25 ± 0.25	0.79 ± 0.08	0.42 ± 0.17	0.81 ± 0.07	0.72 ± 0.16	0.26 ± 0.29	**0.69 ± 0.20**	**0.42** 0.15	**0.49** 0.41
Ms	0.14 ± 0.34	0.72 ± 0.16	0.75 ± 0.11	0.88 ± 0.05	0.56 ± 0.24	0.82 ± 0.08	0.59 ± 0.20	0.77 ± 0.10	0.40 ± 0.21	0.79 ± 0.09	0.05 ± 0.39	0.22 ± 0.36	0.70 ± 0.12	**0.36 ± 0.14**	**0.37** 0.29
Ts	0.06 ± 0.34	0.16 ± 0.28	**0.81** ± 0.08	0.62 ± 0.13	0.58 ± 0.22	0.60 ± 0.15	0.45 ± 0.24	0.65 ± 0.14	0.58 ± 0.16	0.71 ± 0.11	0.45 ± 0.30	0.07 ± 0.33	0.56 ± 0.16	0.49 ± 0.20	**0.49 ± 0.18**

[A] Negative standard error (due to limitations of sampling).

APPENDIX 5: Heritabilities and standard errors ($h^2 \pm SE$) for production traits, and phenotypic correlation coefficients and genetic correlation coefficients $\pm$ SE with pigmentation traits.

Hogget trait	$h^2 \pm SE$ Mean (σ_p)	LPFC	Birth Coat	Hooves	Leg fibres	Face fibres	Face Skin	Ear Fibres
Clean Flc. Wt	0.44 $\pm$ 0.17 3.3 (0.6)	-0.02 -0.35 $\pm$ 0.31 ns	0.12 0.17 $\pm$ 0.29 ns	0.02 0.08 $\pm$ 0.24 ns	0.05 0.22 $\pm$ 0.21 ns	0.08 0.09 $\pm$ 0.34 ns	0.06 0.32 $\pm$ 0.22 ns	0.03 0.27 $\pm$ 0.28 ns
Fibre Diameter	0.47 $\pm$ 0.16 21.2 (1.8)	0.08 -0.67 $\pm$ 0.18 ***	0.04 -0.28 $\pm$ 0.25 ns	-0.04 -0.45 $\pm$ 0.18 *	-0.03 -0.29 $\pm$ 0.20 ns	-0.06 -0.62 $\pm$ 0.20 **	-0.03 -0.15 $\pm$ 0.23 ns	0.02 -0.16 $\pm$ 0.29 ns
Hogget Body Wt.	0.31 $\pm$ 0.14 41.7 (5.3)	-0.11 0.15 $\pm$ 0.35 ns	0.09 0.42 $\pm$ 0.24 ns	0.10 0.39 $\pm$ 0.22 ns	0.12 0.30 $\pm$ 0.22 ns	0.08 0.38 $\pm$ 0.32 ns	0.11 0.30 $\pm$ 0.24 ns	0.04 0.55 $\pm$ 0.23 *

Hogget trait	Ear D Skin	Ear V Skin	Horn Site	Eye Skin	Eye lashes	Nose Skin	Mouth Skin	Tail Skin
Clean Flc. Wt.	0.06 0.60 $\pm$ 0.16 ***	0.04 0.48 $\pm$ 0.19 **	0.01 0.26 $\pm$ 0.22 ns	0.02 0.66 $\pm$ 0.21 **	0.06 0.63 $\pm$ 0.22 **	0.01 0.19 $\pm$ 0.23 ns	0.05 0.15 $\pm$ 0.26 ns	-0.01 0.20 $\pm$ 0.26 ns
Fibre Diameter	-0.02 -0.20 $\pm$ 0.24 ns	-0.07 -0.21 $\pm$ 0.23 ns	-0.07 -0.32 $\pm$ 0.20 ns	-0.02 -0.31 $\pm$ 0.32 ns	-0.01 -0.04 $\pm$ 0.32 ns	-0.04 -0.31 $\pm$ 0.20 ns	-0.05 0.02 $\pm$ 0.26 ns	-0.07 -0.31 $\pm$ 0.23 ns
Hogget Body Wt.	0.06 0.34 $\pm$ 0.25 ns	0.11 0.39 $\pm$ 0.23 ns	0.06 0.34 $\pm$ 0.23 ns	0.05 0.57 $\pm$ 0.29 *	0.10 0.72 $\pm$ 0.16 ***	0.03 0.16 $\pm$ 0.24 ns	0.02 0.24 $\pm$ 0.27 ns	0.13 0.30 $\pm$ 0.26 ns

* $P<0.05$; ** $P<0.01$; *** $P<0.001$; ns not significant.

APPENDIX 6:

Predicted genetic change in clean fleece weight, hogget body weight, average fibre diameter, the $\log_{10}$ concentration of isolated pigmented wool fibres and $\log_{10}$ score for macroscopic pigmentation traits based on WOOLPLAN options for reducing average fibre diameter (Ponzoni 1991).

WOOLPLAN	Mean value	Option 1	Option 2	Option 3
% Gain from Index [A]				
Clean fleece weight		37.4%	6.0%	-1.7 %
Hogget body weight		1.3%	0.4%	0.01%
Fibre diameter		61.3%	92.6%	101.6 %
Predicted genetic gain [A] **(2 generations or 10 years)**		**% of mean**	**% of mean**	**% of mean**
Clean fleece weight	3.34 kg	7.8	2.2	-1.1
Hogget body weight	41.7 kg	1.5	0.7	0.2
Fibre diameter	21.3 μm	-10.9	-14.5	-15.7
Isolated pigmented fibres	0.77	5	10	12
Birth halo-hair	0.11	22	22	22
Hooves	0.49	12	12	12
Leg fibres	0.27	26	20	18
Face fibres	0.03	33	38	38
Face skin	0.25	15	10	10
Ear fibres	0.06	21	20	19
Ear skin dorsal	0.51	14	10	7
Ear skin ventral	0.47	15	10	8
Horn site fibres	0.21	28	19	23
Eye skin	1.06	1	1	1
Eye lashes	0.76	6	5	3
Nose-lips skin	0.49	7	6	5
Inside mouth skin	0.08	4	0	0
Under tail skin	0.55	4	4	2

[A] These estimates are based on parameters assumed by Ponzoni (1987 1988) for WOOLPLAN (clean fleece weight, fibre diameter and body weight) and parameters generated in this thesis for pigmentation traits incorporated into the computer programme of Cunningham (1970).

Fleet, M. R., Foulds, R. A., Pourbeik, T., McInnes, C. B., Smith, D. H., & Burbidge, A. (1995). Pigmentation relationships among young Merino sheep and their processed wool. *Australian Journal of Experimental Agriculture, 35*(3), 343-351.

APPENDIX 8

CULLING VISIBLE FIBRE PIGMENTATION IN MERINO HOGGET SHEEP: EFFECTS ON PRODUCTION AND OTHER PIGMENTATION TRAITS

Malcolm R. Fleet[1], Suzanne I. Mortimer[2] and James R. Gallagher[3]

[1] SARDI Turretfield Research Centre, Rosedale SA 5350
[2] NSW Agriculture, Agricultural Research Centre, Trangie, NSW 2823
[3] The University of Adelaide, Department of Animal Science, Roseworthy SA 5371

SUMMARY

Isolated pigmented fibres occur in some white fleeces and because they are not readily identifiable in greasy wool presale, can result in unpredicted dark fibre faults and financial loss for wool processors. Macroscopic (visible) pigmentation on Merino sheep can indicate a risk of dark fibres. Various combinations of different types of visible fibre pigmentation, scored on animals as lambs and hoggets, can be used as criteria for minimising isolated pigmented fibres in hogget fleeces. The effect of culling against visible fibre pigmentation on production performance and other visible pigmentation traits was examined in a flock of 330 hogget Merinos. Culling ewes with different combinations of types of visible fibre pigmentation had no significant effect on the average production performance for greasy and clean fleece weight, average fibre diameter and off-shears body weight. Such culling can reduce the incidence and mean score of other types of pigmentation (i.e. pigmented skin on the nose-lips, inside the mouth, on the face and ears, around the eyes; hoof pigmentation; and pigmented eyelash fibres other than pronounced). However, there was considerable overlap of these types of visible pigmentation between the culled and selected groups.
Keywords: Pigmentation, production traits, culling, Merino sheep.

INTRODUCTION

Dark fibre is seen as an increasing problem by some customers of Australian wool. A key quality area for the Australian Wool Clip is the elimination of pack fibre, dark fibre (urine stained and melanin pigmented) and non-wool contamination (IWS 1996). Wool fibres darkened by urine stain are controlled by a crutching schedule within Quality Management schemes (e.g. Vandeleur 1993) while the control of melanin pigmented wool requires both sound clip preparation and breeding policies (Fleet 1996).

Recent research (Fleet *et al.* 1995a,b; Fleet 1996) has identified that high concentrations of isolated pigmented wool fibres (i.e. >100 per kg scoured staples) can occur in the fleeces of some hogget Merino sheep. Furthermore, various combinations of visible fibre pigmentation traits can be used as criteria for sheep culling to minimise the risk of isolated pigmented fibres in the hogget wool clip (Fleet *et al.* 1995b). Criterion 4 of Fleet *et al.* (1995b) was the most effective at reducing the incidence of isolated pigmented fibres and involved culling animals with visible fibre pigmentation as hoggets (legs, horn sites, face and ears), pigmented eye lashes if pronounced (>75%) and pigmented fibres as lambs (birthcoat halo-hair, legs, face, horn sites and Australian Piebald spots).

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This paper examines the effect of culling against visible fibre pigmentation on performances of hogget Merino sheep in other visible pigmentation (skin and hooves) and production traits (greasy and clean fleece weight, average fibre diameter and off-shears body weight).

MATERIALS AND METHODS

The phenotypic effects of culling against pigmentation were determined with data from 330 medium Peppin hogget ewes, born in 1985 and 1986 and the progeny of 29 sires, from four flocks of a multiple-bloodline flock (Mortimer and Atkins 1989) located at the Agricultural Research Centre, Trangie. These sheep were scored for a range of visible pigmentation traits as newborn lambs and at hogget age (1.5 years). The hogget production records measured were greasy and clean fleece weight (unskirted), average fibre diameter and off-shears body weight.

The methods of scoring of visible pigmentation traits and fleece measurement are described by Fleet *et al.* (1991; 1995a). Visible pigmentation scores on lambs involved pigmented birthcoat halo-hair, piebald spot, and other pigmented fibre on the face, legs, ears and horn sites in non-fleece areas. As hoggets, the types scored were: pigmented fibre on the face, legs, ears, horn sites and eye lashes; pigment on hooves; and pigmented skin on the nose-lips, face, ears, under-tail, in-mouth and around the eyes. Missing values for some records reduced the number of sheep assessed within some of the culling criteria of Fleet *et al.* (1995b) defined as affected with presence of the following pigmentation types:

Criterion 1: Pigmented birthcoat halo-hair scored on newborn lambs.
Criterion 2: Pigmented hairs on legs, horn sites, face and ears evident at hogget age.
Criterion 3: As for criterion 2, together with the maximum score for pigmented eyelashes (i.e. >75% pigmented).
Criterion 4: As for criterion 2, together with fibre pigmentation scored on lambs (i.e. birthcoat halo-hair, legs, face, ears, horn sites and piebald spots).

Among the 330 hogget fleece samples measured, there were 148 (44.8%) observed to contain one or more pigmented wool fibres and the mean concentration was 252 per kg of scoured staples. The mean concentrations of isolated pigmented fibres reported in Table 1 have been adjusted for differences between observers and include a log transformed value to assist the normalising of these data (Fleet *et al.* 1991).

The data were analysed by least squares analysis of variance in which recognised important effects on production traits were included (i.e. birth-rearing type, dam age, day of birth, flock, year and sire) together with a class, PG, for presence or absence of visible fibre pigmentation as defined by the culling criteria of Fleet *et al.* (1995a). These criteria combined scores for visible fibre pigmentation types as hoggets and, or, as lambs. Least square means were determined after adjustment for significant ($P<0.05$) effects. The effects of culling criterion 4 of Fleet *et al.* (1995b) on production traits and pigmentation are reported while the results of the other culling criteria, though considered, are not detailed.

RESULTS AND DISCUSSION

Table 1 shows the least squares means of the traits within the groups unaffected (104 ewes) and affected (214 ewes) by visible fibre pigmentation as defined by criterion 4 of Fleet *et al.* (1995b) together with the frequency of sheep with no pigmentation (score 0). For each type of pigmentation, there were significant differences ($P<0.05$) between the two groups with the affected (cull) sheep having higher values than the unaffected (selected) sheep. This reflects the general positive phenotypic associations among the various types of pigmentation (Fleet and Mortimer, unpublished data). Note in the unaffected group that skin and hoof pigmentation were common.

Table 1. Means for each trait and frequency of sheep with score 0 (no pigment) for each type of visible pigmentation in groups affected or unaffected by fibre pigmentation as defined by criterion 4 of Fleet *et al.* (1995b)

Visible pigmentation (maximum score)	Unaffected mean	% Score 0	Affected mean	% Score 0
Hoof	2.3 a[A]	60	5.1 b	30
Nose-lips	1.8 a	38	3.0 b	8
In-mouth skin	0.03 a	97	0.49 b	67
Face skin	0.5 a	70	1.5 b	27
Ear dorsal skin	2.1 a	32	4.1 b	10
Ear ventral skin	2.3 a	38	3.8 b	15
Eyes skin	9.7 a	0	11.6 b	0
Eyelash fibre	3.0 a	22	6.8 b	2
Tail skin	2.0 a	10	2.9 b	4
Isolated pigmented fibres				
No. per kg scoured staples	14.7 a		345.3 a	
Log transformed value	0.3 a		1.1 b	
Production traits				
Greasy fleece weight (kg)	4.8 a		4.8 a	
Clean fleece weight (kg)	3.3 a		3.3 a	
Fibre diameter (μm)	19.7 a		20.2 a	
Body weight (kg)	39.4 a		40.2 a	

[A] Means within rows with a different letter (a,b) differ significantly ($P<0.05$).

The outcome of the other culling criteria based on hogget records (i.e. criteria 2 and 3 of Fleet *et al.* 1995b) showed similar trends on the visible pigmentation remaining in both the affected and unaffected groups though were less effective at reducing isolated pigmented fibres (Fleet *et al.* 1995b). Except for pigmented skin in-mouth within criterion 3, the affected group had significantly higher mean scores than the unaffected sheep for the other types of visible pigmentation listed in Table 1.

Criterion 1 of Fleet *et al.* (1995b) was based on birthcoat halo-hair alone and, apart from pigmented eye lashes and pigmented in-mouth skin that were higher in the affected group, the other means for pigmentation types listed in Table 1 were not significantly different between the groups. Birthcoat halo-hair is only temporarily visible on lambs and some fibre shedding can occur before marking (Dry 1975; Fleet, unpublished data) so elimination of this type of fibre pigmentation would also require inspection of newborn lambs.

There were no significant differences ($P<0.05$) in the means for production traits between the affected and unaffected groups as determined with culling criterion 4. The same outcome occurred for the other three culling criteria considered by Fleet *et al.* (1995b). It is concluded that selection against fibre pigmentation (as described by Fleet *et al.* 1995b) would have little effect on the average production of the existing flock. This conclusion reflects the finding of only low phenotypic correlations between pigmentation and production traits (Fleet and Mortimer, unpublished data). However, such selection can impact on progress that can be made toward improving production traits (through reduction of the selection differentials) and there may, possibly, be adverse genetic trends between pigmentation and production traits based on some preliminary estimates of genetic correlations (Fleet and Mortimer, unpublished data). The likely effects of selection against pigmentation on the production of future generations requires clarification (Fleet 1996).

It appears that average performance of hogget ewes in fleece weight, body weight and average fibre diameter of the existing generation will not be altered by culling visible fibre pigmentation to minimise the risk of isolated pigmented wool fibres. However, such culling may reduce to varying degrees other pigmentation (e.g. skin and hooves) not considered in the culling criterion.

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