# OBSERVATIONS ON WHITE SPOTTING IN A DAMARA x MERINO FAMILY

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## SUMMARY
Most sheep breeds in Australia have white coats or when crossed with white Merinos produce progeny with coats that are extensively white. In 1996, the coloured Damara fat-tail breed was introduced and some Merino producers commenced crossbreeding as a diversification from wool production. As part of a trial assessing wool contamination, the opportunity arose to observe white spotting in the progeny of Damara rams mated to Merino ewes. The black Damara ram proved to be heterozygous for characterised genetic variation within the *Extension* locus that encodes a constitutively activated melanocortin-1 receptor responsible for dominant black coat (i.e. $E^D/E^+$). In this paper we show the genetic changes responsible for dominant black also interact to produce greater white spotting on the coats of the tan coloured progeny than on the black lambs. This interaction is discussed in relation to known gene effects in other mammals that could contribute to a better understanding of white coat in sheep.
**Keywords**: White spotting, agouti, melanocortin-1, microphthalmia, sheep

## INTRODUCTION
It is generally assumed that white sheep have evolved from coloured individuals and existing white breeds are the product of human intervention over several thousands of years to enhance the areas of white spotting over the coat (Ryder 1987). White sheep can be considered to have a pre-disposition for phaeomelanin (yellow-red pigment), determined by the allele for white or tan ($A^{Wt}$) of the *Agouti* gene (Parsons *et al.* 1999). In Merino sheep, white spotting genes largely prevent the location of pigment cells (melanocytes) to wool follicles (Fleet *et al.* 2004) and similarly impact on hair follicles and skin areas. White spotting is sometimes associated with reduced reproduction rate or viability (e.g. Nel 1967; Adalsteinsson 1970; Silvers 1979; Malher and Le Chere 1998).

The *Agouti (A)* gene encodes a signal protein (ASP) that interacts with the melanocortin-1 receptor (MC1R), blocking its stimulation by alpha-melanocyte stimulating hormone (α-MSH) and changing *MC1R (or E)* expression (Rouzaud *et al.* 2003). The outcome of the ASP/MC1R interaction is reduced signalling for eumelanin (black-brown pigment) and increased phaeomelanin (tan-red pigment) production (Lu *et al.* 1994). When ASP production is absent, not functional or switched off, then MSH can freely bind with MC1R and the eumelanin pathway can be fully engaged in producing dark pigment. Alternatively, when *MC1R* is changed, so that it continually signals for dark (eumelanin) pigment production, independent of the influences of ASP and MSH, then a dominant black phenotype is produced (Våge *et al.* 1999; Våge *et al.* 2003).

In 1996, the fleece shedding Damara breed was introduced to Australia and some Merino producers commenced crossbreeding as a diversification from wool production. The Damara breed has no restricting standard on coat colouration so a wide range of colours and patterns exist (Du Toit 1995). In this paper we show that the degree of white spotting in a Damara x Merino family is highly influenced by *MC1R* genotypes encoding dominant black ($E^D$) or wild type ($E^+$) colouration.

**MATERIALS AND METHODS**

The animals are from a study that investigated the level of wool contamination arising when Merino ewes rear crossbred Damara lambs and was conducted at Minnipa Agricultural Centre in 1999 (Fleet *et al.* 2001). A black Damara ram was mated to 65 Merino ewes. Lamb-mother relationship was recorded on a daily basis when the lambs were ear tagged, body weighed and scored for white spotting. The ram produced similar numbers of black and white or tan and white progeny. A dominant black segregation pattern in sheep had just been characterised at the molecular level in Norway (Våge *et al.* 1999). A collaboration was established to determine whether the dominant black arising from the primitive hair-fleece Damara breed was conserved in the wool-meat Norwegian Dala breed and to map the location of the *Extension* (*MC1R*) gene using the large Damara x Merino family (Våge *et al.* 2003).

All lambs were assessed for the degree of white spotting and the coat colour(s) were recorded: score 1, completely white; 2, 0.1 – 3% coloured; 3, 4 – 10%; 4, 11 – 25%; 5, 26 – 50%; 6, 51 – 75%; 7, 76 – 90%; 8, 91 – 99%; and 9, no white fibre spots evident. The chi-square statistic was used to assess proportions of white spotting scores in black v. tan progeny (scores 2 – 5 v. scores 6 – 8). Birth weight was analysed by least squares analysis of variance (SAS 2002) including the main effects of birth type (single or multiple born), sex (male or female), coat colour of the progeny (tan and white v. black and white); day of birth fitted as a covariate; interactions between birth type and sex, birth type and coat colour, sex and coat colour; and the random residual error.

**RESULTS AND DISCUSSION**

While white spotting can be associated with reduced reproduction and lamb viability there was no evidence of such effects based on the frequency of lamb colours and birth weights. The mean birth weight of black and white lambs was 4.5kg v. tan and white lambs was 4.7kg ($P = 0.100$). A ratio of 1:1 for black v. tan lambs was expected from a heterozygous ram with black colour being dominant. While more tan than black lambs were born (50 v. 43) the difference was not significant (chi-square$_1$= 0.53; $P>0.05$.) which is consistent with expectation.

Crossing of Merino sheep to a divergent coloured breed reveals the effects of white spotting genes that would otherwise be largely hidden. The black Damara ram presented a differential effect of white spotting on his black v. tan progeny; black progeny had distinctly less white area (Table 1). Comparing scores 2 – 5 v. 6 – 8 for the two colours (i.e. 45 v. 5 for tan and 3 v. 40 for black) produced a chi-square$_1$ = 63.8 ($P<0.001$). The distribution of white spotting among the black lambs was concentrated at score 8, but also a small peak appeared at score 6. The white and tan lambs had scores skewed from the low end of the range. This difference can be compared with recessive black in Merino flocks where expression of tan is more restricted.

**Table 1. White spotting on the progeny**

| Lamb | | Colour score (1 = all white to 9 = solid coloured) | | | | | | | | | No. | Mean |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Colour | Genotype | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | lambs | Score |
| Tan & white | $A^{Wt}/- // E^{+}/E^{+}$ | | 24 | 16 | 5 | 1 | 1 | 1 | 2 | | 50 | 3.0 |
| Black & white | $A^{Wt}/- // E^{D}/E^{+}$ | | | | 1 | 2 | 8 | 3 | 29 | | 43 | 7.2 |

Lauvergne (1975) studied crosses between three French breeds: Solognot (all red at birth); Berrichon (white) and Bizet (dominant black with white blaze, tail end and socks). He suggested that the white spotting evident in these crosses, are usually due to the action of a single gene ($S^{b}$). On a black background, this gene gives a pattern called white spots (white blaze, extremities of legs and tail). On a red background it produces irregular piebaldness with some being white. Moreover, this gene is dominant with incomplete penetrance (50 – 100%) in the heterozygote on a black background while, on a red background it gives irregular piebaldness (with total or sub-total penetrance) in the heterozygotes and almost always total-white colouring in the homozygote. Fleet (1994) provided evidence of the effects of a white spotting gene in the Merino breed that prevents the occurrence of pigmented fibres on the legs and head as well as generally reduce other remnant fibre, skin, hoof and horn pigmentation (Fleet *et al.* 1995).

Why pigment development was less on a tan background than on a black background in these sheep is uncertain. Lamoreux and Russell (1979) found moderately less white spotting occurred in various black mice than in yellow mice genotypes. Woolf (1995) found white leg markings to be more extensive in chesnut (*e/e*) horses (cited by Kijas *et al*. 2001 who characterised *E*-locus variation responsible for black spots in the pig). Based on the current observations in sheep it appears that the MC1R receptor is interacting with other genes to influence the amount of white spotting. Dominant black in sheep involves constitutive activation of the MC1R (Våge *et al.* 1999, 2003), so that signalling for eumelanin production is fully engaged independent of MSH and ASP.

Melanocytes respond to MC1R-signalling from α-MSH by increasing the levels of intracellular adenylate cyclase, producing adenosine monophosphate (cAMP), and protein kinase A; which activate melanogenic enzymes (Abdel-malek *et al.* 2000; Rouzaud *et al.* 2003). Transcription factor microphthalmia (MITF) is produced as a melanocyte specific response to cAMP signal. Elevated MITF protein levels are required for activation of genes encoding tyrosinase (TYR), tyrosinase-related protein-1 (TYRP1) and DOPAchrome tautomerase (DCT or TYRP2); enzymes involved in the synthesis of melanin pigments (Bertolotto *et al.* 1998 a,b; Gaggioli *et al.* 2003; Steingrimsson *et al.* 2004). *MITF* is detected in the neural crest during embryo development (Opdecamp *et al.* 1997), has a key role in the early development of the melanocyte lineage and is implicated in the *KITLG* and *KIT* pathway that can produce similar white spotting (Silvers 1979; Fisher 2000; Steingrimsson *et al.* 2004). Using a cell culture model, Aberdam *et al.* (1998) found that both α-MSH and forskolin (plant extract that stimulates cAMP production) promoted differentiation of melanocytes from melanoblasts, while ASP inhibits this process. In dominant black animals with a constitutive active MC1R there is a generally a high intracellular level of cAMP; in spite of the presence of ASP. The high cAMP-level enhances the production of transcription factor MITF. The increased level of MITF may partially explain the wider colonisation of the skin by melanocytes during foetal development in black v. tan lambs. Other

epidermal factors (e.g. endothelin-1) that can regulate melanocyte proliferation and melanogenesis have also been found to up-regulate *MC1R* expression (Abdel-malek *et al.* 2000). It appears that *MC1R* and *MITF* are key mediators in the prevention of pigmentation in white sheep.

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