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Scrapie-resistant sheep show certain coat colour characteristics

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Summary

Susceptibility to scrapie is known to be associated with polymorphisms at the prion protein (PrP) gene, and this association is the basis of current selective programmes implemented to control scrapie in many countries. However, these programmes might have unintended consequences for other traits that might be associated with PrP genotype. The objective of this study was to investigate the relationship between PrP genotype and coat colour characteristics in two UK native sheep breeds valued for their distinctive coat colour patterns. Coat colour pattern, darkness and spotting and PrP genotype records were available for 11,674 Badgerfaced Welsh Mountain and 2,338 Shetland sheep. The data were analysed with a log–linear model using maximum likelihood. Results showed a strong significant association of PrP genotype with coat colour pattern in Badgerfaced Welsh Mountain and Shetland sheep and with the presence of white spotting in Shetland sheep. Animals with the ARR/ARR genotype (the most scrapie resistant) had higher odds of having a light dorsum and a dark abdomen than the reverse pattern. The implication of these associations is that selection to increase resistance to scrapie based only on PrP genotype could result in change in morphological diversity and affect other associated traits such as fitness.

1. Introduction

Scrapie is the sheep and goat form of transmissible spongiform encephalopathy. Susceptibility to classical scrapie has been shown to be associated with five alleles of the PrP gene (ARR, AHQ, ARH, ARQ and VRQ) that result from polymorphisms at codons 136, 154 and 171 (Goldmann et al., 1994; Hunter et al., 1996). While the ranking of these alleles with respect to scrapie susceptibility varies among breeds, the ARR allele is generally considered as the most resistant and the VRQ allele as the most susceptible (Belt et al., 1995; Baylis et al., 2004). Scrapie control programmes are being implemented in many countries based on selective breeding to eradicate those PrP alleles associated with scrapie susceptibility (Arnold et al., 2002). These programmes have increased the frequency of the ARR allele and reduced the frequency of the VRQ allele (e.g. Warner et al., 2006), but there is a risk that such selection may have negative consequences on other valuable sheep characteristics if the PrP gene has pleiotropic effects or is linked to genes affecting these characteristics.

Sweeney & Hanrahan (2008) reviewed recently published studies of associations between PrP gene and commonly recorded performance traits in sheep and found no convincing evidence of associations between PrP gene and most performance traits. However, based on quantitative trait locus (QTL) and gene mapping studies, they suggested that possible associations with coat colour, health and survival traits are highly likely. Furthermore, some native UK sheep breeders have claimed that the most resistant PrP genotype (ARR/ARR) tends to be more frequent in sheep with certain coat colour patterns (Bell et al., 2005). Although the genetic basis of these putative
associations is unknown, one of the major genes controlling coat colour pattern, the *Agouti* gene, is located on the same chromosome as the *PrP* gene (Parsons *et al.*, 1999b; Beraldi *et al.*, 2006) such that linkage disequilibrium between *Agouti* and *PrP* is possible.

Coat characteristics such as colour and pattern are linked to fitness in wild mammalian populations (Moorcroft *et al.*, 1996; Hoekstra & Nachman, 2003; Gratten *et al.*, 2008), and are also of economic importance in wool-producing sheep breeds (Reni *et al.*, 2008). Also, coat characteristics constitute a major factor in breed characterization of native sheep breeds (Klungland & Våg, 2003; Beraldi *et al.*, 2006). In fact, it is precisely the phenotypic diversity found within these breeds that makes them of interest to breeders and conservationists. Consequently, any loss of diversity associated with selection on *PrP* genotype could have serious implications for their future existence. Here, the objective is to investigate whether selective breeding for scrapie eradication purposes could lead to a change in phenotypic diversity for coat characteristics in two native UK sheep breeds. To do this, associations between *PrP* genotypes and phenotypes of coat colour pattern, colour darkness and spotting were tested.

2. Materials and methods

Coat characteristics (colour pattern, colour darkness and spotting) and *PrP* genotype data were obtained for animals of two native breeds of sheep (Badgerfaced Welsh Mountain and Shetland), raised on commercial farms. Information on the sex, age and farm was also available. Badgerfaced Welsh Mountain animals have two colour patterns named the ‘torddu’ (light dorsal and dark abdominal areas) and the ‘torwen’ (dark dorsal and light abdominal areas) as shown in Fig. 1a and b.

Shetland sheep are more phenotypically diverse, based on coat characteristics. Animals with white, grey, ‘katmoget’, ‘gulmoget’, dark brown, light brown, black and spotted coats were all present in the data. The katmoget and gulmoget patterns (Fig. 1c and d) correspond, respectively, to the torddu and the torwen patterns of Badgerfaced Welsh Mountain sheep. Spotting is defined as having white spots on a coloured background coat.
Coat pattern is under combined non-epistatic control of both the *Agouti* and the *Extension* genes (Vâgé *et al.,* 2003). Coat colour darkness (dark versus light) has been shown to be under the control of the *Brown* gene (Beraldi *et al.,* 2006). Spotting is believed to be controlled by the *Spotting* gene (Renieri *et al.,* 2008). Shetland sheep were thus classified based on the currently known underlying inheritance of coat colour variations into (i) five categories for coat colour pattern (white, grey, katmoget, gulmoget and solid); (ii) two categories for coat colour darkness classes (dark brown and light brown); and (iii) two categories for white spotting (spotted and non-spotted). The category ‘solid’ for coat colour pattern included animals with light brown, dark brown and black coats. Animals with spotted coats were excluded from coat colour pattern classification. The category ‘non-spotted’ included animals with grey, katmoget, gulmoget, dark brown, light brown and black coats. Animals with white coat colour were not scored for spotting since white spots cannot be observed in white animals.

The farmers obtained the *PrP* genotype of the animals in the dataset through participation in the Ram Genotyping Scheme of the National Scrape Plan for Great Britain, in the Rare Breeds Survival Trust Genotype Survey (UK) or through private initiatives. In all cases, the *PrP* genotype was determined by genotyping polymorphisms at three codons (136, 154 and 171) of the *PrP* gene, which give rise to up to five possible *PrP* alleles (ARR, AHQ, ARH, ARQ and VRQ). Genotypes with low frequency were excluded from the analysis. The dataset for analysis included 11 674 and 2338 records for Badgerfaced Welsh Mountain and Shetland sheep, respectively. Tables 1 and 2 show the distribution of the animals by *PrP* genotype and coat characteristics for Badgerfaced Welsh Mountain and Shetland sheep, respectively.

Associations between coat characteristics and *PrP* genotype were tested using a log-linear model for unordered categorical variables (Agresti, 1996) by the method of maximum likelihood using SAS/Logistic Regression (SAS Institute Inc., Cary, NC). The dependent variable was coat colour pattern in Badgerfaced Welsh Mountain (torddu or torwen). For the Shetland sheep, three separate analyses were carried out classifying the animals based on coat colour pattern, coat colour darkness and spotting as described above.

The *PrP* genotype was fitted in the model as a class variable to test its association with coat traits. The model adjusted for possible effects of sex (two levels: male or female) to account for the possibility that coat characteristics are sex-linked traits. The model also included age of the animal (three levels: 1, 2 or 3 years or over) as some coat colours have been shown to change with age (Renieri *et al.,* 1989). Neither the

| Genotype     | Coat colour pattern<sup>a</sup> | Torddu | Torwen |
|--------------|---------------------------------|--------|--------|
| ARR/ARR      | 3088 (2726.10)                  | 405 (766.88) |
| ARR/AHQ      | 1460 (1382.20)                  | 311 (388.82) |
| ARR/ARQ      | 2499 (2520.90)                  | 731 (709.14) |
| ARR/VRQ      | 334 (397.25)                    | 175 (111.75) |
| AHQ/AHQ      | 213 (211.50)                    | 58 (59.49)  |
| AHQ/ARQ      | 638 (710.99)                    | 273 (200.01) |
| AHQ/VRQ      | 82 (119.41)                     | 71 (33.59)  |
| ARQ/ARQ      | 636 (771.09)                    | 352 (216.91) |
| ARQ/VRQ      | 143 (233.36)                    | 156 (65.64) |
| VRQ/VRQ      | 18 (38.24)                      | 31 (10.75)  |
| Total        | 9111                            | 2563    |

<sup>a</sup> Expected numbers were calculated assuming no association between *PrP* genotypes and different coat colours classes.
<sup>b</sup> Torddu: light colour on the dorsal and dark on the abdominal areas; Torwen: dark colour on the dorsal and light on the abdominal areas.

In Badgerfaced Welsh Mountain, there was a highly significant association (*P* < 0.0001) of *PrP* genotype with coat colour pattern. Figure 2 shows the odds ratios comparing ARR/ARR with the other *PrP* genotypes for the occurrence of torddu relative to torwen coat pattern. Animals with the ARR/ARR genotype had higher odds of having the torddu coat pattern than animals with the other *PrP* genotypes. In other words, animals with genotypes other than ARR/ARR were more likely to be torwen rather than torddu. Notably, the largest odds ratio was obtained when comparing ARR/ARR with VRQ/VRQ genotype. Animals with ARR/ARR genotype had 15 times higher odds of having torddu coat pattern compared with animals with VRQ/VRQ genotype.

Figure 2 also shows a clear multiplicative allelic effect on the odds ratio comparing the occurrence of torddu with torwen coat patterns. The largest odds
ratios were when comparing ARR with VRQ carriers followed by ARQ carriers and then AHQ carriers (increase in the magnitude of odds ratios with the increase in susceptibility to scrapie). Substituting either an AHQ or an ARQ allele for an ARR allele in a particular genotype roughly doubles the odds of occurrence of torddu coat colour pattern. Similarly, substituting a VRQ for an ARR allele in a particular genotype increases the odds of occurrence of torddu coat colour pattern by a factor of 4. All the other effects can be obtained as appropriate products of these three allelic effects.

Based on the overall genotype and colour pattern frequency in Badgerfaced Welsh Mountain sheep and assuming no association between the two variables, the expected number of animals with the ARR/ARR genotype and torddu coat pattern is about 2726. However, 362 more animals than expected were found in this category (Table 1). Similarly, 767 animals are expected to have a torwen coat and ARR/ARR genotype, though only 405 were actually present in this category. For animals with the VRQ/VRQ genotype, considerably more torwen and less torddu animals than expected were found.

### Table 2. Distribution of Shetland animals (and expected numbers) by PrP genotype and coat colour and pattern

| Genotype       | Colour pattern | PrP genotype | Colour darkness | Coat spotting |
|----------------|----------------|--------------|-----------------|--------------|
|                | White          | Grey         | Katmoget        | Gulmoget     | Solid        | Dark brown | Light brown | Spotted | Non-spotted |
| ARR/ARR        | 55 (60-29)     | 24 (24-99)   | 169 (105-90)    | 9 (23-00)    | 143 (185-82) | 84 (95-15) | 20 (17-99)  | 67 (65-02) | 345 (346-98) |
| ARR/AHQ        | 23 (16-43)     | 5 (6-81)     | 16 (28-86)      | 9 (6-27)     | 56 (50-64)   | 36 (33-01) | 9 (7-78)    | 46 (20-83) | 86 (111-17) |
| ARR/ARQ        | 116 (117-41)   | 53 (48-66)   | 215 (206-24)    | 32 (44-80)   | 363 (361-89) | 208 (209-07) | 39 (42-73) | 98 (120-10) | 663 (640-90) |
| AHQ/ARQ        | 27 (27-13)     | 6 (11-24)    | 23 (47-66)      | 35 (10-35)   | 89 (83-62)   | 56 (49-84) | 10 (11-42)  | 49 (31-88) | 153 (170-12) |
| ARQ/ARQ        | 69 (70-99)     | 32 (29-42)   | 96 (124-70)     | 27 (27-09)   | 247 (218-80) | 144 (137-22) | 29 (29-93) | 52 (71-65) | 402 (382-35) |
| ARQ/VRQ        | 14 (11-76)     | 6 (4-873)    | 15 (20-65)      | 4 (4-49)     | 39 (36-24)   | 17 (20-71) | 7 (4-15)    | 9 (11-52) | 64 (61-48) |
| Total          | 304            | 126          | 534             | 116          | 937          | 545        | 114         | 321        | 1713        |

^a Expected numbers were calculated assuming no association between PrP genotypes and different coat colours classes.

^b Katmoget: light colour on the dorsal and dark on the abdominal areas; gulmoget: dark colour on the dorsal and light on the abdominal areas; spotted: animals with white spots on a coloured coat background. Solid: included animals with light brown, dark brown and black coats. Non-spotted: included animals with grey, katmoget, gulmoget, dark brown, light brown and black coats.

![Fig. 2. Odds ratios (and 95% confidence limits) of the occurrence of relative to torwen coat patterns comparing genotype with the other PrP genotypes in Badgerfaced Welsh Mountain sheep. Odds ratios with ‘*’ are statistically significant (P < 0.05) from the baseline of 1. An odds ratio greater than 1 indicates that the occurrence of torddu is more likely than torwen for ARR/ARR animals compared with animals with the other PrP genotypes.](image-url)
The first analysis of association of PrP genotype with coat pattern in Shetland sheep (classifying the animals based on Agouti and Extension gene inheritance) was highly significant ($P < 0.0001$). Figure 3 shows the odds ratios comparing ARR/ARR with the other PrP genotypes for the occurrence of katmoget relative to gulmoget coat pattern. Animals with the ARR/ARR genotype had higher odds of having katmoget coat pattern than animals with any other genotype. The largest odds ratio was obtained when comparing ARR/ARR with AHQ/ARQ animals. Other odds ratios comparing the occurrence of other different coat colour patterns are not presented.

There was also a clear multiplicative allelic effect on the odds ratio comparing the substitution of different PrP alleles by ARR for the occurrence of katmoget relative to gulmoget coat colour pattern in Shetland sheep. Figure 3 shows that substituting an AHQ, an ARQ or a VRQ for an ARR allele in a particular genotype increases the odds of occurrence of katmoget by 10, 3 and 2 times, respectively. As an example, substituting ARR/ARR for AHQ/ARQ increases the odds of the occurrence of katmoget by 30 times and that corresponds with a multiplicative allelic effects on the odds ratios (i.e. 10 for AHQ times 3 for ARQ allele substitution).

The expected number of animals with ARR/ARR genotype and katmoget coat colour pattern is 106, assuming no association between the PrP genotype and coat colour pattern (Table 2). However, there were 169 animals in the dataset that belonged to this category (i.e. 63 animals more than expected). Conversely, for gulmoget the expected number of animals in the ARR/ARR category exceeds the observed number (i.e. animals with ARR/ARR genotype are less likely to have gulmoget coat colour pattern).

There was no significant association ($P=0.11$) of PrP genotype and coat colour darkness in Shetland sheep as determined by Brown gene inheritance (analysis 2). The occurrence of dark versus light coat colour did not differ for different PrP genotypes. The analysis of association of PrP genotype with coat spotting in Shetland sheep (analysis 3) revealed a significant association ($P < 0.0001$). Figure 4 shows the odds ratio of having non-spotted versus spotted coats comparing ARR/ARR with the other PrP genotypes. Animals with the ARR/ARR genotype had significantly higher odds (1.63–2.50) of having a non-spotted coat than animals with the ARR/AHQ or AHQ/ARQ genotypes.

4. Discussion

In this study, PrP genotype was found to be significantly associated with coat colour pattern in Badgerfaced Welsh Mountain and Shetland sheep. In both breeds, ARR/ARR animals were found to have higher odds of having light dorsum and dark abdomen than the reverse pattern. There was also a significant association of PrP genotype and coat spotting in Shetland sheep. No association was detected between PrP genotype and coat colour darkness in Shetland. Several PrP genotypes were represented in the data and the animals were distributed among different PrP genotypes and colour classifications with no single-predominant or rare-class (i.e. several cross-classifications by PrP genotype and coat class were represented in the data). Results (not presented) from an extra analysis of Soay sheep also showed no association of PrP genotype coat colour darkness. However, the Soay dataset was too small (137 animals) to reach a reliable conclusion.
It is very unlikely that the significant associations found in this study are due to a disproportionate genetic contribution from one or a few ancestors. For example, Shetland sheep records originated from 90 flocks (mostly small ranging from 1 to 50 records per flock, except for 6 larger flocks). Different colour patterns were present in each flock and no significant effect of farm was found (e.g. katmoget and gulmoget animals were present in 61 and 12 flocks, respectively). The possible interaction effect of PrP genotype with farm was tested and the results were not significant. This may help to exclude the possibility that the observed association is due to population or mating structure.

The statistical model used accounted for the possibility that coat colour characteristics are sex-linked or associated with age (which can be due to genetic selection). Sex did not show significant association with any of the traits studied, indicating that coat colour characteristics are not sex-linked. Age of the animal, however, showed a significant association with coat colour pattern in both Badgerfaced Welsh Mountain and Shetland sheep. Age was also significantly associated with coat spotting in Shetland sheep. Older animals tended to have higher odds of having torddu or katmoget colour patterns and non-spotted coats than torwen or gulmoget and spotted coats compared with younger animals. There is no apparent economic incentive for selecting animals with torddu or katmoget coat patterns as this will have no effect on the value of the animal or its fleece. Therefore, the significant association of coat colour pattern with age is likely to be due to the ongoing selection based on PrP genotype as many of these flocks were part of the GB national scrapie eradication plan. In other words, these results support the association found in this study between PrP genotype and coat colour pattern and spotting (as more older animals were found to be torddu or katmoget with no spotting compared with younger ones) and these same patterns and lack of spotting were found to be more common in ARR carrier animals. No significant association was found between coat colour darkness and PrP genotype in Shetland sheep and this was supported by the lack of association of coat colour darkness and age of the animal.

Results from coat colour gene mapping studies support the association found between PrP genotype and coat colour pattern found here. Coat colour pattern in sheep has been shown to be controlled by the Extension gene located on chromosome 14 and the Agouti gene located on chromosome 13, where the PrP gene is located (Parsons et al., 1999a; Våge et al., 1999, 2003). This may suggest that the association found between PrP gene and coat colour pattern might be due to linkage disequilibrium between PrP and the Agouti genes. In a different breed (Scottish Blackface), an association was also found between PrP genotype and percentage of grey fibres in the fleece (R. M. Sawalha, J. Conington, S. Brotherstone and B. Villanueva, unpublished results). It can be estimated that the distance between PrP and Agouti genes is less than 15 cM (A. Clop, personal communication), using (i) the version SM4.7 of the sheep linkage map; (ii) the sheep linkage map of Lühken et al. (2006) that shows the linkage between microsatellites Huj616, URB58 and CTSBJ12 and PrP gene; and (iii) the virtual sheep genome database.
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(http://www.livestockgenomics.csiro.au/vsheep/) that indicates that microsatellite LS28 is only few kb apart from the Agouti gene. Significant linkage disequilibrium has been found between microsatellite markers separated by more than 60 cM apart in sheep populations (McRae et al., 2002). However, these results have to be taken with caution as the sheep genome map is still poorly constructed.

While the results in the current study clearly show an association of PrP genotype with coat characteristics, it is also possible that current selection practices may affect animal health and performance. For example, in feral Soay sheep, dark coat colour was found to be genetically associated with heavier body weight but also with reduced fitness compared with light coloured coats (Gratten et al., 2008). However, in this particular case, the causal mutation for coat colour has been identified in TYRP1 on chromosome 2 (Gratten et al., 2007). In Scottish Blackface sheep, an association of PrP genotype with body weight and body seasonal tissue mobilization of ewes has been found (Sawalha et al., 2008).

The Agouti gene is associated with adult-onset obesity syndrome, insulin resistance and tumour susceptibility in mice (Yen et al., 1994; Voisey & van Daal, 2002). Although no data were available to test for such effects here, these studies suggest a very real possibility that scrapie eradication programmes based on PrP genotypes might affect not only the diversity of coat characteristics, but also a suite of other traits. The large discrepancy between the observed and expected frequencies of animals with different coat colour patterns may suggests that coat colour pattern and/or PrP genotype is associated with fitness, but no data on animal fitness were available to test this hypothesis. In a previous study, PrP genotype was found to be associated with post-natal lamb mortality in Scottish Blackface sheep (Sawalha et al., 2007). They found that the ARQ allele is more closely associated with higher lamb survival rates than the more resistant ARR or AHQ alleles, currently favoured in scrapie eradication programmes.

The association of PrP genotype and coat colour pattern is particularly important and might have substantial consequences for coat colour diversity as coat colour pattern was associated with the degree of scrapie resistance. For example, in Badgerfaced Welsh Mountain sheep the largest difference in colour pattern distribution was between the most scrapie susceptible genotype (VRQ/VRQ) and the most resistant one (ARR/ARR). It is not possible to infer the underlying coat colour pattern genotypes based on the phenotypic data alone as coat colour pattern is controlled by the Agouti and the Extension genes with multiple alleles involved (Våge et al., 2003). It is possible that the PrP genotype is actually associated with increased heterozygosity of coat colour pattern genotypes and selecting on such genotypes would have no adverse effect on coat colour diversity.

There was also a significant association between PrP genotype and the presence of spotting in Shetland sheep although the magnitude of odds ratios was smaller than that for association of PrP genotype with coat colour pattern. The presence of white spots on a coloured background has been shown to be due to a recessive allele of the Spotting gene (Renieri et al., 2008), but the gene has not been mapped yet in sheep. The association of PrP genotype with coat spotting could indicate that the spotting gene is located on chromosome 13. There was no significant association between PrP genotypes and coat colour darkness in Shetland (or Soay) sheep. Coat colour darkness inheritance has been shown to be under the influence of the Brown gene, which has been mapped to chromosome 2 (Beraldi et al., 2006).

Historically, conservation of domestic animals has received less attention than conservation of wild animals even though domestic species represent a unique genetic resource that once lost cannot be recreated (Jewell, 1971). Rather than being the outcome of evolutionary forces, current sheep breeds are largely the result of artificial selection practices. Native and traditional domestic breeds (such as Shetland and Soay breeds) usually represent an early stage of domestic evolution where they have been selected under widely different environments and so may have acquired characteristics as unique as those under natural selection. Local breeds, particularly those living in isolation in remote areas, are considered an evolutionary resource of particular importance for the study of adaptation to extreme conditions such as seaweed diets and specific parasites and restricted habitats (Ryder, 1976).

There are gene banks such as the National Scrapie Plan Semen Archive that are storing semen with the alleles being eliminated through scrapie eradication programmes (Roughsedge et al., 2006). These banks could be used to restore lost alleles if they were found to be associated with important traits such as coat colour. However, it is desirable that such alleles are not lost in the first place as it is not warranted that the gene banks contain all affected colour patterns. Our study shows that scrapie breeding programmes based on PrP genotype should consider the association with coat colour pattern and spotting in order to ensure sustainable maintenance of the valuable diversity in such traits.

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