AUSTRALIAN PIEBALD: INCOMPLETE PENETRANCE OR INCOMPLETE PICTURE?

L.D. Brash[1], J.H.J. van der Werf[1], Y. Li[2] and B.J. Norris[2]

Australian Sheep Industry Co-operative Research Centre
[1]University of New England, Armidale NSW 2351, Australia
[2]CSIRO Livestock Industries, 306 Carmody Road, St Lucia QLD 4067, Australia.

SUMMARY
Determining the mode of inheritance of the undesirable Australian Piebald condition of Merino sheep is highly relevant to both conventional breeding practice and to current attempts to identify the gene(s) responsible. Despite attracting the interest of animal breeders over decades, there is a scarcity of data and this study shows that the inheritance cannot be conclusively determined on this basis. Considering a range of possible inheritance scenarios will ensure that the gene discovery experiment is designed optimally.
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INTRODUCTION
There are many documented loci associated with pigmentation in sheep. The *Australian Piebald* locus (AsP) in Merino sheep is held responsible for a form of pigmented fleece spots (COGNOSAG 1996). This is characterised by one to several rounded spots of black to grey or chocolate to cinnamon brown covering a proportion of the fleece area ranging from 0.0003 to greater than 0.5 (Brooker and Dolling 1969). Location is apparently random but with a degree of familial resemblance. Australian Piebald creates the risk of contamination of white fleece wool with dark fibres and inflicts further economic losses associated with culling. Australian Piebald phenotypes occur throughout Australian Merino flocks at a low frequency and the condition is known to persist despite culling. This distinct form has not been recorded outside Australia.

Brooker and Dolling (1969) reported that in many respects the occurrence of Australian Piebald phenotypes conformed to the existence of a recessive allele at a single locus: piebald phenotypes occur at a low frequency from unpigmented parents; culling pigmented animals prior to breeding had little effect on occurrence; complex combinations of pigment spots with other forms of pigmentation can occur, showing that *Australian Piebald* alleles were segregating independently to alleles controlling symmetrical pigmentation patterns in Merinos.

However matings among piebald phenotypes resulted in both white and piebald offspring. This does not conform with the expectation of a simple recessive trait in which all offspring would display the recessive character. Brooker and Dolling (1969) recorded 56 progeny of piebald x piebald matings, 40 (0.71) white and 16 (0.29) piebald. These results led to the conclusion that Australian Piebald is controlled by a recessive gene with incomplete penetrance (Brooker and Dolling 1969.)

The Australian Sheep Industry Co-operative Research Centre has a major project with the aim of eliminating genetic causes of pigmentation. As part of this project a Merino pigmentation flock has

been established to produce fully pedigreed half-sibs including piebald phenotypes. These animals will provide the necessary resources for a whole genome scan to determine the location of gene(s) involved in Australian Piebald inheritance. This will be a substantial project in both the field and laboratory phases, so it is important to optimise the mating design to yield the most informative data.

The hypothesis of a recessive gene with incomplete penetrance provides one credible mode of inheritance, but there may also be alternative plausible explanations. The power of the experiment to detect gene(s) must be considered in relation to each mode of inheritance. In this paper a range of putative models of inheritance are tested for plausibility. Due to space constraints, only single-locus, two-allele models are considered here. Models that are found to be satisfactory will then be fully considered in stochastic simulation as a further part of the work.

METHODS

Various modes of Mendelian inheritance were proposed to explain the inheritance of piebald in Merinos. These models are described below while the genotype-phenotype relationship for each is given in Table 1. The alleles are denoted v, x with v dominant to x where relevant. The models were:

1. Simple dominant
2. Simple recessive
3. Recessive with incomplete (0.29) penetrance
4. Dominant with incomplete (0.07) penetrance in heterozygotes
5. Overdominance – trait is expressed only in heterozygotes
6. Overdominance with imprinting – trait is expressed only in the heterozygote and only when the allele is maternally (or paternally) inherited

Table 1. Schematic description of models 1-6 showing white (W) or piebald (P) phenotype resulting from each allelic combination.

Inherited allele	1. v	x	2. v	x	3. v	x
v	P	P	W	W	W	W
x	P	W	W	P	W	W/P

Inherited allele	4. w	x	5. w	x	6. w	x
v	W/P	W/P	W	P	W	P
x	W/P	W	P	W	W	W

Models were evaluated against four documented attributes of the piebald condition:

1. White x white matings can result in white and piebald offspring.
2. Piebald x piebald matings result in white and piebald offspring.
3. Feasibility of the 29% penetrance observed by Brooker and Dolling (1969).
4. Allele frequency is not seriously affected by phenotypic culling.

RESULTS

In Table 2, each model is assessed against the four test criteria. From these results, models 1 and 2 can be discounted for failing to allow for either phenotype to result from matings among like phenotypes.

Table 2. Assessment of inheritance models against known features of Piebald inheritance

Model	Progeny of white x white	Progeny of piebald x piebald	Expected "penetrance"*	Phenotypic half-life** (generation)
1	W	W, P	0.75 – 1.00	<1
2	W, P	P	1.00	7
3	W, P	W, P	0.29	13
4	W, P	W, P	0.29	8
5	W, P	W, P	0.50	<1
6	W, P	W, P	0.25	1

* Apparent penetrance as proportion of piebald progeny expected from piebald x piebald matings.
** Duration of phenotypic culling to reduce piebald incidence from 0.005 to 0.0025.

In Table 2 the expected "penetrance" is calculated as the outcome of piebald x piebald matings, given as the proportion of progeny displaying a piebald phenotype – if white offspring are not possible, a penetrance of 1.00 is given. In some cases the allele frequency is influential resulting in a range of expected penetrance. Note that wherever the expected penetrance is not simply 1.00, departures from the expectation could occur in field data due to sampling error.

Models 1 and 5 show a penetrance far greater than the value of 0.29 observed by Brooker and Dolling (1969). A Chi-squared test showed that the 0.29 observation would be a highly significant departure from an expectation of 0.50 ($p=0.0013$**) and from 0.75 ($p<<0.0001$***). For model 6 the expected penetrance of 0.25 is not significantly different from the observation of 0.29 ($p=0.54$ n. s.) For models 3 and 4 the degree of penetrance has been deliberately selected to comply with the observation, so the results cannot be interpreted as confirming the hypothesis.

In Table 2, the phenotypic half-life is also given. This is the generations of phenotypic culling required to halve the phenotypic incidence from 0.005 to 0.0025. This does not imply that the decline in allele frequency follows a decay curve, but is designed to assist understanding the influence of selection. Model 2 is of a simple recessive trait. We know that this cannot explain piebald inheritance, but it is the mode of inheritance of symmetrical pigmentation types in Merinos, which genes also persist in the population at low frequencies. So it is proposed that the allelic half-life under model two provides a benchmark figure useful for comparing the likely allele persistence with other models.

Models 1, 5 and 6 show a short phenotypic half-life that make them unlikely candidates for piebald inheritance. Models 3 and 4 show a half-life equal or greater to model 2, in keeping with the expectation.

DISCUSSION

Six inheritance models were tested and three models (1, 2 and 5) can be discounted as not satisfying the essential attributes of Australian Piebald. Models 3 and 4 show equal promise in meeting the four test criteria, but both rely on the notion of a random and unobservable penetrance. Model 6 fails the test of allele persistence under culling, but meets the other three requirements and may provide an explanatory model for piebald incidence. For this reason, investigation of more complex models involving imprinting and multiple loci appears to be worthwhile.

This study shows that there is more than one plausible model of inheritance for Australian Piebald. The inheritance should be regarded as an open question and therefore the design of matings in the pigmentation resource flock should ideally suit gene discovery independent of a particular putative model.

Differentiation. Models which meet the four test criteria will be difficult to verify in the field as the results of first-generation matings are virtually identical in each case. Genotyping may give some clues but the quantity of data is likely to be a limitation. However careful planning of second generation matings, subject to the availability of sufficient resources, has the potential to lead to a definitive resolution, according to several pieces of evidence: (a) the presence of imprinting should be revealed by a difference in incidence resulting from reciprocal white x piebald and piebald x white matings; (b) dominance with incomplete penetrance would be revealed by a higher incidence of piebald offspring from second generation piebald x piebald matings, due to the presence of homozygous piebald animals among the parents; and (c) multi-allelic or multi-locus models are likely to show substantial differences in penetrance between families.

Culling and genetic drift. The calculations of allelic half-life suggest that eliminating genetic causes of pigmentation from Merino flocks is an extremely slow and tedious task. Note that the decline in allele frequency may be accelerated by culling the "carrier" relatives of affected individuals, particularly if progeny testing were used. However while allele frequency can be predicted to decline slowly under selection, in reality the actual change in allele frequency will be overwhelmingly under the random influence of genetic drift. There is little prospect of removing pigmentation alleles from the population until a genetic test is available to detect unaffected carriers in the flock.

REFERENCE

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