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# Microsatellite-based genetic variation and differentiation of selected Australian Merino sheep flocks

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ABSTRACT

The genetic variation among three Australian Merino sheep populations was tested using a panel of 28 Microsatellite markers (MS). The first population, a private flock, represented a control population (CR) of self-replacing dams and imported superior fine wool sires. Two other populations were flocks selected for low parasite resistance (LR) and high parasite resistance (HR), respectively. Both flocks originated from the same initial population and had self-replacing breeding plans. The results indicated high genetic variation revealed by measuring the number of alleles and heterozygosity per locus. The high level of within-population genetic variation may have been the result of gene flow, as in the CR population, or breeding structure and selection, as in the LR and HR populations. The level of observed significant inter-population genetic differentiations was also high. The reasons behind the resulted differentiation may be the same as those responsible for within-population genetic variation. In addition, genetic distances confirmed the expected degree of genetic differentiation in that the LR and HR populations were determined to be in a tight population-specific cluster. Nevertheless, evolutionary forces have had an effect on the studied populations as the divergence time was sufficient to enable the formation of distinct genetic structures for all three populations.



## 1. Introduction

The Merino sheep (*Ovis aries*) is one of the oldest known sheep breeds. It has been reported that Merino sheep originated from fine-wool Spanish sheep of the thirteenth century (Piper and Ruvinsky, 1997). Australian Merino sheep are a result of intense selective breeding since their initial introduction to Australia (Massy, 2007). There are three major types of Merino recognized in Australia and known as Australian Merino: the fine-woolled type is kept primarily in the higher rainfall areas of Tasmania, and the tablelands of New South Wales (NSW) and Victoria; the strong-woolled type is kept in lower rainfall areas such as Western Australia; and the medium type, which is the primary type found in NSW, Victoria and Queensland (Banks and Brown, 2009).

Breeding for disease resistance in Merino sheep in Australia has attracted considerable research and development attention. Many reports therefore have been made between breed and within breed variations in resistance to infectious diseases in sheep. Some of these reports described genetic variation in sheep for parasite resistance and procedures for selecting lines of resistant animals for breeding purposes (Woolaston 1992). Several Merino selection lines have been established, some of them were selected for increased and decreased resistance to *H. contortus* along with an unselected line (Woolaston and Piper, 1996). So far, these lines have been under many investigations of immunity, nutrition and genetic studies. However, no available study describes the genetic variation of these lines using available DNA genetic markers, such as microsatellites (MS) after many generations of selection.

The selection processes are leading to a wide range of genetic differences between the breeds under selection in a comparison to non-selected breeds. The MS ability to detect polymorphisms is a unique opportunity to study loci that were under selection. On the other hand, the analysis of MS-based genetic variation is usually motivated by the desire to determine inbreeding levels and to identify the genetic relationship among selected populations. These genetic data can be then used to determine differentiation between subdivided populations, phylogenetic relationships using genetic distances and ultimately the underlying evolutionary his-

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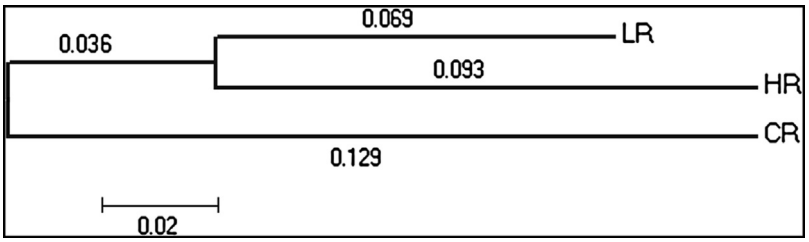


**Fig. 1.** Neighbor-joining phenogram of the three Merino sheep populations. (The numbers show the branch length in genetic distance unit).

tory. This research work aimed to determine genetic variation and differentiation among the selected Merino sheep populations.

## 2. Materials and methods

### 2.1. Sheep populations

Three different populations of Australian Merino sheep were investigated. A private farm flock represented the control population (CR). Dams were self-replacing and superior fine wool sires were purchased from other farms and breeding organisations. Two other populations were flocks maintained by the Commonwealth Scientific and Industrial Research Organization (CSIRO, Chiswick, NSW). One of these flocks was selected for low parasite resistance (LR) and the other for high parasite resistance (HR). Both flocks originated from the same initial population and have been totally separated from each other and other Merino sheep populations since 1976 (Woolaston 1992). The test was performed only on unrelated progeny of year 2000/2001. The number of sampled lambs was 98, 79 and 92 of CR. LR and HR populations, respectively. Tissue samples were taken from lamb ears. The sampling procedure was approved by University of New England Animal Ethics Committee (AEC approval No. 45-2000).

### 2.2. DNA extraction

The lamb tissue samples were digested overnight at 55 °C in 0.5 ml digestion buffer with 200 μg of proteinase K. Following digestion, genomic DNA was extracted from the tissue using phenol/chloroform extraction protocol (Sambrook et al., 1989). After extraction, the DNA pellet was dried for 30 minl in a 37 °C incubator, resuspended in 100 μL of TE buffer and then incubated at 55 °C for 5 min to aid solubilization. DNA samples were diluted to 10 ng/mL.

### 2.3. Microsatellite markers and genotyping

All studied lambs were genotyped for 28 microsatellite (MS) markers located on different chromosomes (Table 1). The lambs were chosen based on DNA-based parentage test performed in first place (Al-Atiyat Raed, 2015). All studied sheep were genotyped for 28 MS from the ovine, caprine and bovine genome located on different chromosomes (Table 1). The panel was designed, developed and used as a part of an automated progeny testing system used in sheep lineage analysis at the McMaster Laboratory-CSIRO, Prospect-Sydney, Australia (Franklin et al., 2000). The MS marker panels were grouped in four sets of fluorescent-labelled primers. In sets one to three, five primer pairs were used in each set for multiplex amplifications. Set four consisted of seven primer pairs. Forward and reverse primers in sets one to three were end-labelled with 6-carboxyfluorescein (6-FAM; blue), tetrachloro-6-carboxyfluorescein (TET; green), or hexachloro-6-carboxyfluorescein (HEX; yellow), respectively. In set four, primers were labelled with only one fluorescent color. The size standard GX-350-6-carboxytetramethylrhodamine (GX-350 TAMRA; red) was used. Each MS panel was used individually in four PCR reactions. PCR reactions of 10 μL were performed in 384-well microlitre PCR plates. The volumes and concentrations of PCR reagents used in the automated genotyping experiments were 3 μL of 10 ng/μL genomic DNA, 1 μL of 4 mM primer mix, 0.8 μL of 25 mM $MgCl_2$, 1 μL of 2 mM 4dNTP's, 1 μL of 10 × Taq polymerase buffer, 0.1 μL of 5 U/μL Taq polymerase and 3.1 μL of Sterile milliQdH$_2$O. Master mixes for each of four MST sets were prepared individually. Sample DNA was loaded into the wells of the PCR plate and then 7 μL of master mix was added. The plate was then placed onto a PTC-200 programmable Thermal Controller (MJ Research, Inc.) using the following cycling parameters: initial denaturation at 95 °C for 2 min, denaturation 94 °C for 45 s, annealing 57 °C for 45 s, extension 72 °C for 60 s, and final extension 72 °C for 7 min. The initial denaturation and final extension were performed for one cycle, whereas denaturation, annealing and extension were repeated for 30 cycles. The PCR products for panels one to three were co-loaded in each well and panel four was loaded in a separate well into the gel using an ABI's 373XL sequencer. The resulted data were extracted into an Excel sheet file, scored and made into the required format for each genetic analysis software.

### 2.4. Genetic Analysis

#### 2.4.1. Genetic variation within populations

Population genetics of the three studied populations was investigated. Heterozygosity, genetic differentiation and genetic distances parameters were analysed for three populations. Most of genetic parameters of the three populations were calculated using the ARLEQUIN (Schneider et al., 2000). The observed heterozygosity is calculated as $\hat{H}_O = \frac{\sum N_{ij}}{N}$, and unbiased expected heterozygosity at each locus was calculated as $\hat{H}_e = \frac{2N}{2N-1}(1 - \sum_{i=1}^{n} \hat{p}_i^{\,2})$ (Nei, 1987), where $N$ is the number of individuals in the sample, $N_{ij}$ is the number of observed heterozygotes, $n$ is the number of alleles and $p_i$ is the frequency of the $i$-th allele. On the other hand, detection of significant departure from Hardy Weinberg Equilibrium (HWE) was analysed using the procedure of Guo and Thompson (1992) which is implemented in ARLEQUIN software package. The calculation of HWE was performed on the basis of allele frequencies. When a population deviated from HWE, a score test was used to examine if the observations was caused by excess or deficit of heterozygous individual.

#### 2.4.2. Genetic population differentiation coefficients

Population differentiation coefficients ($F_{ST}$, $F_{IS}$ and $F_{IT}$) were calculated for studied sheep populations using ARLEQUIN software.

#### 2.4.3. Genetic distances and structure

Genetic distances between populations were measured using the most widely used measure of genetic distances proposed by Nei (1972) implemented in GDA software (Lewis and Zaykin 2001).