GENETIC DIVERSITY IN ALPACAS: CAN INBREEDING EXPLAIN THE HIGH PREVALENCE OF CONGENITAL DEFECTS?

F.C. Jackling[1], J.L. Vaughan[2], M.E. Goddard[3,4] and B.R. Appleton[1]

[1]Department of Genetics, The University of Melbourne, Parkville, VIC, Australia
[2]Cria Genesis, Ocean Grove, VIC, Australia
[3]BioSciences Research Division, Department of Primary Industries Victoria, 1 Park Drive, Bundoora 3083
[4]Faculty of Land and Food Resources, University of Melbourne, Parkville 3010

SUMMARY

Genome research has progressed rapidly in recent years and DNA-based selection tools are now available in a number of domesticated species. To date, advanced genomics technologies have not been developed in alpacas (*Vicugna pacos*). Therefore, breeders select for traits of economic importance (fleece phenotypes) using traditional techniques such as line breeding. Alpacas have experienced a history of population bottlenecks including the mass destruction of alpacas and llamas during the 16th Century, therefore traditional breeding may exacerbate an already depleted gene pool. Alpaca veterinarians report a prevalence of congenital defects much higher than any other livestock species. This study investigated levels of genetic diversity at genome-wide markers in Australian alpacas. Samples have been collected from unrelated individuals with normal and defective phenotypes including choanal atresia, polydactyly, cyclopia, syndactyly, vulval atresia and anal atresia. Multi-locus heterozygosity and inbreeding coefficients were estimated using microsatellite data from 53 or 22 loci. In addition, pedigrees were examined in order to detect pedigree inbreeding. Inbreeding coefficients estimated from genomic data reveal that individuals with congenital defects do not have significantly higher molecular inbreeding levels than healthy individuals. These results suggest that high levels of inbreeding cannot explain the high prevalence of congenital abnormalities in alpacas. This study is the first to report on the genetic variability of Australian alpacas and represents an important first step in the use of genomics to inform alpaca breeding practices.

INTRODUCTION

Modern breeding of alpacas involves the high use of a limited number of elite animals with desirable phenotypes, as is the case with many domesticated animals. The propagation of alleles associated with desirable fleece phenotypes may also propagate alleles associated with deleterious genetic disorders. Inherited diseases have been recognised in a number of livestock species (e.g. Windsor *et al*. 2009; Healy 1996), however the prevalence of congenital defects in alpacas is recognised by veterinarians to be much larger than other livestock species. The actual prevalence of congenital abnormalities is not known and would be difficult to determine as many breeders do not report the birth of an animal with a defect to parties interested in collating defect data such as veterinarians or breed societies.

Alpacas were first introduced into Australia in 1989. The exact number of animals imported to Australia is not known but is estimated to be at least 3000 animals. In 2011, there are more than 117,000 registered alpacas with many more unregistered. The importation of a limited number of alpacas into Australia may have represented a significant bottleneck that may have led to a reduced genetic pool. It is hypothesised that recent line breeding practices have exacerbated an already depleted gene pool and led to the increased prevalence of congenital defects. It is not known whether these disorders are of genetic or environmental origin and the inheritance pattern of these disorders is also not known. The aim of this study was to evaluate genome-wide levels of genetic

diversity in order to determine whether inbreeding is a likely cause of congenital abnormalities in alpacas.

MATERIALS AND METHODS

Blood and tissue samples were collected from healthy animals and animals with a range of congenital defects including choanal atresia, cyclopia, vulval atresia, heart murmur, hypoplastic ovaries, polydactyly, ear dysgenesis, cleft palate, fused toes, fused ears, wry face and anal atresia. DNA was extracted using an Axyprep blood genomics DNA miniprep kit according to manufacturer's instructions. Twenty-five individuals had congenital defects and another 25 were healthy animals. Individuals were judged to be unrelated by examination of pedigrees. Thirty-six individuals (16 normal, 20 defect) were genotyped at 22 loci and 14 individuals (7 normal, 7 defect) were genotyped at 53 loci. Microsatellite markers were identified by radiation hybrid mapping (W. Johnson, NIH). Genomic DNA (50-100ng) was used as template for PCR using 2 µM of forward primer, 2µM reverse primer, 1× Polymerisation buffer (Promega), 2mM $MgCl_2$ and 0.75u *Taq* polymerase (Promega) in a 10µL reaction. Microsatellite markers were amplified using a touch-down protocol as follows: denaturation at 95°C for 3 minutes, followed by ten cycles starting with 94°C for 30 sec, 60°C for 30 sec and 72°C for 1 min. The annealing temperature was decreased by 1°C in each cycle. These ten cycles were then followed by 30 cycles of 94°C for 30 sec, 50°C for 30 sec and 72°C for 1 min. A final extension step was performed at 72°C for 5 min. The PCR products were fluorescently labelled to allow genotyping (Schuelke 2000). The PCR products were then analysed on a 3730 capillary analyser (Applied Biosystems) by Macrogen, Korea and allele sizes scored using GeneMapper 4.0 software (Applied Biosystems).

Pedigree information was accessed through IAR (International Alpaca Registry) database on the Australian Alpaca Association website (http://www.alpaca.asn.au/). Pedigree inbreeding values (F_{PED}) were calculated using Wright's coefficient of relationships (Wright 1917, 1921). Multi-locus heterozygosity was calculated as the proportion of loci that were heterozygous. Inbreeding was estimated from marker information as follows; heterozygous genotypes (ij) were scored as -1 and homozygous genotypes (ii) were given a score of $1-p_i/p_i$, where p_i is the frequency of the allele for which the individual is homozygous. These values were summed across loci and then divided by $n_k - 1$, where n_k is the number of alleles at locus k. Allele frequencies were calculated using all 50 individuals. Student t-tests were used to test for significance differences between defect and healthy animals at genetic diversity measures (MLH and F_{GEN}).

RESULTS AND DISCUSSION

Pedigree inbreeding. Pedigree inbreeding was detected in only two out of 50 individuals, both of which had congenital defects (ear dysgenesis and cleft palate, $F_{PED} = 0.03125$ and polydactyly, $F_{PED} = 0.039$). All other individuals had a pedigree inbreeding coefficient of 0. These pedigree inbreeding coefficients could be greater than 0 due to likely ancestral relatedness in generations further back than available pedigree information. These values may be unrepresentative of true inbreeding as the pedigree relationships are only available since the importation of alpacas into Australia (3-5 generations). However, it has been shown that 4-5 generations are sufficient to accurately detect current inbreeding levels (Balloux *et al*. 2004).

Genome-wide estimates of inbreeding. Descriptive statistics of MLH and F_{GEN} values are provided in Table 1. The main expected effect of inbreeding is reduced heterozygosity, therefore multi-locus heterozygosity values were calculated for all individuals. Large variations in genome-wide heterozygosity were observed between individuals. This is in agreement with findings in other species that individuals with similar inbreeding coefficients have a wide range of

heterozygosity values (Pemberton 2004). Mean multi-locus heterozygosity did not differ significantly between individuals with defects ($\bar{x}$ = 0.685) and individuals without defects ($\bar{x}$ = 0.713) (p = 0.413).

Table 1. Descriptive statistics of multi-locus heterozygosity (MLH) and molecular inbreeding estimates (F_{GEN}) values for individuals with and without congenital abnormalities

	Number of samples	Range of MLH	Mean MLH	Standard deviation of MLH	Range of F_{GEN} values	Mean F_{GEN}	Standard deviation of F_{GEN}
Individuals with defects	25	0.500; 0.944	0.685	0.115	-0.110; 0.127	0.026	0.067
Healthy individuals	25	0.540; 0.894	0.713	0.088	-0.049; 0.136	0.012	0.063

Inbreeding estimates also did not differ significantly between individuals with ($\bar{x}$ = 0.026) and without congenital abnormalities ($\bar{x}$ = 0.012) (p = 0.459). Similarly to MLH values, F_{GEN} values varied to a large extent between individuals (see Figure 1). Inbreeding values calculated with 22 loci and 53 loci were highly correlated (r^2 = 0.973, n=14).

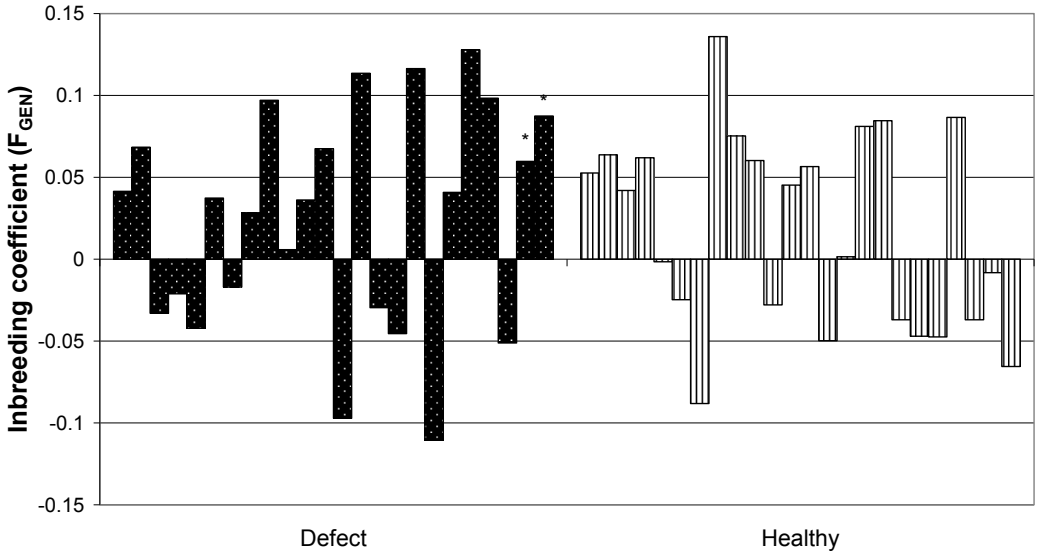

Figure 1. Inbreeding coefficients (F_{GEN}) as estimated from genome-wide microsatellite genotypes in individuals with and without congenital defects. Asterisks (*) highlight individuals with known pedigree inbreeding.

Only two individuals had detectable pedigree inbreeding however these were useful to determine levels of MLH and F_{GEN} that represent individuals with consanguineous pedigrees (see Table 2). Seventeen out of 50 individuals without detected pedigree inbreeding showed F_{GEN} values greater than the equivalent of an F_{PED} value of 0.3125 (F_{GEN} > 0.060). These individuals are hypothesised to have cryptic inbreeding in ancestral generations. This cryptic inbreeding however

does not appear to account for the prevalence of defects in the alpaca population as cryptic inbreeding was detected in both individuals with and without defects.

Table 2. Comparison of pedigree derived inbreeding values (F_{PED}) and estimates of genome-wide heterozygosity (MLH) and inbreeding (F_{GEN}) in two individuals with confirmed pedigree inbreeding

Sample	F_{PED}	MLH	F_{GEN}
Individual with polydactyly	0.039	0.556	0.087
Individual with ear dysgenesis and cleft palate	0.031	0.500	0.060

Microsatellites have disputed usefulness as a measure of genetic diversity (Rousset 2002, Pemberton 2004). This study has examined a small set of markers in order to examine the premise of the hypothesis that inbreeding is the cause of the increased incidence of defects in alpacas. It is expected that the analyses conducted in this study although not exhaustive will be useful in providing some insight into the levels of inbreeding in alpacas. Further research should focus on the genetic mapping of these congenital abnormalities with the aim of developing genetic tests to allow the elimination of these disorders from the alpaca population. Importantly this will require surveillance and reporting of these defects in order to increase sample sizes and provide information on inheritance patterns. A case-control matched defect and normal animals from the same herd and same parents may aid in the dissection of the aetiology of these defects in alpacas.

CONCLUSIONS

This study is the first to investigate genome-wide levels of diversity in alpacas. Although many individuals showed cryptic inbreeding, cryptic relatedness occurred in animals with and without defects. The results of this research suggest that reduction of genome-wide heterozygosity does not explain the high prevalence of defects in alpacas. Alternative hypotheses to be tested include environmental influences and heritable genetic disorders not associated with inbreeding.

ACKNOWLEDGEMENTS

This research was funded by a University of Melbourne Faculty of Science Research Development Grant. Thanks to the Australian Alpaca Association and Alpaca Genomics Australia Pty. Ltd. for their ongoing support for this research and dedication to the genetic improvement of the Australian alpaca stock. A special thanks to Charles Malpas and Andrew Jackling for assistance with spreadsheet calculations. Thanks to Warren Johnson at the National Institute of Health (NIH) for providing microsatellite markers and primers. Thanks also to the alpaca breeders who donated samples and veterinarians for donating time to collect samples.

REFERENCES

Balloux F., Amos W., and Coulson T. (2004) *Molecular Ecology* **13**: 3021.
Healy, P. J. (1996) *Journal of Animal Science* **74**: 917.
Pemberton, J. (2004) *Trends in Ecology and Evolution* **19**: 613.
Rousset, F. (2002) *Heredity* **88**: 371.
Schuelke, M. (2000) *Nature Biotechnology*, **18**: 233.
Windsor, P. A. (2009) *Australian Veterinary Journal*, **87**: 193.
Wright, S. (1917) *Am. Nat.* **51**: 636.
Wright, S. (1921) *Genetics* **6**:111.