Improved Alignment Between The Sheep And Goat Linkage Maps

C. Visser[*], E. Van Marle-Koster[*] and R.P.M.A . Crooijmans†

Introduction

Linkage maps are an essential tool in identifying specific loci associated with phenotypic variation in economically important traits. The current caprine maps available are however limited to the first low-resolution genetic map for the male goat genome constructed by Vaiman *et al.* (1996), followed by a more densely populated map on the male chromosome by Schibler *et al.* (1998). The focus on mapping specific traits of economic importance, such as milk proteins (Barillet (2007); Moioli *et al.* (2007)), polled intersex syndrome (Vaiman *et al.* (1997)) and growth hormones (Gupta *et al.* (2009) has resulted in the creation of unbalanced maps, with an uneven distribution of markers. Specific regions (i.e. the areas flanking the PISRT1 locus) have been very well described and populated densely with microsatellite markers, while other regions were neglected. Although several QTL studies have been performed on small-stock species and partial linkage maps were created as by-products of these studies, these maps are mostly not publicly available (Maddox & Cockett (2007)).

Despite the generally good alignment between the caprine and ovine linkage maps, a relatively large number of discrepancies between both species have been reported (Maddox (2005)), including many inversions in locus orders between the two maps. It is not certain which of these discrepancies are artefacts, and which might be genuine. The use of a small number of microsatellite markers in conjunction with less error checking for the goat map, has lead to a less robust map with a need for further genetic map development. This study aimed to improve the alignment between the ovine and caprine maps by confirming or rejecting previously reported rearrangements of loci order.

Material and methods

The genetic linkage map was constructed by genotyping three generations of Angora goat half-sib offspring, belonging to 12 different families. The families ranged from 44 to 140 offspring, with an average of 88 half-sib offspring per sire. DNA was extracted from whole blood samples using respectively the Qiagen DNEasy Tissue kit at the University of Pretoria and the Invisorb blood mini HTS kit (Invitek) for the XtractorGene (Corbett Robotics) at Wageningen University according to the protocols of the respective manufacturers. Incorrect

[*] Dept Animal and Wildlife Sciences, University of Pretoria, Pretoria, South Africa
† Animal Breeding and Genomics Centre, Wageningen University, Wageningen, The Netherlands

parentage was identified with Cervus 3.0 (Marshall (1998)), and all aberrant individuals were removed from the study.

96 Microsatellite markers were selected from the existing goat map database (http://locus.jouy.inra.fr/cgi-bin/lgbc/mapping/common/intro2.pl?BASE=goat). PCR was performed in a i-cycler (Bio-rad) and T1 Thermocycler (Biometra) using 30ng DNA, 2.94 µl of the ABgene® PCR Master Mix (ABGene, UK) and 0.03 µl each of 40pmol/µl reverse and forward primer. The PCR amplification was conducted in a 6 µl final volume in 384 well PCR plates at the following conditions: 95°C for 5 minutes, followed by 35 cycles of 96°C for 30s, 45s at Annealing Temperature and 90s at 72°C with a final extension step of 10min at 72°C.

Linkage analysis was performed with CRI-MAP 2.4 (Green *et al*. (1989)) compiled for Windows XP. Linkage groups were assigned on the basis that the markers have been mapped to specific goat chromosomes. The "two point' option was used to verify these linkage groups. A LOD threshold of 6 was chosen to detect significant linkage, and lowered in increments (to 1) until all markers could be assigned to a specific chromosome. Linkage groups were further analysed using the "build" option. Markers that could not be placed accurately with this option, were then included in alternative "fixed" orders. The final order (which resulted in the shortest map length) was verified with the "flipsn" option.

Results and discussion

Despite the overall good agreement of the caprine and ovine maps, a large number of discrepancies have been reported between them (Maddox (2005)), including inter-chromosomal re-assignments. Due to the limited development of the goat genetic map since the first loci were mapped, the discrepancies reported were not verified. The marker order generated in this study is in agreement of Schibler *et al.* (1998) except for the marker orders on CHI4, CHI11 and CHI19. The relative marker orders for the chromosomes with potential rearrangements are shown in Figure 1. For these three chromosomal linkage maps our marker order is in agreement with the marker order reported in sheep (SheepMap 4.7 ; http://www.ncbi.nlm.nih.gov/mapview/static/sheepsearch.html). This indicates that the three inversions observed by Schibler *et al.* (1998) were either population specific or incorrect marker assignments and is supported by the reduced genetic length of the linkage map. However, the rearrangement on CHI3 detected by Schibler *et.al.* (1998) is validated in this study, and therefore a true inter-chromosomal rearrangement. The relative map lengths between the studies also differed. This could be explained by the higher average number of informative meiosis (518±179) in our population, compared to 114±70 by Schibler *et al.* (1998) resulting in a more accurate linkage map. Furthermore, Schibler *et al.* (1998) reported results without taking Kosambi's correction into account, while the current study made use of Kosambi's mapping function.

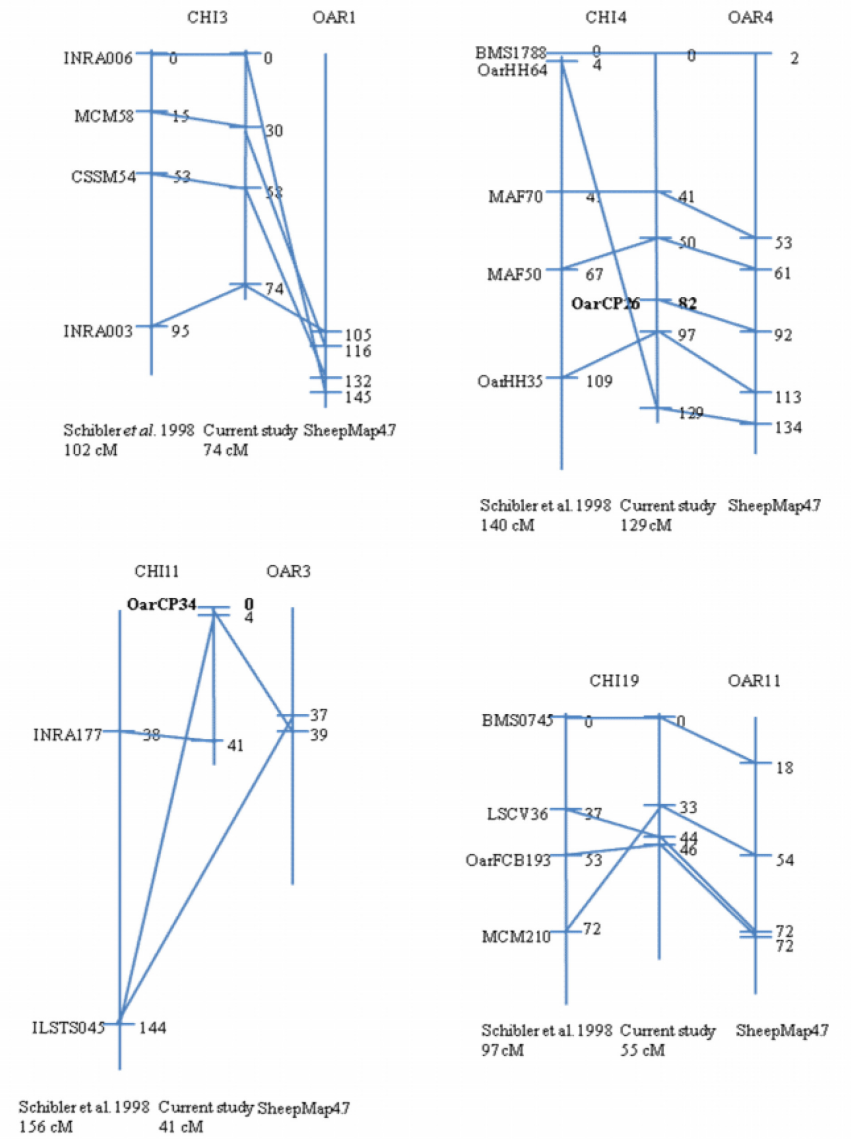

Figure 1: Alignment of marker orders on CHI3, 4, 11 and 19 with orders compared between the current study, the goat map of Schibler *et al.* (1998) and SheepMap4.7 (http://www.ncbi.nlm.nih.gov/mapview/static/sheepsearch.html)

The most common marker used in goat linkage map studies is still microsatellite markers. Single Nucleotide Polymorphisms (SNPs) are becoming the marker of choice in many molecular studies, mainly due to increased automation coupled with low cost (Toro *et al.* (2009)). However, while millions of SNPs have been identified for cattle, and about 5000 potential SNPs have been identified for use in ovine studies, no mapped SNP markers have

been reported for goats (Maddox & Cockett (2007)). Only 1.2% of bovine SNPs were proved to be polymorphic in goats (Maddox & Cockett (2007)), and is thus of little use in goat studies. SNP discovery projects for caprine SNPs are required before these markers can be utilized for molecular research on goats.

Conclusion

The accuracy and coverage of the goat linkage map will be increased significantly once SNPs are available for genome mapping in goats. Until then, microsatellite markers have made an essential contribution to the development of caprine genome maps. The correction of previously reported inversions (compared to the ovine map) will contribute to the improvement and accuracy of the goat map. The improved caprine maps will help with comparative mapping of economically significant loci, which could result in the application of marker assisted selection.

References

Barillet, G. (2007). *Small Rumin Res*. 70: 60-75.

Green, P., Falls, K. and Crooks, S. (1989). Documentation for CRI-MAP, version 2.4. Washington University, St Louis.

Gupta, N., Pandey, A., Malik, G. *et al.* (2009). *Small Rumin Res.* 81: 35-41.

Maddox, J.F. (2005). *Genet. Sel. Evol.* 37 (Suppl 1): S1-S10.

Maddox, J.F. and Cockett, N.E. (2007). *Small Rumin Res.* 70: 4-20.

Marshall, T.C., Slate, J., Kruuk, L.E.B. *et al.* (1998). *Mol Ecol* 7 (5): 639-655.

Moioli, B., Andrea, M.D. and Pilla, F. (2007). *Small Rumin Res.* 68: 179-192.

Schibler, L., Vaiman, D. Oustry, A. *et al.* (1998). *Genome Res* 8: 901-915.

Toro, M.A., Fernandez, J. and Caballero, A. (2009). *Livest Sci* 120: 174-195.

Vaiman, D., Schibler, L., Bourgeois, F. *et al.* (1996). *Genetics* 144: 279-305.

Vaiman, D., Pailhoux, E., Schibler, L. *et al.* (1997). *Theriogenology* 47: 103-109.