

A second-generation linkage map of the sheep genome

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Abstract. A genetic map of *Ovis aries* (haploid $n = 27$) was developed with 519 markers (504 microsatellites) spanning ~3063 cM in 26 autosomal linkage groups and 127 cM (female specific) of the X Chromosome (Chr). Genotypic data were merged from the IMF flock (Crawford et al., Genetics 140, 703, 1995) and the USDA mapping flock. Seventy-three percent (370/504) of the microsatellite markers on the map are common to the USDA-ARS MARC cattle linkage map, with 27 of the common markers derived from sheep. The number of common markers per homologous linkage group ranges from 5 to 22 and spans a total of 2866 cM (sex average) in sheep and 2817 cM in cattle. Marker order within a linkage group was consistent between the two species with limited exceptions. The reported translocation between the telomeric end of bovine Chr 9 (BTA 9) and BTA 14 to form ovine Chr 9 is represented by a 15-cM region containing 5 common markers. The significant genomic conservation of marker order will allow use of linkage maps in both species to facilitate the search for quantitative trait loci (QTLs) in cattle and sheep.

Introduction

Similarity in DNA sequence and microsatellite (ms) locus order among species of ruminant Bovidae were used to develop the current low-resolution genetic map for sheep (232 linked markers; Crawford et al. 1995). Recent genetic maps for cattle (Barendse et al. 1994, 1997; Bishop et al. 1994; Kappes et al. 1997) and goats (Vaiman et al. 1996) developed, in part, with ms of ovine and bovine origin provide further examples of significant homology at individual loci. Information on the similarity of locus order between species is also beginning to emerge (Vaiman et al. 1996). Nineteen ms of ovine origin are included on the IBRP genetic map of cattle (Barendse et al. 1997), 40 on the latest USDA cattle genetic map (Kappes et al. 1997), and 45 on the goat linkage map (Vaiman et al. 1996). In general, locus order appears conserved within the family Bovidae, but discrepancies in linkage group length are apparent (Vaiman et al. 1996), particularly in areas of known rearrangement (Crawford et al. 1995).

A second generation ovine linkage map was constructed primarily with bovine ms. This allowed us to determine whether the degree of similarity in syntenic markers extends to order and in-

terval, in effect creating a framework comparative (linkage) map for the two subfamilies of Bovidae, Bovinae, and Caprinae. Such a map is useful for several reasons. First, using available bovine primers would obviate the expense of developing a large set of random ms of ovine origin. Second, simultaneous construction of linkage maps for the two species, and perhaps even additional closely related species (bison, goat), would be limited only by the degree to which primer pairs would amplify a unique locus across species and the degree of heterozygosity at that locus. Third, numerous aspects of the evolutionary development and divergence of these selected species can be evaluated as additional loci are characterized for marker order and chromosomal location. Finally, the degree to which QTL information is transferable from one species to another within the Bovidae family can be ascertained. The comparative mapping concept of complex phenotypes to orthologous QTL has been clearly demonstrated in cereal grains (Paterson et al. 1995), and comparative analysis among mammalian genomes is progressing (Sonstegard et al. 1997; Sun et al. 1997).

The characterization of >1000 bovine ms loci in sheep was previously reported (de Gortari et al. 1997). In this study, bovine ms were used in a comparative linkage strategy to increase overall coverage and define genetic breakpoints about translocated regions of the sheep genome.

Materials and methods

Data collection. The families used to construct the ovine linkage map have been described previously (Crawford et al. 1995; de Gortari et al. 1997). Briefly, the AgResearch International Mapping Flock (IMF) consists of nine, three-generation full-sib pedigrees with a total of 98 progeny. The USDA reference population (MARC) has 247 backcross progeny produced by mating four F_1 rams (2 Suffolk \times Romanov; 1 Rambouillet \times Romanov, and 1 Romanov \times Rambouillet) to 44 Romanov ewes. Genomic DNA was extracted from white blood cells as described by Miller and associates (1988). Amplification conditions for each primer have been reported (de Gortari et al. 1997). In general, PCR reactions (12 μ l) contained 100 ng template DNA, buffer (50 mM KCl, 1.5 mM $MgCl_2$, 10 mM Tris HCl, pH 9.0), 30 μ M each of three unlabeled dNTPs, 0.45 μ M primer pairs, and 0.35 U Taq DNA polymerase. Radioisotope was incorporated into the PCR product by addition of [α -³²P] dATP 0.1 μ Ci (3000 Ci/mmole) and 3 μ M unlabeled dATP. All amplification conditions included an initial denaturing step of 3 min at 94°C, followed by 30 cycles of 1 min at 94°C, 1 min at the annealing temperature, and 1 min at 72°C. Final extension was for 4 min at 72°C. Reaction products were electrophoresed (40 V/cm) on 7% denaturing polyacrylamide gels with a standard M13mp18 ladder and visualized by autoradiography. Some primer pairs were genotyped preferentially across families of informative sires. Primer sequences not previously reported in Crawford and colleagues (1995) and Freking and coworkers (1997) are referenced in de Gortari and associates (1997) or Kappes and collaborators (1997). When two sets of primer pairs for the same locus were genotyped, the locus name first published was used. Genotypes were independently scored twice and discrepancies resolved.

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Data analysis. The linkage map consists of 402 bovine, 101 ovine, and 1 cervine microsatellites. Loci other than microsatellites included 7 ovine RFLP, 2 bovine proteins, 2 ovine proteins, and 4 erythrocyte antigens. Markers were placed into linkage groups based on two-point lod (≥ 3.0) scores, and the linear order was determined by maximizing the likelihood function within each group by multi-point linkage analysis (CRI-MAP v. 2.4; Green et al. 1990). Markers were added to the linkage groups in decreasing order of informative meioses. Genotypic data from Crawford and colleagues (1995) were merged by genotyping 154 of the markers in both reference populations. Inspection of two-point linkage information and the cattle linkage map were used to identify potential genotypic errors for markers that inflated the linkage group >20 cM. The CHROMPIC option was used to identify unlikely recombination events. Genotypes contributing to these events were amplified and scored a second time to resolve discrepancies and errors. On OAR1, 2, and 7, the linkage groups were divided in order to run the analysis. Common linkage group lengths were calculated from the current map and the USDA MARC cattle map (Kappes et al. 1997), with the two most external common markers used for each linkage group. Three-hundred and seventy of 504 ms (73.4%) are common to the MARC bovine map and the ovine map reported here. Ten additional polymorphic markers with ovine data remain unlinked (lod <3.0 ; meioses range, 12–261).

Results

Map development. In total, 519 markers were linked (485 intervals) on this second-generation genetic map. The map integrates 262 (51%) markers developed at MARC, with 257 (49%) markers from international sources. The two combined reference populations have the potential to produce 760 informative meioses. The mean number of informative meioses per marker was 197 (range 12–624). The autosomal map spanned ~3063 cM. Individual autosomal linkage groups averaged 19 markers (range 8–48) and 117.8 cM (range 49.2 cM, OAR25 to 298 cM, OAR1), as displayed in Table 1. The X Chr had 22 linked ms covering 126.9 cM (female specific). Average interval between adjacent linked markers was ~6.4 cM with 55.5% of the intervals <5 cM and only 3.8% >20 cM. There were 512 unique loci (Table 1). Five loci were described by two sets of primer pairs for the same microsatellite (TGLA58, OAR3, 79.2 cM; MAP2C, OAR11, 102.3 cM; INRA035, OAR12, 98.4 cM; HMH1R, OAR19, 79.5 cM; BOLA-DRB1, OAR20, 34.9 cM). One locus (HBB, OAR15, 50.5 cM) was described by two sets of microsatellite primer pairs and a protein polymorphism. Nineteen microsatellite markers derived from bovine sequences were mapped to ovine chromosomes (Freking et al. 1997), but not placed on the current bovine linkage map (Kappes et al. 1997). Table 1 also illustrates the species source and type of markers used to construct each linkage group.

Although map resolution remains modest (average interval 6.4 cM) and is likely to remain so for the immediate future, we attempted to identify the number of observed double recombinants within .20-cM intervals in each linkage group. The 17 double recombinants identified suggest that either the error rate was low or at this level of resolution we are not yet able to rigorously identify genotypic errors (Rohrer et al. 1996; Kappes et al. 1997). Figure 1 illustrates the 27 linkage groups of the current map. Additional information (primary sequence, annealing temperature, number and size of alleles, etc.) about each locus may be obtained at <http://sol.marc.usda.gov>. All 26 autosomes are oriented with respect to the chromosome (top is p arm for OAR1–3 and centromere for OAR4–26) either by direct physical assignment in sheep or indirectly through the homologous bovine chromosome (Kappes et al. 1997; Lopez-Corrales et al. in preparation).

Conservation of microsatellite markers. Genomic conservation among Bovidae allowed extensive use of ms developed in the bovine. Mean heterozygosity of the 519 markers linked in this ovine map was 68.9% when calculated from the eight F₁ sires

Table 1. Characteristics of the ovine linkage map.

Chromosome Number	Number of Unique Loci	Linkage Group Length (cM)	Number of Bovine ms	Number of Ovine ms	Number of Other Markers
1	50	298.0	38	11	1 ^a
2	40	280.9	32	8	
3	42	293.3	36	7	
4	18	129.2	11	7	
5	17	147.0	14	3	
6	21	144.5	14	3	4 ^b
7	19	139.3	18	1	
8	20	111.0	19	1	
9	20	106.8	16	4	
10	14	77.5	8	4	2 ^c
11	13	126.1	13	1	
12	17	113.6	18	0	
13	14	128.9	10	4	
14	8	80.3	7	1	
15	16	93.2	16	1	1 ^d
16	17	93.7	12	5	
17	22	86.2	13	6	3 ^e
18	19	97.0	13	4	2 ^f
19	19	81.0	16	4	
20	17	64.3	11	6	1 ^g
21	14	68.5	9	4	1 ^h
22	14	72.8	12	2	
23	8	51.1	7	1	
24	11	65.9	8	2	1 ⁱ
25	9	49.2	5	4	
26	11	63.5	7	4	
X	22	126.9	19	3	
Total	512*	3,189.7	402	101	16

^a Ovine protein polymorphism.

^b Ovine RFLP (2); bovine polymorphism; bovine erythrocyte antigen (EAA).

^c Ovine RFLP; bovine EAB.

^d Bovine protein polymorphism.

^e Ovine RFLP (3).

^f Bovine EAM; ovine protein polymorphism.

^g Bovine EAC.

^h Red-deer (cervine) microsatellite.

ⁱ Ovine RFLP.

* Six loci represented by more than one marker.

representing the IMF and MARC pedigrees. However, the mean heterozygosity of a larger sample of bovine-derived autosomal ms in sires of the IMF and MARC ovine families was 45.3% (de Gortari et al. 1997) compared with 60.1% in the sires and dams of the MARC cattle reference population (Bishop et al. 1994). The number of ms in common with a homologous bovine linkage group totaled 370, with a range of 5 to 22 common ms. Twenty-seven of these ms were derived from sheep and the remaining 343 derived from bovine sequences. The common markers span 2866 cM (sex average) in sheep and 2817 cM in cattle. Marker order within a linkage group was consistent between species with limited exceptions. The three metacentric chromosomes in sheep, OAR 1, 2, and 3, are homologous to BTA1 and 3, BTA 2 and 8, and BTA 5 and 11, respectively. The translocation of the telomeric end of BTA9 to BTA14 in sheep (OAR9), previously reported by Crawford and coworkers (1995), is represented by a 15-cM region containing 5 common markers. Figure 2 presents the comparative linkage between BTA9 (the nontranslocated region) and its homolog OAR8. Marker order was inverted only eight times (>2 -cM intervals) when the sheep map was compared with the cattle linkage map developed by Kappes and colleagues (1997). The length of the inversions ranged from 3 to 57 cM. The majority of these are probably not true inversions, but result from a low number of informative meioses that prevented rigorous ordering of loci. Finally, 6 ms (BMS66, OAR3; BMS719, OAR4; BM3627, OAR7; BM4439, OAR8; BMS2840, OAR16; HEL6, OAR23) mapped to sheep linkage groups other than the expected homolog (OAR23, 12, 2, 15, 5, 1, respectively). This was probably owing to amplification of closely related sequences flanking a second locus,

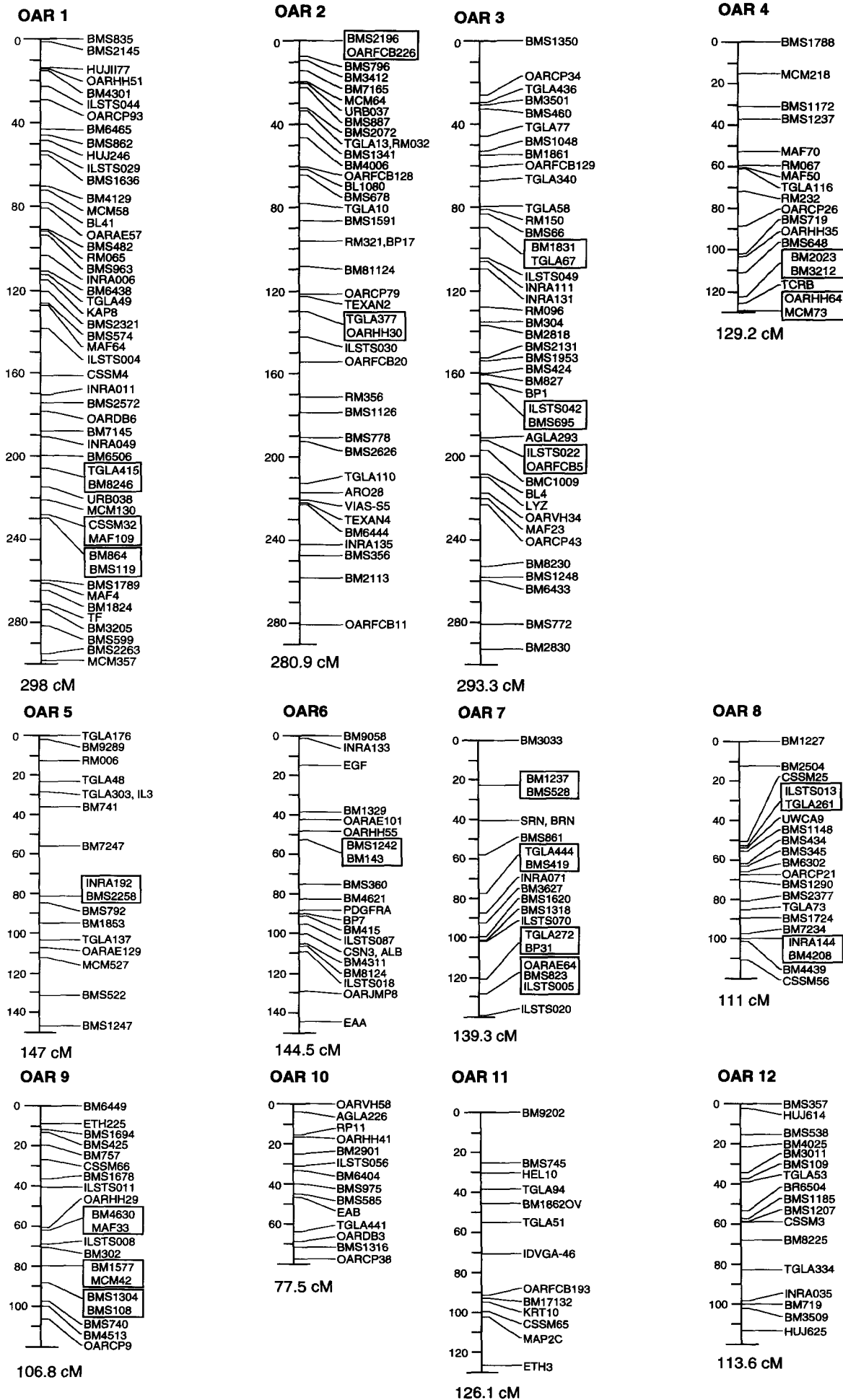


Fig. 1.

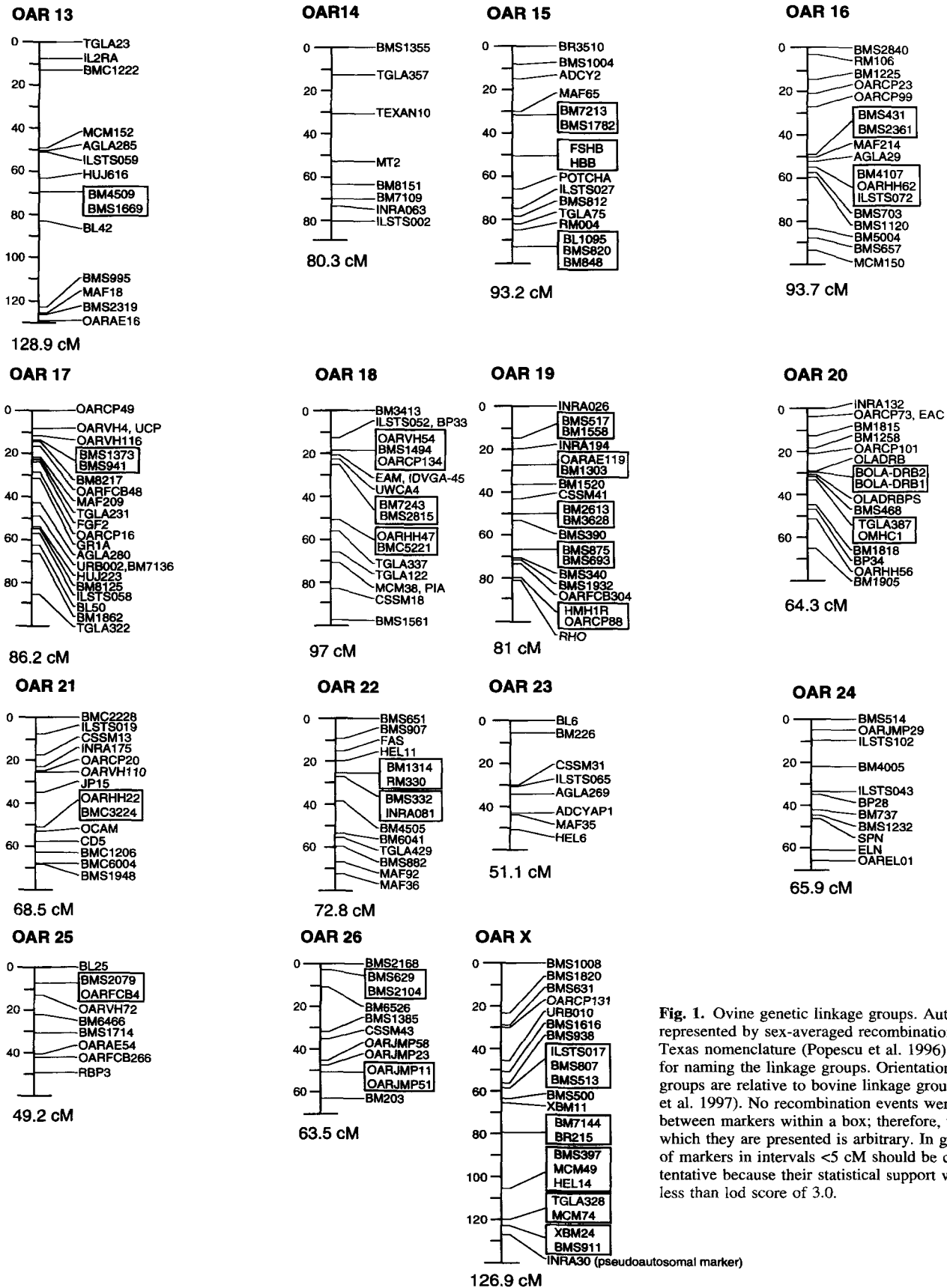


Fig. 1. Ovine genetic linkage groups. Autosomes are represented by sex-averaged recombination rates. The Texas nomenclature (Popescu et al. 1996) was used for naming the linkage groups. Orientation of linkage groups are relative to bovine linkage groups (Kappes et al. 1997). No recombination events were detected between markers within a box; therefore, the order in which they are presented is arbitrary. In general, order of markers in intervals <5 cM should be considered tentative because their statistical support was often less than lod score of 3.0.

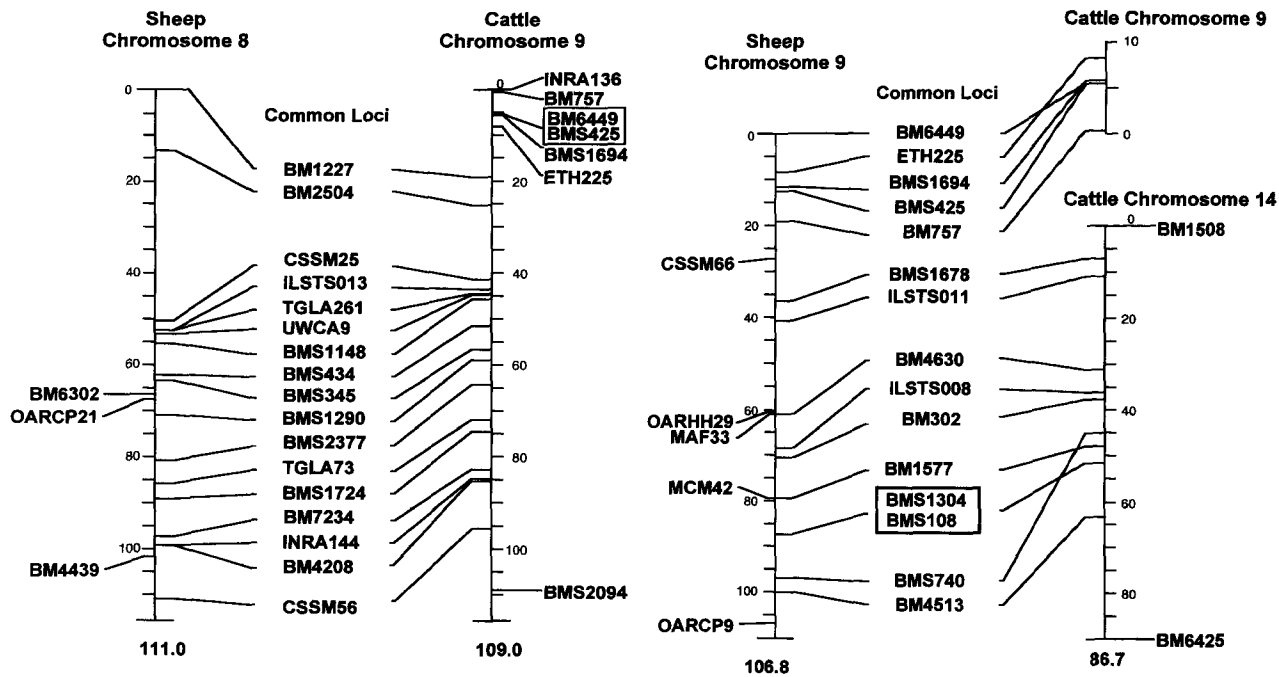


Fig. 2. Current resolutions of the previously reported translocation (Crawford et al. 1995). Ovine linkage groups for Chr 8 and 9 with the homologous bovine linkage groups (Chrs 9 and 14). Loci listed in the center of the linkage groups are common to both maps.

rather than a translocation, because sizes of amplified products were not always consistent across species.

This conservation of marker order and interval is likely to extend to other species within the Bovidae. de Gortari and associates (1997) reported that 67% of bovine primer pairs amplifying a locus in sheep (40% of the 1036 tested) were polymorphic, a value similar to that reported by Vaiman et al. (1996) for the goat. Seventy-seven of 166 (46%) bovine ms that were polymorphic in the goat (Vaiman et al. 1996) are present on the current ovine map.

Discussion

The present map, which includes the genotypes from the IMF flock (Crawford et al. 1995), more than doubles the number of linked ovine markers, increases coverage by ~1000 cM, and consolidates linkage groups to one per chromosome compared with the initial map (Crawford et al. 1995). Our use of bovine ms to construct the initial linkage map for sheep indicated that ~50% of bovine markers were informative, and this information formed the basis for the current map. The ovine map includes 402 bovine primer pairs out of the 504 (80%) linked ms. The current map represents a considerable improvement in resolution (average interval 6.5 cM) and suggests that the overall genetic length of the sheep genome is similar to the bovine genetic length of 2990 cM estimated from 1250 linked markers reported by Kappes et al. (1997). However, the length of the sheep map reported here is less than the length of the bovine map of 3710 cM estimated from 703 linked markers reported by Barendse and coworkers (1997) and considerably less than the bovine genome length (>4000 cM) estimated by Ferretti and associates (1997). Our current estimate of the sheep map length (3190 cM) is closer to the minimum size (~2750 cM) predicted from the Chapman and Bruere (1977) chiasmata data, the estimate for goats provided by Vaiman and coworkers (1996) of 3250 cM including the X Chr, and the chiasmata data of Logue and Harvey (1978) for cattle (~2500 cM) and goats (2485 cM). Chromosome ends are not taken into account in predicting genetic length from chiasmata data; therefore, these values are likely to be biased downward.

At the current level of map resolution, attempts to reduce inflation through continuous checking for genotypic errors with options such as CHROMPIC and comparison of family recombination rates to identify errors at the parental and grandparental level are essential. Direct physical assignment of markers at the ends of individual linkage groups, as reported by Heaton and colleagues (1997) and Lopez-Corrales and collaborators (in preparation), will also help provide a robust estimate of coverage as maps develop.

The genomic conservation between cattle and sheep apparently extends to other members of the Caprinae. Although Vaiman and associates (1996) reported several differences in the location of individual ms between the (male) goat map and cattle and sheep maps, some of these loci were probably the result of amplification of a nonhomologous sequence by heterologous primers, similar to that noted in this report for sheep and cattle. However, in some cases, flanking regions of microsatellites were found by Vaiman and colleagues (1996) to be highly similar when sequenced, yet mapped to nonhomologous chromosomes. This suggests the possibility of a rearrangement(s) between goat and either cattle or sheep. Vaiman and coworkers (1996) discussed the evidence supporting a translocation event from goat Chr 9 to 14 in relation to bovine Chrs 9 and 14. This translocation event is consistent with the event reported in sheep (Crawford et al. 1995). Markers that are monomorphic in the species in which the markers were developed are still potentially useful in linkage map development of other closely related species. This report describes 19 bovine-derived markers not mapped in cattle. These assignments (in sheep) should be interpreted with caution owing to amplification of closely related flanking sequences as discussed above. As homologous loci, these microsatellites may be of value for future marker development upon identification of regions containing QTL across Bovidae.

Concurrent map-based searches for QTL in various livestock species would be enhanced by the development of comparative maps. Evidence of QTL mapping for similar phenotypes to corresponding homologous locations among different cereal grain species has provided strong evidence to suggest orthologous genes were involved in the evolution of these relevant phenotypes (Pa-

terson et al. 1995). This conservation of gene order provides a biological basis for comparative analysis of complex phenotypes among livestock species. The high degree of genomic conservation and ability of heterologous primers to amplify microsatellite loci that maintain a conserved order yield expectations of similar QTL being identified across species. This expectation has particular relevance toward QTL that display more variability in one species than in another, affecting how easily the QTL can be detected. Complex phenotypic traits such as prolificacy, resistance to disease, feed intake, and carcass composition may be investigated in sheep at less cost and over shorter time frames and still have relevance to genetic variability in cattle.

In conclusion, the current sheep map represents a significant increase in resolution that will ultimately enhance the detection of quantitative trait loci for production traits as well as disease susceptibility. It provides an additional set of bovine markers that will undoubtedly prove useful in the development of a comparative linkage map of the Bovidae and, perhaps, other closely related families within the Artiodactyla.

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References

- Barendse W, Armitage SM, Kossarek LM, Shalom A, Kirkpatrick BW, Ryan AM, Clayton D, Li L, Neibergs HL, Zhang N, Grosse WM, Weiss J, Creighton P, McCarthy F, Ron M, Teale AJ, Fries R, McGraw RA, Moore SS, Georges M, Soller M, Womack JE, Hetzel DJS (1994) A genetic linkage map of the bovine genome. *Nat Genet* 6, 227–235
- Barendse W, Vaiman D, Kemp SJ, Sugimoto Y, Armitage SM, Williams JL, Sun HS, Eggen A, Agaba M, Aleyasin SA, Band M, Bishop MD, Buitkamp J, Byrne K, Collins F, Cooper L, Coppettiers W, Denys B, Drinkwater RD, Easterday K, Elduque C, Ennis S, Erhardt G, Ferretti L, Flavin N, Gao Q, Georges M, Gurung R, Harlizius B, Hawkins G, Hetzel J, Hirano T, Hulme D, Jorgensen C, Kessler M, Kirkpatrick BW, Konfortov B, Kostia S, Kuhn C, Lenstra JA, Leveziel H, Lewin HA, Leyhe B, Lil L, Burriel I. Martin, McGraw RA, Miller JR, Moody DE, Moore SS, Nakane S, Nijman II, Olsaker I, Pomp D, Rando A, Ron M, Shalom A, Teale AJ, Thieven U, Urquhart BGD, Vage D-I, Van de Weghe A, Varvio S, Velmala R, Vilkki J, Weikard R, Woodside C, Womack JE, Zanotti M, Zaragoza P (1997) A medium-density genetic linkage map of the bovine genome. *Mamm Genome* 8, 21–28
- Bishop MD, Kappes SM, Keele JW, Stone RT, Sunden SLF, Hawkins GA, Toldo SS, Fries R, Grosz MD, Yoo J, Beattie CW (1994) A genetic linkage map for cattle. *Genetics* 136, 619–639
- Chapman HM, Bruere A (1977) Chromosome morphology during meioses of normal and Robertsonian translocation-carrying rams (*Ovis aries*). *Can J Genet Cytol* 19, 93–102
- Crawford AM, Dodds KG, Ede AJ, Pierson CA, Montgomery GW, Garmonsway HG, Beattie AE, Davies K, Maddox JF, Kappes SM, Stone RT, Nguyen TC, Penty JM, Lord EA, Broom JE, Buitkamp J, Schwaiger W, Epplen JT, Matthew P, Matthews ME, Hulme DJ, Beh KJ, McGraw RA, Beattie CW (1995) An autosomal genetic linkage map of sheep genome. *Genetics* 140, 703–724
- de Gortari MJ, Freking BA, Kappes SM, Leymaster KA, Crawford AM, Stone RT, Beattie CW (1997) Extensive genomic conservation of cattle microsatellite heterozygosity in sheep. *Anim Genet* 28, 274–290
- Ferretti L, Urquhart BGD, Eggen A, Olsaker I, Harlizius B, Castiglioni B, Mezzelani A, Solinas Toldo S, Thieven U, Zhang Y, Morgan ALG, Teres VM, Schwerin M, Burriel I. Martin, Chowdhary BP, Erhardt G, Nijman II, Cribiu EP, Barendse WH, Leveziel H, Fries R, Williams JL (1997) Cosmid-derived markers anchoring the bovine genetic map to physical map. *Mamm Genome* 8, 29–36
- Freking BA, Stone RT, de Gortari MJ, Crawford AM (1997) Chromosomal assignment by linkage of nineteen unassigned bovine microsatellites using ovine reference populations. *Anim Genet*, in press
- Green PK, Falls K, Crooks S (1990) *Documentation for Cri-MAP*, version 2.4. (St. Louis, Mo.: Washington University School of Medicine)
- Heaton MP, Lopez-Corrales NL, Smith TPL, Kappes SM, Beattie CW (1997) Directed cosmid isolation for physical assignment by FISH: PCR-based iterative screening of random genomic libraries. *Anim Biotechnol* 8, 167–177
- Kappes SM, Keele JW, Stone RT, McGraw RA, Sonstegard TS, Smith TPL, Lopez-Corrales, NL, Beattie CW (1997) A second-generation linkage map of the bovine genome. *Genome Res* 7, 235–249
- Logue DN, Harvey MJA (1978) Meiosis and spermatogenesis in bulls heterozygous for a presumptive 1/29 Robertsonian translocation. *J Reprod Fertil* 54, 159–165
- Miller SA, Dykes DD, Polesky HF (1988) A simple salting out procedure for extracting DNA from human nucleated cells. *Nucleic Acids Res* 16, 1215
- Paterson AH, Lin Y-R, Li Z, Schertz KF, Doebley JF, Pinson SRM, Liu S-C, Stansel JW, Irvine JE (1995) Convergent domestication of cereal crops by independent mutations at corresponding genetic loci. *Science* 269, 1714–1718
- Popescu CP, Long S, Riggs P, Womack J, Schmutz S, Fries R, Gallagher DS (1996) Standardization of cattle karyotype nomenclature: report of the committee for the standardization of cattle karyotype. *Cytogenet Cell Genet* 74, 259–261
- Rohrer GA, Alexander LJ, Hu Z, Smith TPL, Keele JW, Beattie CW (1996) A comprehensive map of the porcine genome. *Genome Res* 6, 371–391
- Sonstegard TS, Lopez-Corrales NL, Kappes SM, Beattie CW, Smith TPL (1997) Comparative mapping of human chromosome 2 identifies segments of conserved synteny near the bovine *mh* locus. *Mamm Genome* 8, 751–755
- Sun HS, Cai L, Davis SK, Taylor JF, Doud LD, Bishop MD, Hayes H, Barendse W, Vaiman D, McGraw RA, Hirano T, Sugimoto Y, Kirkpatrick BW (1997) Comparative linkage mapping of human chromosome 13 and bovine chromosome 12. *Genomics* 39, 47–54
- Vaiman D, Schibler L, Bourgeois F, Oustry A, Amigues Y, Cribiu EP (1996) A genetic linkage map of the male goat genome. *Genetics* 144, 279–305