A Deer (Subfamily Cervinae) Genetic Linkage Map and the Evolution of Ruminant Genomes

Jon Slate,*[,1] Tracey C. Van Stijn,[†] Rayna M. Anderson,* K. Mary McEwan,* Nauman J. Maqbool,* Helen C. Mathias,[†] Matthew J. Bixley,* Deirdre R. Stevens,* Adrian J. Molenaar,[‡] Jonathan E. Beever,[§] Susan M. Galloway[†] and Michael L. Tate*

AgResearch, Invermay Agricultural Centre, Mosgiel, New Zealand, [†]AgResearch, Molecular Biology Unit, Department of Biochemistry, University of Otago, Dunedin, New Zealand, [‡]AgResearch, Ruakura Research Centre, Hamilton, New Zealand and [§]Department of Animal Sciences, University of Illinois at Urbana-Champaign, Urbana, Illinois 61801

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ABSTRACT

Comparative maps between ruminant species and humans are increasingly important tools for the discovery of genes underlying economically important traits. In this article we present a primary linkage map of the deer genome derived from an interspecies hybrid between red deer (*Cervus elaphus*) and Père David's deer (*Elaphurus davidianus*). The map is ~2500 cM long and contains >600 markers including both evolutionary conserved type I markers and highly polymorphic type II markers (microsatellites). Comparative mapping by annotation and sequence similarity (COMPASS) was demonstrated to be a useful tool for mapping bovine and ovine ESTs in deer. Using marker order as a phylogenetic character and comparative map information from human, mouse, deer, cattle, and sheep, we reconstructed the karyotype of the ancestral Pecoran mammal and identified the chromosome rearrangements that have occurred in the sheep, cattle, and deer lineages. The deer map and interspecies hybrid pedigrees described here are a valuable resource for (1) predicting the location of orthologs to human genes in ruminants, (2) mapping QTL in farmed and wild deer populations, and (3) ruminant phylogenetic studies.

COMPARATIVE mapping—the comparison of gene location and order between species—has two main purposes. The first is to infer the evolution of genome organization and the second is to exploit information available from a well-mapped species to predict the location of genes in a lesser-mapped species (Wakefield and Graves 1996). Broadly speaking, two classes of markers are used to construct genetic maps (O'Brien *et al.* 1993). The first, termed type I loci, are widely conserved across species but are frequently invariable within a species. The second, termed type II loci, are usually polymorphic within a species, but tend to be less widely conserved (*e.g.*, microsatellites). During the last decade the major motivation for gene mapping in artiodactyls (even-toed ungulates) was to generate a suite of markers suitable for detecting quantitative trait loci (QTL) for production traits. Consequently, livestock genomes have been predominantly mapped using type II markers (Barendse *et al.* 1994; Bishop *et al.* 1994; Crawford *et al.* 1995; Kappes *et al.* 1997; de Gortari *et al.* 1998), and an impressive number of QTL have been identified. However, it is becoming increasingly clear that ruminant gene discovery requires the identification of candidate genes in the human chromosomal region homologous to a QTL (O'Brien *et al.* 1999)—an approach termed positional candidate cloning (Womack and Kata 1995). The majority of ruminant maps are not well suited to this purpose. For example, 370 out of 504 microsatellites placed on the second-generation sheep map were also mapped in cattle (de Gortari *et al.* 1998), yet none of them are known to be conserved in humans. Thus, there is an urgent need for improved comparative maps that include both type II makers used to localize QTL and type I markers to provide a reference between ruminants and humans.

Two recent major breakthroughs have revolutionized cattle-human comparative mapping. First, a cattle-hamster radiation hybrid (RH) panel has been developed enabling researchers to determine the location and order of type I and type II markers (Womack *et al.* 1997). Second, tens of thousands of expressed sequence tags (ESTs) have been generated from cattle cDNA libraries and some hundreds of these have been mapped in RH panels. A new bioinformatics technique termed comparative mapping by annotation and sequence similarity or COMPASS (Ma *et al.* 1998; Band *et al.* 2000; Ozawa *et al.* 2000) uses the basic local alignment search tool (BLAST) algorithm to locate the human ortholog of a cattle EST sequence in public databases. Band *et al.* (2000) report

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[1]*Corresponding author:* AgResearch, Invermay Agricultural Centre, Puddle Alley, Mosgiel, Private Bag 50034, New Zealand.
E-mail: jon.slate@agresearch.co.nz.

a 95% concordance between COMPASS-predicted location and RH map location for over 300 cattle ESTs.

While the recent progress made in cattle genomics has been impressive, other artiodactyl maps predominantly comprise type II markers (*e.g.*, sheep, goats). Efforts are being made to map comparative markers in other species (Schibler *et al.* 1998), but RH panels are currently available for cattle only. An alternative method for mapping type I markers is to utilize interspecific crosses, whereby genes are likely to be polymorphic in backcross or F_2 pedigrees—an approach successfully used to map the mouse genome with many thousands of type I and type II loci during the 1990s (Copeland *et al.* 1993; Dietrich *et al.* 1995). The ability of deer species to hybridize is unparalleled among ruminants, and interspecies hybrids between red deer (*Cervus elaphus*) and Père David's deer (*Elaphurus davidianus*) have previously been identified as an excellent mapping resource (Tate *et al.* 1995). Although the two species are genetically divergent, they do share the same number of chromosomes and very similar karyotypes (Tate *et al.* 1995), suggesting that chromosomal rearrangements are unlikely to distort a map. Human gene probes have been demonstrated to detect variable restriction fragments in hybrid deer, enabling the mapping of type I loci. Furthermore, primers for microsatellites isolated in other ruminants frequently amplify their deer orthologs, so that type II markers can also be mapped in deer and their location can be compared with that in cattle and sheep (Slate *et al.* 1998).

In this article we present a linkage map derived from red deer × Père David's deer interspecies hybrids. Type I and type II loci are included in the map, making it a valuable resource for a variety of purposes. The inclusion of type I markers allows comparison with the human and cattle genomes and also with some model organisms. In contrast the inclusion of type II markers enables comparison with other ruminants currently lacking type I marker maps (*e.g.*, sheep). Both classes of marker are suitable for QTL detection within the mapping pedigree (Goosen *et al.* 1999, 2000), while type II markers will also lend themselves to QTL detection in farmed and wild red deer populations (Slate *et al.* 1999). Additionally, it is shown that bovine and ovine ESTs can be reliably mapped in deer by COMPASS and linkage mapping. Finally, shared marker order between the deer, cattle, sheep, and human genomes is used as a phylogenetic character to present a hypothesis for the evolution of ruminant chromosomes.

MATERIALS AND METHODS

Generation of mapping pedigrees

Seven F_1 Père David's deer × red deer sires were mated (predominantly by artificial insemination) to 267 red deer dams between 1989 and 1995 on two New Zealand deer farms and at Invermay Agricultural Centre. A total of 351 backcross calves were produced, with the number of progeny per stag ranging between 16 and 143. Blood sampling and DNA extraction protocols are described elsewhere (Tate *et al.* 1995). Restriction fragment length variants (RFLVs), protein loci, and 15 of the microsatellites were typed for all 351 progeny, while the remaining markers were typed in a reduced subset of 89 progeny (sired by two of the F_1 stags).

Markers typed

Five classes of marker were typed in the mapping herds. Protocols are described below.

Type I markers: *Protein polymorphisms:* Sixteen protein polymorphisms, showing fixed differences between red deer and Père David's deer were typed using methods described in Tate *et al.* (1992, 1995).

Restriction fragment length variants: We use the term RFLV rather than restriction fragment length polymorphism as these markers tend not to be polymorphic within either parental species. Protocols for typing RFLVs are given elsewhere (Tate *et al.* 1995). Briefly, probes for 149 known mammalian genes were obtained from a variety of sources (see supplementary material at http://www.genetics.org/supplemental). DNA from each animal was cut using six different restriction enzymes. Probes were hybridized to filters containing restricted DNA from three Père David's deer, an F_1 sire, and three red deer, and restriction enzymes giving fixed differences between the two species were identified. The panel of backcross individuals and their sires were screened by the above method using the appropriate restriction enzyme.

ESTs: Ruminant (mainly bovine and ovine) libraries containing cDNAs constructed for EST sequencing were obtained from a variety of sources (see supplementary material at http://www.genetics.org/supplemental). ESTs were sequenced and mapped by linkage mapping and by COMPASS as follows.

DNA sequencing and annotation: Samples were single-pass sequenced using either the T3 (5′-AATTAACCCTCACTAAAGGG-3′)/T7 (5′-GTAATACGACTCACTATAGGGC-3′) or Forward (5′-GTAAAACGACGGCCAGT-3′)/Reverse (5′-GGAAACAGCTATGACCATG-3′) primers on an ABI 377 DNA Sequencer (Perkin-Elmer, Norwalk, CT; Applied Biosystems International, Foster City, CA). Primer sequences were edited from the sequence data prior to comparison with nonredundant sequences in GenBank (http://www.ncbi.nlm.nih.gov/), the human genome working draft (http://www.ncbi.nlm.nih.gov/BLAST/), and the Ensembl human genome working draft "golden path" (http://www.ensembl.org/perl/blastview) using the BLASTN algorithm (Altschul *et al.* 1997). Sequences were tested additionally for similarity against each other. Sequences with strong similarity to mitochondrial DNA or ribosomal DNA were identified as potentially representing DNA contaminants in the cDNA libraries (Ma *et al.* 1998). The name, gene symbol, and GenBank accession number of any sequence matching the test sequence with a threshold expected (E) value of $< e^{-5}$ was noted and considered as a candidate ortholog of the test sequence. The human map location of all potential orthologs was then identified from the UniGene database (http://www.ncbi.nlm.nih.gov/UniGene/), and from comparative maps the expected location in cattle and deer was noted. Map location was predicted with one of three confidence criteria. Where the EST matched only one database sequence with a score of e^{-30} or lower, the database sequence was regarded as a likely ortholog. If more than one database sequence gave similarity scores of e^{-30} or lower, then it was assumed that one hit represented the true ortholog of the EST and the other hits represented paralogs or pseudogenes. Finally, ESTs with a best hit of between e^{-5} and e^{-30}

were regarded as providing tentative evidence for orthology. In addition to using COMPASS to predict the map location of the ESTs, we also mapped them as follows.

Probing of ESTs on filters: Filters from the reduced mapping panel (of 89 progeny) were prepared using the restriction enzymes *Msp*I or *Taq*I and by following a protocol similar to that used to map RFLVs. ESTs were amplified using RedHot Taq (Innovative Sciences). Those giving single bands of the estimated correct size were purified using a High Pure PCR product kit (Roche). Purified product (25 ng) was denatured in a boiling water bath for 5 min and then cooled on ice. The product was then labeled using the RTS RadPrime DNA labeling system (Life Technologies), according to the manufacturer's instructions. Repetitive DNA sequences were blocked on the filters using denatured fish sperm DNA (Roche) prior to hybridization of probes overnight at 63°. Filters were washed for 15 min and 30 min in a solution of 2× SSC, 0.1% SDS, followed by a 10-min wash with 0.5× SSC, 1.0% SDS. Washed filters were exposed to X-OMAT-AR autoradiography film (Kodak) for 1–7 days.

Type II markers: *Microsatellite markers:* Primers for 173 ruminant-derived microsatellite loci were typed, of which 129 loci had been cloned initially in cattle, 19 in sheep, 7 in wapiti (*C. elaphus canadensis*), 9 in red deer (*C. elaphus*), 4 in caribou (*Rangifer tarandus*), 2 in white-tailed deer (*Odocoileus virginianus*), 2 in mule deer (*O. hemionis*), and 1 in gazelle (*Gazella gazella*). Microsatellites were amplified in a 96-well thermocycler (Hybaid) and labeled by direct incorporation of [α-^{32}P]dCTP. Products were run out on a 6% polyacrylamide gel for ~2 hr. More detailed descriptions can be found in Slate *et al.* (1998) and in the supplementary material at http://www.genetics.org/supplemental.

One set of primers (BM1311) amplified what appeared to be more than one microsatellite locus in deer. Thus a total of 174 loci were scored in the mapping panel. Additionally a randomly amplified polymorphism appeared to coamplify with locus VH110.

Amplified fragment length polymorphisms: Genomic DNA (0.5 μg) was double digested at 37° for 3 hr with 5 units each of *Mse*I and *Eco*RI in 5× RL buffer (0.5 M TrisHAc, 0.5 M MgAc, 1.0 M KAc, 1.0 M dithiothreitol, 20 mg/ml BSA). *Eco*RI (biotinylated) and *Mse*I adapters were ligated to the digested DNA at 37° overnight in a reaction mix of 5× RL buffer, 10 mM ATP, T4 ligase, *Eco*RI, and *Mse*I. Following ligation of adapters, a preselective PCR step was performed. Primers were designed to recognize the restriction site plus adapter sequence *plus* an additional adenine base. A 43-cycle touchdown PCR program was performed with the annealing temperature lowered from 59.0° to 50.0° in 0.7° increments every cycle. The final 30 cycles were all at 50°. Product from the preselection reaction was then amplified using primers designed to recognize the restriction site, adapter, plus an additional three to five bases (of which the first was adenine). The PCR protocol was similar to that used for the preselective PCR, except annealing temperatures underwent a touchdown from 69.0° to 60.0° and the *Eco*RI primer was labeled with [γ-^{33}P]dATP. Final PCR products were run on a 6% polyacrlyamide gel for 2 hr. A 10-bp size ladder was run on all gels to size amplified fragment length polymorphisms (AFLPs). Gels were exposed to Kodak X-OMAT-AR film for 1–7 days.

Construction of the linkage map

A linkage map was constructed using the backcross option of MapMaker V3.0 (Lander *et al.* 1987). Initially, markers were assigned to a linkage group using the ASSIGN command and a LOD threshold of 3.0. For rare cases of a marker being assigned to more than one linkage group at LOD ≥ 3.0, then LOD scores were compared. If the difference in LOD scores for the two linkage groups was 3.0 or greater, the marker was assigned to the group with the higher LOD. Marker order on each linkage group was inferred using the THREE POINT (with LOD set to 3.0) and ORDER (thresholds set to LOD = 3.0 and LOD = 2.0) commands. During initial marker order construction the ERROR DETECTION option was set to 1%. A map was constructed for each linkage group using the marker order that gave the highest likelihood. We then used the GENOTYPE option to identify any double recombinants and rechecked the autoradiograms/gels to detect any potential typing errors. Any marker with multiple progeny showing double recombinants with flanking markers that were within 10 cM was removed from the map, and the process was repeated. These markers were considered as candidates for high error rates (for example, due to the Père David's sire allele also being present in the red deer dams), such that their inclusion may have inflated estimates of the total map length. The final map was constructed using the MAP command and ERROR DETECTION set to OFF. The Kosambi mapping function was used to calculate map distances. With a relatively large number of markers on each linkage group it is almost certain that the best two marker orders will be separated by LOD scores of <3.0. However, we also determined the most dense map that could be constructed with order supported by a LOD ≥ 3.0. Markers were successively added to their linkage group in order of informativeness (number of scored progeny) and assigned a location using the TRY option.

Inference of the Pecoran ancestral karyotype

Regions of conserved synteny and marker order between deer and cattle, sheep, human, or mouse were identified, with locus information obtained from the following databases.

Cattle: ArkDB cattle mapping database (http://www.ri.bbsrc.ac.uk/cgi-bin/arkdb/); MARC cattle genome mapping project (http://www.marc.usda.gov/genome/cattle/cattle.html); Bovine Genome database (http://bos.cvm.tamu.edu/bovgbase.html/)

Sheep: ArkDB sheep mapping database (http://www.ri.bbsrc.ac.uk/cgi-bin/arkdb/)

Human: NCBI Unigene resources (http://www.ncbi.nlm.nih.gov/UniGene/)

Mouse: University of Southampton, UK, Genetic Location Database (http://cedar.genetics.soton.ac.uk/public_html/ldb.html); Jackson Laboratory, USA Mouse Genome Database (http://www.informatics.jax.org/searches/mapdata_form.shtml)

All conserved loci met at least one of the stringent criteria for gene homology recommended by the Comparative Gene Mapping Committee (Andersson *et al.* 1996). The order of microsatellite loci was very highly conserved between deer and other ruminants, such that the cattle and sheep homologs of each deer linkage group were readily identified. Thus, it was possible to predict the human homolog of each deer linkage group by reference to the cattle-human comparative map (Band *et al.* 2000). When defining conserved regions between deer and human or mouse, a number of criteria were met. First, where an RFLV probe hybridized to several deer loci, none of the loci were initially declared as orthologs of any locus in another species. However, where additional loci were linked to one of the RFLV loci both in deer and in a comparative species, then orthology was assumed and any other polymorphisms derived from the same probe were regarded as deer paralogs. A small proportion of RFLVs and a greater number of ESTs mapped to a different location in deer to that pre-

dicted from available comparative maps. This was not regarded as evidence for previously undiscovered conserved synteny between ruminants and other species unless supported by more than one marker. While such an approach may result in the failure to detect a number of novel conserved regions between ruminants and humans or mice, we regard this conservative method as preferable to wrongly inferred homology.

By identifying homologous regions in three ruminant species and two outgroups (human and mouse) it was possible to infer the evolutionary history of ruminant chromosomal rearrangements and thus predict the karyotype of the Pecoran ancestor. The principle of Dollo parsimony, widely applied to restriction site data (Swofford *et al.* 1996), was used to infer ancestral gene orders. It was assumed that a particular gene order was sufficiently complex to arise only one time, and if shared by two species then it was also present in their common ancestor. The human order was favored as an outgroup in the parsimony analysis to maximize the number of comparative markers. However, locus order in mouse was also used to resolve ambiguous cases.

RESULTS

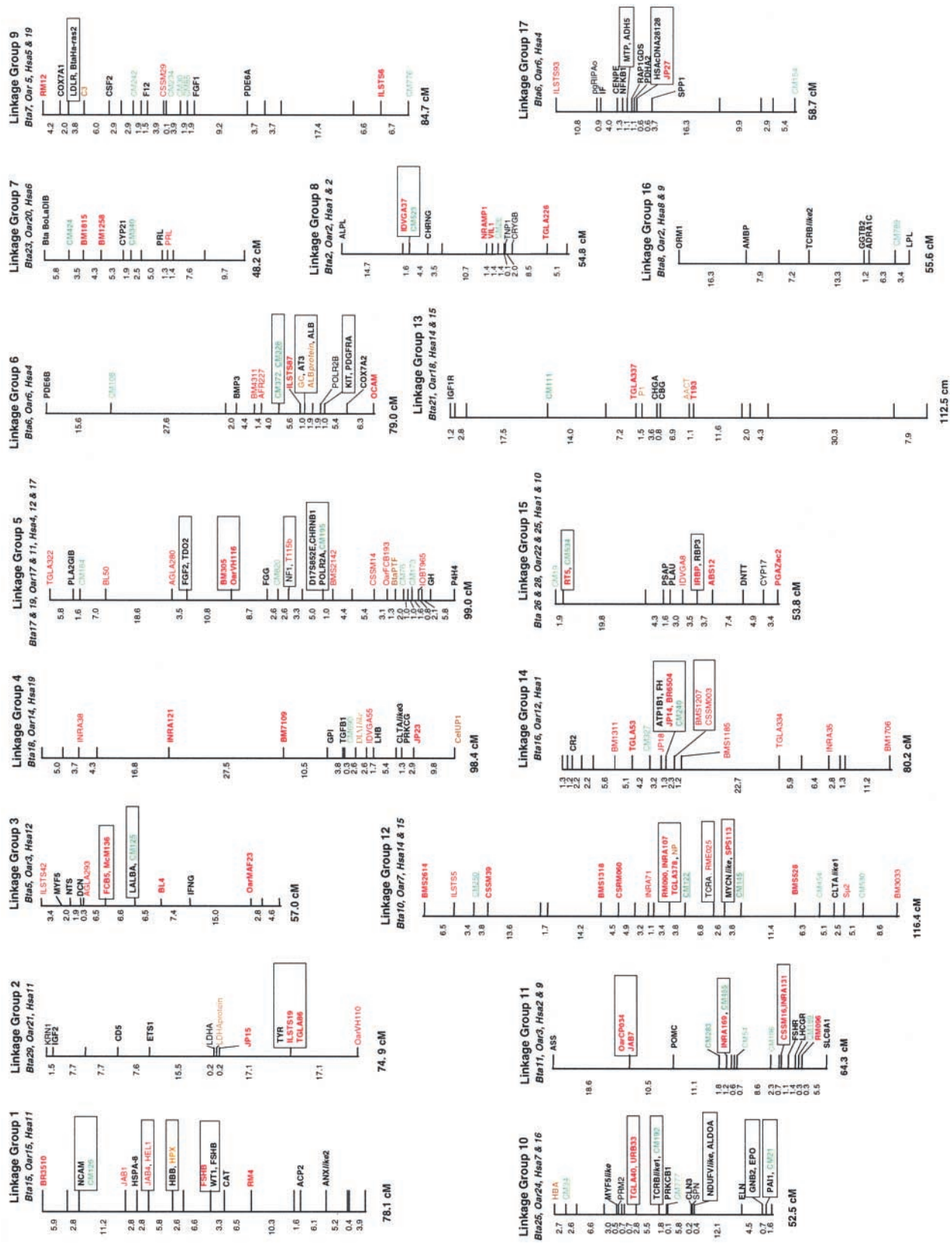
Map summary: The linkage map is shown in Figure 1. Linkage groups are assigned using the notation of Tate (1997) to retain consistency with previously described QTL mapping experiments (Goosen *et al.* 1999, 2000). Currently there is no physical map of red deer, and karyotypes have not been aligned with humans. However, the red deer (and Père David's deer) karyotype of $2n = 68$ (Herzog 1987) is identical to the number of linkage groups derived in this study. Thus, while the term chromosome is not used on the map, a one-to-one correspondence between each linkage group and a deer chromosome is probable. In total 621 markers were mapped, of which 229 were AFLPs, 153 microsatellites, 150 RFLVs, 73 ESTs, and 16 proteins. An additional 93 markers (44 AFLPs, 21 microsatellites, 8 RFLVs, and 20 ESTs) were assigned to a linkage group but not positioned relative to other markers. Ten markers were not assigned to any chromosome, of which 8 were typed in fewer than 50 individuals. As no recombination events were identified between some markers there was a total of 439 intervals. The autosomal map was 2532.3 cM long with a mean interval length of 5.77 cM. Only 9 intervals were greater than 20 cM. The pseudoautosomal region of the Y chromosome was 18.4 cM long and included 8 markers.

Comparison with human and ruminant maps: The majority of the 153 mapped microsatellites had previously been mapped in at least one other ruminant. Eighty-five were previously mapped in sheep (de Gortari *et al.* 1998; Maddox *et al.* 2001) and cattle (Ma *et al.* 1996; Barendse *et al.* 1997; Kappes *et al.* 1997; Band *et al.* 2000). Forty-one were mapped in cattle only and 11 were mapped in sheep only. The remaining 16 loci were cloned from various deer or gazelle species and were not previously mapped in any species. The 149 RFLV probes hybridized to 158 polymorphisms (*i.e.*, some probes gave more than one polymorphism). Among the 158 RFLVs, 88 were mapped previously in both humans and cattle, 67 were mapped in humans only, and 1 was mapped in cattle only. Human and cattle map locations were unavailable for a further 2 RFLVs. Among the 16 protein polymorphisms, 5 were mapped previously in cattle and humans, 9 were mapped previously in humans only, 1 was mapped in cattle only, and the remaining polymorphism was mapped in no other species. The AFLP markers were all unique to the deer map. Thus a total of 169 deer map markers were additionally mapped in humans and 221 were mapped in cattle.

There was a striking degree of conserved synteny between deer and both humans and cattle. Furthermore, marker order was very highly conserved between cattle and deer. For every deer linkage group it was possible to identify the bovine, ovine, and human homolog(s). In total 37 groups of conserved synteny were identified in the deer and cattle maps and 48 conserved synteny groups were found in the deer and human gene maps (Table 1). All but 12 markers were consistent with this model. These "orphan" markers are of special interest because they represent either errors in mapping or assignment of orthologs or, alternatively, new unrecognized areas of conserved synteny.

Five microsatellite markers mapped to locations other than that predicted from their cattle location. The markers were BM4005 (mapped to LG15; expected location, LG10), CSSM65 (mapped to LG18; expected location, LG5), OCAM (mapped to LG6; expected location, LG2), McM136 (mapped to LG3; expected location, LG27), and CSSM26 (mapped to LG33; expected location, LG24). All five markers were assigned to a linkage group on the basis of reasonably high two-point LOD scores (9.28, 4.70, 15.96, 7.99, and 11.74, respectively), although the position of the first two markers relative to other linked markers could not be accurately determined. As all of the markers except McM136 were previously positioned on at least two independent ruminant maps, there is little reason to suspect any error in the expected location. CSSM26 may be correctly mapped in deer, as there is some evidence (see discussion) that LG33 (homologous to Bta2 and Hsa2) also contains

Figure 1.—The red deer genetic map. Linkage groups 1–33 and the pseudoautosomal region of the Y chromosome are shown. Map distances are given in Kosambi centimorgans. RFLVs for gene probes are black, ESTs green, microsatellite loci red, and protein loci brown. Anonymous AFLPs are represented by unlabeled horizontal bars. Where marker order was determined with support LOD ≥ 3.0, the relevant markers are shown in boldface type. Underlined ESTs represent markers where the map location and predicted location from COMPASS were concordant. A more detailed figure can be found as supplementary information on the journal's website at http://www.genetics.org/supplemental.


Linkage Group 1
Bta15, Oar15, Hsa11
78.1 cM
Linkage Group 2
Bta29, Oar21, Hsa11
74.9 cM
Linkage Group 3
Bta5, Oar3, Hsa12
57.0 cM
Linkage Group 4
Bta18, Oar14, Hsa19
98.4 cM
Linkage Group 5
Bta17 & 19, Oar17 & 11, Hsa4, 12 & 17
99.0 cM
Linkage Group 6
Bta6, Oar6, Hsa4
79.0 cM
Linkage Group 7
Bta23, Oar20, Hsa6
48.2 cM
Linkage Group 8
Bta2, Oar2, Hsa1 & 2
54.8 cM
Linkage Group 9
Bta7, Oar 5, Hsa5 & 19
84.7 cM
Linkage Group 10
Bta25, Oar24, Hsa7 & 16
52.5 cM
Linkage Group 11
Bta11, Oar3, Hsa2 & 9
64.3 cM
Linkage Group 12
Bta10, Oar7, Hsa14 & 15
116.4 cM
Linkage Group 13
Bta21, Oar18, Hsa14 & 15
112.5 cM
Linkage Group 14
Bta16, Oar12, Hsa1
80.2 cM
Linkage Group 15
Bta 26 & 28, Oar22 & 25, Hsa1 & 10
53.8 cM
Linkage Group 16
Bta8, Oar2, Hsa8 & 9
55.6 cM
Linkage Group 17
Bta6, Oar6, Hsa4
58.7 cM

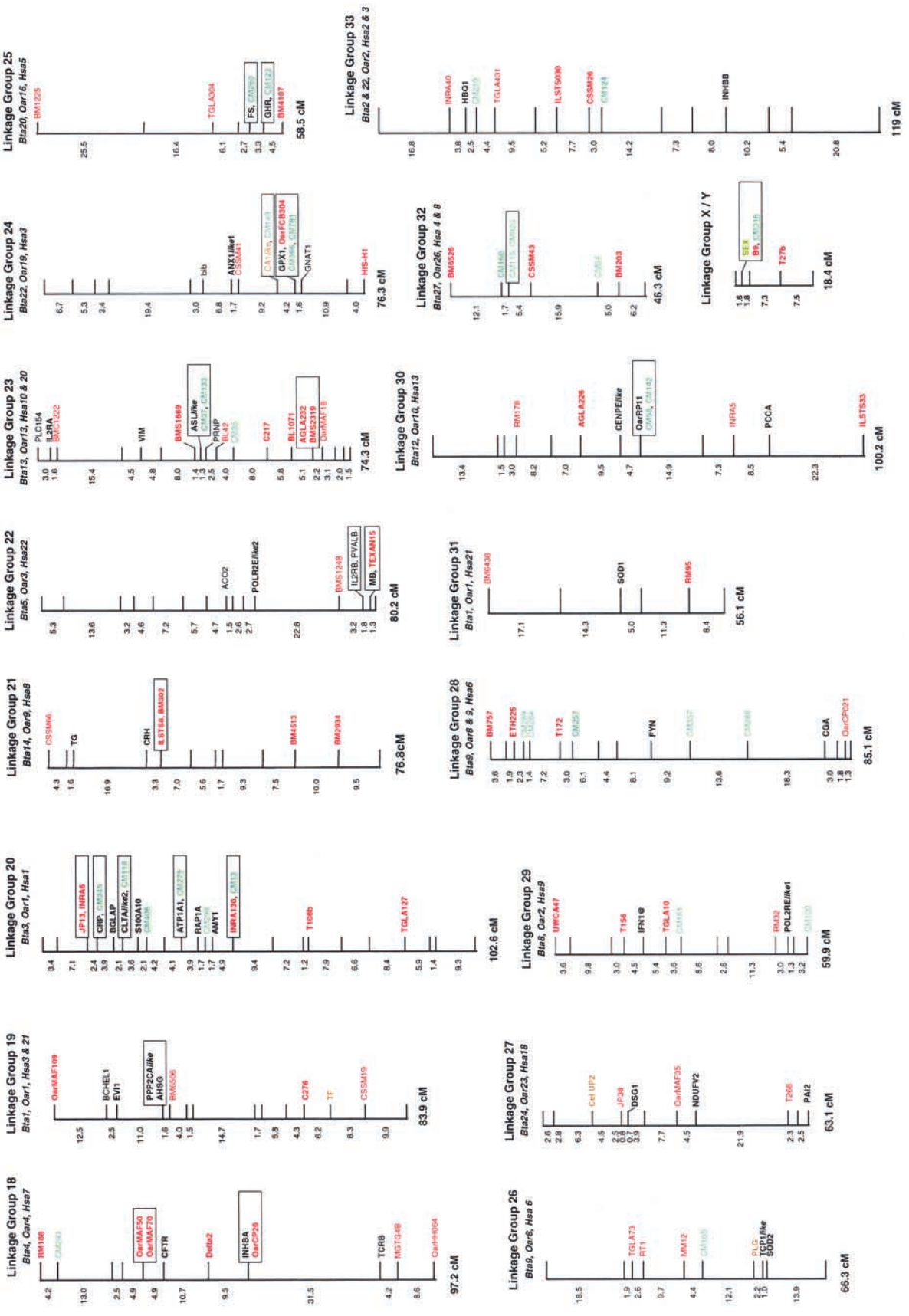


FIGURE 1.—*Continued.*

TABLE 1

Conserved synteny between the Pecoran ancestor and the deer, sheep, cattle, and human genetic maps

Pecoran chromosome	Cattle homolog	Bovid lineage	Sheep homolog	Sheep lineage	Deer homolog	Deer lineage	Human homolog
A1	3		1	Fusion	20		1
A2	1		1		19, 31	Fission and inversion	3, 21
B1	8		2	Fusion	16, 29	Fission	8, 9
B2	2		2		8, 33	Fission and translocation[a]	1, 2, 3
C1	11		3	Fusion	11		2, 9
C2	5		3		3, 22	Fission	12, 22
D	4		4		18		7
E	7		5		9		5, 19
F	6		6		6, 17	Fission	4
G	10		7		12		14, 15
H1	9		8, 9	Translocation	26, 28	Fission	6
H2	14		9		21		8
I	12		10		30		13
J	13		13		23		10, 20
K	16		12		14		1
L	15		15		1		11
M	18		14		4		16, 19
N1	17		17		5	Fusion	4, 12, 22
N2	19		11		5		17
O	20		16		25		5
P	21		18		13		14, 15
Q	22		19		24		3
R	23		20		7		6
S	24		23		27		18
T	25		24		10		7, 16
U	26, 28	Fission	22, 25		15		1, 10
V	27		26		32		4, 8
W	29		21		2		11
X	X		X		X		X

Each inferred Pecoran ancestral chromosome is shown, along with the homologous chromosomes in sheep, cattle, deer, and human. The major chromosomal rearrangements in the lineages leading to bovids (cattle and sheep), sheep, and deer are indicated.

[a] Two markers indicate that a small region of LG33 is syntenic with Bta22 and Hsa2. This translocation in the deer lineage is considered tentative.

a small region homologous to Bta22 and Hsa3. Until independently confirmed, the location of these microsatellites must be considered putative only.

Four RFLV markers mapped to a different linkage group than predicted from their location in humans. These markers (ASL, MYCN, PPP2CA, and TCP1) were given the suffix "-*like*" to denote the inconsistency in comparative locations. Seven RFLV probes produced two or more mappable polymorphisms. One of the polymorphisms mapped to the expected location for four of these loci (MYF5, NDUFV, TCRB, and CENPE). The other polymorphism (*i.e.*, the one that did not map to the expected location) was suffixed with *like*. Polymorphisms produced by the remaining three probes (ANX1, CLTA, and POLR2E) all failed to map to the expected location and were suffixed with *like*1, *like*2, etc.

COMPASS of EST sequences: COMPASS was performed on 93 mapped ESTs in the deer pedigrees. Three of the sequences showed strong homology with ruminant mitochondrial DNA and a further 6 with ribosomal DNA. Eleven sequences did not show significant homology [expected probability (E) $> e^{-5}$] with any sequence in the public databases. Thus a total of 73 sequences were mapped and showed strong homology with database sequence. There was agreement between the COMPASS predicted location and the actual linkage map position for 50 (68.5%) of the sequences. In 29/50 (58.0%) of cases the sequence from the predicted location was either the only hit or best hit *and* had an $E < e^{-30}$. Two ESTs gave significant BLAST hits with database sequence from the expected location at $E < e^{-30}$ but were not the best hit. Nineteen ESTs had BLAST hits with database sequence from the expected location, but with a score of $E < e^{-5}$ rather than $E < e^{-30}$. Of the 23

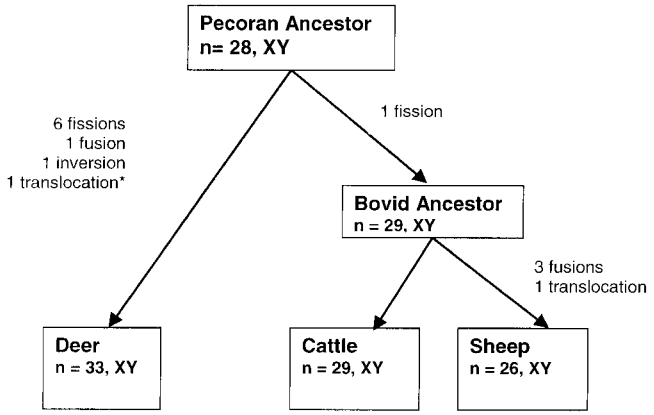


FIGURE 2.—A summary of the evolution of ruminant karyotypes. Cervidae (deer) and Bovidae (cattle, sheep, and goats) are all placed within the higher ruminants (suborder Pecora). Deer first appeared in the fossil record about 20 mya, while sheep and cattle appeared 14 and 7 mya, respectively (SCOTT and JANIS 1993). The major chromosomal rearrangements hypothesized to have occurred between the Pecoran ancestor and the present day sheep, cattle, and deer are shown. *, translocation inferred on the basis of two markers (CSSM26 and CM124).

ESTs that did not map to the location predicted by COMPASS, 11 had hits with a single database entry while another 12 had hits with multiple database entries. It seems probable that some of the discrepancies between the predicted and actual map locations can be attributable to EST probes hybridizing to unknown paralogs of the gene predicted by COMPASS.

Evolution of ruminant chromosomes: Although marker order has been used to identify chromosomal rearrangements during the evolution of individual cattle (GAO and WOMACK 1997; YANG and WOMACK 1998) and pig (JOHANSSON *et al.* 1995) chromosomes, we are unaware of this technique being applied at the genome-wide level. Likewise, comparative ruminant maps have been used to predict the number of rearrangements between ruminant and human genomes (*e.g.*, SCHIBLER *et al.* 1998), but no attempt has been made to determine the actual rearrangement events or in which lineage they occur. We used parsimony analysis of marker order to infer the major rearrangements that have arisen in the cattle, sheep, and deer lineages since a common Pecoran ancestor. The hypothesized Pecoran ancestral chromosomes and their subsequent rearrangements are described in Table 1 and Figure 2, with human, cattle, sheep, and deer homologous chromosomes indicated. The three most complex situations to arise in the parsimony analysis are briefly described as follows.

Pecoran chromosome A2: It is well established that cattle chromosome 1 is homologous to ovine chromosome 1q (CRAWFORD *et al.* 1995; DE GORTARI *et al.* 1998). Comparative markers used in this study and elsewhere (BAND *et al.* 2000) show that the equivalent regions in deer are LG19 and LG31 and in humans are Hsa3 and Hsa21, while the mouse homolog is a single chromosome (Mmu16). However LG19 shows conserved synteny with both Hsa3 *and* Hsa 21. Thus, the most parsimonious state for a mouse/primate/artiodactyl ancestor is a single chromosome, which underwent independent fissions in the human lineage and the deer lineage. The alternative possibility—an ancestral state of two chromosomes—requires two subsequent fusions and a translocation and is considered less likely.

Pecoran chromosome C2: Cattle chromosome 5 is homologous to sheep chromosome 3q. Again, the homologous regions in both deer (LG3 and LG22) and humans (Hsa12 and Hsa22) are represented by two linkage groups. LG3 is homologous to Hsa12 and LG22 is homologous to Hsa22. No markers on deer LG3 are located on Hsa22 and no markers on deer LG22 are located on Hsa12, suggesting that two chromosomes may represent the ancestral state. However, comparison of the deer and mouse maps reveals that ACO2 (deer LG22) maps to Mmu10, as do the comparative markers on deer LG3. Thus, it appears likely that the human/mouse/artiodactyl ancestral state was a single chromosome. This ancestral state was retained by the Pecoran ancestor (C2) and present-day bovidae, while separate fission events have occurred in the lineages leading to rodents, primates, and latterly deer. Note that in this and the previous example the comparison of ruminant gene order with two outgroups resulted in a different outcome than if humans were used as a sole outgroup.

Pecoran chromosomes H1 and H2: It is known that Bta9 is homologous to Oar8 and a small segment of Oar9, while Bta14 is homologous to the remainder of Oar9 (CRAWFORD *et al.* 1995; DE GORTARI *et al.* 1998). Goats appear to share the same arrangement as sheep (VAIMAN *et al.* 1996; SCHIBLER *et al.* 1998). Deer LG26 and LG28 are homologous to Bta9 while deer LG21 is homologous to Bta14. Deer LG28 contained microsatellites that mapped to Oar8 and Oar9, confirming that the bovine marker order is ancestral and that sheep/goat marker order is derived. The simplest hypothesis is that a translocation occurred in the sheep/goat lineage following divergence from cattle. Note that this is separate from the fission in the deer lineage leading to LG26 and LG28.

It has previously been suggested that modern cattle share the same chromosome number ($n = 29$) as the Pecoran ancestor (GALLAGHER *et al.* 1994). However, our analysis demonstrates the Pecoran ancestral karyotype contained 28 autosomal pairs. Deer LG15 is homologous to Hsa10, Bta26 and -28, and Oar22 and -25. Thus the ancestral state is a single chromosome that underwent a fission in the bovid lineage prior to the divergence of cattle and sheep. This fission would have remained undetectable without the availability of the deer map and serves as a good illustration of the increased resolution of comparative mapping when multiple species are used.

Deer LG33 provides another unusual feature of ruminant chromosome evolution. Although translocations appear to have been infrequent during the divergence of ruminant species, a small segment of deer LG33 including markers CSSM26 and CM124 is homologous with Bta22 and Hsa3 (otherwise homologous to LG24). This possible translocation has not been observed between cattle and other ruminants or between humans and any ruminant, suggesting its occurrence during the evolution of the deer lineage.

DISCUSSION

The deer (Cervinae) genetic map and associated mapping herd provides a new resource for gene discovery and comparative genomics in artiodactyls. It has already been used to map QTL for morphometric (Goosen *et al.* 1999) and seasonality traits (Goosen *et al.* 2000) that may be segregating in other ruminant species. However, a potentially greater use may be as a tool to aid the mapping of candidate genes in more economically important species. Mapping type I loci (genes) is problematic in most ruminant species, as RFLVs derived from human or mouse probes tend to be invariant in the mapping population. Although this can be overcome by radiation hybrid mapping in cattle, this represents a single resource that has limited availability. The wide interspecies hybrid described here provides a second ruminant resource for the rapid mapping of any gene for which a probe is available. Location of the gene can then be predicted in other ruminants by comparison with the red deer map—an approach already employed to determine gene order on sheep chromosome 24 (Broom *et al.* 1996).

At 2532 cM the red deer autosomal linkage map is shorter than recent maps of cattle (3532 cM, Barendse *et al.* 1997; 2990 cM, Kappes *et al.* 1997) and sheep (3063 cM, de Gortari *et al.* 1998) and of similar length to the goat linkage map (~2500 cM, Vaiman *et al.* 1996). There are several possible reasons for the map being shorter than that of cattle and sheep. First, it is possible that substantial regions of the deer genome are yet to be mapped. However, comparison with the cattle maps reveal few, if any, chromosomal segments mapped in cattle without a mapped homologous region in deer. Second, it is possible that recombination rates are lower in the deer interspecific hybrid than in other ruminant mapping populations. A similar phenomenon has been suggested in cattle (Yang and Womack 1998) and mouse interspecific hybrids (Hammer *et al.* 1989). Third, the map was derived from male F_1 animals, whereas the majority of other ruminant linkage maps are sex averaged (Barendse *et al.* 1997; Kappes *et al.* 1997; de Gortari *et al.* 1998). There is considerable evidence that recombination rates are lower in males than in females in cattle (Beever *et al.* 1996; Kappes *et al.* 1997) and humans (Matise *et al.* 1994). Fourth, in an attempt to reduce the possibility of map inflation, a strategy was employed whereby markers causing more than one double recombination in a 20-cM interval were not included in the final map. While this approach may lead to the exclusion of a small number of genuinely informative markers it is conservative in that undetected genotyping errors will not contribute to the map length. In summary, we believe that our linkage map offers good coverage of the deer genome. Any regions that are suspected to have poor marker coverage can be mapped rapidly by typing microsatellite (or other) markers from the homologous cattle or sheep regions.

Recently, Band *et al.* (2000) described how COMPASS could predict the location of random cattle EST sequence with 95% accuracy. Here, the concordance between COMPASS and map locations was only 68.5%. There are several possible reasons for this discrepancy. First, Band *et al.* (2000) used selection criteria whereby partial cattle mRNA sequences were excluded from further analyses, as were any sequences that showed significant (expected threshold $\leq e^{-5}$) homology to multiple human paralogs. In this study no such distillation of candidate ESTs was made. Second, we used an alternative mapping strategy to that of Band *et al.* (2000). In that study the authors designed primers to type each EST in a cattle RH panel—an approach that should reliably amplify the intended target. However, we hybridized the ruminant (cattle or sheep) EST against deer filters. Thus, the EST probe may have hybridized to a paralog of the target gene, leading to a discrepancy between the map location and the predicted location. Third, the EST probe may have mapped to the "correct" location, but the relevant region of human-ruminant conserved synteny could remain, as yet, unidentified. Finally, Band *et al.* (2000) made just one "species jump" whereby a map location could be predicted provided the cattle and human sequences were sufficiently similar. In our study two species jumps are involved, as the EST sequence (cattle or sheep) is required to be similar to both human sequence (to predict map location) and also deer sequence (so that the probe will hybridize to the filter). Given all of the above constraints we believe that 68.5% represents an encouraging success rate for mapping ESTs derived from other ruminants to the anticipated region in deer.

Studies of chromosomal evolution (White 1978) have identified a recurrent trend in some lineages in which chromosome rearrangements of the same type tend to predominate. Possibly the most dramatic example lies within the ruminants, where repeated chromosomal fusions have resulted in the $2n = 6$ karyotype of the Indian Muntjac (Yang *et al.* 1997). In contrast, this study shows that the lineage from the Pecoran ancestor to the ancestral deer karyotype (Fontana and Rubini 1990) and to deer of the subfamily Cervidae is characterized by repeated chromosome fission (Table 1; Figure 2).

It is also noteworthy that five of the six Pecoran ances-

tral chromosomes that have undergone fission events in the deer lineage have also undergone independent fusion or translocation events in the sheep lineage. These results support a punctuated model of karyotypic evolution—in which repeated rearrangements of the same type occur for a period of time in a specific lineage. In addition, particular chromosomes may be more prone to rearrangement. In our reconstruction the lineage-specific rearrangements, namely fission in deer and fusion in sheep, are biased toward the larger chromosome arms in the Pecoran ancestor. The mechanisms driving karyotype evolution have been strongly debated (King 1993; Qumsiyeh 1994). Our results appear to be more easily explained by the mechanics of meiosis and chromatin causing particular types of rearrangement to be most frequent in specific lineages (Redi *et al.* 1990). The opposing view that there is selective pressure for fixation of specific mutations in different lineages (Qumsiyeh 1994) seems less likely to explain the lineage-specific mosaic of chromosomal rearrangements observed in the ruminants.

The red deer genome appears to have undergone fewer rearrangements relative to humans than to mouse. A total of 191 type I markers (RFLV and EST) were mapped in both human and deer and between them 66 segments of conserved gene order were identified. In contrast, only 138 markers were mapped in deer and mouse, yet 73 segments of conserved order were found. Thus our data support the assertion that rodent genomes have undergone more rearrangements than have other mammals (Schibler *et al.* 1998; Gellin *et al.* 2000). Previous studies have suggested that internal rearrangements in chromosomes homologous between artiodactyls and humans are relatively common (Johansson *et al.* 1995; Yang *et al.* 1997; Schibler *et al.* 1998; Band *et al.* 2000; Ozawa *et al.* 2000). This study confirms previous findings with deer LGs 2, 3, 5, 9, 10, 11, 14, 15, 18, 20, and 27 all exhibiting a different gene order to their human homolog.

In conclusion, the deer interspecific linkage map is a useful resource for livestock comparative mapping. The ortholog of any human or mouse gene can be mapped rapidly in deer, and the comparative location in economically important species can be inferred. Furthermore, gene order can be used as a character suitable for resolving the major chromosomal rearrangements that have occurred during the radiation of mammals over the previous 60–100 million years.

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