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Acceleration of X-chromosome gene order evolution in the cattle lineage

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The gene order on the X chromosome of eutherians is generally highly conserved, although an increase in the rate of rearrangement has been reported in the rodent lineage. Conservation of the X chromosome is thought to be caused by selection related to maintenance of dosage compensation. However, we herein reveal that the cattle (Btau4.0) lineage has experienced a strong increase in the rate of X-chromosome rearrangement, much stronger than that previously reported for rodents. We also show that this increase is not matched by a similar increase on the autosomes and cannot be explained by assembly errors. Furthermore, we compared the difference in two cattle genome assemblies: Btau4.0 and Btau6.0 (Bos taurus UMD3.1). The results showed a discrepancy between Btau4.0 and Btau6.0 cattle assembly version data, and we believe that Btau6.0 cattle assembly version data are not more reliable than Btau4.0. [BMB Reports 2013; 46(6): 310-315]

INTRODUCTION

The difference in the X-chromosome number between heterogametic and homogametic individuals requires some form of dosage compensation to ensure equal levels of X-linked gene activity. In mammals, dosage compensation occurs in the form of X inactivation. The X inactivation process is initially dependent on an RNA gene, Xist, located in the X inactivation center (XIC) (1). Xist is upregulated in the inactivated chromosome, and gene silencing then occurs in Cis along the chromosome. While Xist silencing may also happen on autosomes, it is most efficient on the X chromosome, leading to the suggestion that X inactivation is boosted by genomic elements on the X chromosome. Because LINE elements, and L1 elements in particular, are much more common on the X chromosome than on autosomes, it has been hypothesized that they serve as boosters of the X inactivation signal (2-4).

Ohno (5) suggested that the establishment of a dosage compensation mechanism in somatic cells of a mammalian ancestor may have conferred constraints on rearrangements between the X chromosome and autosomes during evolution. It was later shown that there is an almost identical set of genes on the X chromosome of many eutherian mammals (6-8). The gene order on the X chromosome is also highly conserved, with only rodents showing large changes in the gene order on the X chromosome (9-11). Comparative maps between cattle and human X chromosomes based on radiation hybrid mapping have shown a highly conserved gene order (12). However, Raudsepp *et al*. (11) reported several evolutionary rearrangements on the cattle X chromosome in the context of an analysis of the horse genome. Here, we estimated the rate of rearrangements on the X chromosome and on autosomes in the cattle lineage to evaluate the degree of conservation of the gene order in cattle. We show that the cattle lineage has experienced an acceleration in the rate of rearrangements on the X chromosome compared with other mammals. We also discuss possible implications of these findings.

Two assemble version in cattle which were Btau4.0 and Btau6.0 (UMD3.1) was many differences. Because of assemble group and method are clearly differences. as a result, Number of SNPs on Btau4.0 and Btau6.0 were different from 46,760 and 48,284 (13). Chromosome size was too striking differences. Among them, X-chromosome size on Btau4.0 and Btau6.0 were striking differences from 83Mbp and 136Mbp (14). Unknown chromosomes information in Btau4.0 was many involved in X-chromosome of Btau6.0 (15).

RESULTS AND DISCUSSION

The most spectacular gene order changes on the X chromosome among boreoeutherian species have occurred in the cow (Artiodactyla) lineage

As seen in Fig. 1, the gene order on the X chromosome of primates, dogs, and horses is conserved almost perfectly. This observation is consistent with previous reports describing the human X chromosome as identical to the putative ancestral mammalian X chromosome (16) and the X chromosome gene order of humans, dogs, and horses being highly conserved (17). As de-

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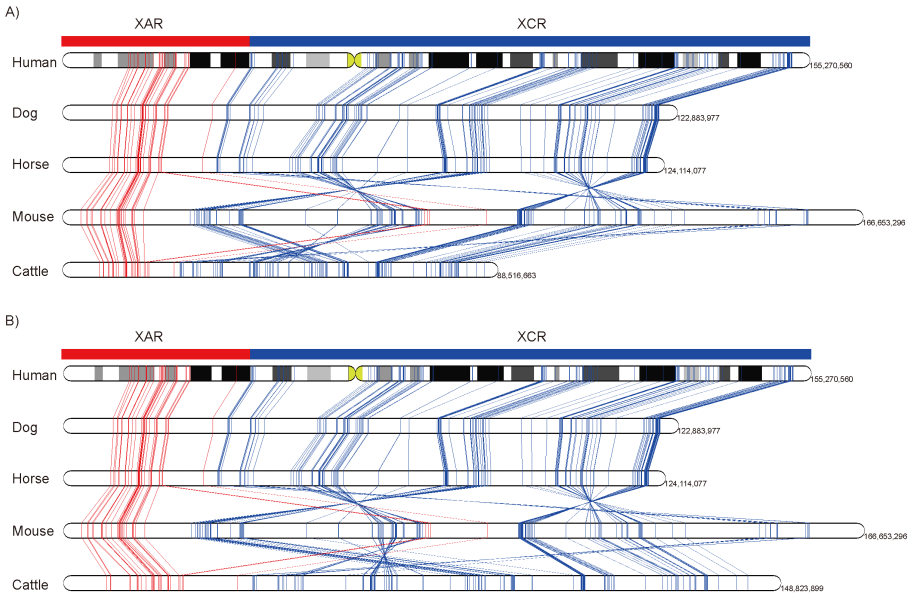

Fig. 1. Gene order of 169 concordant orthologous genes on the X chromosomes between Btau4.0 (A) and Btua6.0 (B). Red lines indicate orthologous genes in the X-added region (XAR), and the blue line indicates orthologous genes in the X-conserved region (XCR) according to annotations of the human chromosome. The number to the right of each chromosome represents the chromosomal length.

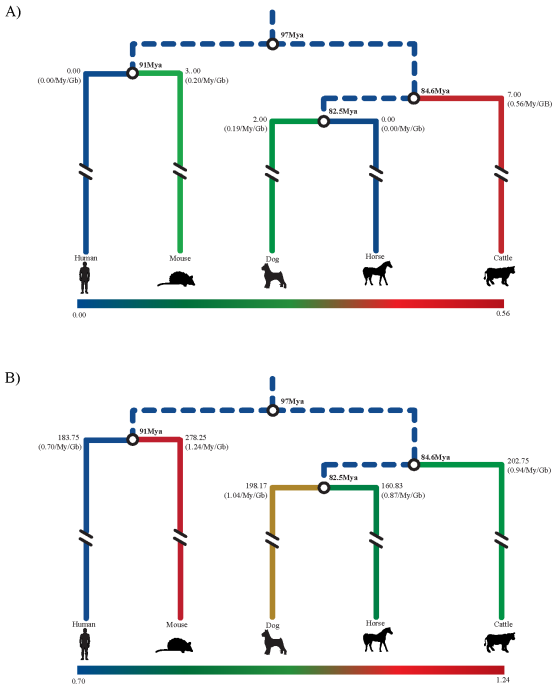

Fig. 2. Rates of unsigned gene order rearrangements on the X chromosomes (A) and autosomes (B) of five mammals. The evolutionary tree is based on the estimation of divergence times provided by the TIMETREE database (http://www.timetree.org/). Gene order distances are based on 169 and 10,196 orthologous gene sets of the mammals on the X chromosome and autosomes, respectively. Rates are depicted as a color gradient from minimum to maximum, and the color scale is shown at bottom. The number on each lineage is the parsimony-inferred number of rearrangements, and the rates are indicated in parentheses. The rate is estimated only for external lineages. Internal lineages are striped.

scribed previously (11, 18), the gene order in the rodent lineage is less conserved on the X chromosome. However, somewhat surprisingly, we found that the cattle lineage is the least conserved among the genomes examined herein. Using the parsimony estimate of the number of rearrangements for our set of markers divided by time as an estimate of rate, we found a rate of 0.56/MY/GB in cattle but a rate that varies between 0 and 0.2/MY/GB in the other species (Fig. 2A). Notably, a similar increase in the rate of rearrangements on autosomes was not observed in cattle (Fig. 2B). Analyses based on signed gene order provided similar X chromosome results in cattle, but not in mice (Supplementary Fig. 1A); and similar autosome results were found in cattle and mice (Supplementary Fig. 1B). The parsimony inferred number of rearrangements will always be an underestimate of the true number. This bias will be stronger for long lineages than for short lineages. However, because the cattle lineage is one of the longest lineages in the phylogeny, this bias cannot explain our results.

A more serious challenge to our results is genome assembly errors. The cattle genome has been sequenced for a male animal (XY), resulting in a sequencing coverage on the X chromosome of only half that of the autosomes. In addition, it is a relatively recently sequenced genome, possibly suggesting that quality might be a concern. To address this problem, we repeated our analyses of the X chromosome using only markers showing a concordant marker order in independently generated genetic and physical maps in cattle. As described in the Methods, we collected 51 concordant markers in terms of orders on linkage and physical maps (Table 1) of the cattle X chromosome. The physical map was generated independently of the linkage map (19) using a radiation hybrid physical map for scaffolding. Using these markers, we again identified a strong increase in the rate of rearrangement

Table 1. A list of the 51 concordant markers of the cattle X chromosome and their corresponding positions in the other mammals. Genome assembly versions of each species are the following: GRCh37 (human), NCBIM37 (mouse), BROADD2 (dog), EquCab2 (horse), and Btau4.0 (cow)

Linkage and physical map positions of cattle X markers			Physical map positions of the other mammals on its X chromosome			
Marker ID	Linkage (cM)	Physical (bp)	Human (bp)	Mouse (bp)	Dog (bp)	Horse (bp)
rs29025873	5.422	419,971	119,567,580	35,762,782	95,480,514	95,696,876
BM6017	6.952	786,886	120,329,845	36,654,163	96,132,843	96,251,754
rs29021780	9.592	2,460,957	122,446,267	38,886,111	97,918,994	97,944,644
rs29024122	13.809	3,020,874	124,344,404	40,790,693	99,639,939	99,533,412
rs29025415	16.436	4,784,486	126,431,537	42,915,524	101,633,382	101,072,231
rs29011414	16.436	5,038,118	126,726,250	43,354,785	101,976,833	101,302,431
rs29010851	18.063	5,991,644	128,445,408	45,051,291	103,635,962	102,756,766
rs29021239	18.954	6,954,481	129,364,024	45,914,211	104,428,272	103,498,632
rs29025917	20.167	7,183,793	130,763,581	48,085,329	105,796,350	104,686,153
rs29025766	22.369	7,587,919	131,308,862	48,324,985	106,168,682	105,042,162
rs29018753	23.998	7,641,801	131,390,738	48,405,132	106,240,240	105,110,581
rs29011997	26.131	8,175,239	132,532,178	49,503,950	107,261,007	106,054,548
rs29017358	35.478	13,069,035	138,700,993	57,379,741	112,661,136	111,131,060
rs29016998	37.046	13,957,313	137,567,579	56,202,263	111,630,518	110,179,432
rs29018113	37.824	15,013,426	141,625,523	60,221,333	114,959,524	113,302,976
BMS1616	38.407	15,388,296	142,262,413	60,974,404	115,528,768	113,841,166
rs29024291	44.526	18,995,391	147,626,248	66,657,221	119,816,429	117,497,090
rs29026580	49.285	23,363,314	153,110,913	71,094,649	124,713,385	122,288,053
rs29021817	52.896	33,009,399	104,511,617	134,770,450	81,788,342	83,042,456
rs29016901	52.896	33,110,101	104,397,823	134,678,137	81,668,366	82,956,487
rs29010811	57.314	34,945,661	107,738,609	137,946,798	85,107,080	85,818,681
rs29019516	57.626	35,776,137	108,858,139	138,720,424	85,903,879	86,518,275
BMS417	57.97	36,022,028	109,092,844	138,927,521	86,123,679	86,731,813
rs29010062	61.177	38,493,795	111,404,982	141,235,588	88,285,080	88,753,545
rs29011155	61.595	39,160,426	111,888,929	141,746,623	88,678,192	89,102,247
rs29012094	61.868	39,452,115	113,162,596	142,878,086	89,871,034	90,173,247
rs29024659	62.61	46,532,067	73,750,952	100,895,440	60,910,480	55,750,418
rs29017241	62.611	47,203,464	73,053,165	100,665,953	60,385,830	55,237,682
rs29017231	62.611	47,333,160	72,904,424	100,588,756	60,309,261	55,130,193
rs29022288	68.375	53,017,688	51,215,660	5,747,095	43,881,291	41,165,104
rs29016346	68.375	55,026,301	49,557,130	7,024,993	42,511,898	39,859,644
rs29013824	70.55	61,365,621	45,646,126	18,759,790	39,516,048	37,131,452
rs29012521	75.505	64,138,634	42,528,553	14,468,741	36,832,019	34,620,018
rs29016964	75.885	64,464,496	42,107,441	13,895,705	36,520,366	34,272,951
rs29017374	75.885	64,682,692	41,895,413	13,582,578	36,340,875	34,106,536
rs29022069	78.885	65,004,007	38,615,868	10,230,968	33,459,528	31,300,136
rs29024547	81.69	66,004,182	36,245,365	76,976,703	31,146,779	29,073,495
rs29025723	82.233	67,093,479	34,611,926	78,386,243	29,601,157	27,578,948
rs29018895	82.628	67,508,196	34,054,866	79,153,416	28,994,368	27,057,957
rs29021970	82.628	67,704,035	33,899,326	79,325,338	28,814,432	26,909,436
rs29027104	82.628	68,020,793	33,601,314	79,747,145	28,538,382	26,639,557
rs29025786	85.354	69,258,118	32,381,945	81,152,721	27,451,945	25,651,581
rs29025641	85.354	69,423,767	32,217,087	81,281,752	27,302,493	25,508,827
HAUT37	92.127	70,006,333	27,019,235	87,574,437	22,201,558	20,694,310
rs29024530	97.554	71,575,314	25,740,727	89,705,205	21,113,807	19,714,984
rs29021100	97.584	75,297,273	20,248,397	155,726,785	16,086,481	15,003,713
rs29018086	97.584	75,423,738	20,105,993	155,873,254	15,947,552	14,874,734
rs29018444	99.32	78,751,552	17,406,849	158,584,285	13,454,083	12,539,478
rs29026155	100.85	80,352,246	15,176,856	161,088,508	11,379,988	10,674,552
rs29016052	105.767	82,919,445	11,793,876	165,092,038	8,266,385	7,751,609
rs29014833	105.767	83,092,462	11,625,720	165,298,627	8,080,354	7,603,480

in the cattle lineage, indicating that possible assembly errors in the cattle X chromosome did not affect our main results (Supplementary Fig. S2 and S3). We conclude that there is a strong increase in the rate of rearrangement on the cattle X chromosome compared with that of other eutherian species that is not matched by a similar increase in the rate of rearrangement on the autosomes.

A number of different factors may explain differences in the rate of rearrangement. The increase in the rodent lineage may, for example, be explained by the short generation time of rodents (20). However, this explanation does not seem to apply to the cattle lineage because artiodactyls do not in general tend to have shorter generation times than other eutherian mammals (21, 22).

In addition, generation-time effects cannot explain the differences between autosomes and X chromosomes. Changes in mutational processes likewise seem unlikely explanations because they should apply to both X chromosomes and autosomes as well. Rather, we should explore potential X-specific explanations.

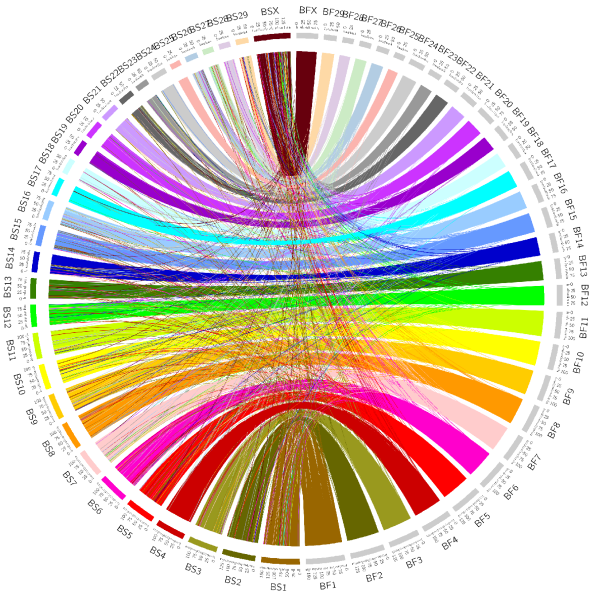

Fig. 3. Genome mapping of Btau4.0 and Btau6.0 using the Circos tool. Chromosome mapping information in Btau4.0 is largely unmatched to that of Btau6.0.

Significant differences in each cattle genome assembly (Btau4.0 and Btau6.0)

We found many differences in the comparison of Btau4.0 and Btau6.0. First, we showed that much of the chromosome mapping information in Btau4.0 is not matched to Btau6.0, as shown by the chromosome X size (Fig. 1) and genome mapping results (Fig. 3) in the two cattle genome assemblies. The X chromosome size in Btau4.0 and Btau6.0 showed a difference of about 61 Mb (88 and 149 Mb, respectively). Orthologous gene sets of mammalian X chromosomes and autosomes were 257 and 11,479 for Btau4.0, respectively, and 372 and 11740 for Btau6.0, respectively. The differences in the X chromosomes and autosomes were 115 and 261, respectively. The result of concordant markers was not similar increases in the X chromosome and autosomes were seen in cattle (Supplementary Fig. 3). However, Btau6.0 differed in that similar increases in the X chromosome and autosomes were seen in cattle, and analyses based on unsigned and signed gene order provided similar results (Supplementary Fig. 4, 5). The results of concordant orthologous gene sets between Btau4.0 and Btau6.0 were similar to the results of Btau4.0 and Btau6.0 in chromosome X, and the Btau4.0 results were similar to the concordant orthologous gene set results in autosomes. However, the Btau6.0 results were not matched to the concordant orthologous gene set results in autosomes. Thus, we assume that Btau6.0 contains more errors and incorrect information than Btau4.0. Consequentially, we have so far determined that the cattle assembly data of Btau4.0 and Btau6.0 are not completely reliable. Thus more care must be taken when using Btau6.0 data than when using Btau4.0 data.

Furthermore, Btau4.6.1, an upgraded version (latest GenBank, latest RefSeq) of Btau4.0, has been released on the NCBI and UCSC websites. We believe that the Btau4.6.1 results are similar to the Btau4.0 results because Btau4.6.1 is an upgraded version of Btau4.0, and is not much different. Thus, the Btau4.6.1 results differ from the Btau6.0 results. We plan to perform more research using the new cattle assembly data.

MATERIALS AND METHODS

Predictions of orthology between species (1 : 1 relationship) were based on the Ensembl (www.ensembl.org) database. We analyzed five species representing five mammalian orders of Eutheria: the human (primate), mouse (rodentia), dog (carnivore), horse (perissodactyla), and cattle (artiodactyla). The genome assemblies used were GRCh37 (human), NCBIM37 (mouse), BROADD2 (dog), EquCab2 (horse), and Btau4.0 (cow). A total of 257 and 11,479 orthologous gene sets for the five species were retrieved from X chromosome and autosomes, respectively. Btau4.0 (cow) was changed to Btau6.0 (cow) in the orthologous gene sets, and a total of 372 and 11,740 orthologous gene sets for the five species were retrieved from the X chromosome and autosomes, respectively. Concordant orthologous gene sets between Btau4.0 (cow) and Btau6.0 (cow) totaling 169 and 10,196 for the five species were retrieved from the X chromosome and autosomes, respectively. Estimates of tree topology and divergence times were retrieved from the TIMETREE database (http://www.timetree.org/).

To calculate the genomic distance between species based on gene order, the genomic distance on the X chromosome and autosomes was calculated by the Hannenhalli and Pevzner (23) algorithm implemented in the GRIMM program (24). To calculate the rate of X chromosomal gene order evolution in each lineage, we use the neighbor-joining (NJ) method with a minor modification. The NJ method (25) is used to reconstruct phylogenies from a matrix (M) of pairwise distances by iterative clustering. Initially, each leaf node is associated with a column and a row of M. In each iteration, a pair of nodes is chosen to cluster based on an optimality criterion calculated from M. The branch lengths of the associated edges are likewise calculated based on M. Of the two nodes chosen to cluster, the rows and columns in M are then eliminated from M, while a row and a column is added corresponding to the new node representing the new cluster. The process is repeated until all nodes have joined a cluster. We used this algorithm to calculate branch lengths based on genomic distances. However, to ensure that the topology was in accordance with that reported in TIMETREE, we disallowed clusters that were in conflict with the TIMETREE topology. In this way, we ensured that the a priori known topology was chosen while using the distances criterion from the NJ algorithm to determine branch lengths in terms of genomic distance. The method provided an estimate of the number of genome rearrangements on each lineage from the pairwise genomic distances.

To address the problem of genome assembly, a set of concordant markers from genetic maps and physical maps were obtained. The genetic map information was from a high-density cattle linkage map. These markers were mapped onto the other mammalian genomes based on the comparative genome alignment information from the UCSC genome browser (http://genome.ucsc.edu/). A total of 51 and 2,634 concordant markers were extracted from the X chromosome and autosomes, respectively. We emphasize that the map by Arias *et al*. (19) was not used in the generation of the physical genome assembly (Btau4.0) and that the two maps, therefore, are independent.

We found concordant genes between Btau4.0 and Btau6.0 by the same method used to find the concordant marker. A total of 169 and 10,196 concordant genes were extracted from the X chromosome and autosomes, respectively.

To determine the L1 content elements of the mammalian genomes, we used the latest available RepeatMasked annotations (http://genome.uscs.edu). To avoid over-counting of fragmented L1, elements with the same name that overlapped the same location were counted as one element.

The genome mapping method in Btau4.0 and Btau6.0 uses the circus of visualization tool (26) after alignment by the LAST program (27). In the LAST method, the first DB was Btau6.0 and the query used was Btau4.0. In the second stage, we edited the alignment data using Python, to generate a simple structure from which duplications had been deleted (selection of high-score values) and merged the small-align set. In the next stage, we used bundlelinks which Circos tutorial program that is make a Circos input file. More details can be found on the Circos Web site (http://circos.ca/).

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