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Cytogenetic Characterization of a Small Evolutionary Rearrangement Involving Chromosomes BTA21 and OAR18

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Abstract
Both cattle (*Bos taurus*) and sheep (*Ovis aries*) belong to the Bovidae family but to different subfamilies, Bovinae and Caprinae, respectively. From a chromosomal point of view, apart from the already known centric fusions (that occurred during the evolutionary process in the Bovidae family) and the small differences in the chromosome classification, the 2 karyotypes are very similar in banding patterns. In this study, the combination of bioinformatics techniques and physical mapping of DNA markers enabled the identification of a micro-rearrangement, a small inversion involving bovine chromosome 21 (BTA21) and the corresponding sheep chromosome 18 (OAR18). The aim of this study was the cytogenetic characterization of this difference in genomic assemblies between cattle and sheep in this single chromosome region. To verify the inversion in FISH experiments, we used the BACs 442H08 and 222H03 from the INRA library and BACs 134H22 and 436P08 from the sheep-specific CHORI library. The results confirmed the presence of the inverted fragment in sheep compared to the cattle genome. Genomic rearrangements may have consequences depending on their influence on gene activity, but in this case no gene or transcribed DNA portion seemed to be involved. In conclusion, we showed for the first time, concerning autosomes, that besides the already known centric fusions also other differences exist between the bovine and sheep karyotypes. Furthermore, we demonstrated that the combination of a bioinformatics approach and physical mapping is a valid tool for the identification of currently unknown rearrangements between related species.

Both cattle (*Bos taurus*, BTA) and sheep (*Ovis aries*, OAR) belong to the Bovidae family, which appeared for the first time at around 23 Mya [Vrba, 1979; Kingdon, 1989]. However, these 2 species belong to different subfamilies: the first one to the Bovinae, whereas the latter one belongs to the Caprinae, which also includes goats (*Capra hircus*, CHI). The closest ancestor of cattle and sheep dates back to 19.7–21.5 Mya [Hassanin et al., 2012].

From a chromosomal point of view, excluding the sex chromosomes, cattle and sheep have the same fundamental chromosomal number (FN = 58) but different diploid numbers: 2n = 60 for cattle and 2n = 54 for sheep. This difference is due to 3 autosomal centric fusions that occurred during the evolutionary process in the Bovidae family. As a matter of fact, in sheep, chromosome 1 originated from the fusion of the homologous bovine chro-

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Table 1. Localization of the BACs used in FISH experiments in the different genome assemblies considered

BAC	Library	GenBank accession No. for BAC end sequences	Cattle genome[a]			Sheep genome[a]	
			UMD_3.1.1	Btau_5.0.1	ARS-UCD1.2	Oar_v4.0	Oar_rambouillet_v1.0
442H08	INRA Bt	CR802698/CR802697	BTA21:24,235,116	BTA21:24,311,989	BTA21:23,770,377	OAR18:23,710,554	OAR18:22,245,007
222H03	INRA Bt	CR794510/CR794509	BTA21:25,381,219	BTA21:25,456,564	BTA21:24,908,782	OAR18:22,612,220	OAR18:21,078,939
436P08	CH-243	DU244225/DU238393	BTA21:24,013,609	BTA21:24,092,116	BTA21:23,549,905	OAR18:23,922,021	OAR18:22,496,753
134H22	CH-243	DU301576/DU296836	BTA21:25,419,499	BTA21:25,494,844	BTA21:24,947,060	OAR18:22,576,299	OAR18:21,029,781
6F18	CH-240	CC771412/CC771335	BTA21:25,474,818	BTA21:25,550,163	BTA21:25,002,386	OAR18:22,557,770[b]	OAR18:21,203,904[b]
319I16	CH-240	CC487916/CC487826	BTA21:25,659,527	BTA21:25,734,792	BTA21:25,186,043	OAR18:24,167,070	OAR18:23,036,216

[a] The central positions of the BACs are reported. [b] The central position of the most proximal portion of 6F18 BAC is indicated (see Fig. 2b).

mosomes BTA1 and BTA3, chromosome 2 from BTA2 and BTA8 fusion, and chromosome 3 from BTA5 and BTA11 fusion [Iannuzzi et al., 2009; Pauciullo et al., 2014]. A similar situation has been reported in buffalo (*Bubalus bubalis*, 2n = 50). In this species, the first 5 chromosomes result from the fusion of 10 different cattle chromosomes [Iannuzzi, 1994]. It is a common opinion that centric fusions represent the most frequent karyotype evolutionary mechanism in the Bovidae family. Beyond the centric fusions and the small differences in the chromosome classification, the bovine and ovine karyotypes are very similar according to their banding patterns [Iannuzzi and Di Meo, 1995]. Nevertheless, cytogenetic analysis by high-resolution banding is no longer considered sufficiently detailed for the detection of some small chromosomal rearrangements, such as inversions of small regions of the genome. Conversely, the combination of different bioinformatics techniques and physical mapping of DNA markers allows for more precise analyses. In fact, this kind of approach has recently demonstrated the existence of a small karyotype divergence between cattle and goat [De Lorenzi et al., 2015].

In this study, we applied the same methodology (bioinformatics techniques and physical mapping) in comparing the complete bovine and sheep genomes. The results obtained highlighted a possible divergence in a small region involving bovine chromosome 21 (BTA21) and the corresponding sheep chromosome 18 (OAR18). The aim of this study was the cytogenetic characterization of this difference in genomic assemblies between cattle and sheep in this single chromosome region.

Materials and Methods

Cell Cultures

Peripheral blood lymphocyte cultures were performed following standard methods [Iannuzzi and Di Berardino, 2008] to obtain bovine, goat, sheep, and water buffalo metaphases. Cultures were incubated for 72 h at 37°C, and colcemid was added 60 min before the cells were harvested. Sheep metaphases were also obtained from fibroblast cultures following the method reported by Iuso et al. [2015].

FISH Experiments

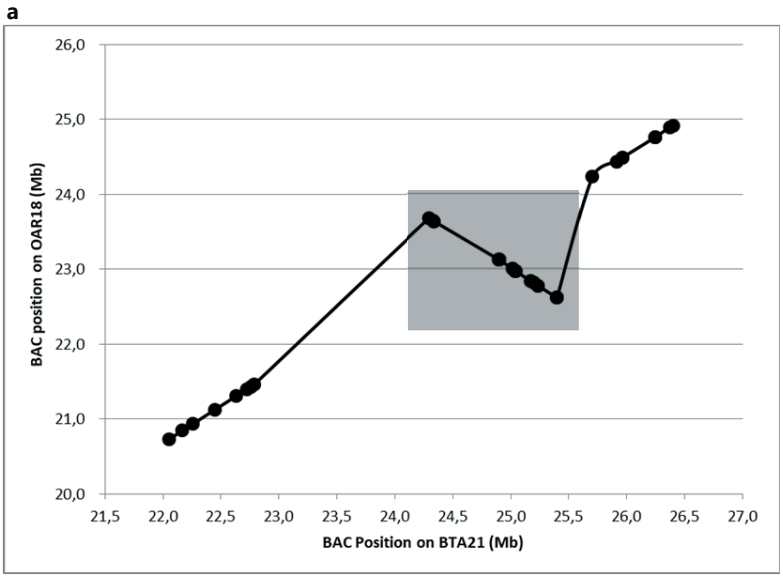
BACs from both the INRA Bt library [Eggen et al., 2001] as well as CHORI CH-243 and CHORI CH-240 libraries [Osoegawa et al., 1998] were used as probes (Table 1). DNA was extracted according to the method described on the CHORI website (http://bacpac.chori.org/) after an overnight growth at 37°C in 3 mL Luria Broth (LB) supplemented with 15 μg chloramphenicol. For each FISH experiment, 250 ng DNA was labeled, and FISH was performed as reported in De Lorenzi et al. [2017].

Bioinformatics Analysis

The data used to identify the supposed inversion were obtained according to the protocol of De Lorenzi et al. [2015]. Briefly, BAC end sequences (BES) from the INRA Bt BAC library [Eggen et al., 2001] were used as e-probes to compare the cattle, sheep, and goat genomes. The initial analysis was performed with 24,743 BACs and the corresponding 49,486 BES. Sequences were downloaded from the NCBI GSS database. The considered genomic assemblies were cattle UMD_3.1.1 and sheep Oar_v4.0. These sequences underwent stringent quality control to eliminate those sequences that could have produced incongruent results. The localization of e-probes on the genomes was performed using the BLAST-like Alignment Tool (BLAT) software [Kent, 2002]. Identification of the regions involved in the evolutionary break points (EBPs) at the molecular level was carried out using BLAT software [Kent, 2002].

Results and Discussion

The bioinformatics analysis highlighted the presence of a small inversion between the cattle and sheep genomes. The supposed 1.2-Mb inversion would have involved the BTA21 24.2–25.4-Mb region. This segment appeared to be inverted in the homologous OAR18 23.6–22.6-Mb genomic region (Fig. 1a). The genomic positions of the BACs considered in the bioinformatics analysis are



b

BAC	BTA CHR	BTA Mb	OAR Mb	OAR CHR
246G11	21	22,1	20,7	18
623G12	21	22,2	20,8	18
197E09	21	22,3	20,9	18
378B05	21	22,4	21,1	18
558E12	21	22,6	21,3	18
974C04	21	22,7	21,4	18
576F07	21	22,8	21,4	18
449D06	21	22,8	21,5	18
442H08	21	24,3	23,7	18
842H05	21	24,3	23,6	18
6C10	21	24,3	23,6	18
113C04	21	24,9	23,1	18
390E10	21	24,9	23,1	18
549D04	21	25,0	23,0	18
202E06	21	25,0	23,0	18
332G11	21	25,0	23,0	18
265A09	21	25,2	22,8	18
151B12	21	25,2	22,8	18
298D10	21	25,2	22,8	18
234F10	21	25,4	22,6	18
222H03	21	25,4	22,6	18
328E02	21	25,7	24,2	18
1069D05	21	25,9	24,4	18
230C01	21	26,0	24,5	18
230G06	21	26,0	24,5	18
1079A10	21	26,2	24,8	18
1079D06	21	26,2	24,8	18
272B06	21	26,4	24,9	18
496B07	21	26,4	24,9	18

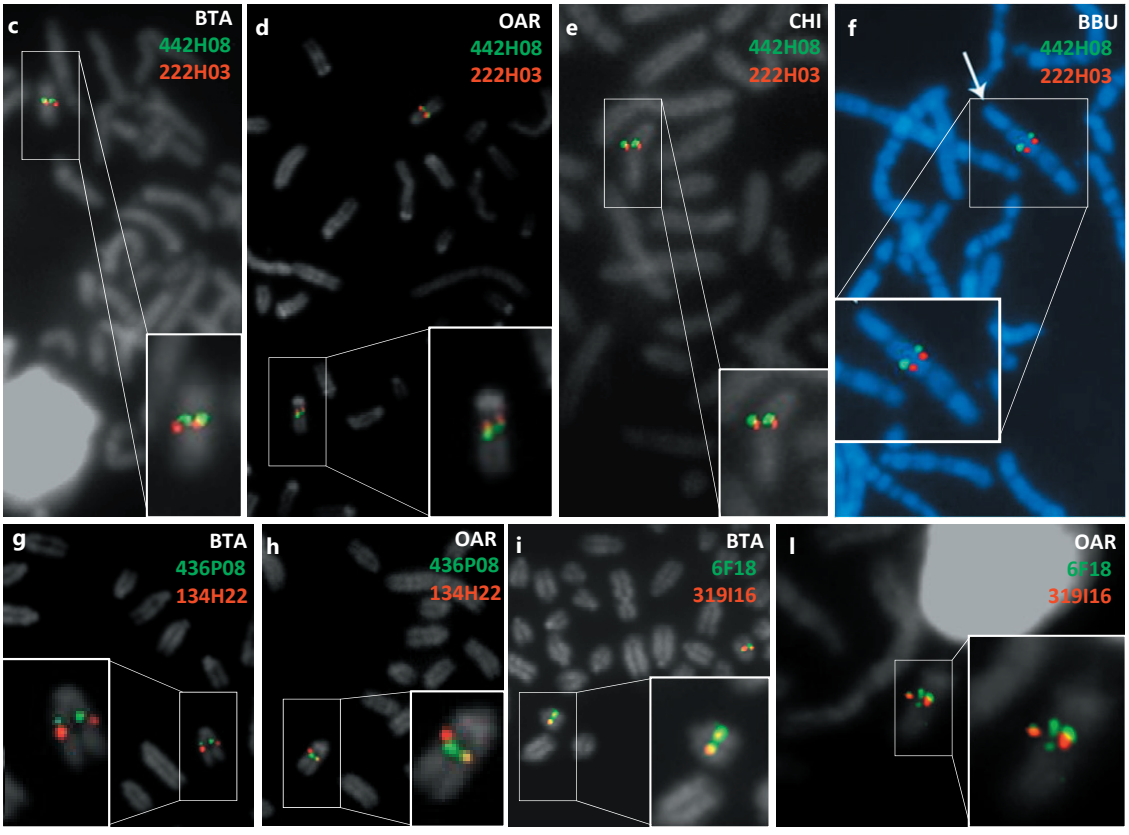


Fig. 1. a Graphic representation of the correspondence between bovine chromosome BTA21 21.5–27.0 Mb and sheep chromosome OAR18 20.0–26.0 Mb. BAC end sequences were used as probes. The unexpected inverted genome region is evidenced in the gray box. **b** List of the BACs used as probes in the bioinformatics analysis and their position on BTA21 and OAR18 chromosomes. The inverted region is shown in gray. **c–l** FISH on cattle (**c**, **g**, **i**), sheep (**d**, **h**, **l**), goat (**e**), and water buffalo (**f**) metaphases with the probes indicated in the upper right corners. The BACs shown in red were marked with Cy3, whereas those shown in green were labeled with biotin and detected with avidin-FITC.

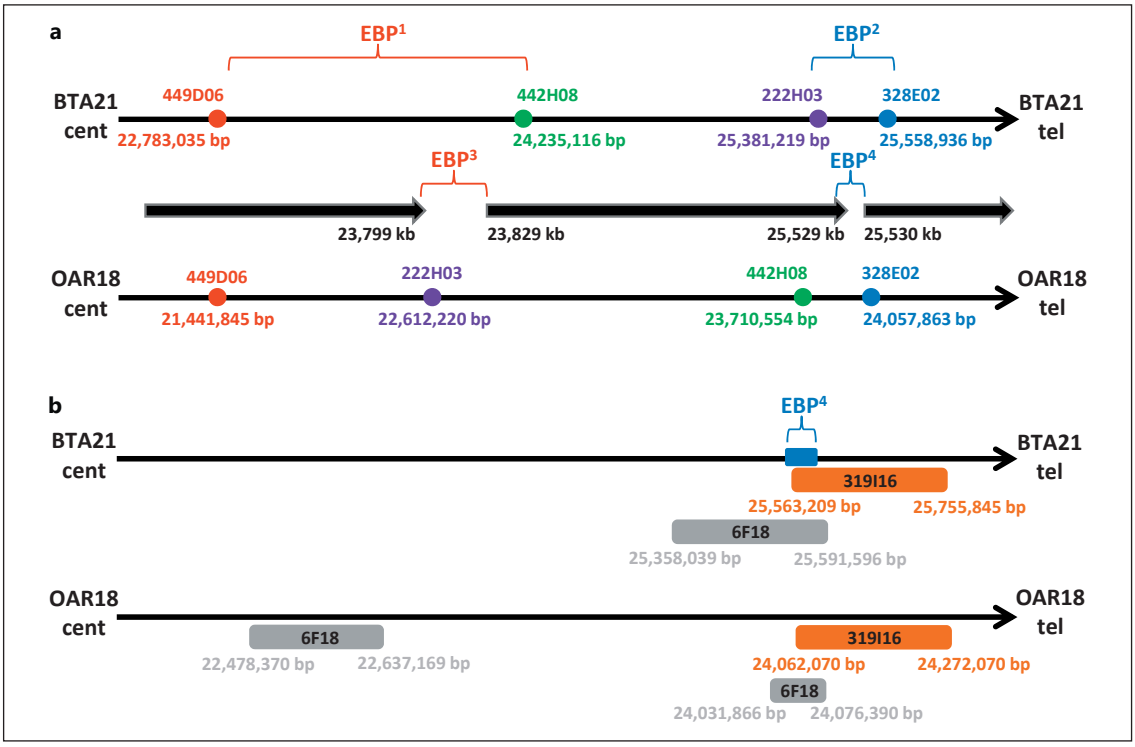


Fig. 2. a Graphic representation of the position of the BACs that delimit the evolutionary break points (EBPs) on bovine chromosome 21 and the corresponding sheep chromosome 18. In the middle, the presumed position of the 2 EBPs (proximal and distal) identified by the bioinformatics analysis is reported (black fat arrow). **b** Graphic representation of the BACs 6F18 and 319I16 used to visually highlight their separation in sheep following the event of break and inversion. The genomic regions including the proximal[1] and distal[2] EBPs obtained from the bioinformatics analysis are indicated. The genomic regions including the proximal[3] and distal[4] EBPs at molecular level obtained from the BLAT analysis are indicated.

reported in Figure 1b. Furthermore, this supposed discrepancy between cattle and sheep is also visible by analyzing recent genomic assemblies (Table 1). Considering the bovine database, the assumed inversion, involving 13 BACs and 2 EBPs of the inverted fragment in sheep, included the regions between 22,800 kb (BAC 449D06) and 24,300 kb (442H08) for the proximal EBP and between 25,400 kb (BACs 222H03 and 234F10) and 25,700 kb (BAC 328E02) for the distal EBP (Fig. 1b).

To verify or refute the inversion highlighted by the bioinformatics approach, we used 2 BACs, 442H08 and 222H03, from the INRA library in the FISH experiments. The results confirmed the presence of the inverted fragment in sheep compared to the cattle genome (Fig. 1c, d). Moreover, we tested the same BACs on goat and water buffalo (*B. bubalis*) but found no variation (Fig. 1e, f). Considering the positions of the BACs in the genome assemblies of water buffalo (UOA_WB1) and goat (CHI_1 and ARS1), the assembly of this genomic portion in goat coincides with that seen in FISH experiments, but this was not observed in the buffalo. It is suggested that the assembly of this region is not correct in the buffalo. As a further confirmation of the accuracy of our data, we decided to prove the presence of the inversion using BACs 134H22 and 436P08 belonging to the sheep-specific library CHORI-243. FISH experiments with these last probes gave results comparable to the previous ones (Fig. 1g, h).

In view of the importance of the result obtained, we carried out another approach with the purpose of identifying the position of the EBPs at the molecular level.

Using several BLAT analyses (additional information available on request), we defined the presumed position of the 2 EBPs (Fig. 2). Considering the cattle genome (UMD_3.1.1 genome assembly) versus the sheep genome (Oar_v4.0 genome assembly), the proximal EBP is between 23,799 and 23,829 kb. For the distal region, the EBP is between 25,529 and 25,530 kb (Fig. 2a).

The BACs 6F18 and 319I16 have also been identified in the distal EBP area (Fig. 2b). These 2 BACs have been identified by BLAT analysis, and their positions are reported in Table 1. They are partially overlapping (28 kb) and 6F18 broke as a consequence of the evolutionary event. Using these BACs simultaneously on cattle and sheep metaphases, in cattle, the hybridization signals are practically overlapping (Fig. 1i), while in sheep (in which the breaks and inversion event occurred), the signals are separated (Fig. 1l).

Excluding the centric fusions described above, cattle, sheep, and goat present the following autosomal divergences: (1) translocation of a small subcentromeric portion of BTA9 to the proximal region of CHI14 and the homologous sheep chromosome OAR9 [de Gortari et al., 1998; Iannuzzi et al., 2001, 2009]; this translocation was also reported and characterized by Iannuzzi et al. [2001] and by De Lorenzi et al. [2015]; (2) a 7.4-Mb chromosomal inversion in CHI13 compared with the homologous BTA13 [De Lorenzi et al., 2015]; and (3) a small inversion involving BTA21 and the corresponding homologous OAR18, as reported in the present study. Much more complex were the divergences which differentiated the sex chromosomes in bovids during karyotype evolution, especially the X chromosome [reviewed in Iannuzzi et al., 2009].

From an evolutionary point of view, the presence of the reported rearrangement can influence the activity of some genetic factors. For example, it has been shown that a chromosomal break event close to the *SCNN1B* gene in pig is responsible for a limited ability to taste NaCl [Groenen et al., 2012]. Another example of how a chromosomal inversion can affect the activity of a gene is given by the genetic mechanism that leads to the formation of the phenotype called the tobiano white-spotting pattern in horse. This phenotype is associated with a chromosomal inversion in equine chromosome 3 that jeopardizes the action of the *KIT* gene [Brooks et al., 2007]. Finally, chromosomal inversions can lead to reproductive disorders in the same species [Morin et al., 2017] and reproductive barriers between species because they negatively affect pairing and synapsis in meiosis [Noor et al., 2001].

Bioinformatics analysis showed that near the break points of the inverted fragment, there are no protein or ncRNA genes.

Considering the proximal EBP (23,799,700–23,829,100 bp), the closest transcribed genetic element is located 35 kb upstream the EBP in tail-to-tail orientation. This gene is homologous to human *C15orf40*, and the presence of several cattle expressed sequence tags (i.e., DV893393) demonstrates that it is actively transcribed in different tissues. Considering the distal EBP (25,529,400–25,530,100 bp), 2 genes could be involved: *MORF4L1* (located 121 kb downstream, tail-to-tail orientation) and *BTBD1* (11 kb upstream, but head-to-tail orientation). Both are expressed in several tissues, and no mutation is known to date. Therefore, it is not possible to predict the effect of a possible alteration of their activity.

In conclusion, regarding autosomes, our cytogenetic analysis confirmed for the first time that there are other differences between bovine and sheep karyotypes, in addition to the already known centric fusions. Furthermore, we demonstrated that the combination of a bioinformatics approach and physical mapping by FISH analysis results in a valid tool for the identification of currently unknown rearrangements between related species. Finally, it is important to highlight that in an age of massive and high-throughput sequencing, the FISH technique still remains an important tool for testing the accuracy of genome assemblies and for further confirmation of genomic alterations identified by other methodologies.

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Statement of Ethics

For this study, no animals were used. The cytological preparations used in FISH experiments were already available in our laboratory from routine analyses previously authorized and carried out.

Disclosure Statement

The authors have no conflicts of interest to declare.

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Author Contributions

L.D.L and A.I. performed the FISH experiments. A.P. performed the cell cultures of sheep fibroblasts. P.P. obtained and analyzed the bioinformatics data, identified and studied the breakpoint regions at the molecular level. All authors participated in writing the manuscript.

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