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New cryptic karyotypic differences between cattle (*Bos taurus*) and goat (*Capra hircus*)

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Abstract Cattle (*Bos taurus*) and goat (*Capra hircus*) belong to the Bovidae family, and they share a common ancestor 19.7–21.5 Ma ago (MYA). The Bovidae family apparently experienced a rapid species radiation in the middle Miocene. The present day cattle and goat possess the same diploid chromosome number (2n=60) and structurally similar autosomes, except that a small subcentromeric portion of cattle chromosome nine has been translocated to goat chromosome 14. In this study, we adopted a new strategy that involves the use of bioinformatics approach to detect unknown cryptic chromosome divergences between cattle and goat using and subsequent validation using the fluorescence in situ hybridization (FISH) of bacterial artificial chromosome clones. We identified two hypothetical discrepancies between the cattle and goat genome assemblies: an inversion in the goat chromosome 13 and a transposition in the goat chromosome 6. The FISH technique allowed clear validation of the existence of a new 7.4 Mb chromosomal inversion in the goat chromosome 13. Regarding the transposition in the goat chromosome six, FISH analyses revealed that the cattle and goat genomes shared the same organization, with the assembly of the goat genome being the correct one. Moreover, we defined, for the first time, the size and orientation of the translocated fragment involved in the evolutionary translocation between cattle chromosomes 9 and goat chromosome 14. Our results suggest that bioinformatics represents an efficient method for detecting cryptic chromosome divergences among species.

Keywords Cattle · Goat · Chromosomes · Fish

Abbreviations

MYA	Million years ago
FISH	Fluorescent in situ hybridization
CHI	Capra hircus
BTA	Bos taurus
Mb	Megabase
BAC	Bacteria artificial chromosome
INRA	Institut nationale de la recherche agronomique
Kb	Kilobase
CNV	Copy number variation
EBP	Evolutionary break point

Introduction

Both cattle (*Bos taurus*) and goat (*Capra hircus*) belong to family Bovidae, order Cetartiodactyla, suborder

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Ruminantia. The Bovidae family can be divided in two subfamilies: Bovinae and Antilopinae. Bovinae includes three tribes: Bovini, Tragelaphini, and Boselaphini, while Antilopinae includes nine tribes: Aepycerotini, Neotragini, Antilopini, Reducini, Oreotragini, Cephalophini, Alcelaphini, Hippotragini, and Caprini (Hassanin et al. 2012). Cattle belong to the Bovini tribe, whereas goat belongs to the Caprini tribe (Fig. 1).

The common ancestor belonging to Cetartiodactyla has been dated back to 60–77 Ma ago (MYA) (Arnason and Gullberg 1996; Bininda-Emonds et al. 2007), while the first presence of Bovidae has been set at 23 MYA (Vrba 1979; Kingdom 1989). The closest ancestor of cattle and goat has been traced back to the phylogenetic separation between Bovinae and Antilopinae, which is dated in the early Miocene between 19.7 and 21.5 MYA (Hassanin et al. 2012). After this separation, a rapid speciation involving the family Bovidae probably occurred in the middle Miocene. Currently, the Bovidae family consists of more than 149 species, including the most important zoo-economic species such as cattle, sheep, goats, buffalo, and zebu. From the chromosomal perspective, all bovid species possess similar fundamental chromosome numbers (58–62), but highly variable diploid numbers (30–60). This difference may be largely due to the high incidence of autosomal centric fusions that occurred during the evolutionary process of the Bovidae family (Iannuzzi et al. 2009). Cattle and goat possess the same diploid number (2n=60) and structurally similar autosomes; to date, only one small karyotypic difference has been detected, that is, a small translocation, with the subcentromeric portion of cattle chromosome 9 (BTA9) being translocated to the goat chromosome 14 (CHI14) (ISCNDB 2000). This rearrangement was first demonstrated by linkage analysis (de Gortari et al. 1998) and later confirmed by FISH-mapping (Iannuzzi et al. 2001). This translocation could represent the leading ramification of Bovinae and Antilopinae divergence (Buckland and Evans 1978). However, to date no information is available regarding the size of the translocated fragment.

In this study, we report a new strategy that allows us to detect cryptic chromosome divergences between cattle and goat using bioinformatic and FISH-mapping approaches.

Materials and methods

Bioinformatics

Efficient E-probes were produced for comparing the cattle and goat genomes using the bacterial artificial chromosome (BAC) END sequences (ENDs) as probes because (a) we believed that the two ENDs of a single BAC (referred to as TJ and TV) belong to the same DNA sequence as chimeric cloning is extremely rare in the BACs library construction; (b) in a well-assembled genome around the BAC region, the two ENDs of a single BAC must be oriented in opposite direction; and (c) the two ENDs must be at a distance >30 and <300 Kb as this size represents the most common size of an insert in the BAC library. We downloaded all of the ENDs belonging to the INRA Cattle BAC Library from the GSS database (NCBI), retaining only those BACs that showed both ENDs (discarding the single END). The analysis was performed with 24,743 BACs and the corresponding 49,486 ENDs.

To locate the sequence of each BAC in the cattle and goat genomes, we performed a local alignment of each BAC-END on the whole cattle genome and on the whole goat genome by using BLAT (Kent 2002). To accelerate the process, we used a local client/server version of BLAT. When BLAT is used to search for a particular sequence in a genome, the first step before the alignment is the indexation of the genome. Indexation is an internal strategy of the algorithm, an automatic process that is completed within few seconds. There are two ways of aligning few sequences: using a web interface or by installing a local version of BLAT in a computer. However, if several thousands of sequences are to be aligned, this process would be critically compromised by time consumption as the genome is indexed before each query or alignment. Building an indexed genome database and maintaining it in memory throughout the entire process is possible by using a client/server version of BLAT that is installed in the local server. Thus, the genome is indexed only once at the beginning, following which each query can access the server by passing the need to index the genome for each query. The options used for the alignments were, "tileSize=11, minMatch=4, stepSize=5, repMatch=2253"; these values are similar to the default values suggested by BLAT developers for aligning the DNA sequences, albeit with slight modification to increase the stringency conditions of the alignment. Often, each query yields

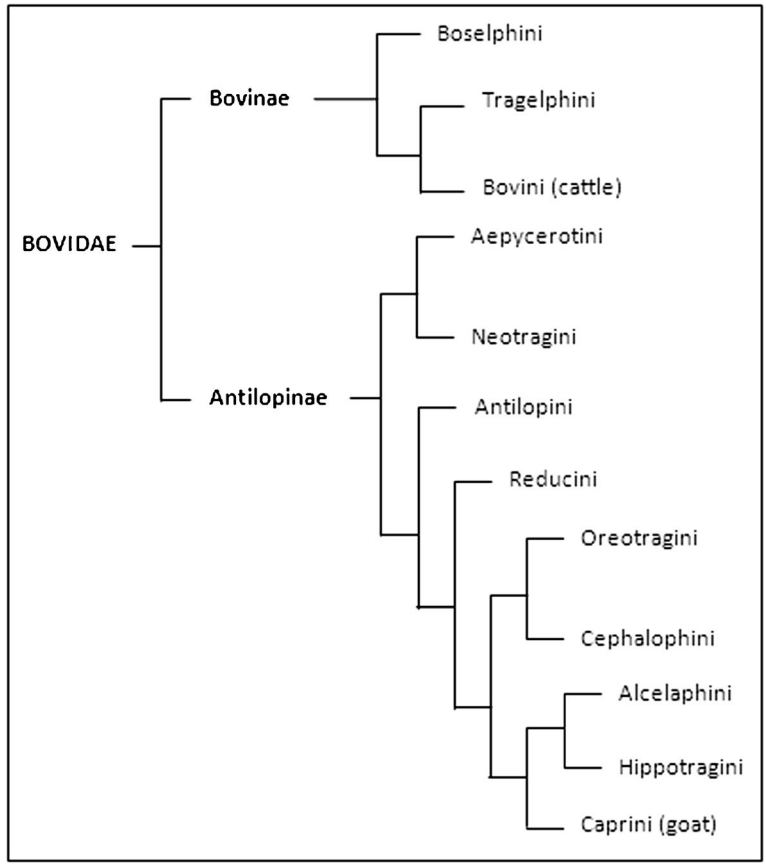

Fig. 1 Evolution tree of Bovidae family. The figure reports the position of the 12 tribes present in the Bovidae family in a evolutionary tree (adapted from Hassanin et al. 2012). The distances between tribes are not representative of the evolution time

several alignments of the query sequence with different regions in the reference genome. We retained only those alignments that have 99.5 % or more identity. The output file for each alignment was further processed to investigate whether the two ENDs of each BAC fulfilled the requirement of being in the opposite orientation in the same chromosome and at a distance of 30–300 Kb. The bioinformatic pipeline is depicted in Fig. 2.

Fluorescent in situ hybridization (FISH)

FISH experiments were performed according to the protocol described by De Lorenzi et al. (2014). The BACs used as probes belonged to the INRA Library (Eggen et al. 2001) and are reported in Table 1.

Genome version

To identify the position of the BACs, we used the following freely available genome assembly: bosTau6 for cattle (http://genome.ucsc.edu; Zimin et al. 2009)

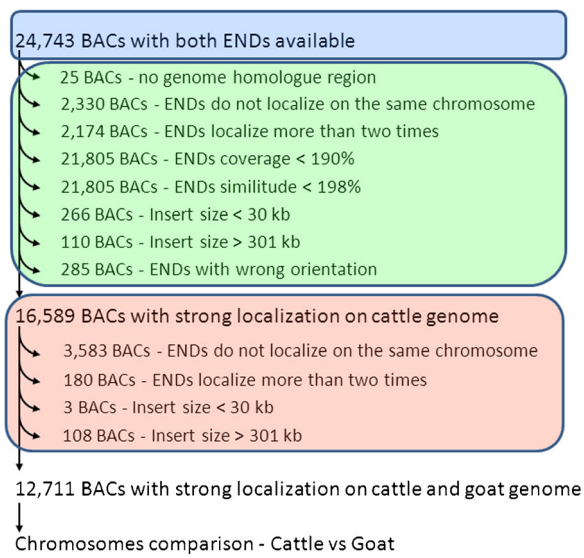

Fig. 2 Bioinformatics pipeline. The figure summarizes the passage performed in order to identify the useful BACs. The data contained in the *blue box* were obtained from GSS database (NCBI). The *green box* identifies the operations performed against the cattle genome assembly. The *red box* identifies the operations performed against the goat genome assembly

Table 1 BACs used in FISH experiments

BAC	ENDs localization on cattle genome	Insert size (Kb)
713F08	chr6: 110,531,444–110,405,587	125
246G01	chr6: 106,566,883–106,677,427	109
307H04	chr9: 5,167,931–5,289,771	120
636E01	chr9:12,845,616–12,984,234	137
212F04	chr13: 11,539,602–11,651,931	111
513C11	chr9: 13,250,122–13,376,091	124
918D08	chr13: 16,541,513–16,697,543	155
474A12	chr9: 86,581,359–86,732,461	119
456B03	chr14: 74,860,506–75,006,749	144

and genome browser v1.0 for goat (http://goat.kiz.ac.cn/GGD/index; Dong et al. 2013).

Statistical analysis

Data regarding the average distance (Kb) between consecutive BACs were statistically analyzed using the GLM procedure of the SAS package (2008).

Results

BACs localization on the cattle genome

Initially 24,743 BACs were subject to the in silico analysis. Twenty-five BACs were discarded as both ENDs of each of these 25 clones showed no chromosomal localization in cattle; another 2330 BACs were also discarded as they each showed ENDs localization on two different cattle chromosomes. For reliable E-probes, each END must have only one genomic localization; we thus discarded a further 2174 BACs as they showed more than one "strong" genomic localizations for at least one END. After applying the coverage filter (i.e., % of END bps that match with the identified genome region) of the ENDs, 1805 BACs were discarded as their ENDs showed coverage of <190 % (each END can show a maximum coverage of 100 %). The remaining BACs were further filtered according to their similitude (i.e., % of END bps identical to the genome region). Only those BACs whose ENDs had a combined value of similitude >198 % (max possible= 200 %) were maintained, this process led to 1158 BACs being discarded. Finally, a total of 266 BACs with an insert size (measured as the distance among the ENDs localized on the genome) of <30 Kb, 110 BACs with an insert size >301 Kb, and 285 BACs with ENDS not oriented in opposite orientation were discarded. After the abovementioned filtering procedures were completed, we obtained a total of 16,589 BACs with extremely reliable localizations on the cattle genome. In order to generate BACs representative of all cattle genomes, we observed their distribution on each chromosome. Table 2 depicts the BAC distribution data on single chromosomes. Considering the whole genome, we had a BAC every 160 Kb, and almost all of the chromosomes showed comparable density. However, the distribution of the BACs on individual chromosomes does not appear to be statistically uniform. BTAX and BTA15 had greater BAC distance average, whereas BTA25 and BTA26 had lesser distance average. The results of the statistical analysis are shown in Table 2.

BACs localization on goat genome

In order to obtain a reliable localization of the BACs on goat genome, we conducted an analysis and a subsequent check control as reported earlier for cattle. We discarded 3583 BACs because their ENDs were localized on two different chromosomes as well as 184 BACs as they possessed at least one END with more than one localization on the goat genome. Finally, we discarded 3 BACs with a "goat" insert size of <30 Kb as well as 108 BACs with an insert size of >301 Kb. At the end of this process, we obtained 12,711 BACs, each with a reliable localization on both the cattle and goat genomes, which allowed a good comparison between the two genomes.

Visualization of the differences

Cattle and goat showed similar karyotype (excluding the sexual chromosomes), with the only difference being the BTA9;14 small translocation. To reveal the potential cryptic differences present between the two genomes, for each single chromosome, we represented the position of each BAC using a Cartesian graph (Dong et al. 2013). When all BACs were located in an analogous position within the same chromosome, a straight line appeared (Fig. 3a). Following this procedure, we identified three hypothetical autosomal discrepancies between the two genomes, including the previously reported BTA9;14 translocation (Fig. 3b); an inversion detected in chromosome 13 of goat (CHI13-, Fig. 3c), and a transposition in goat chromosome 6 (CHI6-,

Table 2 BACs distribution on cattle genome

BTA[a]	BACs[b]	Chr size[c]	average distance[d]	Stat[e]
1	891	158,337,067	177	c
2	868	137,060,424	157	cd
3	781	121,430,405	155	d
4	756	120,829,699	158	cd
5	855	121,191,424	141	de
6	684	119,458,736	174	cd
7	907	112,638,659	124	ef
8	700	113,384,836	160	cd
9	643	105,708,250	164	cd
10	622	104,305,016	167	cd
11	752	107,310,763	142	de
12	480	91,163,125	189	bc
13	470	84,240,350	176	cd
14	491	84,648,390	169	cd
15	393	85,296,676	213	b
16	524	81,724,687	155	cd
17	455	75,158,596	164	cd
18	386	66,004,023	170	cd
19	458	64,057,457	138	def
20	468	72,042,655	152	d
21	406	71,599,096	176	cd
22	420	61,435,874	144	de
23	304	52,530,062	172	cd
24	359	62,714,930	172	cd
25	381	42,904,170	111	f
26	659	51,681,464	78	g
27	266	45,407,902	169	cd
28	280	46,312,546	162	cd
29	322	51,505,224	158	cd
X	608	148,823,899	245	a
W,G,[f]	16,589[g]	2,660,906,405[h]	160[i]	

[a] Cattle chromosome

[b] Number of BACs localized on chromosome

[c] Chromosome size (retrieved from UCSC genome browser)

[d] Average distance between BACs in Kb

[e] Different letters on the same row=$P<0,05$

[f] Whole genome, Y chromosome excluded

[g] Total BACs mapped on cattle genome

[h] Total cattle genome dimension, Y chromosome not included

[i] Average distance on whole genome expressed in Kb

Fig. 3d). As expected, more complex structural differences were also detected between the cattle and goat X chromosomes assembly. However, the description and validation of such complex rearrangements were beyond the scope of the current paper.

BTA9;14 translocation

From the information provided by the localization of the BACs in the centromeric region of BTA9, we assumed that the translocated portion was also inverted in the goat chromosome 14 and also that this region was 13-Mb long (Fig. 3b; Table 3). The use of FISH technology demonstrated that both assumptions were correct. In particular, using the BAC 307H04 and 636E01, we verified that the translocated portion of BTA9 was also inverted in the goat genome. In cattle, FISH experiments were performed on well-spread metaphases belonging to a rcp9;11 carrier to identify the BTA9 (De Lorenzi et al. 2007); in goat, the CHI14 was identified along with the BAC 456B03 as a marker (Fig. 4a, b). Then, using BAC 513C11, we highlighted that the length of the translocated fragment was 12.8 and 13.2 Mb (Fig. 4c, d). Goat chromosome 9 was identified using BAC 474A12 as a marker.

Goat chromosome 13 inversion

Observing the localization of the BACs in the cattle and goat genomes, we could conclude that a 5.3-Mb long region was inverted in the goat genome in comparison with the cattle genome (Fig. 3c; Table 4). We verified this hypothesis by FISH. Using the BACs 918D08 and 212F04 as probes, we demonstrated that the fragment was actually inverted in the goat when compared with the cattle (Fig. 4e, f).

Goat chromosome 6 transposition

Finally, we considered the hypothetical transposition evidenced by the bioinformatic procedure on the goat chromosome 6 (Fig. 3d; Table 5). A short genome fragment of length 3.3 Mb that was believed to be close to the telomere in cattle was found to be positioned in the telomeric position in goat. Using BACs 713F08 and 246G1, we found no differences between the cattle and goat genomes and, surprisingly, we found that the genome assembly reported for goat represents the correct one (Fig. 4g, h).

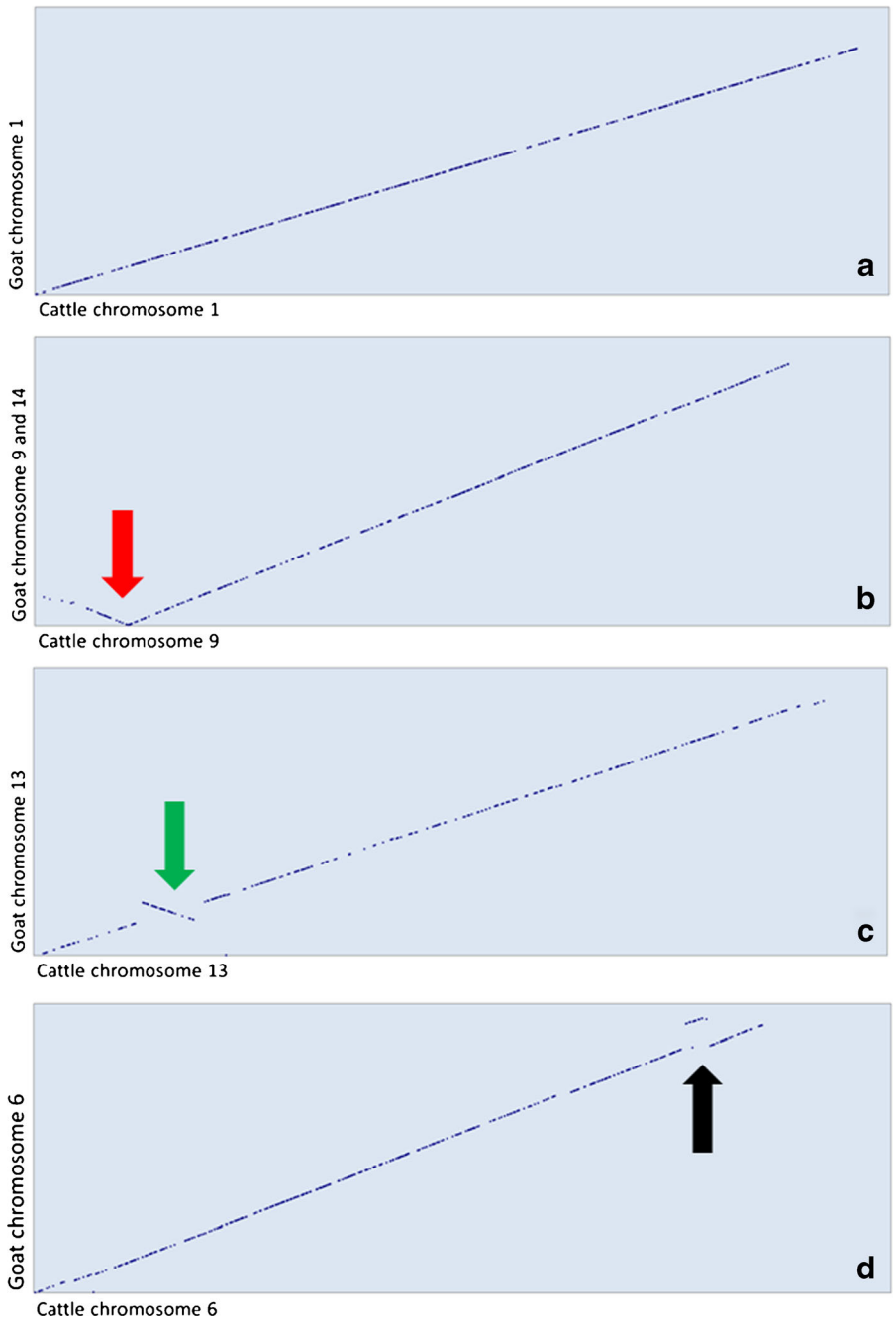

Fig. 3 Output of the bioinformatic analysis. The figure reports the graphic output of the comparison among BACs localization on cattle and goat genomes. **a** Each *dot* represents a BAC. In the case of a perfect correlation between cattle (*x axis*) and goat (*y axis*) position of BACs a straight line appears. **b** Visualization of the BTA9;14 translocation in goat; the *red arrow* shows that cattle genome portion is translocated on CHI14. The orientation of the line in this portion, inverted compared to the remaining part, indicates that the fragment is also inverted. **c** Visualization of the inversion of CHI13. The *green arrow* indicates the portion of the cattle genome that appears to be inverted in goat genome. **d** Visualization of the transposition of CHI6. The *black arrow* indicates the portion of the cattle genome that seems to be transposed at the end of chromosome 6 of goat

Table 3 BACs localized in goat BTA9;14 translocation

BAC[a]	BTA[b]		CHI[c]	
30F12	9	2,191,741	*14*	*8,988,612*
307H04	9	5,167,931	*14*	*7,667,336*
57B09	9	7,434,863	*14*	*5,815,443*
236A12	9	7,460,167	*14*	*5,793,343*
421E03	9	7,489,073	*14*	*5,762,019*
443E09	9	7,489,073	*14*	*5,762,019*
883H01	9	7,601,074	*14*	*5,647,118*
926G11	9	7,977,247	*14*	*5,344,255*
296E04	9	8,054,242	*14*	*5,269,299*
882E06	9	8,892,116	*14*	*4,435,901*
441E06	9	9,137,916	*14*	*4,137,345*
533F06	9	9,807,418	*14*	*3,451,846*
543H09	9	10,069,572	*14*	*3,207,768*
36G08	9	11,537,646	*14*	*1,709,208*
572G11	9	11,963,422	*14*	*1,282,595*
636E01	9	12,845,616	*14*	*382,288*
513C11	9	13,250,122	9	234,227
601B03	9	13,283,260	9	259,579
1058F01	9	13,341,182	9	317,740
572F01	9	13,364,333	9	342,379
386A05	9	13,375,810	9	355,871
923F07	9	13,419,534	9	402,425
248G03	9	13,542,476	9	528,284
11E03	9	14,088,920	9	1,087,873
194E09	9	14,340,423	9	1,320,650

The translocated portion in goat is in italics

[a] BAC name

[b] Cattle chromosome localization

[c] Goat chromosome localization

Discussion

During the BAC mapping on the cattle genome using the bioinformatic approach, we discarded 8154 BACs that represented 33 % of the available total number of BACs (24,743). These BACs did not pass the control checks. Although it is possible that some of these BACs that possessed good localization got eliminated due to the highly restrictive filters we applied. Considering that the cattle genome assembly we used (UMD3.1) contains some gaps (estimated at approximately 8 %; Zimin et al. 2009), some of the discarded BACs probably map to these unknown genomic regions. Some BACs could be discarded as their ENDs were localized in the repetitive regions, in the CNV regions, or on the Y chromosome.

More than 100 BACs were discarded as they showed an insert size of >301 Kb. Some of the BACs showed an insert size of 350–400 Kb that was compatible with the BAC library construction, although others showed an insert size of >1 Mb, a condition that is incompatible with the library construction procedure. Moreover, in the cattle genome, we discarded 285 BACs with ENDs that were not in opposite orientation. In these two last cases, the most probable hypothesis is an incorrect genome assembly in the concerned genomic region due to the use of human synteny in cattle genome assembly. Finally, unexpectedly, the distribution of the BACs on single chromosomes was not statistically similar. The reason for this observation is intriguing, with a plausible explanation that these particular cattle chromosomes probably contain fewer repetitive sequences and, therefore, fewer BACs were discarded at the check-control level.

There were some interesting conclusions for the localization of the 16,589 BACs with good cattle localization on the goat genome. First, 3583 BACs showed good localization of the two ENDs on two different goat chromosomes (in this case, one END coincided with cattle localization). This observation can be explained by the following two hypothetical reasons: either incorrectly assembled goat genome or localization of the BACs at an evolutionary breakpoint between cattle and goat. We believe that the second hypothesis is extremely unlikely considering the high degree of similarity between the cattle and goat genomes as well as the large number of presumed differences between them. Furthermore, this hypothesis is not supported by the strategy that is applied to the construction of the goat genome assembly, whereby the 315 super-scaffolds were constructed based on the physical map generated by optical mapping, but 302 of the 315 super-scaffolds were anchored onto 30 goat pseudo-chromosomes using cattle synteny information obtained from cattle genome assemblies Bta4.0 and UMD3.1 (see Dong et al. 2013 for detail). This means that our bioinformatic approach could identify only such structural differences between cattle and goats that are contained in the super-scaffolds. However, because the super-scaffold was assembled without using any cattle synteny information, the cryptic rearrangements we detected could reflect the true karyotype divergence between the two species if we assume the genome assemblies are correct.

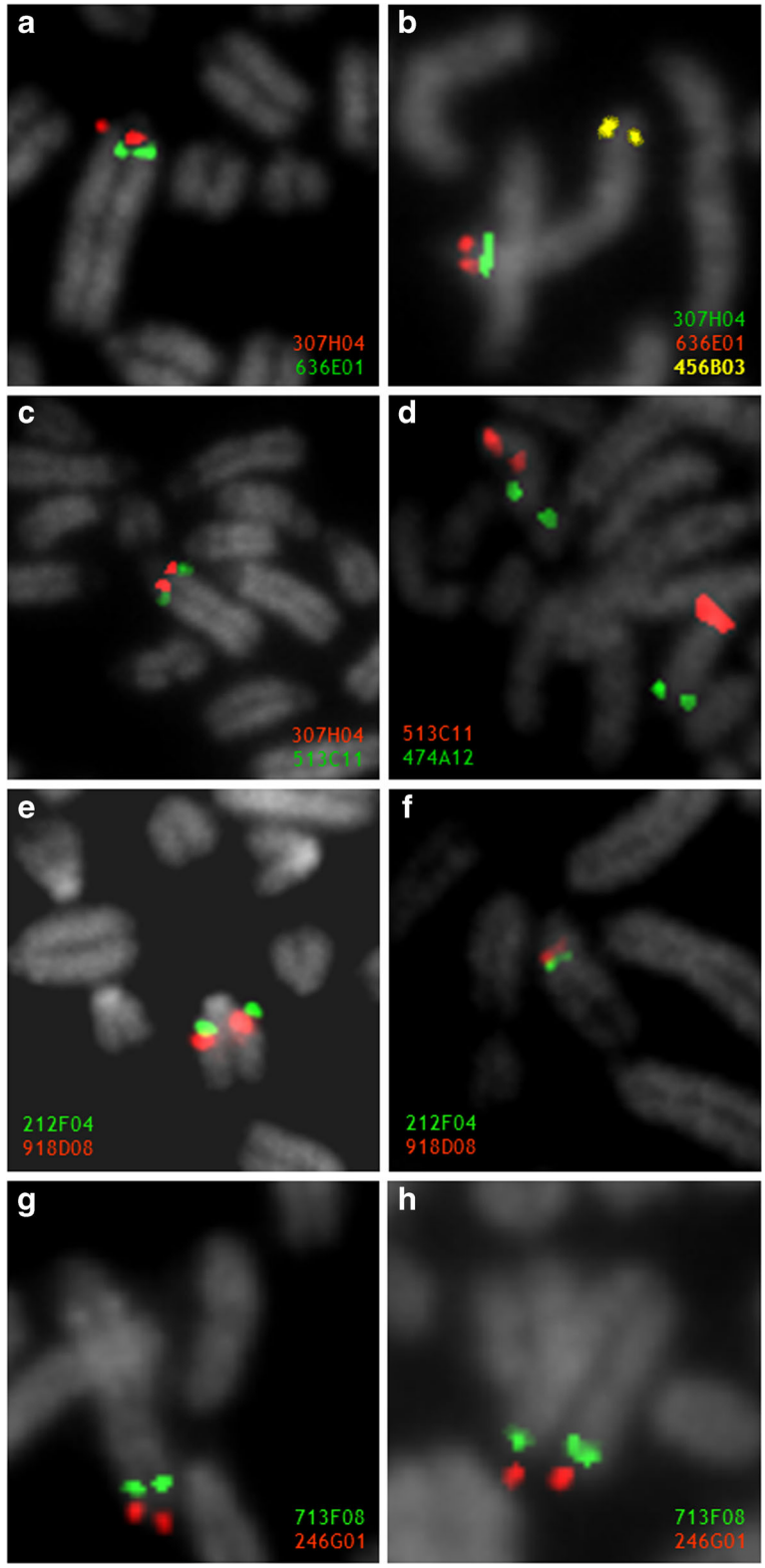

Fig. 4 FISH analyses. The figure reports the results obtained after FISH experiments performed to confirm or denied the bioinformatic outputs. In all images the name of the BACs corresponds to FISH signals. **a**, **c**, **e**, **g** FISH on cattle. **b**, **d**, **f**, **h** FISH on goat. **a**–**d** FISH to analyzes the BTA9;14 translocation. **e**, **f** FISH to analyze the CHI13 inversion. **g**, **h** FISH to analyze the CHI6 transposition

Table 4 BACs localized in goat CHI13 inversion

BAC[a]	BTA[b]		CHI[c]	
120F04	13	9,199,995	13	8,429,715
546F08	13	9,250,916	13	8,482,267
773F01	13	9,397,237	13	8,634,703
94E10	13	9,398,325	13	8,635,641
559A01	13	9,398,463	13	8,636,095
217H03	13	9,693,982	13	8,937,201
957H10	13	10,349,099	13	9,612,613
148A10	13	10,533,657	13	9,784,369
714G12	13	10,676,082	13	9,922,151
980G07	13	10,685,991	13	9,931,945
212F04	13	11,539,602	*13*	*16,453,751*
353C03	13	11,546,470	*13*	*16,447,094*
406E03	13	11,861,952	*13*	*16,140,697*
90C11	13	11,991,013	*13*	*16,012,420*
476E12	13	12,072,433	*13*	*15,932,807*
104B01	13	12,099,209	*13*	*15,903,497*
480E06	13	12,490,591	*13*	*15,522,555*
314B09	13	12,527,465	*13*	*15,483,376*
560H04	13	12,626,506	*13*	*15,387,568*
779H03	13	12,784,547	*13*	*15,237,834*
471C07	13	12,808,582	*13*	*15,213,920*
456A02	13	12,887,884	*13*	*15,128,940*
382H03	13	13,051,295	*13*	*14,968,547*
227D03	13	13,493,959	*13*	*14,526,609*
235H02	13	13,493,960	*13*	*14,526,609*
885D08	13	13,508,354	*13*	*14,515,265*
887F08	13	13,508,354	*13*	*14,515,265*
28E03	13	13,626,696	*13*	*14,397,931*
312E02	13	13,659,088	*13*	*14,366,468*
83H11	13	13,869,485	*13*	*14,156,316*
205H09	13	14,240,933	*13*	*13,775,086*
404D05	13	14,339,816	*13*	*13,676,763*
183H07	13	14,488,203	*13*	*13,532,571*
492A12	13	14,947,115	*13*	*13,065,300*
274E12	13	15,723,798	*13*	*12,271,925*
918D08	13	16,541,513	*13*	*11,460,368*
224A04	13	16,805,420	*13*	*11,210,842*
211C10	13	18,065,893	13	16,725,406
987C01	13	18,355,336	13	17,014,195
476H07	13	18,435,436	13	17,092,983
628F07	13	18,443,690	13	17,101,195
983D10	13	18,810,769	13	17,463,586
553A05	13	18,831,983	13	17,484,332

The inverted portion in goat is in italics

[a] BAC name

[b] Cattle chromosome localization

[c] Goat chromosome localization

Table 5 BACs localized in goat CHI6 transposition

BAC[a]	BTA[b]		CHI[c]	
470H11	6	104,522,225	6	99,864,046
63C08	6	104,628,726	6	99,968,896
251D02	6	104,726,314	6	100,064,665
595B07	6	105,157,085	6	100,495,514
607G07	6	105,157,086	6	100,495,514
25E06	6	105,464,841	6	100,777,362
487F04	6	105,694,027	6	101,003,312
246G01	6	106,566,883	*6*	*111,586,265*
493G04	6	106,667,202	*6*	*111,708,068*
395E09	6	107,080,171	*6*	*112,130,155*
102D09	6	107,572,332	*6*	*102,025,962*
426C07	6	108,011,768	*6*	*112,822,603*
140G08	6	108,589,319	*6*	*113,300,412*
116A06	6	108,589,319	*6*	*113,300,412*
991B10	6	109,108,320	*6*	*113,827,211*
415C11	6	109,855,551	*6*	*113,443,474*
713F08	6	110,531,444	6	102,730,824
195B10	6	110,792,790	6	102,991,459
515G05	6	110,815,527	6	103,013,552
888C05	6	111,126,473	6	103,305,277
272D11	6	111,142,834	6	103,829,347
1053D05	6	112,164,972	6	104,380,057
810C06	6	112,371,994	6	104,579,600
374G08	6	112,404,921	6	104,610,332
1095B09	6	113,016,070	6	105,193,380
55B03	6	113,193,862	6	105,375,085
533B05	6	113,213,822	6	105,392,620
153F03	6	113,215,318	6	105,394,064
1004G10	6	113,685,417	6	105,873,957
1004H02	6	113,714,606	6	105,906,791
710G05	6	114,094,155	6	106,283,924
70E12	6	114,932,430	6	107,174,546
984B12	6	115,165,859	6	107,415,962
192E09	6	115,693,510	6	107,941,105
444E07	6	116,140,506	6	108,363,885
311A01	6	116,225,315	6	108,447,814
12C12	6	116,255,971	6	108,477,789
347G12	6	116,360,258	6	108,582,816
140B09	6	116,445,137	6	108,666,203
188G06	6	117,164,264	6	109,261,525
622B09	6	117,301,728	6	109,404,602
394E12	6	118,512,956	6	110,527,906
534A02	6	118,697,343	6	110,715,138
546A01	6	118,855,070	6	110,842,087

Table 5 (continued)

BAC[a]	BTA[b]		CHI[c]	
543D10	6	118,855,070	6	110,842,104
865H10	6	118,855,234	6	110,842,251
249H02	6	118,856,740	6	110,843,930
186F10	6	119,027,801	6	111,046,220

The transposed portion in goat is in italics

[a] BAC name

[b] Cattle chromosome localization

[c] Goat chromosome localization

To summarize, we used a bioinformatic approach in this study to obtain a total of 12,711 BACs with a strong localization in the genomes of cattle and goat. We also obtained information that can facilitate improvement of the cattle and goat genomes in the future. Moreover, our strategy enabled us to define, for the first time, the size and orientation of the translocated fragment involved in the BTA9;14 evolutionary rearrangement. Until recently, the inversion of the fragment was only suspected, but not confirmed (Iannuzzi et al. 2001), and its size was unknown. Another interesting discrepancy that we detected was the hypothetical transposition that occurred in the goat chromosome 6. In this case, the FISH analyses revealed that both the cattle and goat genomes shared the same organization, and that the assembly of the goat genome was the correct one. This finding is surprising as the cattle genome is generally believed to be more accurate than the goat genome.

Notably, we identified a new chromosomal divergence between cattle and goat: an inversion of 7.4 Mb in the goat chromosome 13 with respect to its cattle homologue chromosome (BTA14). This discovery represents the starting point for new areas of research, including research on the effect of this inversion on the goat phenotype. The two rearrangement breakpoints (RBP) were included in the regions of 853,611 bp (10,685,991–11,539,602 bp) and 1,260,473 bp (16,805,420–18,065,993 bp) for the centromeric and telomeric RBPs, respectively. The first RBP includes three genes (*SNRPB2*, *OTOR*, and *NANP*), whereas the second one includes 9 genes (*PRKCQ*, *PFKFB3*, *RBM17*, *IL2RA*, *FBXO18*, *ANKRD26*, *YME1L1*, *MASTL*, and *ACBD5*). Further investigations are warranted to identify the precise point where the RBPs occurred, as we cannot exclude that this event interfered with the regulation of one or more of these genes. For example, in pig *SCNN1B*, a genetic factor involved in the perception of salty taste was located in a porcine-specific evolutionary breakpoint (EBP), and this genomic rearrangement jeopardized the ability to perceive the taste of salt (Groenen et al. 2012). The inverted fragment includes 17 genes (*CCDC3*, *CDC123*, *NUDT5*, *SEC61A2*, *DHTKD1*, *PROSER2*, *ECHDC3*, *USP6NL*, *CELF2*, *MIR7861*, *VAMP7*, *GATA3*, *ATP5C1*, *KIN*, *ITIH2*, *ITIH5*, and *SFMBT2*). Further studies are required to understand whether this inversion is responsible for the perturbation of gene expression in goat of one or more of these genes.

Another question that needs to be addressed by future study concerns whether the inversion is present only in goat or whether other Antilopinae tribes also show the same genomic rearrangement. The preliminary bioinformatic result indicates that this inversion is also present in sheep (*Ovis aries*), although further investigation is needed to highlight its occurrence in other species.

In conclusion, we applied a new bioinformatic strategy to identify the new cryptic rearrangements between cattle and goats and validated their existence in goat, with respect to cattle, using the FISH technique.

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