Constitutive heterochromatin distribution in pig *(Sus scrofa)* chromosomes

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SUMMARY - Constitutive heterochromatin (HC = C-banding) distribution was studied in pig *(Sus 5crofa)* chromosomes from 20 animals belonging to Cinta Senese and Calabrese breeds raised in southern Italy. The use of CBG-banding, sequential GBG/CBA-banding and sequential GBG/Ag-NOR/CBA-banding techniques allowed more detailed characterization of C-banding patterns in pig chromosomes (SSC). The following features were noticed: (a) all autosomes and the X-chromosome showed centromeric C-positive bands; (b) the entire q-arm and proximal part of the p-arm y chromosome were C-positive: (c) clear interstitial C-positive bands were noticed in SSC1q17, SSC3p14 and SSC16q21; (d) the nucleolusorganizer (NO) chromosome 10 showed two distinct HC-blocks very far apart in both arms with large, polymorphic (different size) NORs between the chromosome pair, while NO-chromosome 8 showed only one C-positive band (the smallest) in the q-arms; (e) C-band polymorphism was observed between and within chromosome pairs also in related subjects (three generations were followed); (f) the C-banding patterns are inherited in Mendelian fashion; (g) the most common C-band polymorphic chromosomes pairs were SSC 1, 7, 9, 11, 12, 16, 17 and 18.

Key words: pig, chromosomes, C-banding, G-banding.

INTRODUCTION

Constitutive heterochromatin (HC = C-bands) has been reported to be highly polymorphic in mammalian chromosomes, including in pig where all chromosomes have been reported to be C-positive (SYSA 1980; LIN *et at.* 1982; HANSEN 1982; GLAHN-LUFT *et at.* 1982; SWITONSKI *et al.* 1983). Also intersti

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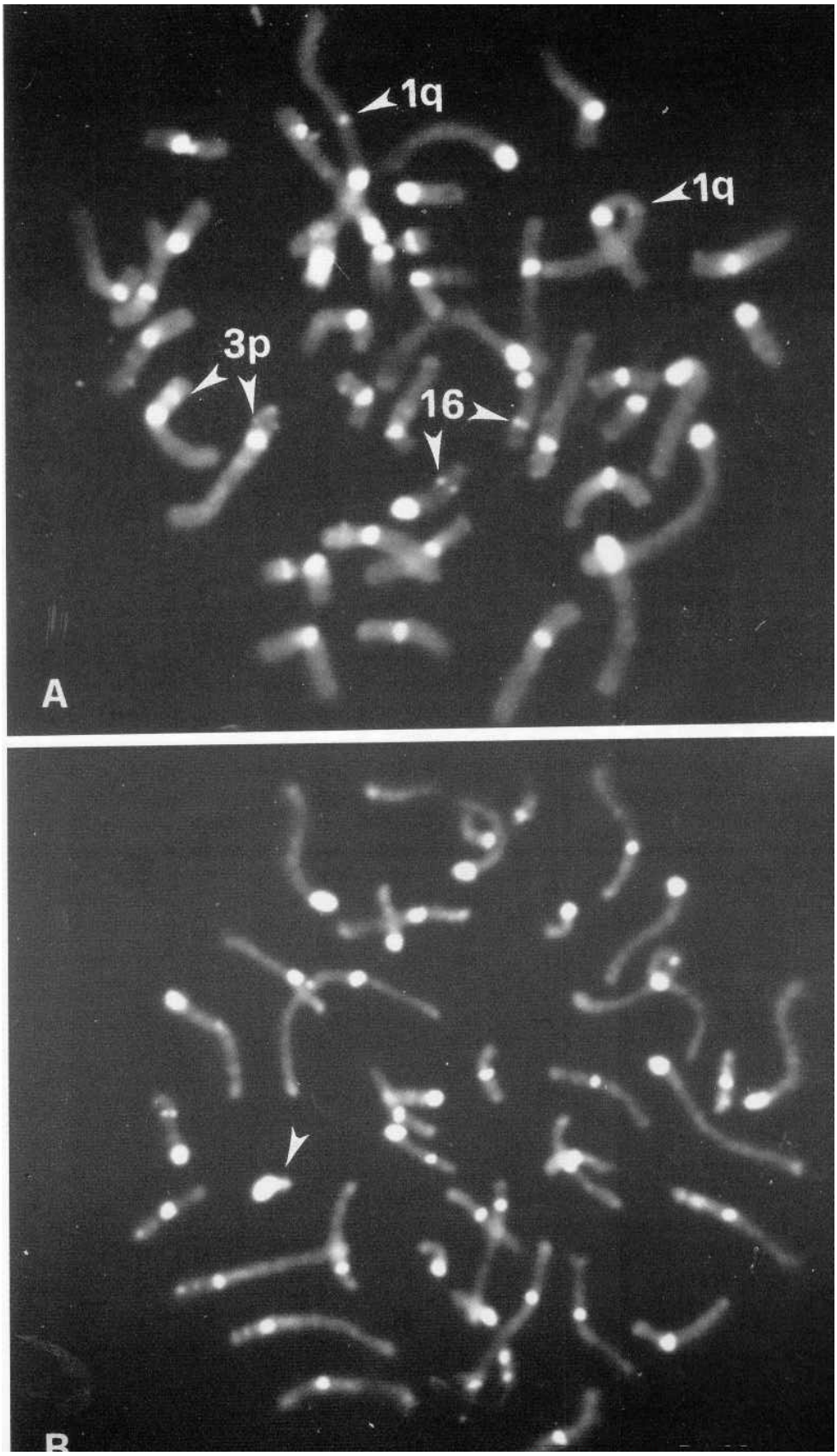
tial HC has been found in pig chromosome 16 (HANSEN 1982; GLAHN-LUFT *et al.* 1982). The only previous study which analyzed the C-banding patterns on pre-identified pig chromosomes by using the Q-banding technique was that of HANSEN (1982). This author found C-band polymorphism in many chromosome pairs. Furthermore, he found that the C-banding technique was unsuitable for identifying C-band polymorphism of homologous chromosomes, probably due to technical errors.

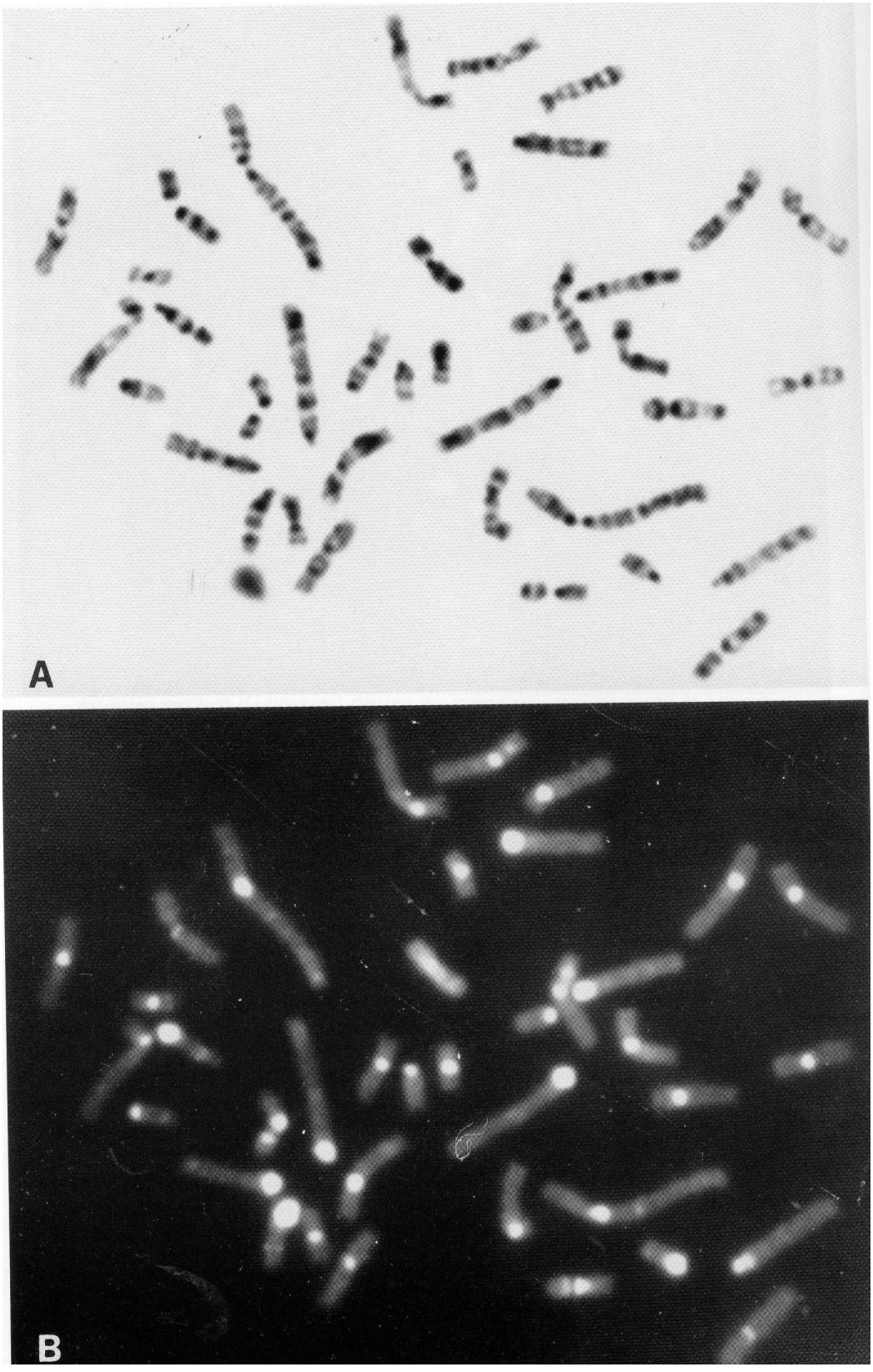
In the present study we show the HC-distribution in pig chromosomes (SSC) from two Italian local breeds by using different banding techniques, also in sequential procedures, which allowed more detailed characterization of C-banding pattern chromosomes in this important species, and demonstrate the suitability of the C-banding technique to study HC-polymorphism between and within chromosome pairs.

MATERIALS AND METHODS

Twenty Italian pigs from Cinta Senese (central Italy) and Calabrese (southern Italy) breeds, raised at the Cons DABI, Circello (Benevento), which is also the National Focal Point of the F AO in Italy for domestic animals, were used for this study. Peripheral blood lymphocytes were cultured for about 72 h in McCoy's 5A modified medium (Gibco) and treated for early-BrdU incorporation as follows: in the morning of the third culture day, BrdU (15 ìg/ml) was added to the cell cultures and removed 3 hours later after centrifugation and washing once with Puck saline solution (pH = 7.0). Then cells were recovered in the same medium containing Thymidine (10J.lg/ml) for 5 hours with colcemid (0.01 ìg/ml) treatment for 1 hour. This allowed replicating G-banding patterns to be obtained. Slides were treated for both CBG- and CBA-banding (SUMNER 1972). For CBA-banding, slides were treated and stained with acridine orange as previously reported (DI MEO *et al.* 1995). Sequential CBG/CBA-banding, GBG/CBA-banding and GBG/Ag-NOR/CBA-banding techniques were also performed. For sequential GBG/CBA-banding and sequential GBG/Ag-NOR/CBA-banding techniques, the following procedures were followed: slides were stained for 20 minutes with Hoescht 33258 (50 ìg/ml), washed in distilled water, mounted in 2xSSC (l ml, pH = 7.0) with coverslip and exposed for 30 minutes under U . V. light (at a distance of 4 cm). Then slides were washed in distilled water, treated in 2xSSC at 60° C for 1 hour, immersed in 2xSSC at room temperature, dehydrated in an alcohol series (50%, 70% and 95%) and stained with Giemsa (8% in phosphate buffer pH = 7.0) for 30 minutes. The best banded preparations were photographed and then slides were destained in alcohol, washed in tap and distilled water and finally treated for the CBA-

Fig. 1. - Female (A) and male (B) pig early-metaphase cells treated for CBA-banding technique. Note the interstitial C-bands in SSC1q, SSC3p and SSC16 (arrows in A). Chromosome y was entirely heterochromatic in the q-arms and proximal part of the p-arms (arrow in B).


1q
1q
3p
16
A
B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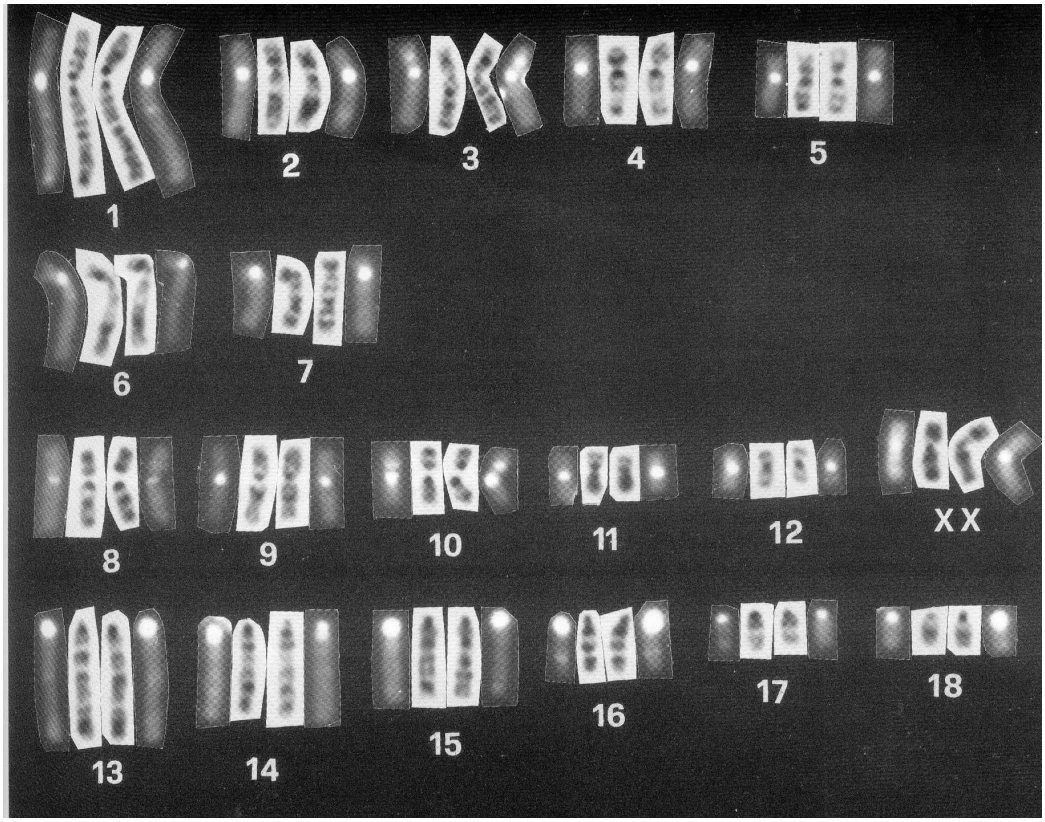

Fig. 2. - Sequential GBG/CBA-banding in pig female early-metaphase (A-B) with relative karyotype (C). Notice that the NO-chromosome 8 shows a single HC-block located in the proximal q-arm centromeric region while the NO-chromosome 10 shows two separated HC-blocks in both arms (C). Notice also the HC-polymorphism in SSC 6, 8, 11, 12, and 18, as well as the interstitial C-positive bands in SSC1q17, SSC3p14 and SSC16q21.

banding technique (sequential GBG/CBA-banding) following SUMNER'S (1972) protocol or treated for Ag-NOR staining before CBA-banding (sequential GBG/Ag-NOR/CBA-banding). Pig chromosomes were arranged according to the standard karyotype (GUSTAVSSON 1988).

RESULT AND DISCUSSION

The CBA-banding technique was more effective than CBG-banding, as demonstrated also by sequential CBG/CBA-staining of the same metaphases. In particular, the HC-blocks in biarmed chromosomes were more prominent than those obtained by using CBG-banding. This can in part explain the results obtained by HANSEN (1982) who reported C-negative or indistinct C-banding in some biarmed chromosomes by using the CBG-banding technique. However, LIN *et at.* (1982) found all pig chromosomes to be C-positive by using the CBG-banding technique (normal procedure) , and different types of C-bands by using A-T or G-C specific ligands. In particular, (a) C-band positive and A-T rich heterochromatin was located in the centromeric region of acrocentric chromosomes and (b) faint C-band positive and G-C rich heterochromatin was located mainly in the centromeric region of biarmed chromosomes. In our studies all pig chromosome subjects we examined were CBA-band positive (Fig. 1), including the sex chromosomes with the y -chromosome being entirely heterochromatic in the q-arms and proximal part of the p-arms (Fig. 1B). This in part agrees with LIN *et al.* (1982) who reported the y -chromosome C-band positive in the q-arms.

Interstitial C-positive bands were observed in SSC 1q, SSC3p and SSC 16 (Figs. 1 and 2), while in previous papers interstitial C-bands were noticed only in SSC16 (GLAHN-LUFT *et at.* 1982; HANSEN *et a!.* 1982). However, these authors did not precisely indicate the band location of this interstitial C-positive band in SSC 16. The use of sequential GBG-/CBA-banding techniques (Fig. 2) allowed us to study the HC-distribution between and within the karyotypes of all animals and to precisely localize the observed interstitial C-positive bands in SSC1q17 (G-negative), SSC3p14 (G-positive) and SSC16q21 (G-negative). Furthermore, the nucleolus organizer (NO) chromosomes 8 and 10 showed different C-banding patterns: SSC8 showed only one HC-block in the proximal q-arm centromeric region, while SSC10 showed two distinct HC-blocks, often very far from each other, in both chromosome arms (Fig. 2). To verify this phenomenon we sequentially treated slides for GBG-/Ag-NOR/GBA-banding techniques (Fig. 3). Essentially, the distances between the two HC-blocks in SSC10 were due to NOR-polymorphism (different size of Ag-NOR staining). In SSC8, even when the NORs were polymorphic, only one HC-block (the smallest of the pig chromosome complement) was detected. Previous papers did not report any C-banding patterns related to the NOR-polymorphism of SSC8 and SSC10. This particular polymorphism (both in the NORs and HC) should be better investigated in these two chromosomes.

The C-banding patterns found in different cells from the same animal were repetitive, since the same HC-polymorphism (between and within cromosome pairs) was found. This has previously also been found in cattle (DI BERARDINO *et a!.* 1980; DI MEO *et a!.* 1990) and river buffalo (DI MEO *et a!.*

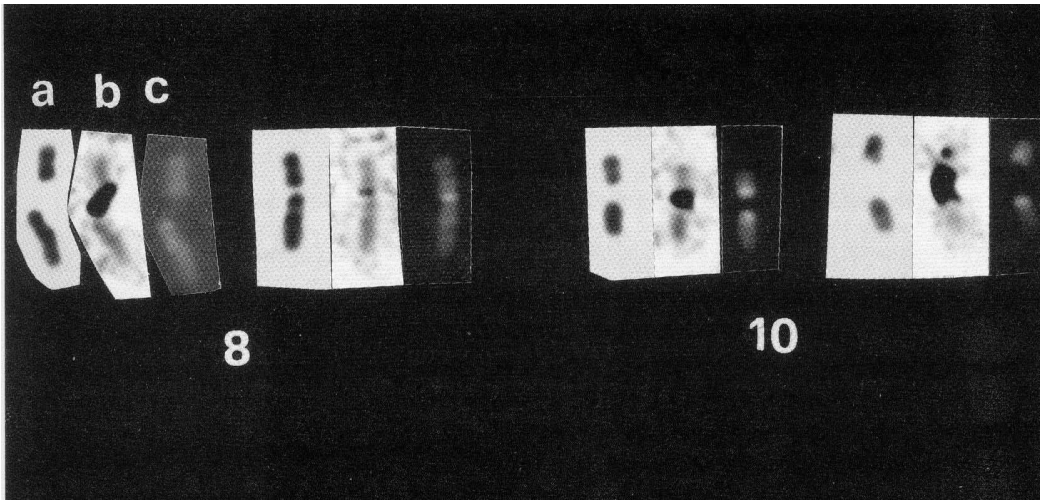

Fig. 3. - NO-chromosomes 8 and 10 drawn from the same cell treated for GBG-/AG-NOR-/CBA-banding techniques (a, b and c, respectively). Note that the distance between the two HC-blocks in SSC10 was due to the NOR-polymorphism. In SSC8 only one HC-block was observed even when polymorphic NORs were present.

1995). For this reason, only one good GBG/CBA-banded karyotype for each animal was sufficient to study the HC-distribution among different animal karyotypes. A clear HC-polymorphism was observed in all the animal karyotypes we examined. However, the chromosome pairs which were found to be particularly polymorphic in their C-bands were SSC 1,7 ,9, 11, 12, 16, 17 and 18 which can be considered as marker chromosomes.

We examined the HC-distribution on 13 subjects from three different generations in the Calabrese breed. We found an HC-polymorphism among all animals, even considering that they were highly related. Indeed, many chromosome pairs which were found to be heteromorphic in their C-banding, were only partially conserved in the progeny due to different meiotic chromosome segregations.

We also tried to follow the HC-block sizes in the karyotypes which we examined along the three generations. We observed a Mendelian segregation of C-banding patterns, since the C-band sizes were conserved in the progeny. This behavior, together with the HC-polymorphism between and within the chromosome pairs, make this cytogenetic technique very useful in livestock selection programs. In fact, it should be very interesting and practical to find positive relationships between the HC-polymorphism and quantitative traits in domestic animal populations so to use the C-banding technique in genetic

assisted selection together to other techniques, as those using molecular genetics (microsatellites). Furthermore, the polymorphism of C-banding, especially if used on pre-identified chromosomes, could be a useful tool to study, from a chromosomal point of view, the biodiversity between and within breeds from the same species.

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