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C-Banded Karyotype and Nucleolar Organizer Regions (NORs) of Wild Boar, *Sus scrofa* (Artiodactyla: Suidae) from Anatolia

Atilla ARSLAN[1], İrfan ALBAYRAK[2]

[1]Department of Biology, Faculty of Science and Arts, Selçuk University, 42031 Konya - TURKEY

[2]Department of Biology, Faculty of Science and Arts, Kırıkkale University, 71450 Kırıkkale - TURKEY

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Abstract: The present study reports the karyotype, C-banding, and nucleolar organizer regions (NORs) of 6 *Sus scrofa* (Linnaeus, 1758) males from Anatolia. The karyotype of *S. scrofa* comprised (2n) 38 chromosomes, the number of chromosomal arms (FN) was 64, and the number of autosomal arms (FNa) was 60. C-positive regions appeared to be restricted to the centromeric regions of autosomes 1, and 13-18, and the entire long arm of the Y chromosome. Some autosomes had very slight C-bands. The X chromosome appeared to be entirely euchromatic. NORs were identified by the silver-staining technique and were observed on secondary constriction sites of 2 metacentric chromosomes pairs.

Key Words: *Sus scrofa*, wild boar, cytogenetic, Anatolia

Anadolu'daki Yaban Domuzu, *Sus scrofa* (Artiodactyla: Suidae)'nın C-bantlı Karyotipi ve Nükleolar Organizatör Bölgeleri (NORs)

Özet: Bu çalışmada Anadolu'daki altı erkek *Sus scrofa* (Linnaeus, 1758) örneğinin karyotip, C-bant ve nükleolar organizatör bölgelerinin (NORs) özellikleri araştırıldı. *S. scrofa*'nın karyotipinin (2n) 38 kromozomdan oluştuğu, temel kromozom kol sayısı (FN) 64 ve otozomal kromozom kol sayısı (FNa) 60 olduğu gözlendi. C-pozitif bölgeler, 1, 13-18 nolu kromozomların sentromerik bölgelerinde ve Y kromozomun uzun kolunun tamamında göründü. Bazı otozomlar çok hafif C-bantlara sahiptir. X kromozomun tamanının ökromatik tipte olduğu belirlendi. Gümüş boyama tekniği ile tanımlanan nükleolar organizatör bölgeleri (NORs), iki çift metasentrik kromozomdaki ikincil boğum yerlerinde bulundu.

Anahtar Sözcükler: *Sus scrofa*, yaban domuzu, sitogenetik, Anadolu

Introduction

Turan (1) stated that *Sus scrofa* Linnaeus, 1758 is found throughout Turkey. Mursaloğlu (2) reported that the wild boar in Anatolia is represented by *Sus scrofa libycus* Gray, 1868. Karyological studies around the world show that the diploid chromosome number of the domestic pig (*Sus scrofa domestica*) and the wild boar (*Sus scrofa scrofa*) ranges from 36 to 38 (3-12). The karyology of *S. scrofa* from Turkey was reported by Albayrak and İnci (13), but they did not report any chromosomal polymorphisms in *S. scrofa*. Nonetheless, chromosome number polymorphisms have been frequently recorded in wild boar populations as a result of centric fusion (3,8,12). The distribution of constitutive heterochromatin (C-band) in the chromosomes of domestic pigs and wild boar has been studied by Sysa (14) and Hansen (15). Nucleolar organizer regions (NORs) of this species have also been determined by some authors using fluorescence *in situ* hybridization (FISH) and silver staining techniques (16-21).

Herein, we present the C-banding and Ag-NORs data for *S. scrofa* from Turkey, and a comparison of these results with those from other parts of the world.

Materials and Methods

The study animals (6 males) were collected from Kırıkkale Province in 2007. Karyotype preparations were obtained from the bone marrow of the colchicined animals (22), followed by conventional Giemsa staining. Constitutive heterochromatin and NORs were detected to identify pairs of each autosomal and sexual chromosome via C-banding (23) and Ag-NOR staining (24), respectively. Chromosome morphology was established by calculating centromeric indexes. In total, 10-20 slides were prepared from each animal for analysis, and a minimum of 20 well-spread metaphase plates were also analyzed from each animal. Some standard voucher specimens (skins and skulls) were deposited in the Department of Biology, Faculty of Science and Arts, Selçuk University, Konya, Turkey.

Results and Discussion

The karyotype of Turkish *S. scrofa* comprises 38 chromosomes. The number of autosomal arms (FNa) is 60 and the number of fundamental arms (FN) is 64. Twelve autosomal pairs are bi-armed (nos. 1-12) and 6 pairs are acrocentric (nos. 13-18). The X chromosome is a medium-sized submetacentric and the Y chromosome is a small metacentric (Figure 1). We know that for populations studied elsewhere, diploid chromosome numbers of 36 and 37 were recorded from the USA, Italy, the Netherlands, and Poland (7,11,12,25). According to previous reports (3,8,12), the causes of this chromosomal polymorphism could perhaps be attributed to a Robertsonian translocation (i.e. centric fusion) occurring between 1 or 2 pairs of acrocentric chromosomes. All previous reports indicated (2n = 36, 37, and 38) the same number (60) of autosomal arms (FNa). Centric fusion was not observed neither in the present study nor in the study performed by Albayrak and İnci (13) on Turkish wild boar.

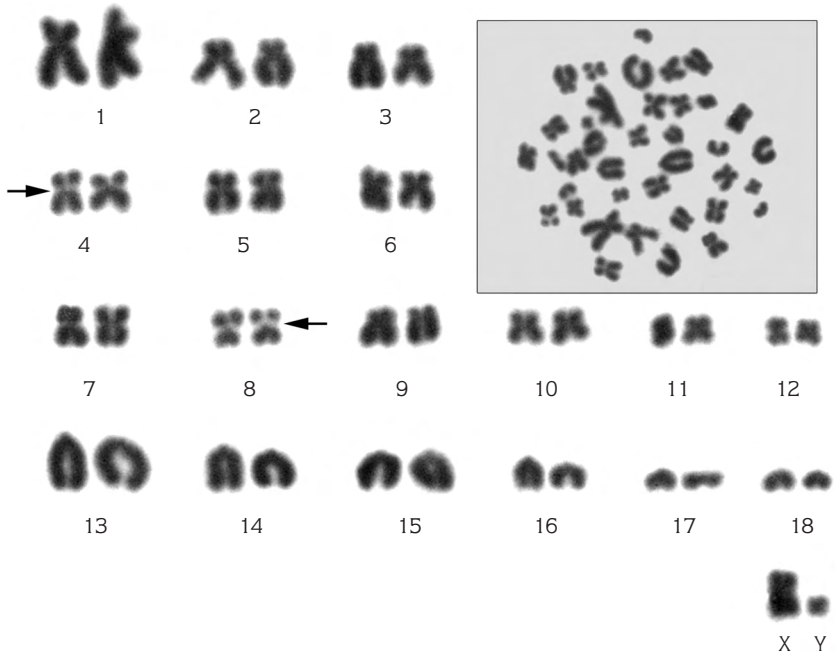

Figure 1. Metaphase spread and karyotype of Turkish *Sus scrofa*. Arrow indicates the secondary constrictions (NOR).

C-bands are located at the centromeric regions of submetacentric and acrocentric chromosomes nos. 1 and 13-18, as well as the entire long arm of the Y chromosome. Some autosomes have only very slight centromeric C-bands. The X chromosome appears to be entirely euchromatic. The C-bands of acrocentric chromosomes were larger than those on chromosome no. 1 (Figure 2). In the domestic pig the centromeric regions of all chromosomes have been reported to be C-positive (14,15). Furthermore, interstitial C-bands were observed in a chromosome pair by Hansen (15). Telomeric C-bands have also been identified by Adega et al. (26) in the domestic pig; however, neither interstitial nor telomeric C-bands were determined in our specimens. These results show that (with the exclusion of chromosome no. 1) the centromere regions of bi-armed chromosomes have been completed by a reduction in heterochromatin blocks. According to Adega et al. (26), the classic C-banding technique used in cytogenetic studies detects the major constitutive heterochromatin blocks in pig chromosomes; however, the classical method has also been used in studies by Sysa (22) and Hansen (23).

Using silver-nitrate staining we determined that the NORs are localized at the secondary constriction sites of 2 metacentric chromosome pairs (nos. 4 and 8). Active NOR signals were observed to be different in each chromosome, in both the conventional karyotype and Ag-stained metaphase (Figures 1 and 3). In the present study metacentric and submetacentric chromosomes were not karyotyped separately; therefore, chromosomes were numbered as mixed so that in our results chromosomes 4 and 8 corresponded to chromosomes 8 and 10, respectively, in other reports. Most researchers have reported a preferential staining of the NORs on chromosome 10. Mellink et al. (27), however, observed a high incidence of Ag-stained NORs on chromosome no. 8 in wild *Sus* species (*S. scrofa scrofa, S. scrofa vittatus, S. verrucosus, S. celebensis*, and *S. salvanius*). It has been revealed by the FISH technique that domestic pig rDNA is located either in the secondary constrictions of both chromosome pairs 10 and 8 (27) or only in the secondary constrictions of chromosome 10 (28). Mellink et al. (21) determined that the signal on chromosome 10 can be stronger than that in chromosome 8, as in the present study.

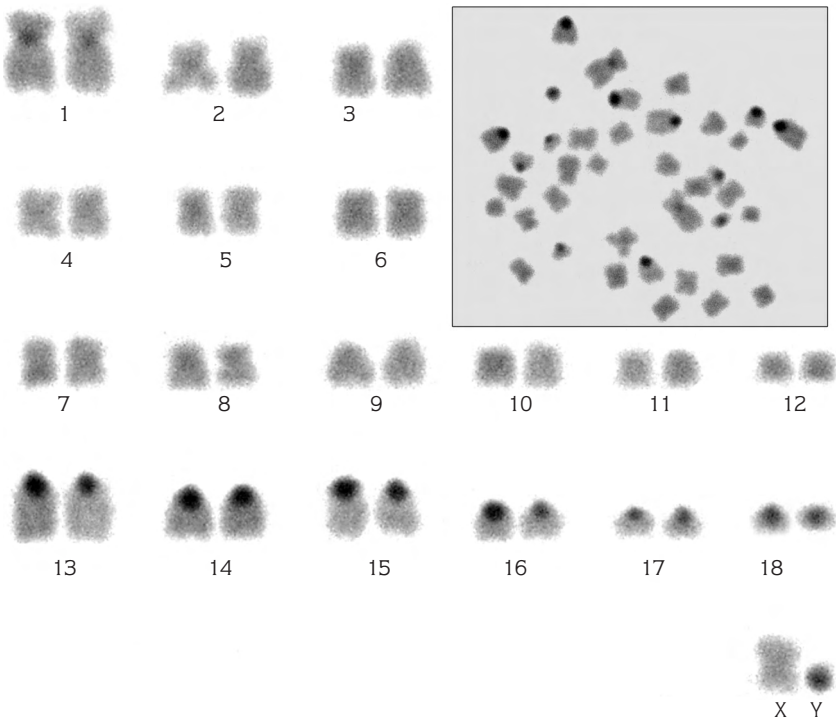

Figure 2. Metaphase spread and C-banded karyotype of Turkish *Sus scrofa*.

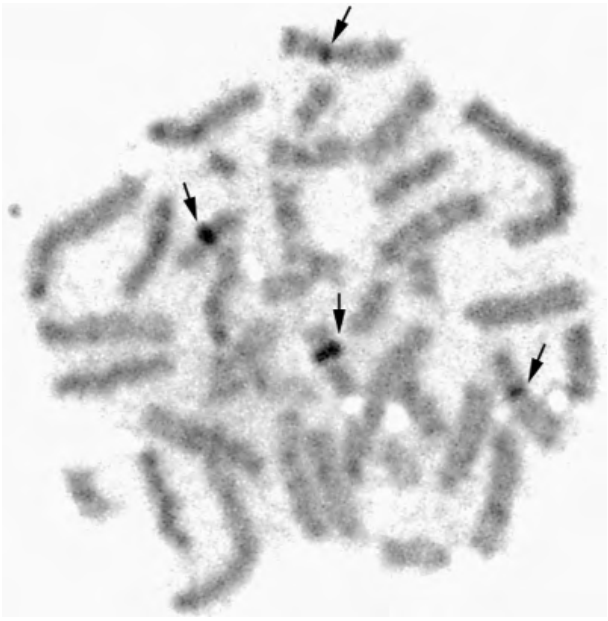

Figure 3. Silver-stained metaphases of Turkish *Sus scrofa*. Arrow indicates the Ag-NOR.

Consequently, the localization of NORs obtained from Turkish *Sus scrofa* is similar to nominative and non-nominative species. Although Turkish *Sus scrofa libycus* is known to be an ancestor to all domestic pigs, it is clearly different than others, because centromeric C-bands were not seen in most bi-armed chromosomes of the Turkish specimens that have been studied to date.

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Corresponding Author:
Atilla ARSLAN
Department of Biology,
Faculty of Science and Arts,
University of Selçuk,
Konya - TURKEY
E-mail: aarslan@selcuk.edu.tr

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