

## Chromosomal localization and activity of nucleolar organizer regions in the dog (*Canis familiaris*)

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**Abstract** – Using fluorescent in situ hybridization on Q-banded chromosomes, nucleolar organizer regions (NORs) were localized on canine chromosomes 7qter, 17qter, Yqter and also terminally on a small autosome, not yet included in the dog standard karyotype. The analysis of NOR activity was carried out on Ag-I stained metaphases originating from 27 individuals (10 males and 17 females). The mean number of silver stained NORs was 6.0 in males and 5.3 in females and the modal number was 7 and 6, respectively. Interindividual variability of the mean and modal numbers of silver NORs was observed. The lowest individual mean number of NORs was 4.2 and the highest 7.0, and the modal value ranged from 4 to 7. © Inra/Elsevier, Paris

**NOR / dog / fluorescent in situ hybridization / silver staining**

**Résumé** – Localisation chromosomique et activité d'organismes nucléolaires chez le chien (*Canis familiaris*). Les organisateurs nucléolaires (NORs) des chromosomes visualisés en bandes Q ont été localisés par hybridation fluorescente in situ sur les chromosomes canins 7qter, 17qter, Yqter et ainsi qu'à l'extrémité d'un petit autosome, qui n'est pas encore inclus dans le caryotype standard du chien. L'activité des organisateurs nucléolaires a été mise en évidence sur des métaphases colorées par l'Ag-I provenant de 27 individus (10 mâles et 17 femelles). Il a été trouvé que le nombre moyen d'organismes nucléolaires colorés par l'argent était de 6 chez les mâles et de 5,3 chez les femelles et que la valeur modale était respectivement 7 et 6. Une variabilité interindividuelle du nombre moyen et de la valeur modale a été observée pour les organisateurs nucléolaires colorés par l'argent. Le nombre moyen le plus bas d'organismes nucléolaires chez un individu était de 4,2 et le plus élevé de 7, la valeur modale se situant entre 4 et 7. © Inra/Elsevier, Paris

**NOR / chien / hybridation fluorescente in situ / coloration par l'argent**

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## 1. INTRODUCTION

In the nucleolar organizer regions (NORs), there are genes for 5.8S, 18S and 28S ribosomal RNA. These regions can be visualized by the silver staining method or in situ hybridization with the use of appropriate molecular probes.

Silver staining reveals transcriptionally active NORs, since precipitation of metallic silver occurs only in the regions where acid proteins are present [15]. On the other hand, in situ hybridization visualizes all (active and inactive) NORs which are present in a karyotype.

In the family Canidae, chromosomal localization of NORs is known for two species with an established standard karyotype: the blue fox, *Alopex lagopus* [8] and the silver fox, *Vulpes fulvus* [9]. Recently, a partial standard karyotype of the dog, comprising the 21 largest autosome pairs and sex chromosomes, was established [18]. This made the development of the physical mapping of the canine genome possible.

The objective of the present study was the chromosomal localization of NORs in the dog karyotype and the analysis of their activity. This study is a contribution to a joint effort within the DogMap programme established in 1993, which aimed at the development of a low density marker map of the dog genome [13].

## 2. MATERIAL AND METHODS

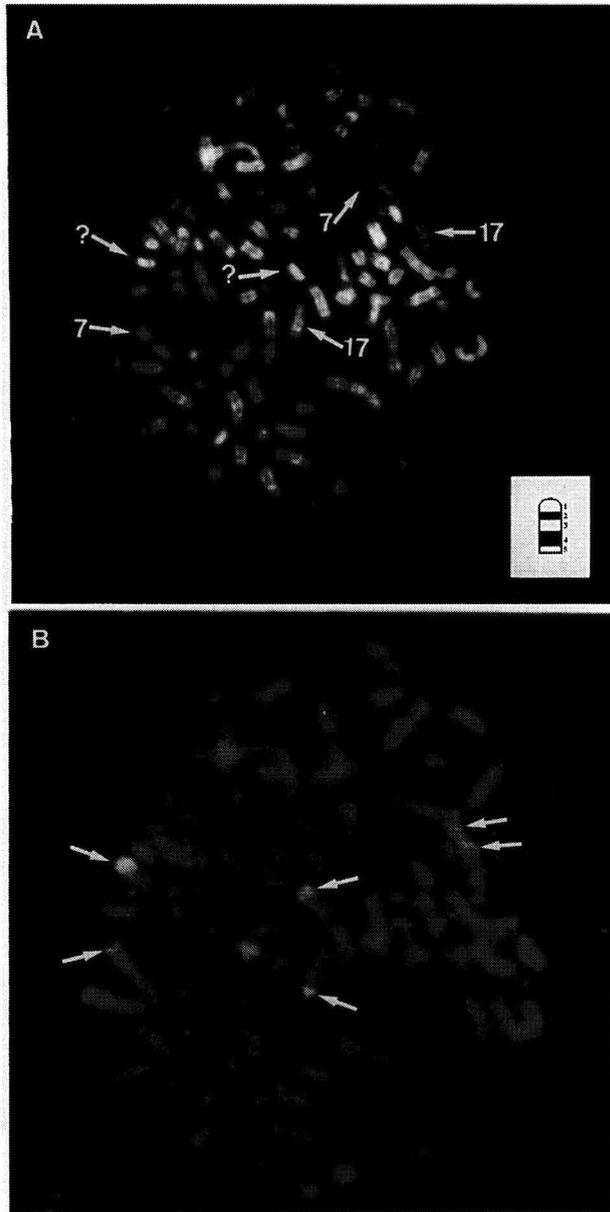
The somatic chromosomes were studied in cultured peripheral blood lymphocytes collected from 27 crossbred dogs.

Chromosomal localization of NORs was carried out with the use of fluorescent in situ hybridization (FISH) on pre-identified Q-banded chromosomes. For FISH experiments, a 28S rDNA human probe [16] was labelled with the use of biotin-16-dUTP. Hybridization of the probe was carried out without competitor DNA and the Avidin-FITC system was applied to detect the hybridization signals. Chromosomes were identified according to the nomenclature presented in the G-banded partial standard karyotype [18]. It was assumed that Q-banding patterns of dog chromosomes were similar to the GTG ones.

The activity of NORs was studied for 27 individuals (10 females and 17 males). For each individual 8 to 10 silver stained metaphases were analysed. In the case of a single female, 12 metaphases were recorded. Active NORs were visualized by the Ag-I banding technique [3].

## 3. RESULTS

FISH experiments revealed NORs on three autosome pairs (*figure 1*) and on the Y chromosome. The Q-banding pattern indicated that NORs were localized in the terminal part of the q arms of chromosome pairs: 7 and 17. The third chromosome pair, also bearing NORs in the terminal part of the q arm, belongs to a group of small autosomes not yet included in the canine standard karyotype. The banding pattern of this chromosome is shown in *figure 1A*. In the male karyotype, a NOR occurred also in the terminal part of the long arm of the Y chromosome.



**Figure 1.** A Q-banded metaphase of a female. Insert shows a diagrammatic representation of the Q/G-banding pattern of the small autosome (?→) bearing NORs; B fluorescent in situ hybridization of the plasmid 28S rDNA probe on the same metaphase. NOR-bearing chromosomes are indicated by arrows.

A detailed analysis of the silver deposit distribution on the chromosomes of 27 individuals showed that all seven NORs were transcriptionally active (*table I*). The activity of NORs was similar in all three autosome pairs. The majority of metaphases (approx. 79 %) demonstrated that both NORs of a chromosome pair were active. The NOR on the Y chromosome showed a similar pattern of activity.

**Table I.** Distribution of silver stained NORs on canine chromosomes.

Chromosome	Total number of metaphases	Silver deposits on		Lack of silver deposits
		Both chromosomes	One chromosome	
7	266	202 (76 %)	56 (21 %)	8 (3 %)
17	266	222 (83 %)	39 (15 %)	5 (2 %)
Unidentified	266	204 (77 %)	53 (20 %)	9 (3 %)
Y	164	–	130 (79 %)	34 (21 %)

Analysis of the number of silver NORs among the 27 dogs showed a wide range of variation (*table II*). The individual mean number of silver deposits varied from 4.2 to 6.0 in females and from 4.8 to 7.0 in males. The mean number of NORs calculated for all males was higher (6.0) than for females (5.3). This was mainly due to the NOR activity on the Y chromosome. Similar variability was found for the modal number, i.e. the most frequently observed number of silver NORs.

**Table II.** Interindividual variability of the number of silver NORs.

Sex	No. of animals	Mean ( $\bar{x}$ )		Modal number	
		All individuals	Interindividual range <sup>a</sup>	All individuals	Interindividual range <sup>a</sup>
Females	10	5.3 (1.06) <sup>b</sup>	4.2–6.0	6	5–6
Males	17	6.0 (1.13) <sup>b</sup>	4.8–7.0	7	4–7

<sup>a</sup> The range shows the lowest and the highest individual mean or modal number of silver NORs found among studied animals; <sup>b</sup> the standard deviations are indicated in parentheses.

#### 4. DISCUSSION

Nucleolar organizer regions in the dog karyotype were studied by several authors. Unfortunately, the obtained results were not consistent. Several reports showed the occurrence of silver stained NORs in the terminal part of the long arms of three autosome pairs and also on the Y chromosome [6, 11, 12]. Other reports based on

silver staining suggested, however, a higher number of chromosome pairs bearing NORs [1, 5, 14]. Recently, the in situ hybridization approach was applied. Using a 18S + 28S rDNA human probe, Mäkinen et al. [10] localized NORs on G-banded chromosomes 7, 17, a small unidentified autosome and the Y chromosome. Also, Fischer et al. [2] assigned NORs by the FISH method in a female karyotype to three G-banded autosome pairs: 7, 8 and a small unidentified autosome. Our study, using the QFQ/FISH approach, confirmed the results obtained by Mäkinen et al. [10]. Moreover, we present the Q/G pattern of the small autosome, not yet included in the canine standard karyotype.

The analysis of silver stained metaphases of 27 individuals revealed high transcriptional activity of the NORs. Similar results were presented by Pathak et al. [12] for a single male and Graphodatsky [4] for three males and one female. However, in the latter study the Y chromosome was not indicated as a NOR-bearing one. Our study also showed that activity of NORs demonstrated a wide interindividual variability. The individual mean number of NORs ranged between 4.2 and 7.0 in this study.

The distribution of silver stained NORs has also been analysed in other canids. It was found that NORs were present on seven chromosomes, including the Y chromosome, in the raccoon dog, *Nyctereutes procyonoides* [7]. In the karyotype of the silver fox, *Vulpes fulvus*, there are three autosome pairs bearing NORs which are localized terminally on chromosomes 8q, 9q and 13q [8]. The analysis of their activity showed that the mean number of silver stained NORs was 5.2, but the modal number was 6 [17]. The highest number of NORs was found in the karyotype of the blue fox, *Alopex lagopus*. There are six chromosome pairs with NORs. They were localized terminally on the short arm of chromosomes 13, 15, 17, 18, 20 and 22 [9].

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