



Differential karyotype profiling of three popular breeds of dogs in India

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The description of the domestic dog (*Canis lupus familiaris*) as man's best friend is a conception that is derivative from ages of co-existence and friendship. Dog breeds are companion animals primarily employed for guarding the farm and farmhouses, shepherding the livestock species. The 2.4 GB of DNA comprising the haploid nuclear genome of the (female) domestic dog are apportioned into 38 pairs of acrocentric autosomes and two metacentric sex chromosomes (Breen 2008). Cytogenetic studies may help categorize the chromosomal abnormalities which may prime to generative failures or infertility-related difficulties mainly in males.

The studies of the meiotic cells of the dog has determined the presence of 78 chromosomes (Minouchi *et al.* 1928) and later confirmed by using cultured peripheral lymphocytes (Gustavsson *et al.* 1964). The karyotype comprises 38 pairs of acrocentric autosomes, a large sub-metacentric X chromosome, and a small metacentric Y chromosome. Based on genome sequence data, dog chromosome 1 (CFA 1), is the largest autosome, with ~ 125 Mb in size (Lindblad-Toh *et al.* 2005) and is, therefore, smaller than human chromosome 12, with all but the five largest dog chromosomes (CFA 1–5) being smaller in Mb size than human chromosome 18.

Divergence among the canidae with respect to the common ancestor is reported to have commenced ~ 7–10 million years ago (Wayne *et al.* 1993). Earlier studies indicated the two major groupings, the 'dog-like' and 'fox-like' canids (Bininda-Emonds *et al.* 1999) (Graphodatsky *et al.* 2001). More latest genetic data, including data created as part of the project, dog genome sequence (Lindblad-Toh *et al.* 2005), have directed that the family may be refined into four major phylogenetic groups represented by the fox-like canids (including the raccoon dog), the gray and island fox species, the South American canids and the wolf-like canids (including the domestic dog) (Ostrander 2007). The karyotype design of the Canidae ranges from $2n = 34$ in the red fox (*Vulpes*

vulpes) to $2n = 78$ in the wolflike canids (Wayne 1993).

More than 1000 dog data were recorded while visiting Guru Angad Dev Veterinary and Animal Sciences University, Ludhiana, Punjab hospital from the Out Patient Department Cards.

Labrador, Pug, and German shepherd are the most prevalent breeds of the region (Table 1). The blood samples were collected aseptically from the above mentioned three breeds for cytogenetic profiling. The blood was subjected to the separation of lymphocyte cells.

The collected data were analyzed to find out the top three most common breeds of dogs prevailing in the region. The peripheral blood samples were collected aseptically from the cephalic vein or saphenous vein of those three popular breeds of dog and local indigenous dogs in a heparinized tube. Short term peripheral blood lymphocyte culture technique (Moorhead *et al.* 1960) was followed with few modifications. The cultures were set up in a sterile culture tube by placing 0.3 to 0.5 ml of white blood cells (buffy coat) in 6 ml of Ham's F-Medium supplemented with L-glutamine, 1.0 ml fetal bovine serum, and Pokeweed mitogen. The cells were cultured by incubation at 37°C for 70–72 h. The cells were harvested by the addition of colchicine. The cell suspension was incubated in pre-warmed at 37°C in hypotonic solution (0.075 M potassium chloride) for 8–10 min and fixed in Carnoy's fixative methanol and acetic acid (3:1). Further, cells were treated to make their cell wall brittle to get burst against the slide, while dropping from a specific height. Slides were air-dried and stained by Giemsa for the visualization of chromosomes under a microscope prepared in a clean pre-chilled glass slide and air-dried.

The chromosomes G-banding was carried out using trypsin digestion protocol (Barch *et al.* 1991). The slides were observed under a microscope (Mag-cam DC 5) and decent metaphase spreads were photographed for developing banded karyotypes.

Labrador (29.64%), Pug (17.20%) and German shephard (11.89%) were the most common breeds in this region. Chromosome analysis of four breeds of dogs revealed the diploid number ($2n$) as 78, with 38 pairs of acrocentric autosomes, one large sub-metacentric X-chromosome, and a small sub-metacentric Y-chromosome.

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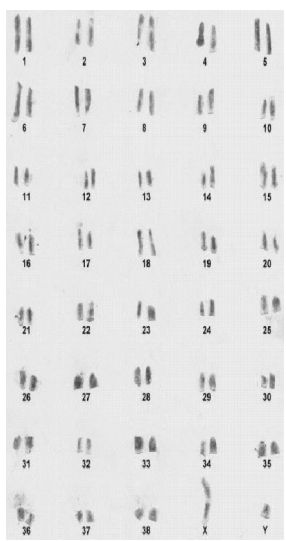
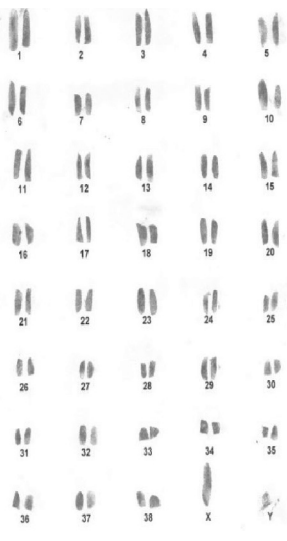
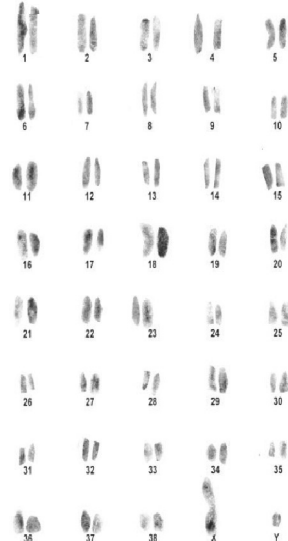
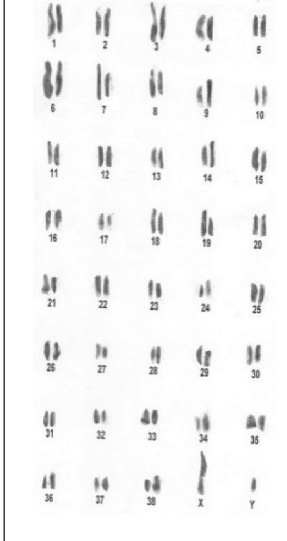
Karyotyping Labrador male	Karyotyping Pug male	Karyotyping German Shepherd male	Karyotyping Indigenous male
			
Karyotype (G-banding) of Labrador adult male	Karyotype (G-banding) of Pug adult male	Karyotype (G-banding) of German Shepherd adult male	Karyotype (G-banding) of Indigenous adult male

Fig. 1. Karyology of four breeds (Labrador, Pug, German shepherd, Indigenous) of dog.

Table 1. Top 10 breeds with their frequency

Breed Name	Total No.
Labrador	324
Pug	188
German Shepherd	130
Pomeranian	82
Rottweiler	61
Pit-bull	52
American Bully	35
Beagle	25
Dachshund	25
Spitz	23

The size of the acrocentric chromosomes gradually decreased from the first pair to the thirty-eighth pair of autosomes in both the sex. Chromosome analyses of domestic dogs were in agreement with the earlier reports (Suryawanshi *et al.* 2004). Sex chromosome pair is represented by two large metacentric X in females and the Y chromosome has metacentric morphology in males and is the shortest one in the complement (Topashka *et al.* 2009). The importance of cytogenetic abnormalities in clinical diagnosis and response has been well established in particular for hematological malignancies with the diagnostic tools being microscopy-based karyotyping (Hans 2019).

SUMMARY

The present investigation aims to study the karyology of the three most popular dog breeds as well as indigenous local dog. In this study, we identified the most popular dog

breeds of the Punjab region which are maintained as companion animals, or for guarding. Metaphase plates were prepared after culturing of lymphocytes isolated from heparinized blood collected from the identified three most popular canine breeds. The isolated lymphocyte cells were cultured for 70–72 h following the cell cycle arrest at metaphase. The G-banding of the chromosomes was done by Giemsa staining through a standard protocol. The most popular three breeds of dog in the sub-tropical region were Labrador, the German Shepherd, and Pug. There were no significant distinguishable differences between the karyotypes of the dog breeds studied. This study gives insight into karyology information, which can be beneficial to the researchers, dog breeders, and kennel clubs. Moreover, it provides information about chromosomal abnormalities which may lead to the study of various fertility, growth, and phenotypic abnormalities problems in dog breeds.

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