Scanning Microscopy

Volume 2 | Number 3 Article 35

3-18-1988

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Niiro, G. K. and Seed, T. M. (1988) "SEM of Canine Chromosomes: Normal Structure and the Effects of Whole-Body Irradiation," *Scanning Microscopy*: Vol. 2 : No. 3 , Article 35.
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Scanning Microscopy, Vol. 2, No. 3, 1988 (Pages 1593-1598)
Scanning Microscopy International, Chicago (AMF O'Hare), IL 60666 USA

SEM OF CANINE CHROMOSOMES: NORMAL STRUCTURE AND THE EFFECTS OF WHOLE-BODY IRRADIATION

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(Received for publication September 25, 1987, and in revised form March 18, 1988)

Abstract

Canine chromosomes are not only numerous (38 autosomal pairs), but they are small (compared to human chromosomes) and morphologically similar as well. Analysis of the canine karyotype by light microscopy (LM) of banded chromosomes is, thus, difficult, and the literature on the canine karyotype is scanty. In this study, we describe examination of chromosomes from normal and chronically irradiated dogs with the scanning electron microscope (SEM). Metaphase chromosomes from bone marrow aspirates were Giemsa-banded with either 0.025% trypsin alone or 0.1% trypsin preceded by 10% H_2O_2 and prepared for SEM. Examination of chromosomes from normal dogs revealed cylindrical chromosome profiles with well-defined chromatids and centromeres. The chromosome arms were consistently marked by periodic grooves that had complementary structures on sister chromatids and may represent the trypsin-sensitive chromatic regions. The quality of the preservation varied from preparation to preparation and depended on the concentration and time of trypsin treatment. Chromosomes from irradiated dogs revealed translocations, deletions, and gaps. We conclude that SEM produces images superior to LM images of canine chromosomes; SEM images can be used not only to identify individual chromosomes, but also to identify genetic lesions in the chromosomes of chronically irradiated dogs. We further conclude that the two Giemsa-banding protocols used in the present study produced variable results, although 0.025% trypsin alone appeared to give better and more consistent results than 0.1% trypsin preceded by 10% H_2O_2.

KEY WORDS: Chromosomes, canine gamma irradiation, scanning electron microscopy, cytogenetics.

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Introduction

Since the first report of a consistent structural chromosomal abnormality associated with chronic myelocytic leukemia, the so-called Philadelphia chromosome (Ph^1) described by Nowell and Hungerford in 1960, similar abnormalities in other cancers have been reported [see reviews by LeBeau and Rowley (1986) and Rowley (1983)]. The abnormalities identified so far are the gain of a part or all of a chromosome, loss of a part or all of a chromosome, and translocations. These abnormalities have been identified mainly by light microscopy (LM) with the use of novel banding techniques. However, further advancements in the study of chromosomal abnormalities may depend on observing chromosomes with instruments of higher resolution. A logical choice would be the scanning electron microscope (SEM), which has already demonstrated its usefulness in elucidating the structure of the fragile X chromosome (Harrison et al., 1983) and of chromosomal gaps (Chernos et al., 1986). Furthermore, SEM could prove to be useful for analysis of complex karyotypes such as the dog's. The canine karyotype consists of 38 pairs of morphologically similar autosomes that are mostly of the acrocentric or telocentric type (Moore, 1970). Even with the advent of novel chromosomal banding techniques, the size and similarity of canine chromosomes make their identification extremely difficult. This situation is reflected in the limited number of studies published on canine karyotype analysis by Giemsa-banding (G-banding) (Manolache et al., 1976; Selden et al., 1975).

The scanning electron microscope has been applied to the study of chromosome structure for more than 20 years. However, until recently, the images produced by the technique have not led to a clear understanding of chromosomal morphology. One reason for this failure has been the wide variation in preparative techniques used to isolate metaphase chromosomes for SEM study (Allen et al., 1986b). Recently, techniques developed in the laboratory of Allen (Allen et al., 1986a; Harrison et al., 1981, 1982, 1985) and based on routine methods used in LM have produced more consistent SEM micrographs.

The aim of the present study was to apply these techniques to the cytogenetic study of dogs with radiation-induced leukemia. The dog model of radiation-induced leukemia has been previously described (Fritz et al., 1985; Seed et al., 1977, 1982, 1985). The data presented here represent preliminary findings on chromosomal abnormalities of these leukemic dogs. This study also appears to be the first to report the use of SEM to observe canine chromosomes.

Materials and Methods

Animals and Irradiation Protocols

Outbred beagles used in this study were derived from the closed colony at Argonne National Laboratory described previously (Seed et al., 1985). These dogs were exposed to whole-body irradiation for 22 h each day from a ^{60}Co gamma-ray source (Gamma Beam 150, Atomic Energy of Canada, Ltd., Ottawa). The total dose was 1 to 10 rad/day.

Preparation of Metaphase Spreads and Giemsa-Banding

Preparation of metaphase spreads and subsequent G-banding of the chromosomes were done according to methods modified from the technique of Testa and Rowley (1981). Mononuclear cells were separated from bone marrow aspirates taken from the humerus (Seed et al., 1982). These cells were incubated for 10-20 min at 37°C in Medium 199 (M. A. Bioproducts, Whittaker Corp., Walkersville, MD) containing 10% horse serum, to which 2.5 µg/mL colchicine (E. Lily, Indianapolis, IN) were added. The cells were then incubated in 75 mM KCl for 10 min at 37°C and fixed with three changes of 3:1 ethanol:acetic acid. The cells were then dropped onto circular plastic coverslips and air-dried slowly under high humidity.

The spreads were aged for 24 h at room temperature plus an additional 10 min at 70°C and were subsequently G-banded by one of two protocols. The first protocol consisted of a 14-s immersion in 10% H_2O_2, followed by a brief rinse in distilled water and a 12-s immersion in 0.1% trypsin (trypsin 1:250, Difco Labs., Detroit, MI) in Isoton (Coulter Diagnostics, Hialeah, FL). The chromosomes were then fixed in 2% glutaraldehyde buffered with 0.1 M sodium cacodylate for a minimum of 2 h at 4°C. The second protocol consisted simply of a 10-s immersion in 0.025% trypsin; no H_2O_2 was used. After trypsin treatment, the spreads were placed in buffered 2% glutaraldehyde.

Preparation for Scanning Electron Microscopy and Morphometry

Metaphase spreads were prepared for SEM by using a method modified from the technique of Harrison et al. (1981). Following G-banding and fixation in glutaraldehyde, the metaphase spreads were quickly rinsed in buffer and post-fixed with 1% OsO_4 in buffer for 10 min. After three rinses with distilled H_2O, the spreads were treated with a saturated solution of thiocarbohydrazide (TCH) for 5 min. After three rinses in distilled H_2O and another 10 min of treatment in 1% OsO_4, the TCH-OsO_4 treatment was repeated. The chromosomes were then dehydrated with increasing concentrations of ethanol and critical-point dried from Freon 13, with Freon 113 as the intermediate fluid. The metaphase spreads were then lightly sputter-coated with gold before examination in a Cambridge Stereoscan Mark IIa or a JEOL JSM-840a SEM, both operated at 10 kV.

Chromosome lengths were measured from micrographs on an Apple II plus graphics tablet with the Stereometric Measurement and Analysis computer program (Scientific Micro Programs).

Results

Comparison of Giemsa-Banding Protocols

Although the two banding methods used in this study produced variable results, the milder banding treatment of 0.025% trypsin alone appeared to produce better, more consistent spreads (Fig. 1a) in terms of well-preserved morphology and distinct patterns of chromatid grooves (see below). In contrast, the harsher treatment of 0.1% trypsin preceded by 10% H_2O_2 tended to yield more spreads with poor morphology and chromosomes with indistinct patterns of chromatid grooves Fig. 1b). An example of undesirable results is shown in Fig. 1b; such chromosomes often had an "overdigested" appearance, with numerous connections between individual chromosomes and between sister chromatids. Most importantly, the grooves seen in Fig. 1a were not evident.

Morphology of Chromosomes from Control Dogs

In the best preparations, canine chromosomes appeared as relatively smooth, cylindrical profiles with well-defined chromatids and centromeres (Figs. 2a and 2b). A consistent observation was the presence of indentations or grooves periodically placed along the length of the chromosome arms that subdivided the arms into parallel segments. Corresponding indentations were found along both sister chromatids.

Morphology of Chromosomes from Irradiated Dogs

The overall appearance of chromosomes from irradiated dogs was much like that of control dogs; i.e., chromosomes were cylindrical with well-defined chromatids, centromeres, and grooves (Fig. 3a). In contrast to chromosomal spreads from control dogs, however, spreads from irradiated dogs commonly contained abnormal chromosomes that were readily detected and highlighted by SEM. These abnormalities included translocations (Fig. 3b), fragile tips (Fig. 3c), and deletions (Fig. 3d). Further, canine chromosomes examined by SEM were readily counted and measured (Table 1), giving data that compare favorably to LM-based data. In terms of chromosome length, the effect of chronic irradiation is manifested by a gain in percent of total genomic complement in chromosomes of families A and B and by losses in family G, as well as in the sex chromosome group (Table 1).

Discussion

The results we have obtained on dog chromosomes are similar to those obtained on human chromosomes (Harrison et al., 1981). Of special

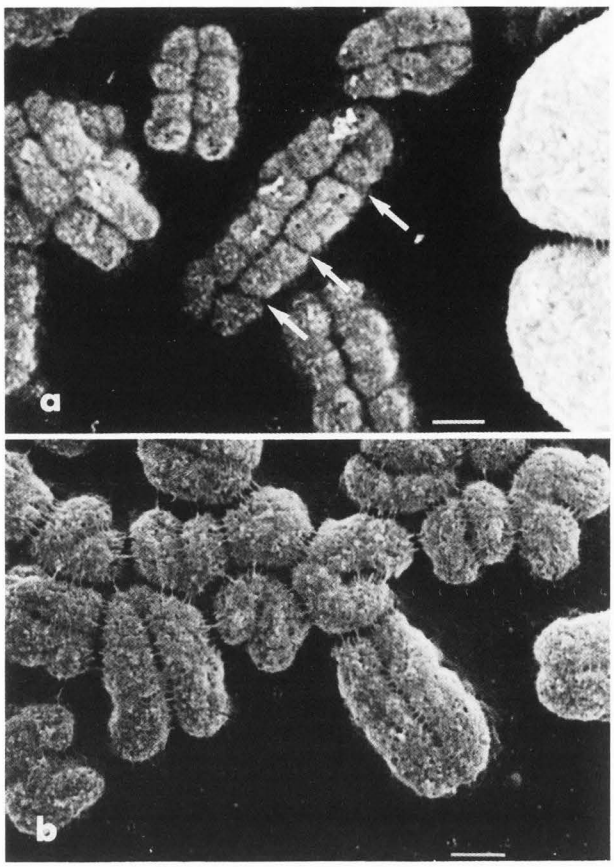

Fig. 1. Comparison of canine chromosomes after (a) treatment with 0.025% trypsin alone and (b) treatment with 0.1% trypsin preceded by 10% H_2O_2. Note the appearance of grooves (arrows) in 1a; these are not evident in 1b. Bar = 1 μm.

interest are the indentations or grooves on the chromosome arms. Harrison et al. (1981) have demonstrated that the grooves correspond to the G-positive bands seen by LM. A preliminary comparison between LM and SEM images of the first canine chromosome pairs indicates that the SEM grooves and LM G-positive bands correspond. Because of its higher resolving power, we feel that SEM analysis can lead more quickly to a precise canine karyotype than will conventional LM analysis. In general, SEM analysis seems likely to lead to a better understanding of overall chromosome structure (Mullinger and Johnson, 1987).

Our two banding protocols produced variable results. That is, well-preserved morphology and distinct circumferential grooves, as well as poor morphology and indistinct grooves, resulted from both treatment with 0.1% trypsin preceded by 10% H_2O_2 and treatment with 0.025% trypsin alone. However, we feel that the milder treatment, with 0.025% trypsin alone, produces more consistent results. We have not ruled out the future possibility of using treatments intermediate to the two we used in this study.

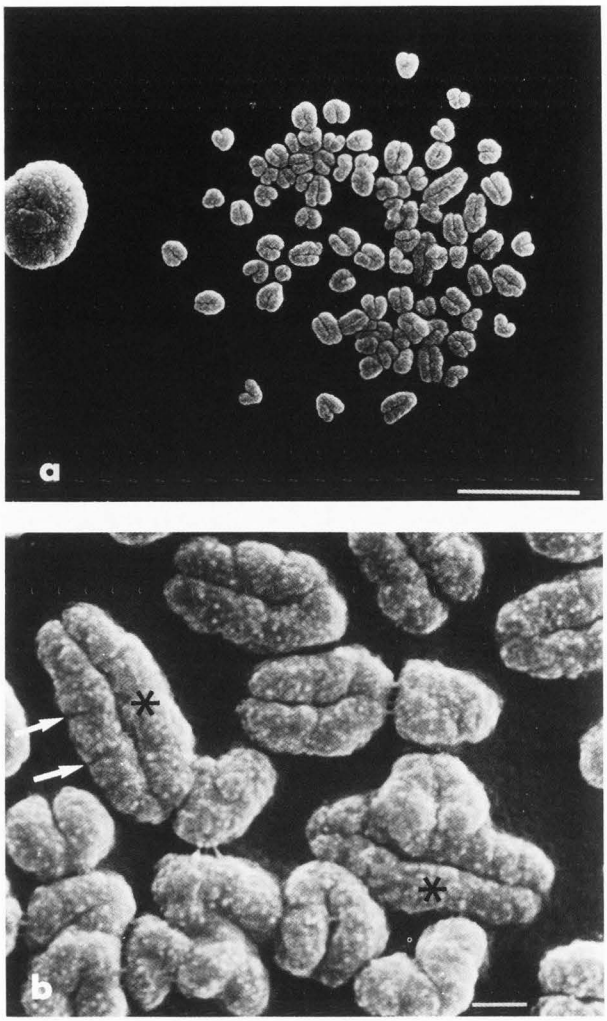

Fig. 2. Morphology of chromosomes from control dogs. (a) Low-power micrograph showing entire metaphase spread. Treatment with 0.1% trypsin preceded by 10% H_2O_2. Bar = 10 μm. (b) Higher-power view of the same spread showing pair of chromosome 1 (*). Note the cylindrical profile and grooves (arrows). Bar = 1 μm.

The initial examination by SEM of chromosomes from chronically irradiated dogs has highlighted a number of prominent lesions. Because of the greatly increased resolution provided by SEM, the mapping and characterization of critical, radiation-induced chromosomal lesions will be facilitated.

In conclusion, we have shown that SEM is a useful tool for the analysis of canine chromosomes. It appears that with some "fine-tuning" of the methods, individual canine chromosomes and chromosomal abnormalities can be identified by SEM with precision and reliability.

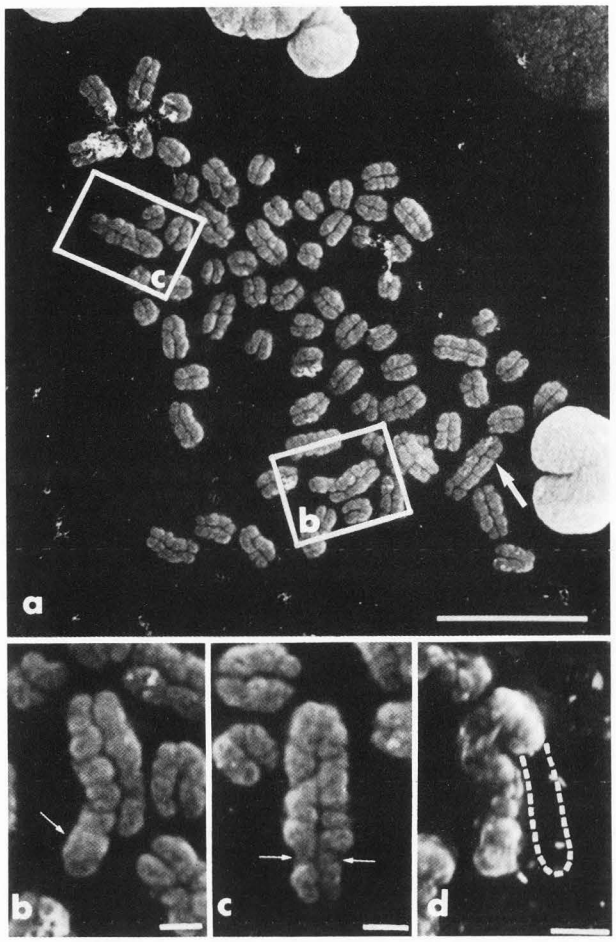

Fig. 3. Morphology of chromosomes from irradiated dogs. (a) Low-power micrograph showing entire metaphase spread appearing much like Fig. 2a. Arrow points to chromosome 1. Bar = 10 μm. Higher-power view of enclosed boxes is shown in (b) and (c). (b) Chromosome from same spread with possible translocation. The shorter chromatid is the normal length. Bar = 1 μm. (c) Other chromosome 1 from same spread showing fragile tips (arrows). Bar = 1 μm. (d) Chromosome from different spread showing possible deletion. The missing portion is outlined with white dots. Bar = 1 μm.

Acknowledgements

The authors would like to express their gratitude to Dr. J. A. McNulty and Ms. L. Fox of the Department of Anatomy, Loyola University Stritch School of Medicine, for the generous use of their scanning electron microscope and for their excellent technical assistance. The technical assistance of Ms. L. V. Kaspar and the secretarial help of Ms. M. C. D'Arpa are gratefully acknowledged.

This work was supported by the U.S. Department of Energy, Office of Health and Environmental Research, under contract No. W-31-109-ENG-38.

Table 1. Canine chromosome lengths measured by SEM as a percentage of haploid complement.

Chromosome no.	Family (Common lesions, if present)	% Total genomic length	
		Control (N = 4)	Irradiated (N = 4)
1	A	4.93 ± 0.51	5.60 ± 0.57
2	B	3.87 ± 0.13	4.76 ± 0.54
3	(q-arm	3.70 ± 0.05	4.25 ± 0.28
4	extensions,	3.60 ± 0.06	4.05 ± 0.30
5	translocations)	3.52 ± 0.05	3.95 ± 0.27
6		3.42 ± 0.05	3.69 ± 0.30
7		3.31 ± 0.06	3.53 ± 0.32
8		3.20 ± 0.08	3.44 ± 0.32
9	C	3.09 ± 0.05	3.35 ± 0.28
10		3.00 ± 0.10	3.31 ± 0.27
11		2.94 ± 0.09	3.26 ± 0.22
12		2.91 ± 0.07	3.23 ± 0.18
13		2.84 ± 0.10	3.13 ± 0.13
14		2.81 ± 0.12	2.97 ± 0.12
15		2.76 ± 0.15	2.85 ± 0.20
16		2.72 ± 0.13	2.77 ± 0.24
17	D	2.70 ± 0.13	2.70 ± 0.22
18		2.68 ± 0.12	2.57 ± 0.09
19		2.53 ± 0.07	2.53 ± 0.06
20		2.48 ± 0.06	2.49 ± 0.01
21		2.39 ± 0.05	2.46 ± 0.00
22		2.35 ± 0.07	2.45 ± 0.00
23		2.27 ± 0.07	2.42 ± 0.00
24		2.21 ± 0.10	2.31 ± 0.01
25		2.15 ± 0.09	2.18 ± 0.01
26	E	2.11 ± 0.09	2.03 ± 0.04
27		2.10 ± 0.08	1.96 ± 0.09
28		2.07 ± 0.06	1.91 ± 0.07
29		2.04 ± 0.03	1.85 ± 0.01
30		1.97 ± 0.09	1.81 ± 0.04
31		1.88 ± 0.19	1.81 ± 0.04
32		1.80 ± 0.19	1.68 ± 0.18
33	F	1.75 ± 0.17	1.63 ± 0.22
34		1.67 ± 0.16	1.54 ± 0.25
35		1.60 ± 0.18	1.57 ± 0.00
36	G	1.50 ± 0.10	1.32 ± 0.00
37		1.40 ± 0.04	- -
38	(Loss)	1.31 ± 0.03	1.18 ± 0.00
X	SX	2.24 ± 0.11	2.65 ± 0.00
Y	(Loss)	0.57 ± 0.09	0.43 ± 0.00

References

Allen TD, Jack EM, Harrison CJ, Claugher D (1986a) Scanning electron microscopy of human metaphase chromosomes. Scanning Electron Microsc. 1986; I:301-308.

Allen TD, Jack EM, Harrison CT, Claugher D, Harris R (1986b) Human metaphase chromosome preparation for scanning electron microscopy: A consideration of inherent problems. In: Science of Biological Specimen Preparation, Proc. of the 4th Pfefferkorn Conference, Grand Canyon, NM, Mueller et al. (eds), SEM, Inc., AMF O'Hare IL 60666, 299-307.

Chernos JE, Rattner JB, Martin RH (1986) An investigation of human sperm pronucleus chromosome "gaps" using scanning electron microscopy. Cytogenet. Cell Genet. 42, 57-61.

Fritz TE, Tolle DV, Seed TM (1985) The preleukemic syndrome in radiation-induced myelogenous leukemia and related myeloproliferative disorders. In The Preleukemic Syndrome, Bagby Jr GC (ed), CRC Press, Inc., Boca Raton, 87-100.

Harrison CJ, Britch M, Allen TD, Harris R (1981) Scanning electron microscopy of the G-banded human karyotype. Exp. Cell Res. 134, 141-153.

Harrison CJ, Allen TD, Britch M, Harris R (1982) High-resolution scanning electron microscopy of human metaphase chromosomes. J. Cell Sci. 56, 409-422.

Harrison CJ, Jack EM, Allen TD, Harris R (1983) The fragile X: A scanning electron microscope study. J. Med. Genet. 20, 280-285.

Harrison CJ, Jack EM, Allen TD, Harris R (1985) Light and scanning electron microscopy of the same metaphase chromosomes. J. Cell Sci. 77, 143-153.

LeBeau MM, Rowley JD (1986) Chromosomal abnormalities in leukemia and lymphoma: Clinical and biological significance. In Advances in Human Genetics, Harris H, Hirschhorn K (eds), Plenum Press, New York, 1-54.

Manolache M, Ross WM, Schmid M (1976) Banding analysis of the somatic chromosomes of the domestic dog (Canis familiaris). Can. J. Genet. Cytol. 18, 513-518.

Moore Sr W (1970) Cytogenetics. In The Beagle as an Experimental Dog, Anderson AC (ed) with Good LS, Technical Editor, Iowa State University Press, Ames, IA, 510-519.

Mullinger AM and Johnson RT (1987) Disassembly of the mammalian metaphase chromosome into its subunits: Studies with ultraviolet light and repair synthesis inhibitors. J. Cell Sci. 87, 55-69.

Nowell P, Hungerford DA (1960) A minute chromosome in human chronic granulocytic leukemia. Science 132, 1197.

Rowley JD (1983) Consistent chromosome abnormalities in human leukemia and lymphoma. Cancer Invest. 1, 267-280.

Seed TM, Kaspar LV, Fritz TE, Tolle DV (1985) Cellular responses in chronic radiation leukemogenesis. In Carcinogenesis, Vol. 10, Huberman E, Barr SH (eds), Raven Press, New York, 363-379.

Seed TM, Kaspar LV, Tolle DV, Fritz TE (1982) Hematologic predisposition and survival time under continuous gamma irradiation: Responses mediated by altered radiosensitivity of hemopoietic progenitors. Exp. Hematol. 10, 232-248.

Seed TM, Tolle DV, Fritz TE, Devine R, Poole CM, Norris WP (1977) Irradiation induced erythroleukemia and myelogenous leukemia in the beagle dog: Hematology and ultrastructure. Blood 50, 1061-1079.

Selden SR, Moorhead PS, Dehlert ML, Patterson DF (1975) The Giemsa banding pattern of the canine karyotype. Cytogenet. Cell Genet. 15, 380-387.

Testa JR, Rowley JD (1981) Chromosomes in leukemia and lymphoma with special emphasis on methodology. In Leukemic Cell, Catovsky D (ed), Churchhill Livingstone, New York, 184-201.

Discussion with Reviewers

N. Wang: The chromosomes in Fig. 1a are generally longer than those in Fig. 1b. Do you think this difference in length has anything to do with the appearance of the groove?
Authors: Chromosomes may respond differently to trypsin treatment depending upon their degree of contraction. Less contracted chromosomes have more of their structure exposed, permitting either enhanced or faster trypsin digestion or both. One might speculate that less contracted chromosomes would display more prominent grooves.

Reviewer III: Would actual sizes of chromosomes be more useful than % genomic length?
Authors: We would have included the actual lengths, however, we were most interested in whether individual chromosomes have been affected to a greater or lesser degree than the karyotype as a whole. We felt that this sort of data is best demonstrated by % genomic length analysis.

J. Rowley: Why are the authors sure that Fig. 3b is a translocation? Why isn't it a chromatid deletion? How do the authors know (see figure legend) that the shorter chromatid is the normal length?
Authors: The first chromosome pair of the canine karyotype is considerably longer than the second pair (Selden et al., 1975). On this basis, we can identify the first pair in Fig. 3a as the chromosome in the box labeled c and that identified by the arrow. Thus, it would appear that the normal length of the chromosome in question is that of the shorter chromatid.

J. Rowley: How certain are the authors that the x chromosome in the control is 2.24% rather than some other chromosome in Group D or E?
Authors: The x chromosome is metacentric (Selden et al., 1975) and can be reliably identified since the other chromosomes are acrocentric or telocentric.

J. Rowley: How precise and reliable are the data in Table 1? Is it premature to publish this table?
Authors: The number of SEM chromosome spreads analyzed and measured are, indeed, small, thus placing limits on the degree of confidence one places on specific chromosomes. However, part of the intent of this study was to demonstrate the utility of SEM to the study of canine cytogenetics. The SEM not only has a higher resolving power that may make identification of chromosomes more precise, quantitative data collected by SEM such as that shown in Table 1 compares favorably with LM-based data.

T. D. Allen: You indicate in Fig. 1a the complementary position of grooves in sister chromatids, which is readily apparent. However, the pair of grooves directly above the central arrow appears to run at a different angle to the long axis on each chromatid. Do you have any explanation for this finding? Does this indicate a gyre of final spiralisation in chromatid condensation?
Authors: We have also noted your obversation and it certainly makes for tempting speculation. However, without seeing more of the chromosomes, i.e., the other side, the answer to your question remains hidden.

T. D. Allen: This study is perhaps one of the first SEM studies of a karyotype with typically acrocentric chromosomes. It is intriguing to see some variation, c.f., the acrocentric next to the chromosome with a deletion in Fig. 3b, which had "U" configuration, with several in Fig. 3a, where sister chromatids appear to lie side by side without any common structure or centromere. Would the authors like to comment on this finding?
Authors: At the present time, we believe that the "true" configuration is that where the sister chromatids appear to lack any common structure. In most of the "U" acrocentrics one can see a faint or sometimes obscured line of separation between the sister chromatids.