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Canine cytogenetics – from band to basepair

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Abstract. Humans and dogs have coexisted for thousands of years, during which time we have developed a unique bond, centered on companionship. Along the way, we have developed purebred dog breeds in a manner that has resulted unfortunately in many of them being affected by serious genetic disorders, including cancers. With serendipity and irony the unique genetic architecture of the 21st century genome of Man's best friend may ultimately provide many of the keys to unlock some of nature's most intriguing biological puzzles. Canine cytogenetics has advanced significantly over the past 10 years, spurred on largely by the surge of interest in the dog as a biomedical model for genetic disease and the availability of advanced genomics resources. As such the role of canine cytogenetics has moved rapidly from one that served initially to define the gross genomic organization of the canine genome and provide a reliable means to determine the chromosomal location of individual genes, to one that enabled the assembled sequence of the canine genome to be anchored to the karyotype. Canine cytogenetics now presents the biomedical research community with a means to assist in our search for a greater understanding of how genome architectures altered during speciation and in our search for genes associated with cancers that affect both dogs and humans. The cytogenetics 'toolbox' for the dog is now loaded. This review aims to provide a summary of some of the recent advancements in canine cytogenetics.

The description of the domestic dog (*Canis familiaris*) as Man's best friend is a concept that is derived from millennia of co-existence and companionship. The bond between these two species is perhaps unlike that shared by any other two mammalian species. The availability of high quality genome resources for both human and dog has taken the relationship to a new level and comparative studies of their genomes are providing a plethora of new insights into the underlying biomedical similarities between these two species. Prerequisite to an understanding of normal genome organization of a species is the ability to define gross genome architecture. The 2.4 Gb of DNA comprising the haploid nuclear genome of the (female) domestic dog are partitioned into 38 pairs of acrocentric autosomes and two metacentric sex chromosomes (Fig. 1). The gross morphological similarity shared by many of the autosomes of the dog continues to present significant challenges to chromosome identification using conventional cytogenetics. The development of reagents and resources for molecular cytogenetic analyses of the canine genome has played a pivotal role in the generation of comprehensive and effective genome and comparative maps for the dog. Development and use of this molecular cytogenetics 'toolbox' allowed the development of a standardized chromosome nomenclature that led subsequently to the generation of integrated genome maps and played a key role in the anchoring of the canine genome assembly. In addition, molecular cytogenetics has allowed the gross genome architecture of the dog to be compared to other mammalian species and has also facilitated the development of means to assess genome changes associated with a variety of canine cancers. This article will review the development and application of these cytogenetic reagents and approaches.

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Table 1. Physical size of the chromosomes comprising the canine genome, derived from the earlier study of flow sorted chromosomes (Langford et al., 1996) and by the more recent canine genome sequence (Lindblad-Toh et al., 2005)

Chromosome	~ Size in Mb derived from flow sorting	~ Size in Mb derived from the genome assembly
CFA 01	135	125
CFA 02	100	88
CFA 03	104	94
CFA 04	102	91
CFA 05	99	91
CFA 06	88	80
CFA 07	95	83
CFA 08	87	77
CFA 09	79	64
CFA 10	81	72
CFA 11	87	77
CFA 12	86	75
CFA 13	76	66
CFA 14	73	63
CFA 15	76	67
CFA 16	74	62
CFA 17	81	67
CFA 18	67	58
CFA 19	67	56
CFA 20	68	61
CFA 21	63	54
CFA 22	72	64
CFA 23	63	55
CFA 24	55	50
CFA 25	60	54
CFA 26	48	42
CFA 27	57	48
CFA 28	55	44
CFA 29	51	44
CFA 30	47	43
CFA 31	50	42
CFA 32	51	41
CFA 33	41	34
CFA 34	50	45
CFA 35	38	29
CFA 36	41	33
CFA 37	40	33
CFA 38	38	26
CFA X	137	126
CFA Y	27	–
Total	2,782[a]	2,424

[a] Total size of haploid female genome indicated.

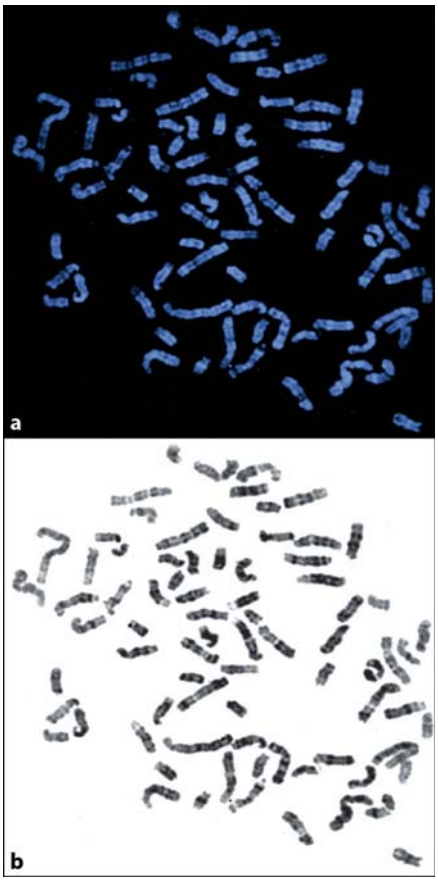

Fig. 1. The dog karyotype. (**a**) DAPI stained and (**b**) enhanced DAPI banded metaphase preparation of the domestic dog, with 38 pairs of acrocentric autosomes and a pair of metacentric sex chromosomes.

Development of a standard karyotype for the dog

The presence of 78 chromosomes in cells of the dog was determined by studies of meiotic cells (Minouchi, 1928) and later confirmed using cultured peripheral lymphocytes (Gustavsson, 1964). The karyotype comprises 38 pairs of acrocentric autosomes, a large sub-metacentric X chromosome and a small metacentric Y chromosome (Fig. 1). Based on genome sequence data, the largest autosome, dog chromosome 1 (CFA 1) is ~125 Mb in size (Lindblad-Toh et al., 2005) and is thus 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 (Table 1). Evaluation of unbanded chromosome preparations of the dog allows precise identification only for the sex chromosomes (due to their size and morphology) and chromosomes 1 and 38 (by virtue of their size). The gradual decrease in size of the remainder of the karyotype renders reliable recognition of conventionally stained homologous pairs almost impossible.

For many mammalian species, the development of chromosome banding techniques in the early 1970s provided the ability to identify homologous chromosome pairs and led to the subsequent development of internationally recognized chromosome numbering and banding nomenclatures. A variety of banded karyotypes for the dog have been published over the past 30 years and these are summarized in Table 2. It became clear that, at least for many of the smaller dog autosomes, chromosome banding patterns alone did not offer sufficient discrimination to describe the complete karyotype of the dog with confidence. Consequently, to ensure consistency in chromosome description, a 'Committee

Table 2. Published banded karyotypes of the domestic dog

Author (year)	Banding
Selden et al. (1975)	GTG
Manolache et al. (1976)	GTG
Howard-Peebles and Pryor (1980)	R
Fujinaga et al. (1989)	GTG
Poulsen et al. (1990)	R
Stone et al. (1991)	GTG
Moreno-Millan et al. (1991)	R
Graphodatsky et al. (1995)	GTG
Langford et al. (1996)	DAPI
Reimann et al. (1996)	GTG
Switonski et al. (1996)[a]	GTG
Christian et al. (1998)	digital
Breen et al. (1998)[b]	DAPI/GTG
Breen et al. (1999)[c]	DAPI
Yang et al. (1999)	DAPI
Graphodatsky et al. (2000)	DAPI

[a] CFA 1–21 were defined by the use of whole chromosomes paint probes generated by Langford et al. (1996), with the chromosome numbering based on Selden et al. (1975).
[b] CFA 22–38 were defined by the whole chromosomes paint probes generated by Langford et al. (1996) and ordered according the recommendation of the 'Committee for the Standardization of Dog Karyotype'.
[c] All chromosomes were defined by the use of whole chromosomes paint probes generated by Langford et al. (1996) and ordered according the recommendation of the 'Committee for the Standardization of Dog Karyotype'.

for the Standardization of the Karyotype of the Domestic Dog' was established in the early 1990s under the auspices of the DogMap Workshop. This committee was charged with proposing a chromosome nomenclature of the dog karyotype that would be accepted on the international arena. Using conventional cytogenetics the committee was able to reach a consensus on the identification of dog chromosomes 1–21 (CFA 1–21), but concluded that a complete standardized karyotype would require the use of molecular cytogenetic reagents (Switonski et al., 1996). Simultaneously, Langford et al. (1996) had used high-resolution bivariate flow sorting of dog chromosomes to generate a panel of whole chromosome paint probes (WCPP) for fluorescence in situ hybridization (FISH) analysis of the dog. These WCPP provided the essential common resource to members of the committee, and the joint efforts of many researchers led subsequently to the development of a consensus chromosome nomenclature for CFA 21–38 (Breen et al., 1998). The combined chromosome numbering system of all chromosomes of the dog that was proposed by the committee (Switonski et al., 1996; Breen et al., 1998, 1999) was later endorsed by the 'International Society of Animal Genetics' (ISAG) DogMap workshop held in Minneapolis, July 2000. At that time, the physical size of each dog chromosomes was estimated by inference from bivariate-chromosome sorting with mixtures of human and dog chromosomes (Langford et al., 1996; Breen et al., 1999) and suggested that the haploid genome of a female dog was in the region of 2,800 Mb (Table 1). It is interesting to note that while the dog karyotype was initially assembled based on conventional microscopic assessment of chromosome size, we know now that the actual physical sizes of the dog chromosomes bear little resemblance to their order in the established karyotype. Despite the introduction of a more sophisticated means of identification, the initial ordering of the karyotype was left as was and so the continued use of more sophisticated tools is still essential for reliable dog chromosome identification.

The introduction of canine molecular cytogenetics

The generation of whole chromosome paint probes and larger insert genomic libraries (bacteriophage and cosmid) saw the introduction of canine molecular cytogenetics. Simultaneously, the development of sophisticated cooled charged coupled device cameras, refinement of fluorescence filter band widths and the introduction of advanced multi-plane imaging software facilitated the development of multicolor FISH analysis. As a consequence more laboratories began to adopt fluorochrome based banding techniques (e.g. DAPI banding) to allow concurrent chromosome identification during FISH analyses, rather than the need to perform pre- or post hybridization chromosome banding. Sequential chromosome painting analysis with fluorescently labeled chromosome paint probes (Langford et al., 1996) onto single metaphases produced a series of complete DAPI banded karyotypes of the dog in which the chromosome numbering followed that adopted by the standardization committee (Breen et al., 1999). To allow accurate assignment of FISH mapped single locus probes (SLPs) to DAPI banded dog chromosomes, a 460-band ideogram of the dog karyotype with five grey levels was also produced (Breen et al., 1999).

Independently, a second set of dog chromosome paint probes was generated (Yang et al., 1999) and a detailed comparative chromosome painting approach (dog vs. red fox vs. human chromosomes) was applied in order to identify the dog chromosomes indirectly. The authors were able to integrate their chromosome nomenclature with that of the standardization committee for all but ten of the smaller autosomes (Graphodatsky et al., 2000). These ten discrepancies are shown in Table 3. A second DAPI banding pattern for the whole karyotype of the dog was generated in the form of a black and white ideogram. The nomenclature of the standardization committee was subsequently used to define the chromosomes in the 7.6× canine genome assembly (Lindblad-Toh et al., 2005) and so use of the nomenclature proposed by Yang et al. (1999), which has been used subsequently by Graphodatsky et al. (2000), requires numerical conversion of these ten autosomes to be compliant with the genome assembly data.

Table 3. Dog chromosome nomenclature differences between the studies of Breen et al. (1999) and Yang et al. (1999)

Breen et al. (1999)	Yang et al. (1999)
25	28
27	29
28	31
29	34
30	25
31	27
33	30
34	35
35	37
37	33

The role of canine molecular cytogenetics in genome mapping

Following the establishment of a standardized chromosome nomenclature, the use of multicolor FISH technology in canine genomics opened the door to expedite the physical mapping of numerous clones that contained key elements of emerging genome maps. In the first integrated genome map of the dog (Breen et al., 2001), 266 cosmid clones, each containing a polymorphic microsatellite marker, were used as chromosome-specific single locus probes (SLPs) to define their cytogenetic location. Distributed across all chromosomes these clones thus served as key cytogenetic points and enabled the 1,800 marker integrated meiotic/radiation hybrid (RH) map of the canine genome (Breen et al., 2001) as well as the subsequent 3,279 marker (Guyon et al., 2003) and 4,249 marker (Breen et al., 2004) RH maps of the dog to be firmly anchored to the karyotype. At the cytogenetic level, this 4,249 RH map reported the precise location of 1,000 canine bacterial artificial chromosome (BAC) clones (see below), each of which had been ordered along the length of each chromosome using stepwise multicolor SLP analysis. Of these 1,000 BAC clones, 804 were in common with the RH map. The use of a common set of markers for both the cytogenetic and the RH maps ensured that the integrated map was firmly anchored at multiple locations along the length of each chromosome, providing a very high level of confidence in marker order for this and the subsequent 10,000 marker RH map (Hitte et al., 2005). Molecular cytogenetics played a valuable role in anchoring the 7.6× genome assembly; indirectly via the use of cytogenetically-anchored RH map data as a means to provide useful ordering information, and directly as a tool to determine the precise location of sequences directly on chromosomes.

The development of additional reagents to enhance the growing canine molecular cytogenetics 'toolbox' became available with the development of two BAC libraries. The first, RPCI-81 (Li et al., 1999) was constructed from a male Doberman Pinscher and represents 8.1× genome coverage with an average insert size of 155 kb (http://bacpac.chori.org/mcanine81.htm). Numerous groups have used this library as a source of large insert genomic clones that have been used as SLPs to determine the precise cytogenetic location of many loci within the canine genome. In addition to the ~1,000 clones mapped as part of the development of the canine RH-map (Breen et al., 2004) numerous BACs were selected by screening the library specifically to obtain clones containing genes of interest. Many of these clones, which were FISH mapped to determine their cytogenetic location, contained genes associated with disease (e.g. van de Sluis et al., 1999, 2001; Wagner et al., 1999; Thomas et al., 2001a; Lozier et al., 2002; Rak et al., 2002, 2003; van den Berg et al., 2003, 2004; Wagner, 2003; Klukowska et al., 2004a, b; Stabej et al., 2004, 2005; Debenham et al., 2005; Philipp et al., 2005; also see http://faculty.vetmed.ucdavis.edu/Faculty/lalyons/Sites/CompGenBACsDOG.htm) and cancer (Thomas et al., 2003a, b). Additional clones from the RPCI-81 library have been used as an aid to visualize comparative genome organization (e.g. Szczerbal et al., 2003; Yudkin et al., 2007).

Specifically for molecular cytogenetic studies, clones from the RPCI-81 BAC library have been used to generate panels of chromosome-specific FISH probes. A set of 41 clones was described by Thomas et al. (2003c) that may be used to identify conclusively each dog chromosome according to the position of each clone on the corresponding chromosome. Also from this library, a panel of 80 BAC clones was assembled in groups of 1–3 clones per dog autosome that, when labeled/detected with one of five fluorochromes in a single hybridization, is reported to identify simultaneously all dog chromosomes within a metaphase spread (Courtay-Cahen et al., 2007). Both these chromosome-specific panels are useful for identifying chromosomes that are considered structurally normal. As such, these panels may be used for chromosome identification following metaphase-based comparative genomic hybridization (CGH) analysis. As part of the cytogenetic anchoring of dog RH maps, over 1,000 BAC clones from the RPCI-81 library were cytogenetically assigned using multicolor FISH analysis and details of each assignment were reported (Breen et al., 2004) (http://www.cvm.ncsu.edu/mbs/breen/dog_map.htm). Using these clones, Thomas et al. (2005) generated a genome-wide BAC microarray, with ~2 Mb resolution and demonstrated how this array may be used for detecting copy number changes in canine cancers using array comparative genomic hybridization (see below). Use of a cytogenetically characterized array overcomes the need for identification of individual dog chromosomes and allows for a much greater throughput of cases.

Genome integrated canine cytogenetics – band to base pair

A second dog BAC library, CHORI-82, was constructed from a female Boxer, 'Tasha', the same female whose DNA was used to generate the 7.6× canine genome assembly (http://bacpac.chori.org/library.php?id=253). This library

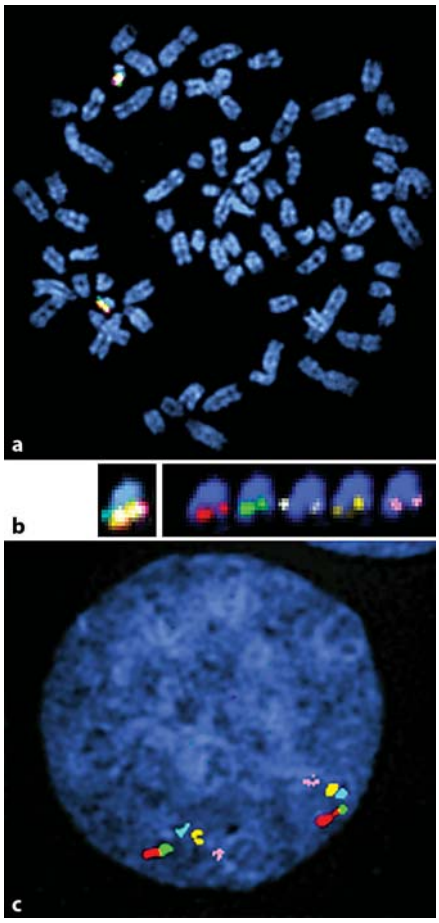

Fig. 2. Application of multicolor FISH to determining genome assembly location. Using the canine genome assembly five canine BAC clones were selected at 1 Mb intervals from the telomeric end of CFA 37. (**a**) Metaphase spread of a dog showing co-localization of the five differentially labeled canine BAC clones to the telomeric end of CFA 37. At this resolution while all five clones may be shown to map to CFA 37, their order is unable to be resolved, even when displayed as separate color planes as shown in (**b**). (**c**) Interphase FISH of the same five BACs showing clear and consistent order of the five clones on both homologues.

represents 10× coverage of the canine genome and contains ~198,000 clones with an average insert size of 172 kb. As part of the development of the 7.6× canine genome sequence, BAC end sequences were generated from the clones comprising this library and so the library represents a valuable cytogenetics resource of genome assembly-integrated clones. Using web-based resources such as the UCSC Genome Browser (http://genome.ucsc.edu/cgi-bin/hgGateway) CHORI-82 clones may be selected, based either on their position within the genome or their specific gene/sequence content, and used as SLPs in FISH analysis. Using this process chromosome tiling sets of arrays have been generated with clone spacing at 10 Mb resolution (Thomas et al., 2007) and 1 Mb resolution (Thomas et al., submitted).

In molecular cytogenetics of any species the accepted resolution of metaphase FISH is in the region of 2–4 Mb, assuming that the chromosome spreads are elongated and the probe signals are discrete. In general the more common use of mid-metaphase preparations reduces this resolution to approx 4–6 Mb and so precise ordering of clones that are spaced by smaller distances requires the use of interphase FISH analysis (Fig. 2). In developing the genome wide, assembly-integrated BAC panels, Thomas et al. (submitted) were able to determine that over 95% of the 2,122 clones mapped by FISH did so with a unique cytogenetic location and to the region of the chromosome indicated by the genome assembly. This figure lends high confidence to the structural integrity of the assembled genome and confidence to selecting clones from the genome browsers.

As with other species, the availability of BAC clones that are integrated into the genome assembly launched canine molecular cytogenetics into a new arena – observations of microscopically visualized chromosome aberrations may be rapidly translated to aberrations of specific regions of the genome sequence. With this in mind, genome integrated canine BAC arrays have been developed with the panels of genome-anchored BACs described above. The first is a genome-wide BAC array comprising 275 clones spaced at intervals of approximately 10 Mb and also contains clones representing the dog orthologues of 31 genes implicated in human cancers (Thomas et al., 2007). This array has been shown to be of use for first pass assessment of gross genome-wide DNA copy number variation in canine tumors and provides a similar resolution to that produced by metaphase based CGH analysis, albeit without the need to be able to identify the dog chromosomes. A higher resolution genome-wide array has also been generated which contains 2,122 clones and represents a typical resolution of ~1 Mb (Thomas et al., submitted). In both cases, each clone included on the array has been used as an SLP to verify that it a) has a unique cytogenetic location and b) maps to the chromosome region indicated by the assembly. The results of this study indicated that, with a few exceptions, the 7.6× canine genome assembly is able to act as a robust source of CHORI-82 clones that may be used for canine cytogenetics.

Canine cytogenetics and evolution

The domestic dog belongs to the Canidae, a family that is believed to have diverged from other carnivore families ~50–60 million years ago. Within the extant Canidae, divergence from a common ancestor is reported to have commenced ~7–10 million years ago (Wayne, 1993). Previous studies indicated that the family is divided into two major groupings, the 'dog-like' and 'fox-like' canids (Bininda-Emonds et al., 1999; Graphodatsky et al., 2001). More recent genetic data, including data generated as part of the dog genome sequence project (Lindblad-Toh et al., 2005), have suggested that the family may be refined into four major phylogenetic groups represented by the fox-like canids (including

the racoon dog), the gray and island fox species, the South American canids and the wolf-like canids (including the domestic dog) (Ostrander, 2007). Cytogenetic studies of the 34 extant species comprising the Canidae have revealed considerable variation in chromosome number and morphology. The karyotype architecture of the Canidae ranges from 2n = 34 in the red fox (*Vulpes vulpes*) to 2n = 78 in the wolf-like canids, including the domestic dog (*Canis familiaris*) (Wayne, 1993). Canid karyotypes with lower chromosome numbers contain numerous bi-armed autosomes, while the autosomes of the wolf-like canids are all acrocentric. The family has thus undergone a relatively high rate of karyotype evolution and offers an exciting opportunity to use detailed cytogenetic evaluation, with both conventional and molecular approaches, to assess chromosomal evolution.

Whole chromosome reciprocal chromosome painting and comparative chromosome banding have been performed on numerous species within the Canidae. In addition to the domestic dog (2n = 78) these studies have primarily involved the fox-like canids such as the Red Fox (*Vulpes vulpes*, 2n = 34 + B [0–8]), Chinese Raccoon Dog (*Nyctereutes procyonoides procyonoides*, 2n = 54 + B [0–4]), Japanese Raccoon Dog (*Nytereutes procyonoides procyonoides*, 2n = 38 + B [0–8]), and Arctic Fox (*Alopex lagopus*, 2n = 48–50). These studies suggest an apparent gross pattern of whole-arm rearrangements during speciation within the Canidae (Yang et al., 1999; Graphodatsky et al., 2000, 2001; Nie et al., 2003). Notable exceptions to the presence of single segment shuffling was evident for dog chromosomes 1, 13 and 19, each of which corresponds to two chromosome segments of the Red Fox, Raccoon dog and Arctic Fox, and while dog chromosome 18 is represented by a single conserved segment in the Raccoon Dog, it also is split across two segments in the Red Fox and Arctic Fox (Graphodatsky et al., 2000, 2001).

An interesting feature of the karyotypes of several species within the Canidae is the presence of B chromosomes, supernumerary chromosomes within a karyotype that may vary in their number and have been described in a wide range of species, primarily plants and insects (Jones, 1975; Jones and Diez, 2004). The role of B chromosomes and their variability in number is still unknown, though in some non-mammalian species it has been suggested that B chromosome numbers may contribute to the formation of aberrant meiotic products (Camacho et al., 2000, 2004). Early studies of B chromosomes in the Raccoon dog indicated that they were composed of telomeric-like sequences (Wurster-Hill et al., 1988), while more recent studies indicated the presence of nucleolar organizer region (NOR)-like sequences (Szczerbal and Switonski, 2003). B chromosomes of the red fox, however, have been shown to be rich in centromeric-like sequences (Yang et al., 1999). FISH analysis, using chromosome paints and/or single locus probes has suggested that canid B chromosomes may share ancestry with small (<1 Mb) autosomal DNA segments and thus contain active genes (Trifonov et al., 2002; Graphodatsky et al., 2005; Yudkin et al., 2007). A more detailed assessment of the genome organization of these supernumerary chromosomes will be needed to further evaluate their potential significance. The dog genome sequence will undoubtedly play a key role in furthering these studies by providing a common reference point for the other canids.

Comparative maps from a wide range of mammalian species are providing key information about the dynamics of chromosome evolution (Murphy et al., 2005). For example, cytogenetic resources developed for the dog have also played a role in extending comparative cytogenetics beyond the Canidae by expanding cross-species painting studies to include members of the Felidae and Ursidae, both of which also belong to the order Carnivora (Tian et al., 2004).

Canine clinical cytogenetics and cancer

The presence of gross cytogenetic changes in non-neoplastic cells has been reported only rarely in the domestic dog. The most common chromosome abnormality reported in non-cancer specimens involves the sex chromosomes. Almost two decades ago, conventional cytogenetics was used to investigate dogs with sexual development disorders and revealed that several possessed X chromosome aneuploidy (79,XXY) or were chimeras (78,XX/78,XY) (reviewed by Mellink and Bosma, 1989). In addition infertile bitches have been shown, using both conventional cytogenetic and molecular cytogenetics, to present with X chromosome aneuploidy (Schelling et al., 2001; Switonski et al., 2003a, b). Molecular cytogenetic analysis of dogs presenting Duchenne muscular dystrophy (DMD) revealed that, as in humans, such cases presented with a small deletion of a region of Xp21, containing the canine *DMD* gene (Schatzberg et al., 1999). The availability of high-resolution cytogenetic resources for the dog will allow a more detailed evaluation of more subtle changes in dogs with unusual syndromes.

Clonal chromosome aberrations have been described in almost 27,000 human neoplasms that collectively represent approximately 75 different types of cancer (http://cgap.nci.nih.gov/Chromosomes/Mitelman). There is broad acceptance that the accurate identification of recurring chromosome abnormalities in malignant cells provides opportunities to increase the sophistication of diagnosis, sub-classification and prognosis of neoplastic disorders. In human medicine, the identity of cytogenetic aberrations has been shown to also assist in the localization of cancer-associated genes and even selection of the most appropriate therapeutic approach. The application of molecular cytogenetics to the analysis of human neoplasia has revolutionized the way in which we interrogate tumor cells for cytogenetic changes, whether they are numerical or structural in nature.

Veterinary medicine has provided a wealth of information about the clinical and pathological presentation of numerous canine cancers. Despite millions of years of divergent evolution, the high degree of similarity between human and dog also extends to their genome sequences (Lindblad-Toh et al., 2005). As pets, dogs (and thus their genomes) are exposed to the same environmental influences as humans. It is not surprising therefore that there are considerable

pathophysiological similarities shared by many forms of human and canine cancer (Withrow and Vail, 2006). In addition, the demographic history and genetic structure of many purebred domestic dogs has resulted in the development of distinct genetic isolates or breeds, which have been shown to have highly reduced levels of genetic variation and specific association with genetic disease (Ostrander and Giniger, 1997; Ostrander and Kruglyak, 2000; Parker et al., 2004; Sutter and Ostrander, 2004; Sutter et al., 2004; Ostrander, 2007). In terms of cancer incidence, it is well known that purebred dog breeds are associated with differing susceptibility to specific malignancies (Priester and McKay, 1980; Patterson, 2000; Modiano et al., 2005). Such breed-associated predisposition suggests that selected breeds of dog are inheriting 'at risk' alleles for very few genes, perhaps even a single gene, with a profound effect. These features, combined with the sophisticated genomic resources now available for the dog, have placed the dog in a position of high visibility as a model system for cancer research.

The combination of pathophysiological and genetic similarity shared between human and dog led to the hypothesis that canine tumors may contain the natural variety of chromosome aberrations that are observed in human cancers, a feature that is not evident with induced rodent tumors. Molecular cytogenetic studies of canine cancers have demonstrated that this is indeed the case. For example, in studying canine hematopoetic malignancies it has been shown that cells isolated from human and dog tumors of comparable malignancies share several evolutionarily conserved cytogenetic changes (Thomas et al., 2003c; Breen and Modiano, 2008). As is the case in human cancers, a detailed cytogenetic investigation of canine tumors may therefore reveal the identity of recurrent changes that are of diagnostic and/or prognostic significance. To further this concept, evaluation of cytogenetic changes in canine tumors has been performed using a combination of direct cytogenetic analysis of tumor karyotypes from individual cells, using both conventional and molecular cytogenetics and indirect analysis using DNA from the tumor cell population in comparative genomic hybridization.

Direct analysis – chromosome paints and single locus probes

The difficulties associated with identification of normal dog chromosomes (see above) are exacerbated when such chromosomes undergo structural changes and so evaluation of complex canine tumor karyotypes using G-banding or DAPI banding alone is not a realistic option. While there were numerous early studies aimed at evaluating chromosome changes in canine cancers, the limitations of using chromosome banding techniques were very evident. Consequently, early conventional cytogenetic analyses of canine cancer karyotypes were generally restricted to very few cases. Further, while such reports were able to describe the presence of additional chromosomes and/or aberrant chromosome structures, they were unable to identify conclusively which chromosomes were involved in the aberration (Basrur and Gilman, 1966; Whang-Peng, 1969; Miles et al., 1970; Idowu, 1976; Grindem and Buoen, 1986; Mayr et al., 1990, 1992; Stone et al., 1991). The development of molecular cytogenetics reagents for the dog, however, permits detailed direct analysis of canine tumor genomes by FISH analysis. The use of chromosome paint probes and/or cytogenetically characterized single locus probes allows a rapid assessment of the gross numerical and structural characteristics of chromosomes within individual tumor cells. Chromosome painting analysis is widely used to unravel the complex nature of chromosome rearrangements and to determine chromosomal organization in the karyotypes of human cancers and syndromes (see for example, Blennow, 2004; Langer et al., 2004). The availability of high quality chromosome paints for application to canine tumor cytogenetics (Langford et al., 1996; Yang et al., 1999) has enabled this approach to be used similarly in the investigation of karyotypic abnormalities in canine tumors including mammary carcinoma (Tap et al., 1998), lymphoma (Thomas et al., 2001b) and soft tissue sarcoma (Milne et al., 2004). The development of multiplex-FISH (M-FISH) (Speicher et al., 1996) and spectral karyotyping (Schrock et al., 1996) for human cytogenetics has led to many new cytogenetic discoveries over the past decade due to their ability to increase the amount of genomic data that can be generated in a single experiment. To identify all 40 chromosome types (38 autosomes + X + Y) of the dog in a single FISH reaction with different colors, i.e. 40 color M-FISH, would require the use of six spectrally resolvable fluorochromes, which is beyond the scope of many laboratories. To overcome this limitation, Milne et al. (2004) used a robust seven-color system in their study of canine sarcomas and established karyotype coverage over a series of six different separate FISH reactions.

Though of broad use, chromosome paint probes generated for the dog have not been widely available. To overcome this, panels of cytogenetically verified chromosome-specific single locus probes have been generated as described above (Thomas et al., 2003c, 2007; Courtay-Cahen et al., 2007). Since these SLP panels are generated from commercially available canine BAC libraries, and the identity of each clone used is publicly available, it is a simple process for any cytogenetics laboratory to purchase these clones and generate their own SLP panels. The most recent of these chromosome-specific SLP panels was generated by selection of clones from the canine genome assembly (see above) and offered an opportunity to tile aberrant chromosomes in tumor karyotypes and refine the precise region of chromosome breakpoints in tumor karyotypes beyond the resolution possible with chromosome paint probes (Breen and Modiano, 2008).

Indirect analysis – comparative genomic hybridization

Comparative genomic hybridization (CGH) is a molecular cytogenetic approach to screen an entire genome for regions of copy number aberration (CNAs) in a single experiment (Kallioniemi et al., 1992, 1994). Metaphase-based CGH was used to identify CNAs in a range of human malignancies including cancers of the brain (Petersen et al., 2000; Wiltshire et al., 2000; Scheil et al., 2001), prostate

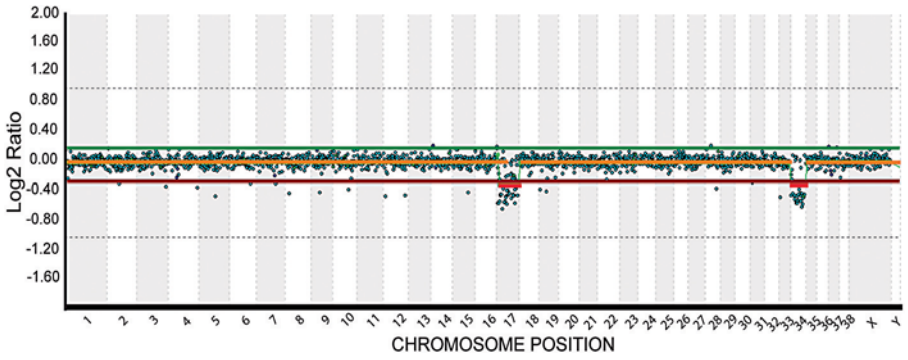

Fig. 3. Whole genome aCGH profile of a canine tumor. These data were obtained using a 1 Mb assembly-integrated dog BAC array (Thomas et al., submitted). Data are plotted as the median, block-normalized and background-subtracted $\log_2$ ratio of replicate spots for each of 2,122 BAC clones on the 1 Mb array. Log_2 ratios representing genomic gain and loss are indicated by horizontal bars above (thin green line) and below (thin red line) the midline representing normal copy number. Using the aCGH Smooth algorithm (Jong et al., 2004), the chromosome copy-number status line appears as orange where no major genomic imbalances were apparent and as either red or green in regions where genomic imbalances were apparent (red = loss, green = gain). This profile indicates whole chromosome loss for CFA 17 and CFA 34. In addition there are several additional copy number aberrations of isolated clones. Validation of these inferred copy number changes is verified by using the BAC clones on the array to perform single locus probe analysis of the tumor cells. This process provides a means to assess that distribution of locus copy number within the actual tumor cell population (as shown in Fig. 4).

(Cher et al., 1994), breast (Tirkkonen et al., 1998; Rodriguez et al., 2000), bladder (Obermann et al., 2003), thyroid (Wreesmann et al., 2002), skin (Bastian et al., 1998) and hematopoetic system (Allen et al., 2002; Berglund et al., 2002; Franke et al., 2002). Chromosome-based CGH was developed for application to canine tumors (Dunn et al., 2000; Thomas et al., 2001b) and has been used to demonstrate that recurrent chromosome aberrations are present in canine lymphoma (Thomas et al., 2003c). However, as with other species, canine metaphase-based CGH presents two key limitations to high throughput: 1) the approach requires conclusive identification of dog metaphase chromosomes, necessitating a secondary analysis with chromosome specific SLPs (Dunn et al., 2000; Thomas et al., 2003c; Courtay-Cahen et al., 2007); 2) the use of metaphase chromosomes as the target limits the ability to detect copy number changes to those that are generally greater than 5–10 Mb (Pinkel et al., 1998; Lichter et al., 2000).

The recent development of microarray-based CGH (aCGH) analysis overcomes many of the limitations of conventional metaphase-based CGH, offering increased resolution that is dependent solely on clone spacing. Array CGH has been used to describe a wide variety of DNA copy number changes in human cancers, providing a new and valuable insight into the underlying gene involvement of tumors (e.g. Bejjani and Shaffer, 2006; Shaffer and Bejjani, 2006; Gunn et al., 2007). Array-based CGH analysis has been developed for application to the dog, based on the use of degenerate-oligonucleotide-primed PCR products from canine BAC clones, each of which has been assigned to a precise cytogenetic location using FISH analysis (see above). Preliminary use of these arrays, as shown in Fig. 3, has demonstrated that for canine cytogenetics this approach offers an exciting opportunity for significant improvement in resolution as well as throughput of canine cancer specimens

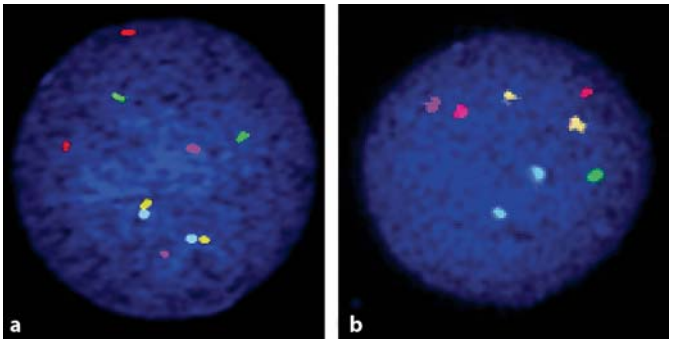

Fig. 4. SLP analysis of a canine tumor. The data revealed dual monosomy and indication of a translocation event. In Fig. 3, the aCGH data indicated a DNA copy number loss for CFA 17 and 34. Five BAC clones were used to generate multicolor single locus probe data for this case including a clone each of CFA 17 (purple) and CFA 34 (green) and two clones spaced at 1Mb on CFA 20. (**a**) Co-hybridization of these five clones to an interphase nucleus from a normal canine cell, demonstrating the presence of a normal copy number (n = 2) for each of the five clones and also the close association of the two clones from CFA 20 (shown in aqua and yellow). (**b**) Co-hybridization of the same five BAC clones to an interphase nucleus of a cell from the tumor profiles in Fig. 3 revealed that while three of the five probes had normal (n = 2) copy number (red, yellow and aqua), the two probes representing CFA 17 (purple) and CFA 34 (green) were present as one copy, confirming the copy number loss of these two chromosomes indicated by the aCGH profile in **a**. In addition, the two clones on CFA 20 (yellow and aqua) are now no longer closely associated in this tumor cell, indicating the presence of chromosome break in the 1 Mb interval between these two clones.

(Thomas et al., 2007 and submitted). The use of integrated BAC arrays allows rapid translation from cytogenetic aberrations to genome sequence aberrations. In combination with reliable clinical and pathological data of canine cancer patients, canine cytogenetics should therefore develop an

approach that will provide help to establish more accurate classification of canine tumors and so advance our aim to enhance the clinical management of our pet dogs.

The availability of a high density genome wide, genome integrated, cytogenetically characterized panel of canine BAC clones also provides the basis for the selection of clones for use in the characterization of both numerical and structural chromosomal changes associated with cancers. Individual clones may be used to determine DNA copy number variations within tumor cell populations and pooled clones may be used to visualize karyotype reassembly presenting in such cells (Fig. 4). The identity of recurrent cytogenetic changes in canine cancers should lead to the discovery of those changes that are associated with diagnosis and prognosis and thus open the door for clinical cytogenetics to become a routine component of the clinical management of dogs presenting with cancers.

Summary

The use of genome-integrated cytogenetic resources such as those described above have advanced canine cytogenetics to a stage that will allow a much more detailed evaluation of mammalian chromosomal evolution. Simultaneously the field has reached a point where aberrant chromosomal regions may be translated to specific regions of the canine genome and may identify genes of significance in canine cancers. As with human patients, we anticipate that new therapies offered for our pet dogs will become available. The ability to characterize response-associated cytogenetic changes will be a valuable aid to the refinement of prognosis and thus the clinical management of patients.

The pathophysiological and genome similarities between dog and human cancers may also lead to genes being discovered in studies of canine cancers that are also of major significance in human cancers. It seems that the unique bond shared between human and dog extends now beyond one with an emotional basis; it is now a bond that is at least in part fortified by the biomedical relationship that we share. For thousands of years the bark of our dogs has provided an early warning system for our safety. In the 21st century that role may extend, with studies of the canine genome aiding our development of early warning systems for our health. The domestic dog and now its genome, quite rightly, continue to be Man's best friend.

References

Allen JE, Hough RE, Goepel JR, Bottomley S, Wilson GA, et al: Identification of novel regions of amplification and deletion within mantle cell lymphoma DNA by comparative genomic hybridization. Brit J Haematol 116:291–298 (2002).

Basrur PK, Gilman JP: Chromosome studies in canine lymphosarcoma. Cornell Vet 56:451–469 (1966).

Bastian BC, LeBoit PE, Hamm H, Brocker EB, Pinkel D: Chromosomal gains and losses in primary cutaneous melanomas detected by comparative genomic hybridization. Cancer Res 58: 2170–2175 (1998).

Bejjani BA, Shaffer LG: Application of array-based comparative genomic hybridization to clinical diagnostics. J Mol Diagn 8:528–533 (2006).

Berglund M, Enblad G, Flordal E, Lui WO, Backlin C, et al: Chromosomal imbalances in diffuse large B-cell lymphoma detected by comparative genomic hybridization. Modern Pathol 15:807–816 (2002).

Bininda-Emonds OR, Gittleman JL, Purvis A: Building large trees by combining phylogenetic information: a complete phylogeny of the extant Carnivora (Mammalia). Biol Rev Camb Phil Soc 74:143–175 (1999).

Blennow E: Reverse painting highlights the origin of chromosome aberrations. Chromosome Res 12:25–33 (2004).

Breen M, Modiano JF: Evolutionarily conserved cytogenetic changes in hematologic malignancies of dogs and humans – Man and his best friend share more than companionship. Chromosome Res 16:145–154 (2008).

Breen M, Reimann N, Bosma AA, Landon D, Zijlstra C, et al: Standardisation of the chromosome nos. 22–38 of the dog (*Canis familiaris*) with the use of chromosome painting probes, in Proceedings of the 13th European Colloquium on Cytogenetics of Domestic Animals (Budapest, 1998).

Breen M, Bullerdiek J, Langford CF: The DAPI banded karyotype of the domestic dog (*Canis familiaris*) generated using chromosome-specific paint probes. Chromosome Res 7:401–406 (1999).

Breen M, Jouquand S, Renier C, Mellersh CS, Hitte C, et al: Chromosome-specific single-locus FISH probes allow anchorage of an 1800-marker integrated radiation-hybrid/linkage map of the domestic dog genome to all chromosomes. Genome Res 11:1784–1795 (2001).

Breen M, Hitte C, Lorentzen TD, Thomas R, Cadieu E, et al: An integrated 4249 marker FISH/RH map of the canine genome. BMC Genomics 5: 65 (2004).

Camacho JP, Sharbel TF, Beukeboom LW: B-chromosome evolution. Phil Trans R Soc Lond B Biol Sci 355:163–178 (2000).

Camacho JP, Perfectti F, Teruel M, Lopez-Leon MD, Cabrero J: The odd-even effect in mitotically unstable B chromosomes in grasshoppers. Cytogenet Genome Res 106:325–331 (2004).

Cher ML, MacGrogan D, Bookstein R, Brown JA, Jenkins RB, Jensen RH: Comparative genomic hybridization, allelic imbalance, and fluorescence in situ hybridization on chromosome 8 in prostate cancer. Genes Chromosomes Cancer 11:153–162 (1994).

Christian A, McNiel E, Robinson J, Drabek R, LaRue S, et al: A versatile image analysis approach for simultaneous chromosome identification and localization of FISH probes. Cytogenet Cell Genet 82:172–179 (1998).

Courtay-Cahen C, Griffiths LA, Hudson R, Starkey M: Extensive coloured identification of dog chromosomes to support karyotype studies: the colour code. Cytogenet Genome Res 116:198–204 (2007).

Debenham SL, Hart EA, Ashurst JL, Howe KL, Quail MA, et al: Genomic sequence of the class II region of the canine MHC: comparison with the MHC of other mammalian species. Genomics 85:48–59 (2005).

Dunn KA, Thomas R, Binns MM, Breen M: Comparative genomic hybridization (CGH) in dogs – application to the study of a canine glial tumour cell line. Vet J 160:77–82 (2000).

Franke S, Wlodarska I, Maes B, Vandenberghe P, Achten R, et al: Comparative genomic hybridization pattern distinguishes T-cell/histiocyte-rich B-cell lymphoma from nodular lymphocyte predominance Hodgkin's lymphoma. Am J Pathol 161:1861–1867 (2002).

Fujinaga T, Yamashita M, Yoshida MC, Mizuno S, Tajima M, et al: The banding patterns of normal canine chromosomes. Nippon Juigaku Zasshi 51:294–299 (1989).

Graphodatsky AS, Beklemisheva VR, Dolf G: High-resolution GTG-banding patterns of dog and silver fox chromosomes: description and comparative analysis. Cytogenet Cell Genet 69:226–231 (1995).

Graphodatsky AS, Yang F, O'Brien PC, Serdukova N, Milne BS, et al: A comparative chromosome map of the Arctic fox, red fox and dog defined by chromosome painting and high resolution G-banding. Chromosome Res 8:253–263 (2000).

Graphodatsky AS, Yang F, O'Brien PC, Perelman P, Milne BS, et al: Phylogenetic implications of the 38 putative ancestral chromosome segments for four canid species. Cytogenet Cell Genet 92: 243–247 (2001).

Graphodatsky AS, Kukekova AV, Yudkin DV, Trifonov VA, Vorobieva NV, et al: The proto-oncogene *C-KIT* maps to canid B-chromosomes. Chromosome Res 13:113–122 (2005).
Grindem CB, Buoen LC: Cytogenetic analysis of leukaemic cells in the dog. A report of 10 cases and a review of the literature. J Comp Pathol 96: 623–635 (1986).
Gunn SR, Robetorye RS, Mohammed MS: Comparative genomic hybridization arrays in clinical pathology: progress and challenges. Mol Diagn Ther 11:73–77 (2007).
Gustavsson I: The chromosomes of the dog. Hereditas 51:187–189 (1964).
Guyon R, Lorentzen TD, Hitte C, Kim L, Cadieu E, et al: A 1 Mb resolution radiation hybrid map of the canine genome. Proc Natl Acad Sci USA 100:5296–5301 (2003).
Hitte C, Madeoy J, Kirkness EF, Priat C, Lorentzen TD, et al: Facilitating genome navigation: survey sequencing and dense radiation-hybrid gene mapping. Nat Rev Genet 6:643–648 (2005).
Howard-Peebles PN, Pryor JC: The R-banding pattern of the canine karyotype. J Hered 71:361–362 (1980).
Idowu L: Observations on the chromosomes of a lymphosarcoma in a dog. Vet Rec 99:103 (1976).
Jones RN: B-chromosome systems in flowering plants and animal species. Int Rev Cytol 40:1–100 (1975).
Jones RN, Diez M: The B chromosome database. Cytogenet Genome Res 106:149–150 (2004).
Jong K, Marchiori E, Meijer G, Vaart AV, Ylstra B: Breakpoint identification and smoothing of array comparative genomic hybridization data. Bioinformatics 20:3636–3637 (2004).
Kallioniemi A, Kallioniemi OP, Sudar D, Rutovitz D, Gray JW, et al: Comparative genomic hybridization for molecular cytogenetic analysis of solid tumors. Science 258:818–821 (1992).
Kallioniemi OP, Kallioniemi A, Piper J, Isola J, Waldman FM, et al: Optimizing comparative genomic hybridization for analysis of DNA sequence copy number changes in solid tumors. Genes Chromosomes Cancer 10:231–243 (1994).
Klukowska J, Szczerbal I, Wengi-Piasecka A, Switonski M, Schelling C, et al: Characterization and mapping of canine microsatellites isolated from BAC clones harbouring DNA sequences homologous to seven human genes. Anim Genet 35:404–407 (2004a).
Klukowska J, Szczerbal I, Wengi-Piasecka A, Switonski M, Schelling C, et al: Identification of two polymorphic microsatellites in a canine BAC clone harbouring a putative canine *MAOA* gene. Anim Genet 35:75–76 (2004b).
Langer S, Kraus J, Jentsch I, Speicher MR: Multicolor chromosome painting in diagnostic and research applications. Chromosome Res 12:15–23 (2004).
Langford CF, Fischer PE, Binns MM, Holmes NG, Carter NP: Chromosome-specific paints from a high-resolution flow karyotype of the dog. Chromosome Res 4:115–123 (1996).
Li R, Mignot E, Faraco J, Kadotani H, Cantanese J, et al: Construction and characterization of an eightfold redundant dog genomic bacterial artificial chromosome library. Genomics 58:9–17 (1999).
Lichter P, Joos S, Bentz M, Lampel S: Comparative genomic hybridization: uses and limitations. Semin Hematol 37:348–357 (2000).
Lindblad-Toh KCM, Wade TS, Mikkelsen EK, Karlsson DB, Jaffe M, et al: Genome sequence, comparative analysis and haplotype structure of the domestic dog. Nature 438:803–819 (2005).
Lozier JN, Dutra A, Pak E, Zhou N, Zheng Z, et al: The Chapel Hill hemophilia A dog colony exhibits a factor VIII gene inversion. Proc Natl Acad Sci USA 99:12991–12996 (2002).
Manolache M, Ross WM, Schmid M: Banding analysis of the somatic chromosomes of the domestic dog (*Canis familiaris*). Can J Genet Cytol 18: 513–518 (1976).
Mayr B, Swidersky W, Schleger W, Reifinger M: Cytogenetic characterization of a canine haemangiopericytoma. Br Vet J 146:260–263 (1990).
Mayr B, Kramberger-Kaplan E, Loupal G, Schleger W: Analysis of complex cytogenetic alterations in three canine mammary sarcomas. Res Vet Sci 53:205–211 (1992).
Mellink CNM, Bosma AA: The karyotype of the dog (*Canis familiaris* L.), in Halnan CRE (ed): Cytogenetics of Animals, pp 151–158 (CAB International, Wallingford 1989).
Miles CP, Moldavannu G, Miller DG, Moore A: Chromosome analysis of canine lymphosarcoma: two cases involving probable centric fusion. Am J Vet Res 31:783–790 (1970).
Milne BS, Hoather T, O'Brien PCM, Yang F, Ferguson-Smith MA, Dobson J, Sargan D: Karyotype of canine soft tissue sarcomas: a multicolor, multi-species approach to canine chromosome painting. Chromosome Res 12:825–835 (2004).
Minouchi O: The spermatogenesis of the dog, with special reference to meiosis. Jpn J Zool 1:255–268 (1928).
Modiano J, Breen M, Avery A, London C: Breed specific canine lymphoproliferative diseases, in Ostrander EA, Giger U, Lindblad-Toh K (eds): The Dog and Its Genome, pp 439–450 (CSH Press, Cold Spring Harbor 2005)
Moreno-Millan M, Rodero A, Alonso FJ, Falcon C: Contribution to the establishment of the R-banded karyotype in dogs. Genet Sel Evol 23: 179s–182s (1991).
Murphy WJ, Larkin DM, Everts-Van der Wind A, Bourgue G, Tesler G, et al: Dynamics of mammalian chromosome evolution inferred from multispecies comparative maps. Science 309: 613–617 (2005).
Nie W, Wang J, Perelman P, Graphodatsky AS, Yang F: Comparative chromosome painting defines the karyotypic relationships among the domestic dog, Chinese raccoon dog and Japanese raccoon dog. Chromosome Res 11:735–740 (2003).
Obermann EC, Junker K, Stoehr R, Dietmaier W, Zaak D, et al: Frequent genetic alterations in flat urothelial hyperplasias and concomitant papillary bladder cancer as detected by CGH, LOH, and FISH analyses. J Pathol 199:50–57 (2003).
Ostrander EA: Genetics and the shape of dogs. Am Sci 95:406–413 (2007).
Ostrander EA, Giniger E: Semper fidelis: what man's best friend can teach us about human biology and disease. Am J Hum Genet 61:475–480 (1997).
Ostrander EA, Kruglyak L: Unleashing the canine genome. Genome Res 10:1271–1274 (2000).
Parker H, Kim L, Sutter N, Carlson S, Lorentzen T, et al: Genetic structure of the purebred domestic dog. Science 304:1160–1164 (2004).
Patterson DF: Canine genetic disease information system: A computerized knowledge base of genetic diseases in the dog (Mosby-Harcourt, St. Louis 2000).
Petersen I, Hidalgo A, Petersen S, Schluns K, Schewe C, et al: Chromosomal imbalances in brain metastases of solid tumors. Brain Pathol 10:395–401 (2000).
Philipp U, Quignon P, Scott A, Andre C, Breen M, Leeb T: Chromosomal assignment of the canine melanophilin gene (*MLPH*): a candidate gene for coat color dilution in Pinschers. J Hered 96: 774–776 (2005).
Pinkel D, Segraves R, Sudar D, Clark S, Poole I, et al: High resolution analysis of DNA copy number variation using comparative genomic hybridization to microarrays. Nat Genet 20:207–211 (1998).
Poulsen BS, Shibasaki Y, Ikeuchi T, Ronne M: Banding studies in *Canis familiaris*. I. Replication patterns in karyotypes from lymphocyte cultures. Cytobios 62:161–165 (1990).
Priester WA, McKay FW: The occurrence of tumors in domestic animals. Natl Cancer Inst Monogr 54:157–159 (1980).
Rak SG, Drogemuller C, Kuiper H, Leeb T, Quignon P, Andre C, Distl O: Cloning and chromosomal localization of *MYO15A* to chromosome 5 of the dog (*Canis familiaris*). Chromosome Res 10: 407–410 (2002).
Rak SG, Drogemuller C, Leeb T, Quignon P, Andre C, et al: Chromosomal assignment of 20 candidate genes for canine congenital sensorineural deafness by FISH and RH mapping. Cytogenet Genome Res 101:130–135 (2003).
Reimann N, Bartnitzke S, Bullerdiek J, Schmitz U, Rogalla P, et al: An extended nomenclature of the canine karyotype. Cytogenet Cell Genet 73: 140–144 (1996).
Rodriguez C, Causse A, Ursule E, Theillet C: At least five regions of imbalance on 6q in breast tumors, combining losses and gains. Genes Chromosomes Cancer 27:76–84 (2000).
Schatzberg SJ, Olby NJ, Breen M, Anderson LV, Langford CF, et al: Molecular analysis of a spontaneous dystrophin 'knockout' dog. Neuromuscular Disord 9:289–295 (1999).
Scheil S, Bruderlein S, Eicker M, Herms J, Herold-Mende C, Steiner HH, et al: Low frequency of chromosomal imbalances in anaplastic ependymomas as detected by comparative genomic hybridization. Brain Pathol 11:133–143 (2001).
Schelling C, Pienkowska A, Arnold S, Hauser B, Switonski M: A male to female sex-reversed dog with a reciprocal translocation. J Reprod Fertil Suppl 57:435–438 (2001).
Schrock E, du Manoir S, Veldman T, Schoell B, Wienberg J, et al: Multicolor spectral karyotyping of human chromosomes. Science 273:494–497 (1996).
Selden JR, Moorhead PS, Oehlert ML, Patterson DF: The Giemsa banding pattern of the canine karyotype. Cytogenet Cell Genet 15:380–387 (1975).
Shaffer LG, Bejjani BA: Medical applications of array CGH and the transformation of clinical cytogenetics. Cytogenet Genome Res 115:303–309 (2006).
Speicher MR, Gwyn Ballard S, Ward DC: Karyotyping human chromosomes by combinatorial multi-fluor FISH. Nat Genet 12:368–375 (1996).
Stabej P, Imholz S, Versteeg SA, Zijlstra C, Stokhof AA, et al: Characterization of the canine desmin (*DES*) gene and evaluation as a candidate gene for dilated cardiomyopathy in the Dobermann. Gene 340:241–249 (2004).
Stabej P, Leegwate PA, Imholz S, Versteeg SA, Zijlstra C, et al: The canine sarcoglycan delta gene: BAC clone contig assembly, chromosome assignment and interrogation as a candidate gene for dilated cardiomyopathy in Dobermann dogs. Cytogenet Genome Res 111:140–146 (2005).
Stone DM, Jacky PB, Prieur DJ: Cytogenetic evaluation of four canine mast cell tumors. Cancer Genet Cytogenet 53:105–112 (1991).

Stone DM, Jacky PB, Prieur DJ: The Giemsa banding pattern of canine chromosomes, using cell synchronization technique. Genome 34:407–412 (1991).
Sutter NB, Ostrander EA: Dog star rising: the canine genetic system. Nat Rev Genet 5:900–910 (2004).
Sutter NB, Eberle MA, Parker HG, Pullar BJ, Kirkness EF, et al: Extensive and breed-specific linkage disequilibrium in *Canis familiaris*. Genome Res 14:2388–2396 (2004).
Switonski M, Reimann N, Bosma AA, Long S, Bartnitzke S, et al: Report on the progress of standardization of the G-banded canine (*Canis familiaris*) karyotype. Committee for the Standardized Karyotype of the Dog (*Canis familiaris*). Chromosome Res 4:306–309 (1996).
Switonski M, Szczerbal I, Grewling J, Antosik P, Nizanski W, Yang F: Two cases of infertile bitches with 78,XX/77,X mosaic karyotype: a need for cytogenetic evaluation of dogs with reproductive disorders. J Hered 94:65–68 (2003a).
Switonski M, Szczerbal I, Skorczyk A, Yang F, Antosik P: Robertsonian translocation (8;14) in an infertile bitch (*Canis familiaris*). J Appl Genet 44:525–527 (2003b).
Szczerbal I, Switonski M: B chromosomes of the Chinese raccoon dog (*Nyctereutes procyonoides procynoides* Gray) contain inactive NOR-like sequences. Caryologica 56:209–212 (2003).
Szczerbal I, Rogalska-Niznik N, Klukowska J, Schelling C, Dolf G, Switonski M: Comparative chromosomal localization of the canine-derived BAC clones containing *LEP* and *IGF1* genes in four species of the family Canidae. Cytogenet Genome Res 102:264–266 (2003).
Tap OT, Rutteman GR, Zijlstra C, de Haan NA, Bosma AA: Analysis of chromosome aberrations in a mammary carcinoma cell line from a dog by using canine painting probes. Cytogenet Cell Genet 82:75–79 (1998).
Thomas R, Breen M, Deloukas P, Holmes NG, Binns MM: An integrated cytogenetic, radiation-hybrid, and comparative map of dog chromosome 5. Mamm Genome 12:371–375 (2001a).
Thomas R, Smith KC, Gould R, Gower SM, Binns MM, Breen M: Molecular cytogenetic analysis of a novel high-grade T-lymphoblastic lymphoma demonstrating co-expression of CD3 and CD79a cell markers. Chromosome Res 8:649–657 (2001b).
Thomas R, Bridge W, Benke K, Breen M: Isolation and chromosomal assignment of canine genomic BAC clones representing 25 cancer related canine genes. Cytogenet Genome Res 102:249–253 (2003a).
Thomas R, Fiegler H, Ostrander EA, Galibert F, Carter NP, Breen M: A canine cancer-gene microarray for CGH analysis of tumors. Cytogenet Genome Res 102:254–260 (2003b).
Thomas R, Smith KC, Ostrander EA, Galibert F, Breen M: Chromosome aberrations in canine multicentric lymphomas detected with comparative genomic hybridisation and a panel of single locus probes. Brit J Cancer 89:1530–1537 (2003c).
Thomas R, Scott A, Langford CF, Fosmire SP, Jubala CM, et al: Construction of a 2 Mb resolution BAC microarray for CGH analysis of canine tumors. Genome Res 15:1831–1837 (2005).
Thomas R, Duke SE, Bloom SK, Breen TE, Young AC, et al: A cytogenetically characterized, genome-anchored 10 Mb BAC set and CGH array for the domestic dog. J Hered 98:474–484 (2007).
Tian Y, Nie W, Wang J, Ferguson-Smith MA, Yang F: Chromosome evolution in bears: reconstructing phylogenetic relationships by cross-species chromosome painting. Chromosome Res 12:55–63 (2004).
Tirkkonen M, Tanner M, Karhu R, Kallioniemi A, Isola J, Kallioniemi OP: Molecular cytogenetics of primary breast cancer by CGH. Genes Chromosomes Cancer 21:177–184 (1998).
Trifonov VA, Perelman PL, Kawada SI, Iwasa MA, Oda SI, Graphodatsky AS: Complex structure of B-chromosomes in two mammalian species: *Apodemus peninsulae* (Rodentia) and *Nyctereutes procyonoides* (Carnivora). Chromosome Res 10:109–116 (2002).
van den Berg L, Versteeg SA, van Oost BA:. Isolation and characterization of the canine serotonin receptor 1A gene (*htr1A*). J Hered 94:49–56 (2003).
van den Berg L, Imholz S, Versteeg SA, Leegwater PA, Zijlstra C, et al: Isolation and characterization of the canine serotonin receptor 1B gene (*htr1B*). Gene 326:131–139 (2004).
van de Sluis BJ, Breen M, Nanji M, van Wolferen M, de Jong P, et al: Genetic mapping of the copper toxicosis locus in Bedlington terriers to dog chromosome 10, in a region syntenic to human chromosome region 2p13-p16. Hum Mol Genet 8:501–507 (1999).
van de Sluis B, Nanji MS, Breen M, Pearson PL, Oost BA, et al: Characterization and chromosomal localization of five canine *ATOX1* pseudogenes. Cytogenet Cell Genet 93:105–108 (2001).
Wagner JL: Molecular organization of the canine major histocompatibility complex. J Hered 94:23–26 (2003).
Wagner JL, Burnett RC, Storb R: Organization of the canine major histocompatibility complex: current perspectives. J Hered 90:35–38 (1999).
Wayne RK: Molecular evolution of the dog family. Trends Genet 9:218–224 (1993).
Whang-Peng J: Chromosome studies in various neoplasms of domestic animals. Natl Cancer Inst Monogr 32:117–120 (1969).
Wiltshire RN, Rasheed BK, Friedman HS, Friedman AH, Bigner SH: Comparative genetic patterns of glioblastoma multiforme: potential diagnostic tool for tumor classification. Neuro Oncol 2:164–173 (2000).
Withrow SJ, Vail D: Small animal clinical oncology, (WB Saunders, Philadelphia 2006).
Wreesmann VB, Ghossein RA, Patel SG, Harris CP, Schnaser EA, et al: Genome-wide appraisal of thyroid cancer progression. Am J Pathol 161:1549–1556 (2002).
Wurster-Hill DH, Ward OG, Davis BH, Park JP, Moyzis RK, MeyneJ: Fragile sites, telomeric DNA sequences, B chromosomes, and DNA content in raccoon dogs, *Nyctereutes procyonoides*, with comparative notes on foxes, coyote, wolf, and raccoon. Cytogenet Cell Genet 49:278–281 (1988).
Yang F, O'Brien PC, Milne BS, Graphodatsky AS, Solanky N, et al: A complete comparative chromosome map for the dog, red fox, and human and its integration with canine genetic maps. Genomics 62:189–202 (1999).
Yudkin DV, Trifonov VA, Kukekova AV, Vorobieva NV, Rubtsova NV, et al: Mapping of *KIT* adjacent sequences on canid autosomes and B chromosomes. Cytogenet Genome Res 116:100–103 (2007).