Chromosomal Mapping of Canine-Derived BAC Clones to the Red Fox and American Mink Genomes

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Abstract

High-quality sequencing of the dog (Canis lupus familiaris) genome has enabled enormous progress in genetic mapping of canine phenotypic variation. The red fox (Vulpes vulpes), another canid species, also exhibits a wide range of variation in coat color, morphology, and behavior. Although the fox genome has not yet been sequenced, canine genomic resources have been used to construct a meiotic linkage map of the red fox genome and begin genetic mapping in foxes. However, a more detailed gene-specific comparative map between the dog and fox genomes is required to establish gene order within homologous regions of dog and fox chromosomes and to refine breakpoints between homologous chromosomes of the 2 species. In the current study, we tested whether canine-derived gene–containing bacterial artificial chromosome (BAC) clones can be routinely used to build a gene-specific map of the red fox genome. Forty canine BAC clones were mapped to the red fox genome by fluorescence in situ hybridization (FISH). Each clone was uniquely assigned to a single fox chromosome, and the locations of 38 clones agreed with cytogenetic predictions. These results clearly demonstrate the utility of FISH mapping for construction of a whole-genome gene-specific map of the red fox. The further possibility of using canine BAC clones to map genes in the American mink (Mustela vison) genome was also explored. Much lower success was obtained for this more distantly related farm-bred species, although a few BAC clones were mapped to the predicted chromosomal locations.

Key words: Canis lupus familiaris, comparative genomics, FISH, Mustela vison, Vulpes vulpes

The phenotypic variation observed in domestic and farm-bred fur animals is striking, but its genetic basis is far from being well understood. New genetic tools and genome sequences (http://www.broad.mit.edu/mammals/) create an opportunity for the analysis of monogenic and complex phenotypes in dog, cat, and livestock species (reviewed in Georges 2007; Karlsson et al. 2007; Pontius et al. 2007; Wayne and Ostrander 2007). Furthermore, information from well-studied genomes can be applied for comparative analysis and genetic mapping of traits of interest in closely related species (de Gortari et al. 1998; Rogers et al. 2000, 2006; Reed et al. 2005; Anistoroaei et al. 2007). Here, we explore an opportunity to use canine genomic tools for assignment of gene-specific markers in the genomes of 2 farm-bred species, the red fox (Vulpes vulpes) and American mink (Mustela vison), in which artificial selection has produced a variety of coat color, morphological, and behavioral phenotypes (Shackelford 1949, 1949; Belyaev et al. 1975; Robinson 1975; Nes et al. 1988; Våge et al. 1997; Trut 1999, 2001; Kharlamova et al. 2000, 2007; Trapezov et al. 2008).

Canids (dog and fox) and mustelids (mink) belong to the same Canoidea branch of the Carnivora and diverged from a common ancestor about 40 million years ago (Ma), whereas red fox and dog, members of the Canidae family, diverged about 10 Ma (Wayne et al. 1997; Bininda-Emonds et al. 1999). The dog karyotype (Canis lupus familiaris) comprises 37 pairs of acrocentric autosomes and a pair of sex chromosomes, whereas the red fox has 16 pairs of metacentric autosomes, a pair of sex chromosomes, and 0–8...
Table 1. FISH localization of canine gene–containing BAC clones in the genomes of the silver fox and American mink

<table>
<thead>
<tr>
<th>Gene name</th>
<th>BAC clone</th>
<th>Fox FISH mapping (VVU)</th>
<th>Mink FISH mapping (MVI)</th>
<th>Fox CanFam2 canine ortholog location</th>
<th>Mink CanFam2 canine ortholog location</th>
<th>Dog chromosome based on RH mapping data (CFA)</th>
<th>Closest RH markers</th>
<th>∟</th>
<th>LOD</th>
<th>Marker location on canine FISH-RH–integrated map, cr (Breen et al. 2004)</th>
<th>Reference</th>
<th>Genes that were previously mapped in the red fox by FISH</th>
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<td>T-brachyury protein (T gene)</td>
<td>90-18</td>
<td>1p1.5–2.6</td>
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<td>CFA1</td>
<td>FH3993</td>
<td>0.09</td>
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<td>AJ245513; Haworth et al. (2001)</td>
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<td>HTR1B</td>
<td>18-L8</td>
<td>1q3.5–3.6</td>
<td>1p26–27</td>
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<td>CFA12 (van den Berg et al. 2004)</td>
<td>FH3284, BAC_372-C2</td>
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<td>16.75</td>
<td>1164</td>
<td>699</td>
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<tr>
<td>CRHR1</td>
<td>189-B1</td>
<td>2p2.10</td>
<td></td>
<td>Chr 9: 12, 791, 000-12, 841, 243</td>
<td>CFA9</td>
<td>EST5H8, BAC_372-C21</td>
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<td>GRIN2C</td>
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<td>2p2.10</td>
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<td>CFA9</td>
<td>CCL2, GALK1</td>
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<td>10.93</td>
<td>690</td>
<td>This study n</td>
<td>Sidjanin et al. (2003)</td>
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<td>Tbx2 and Tbx4</td>
<td>327-P20</td>
<td>2p2.3–2.7</td>
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<td>TBX2—Chr 9: 38, 559, 410-38, 567, 853</td>
<td>CFA9</td>
<td>SRP68, FH2846</td>
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<td>8.09</td>
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<td>SLC6A4</td>
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<td>CFA9</td>
<td>EST27D2</td>
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<td>19.82</td>
<td>820</td>
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<td>SLC6A3</td>
<td>89-K1</td>
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<td>Chr 34: 14, 216, 551-14, 251, 928; Chr 2: 63, 201, 559-63, 242; 406 (CFA2 less likely)</td>
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<td>FH3235, REN128H16</td>
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<td>DBH</td>
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<td>AHTH211, LEI-2D2</td>
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<td>C-KIT</td>
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<td>2p1.1</td>
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<td>Chr 13: 50, 040, 774-50, 124, 361</td>
<td>CFA13</td>
<td>REN177B24, REN126A23</td>
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### Table 1. Continued

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<tr>
<th>Gene name</th>
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<th>Fox FISH mapping (VVU)</th>
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<th>CanFam2 canine ortholog location</th>
<th>Dog chromosome based on RH mapping data (CFA)</th>
<th>Closest RH markers</th>
<th>LOD</th>
<th>Marker location on canine FISH-RH–integrated map, cR (Breen et al. 2004)</th>
<th>Reference</th>
<th>Genes that were previously mapped in the red fox by FISH</th>
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<td>GAD1</td>
<td>130-12</td>
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<td>Chr 36: 18, 369, 306-18, 412, 283</td>
<td>CFA36</td>
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<td>cR, 130-12: 1, 070, 784, 957-1, 070, 647</td>
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<td>CYP3A26</td>
<td>168-J21</td>
<td>3q1.5</td>
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<td>Chr 6: 12, 784, 228-12, 821, 910</td>
<td>CFA6</td>
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<td>cR, 168-J21: 1, 070, 784, 957-1, 070, 647</td>
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<td>Chr 4: 25, 492, 895-25, 781, 226</td>
<td>CFA4</td>
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<td>cR, 99-C20: 1, 070, 784, 957-1, 070, 647</td>
<td>Kuiper et al. (2002)</td>
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<td>DRD1</td>
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<td>1q31–33</td>
<td>Chr 4: 30, 803, 406-60, 812, 281</td>
<td>CFA4</td>
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<td>cR, 84-B18: 1, 070, 784, 957-1, 070, 647</td>
<td>AF286877; van de Sluis et al. (2001)</td>
<td>AF286877; van de Sluis et al. (2001)</td>
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<td>GABRG2</td>
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<td>CFA4</td>
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<td>S44</td>
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<td>Plus CFA18, 19, 20, and 24</td>
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<th>Closest RH markers</th>
<th>Marker location on canine FISH-RH–integrated map, cR (Breen et al. 2004)</th>
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<th>Selected candidate genes</th>
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<td>CFA22</td>
<td>DCT</td>
<td>FH2662</td>
<td>0.03 20.52 592</td>
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<td>HoxA</td>
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<td>7p2.3</td>
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<td>GNGTI</td>
<td>7q1.1–1.3</td>
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<td>SHARP1 (BHLHB3)</td>
<td>115-A9</td>
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<td>ST8SL1</td>
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<td>8p1.1–1.2</td>
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<td>Chr 27: 24, 475, 788-24, 480, 605</td>
<td>CFA27</td>
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<td>STS150j06</td>
<td>0.34 7.73 379</td>
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<tr>
<td>UGT1A6</td>
<td>252-I15</td>
<td>9p2.3–2.5</td>
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<td>Chr 25: 48, 138, 801-48, 813, 812</td>
<td>CFA25</td>
<td>D04813</td>
<td>ALPI</td>
<td>0.14 17.65 502</td>
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<td>102-D19</td>
<td>9q1.2–p1.1</td>
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<td>CFA20</td>
<td>FH4027</td>
<td>REN55P21</td>
<td>0.21 17.12 226</td>
<td>This study</td>
<td>n</td>
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<td>IGF1</td>
<td>126-J3</td>
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<td>CFA15</td>
<td>EST13G6</td>
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<td>TPH1</td>
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<td>REN143N23</td>
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<td>This study</td>
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<td>TECTA</td>
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<td>12q2.1–2.5</td>
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<td>MYO15A</td>
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<td>8p11</td>
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<td>REN78M01</td>
<td>BAC_375-M3</td>
<td>0.06 19.17 397</td>
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**Table 1.** Chromosomal Mapping of Canine-Derived BAC Clones
<table>
<thead>
<tr>
<th>Gene name</th>
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<th>Fox FISH mapping (VU)</th>
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<th>CanFam2 canine ortholog location</th>
<th>Dog chromosome based on RH mapping data (CFA)</th>
<th>Closest RH markers</th>
<th>θ</th>
<th>LOD</th>
<th>Marker location on canine FISH-RH–integrated map, cR (Breen et al. 2004)</th>
<th>Reference</th>
<th>Genes that were previously mapped in the red fox by FISH</th>
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<td>130-L5</td>
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<td>933</td>
<td>This study</td>
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<td>CNGB3</td>
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<td>13q1.1</td>
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<td>Chr 29: 35, 752, 880–35, 896, 148</td>
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<td>Factor VIII (F8:A)</td>
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The location of the same genes in the CanFam2 canine genome assembly and on the canine RH map 5000 cR is indicated. “n,” “c,” and “m” indicate genes known to be involved in neurobiology and behavior, coat color, and morphology, respectively. BAC clone 15-D10 contains at least 2 genes OTK and AVP, and BAC clone 327-P20 contains genes Tbx2 and Tbx4. LOD, logarithm of the odds.
B chromosomes (Wipf and Scackeford 1942; Gustavsson 1964; Belyaev et al. 1974). The American mink’s karyotype presents 14 pairs of bi-armed chromosomes plus a pair of sex chromosomes (Mandahl and Fredga 1975; Christensen et al. 1996). The cytogenetic relationships among dog, red fox, and American mink genomes is well understood, as fluorescence in situ hybridization (FISH) with whole-chromosome probes established the correspondence of chromosomal arms between the dog and fox, and the dog and mink karyotypes (Yang et al. 1999, 2000; Graphodatsky, Yang, O’Brien et al. 2000; Graphodatsky, Yang, Serdukova et al. 2000; Graphodatsky et al. 2001, 2002).

Among the Canidae, the genome of the dog has been the most intensely studied to date. Genomic tools available for such studies include 2 bacterial artificial chromosome (BAC) libraries; meiotic, radiation hybrid (RH), and single nucleotide polymorphism maps; and an assembled genome sequence anchoring these resources (Werner et al. 1999; Breen et al. 2001, 2004; Kirkness et al. 2003; Hitte et al. 2005; Lindblad-Toh et al. 2005). The evolutionary closeness of the dog and fox provides an opportunity to apply canine genomic tools to study the fox genome. Recently, a meiotic linkage map of the silver fox (a coat color variant of the red fox) was constructed using dog-derived microsatellite markers (Kukkevka et al. 2004, 2007). Alignment of the fox map against the dog genome identified highly conserved marker order between orthologous regions of dog and fox chromosomes.

Although some gene-specific markers have been placed on the fox meiotic linkage map, this approach has several limitations. Finding gene location by linkage mapping requires identification of gene-specific polymorphic markers and genotyping of these markers in reference 3-generation pedigrees. Furthermore, because alignment of the fox meiotic linkage map against the dog genome has identified pericentromeric regions of recombination suppression in fox chromosomes, marker order in these regions cannot be resolved unambiguously by meiotic linkage mapping.

Cross-species FISH mapping of BAC clones has been used previously for comparative mapping (Di Meo et al. 2003; Larkin et al. 2006; Davis et al. 2008), and several canine BAC clones have been mapped in the genome of the red fox and other canid species (Szczersbal et al. 2003, 2006; Graphodatsky et al. 2005; Klukowska-Rötzler et al. 2005). Two canine genome FISH maps are available, both anchored to the dog sequence assembly, with resolutions of 2 and 10 Mb, respectively (Thomas et al. 2005, 2007). A comparable FISH map of the fox genome would allow alignment to the dog genome at a high order of resolution than previously possible and provide a useful tool for genetic studies in foxes.

In the present study, 40 gene-containing canine BAC clones were selected to test whether such clones can be routinely used for gene mapping in foxes and were further evaluated to explore whether canine genomic tools might also be used to assign gene-specific markers in the genome of the American mink (M. vison), a more distantly related farm-bred species. The clones included 21 containing genes implicated in coat color, neurobiology, and skeletal development and selected as potential candidate genes for phenotypes segregating in these species.

Materials and Methods

Selection of Canine BAC Clones for Comparative Mapping

Forty clones (Table 1) were selected from the RPCI-81 canine 8.1-fold BAC library (http://www.chori.org/bacpac/mcanine81.htm). Nineteen of the selected clones had been previously identified as containing specific genes (Table 1), and a further 21 clones, each positive to a single gene-specific probe, were identified specifically for this study (Table 1) by screening the library as previously described (Kukkevka et al. 2003). BAC DNA was extracted by alkaline lysis (Birnboim and Doly 1979). Polymerase chain reaction (PCR) amplification of BAC DNA with gene-specific primers (Supplementary Table 1) and sequencing were used to confirm that each selected clone contained the correct corresponding gene.

Slide and Probe Preparation

Metaphase chromosomal spreads were prepared from primary fibroblast and peripheral blood lymphocyte cultures of red fox (V. vulpes) and American mink (M. vison) as described previously (Yang et al. 1999; Graphodatsky, Yang, O’Brien et al. 2000; Graphodatsky, Yang, Serdukova et al. 2000; Graphodatsky et al. 2002; Yudkin et al. 2007).

FISH Mapping

FISH on chromosomal spreads of red fox and American mink was performed using a standard protocol (Yang et al. 1999; Graphodatsky, Yang, O’Brien et al. 2000; Graphodatsky, Yang, Serdukova et al. 2000). Canine BAC clones were labeled by NICK Translation Kit (Invitrogen, Carlsbad, CA).

RH Mapping of Selected Genes on Canine 5000-centiray Panel

The 5000-centiray (cR) canine RH panel (Priat et al. 1998) was used for RH mapping of selected genes on dog chromosomes. Gene-specific primers were designed using sequences retrieved from the Trace Archive database (Supplementary Table 1). Primers for each gene were tested first on the DNA of dog and hamster, and only primer pairs that amplified the expected size product from canine but not hamster DNA were used for RH mapping. Each marker was amplified on the RH panel under the following conditions: 96°C for 2 min; 30 cycles of 96°C (20 s), 58°C (20 s), and 72°C (20 s); and a final extension at 72°C for 5 min. All PCRs were performed using 25 ng of genomic DNA from each cell line in a final volume 15 μl, and the products were separated on a 1.8% agarose gel. PCR products were visualized by ethidium bromide staining. All markers were scored, and the complete marker set was analyzed together with 3270 markers previously mapped at
the 5000-rad panel (http://www-recomgen.univ-rennes1.fr/Dogs/; Breen et al. 2004) using MultiMap (Matise et al. 1994). Two-point linkage analysis was performed for each selected gene to determine its nearest markers and chromosomal location. Presence of gene-specific amplicons produced for RH mapping in the corresponding canine BAC clones was confirmed by PCR.

**Localization of Selected Genes in CanFam2**

To identify positions of selected genes in the 7.6 × sequence assembly of the dog genome (CanFam2), canine orthologs were located in the canine assembly by 1) gene names and 2) RH primer sequences mapped in silico using resources of the University of Southern California, Santa Cruz, Genome Bioinformatics Site (http://genome.ucsc.edu/).

**Results**

**Localization of Selected Genes in the Dog Genome**

**RH Mapping**

Two-point linkage analysis of 38 canine genes with 3270 markers previously mapped on RH map 5000 cR identified the chromosomal location of 36 genes (Table 1). For each tested gene, the position of at least 5 RH markers that showed linkage with the highest support was identified on the RH5000 map (Breen et al. 2004). Genes were assigned to a particular chromosome when all such markers mapped to the same chromosomal region. The chromosomal locations of the 2 closest markers for each mapped gene on the RH5000 map (Breen et al. 2004) are listed in the Table 1. RH-mapped genes were assigned to 19 dog chromosomes with 8 chromosomes containing more than one gene (Table 1). The gene AVP has not been mapped; all primer pairs designed for this gene produced similar size band on hamster DNA. Location of HOXA could not be determined unambiguously. Two genes (PAX3 and KDR) have not been mapped in this study, but location of these genes on the RH5000 map has been identified previously (Lowe et al. 2003; Breen et al. 2004).

**Localization of Selected Genes in CanFam2**

The unique locations of 35 selected genes in the CanFam2 assembly were identified using gene name as a query (Table 1). Five genes (SLC6A3, SLC25A3, ATOX1 pseudogene, PAX3, and HOXA) were assigned to more than one chromosome in CanFam2 (Table 1). Four of these genes (SLC6A3, SLC25A3, ATOX1 pseudogene, and PAX3) were localized uniquely in CanFam2 using in silico PCR with primer pairs used for BAC screening or RH mapping. Location of HOXA could not be determined unambiguously.

**Figure 1.** Examples of FISH mapping (left) of canine-derived BAC clones onto G-banded (right) metaphases of the red fox (VVU) and American mink (MVI). Localization of the TYRP2 onto VVU6p1.3–1.4 (a) and MYO15A onto MVI8p12–13 (b). Scale bar—10 μm.
For 38 genes, the canine chromosomal locations on CanFam2 and the canine RH map were in agreement (Table 1). \textit{SLC6A3} and \textit{AVP} were the exceptions: \textit{SLC6A3} was assigned to CFA22 by RH mapping but is most likely located on CFA34 in CanFam2, and \textit{AVP} was identified on CanFam2 but not on the RH Map.

Localization of Canine BAC Clones to Fox Chromosomes by FISH

All 40 canine gene–containing BAC clones were uniquely mapped to fox chromosomes by FISH. BAC clones were assigned to 15 of 17 fox chromosomes (Table 1 and Figure 1a). More than one BAC clone were assigned to 13 fox chromosomes: Two BACs were assigned to VVU1, 6, 7, 8, 9, 10, 13, 14, and X; 3 BACs were assigned to VVU3 and 12; 4 BACs were assigned to VVU4; and 10 BACs were assigned to VVU2 (Table 1 and Figure 2).

Comparative locations of 38 genes on the fox and dog chromosomes were in agreement with comparative cytogenetic data. Localization of a BAC clone containing \textit{SLC6A3} gene on VVU2 was in disagreement with both the most likely location of this gene in CanFam2 (CFA34) and its location predicted by RH mapping (CFA22). Because \textit{HOXA} has not been identified in the dog genome, we could not make a comparison between dog and fox for this gene. The BAC containing the gene \textit{C-KIT} localized to both VVU2 and B chromosomes as described previously for other \textit{C-KIT} BAC clones (Graphodatsky et al. 2005).

These newly located genes, together with those previously mapped in the fox genome using hybrid cell lines (Rubtsov et al. 1988, 1998), increased the total number of gene-specific markers in the fox genome to 77 (Supplementary Table 2).

Localization of Canine BACs to Mink Chromosomes

Four BAC clones, containing the genes \textit{HTR1B}, \textit{GNGT1}, \textit{MYO15A} and \textit{ATOX1} pseudogene, were uniquely assigned to mink chromosomes (Figures 1b and 3) and in agreement with comparative cytogenetic data for mink and dog genomes (Figure 3 and Table 1). In each case, these 4 gene-containing BAC clones identified segments of mink chromosomes that were orthologous to the corresponding fox chromosomes (Figure 2, Table 1).
Forty canine-derived BAC clones containing known genes were uniquely assigned to fox chromosomes by FISH. Among selected BAC clones, 15 contain genes implicated in neurobiology and behavior, 5 clones contain genes involved in coat color in mammals, and at least 1 clone contains a gene important for morphology. Selection of these genes for FISH mapping in the fox genome was predetermined by the needs of ongoing genetic studies in foxes. The rest of the clones used in this study represent previously characterized BAC clones selected to increase the number of probes. When BAC clones for FISH mapping were selected for this study, the RPCI-81 BAC library was the only available well-characterized canine BAC library. In contrast to the canine library CHORI-82 that was subsequently used for the dog genome sequencing (Lindblad-Toh et al. 2005), RPCI-81 clones are not anchored in the canine sequence assembly, and the location of each clone in the dog genome had to be identified de novo. To do so, both in silico localization of these genes in CanFam2 and RH mapping were used. The chromosomal localization of 36 of these genes in the dog was in agreement for the 2 methods. Location of 2 genes was in disagreement (Table 1). The HOXA4 gene was not uniquely localized in the dog genome by either method. The BAC clone containing HOXA4 gene had been identified by screening the RPCI-81 BAC library with the probe AF103746 (National Center for Biotechnology Information database; Li et al. 1999). This probe is 118 bp long and most likely does correspond to the HOXA4 gene on CFA14, although the statistical support for this location is not significantly higher than for several other HOXA4 genes. RH mapping with primers based on AF103746 did not identify a unique location for this gene in the dog genome either. For this reason, this gene was excluded from the comparative analysis of the dog and fox.

Comparison of gene locations between the fox and dog genomes was performed to test whether gene locations identified in the fox genome by FISH agreed with their chromosomal locations predicted from comparative cytogenetic data and alignment of the fox meiotic linkage map to the dog genome. The locations of 38 genes mapped in the fox genome agreed with these predictions. Location of one gene, SLC6A3, was in disagreement. The most likely location of this gene in CanFam2 is on CFA34, which is homologous to fox chromosome 3, but the BAC 89-K1 carrying SLC6A3 gene was mapped to the fox chromosome 2, close to the SLC6A4 gene. Similarity between the coding regions of these 2 genes or chimeric structure of the BAC 89-K1 can contribute to unpredicted localization of this gene. Alternatively, but less likely, the SLC6A4 mapping results might also indicate a small insertion in VVU2 corresponding to part of CFA34 that has not yet been identified.

Previously, FISH localization of 3 canine BAC clones in 4 canids—the dog, red fox, arctic fox, and Chinese raccoon dog—has been reported (Szczerebalski et al. 2003; Klukowska-Rötzler et al. 2005). These clones were selected from a different dog BAC library (http://www.dogmap.ch/getclone.html, Schelling et al. 2002), but 2 of them contain genes included in the current study (IGF1 and HTR2A). Our results confirmed localization of the IGF1 gene onto VVU10q chromosome and HTR2A on VVU6p.

In the current study, 2 genes (TYRP2 and HTR2A) were mapped to the short arm of fox chromosome 6, which is homologous to CFA22. TYRP2 mapped close to the centromere, whereas HTR2A mapped close to the telomere. According to the CanFam2 annotation, the location of these genes on CFA22 is opposite, TYRP2 being near the dog telomere, whereas HTR2A is located close to the centromere. The relative positions of HTR2A and TYRP2 genes on VVU6 indicate that the segment homologous to CFA22 has the opposite orientation in VVU6 relative to that reported by comparative chromosomal painting (Graphodatsky et al. 2001, Figure 2). This observation is also supported by previous FISH mapping results for HTR2A (Szczerebalski et al. 2006) and by alignment of the fox meiotic linkage map against the dog genome (Kukkova et al. 2007).

Previously, more than 40 gene-specific markers have been localized in the fox genome using a panel of somatic cell hybrids (Rubtsov et al. 1988, 1998). These data, together with mapping data reported in Szczerebalski et al. (2003, 2006), and FISH mapping results from the current study bring the number of genes mapped onto specific fox chromosomes to 77 (Supplementary Table 2).

In the current study, we found that FISH mapping with canine-derived BAC clones is an efficient approach for mapping genes in the silver fox genome, as all 40 canine BAC clones used in our study were uniquely assigned to fox chromosomes. Locations of 38 genes were in agreement with the cytogenetic predictions; locations of 2 genes were inconclusive. This is not unexpected as there is some
inconsistency even when canine BAC clones are FISH mapped in the dog (Breen et al. 2004). When an unpredicted location of a BAC clone is identified, additional BAC clones from the same genomic region should be mapped to understand whether observed results were caused by chimeric BAC structure, sequence similarity between different genes, or chromosomal insertions that were not detected previously. Results of this pilot project indicate an opportunity for large-scale mapping of the fox genome with canine-derived BAC clones. BAC clones from the CHORI-82 library are well characterized and anchored in the CanFam2 dog genome assembly (Lindblad-Toh et al. 2005). Mapping of the dog genome with panels of CHORI-82 BAC clones demonstrated that multicolor FISH allows construction of a gene map of the dog genome with the resolution up to 2 Mb (Tomases et al. 2005, 2007). A high-density gene map of the fox genome constructed using multicolor FISH would allow high-resolution comparison of the chromosomal regions between fox, dog, and human, refining breakpoints between homologous chromosomes, characterization of the telomeric ends of fox chromosomes, and establishing gene order between homologous regions of dog and fox chromosomes. A fox–dog comparative gene map will be an important resource for genetic studies in foxes, allowing localization of fox-mapped loci in the dog genome, selection of canine microsatellite markers for fine mapping of these loci in foxes, and identification of positional candidate genes. Furthermore, construction of high-resolution comparative FISH maps for different canid species will provide new insights into the karyotypic evolution of the Canidae.

FISH mapping results for canine-derived BAC clones in the American mink were less successful. Only 4 canine BAC clones were uniquely assigned to mink chromosomes (Figure 3, Table 1). Previous reports of FISH mapping of bovine-derived BAC clones in the genome of American mink were more encouraging (Larkin et al. 2006). These different success rates may represent differences in protocol or gene selection. A BAC library of the European ferret (Mustela furo) that has recently become available (https://bacpac.chori.org/library.php?id=330), together with the first meiotic linkage map of the American mink (Anistoroaei et al. 2007), should provide valuable resources for genetic studies in Mustela. Locations of the 4 genes mapped by FISH in mink were in agreement with cytogenetic predictions based on the established homology between the dog and mink chromosomes (Yang et al. 1999; Graphodatsky, Yang, Serdukova et al. 2000; Graphodatsky et al. 2002). The fact that these 4 probes identified regions of the mink and the fox genomes, which were homologous to the same dog chromosomes, demonstrates that there is utility for this method of comparative mapping and gene identification in the 3 species.

Supplementary Material
Supplementary Tables 1 and 2 can be found at http://www.jhered.oxfordjournals.org/.

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