Second-Generation Integrated Genetic Linkage/Radiation Hybrid Maps of the Domestic Cat (*Felis catus*)

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Abstract

We report construction of second-generation integrated genetic linkage and radiation hybrid (RH) maps in the domestic cat (*Felis catus*) that exhibit a high level of marker concordance and provide near-full genome coverage. A total of 864 markers, including 585 coding loci (type I markers) and 279 polymorphic microsatellite loci (type II markers), are now mapped in the cat genome. We generated the genetic linkage map utilizing a multigeneration interspecies backcross pedigree between the domestic cat and the Asian leopard cat (*Prionailurus bengalensis*). Eighty-one type I markers were integrated with 247 type II markers from a first-generation map to generate a map of 328 loci (320 autosomal and 8 X-linked) distributed in 47 linkage groups, with an average intermarker spacing of 8 cM. Genome coverage spans approximately 2,650 cM, allowing an estimate for the genetic length of the sex-averaged map as 3,300 cM. The 834-locus second-generation domestic cat RH map was generated from the incorporation of 579 type I and 255 type II loci. Type I markers were added using targeted selection to cover either genomic regions underrepresented in the first-generation map or to refine breakpoints in human/feline synteny. The integrated linkage and RH maps reveal approximately 110 conserved segments ordered between the human and feline genomes, and provide extensive anchored reference marker homologues that connect to the more gene dense human and mouse sequence maps, suitable for positional cloning applications.

The earliest fossil records that show companionship between man and cat date from approximately 6,000 years ago in Cyprus (Budiansky 2002). The presence of cat remains in the neolithic settlement of Khirokitia, an isolated island habitat with no native wild cats (*Felis silvestris*), suggests that the animal was brought to the island by early human settlers (Davis 1989). Initially, cats were likely useful in controlling rodent infestation; they subsequently expanded their role and range as guards in Buddhist temples in Japan, "mousers" on Phoenician ships, and to a position of near reverence in Egypt (2000 BC).

Today, some 60 million cats reside in homes in the United States (Pet Food Institute, Washington, DC), where the 2000 census lists some 106 million households. Approximately 37 cat breeds are recognized by the two largest cat registries in the United States (Cat Fanciers' Association [CFA] and The International Cat Association [TICA]). The majority of breeds have been derived within

the past few hundred years and many within the last hundred years. Artificial selection of perhaps a dozen loci has generated animals with prized phenotypic distinctiveness in coat color, hair type, and morphology (Robinson 1991). High veterinary surveillance has identified some 258 hereditary disorders in cat breeds (Nicholas et al. 1998), many analogous to human hereditary disorders. While some of these pathologies have been characterized (Fyfe et al. 1992; Gilbert et al. 1988; Jackson et al. 1992), the majority of the genetic factors which give rise to these disorders have yet to be mapped and characterized on a molecular genetic level.

We have been interested in the domestic cat as an animal model for genetic analysis (O'Brien and Nash 1982; O'Brien et al. 1997a,b, 2002) with the potential to contribute to our understanding of human hereditary disease analogues, neoplasia, genetic factors associated with host response to infectious disease, and mammalian genome evolution. The first physical maps of the cat (O'Brien and Nash 1982), reciprocal chromosome painting analyses (Rettenberger et al. 1995; Wienberg et al. 1997), and a radiation hybrid (RH) map including 424 type I (coding) loci and 176 type II (polymorphic) loci (Murphy et al. 2000) have revealed that the cat genome shares among the highest level of syntenic conservation with human as compared to other non primate mammalian species' genomes (O'Brien et al. 1999a,b). This suggests that the human and cat likely model a primitive ancestral mammalian genomic organization (Murphy et al. 2001c; O'Brien et al. 1999a,b).

We present here second-generation integrated type I and II physical (RH) and genetic linkage maps of the domestic cat genome. The genetic linkage map is a first integration of coding and polymorphic loci, adding 81 type I coding loci to 247 type II microsatellite loci from the previous feline linkage map (Menotti-Raymond et al. 1999). The second-generation RH map integrates 579 type I and 255 type II loci. The map refines the first-generation integration of genetic linkage and radiation hybrid maps of the cat (Sun et al. 2001), providing increased resolution, orientation and comparative anchor points for the feline genetic map. The revised maps offer increased precision for mapping of feline phenotypes as well as higher resolution of comparative genome organization in mammals.

Materials and Methods

Pedigree

Mendelian inheritance was analyzed in a multigeneration interspecies cross between the domestic cat (*Felis catus*) and the Asian leopard cat (*Prionailurus bengalensis*) (Menotti-Raymond et al. 1999). We utilized an interspecies cross to increase the number of DNA sequence differences in type I coding loci between the species that could be tracked in the pedigree. Three separate pedigrees were utilized. Family 1 included 17 domestic cats, 14 F_1 females, 8 leopard cat males, 48 B × D offspring (kittens from F_1 females backcrossed to a domestic cat) and 8 B × L offspring (kittens from F_1 females backcrossed to a leopard cat). Families 2 and 3 provided an F_1 female and four and three B × D siblings, respectively. Recombination estimates for type I markers were derived from meiotic events which occurred solely in F_1 hybrid females by scoring in their 66 progeny. As type II markers could be polymorphic in domestic cats, leopard cats, and F_1 hybrid females, meiotic events could maximally be scored in 63 F_1 hybrid females, 13 domestic cat females, 6 leopard cat females, 52 domestic cat males, and 37 leopard cat males. Details of the pedigree and pedigree structure for family 1 are available in Menotti-Raymond et al. (1999). DNA was extracted from leukocytes or fibroblast tissue culture cell lines established for each animal (Modi and O'Brien 1988) using standard proteinase K/phenol/chloroform extraction protocols (Sambrook et al. 1989).

Linkage Map Primer Development and Genotyping

Primers utilized for amplification of 75 of the type I loci incorporated in the linkage map have been previously reported (Murphy et al. 2000). Primer pairs for six additional loci can be found with supplemental information on the Web site: http://home.ncifcrf.gov/ccr/lgd. PCR amplifications for genotyping of type I loci for the genetic linkage map were performed in 15 μl reaction volumes containing 1X Applied Biosystems (ABI) Gene Amp PCR II buffer containing 10 mM Tris-HCl (pH 8.3) and 50 mM potassium chloride (KCl); 1.5 mM magnesium chloride (MgCl$_2$), 250 μM of three deoxyribonucleoside 5′-triphosphates (dATP, dGTP, and dTTP), 125 μM of dCTP (Pharmacia), 0.04 μl of [α-^{32}P]dCTP at 3,000 Ci/mmol (New England Nuclear), 0.4 U of *AmpliTaq* gold DNA polymerase (Perkin-Elmer Cetus, Norwalk, CT), 0.8 μM final primer concentration of forward and reverse primer (Life Technologies, Frederick, MD), and 50 ng of DNA. Reactions were amplified in Perkin-Elmer 9700 thermal cyclers as described (Murphy et al. 1999). Samples were diluted 5:1 with formamide loading dye (Ravnik-Glavac et al. 1994), denatured for 4 min at 95°C and 3 μl of product loaded per lane. Amplification of PCRs for genotyping of type I loci for the RH map were performed as reported (Murphy et al. 2000).

SSCP Screening

Products were examined for domestic cat/leopard cat polymorphism using three sets of gel conditions: 6.5% nondenaturing polyacrylamide gels (monomer to cross-linker ratio of 37.5:1) (Bio-Rad) using one of three buffers: 1 × TBE (pH 8.3), 1 × TBE + 5% glycerol (Ravnik-Glavac et al. 1994), or 1 × TME (pH 6.8) (Hayashi et al. 1998). Gels were subjected to electrophoresis at 4°C at either 50 W for 4 h or 20W for 10 h. Gels were dried and exposed to Kodak X-OMAT film overnight. Genotypes were scored by two people independently and any inconsistencies between the two scorings were reexamined.

PCR/RFLP Genotyping of Type I Loci for the Genetic Linkage Map

PCR products of type I loci were sequenced in the domestic cat and Asian leopard cat using ABI Big-Dye terminator (PE Applied Biosystems, Foster City, CA) chemistry on an ABI 377 sequencer apparatus. Sequences were analyzed with Sequencher (version 4.1; Gene Codes Corp., Ann Arbor, MI) to identify interspecific sequence differences which specified natural restriction fragment length polymorphism (RFLP) sites or around which RFLP sites could be generated through primer design. Ten PCR-RFLP assays were designed (Table 1) and genotyped in the interspecies pedigree. To improve sensitivity of the assay to amplification in hybrid animals and detection of "hybrid nulls" (Menotti-Raymond et al. 1999), PCR products were fluorescently tagged and precisely sized following digestion with an ABI 377 sequencing apparatus. One primer of the pair was tagged with a fluorescent dye phosphoramidite. In addition, the RFLP site and a pig-tail (GTTCTT) were appended to the 5′ end of the unlabeled primer as a control to monitor for complete digestion of the sample and to prevent addition of an untemplated dATP to the PCR product (Brownstein et al. 1996; Smith et al. 1995). With the exception of type I loci *FGFR4* and *IFNG*, the sequence was modified in the primer to introduce a restriction endonuclease site at the domestic cat/leopard cat polymorphism.

Reactions were amplified as stated above with the exception that the final concentration of $MgCl_2$ was 2.0 mM, the final concentration of dCTP was as the other dNTPs, and radiolabeled isotope was not used. Digestion of the PCR product was performed in a 10 µl reaction consisting of 5 µl of PCR product, 3 µl of sterile deionized water, 1 µl of restriction endonuclease (New England Biolabs) and 1 µl of 10 × buffer (supplied by the manufacturer). Following digestion for 2 h at 37°C (with the exception of *BstU*1 digest at 60°C), products were subjected to electrophoresis in an ABI 377 apparatus and sized as described (Menotti-Raymond et al. 1999).

Linkage Map Construction

We performed linkage analysis as described (Menotti-Raymond et al. 1999) with the following changes. Initial LOD score screening was performed with CRI-MAP (version 2.4) (Lander and Green 1987) because tests showed that even though the LOD scores it computes are not exact, they are good approximations on this cat pedigree. A type I marker was considered linked to a second if a two-locus LOD score of ≥3.5 was achieved. We used the conservative 3.5 threshold here instead of the standard 3.0 threshold (Ott 1991) because the type I markers are less informative and because at least one case with a 3.3 LOD score (between FCA091 and FCA069) erroneously indicated linkage. To find an order in each linkage group FIRSTORD (Curtis 1994) and CRI-MAP (version 2.4) (Lander and Green 1987) were used. We considered LOD score differences of 1.0, 2.0, and 3.0, corresponding to odds ratios of 10:1, 100:1, 1000:1, when choosing among alternative genetic map orders. A LOD score threshold of 3.0 was used traditionally for constructing genetic maps in a setting where there was no other evidence regarding marker order. In this context, we are comparing a genetic map to an RH map based on two disjoint types of laboratory data. Therefore, it is useful to know to what extent the two sets of evidence for marker order agree or disagree on each chromosome, especially in those regions where neither map is very certain.

Isolation of Tetranucleotide Microsatellite Loci for Radiation Hybrid Mapping

Tetranucleotide repeat loci were isolated from genomic DNA of a male domestic cat (NCI Fca215) as previously described (Sarno et al. 2000) from construction of a microsatellite enriched library (Menotti-Raymond et al., submitted) using capture hybridization techniques.

RH Genotyping

Feline sequence tagged sites (STSs) were designed from a number of sources, including feline mRNAs deposited in GenBank, sequences derived from conserved primer design and amplification (http://home.ncifcrf.gov/ccr/lgd), CATS primer-like derived sequences (Lyons et al. 1997; Murphy et al., submitted), and feline expressed sequence tags (ESTs) (Murphy et al. 2000). Each STS was genotyped via PCR-based screening of the 93-clone feline RH panel (Murphy et al. 1999). STSs were amplified under a standard PCR profile using *Taq*-Gold DNA polymerase in 10 µl reactions containing 1.5 mM $MgCl_2$ as described (Murphy et al. 2000). Microsatellites were amplified (Menotti-Raymond et al. 1999) and genotyped in the domestic cat RH panel as previously reported (Murphy et al. 2000).

RH Map Construction

We identified linkage groups using the program RH2PT in the software package RHMAP (Boehnke et al. 1991). An initial LOD threshold of 8.0 was used and then subsequent groups assembled using decreasing thresholds of 4.0 or 6.0 guided by previous mapping results (Murphy et al. 2000) and physically mapped loci (Menotti-Raymond et al. 1999; O'Brien et al. 1997b). This procedure resulted in contiguous linkage groups that defined each arm of metacentric chromosomes and entire acrocentric chromosomes, with centromeres being identified using marked retention frequency spikes as have been revealed in previous studies (Murphy et al. 2000; Stewart et al. 1997). To order the markers in the RH map, we primarily used a reduction from the problem of RH mapping to the traveling salesman problem (TSP), which is widely studied in combinatorial optimization (Ben-Dor and Chor 1997). An important virtue of this approach is that we can use CONCORDE (Applegate et al. 1998), an existing highly sophisticated software package for the TSP that quickly finds optima better than those found by software packages specifically for RH mapping (Agarwala et al. 2000). There

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Table 1. Type I loci genotyped using PCR/RFLP assays

| | PCR/RFLP assay | | | | PCR conditions | | | |
Locus	Polymorphism	Uncut prdt	Pbe	Fca	PCR anneal	Modifications	Forward primer	Reverse primer
ADRA1B	Eag I	169	139	157	53°C	Taq, 1 mM MgCl$_2$	GTTTCTTCGGCCGTGCTGCCCTTC-TCGGC	FAM-AGTAGGCGCACCCCAATGTAG
CAMK4	BstZ107I	205	176	196	53°C	Taq	FAM-ACCACTCAAAATCGGTGAGG	GTTTCTCGTATACGGCACTGATTC-CAGAAAGTA
CHRNA1	BstUI	206	178	197	55°C		FAM-TTCGTGACTTGAAACCCACA	GTTTCTTCGCGTAACATCCCTTGGCTCGC
CYP19	Msp I	200	150	190	55°C		GTTTCTTCCGGCGCAAAGCCTTAC-AGGATGA	FAM-TGACATTTTTAGGAGTGGCAC
FGFR4	Hae III	106	98	75	48°C	Taq, 1.5 mM MgCl$_2$	FAM-CCTCGAATGGGCACATTTAC	GTTTCTGGCCCAGGTCTCTTCTTCTCAGT
IFNG	10bp Pbe deletion	NA	90	100	55°C		AGCCCTCTCCCCTTCTTCTT	TET-TTAAATTGGCTGCCTAGTTGG
ODC1	Rsa I	180	136	171	55°C		GTTTCTTGTACCAAATCCTTGTA-AACAAGTGTCTCAG	TET-ACAACCTGCAGACCCCATAC
PAH	Nsi I	216	186	208	55°C	Taq	GTTTCTTATGCATACAAGGAGAT-CCATAAATGC	FAM-TGTGGATGGCAGTGTACAAGA
PLAU	Ava I	233	150	222	55°C		GTTTCTTCTCGAGATGCAGCTGT-CCAAAGAAA	TET-TTGTGCAGCCACAGATTCAT
PTHLH	Xma I	215	205	181	55°C	Taq	TET-CCTGATTGCAGAAATCCACA	GTTTCTTCCCGGGTTTACCCTTCT-TTTTCTTCCC

Polymorphism: Restriction endonuclease site identifying polymorphism between domestic cat and leopard cat. Uncut prdt: Size of uncut PCR product in base pairs (bp). Pbe: Size of labeled PCR digestion product in leopard cat, also in bp. Fca: size of labeled PCR digestion product in domestic cat, also in bp. Modifications: Exceptions to PCR conditions stated in Materials and Methods section. FAM. TET: fluorescent phosphoramidite tag. NA: not applicable.

are reductions from RH mapping to TSP for either the maximum likelihood (MLE) or minimum obligate chromosome breaks (OCB) criteria. We solved instances for both criteria, although we relied primarily on the MLE solutions because MLE is used more widely in RH mapping, and the genetic map uses the maximum likelihood criterion. The formal reduction from RH mapping to TSP assumes simplistically that there are no two's (uncertain retention) in the vector data and that the retention frequency is uniform. To treat the problem of two entries robustly, we used previously devised multiple weighting schemes, three for MLE, two for OCB that treat the two vector entries in different plausible ways (Agarwala et al. 2000). In two regions where the retention frequency was especially variable across nearby markers, we used RHMAP (Boehnke et al. 1991) because it accounts for unequal retention frequency in the cost function.

We selected an initial set of markers by first removing markers that were defined as "too close" (i.e., within a certain number of obligate breaks from a nearby marker) or contained too many ambiguities (positions scored as two in the RH vector). Using the remaining markers, we reduced each chromosome arm marker set to 5 instances of TSP, solved those using CONCORDE, and translated the TSP solutions back to RH maps (Agarwala et al. 2000). We then defined "framework markers" as those that were ordered consistently by all three interpretations of the MLE criterion using the map integration approach in Agarwala et al. (2000), but we also noted those loci that got placed differently by MLE and OCB. We then placed the markers that got initially set aside for being "too close" into the constrained framework map using the RHMAXLIK program in the RHMAP software package (Boehnke et al. 1991) with the MLE criterion. We then took the set of markers that were set aside either for having too many two entries or because their placement differed in the three different MLE TSP solutions and placed them into "bins" between adjacent markers or at the end of a chromosome arm. To choose the best bin we considered adding each marker to the post-RHMAXLIK map before the first marker, between each pair of markers, and after the last marker using the "base MLE" criterion (as defined in Agarwala et al. 2000); we assigned each marker

to the most likely bin. See supporting information at the Web site: http://home.ncifcrf.gov/ccr/lgd.

Comparative Mapping Data

Human comparative mapping locus positions were derived from the NCBI sequence assembly (build 30) of the human genome.

Results

We have constructed and integrated second-generation linkage and RH maps of the domestic cat. An interspecies pedigree between the domestic cat (*Felis catus*) and the Asian leopard cat (*Prionailurus bengalensis*) was generated for construction of a type I/type II linkage map in order to increase the polymorphic content in coding loci that could be tracked in meiotic recombination products (Menotti-Raymond et al. 1999). Initially, a large-scale screening of candidate type I PCR products was performed to identify loci exhibiting polymorphism between the two species. Two methodologies were utilized: detection of sequence polymorphisms using single-stranded conformation polymorphism screening procedures, and direct comparison of sequences of gene homologues in the two species.

PCR products of 461 type I loci (Murphy et al. 2000) were screened for mobility polymorphism using SSCP techniques (Ravnik-Glavac et al. 1994). Three sets of gel conditions were utilized in the SSCP screening in order to improve detection of sequence polymorphisms. The majority of PCR products were short products between 100 and 300 base pairs, and generally polymorphisms were detectable under all three gel conditions. A proportion of the polymorphisms were detectable with a single buffer system, while 18%, 12%, and 8% of the products exhibited polymorphism exclusively using $1 \times$ TME, $1 \times$ TBE, or $1 \times$ TBE + 5% glycerol, respectively. In addition, PCR/RFLP assays (Lander and Botstein 1986) were designed around sequence polymorphisms identified from comparison of homologous sequences of domestic cat and leopard cat PCR products.

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Figure 1. Feline genetic linkage (GL) and radiation hybrid (RH) maps and human sequence-based comparative maps. Feline chromosome names (A1, A2, etc.) are listed above each GL and RH map. Loci mapped in common between the RH and GL maps are connected with dashed lines. Microsatellites are denoted by the prefix FCA or an F, followed by a number. Type I coding loci are given by their human gene homologue symbols, or in the case of feline ESTs, preceded by an Fc, and followed by the human homologue's UniGene cluster identifier (see associated data at http://lgd.nci.nih.gov). GL framework distances (sex-averaged) are given in centiMorgans, with intermarker distances shown to the right, and summed for each chromosome below. Genetic linkage map loci labeled in blue are ordered at odds $\geq$ 100:1, those in black < 100:1. The RH map centiRay distance scale is represented by each bar corresponding to 200 cR_{5000}. Binned loci are denoted by vertical bars spanning the most likely framework map interval. Genetic markers physically localized to cat chromosomes using a rodent/cat somatic cell hybrid panel (O'Brien et al. 1997a) are underlined. Blocks of conserved order in the human genome, inferred from the cat RH map orders, are shown to the right of each RH map and are bounded by their chromosomal physical coordinates (in Mb) in the human genome sequence assembly (NCBI build 30). A full listing of physical locations for each human homologue can be found at http://lgd.nci.nih.gov

Genetic Linkage Map Using Interspecies Backcross

Approximately 392 primer pairs amplified well in domestic cat, leopard cat and interspecies hybrid DNA. Of these, primer pairs that did not amplify or amplified poorly in the leopard cat (approximately 10%), and primer pairs that exhibited the "hybrid null" condition (5%), in which primers designed from domestic cat sequence amplify either species independently, but preferentially amplify domestic cat DNA in the interspecies hybrid samples (Menotti-Raymond et al. 1999), were dropped as candidate loci. One hundred nine loci (28%) were identified which demonstrated polymorphism between the domestic cat and leopard cat, 98 of these products detectable by SSCP and 11 by PCR-RFLP assays. DNA from 118 animals of the interspecies pedigree were genotyped and scored independently by two investigators. Following genotyping, 81 of the type I markers (70 type I markers by SSCP, 10 by PCR/RFLP assays, and 1 locus, *GBE*1, using a polymorphic microsatellite [Fyfe et al. 1992]) were analyzed with 247 type II loci of the first generation linkage map of the domestic cat to create an integrated type I/type II linkage map of 328 markers. Primer pairs for the 81 type I loci included 39 feline ESTs (Murphy et al. 2000), 16 CATS (Lyons et al. 1997) and "new CATS" (Murphy et al. 2000), 12 feline sequences deposited in GenBank (Murphy et al. 2000), 3 TOASTS (Jiang et al. 1998), 10 from modification of CATS primers for PCR/RFLP (Table 1), and a microsatellite in the intron of *GBE*1 (Fyfe et al. 1992). Primer pairs for 81 type I loci are listed in Table 1, Murphy et al. (2000), and on the LGD Web site: http://lgd.nci.nih.gov.

Figure 1 presents the integrated type I/ type II genetic linkage map. As a result of analysis using FIRSTORD and CRI-MAP (Curtis 1994; Lander and Green 1987), 319 type I and type II markers linked to at least one other marker creating 47 linkage groups that were mapped by RH and somatic cell hybrid panels to the 18 autosomes and the X chromosome.

Type I markers were added to all of the autosomes and the X chromosome with the exception of chromosome B1 (Table 2). Six type I markers (*HMG1L10*, *ADRA1B*, *DKFZP586F2423*, *SLC8A1*, *MYCN*, *FTL*) were mapped in the linkage map which have not been mapped in the domestic cat RH panel (Murphy et al. 2000) (Figure 1). These additional markers confirm expectations relative to conservation of synteny observed between human and cat (Murphy et al. 2000; Rettenberger et al. 1995; Wienberg et al. 1997). Eleven singleton microsatellites from the previous map (FCA327, FCA088, FCA334, FCA667, FCA356, F41, FCA137, FCA476, FCA031, FCA311, FCA001) were integrated into linkage groups consistent with their previous physical assignment. Nine unlinked microsatellites, physically mapped in the first-generation genetic linkage map, were not incorporated into linkage groups. Among these nine singletons assigned to a chromosome, four markers (FCA129 on A1, FCA357 on A1, FCA493 on A3, FCA193 on D1) have pairwise LOD scores greater than 2.0 with at least one marker in a linkage group on the chromosome to which they are assigned, one marker (FCA181) has its only pairwise

LOD score greater than 2.0 with another singleton (FCA493) also on A3. For these five singletons, every pairwise LOD score greater than 2.0 is to a marker on the assigned chromosome. For the other four assigned singletons (FCA214, FCA247, FCA538, FCA113), the best pairwise LOD scores range from 1.55 to 1.77 and are always to a marker in a linkage group on a different chromosome. Among these nine singletons, only FCA357 has an especially low number of meioses (16 fully informative), so that factor cannot explain our inability to place these nine markers on the linkage map. The mapped location of eight of the nine singletons has been confirmed in the RH map; one locus (FCA538) located on chromosome D3 could not be mapped in the RH panel. Our estimates of recombination distances for eight of the singletons to adjacent markers, based on physical location in the RH map and cR/cM ratios, would suggest the likelihood of their linkage to an adjacent marker. However, we note that six of the nine markers map in the RH map to telomeric regions, areas which often exhibit expanded recombination distances. Twenty-eight genotyped type I loci were not incorporated into the map due either to lack of linkage to another locus with a sufficiently high LOD score, genotyping patterns inconsistent with Mendelian laws of inheritance, or linkage inconsistent with RH mapping data and cat-human conserved synteny expectations (O'Brien et al. 1997a,b; Rettenberger et al. 1995; Wienberg et al. 1997).

We have physically reassigned six small linkage groups which were tentatively assigned in the first linkage map (Menotti-Raymond et al. 1999). In the first-generation map, chromosomal assignment of linkage groups was based on PCR assays in a cat rodent somatic cell hybrid panel which had poor representation of E2 and F1, leading to mapping failure for these two chromosomes (Menotti-Raymond et al. 1999; O'Brien et al. 1997a). Three groups have been reassigned to chromosome E2 (FCA309, FCA058, FCA531, FCA665; FCA096, FCA075, FCA085, FCA586, FCA032; FCA070, FCA589) and two to F1 (FCA191, FCA120; FCA223, FCA344). An additional linkage group has been reassigned to E3 (FCA466-FCA628). A segment of a linkage group on A1 (FCA024, FCA571, F146, FCA153, FCA229, FCA499, F42, FCA280, F53, FCA176, FCA274) has been reassigned to chromosome D4 due to previous spurious linkage through FCA004. The highly reduced number of informative meioses observed for FCA004 likely contributed to generation of this chimeric linkage group. FCA004, FCA091, and FCA014 have been dropped from this generation linkage map due to conflicting linkage results. These markers are mapped in the RH map.

To approximate the genetic length of the feline map, we took into account four terms: the sum of the lengths of the linkage groups, the distances between the last marker on a chromosome and the end of the chromosome, the distance between the 28 adjacent pairs of linkage groups on the same chromosome, and the distance between the nine singletons and some linkage group. The sum of the lengths of the linkage groups is 2646cM (Table 2). To estimate the distance to the end of each chromosome we took the average intermarker spacing among adjacent linked markers, which is

Table 2. Feline genetic linkage/RH map statistics

Chromosome	No. of loci–linkage map		No. of loci–RH map		Genetic length	RH length	cR/cM ratio
	Type I	Type II	Type I	Type II			
A1	7	30	37	29	242	1484	6.1
A2	6	16	44	18	193	1453	7.5
A3	4	15	33	12	151	1337	8.9
B1	4	25	29	24	263	1459	5.5
B2	0	5	38	7	39	1137	29.2
B3	5	9	47	10	156	1102	7.1
B4	10	14	39	16	187	1078	5.8
C1	5	16	49	19	209	1769	8.5
C2	2	17	29	15	166	1074	6.5
D1	4	13	37	12	115	1066	9.3
D2	6	13	20	14	140	905	6.5
D3	6	18	20	16	206	1012	4.9
D4	2	9	16	14	100	935	9.4
E1	5	9	33	9	76	853	11.2
E2	5	11	19	10	121	561	4.6
E3	6	4	24	5	67	668	10.0
F1	2	4	17	3	53	501	9.5
F2	1	13	7	14	127	480	3.8
X	1	7	33	7	35	844	24.1
Y	0	0	8	0	0	170	NA
Total	81	248	579	254	2646	19888	7.5

7.8 cM, and divided by two, to a get a total contribution of 19 (chromosomes) $\times$ 2 (ends/chromsomes) $\times$ 3.9 cM = 148 cM. We believe that the end markers on adjacent linkage groups are unlikely to be close, since they were not detected to be linked with a high LOD score, and we detected many linkages greater than 10 cM. Therefore we estimated the intergroup distance by taking the average of the intermarker distances between markers that are adjacent in a linkage group, but at least 5 cM apart, which is 13.3 cM. The third term in the distance estimate is $28 \times 13.3 = 372$. Similarly we estimated the last term as $9 \times 13.3 = 120$. The total of the four terms is approximately 3300 cM ($2646 + 148 + 372 + 120 = 3286$).

RH Map

Two hundred thirty-four additional loci were genotyped on the 93-clone feline RH panel. Of these, 48 were dinucleotide repeat loci developed for the first-generation linkage map (Menotti-Raymond et al. 1999) that were not placed in the first iteration of the feline RH map (Murphy et al. 2000). Thirty-one additional tetranucleotide repeat loci were also genotyped in the RH panel (Menotti-Raymond et al., submitted). We developed 164 new type I markers for targeted mapping of chromosomes with poor type I marker representation and to help refine syntenic boundaries. These were derived from a number of sources, including CATS (Lyons et al. 1997), TOASTS (Jiang et al. 1998), feline mRNAs deposited in GenBank, and ESTs derived from previously developed cDNA libraries (Murphy et al. 2000). In addition, we designed 51 conserved comparative CATS-like primers (Murphy et al. 2001a,b; Murphy et al. submitted)

(http://home.ncifcrf.gov/ccr/lgd/) to amplify and design feline-specific STSs from chromosomes with poor type I marker coverage. Nine type I loci placed on the previous version of the RH map were excluded due to suspect homology based on additional BLAST searches. Most of these loci exhibited reduced or elevated retention frequencies relative to adjacent markers and did not conform to expectations of syntenic order, resulting in map expansion.

The expanded RH map covers all feline chromosomes, with an estimated length of 19,888 centirays (Table 2, supplementary data at http://home.ncifcrf.gov/ccr/lgd/). Seven hundred twelve loci (85% of all RH loci) were positioned in the framework maps, 63 tightly linked markers positioned on the framework using RHMAP, and 59 markers placed in respective intervals using the described binning strategy. The average genomewide retention frequency remained at 0.39, consistent with previous findings (Murphy et al. 1999, 2000). A single gap exists on the short arm of chromosome E1, though this gap is spanned by linked genetic markers (see below).

The Integrated Linkage-RH Map

Figure 1 illustrates the integration of the 328 marker linkage map with 834 markers ordered in a 5000 rad radiation hybrid analysis of domestic cat chromosomes (Murphy et al. 2000). This map further expands integration of the genetic linkage and cat RH maps demonstrated by Sun et al. (2001). With near-full genome coverage, the RH map provides orientation and order for the multiple interchromosomal groups in the genetic linkage map. It also provides physical assignment for several linkage groups on the basis of their representation in

RH groups of type I markers with human homologues on chromosomes that are consistent with previous reciprocal cat-human chromosome painting-based syntenies. The genetic linkage map provides confirmation of RH marker order, physical assignment of linkage groups from somatic cell hybrid mapping, and helps to bridge a gap in the chromosome E1 RH map.

We observed an average cR:cM ratio of 7.5 across the 19 feline chromosomes (Table 2). Most chromosomes exhibit a high concordance in marker order between the genetic linkage and RH maps. Where the maps differ, rotation of pairs of closely linked loci that were ordered with poor confidence in one or both maps describes the vast majority of discrepancies in marker order. Chromosomes A2 and B4 exhibit a higher level of marker order discrepancy. Statistical confidence in marker order is not high in the linkage map for chromosomes A2 and B4, as the average number of informative meioses for loci on these chromosomes was reduced (45) in the first-generation map relative to the average number of meioses observed across all chromosomes (87). Small, undetected cytogenetic rearrangements in these chromosomes between the two species may contribute to our inability to order loci.

Comparative Mapping

The number of observed conserved segments ordered (CSOs) between the cat and human genomes is approximately 110, and 86 CSOs when singleton-based segments are ignored. These observations are very similar to previous parallel RH comparisons between the cat and human genome (Murphy et al. 2000) where 100 CSOs were both counted and estimated using Nadeau and Taylor (1984) methods. Feline type 1 marker density increased markedly on several cat chromosomes, particularly chromosomes A2, B3, C1, C2, D1, and E3, resulting in increased parallel genome alignment precision.

We dropped several single-marker-based conserved segments from the previous map as they resulted from erroneous homology assessment, and further violated predictions based on chromosome painting studies (Rettenberger et al. 1995; Wienberg et al. 1997). However additional targeted mapping confirmed some small CSOs identified from just a single locus in the previous RH map. For example, two genes from human chromosome (Hsa) 1q42-44, *CHRM*3 and *LGALS*8, were found linked to chromosome D2, which also exhibits synteny with Hsa10q. The dog and cattle genomes exhibit this similar chromosome association between Hsa 1q42-44 and 10q (Band et al. 2000; Breen et al. 2001; Yang et al. 2000). Further characterization of the disposition of this chromosome association in other mammalian genomes might identify this as a useful phylogenomic marker.

Discussion

We have generated second-generation genetic linkage and RH maps of the domestic cat to identify genes controlling heritable disease phenotypes and refine the evolutionary history of mammalian genomes. The linkage and RH maps, both integrating coding genes (type I) and polymorphic microsatellite markers (type II), together include 864 loci spanning all 18 autosomes and the X chromosome, in addition to Y chromosome coverage in the radiation hybrid map. We observed an average intragroup intermarker spacing of 8 cM over an estimated genome length of 3300 cM, with an average centiMorgan:centiRay ratio of 7.5 cR_{5000}/cM. Due to the interspecies nature of the pedigree, quantitative distances between markers may be somewhat distorted. In addition, our estimate of the total map length in the domestic cat is based on presumptions from the hybrid pedigree. The only way to test the recombination distances in the separate species would be to construct parallel maps in single-species pedigrees. The interspecies pedigree was used primarily to maximize the number of type I loci which could be incorporated with the polymorphic type II markers. We believe that marker order is reproducible in the domestic cat, as it is corroborated by the high degree of concordance between the linkage and RH maps in this article. The addition of type I markers to the linkage map offers additional cross reference to the gene dense maps of human and mouse for comparative mapping purposes while further connecting and referencing the gene-rich RH map of the cat (Murphy et al. 2000; Sun et al. 2001).

Two hundred ninety-three markers are mapped in common between the domestic cat linkage and RH maps, including 70 type I and 223 type II loci (Figure 1). In general, the RH and linkage maps exhibit a high concordance of marker order. The map further affirms a high degree of conserved synteny between the genomes of human and cat, previously characterized in physical maps of the cat (Murphy et al. 2000; O'Brien et al. 1997a; O'Brien and Nash 1982) and human/cat reciprocal chromosome painting patterns (Rettenberger et al. 1995; Wienberg et al. 1997). The addition of 155 new type I markers, though targeted at regions of low marker density, reveals 110 conserved ordered segments between the cat and human genomes, roughly consistent with the previous estimates and predictions using the Nadeau-Taylor (1984) method (Murphy et al. 2000). This finding suggests that, as probably all large conserved segments have been detected, continued feline marker development should target and refine rearrangement breakpoints and further inspection of single-locus discrepancies with predicted cat-human synteny.

The characterization of polymorphism in a locus in parental generations is prerequisite for inclusion of the marker in a genetic recombination map. Our rationale behind an interspecies cross was to increase type I product polymorphism content, a technique used in the generation of a number of species' maps (Brady et al. 1997; Marklund et al. 1996; Riggs et al. 1997). In the mouse, more than 70% of 3′ UTR PCR products characterized polymorphism between C57BL/6J and *Mus spretus* (Brady et al. 1997), while 40% of loci examined exhibited SSC polymorphism between different species of pig (Jorgensen et al. 1997). Therefore we anticipated a much higher rate of polymorphism between

the domestic cat and leopard cat than observed (28%). Evolutionary divergence times estimated between the mouse species, some 2–4 million years (Ferris et al. 1983), is well within the divergence time estimated between the domestic cat and Asian leopard cat (Johnson and O'Brien 1997). The very high incidence of polymorphism in the mouse 3′ UTRs may well be a reflection of increased rates of nucleotide substitution frequently reported in murid rodents relative to other mammalian species (Li 1997; Wu and Li 1985).

In conclusion, the addition of type I loci to the genetic linkage map of the cat provides additional support for the high level of conserved synteny observed between the cat and human genomes in physical and RH maps of the cat. It also provides increased resolution between the integrated linkage and RH maps of the cat. In addition, it offers important comparative reference for the mapping and positional cloning of interesting phenotypes in the cat genome, including heritable and infectious diseases, coat color and pattern morphologies.

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