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# THE FELINE GENOME PROJECT[1]

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■ **Abstract** The compilation of a dense gene map and eventually a whole genome sequence (WGS) of the domestic cat holds considerable value for human genome annotation, for veterinary medicine, and for insight into the evolution of genome organization among mammals. Human association and veterinary studies of the cat, its domestic breeds, and its charismatic wild relatives of the family Felidae have rendered the species a powerful model for human hereditary diseases, for infectious disease agents, for adaptive evolutionary divergence, for conservation genetics, and for forensic applications. Here we review the advantages, rationale, and present strategy of a feline genome project, and we describe the disease models, comparative genomics, and biological applications posed by the full resolution of the cat's genome.

CONTENTS

INTRODUCTION . . . . . 657
THE CASE FOR A FELINE GENOME PROJECT . . . . . 658
PROGRESS IN ASSEMBLING THE FELINE GENE MAP . . . . . 663
THE CAT AS AN INDEX FOR COMPARATIVE GENOMICS . . . . . 667
PROGRESS IN FELINE GENETIC DISEASE MODELS . . . . . 671
A ROLE FOR THE CAT GENOME IN FORENSICS . . . . . 673
CONCLUSIONS AND FUTURE PROSPECTS . . . . . 674

## INTRODUCTION

In the century since the rediscovery and replication of Mendel's seminal tenets of hereditary transmission by Hugo DeVries, Carl Correns, and Erick von Tschermak in 1900, the field of genetics has blossomed into a defining discipline of biology. A half-century of deductive genetic experimentation followed by a generation of advances in molecular biology laid the conceptual groundwork for the modern

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genomics era. Genetic maps of microorganisms, plants, and animals were detailed, leading to whole genome sequencing (WGS) of traditional model organisms including *Escherichia coli*, yeast, *Drosophila*, *Arabidopsis*, *Caenorhabditis elegans*, rice, mouse, and human. The prospect of WGS analysis of the genomes of selected species is providing an unprecedented view of the genetic instructions that specify development, distinctiveness, adaptation, and reproduction. The combination of automated DNA sequencing, gene annotation algorithms, and other computational routines hold exceptional promise for explaining gene action, gene retention, and gene interactions in virtually any species we choose to study.

Among mammals, genomic advances have been driven by the completion of the human and mouse full genome sequences (30, 36, 117). The next mammal whose WGS is under way is the rat, a powerful biomedical model for hypertension, diabetes, and other complex polygenic human diseases (http://rgd.mcw.edu) (170). Close behind in gene map and WGS development are the close primate relatives of man, chimpanzee and rhesus macaque, as well as agriculturally important species, cow, pig, and chicken, which will surely follow (136). In parallel, the companion animal species, domestic dog and cat, have stimulated increasing enthusiasm for genome study because of important advantages that these species bring to biomedical genomic research (16, 137, 140). In this review, we describe the rationale, progress, applications, and potential of the genome project targeting the domestic cat, *Felis catus*.

The Feline Genome Project began two decades ago when the first gene map of the species was published and compared to the developing human gene map of that time (138). We selected the cat because it offered a particularly good model for feline transmissible cancer, caused by Feline Leukemia virus (FeLV) (60, 61). We were hoping to develop a new tool to study genetic and infectious disease and also to add an additional mammalian order to the long-term project of studying the mammalian radiations by tracking the changes and adaptations in the genomes of modern mammals (134, 137). The genomic advances and promise for comparative genetics offered by the cat genome project offer a cogent reason for emphasis and eventual WGS for the cat.

## THE CASE FOR A FELINE GENOME PROJECT

The cost of WGS is high, estimated at 50 million U.S. dollars to complete a mammalian genome sequence of 2.7–3.2 billion bases at 5X coverage, comparable to the size of the murine and human genomes, respectively (59, 76, 117, 136). Thus choosing a species for WGS requires considerable biological utility, particularly for human biomedical inference, since the largest funding agencies support health-related research. Once the principal mouse and rat WGS are achieved, there are a number of advantages for WGS of additional mammalian species (136). The most compelling features for targeting the domestic cat are described below.

There are approximately 65 million cats in the United States and several times that number worldwide, so many that overpopulation of feral cats is

considered a serious nuisance in many countries (3, 6, 11, 147). The contributing reasons for the population increase include mankind's fascination and domestication of the species combined with a relatively high fecundity. Ease of breeding plus our historic adulation and domestication of cats increase their potential as a genomic model for medical and biological application. Our personal affinity for companion animals, notably cats and dogs, provides a medical surveillance matched only by human biology. The world's veterinary schools produce hundreds of practitioners each year, most of whom carefully document genetic and chronic diseases of our pets. The result is a comprehensive veterinary literature that has described over 258 feline genetic diseases and 437 canine genetic diseases (http://www.angis.org.au/Databases/BIRX/omia/). Approximately half of these diseases have established homology with human genetic defects. A list of feline homologs of single-gene defects found in humans is presented in Table 1. The clinical and physiological study of these feline hereditary diseases provides a strong comparative medicine opportunity for prevention, diagnostic, and treatment studies in a laboratory setting.

The cat also has provided several invaluable models for infectious disease. These include endemic feline leukemia virus and feline sarcoma virus, Type C retroviruses that interact with cellular oncogenes to induce leukemia, lymphoma, and sarcoma (60, 61). Historically, many of the human oncogenes that define signal transduction pathways were originally discovered in the context of FeLV interaction in cat models. The cat provides the only naturally occurring model for human AIDS pathogenesis in its endemic fatal transmissible feline immunodeficiency virus (FIV) (97a, 148, 184). Similar to its close phylogenetic relative HIV, FIV induces CD4-T lymphocyte depletion in affected cats, immune system collapse, and susceptibility to adventitious microbial agents as a prelude to wasting disease and death (148). Interestingly, over 20 wildcat species (including lions, leopards, cheetahs, ocelots, pumas, and other big cats) are epidemic with their own species-specific strain of FIV (18, 24, 25, 142). In contrast to domestic cats, the endemic FIV strains do not appear to cause acute immunodeficiency in the wildcat species, perhaps a consequence of historic natural selection of host genetic resistance to the fatal virus (25, 134).

The feline panleukopenia (distemper) virus has revealed a natural history parable in its abrupt transformation of the cat virus to an epidemic, fatal canine parvovirus that emerged in the world's puppy population in 1978 (145). In another chilling episode, the canine distemper virus, which is normally restricted to canid species, precipitously adapted to and decimated a large African lion population in 1994, killing one third of the lions in the Serengeti ecosystem within a nine-month period (156). A clear involvement of host defense mechanisms in these and other infectious disease outbreaks renders the cats and their pathogens an excellent candidate species for characterizing the interaction of microbial adaptation and host disease gene defenses. Given the critical importance of infectious disease in scores of chronic and acute human disease, there are powerful research opportunities in the cat family (45, 134).

**TABLE 1** Hereditary human diseases with feline models

| Phenotype | Human candidate loci | Human locus | Feline chromosomal position | Inheritance pattern in cats | Ref. |
|---|---|---|---|---|---|
| ALBINISM, OCULOCUTANEOUS TYPE I | *TYR* | 11q14-q21 | D1 pcen | AR | 97 |
| CARDIOMYOPATHY, HYPERTROPHIC | SEVERAL | | | AD | 91 |
| CEREBELLAR DEGENERATION | SEVERAL | | | AR | 75 |
| CEROID LIPOFUSCINOSIS | *CLN1-6* | | | | 180 |
| CHEDIAK-HIGASHI SYNDROME | *CHS1* | 1q42.1-q42.2 | D2 | AR | 150 |
| DEAFNESS | SEVERAL | | | | 70 |
| DIABETES MELLITUS, TYPE I | *IDDM1* | 6p21.3 | B2 cen | | 89 |
| DIABETES MELLITUS, TYPE II | *IDDM2* | 11p15.5 | D1q | | 102 |
| DWARFISM | *ACH* | 4p16.3 | B1q | AD | 69 |
| EHLERS-DANLOS SYNDROME, TYPE VII | *ADAMTS2* | 5q23 | A1q | AR | 94 |
| FACTOR X DEFICIENCY | *F10* | 13q34 | A1p | | 57 |
| FACTOR XII DEFICIENCY | *F12* | 5q33-qter | A1q | AR | 88 |
| G6PD DEFICIENCY | *G6PD* | Xq28 | Xq | XR | 167 |
| GANGLIOSIDOSIS, GM1 | **GLB1* | 3p21.3 | C2q | AR | 38 |
| GANGLIOSIDOSIS, GM2 | **HEXB* | 5q13 | A1q | AR | 116*** |
| GLYCOGEN STORAGE DISEASE II | *GAA* | 17q25.2-q25.3 | E1q | | 153 |
| GLYCOGEN STORAGE DISEASE IV | **GBE1* | 3p12 | C2q | AR | 50, 51 |
| HAEMOPHILIA A | *F8* | Xq28 | Xq | X | 98 |
| HAEMOPHILIA B | *F9* | Xq27.1-q27.2 | Xq | X | 104 |
| HYPERLIPOPROTEINAEMIA | **LPL* | 8p22 | B1 pcen | AR | 55, 99*** |
| LUPUS ERYTHEMATOSUS | SEVERAL | | | UNK | 174 |

| | | | | | |
|---|---|---|---|---|---|
| MANNOSIDOSIS, ALPHA | **MAN2B1* | 19cen-q12 | E2p | AR | 10, 21, 165, 176*** |
| MONO-CRYPTORCHIDISM | NC | | | UNK | 114 |
| MUCOLIPIDOSIS II | *GNPTA* | 4q21-q23 | B1q | AR | 14 |
| MUCOPOLYSACCHARIDOSIS I | **IDUA* | 4p16.3 | B1q** | AR | 64, 68, 62a*** |
| MUCOPOLYSACCHARIDOSIS VI | **ARSB* | 5q11-q13 | A1q | AR | 32, 37, 46, 54, 66, 67, 81, 192, 107*** |
| MUCOPOLYSACCHARIDOSIS VII | **GUSB* | 7q21.11 | E3cen | AR | 56, 52*** |
| MUSCULAR DYSTROPHY, DMD, BECKER | **DMD* | Xp21.2 | Xp | X | 23, 53, 185 |
| NEUROAXONAL DYSTROPHY | NC | | | UNK | 177 |
| NIEMANN-PICK DISEASE, TYPE C | *NPC1* | 18q11-q12 | D3q | AR | 17, 101*** |
| PELGER-HUET ANOMALY | NC | | | UNK | 179 |
| POLYCYSTIC KIDNEY DISEASE | *PKD1,2,3* | 16p13.31-p13, 4q21-q23 | E3q, B1q | AD | 12*** |
| POLYDACTYLY | SEVERAL | | | AD | 35 |
| PROGRESSIVE RETINAL ATROPHY | SEVERAL | | | AR | 157 |
| ROD-CONE DYSPLASIA | SEVERAL | | | AD | 96 |
| SPINA BIFIDA | Mouse *T/t locus* | | | AD | 90 |
| SPINAL MUSCULAR ATROPHY, TYPE III | *SMN1* | 5q12.2-q13.3 | A1q | AR | 49*** |
| TESTICULAR FEMINIZATION | *DHTR* | Xq11-q12 | Xq | X | 95 |
| URTICARIA PIGMENTOSA | NC | | | | 175 |
| VITAMIN-K-DEPENDENT COAGULATION DEFECT | *GGCX* | 2p12 | A3q | | 163 |

UNK: Unreported pattern of inheritance, but heritable.

NC: No gene identified in human.

*Mutational mechanism identified in the cat.

**Tentative assignment.

***Reported breeding colony.

The cat also possesses several advantages from a comparative genomics perspective. Gene mapping and chromosome painting experiments have shown that the feline genome, which is composed of 19 chromosome pairs, is extensively conserved in gene content (conserved synteny) and G-banded chromosome appearance among other Felidae species, among other carnivore species, and indeed across many placental mammals (115, 124, 137, 138, 152, 182). The extent of chromosome segment conservation between the cat and human genomes is among the highest observed between mammalian orders (120, 121, 137, 152, 182). For example, the feline genome assembly is 3 to 4 times less rearranged relative to the human genome than are the genomes of murid rodent species (mouse and rats) (120, 121, 137). Overall, there seems to have been an extremely slow rate of chromosome translocation exchange between cats and primates. The remarkable colinear parallel of the cat and human genome provides an opportunity to inspect rather long stretches of conserved synteny between the two species, as well as the patterns and details of global reshuffling that are apparent in other lineages.

The domestic cat is one of 37 species of the Felidae family, itself one of 11 Carnivore families. The Felidae family dates back to around 15 mya (84), leading to the adaptive occupation of ecological niches throughout the world. The relative success of these majestic predators combined with humankind's fascination with the great cats for thousands of years has produced an extensive literature on human-cat interactions. Several species have been the object of long-term field ecology projects, and most can be observed and sampled in zoological collections. Thus, biological specimens are accessible in zoos, from field projects, and museums. In the past two decades, scientists at NCI's Laboratory of Genomic Diversity, in cooperation with scientists from the Smithsonian's NOAHS Center, have assembled over 40,000 tissue specimens from cats and their wild relatives collected across the world. This collection is unprecedented in its scope and utility for population-based research inquiries of free-ranging species (132, 133).

Further, the genomes of the Felidae family species are nearly identical to the domestic cat, with 15 of 19 domestic cat chromosomes invariant among all the other Felidae species (115, 190, 191). The genetic tools and resources developed for the domestic cat (e.g., microsatellites, coding gene PCR primers, libraries, etc.; see below) are readily applied to the study of wildcat species (34, 41, 42, 113, 172). Application of these molecular genetic tools and resulting evolutionary inferences has provided considerable insight into the history and peril of endangered Felidae species (cheetahs, pumas, lions, tigers, and others), laying the groundwork for the important new discipline of conservation genetics (33, 41, 42, 48, 83–86, 112, 132, 133, 172).

There are a number of practical advantages to a domestic cat model as well. The cats breed well in a captive setting and domestication dating back between 6000 to 8000 years ago has produced nearly 40 recognized breeds that have experienced moderate degrees of inbreeding and artificial selection across their recent ancestry (47, 71). The breeds provide recent phylogenetic lineages that capture different combinations of coat color, coat length, patterning, appearance, and

behavioral traits suitable for genetic analysis (155). Modern breeds reflect different combinations at around 12 monogenic coat color trait loci, most with homologous counterparts in coat color genes of mouse and other domestic species (154). The same gene homologs of pigmentation loci in other mammalian species have been implicated in anemia, sterility, and neurological and metabolic disorders (7, 8, 77). Further, the history of modest inbreeding in cat breeds supplies important populations ideal for linkage disequilibrium mapping of complex quantitative characters as have also been recognized in dog breeds (143). Dense genetic maps combined with existing cat pedigrees offer a rare opportunity to interpret a large body of hereditary trait inference.

The cat's reproductive apparatus and physiology have been extensively studied, leading to a fascinating comparative database that describes hormonal, behavioral, and reproductive distinctions among Felidae species (19, 73, 183). The strikingly different reproductive strategies seen among different cat species (e.g., induced or spontaneous ovulation, hormone ratios, mating systems, variable sperm quality, etc.) illustrate the adaptive coevolution of reproductive physiology, sexual selection, and behavioral ecology in graphic, well-studied situations (22, 27, 144). The experimental knowledge of cat reproduction has allowed considerable advances in assisted reproduction in cat species, notably artificial insemination, sperm, and embryo cryo-presentation, and in vitro fertilization (19, 40, 74, 80, 187, 189). Embryo transfer technology has led in December 2001 to the birth of CC, the first cloned domestic cat (160). The development of nuclear cloning for cats nearly ensures the likelihood of stem cell development and therapy, as well as the prospect of gene-specific knockout technology in the species, so powerful in rodent models (78, 87, 171).

Finally, the cat has evolved within the mammal superorder Laurasiatheria, one of four mammal clades that predated the radiation of modern mammals (118, 136). The three mammalian species already scheduled for WGS, human, rat, and mouse, are all members of a different clade, Euarchontoglires. For this reason, the cat genome with its conserved syntenic organization relative to human would represent a significant genomic expansion of the evolutionary diversity present among modern mammals. This divergence offers considerable breadth in sampling available genetic diversity among the living species of mammals.

## PROGRESS IN ASSEMBLING THE FELINE GENE MAP

The domestic cat carries 18 autosomal pairs, X and Y chromosomes, in a genome containing around $3 \times 10^9$ nucleotides, comparable to the human and mouse genomes. Early feline gene maps employed somatic cell hybrid panels derived from fusion of cat lymphocytes and genetically selectable rodent cell lines (135, 138). A combination of somatic cell hybrid mapping coupled with FISH mapping of heterologous molecular clones to cat metaphase chromosomes led to a skeleton map of 105 coding genes (135). Comparison of the linkage arrangement of cat genes to their human counterparts revealed a high degree of conserved

synteny, strings of homologous genes on a single chromosome in both species (135, 138, 140, 141). The extended genome conservation revealed by the gene map comparisons of cat and man was affirmed, virtually by direct observation using chromosome painting methods, or Zoo-FISH (152, 182). By hybridizing fluorescent-labeled chromosomes isolated from human chromosome libraries to cat metaphase chromosomes, the precise regions or segments of gene sequence homology in the cat genomes for each human chromosome were identified (140, 152, 182). The reciprocal human conserved syntenic segments were demarcated by painting human metaphase chromosome spreads with individual flow-sorted cat chromosomes (182).

The chromosome painting procedures identified 32 contiguous cat chromosome segments that were homologous to single human chromosomes and 30 conserved human chromosome segments that were painted by a single cat chromosome probe. These initial comparisons suggested that as few as 13 scissor-cuts could rearrange the cat genome into the human genome or vice versa. This value is lower than similar Zoo-FISH comparisons between other mammal species and the human genome. For example, the cattle genome would require 27 scissor cuts to reassemble it to the human genome arrangement. Horse would require 34 cuts, pig 28, dog 45, and mouse 160 cuts (28, 137). If we postulate a date of approximately 90 million years as the age of the common ancestor of humans and cats (43, 118), then it takes an average of 14 million years for a single translocation exchange to occur. This is among the slowest rate observed among all the mammalian genome comparisons with humans reported and emphasizes the highly conservative "default" mode of genome evaluation documented in many primate and carnivore species (137).

The Zoo-FISH methodology is limited by its inability to resolve homologous chromosome segments less than 5 Mb and also by its failure to reveal intrachromosomal inversion rearrangements within studied mammalian genomes. To increase the comparative genomic resolution of such inversions as well as to achieve higher power in the phenotype mapping, the cat map was expanded in several ways to provide a dense representation of both Type I coding genes and Type II hypervariable microsatellite markers (108, 121, 166). Mapping the coding genes is critical for establishing homologs to the full-length whole genome sequence (WGS) maps of human and mouse. Type II microsatellite locus markers are required to map feline phenotypes to specific genomic locations. Three mapping resources (Table 2), each with particular advantages were developed: (*a*) an interspecies backcross (ISB) between domestic cats and a closely related species Asian leopard cat (*Prionailarus bengalensis*) (Figure 1); (*b*) a domestic cat pedigree established from outbred cats by Nestlé-Purina PetCare (St. Louis, MO), and segregating six coat color loci plus other tractable phenotypes; (*c*) a 5000-rad radiation hybrid (RH) panel of the domestic cat that allows for physical mapping to a resolution of less than a megabase (119, 121). First- and second-generation linkage and physical maps using each of these tools have been developed (108, 109, 121, 166) to produce a feline genetic map integrating (*a*) comparative anchor–Type I loci for alignment with human and mouse genomes (103, 121), (*b*) microsatellite loci

**TABLE 2** Progress in feline gene mapping, December 2002

| | Type I coding genes | Type II microsatellite loci | Other | Total markers | Average marker density |
|---|---|---|---|---|---|
| I. Linkage map—ISB* | 81 | 248 | 0 | 329 | 10 cM |
| II. Linkage map—Nestlé Purina | 0 | 705 | 0 | 705 | 4.7 cM |
| III. Radiation hybrid map-5000 rad | 775 | 954 | 11 | 1740 | 1.9 cM |
| IV. Integrated map | 784 | 1086 | 11 | 1881 | 1.8 cM |

*ISB—Interspecies backcross, see Figure 1.

placed on average 11 cM apart (108), and (*c*) selected genes with important phenotypes. A summary of the most recent maps, including markers as of December 2002, is presented in Table 2.

The ISB pedigree, composed of 108 individuals and 66 informative meioses, was constructed to maximize the chance of obtaining genetic variants between Type I loci, as was demonstrated in building the mouse gene map (29, 108, 109). To date we have placed 81 Type I markers on the linkage map. In addition, 248 microsatellites were mapped on the linkage map from a larger group of 600 characterized microsatellites to provide highly informative loci for phenotype mapping. The sex-averaged length of the feline genome was estimated from the ISB at 3300 cM, with an average density of 10 cM between each marker. Forty-seven linkage groups were physically mapped to cat chromosomes by using the cat-rodent somatic cell hybrid panel (135), the radiation hybrid mapping data, or by conserved synteny with the human genome (121). A separate intraspecific pedigree, developed in collaboration with Nestlé-Purina, utilizes 256 cats with 483 meiosis derived from 27 founders. A total of 705 microsatellite loci are mapped on this pedigree, which provides an average density of one marker per 4.7 cM.

The 5000 rad RH map consists of a panel of 93 hybrid lines analyzed for concordant retention of markers to develop a higher density ordered physical map of Type I and Type II markers. The RH map contains 775 Type I markers (density = 4.3 cM/Type I marker) and 954 Type II markers (density = 3.5 cM/marker). The Type I markers are derived from a variety of sources including those detected by the Comparative Anchor Tagged Sequence (CATS) primer design method (82, 103), feline mRNAs deposited in GenBank, human expressed sequence tags (ESTs), and feline ESTs matched to human or mouse Type 1 coding genes (109, 121).

By comparing the linkage order derived from the ISB and the RH map, an alignment of the markers mapped in two or three approaches can be used to develop an integrated map (Figure 2). The derived marker order derived from the ISB and RH map are remarkably concordant, providing a high confidence in integration strategy. The current integrated RH-linkage map contains a total of 1881 markers,

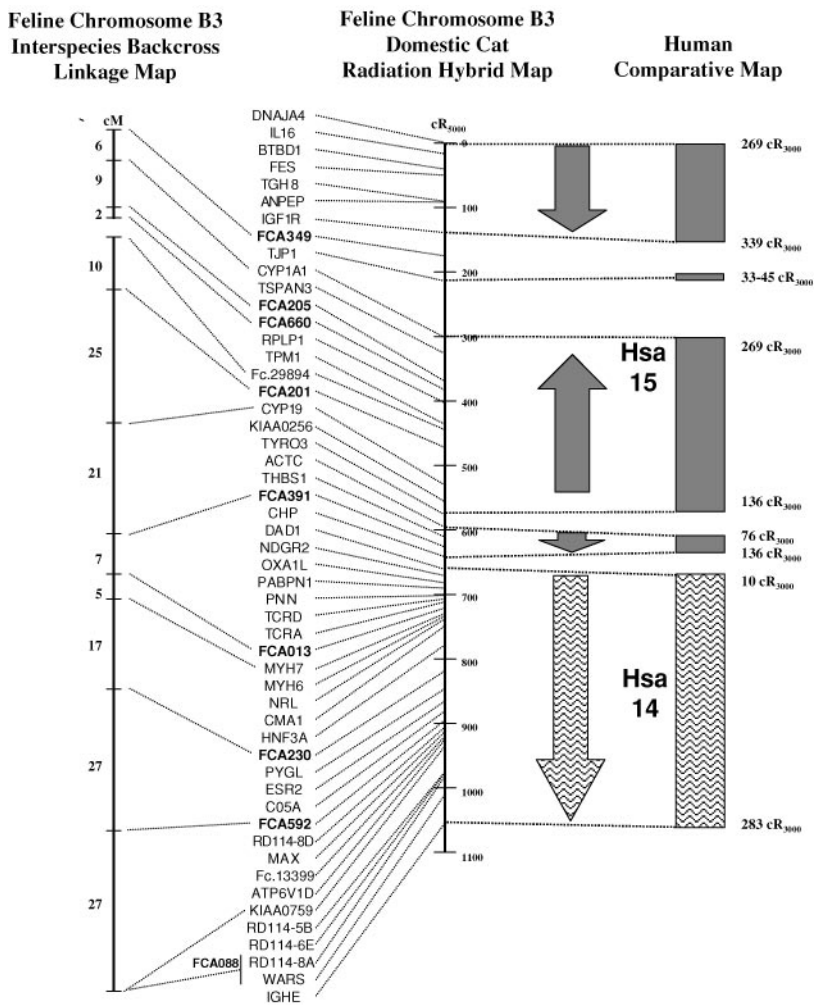


**Figure 2** Comparison of the feline chromosome B3 maps derived from the feline interspecies backcross linkage panel (*left*), feline radiation hybrid (RH) panel (*center*) compared with the human genome (*right*). Feline microsatellite markers are shown in bold, while Type I coding markers are shown in regular type. The patterned and shaded blocks for the human genome denote regions of conserved order with the adjoining feline chromosome (demarcated by *dashed connectors*), based on human radiation hybrid maps. Shaded blocks are homologous to human chromosome 15, while patterned blocks are homologous to human chromosome 14. Human Genebridge4 RH centiray positions are shown to the right of each block. The orientation of the collinear homology segments are depicted by arrows.

an average interval of 1.8 cM or 16.8 cR between each marker. Updated genetic maps of the integrated marker map can be viewed at (www.lgd.nci.nih.gov). The integrated gene map provides a powerful tool both for tracking cat phenotypes and comparing the processes that mold genome organizations that determined the evolution of mammals (120, 137, 140).

## THE CAT AS AN INDEX FOR COMPARATIVE GENOMICS

The rapid development of comparative genomic data is now beginning to reveal some important evolutionary features around the organization of modern genomes. Gene maps and chromosome painting clearly indicate a bimodal pattern of genome conservation among placental mammals. The ancestral or default pattern of genome rearrangement is very slow, roughly one translocation exchange every 14 million years. The slow rate is evident in Felidae species, humans, many primates, and also in other mammals such as mink, ferret, dolphin, and shrews (120, 137). Nested within and among these conserved genomic lineages, however, are species whose genomes have been reshuffled moderately (e.g., pigs and cows) and other mammalian lineages where the genome reshuffling is even more extensive (mouse, rat, gibbons, New World monkeys, dogs, and bears) (79, 120, 122, 123, 125, 181).

Once the unequal rates of chromosome exchange among different lineages were appreciated it became possible to use evolutionary principles to reconstruct the ancestral genome organization and to interpret the genomic changes that have occurred on each mammal lineage. By identifying certain common human homologue combinations (fusions) or separations (fissions), it has been possible to postulate the disposition of the primitive common ancestor of primates (139), of carnivores (120), and of all placental mammals (120), based on maximum parsimony (Figure 3). The ancestral placental mammal genome consisted of 24 autosomes plus X and Y, and included 32 ancestral conserved syntenies or segments, compared to the human genome (Figure 3*a*). Similar imputations from comparative mapping and painting have allowed postulation of the common ancestor of all carnivore species (Figure 3*b*). That genome has 21 chromosome pairs and is composed of 26 conserved syntenies as compared with the ancestral mammal, 23 conserved syntenies with the modern cat and 34 conserved syntenies with the human genome. Since human and cat both display the conserved "slow" pattern of genome evolution, apparent chromosome exchanges are remarkably few in number. By contrast, the extensive genomic exchanges that occur in the "fast" lineage species (dogs, bears, gibbons, New World monkeys) are attributed to more recent reshuffling events that occurred abruptly (in evolutionary terms), subsequent to divergence from the common ancestor but before divergence into the modern genomically divergent bears, dogs, and other species (123, 125, 137). This point is illustrated by Table 3 where the number of conserved segments (unordered), revealed by painting human chromosome probes onto karyotypes of 15 representative mammal species from seven placental orders, were used to

**TABLE 3** Variation in genomic evolutionary rate across placental mammals

| Species | Zoo-FISH conserved segments with placental mammal ancestor[a] | Haploid number | # of rearrangements relative to ancestral placental mammal genome[b] | Whole genomic rate[c] |
|---|---|---|---|---|
| Dolphin | 25 | 22 | 3 | 0.034 |
| Cat | 27 | 20 | 6 | 0.068 |
| Human | 29 | 23 | 6 | 0.068 |
| Macaque | 28 | 21 | 7 | 0.080 |
| Mink | 26 | 15 | 10 | 0.114 |
| Lemur | 35 | 30 | 10 | 0.114 |
| Tree shrew | 35 | 31 | 10 | 0.114 |
| Horse | 40 | 32 | 15 | 0.170 |
| Cow | 41 | 30 | 16 | 0.182 |
| Bat | 35 | 16 | 19 | 0.216 |
| Shrew | 30 | 10 | 20 | 0.227 |
| Pig | 41 | 19 | 22 | 0.250 |
| Gibbon | 51 | 22 | 26 | 0.295 |
| Spider monkey | 48 | 17 | 30 | 0.341 |
| Dog | 64 | 39 | 39 | 0.443 |
| Rat[d] | 85 | 20 | 65 | 0.739 |
| Mouse[d] | 103 | 20 | 83 | 0.943 |

[a]References (140, 181).

[b]Equal to the number of conserved unordered segments minus the lower haploid number of the compared species (137). Estimated based on chromosome painting data using human probes.

[c]Equal to the number of rearrangements relative to ancestral placental mammal genome divided by the estimated time of divergence from the ancestral Boreoeutherian mammal [~88 mya (43, 118)].

[d]Estimated from gene mapping data, excluding smaller segments defined by one or two genes to be more comparable to estimates derived from Zoo-FISH alone.

compute the genomic rate of translocation exchange based upon recent phylogenetic relationships and dating (43, 118). The disparate rates of chromosome translocation are evident by a 12-fold difference between the slowest species (dolphin) and the fastest (dog) (Table 3).

As mentioned above, genome comparisons derived from chromosome painting and banding homologies can reveal neither small (<5 cm) conserved segments nor intra-chromosomal inversions. Painting provides a considerable underestimate as shown by the analysis of 353 feline Type I markers with the human map (121). In that analysis we discerned 100 conserved segment orders—CSOs—between human and cat (Figure 4), nearly three times as many as the 30–32 conserved syntenies (140, 152, 182) revealed by chromosome painting (Table 4). A similar result was also obtained with comparison of ordered Type I gene maps of

**TABLE 4** Conserved segments estimated using different mapping technologies

| Species comparison | Conserved segments: Zoo-FISH | Conserved segments: Unordered via mapping | Conserved segments: Ordered via mapping |
|---|---|---|---|
| Human-cat | 30–32 | 100 | |
| Human-cow | 50 | 82 | 149 |
| Human-rat | NA | 152 | 190 |
| Human-mouse | NA | 180 | 200 |



cow to human (Table 4). This increasing focus of CSOs over paint-conserved syntenies suggests there are twice as many interstitial inversions produced as translocations in these lineages (Figure 4). These higher-level resolution comparisons increase the precision of conserved segment identification to approach the number of conserved segments predicted by the theoretical calculations (121, 122). They also would suggest that about 500 ordered Type I markers would be sufficient to reveal >90% of the conserved syntenic segments between any two mammalian species with slow to moderate rates of genomic exchange.

The most precise comparisons of mammalian genomes will derive from aligned homologs of DNA sequences, eventually across the entire genome. As has been recently demonstrated, mouse and human WGS comparisons reveal the full spectrum of gene inclusion, gene birth and death, transpositions, repetitive element expansions, and conserved syntenies (30, 36, 59, 117). A provocative glimpse of a three species genome sequence comparisons has been achieved with the recent full sequence comparison of the major histocompatibility complex (MHC) class II sequence of human *HLA*, mouse *H2*, and cat *FLA* (Figure 5) (193). The human *HLA* region consists of 224 genes of which 128 are expressed while 96 are pseudogenes (113a). Nearly half of the *HLA* genes play a role in immune defenses and about 50% of the HLA sequences consist of repetitive elements (LINES, SINES, LTRs, and STRs). Sequence alignment of human mouse and cat MHC class II region homologs (Figure 5) revealed several fascinating evolutionary features (1, 193). First, the three species differ considerably in the gene cluster length with *HLA*-998kb, *FLA*-758kb, and *H2*-495kb. The three species each contained around 35 functional genes but differed markedly in gene disposition and pseudogene numbers: *HLA* has 23 pseudogenes, *FLA* has 7, and H2 has 5 within the same region. In addition, cats have appreciable differences in their class II gene families. The *DQ* family is absent and *DP* genes are vestigial, represented by two pseudogenes. The loss of *DP* and *DQ* genes is compensated for in cats by an expansion of the *DR* region to seven modern *DR* genes derived from gene duplication and inversion events in the history of this family (193). The extinction of *DP* and *DQ* gene function in the cat is a likely explanation for the rather inefficient humoral response to maternal antigens (149) or to graft rejection seen in domestic cats (186). Additional differences in pseudogene transposition, retro element density,

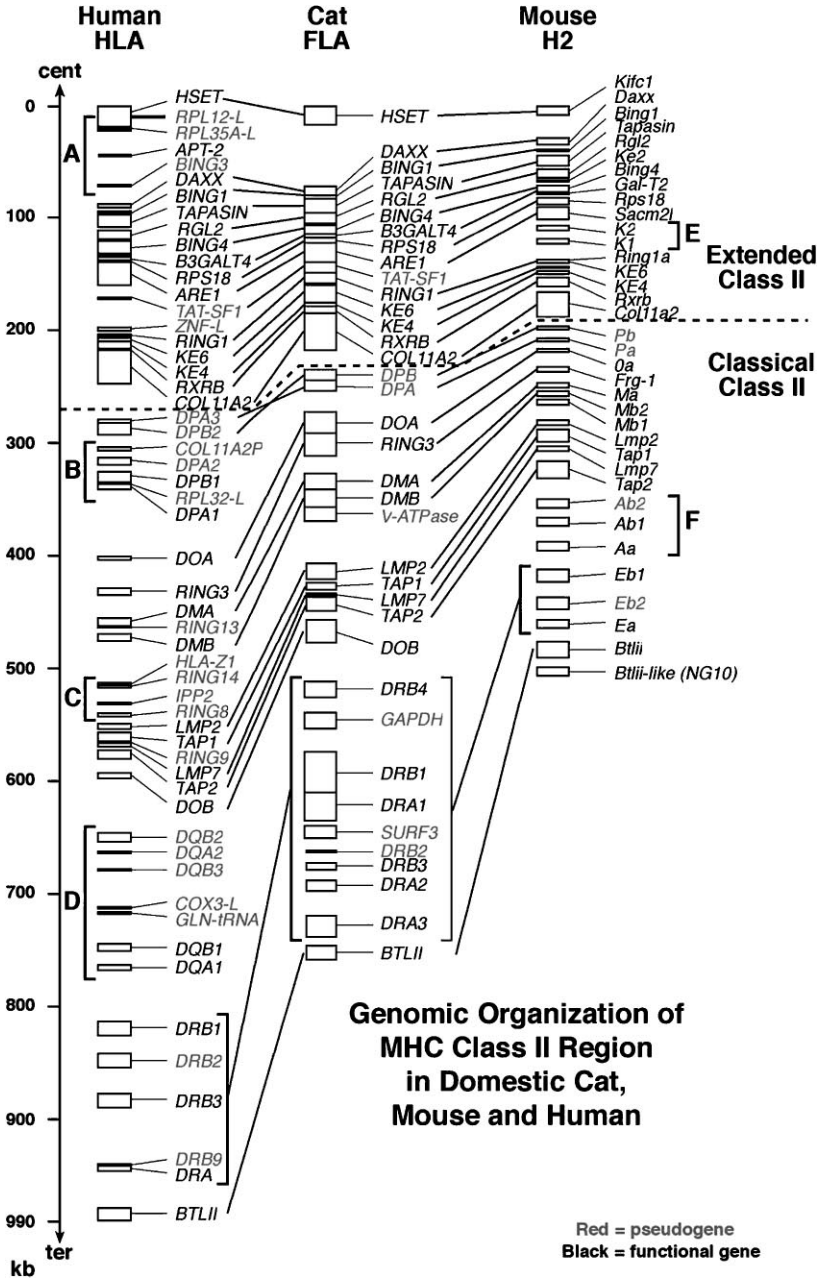


**Figure 5** Comparative genomic organization at the MHC Class II region in mouse, domestic cat, and human (193). Brackets (*A–C*) indicate gene segments in human, but absent in mouse and cat. *D* is a human gene segment absent in cat. *E* represents a mouse gene segment absent in human and cat. *F* is a mouse segment absent in cat.

length expansion, and conserved sequence blocks provide a tantalizing portrait of the adaptive events that have shaped this important genomic region in the cats, mice, and men.

## PROGRESS IN FELINE GENETIC DISEASE MODELS



Some 258 hereditary pathologies have been reported in the domestic cat (http://www.angis.org.au/Databases/BIRX/omia/), largely due to intensive medical surveillance of cats in the veterinary profession. To date, disease-causing mutations have been characterized in nine cat genes, although several other pathologies have been well characterized on a biochemical or protein level, including Niemann-Pick Type C, spinal muscular atrophy, Chediak-Higashi syndrome, dwarfism, hypertrophic cardiomyopathy, and mucolipidosis (Table 1). The largest representation comes from lysosomal storage enzyme disorders that arise from defects in genes that play a role in degradation of macromolecules such as mucopolysaccharides by lysosomes. As most lysosomal enzymes are secreted and can be taken up by neighboring cells (128, 168), an array of corrective therapeutic strategies has been proposed and many of these have been examined in the cat, including enzyme replacement, bone marrow transplantation, and gene therapy (54, 62, 99, 165, 178, 188). Feline models have been important in elucidating molecular pathogenesis as well as in playing a critical role in evaluating and optimizing therapeutic strategies prior to clinical trials in humans.

The cat is a model for mucopolysaccharidosis Types I, VI, and VII disorders, which result from lysosomal enzyme deficiencies involved in mucopolysaccharide degradation. Mucopolysaccharidosis Type I (MPS I), which results from deficient activity of the enzyme alpha-L-iduronidase (IDUA) (130), can lead to mental retardation, growth abnormalities, and shortened lifespan in humans (129). Naturally occurring models have been characterized in the cat (64, 65) and dog (164). A 3-base pair (bp) in-frame mutation characterized in the feline *IDUA* gene of affected individuals (68) results in deletion of an aspartic acid residue highly conserved in human, dog, cat, and mouse. The cat model provides an ideal system to study mechanisms of brain neurodegeneration and neural-directed strategies, especially given a large body of pre-existing literature on cat neurology.

MPS VI or Marteaux-Lamy disease, a deficiency for activity of arylsulfatase B (ARSB), is associated with growth retardation, coarse facial features, and skeletal deformities in humans and cat (31, 66, 67, 81). A missense mutation in *MPS VI* in affected cats results in a nonsynonymous substitution (L476P) (192) in a residue conserved across six other sulfatases, suggesting its critical enzymatic role. Two other independent mutations have been identified (32, 37). Affected cats respond to allogeneic bone marrow transplantation (54), while in vivo studies have demonstrated retroviral-mediated correction of MPS VI-deficient fibroblasts, chondrocytes, and bone marrow cells in both humans and cat (46).

MPS VII results from deficiency of beta glucuronidase (GUSB) (130), which in humans manifests as cartilaginous and bony malformations, growth and mental

retardation, abdominal organ enlargement, and corneal clouding (130). Naturally occurring animal models have been described in mice (13), dogs (63), and cat (56). The molecular basis for feline MPS VII (52) results from a nonsynonymous substitution (E351K) in a highly conserved amino acid, likely involved in maintaining protein conformation. Enzymatic activity has been restored in fibroblasts and restored by retroviral gene transfer of rat beta-glucuronidase cDNA. As GUSB is an essential housekeeping enzyme, this feline model is important for examination of exogenous genes and gene product delivery to a variety of tissue types, and could prove especially valuable in light of the extensive research conducted on the anatomy and physiology of the cat central nervous and visual systems.

Deficiency of lysosomal alpha mannosidase leads to accumulation of mannose-rich oligosaccharides (169), leading to mental retardation, recurrent infections, skeletal changes, and hearing impairment (21). A 4-bp deletion leads to a frameshift mutation and premature termination codon in affected Persian cats (10). Mutational molecular heterogeneity has been demonstrated in a domestic long-haired cat. The feline model has served as a powerful model for bone marrow transplantation (BMT) of lysosomal storage diseases, exhibiting dramatic improvement of $\alpha$-mannosidase activity in brain tissue of affected Persian cats (62, 178). These results have provided direct evidence of the efficacy of BMT as corrective strategy for neuronal storage diseases of the CNS and the potential of hematopoietic stem cells as corrective strategy for lysosomal storage disorders. Retroviral constructs of a human cDNA have also been demonstrated to correct enzymatic activity in deficient human and feline fibroblasts (165).

Glycogen storage disease Type IV is a rare disorder of glycogen metabolism caused by deficiency in glycogen branching enzyme (151). Glycogen deposits, found in numerous tissues, result in a failure to thrive and death from cirrhosis (151). The Norwegian Forest cat is the only animal model reported for this pathology (50). The feline mutation mechanism has been identified as a complex rearrangement resulting in the deletion of a 172-bp exon (51).

Lipoprotein lipase (LPL) is a crucial enzyme involved in the regulation of lipoprotein and lipid metabolism (20). Cats with LPL deficiency share remarkably similar phenotype to humans, including severe pancreatitis, chylomicronemia, and failure to thrive (55). A nonsynonymous substitution in feline LPL (Gly412 Arg) results in LPL deficiency in affected cats (55). Cats could prove to be a most valuable animal model of LPL deficiency as, of the numerous animal model systems examined including the mouse, the cat most closely resembles the lipoprotein pattern and lipid transport system of humans. This feline model offers great potential as an in vivo system to examine increased triglyceride levels associated with LPL deficicency on atherosclerosis (55).

A separate class of lysosomal storage disorder characterized in the cat are the gangliosidoses, GM1 and GM2, heritable neurodegenerative diseases. Excessive neuronal accumulation of the gangliosides GM1 and GM2 results from deficiency of lysosomal beta-galactosidase (BGAL) and hexosaminidase (HEX) A and B activity, respectively (131), leading to neuronal distortion and degeneration.

Feline models have been especially important in characterizing the pathobiology and molecular biology of these diseases. The mutational mechanism of GM1 in the cat results from a nonsynonymous substitution (Arg to Prol at base 1486) with subsequent loss of hydrolytic activity (4). GM2-gangliosidosis has been characterized in two independent cat models (58, 116) exhibiting remarkably similar pathology to human Sandhoff's disease (131). In affected cats, deletion of a cytosine residue results in a frameshift and premature stop codon (116), while a 25-bp inversion within the reading frame was characterized in a non-bred domestic cat (4). Limited reduction in GM2 neuronal storage has been reported following bone marrow therapy (178). Feline models will be critical in the development of therapeutic strategies for these disorders. Whereas acid beta-galactosidase deficiency has been corrected in human fibroblasts by retroviral mediated gene transfer (158), gene therapy of the CNS presents a challenging front as corrective retroviral constructs require mitotically dividing cells for integration and expression (26). On this note, targeted delivery of hexosaminidase A, covalently bound to the nontoxic fragment C of tetanus toxin, increased in vitro enzyme binding and uptake by cultured brain cells from *GM2*-affected cats (39).

X-linked muscular dystrophy in man is characterized by progressive degeneration of skeletal and cardiac muscle. Mutations, which have been exhaustively characterized in this disorder in man, lead to either absence or abnormality in the protein product dystrophin (72, 92). Models for X-linked muscular dystrophy have been characterized in mouse (161), cat (53), and dog (159). A deletion in the dystrophin muscle promoter characterized in the cat eliminates expression of muscle and Purkinje neuronal dystrophin isoforms (185). The marked clinical heterogeneity observed in these models, from severe disability exhibited in man and dog, to little muscle fibrosis and an actual regenerative process leading to muscle hypertrophy in mouse and cat (2, 23, 53), could be important in characterizing immediate and secondary consequences of the lack of dystrophin (146) and points out the importance of multiple animal models.

## A ROLE FOR THE CAT GENOME IN FORENSICS

The use of DNA markers to identify sources of biological traces left at crime scenes is heralded as the most important advance in the forensic sciences since fingerprinting. The report of microsatellite loci as a source of polymorphism in human DNA has revolutionized the forensic community in the past several years (44a, 127). Forensic DNA typing with human microsatellite loci has become widespread and is now routinely used in hundreds of public and private crime laboratories in the United States and throughout the world. The feasibility of genotyping multiple microsatellite loci in PCR-based multiplex analysis with as little as a single nanogram of genomic DNA allows forensic accession to biological materials previously considered inappropriate because of the age of the sample, quality, or quantity of DNA yield. These technological developments have also made realistic the genetic screening of trace biological specimens of animal tissues, particularly

hairs and blood, from individual animals including pets inadvertently left at crime scenes (110). The definition of species-specific microsatellite maps, such as those of cats and dogs, have made such forensic assessments an important new tool in forensic laboratories (5, 15, 16, 106, 108).

Utilizing microsatellite genotypic characterization of forensic hairs from a pet cat, we contributed to the establishment of a legal precedent for employing genetic individualization of animal tissue in homicide cases (110, 111). With support from the National Institutes of Justice, we have expanded this application by developing a forensic typing system for the cat and a genetic database of cat breeds for the genetic individualization of domestic cat tissue specimen.

A set of 11 tetranucleotide microsatellite loci were selected for a forensic panel based on distribution in the cat genome (121), heterozygosity observed across a reference panel of 29 cat breeds (5–10 animals/breed, $n = 230$), and Mendelian inheritance testing. The number of alleles observed for the panel in the 230 animals genotyped ranged from 9 to 33, with an average of 17 alleles/locus. Average locus heterozygosities across the 29 breeds for the independent loci ranged from 0.78 to 0.95, with an average locus heterozygosity for the forensic panel of 0.90. Breed-specific locus heterozygosities for the 11 loci ranged from 0.61 (Birman) to 0.86 (Norwegian Forest cat). A multiplex genotyping is under validation in a panel of approximately 1300 cats representing 37 of the major recognized cat breeds in the United States to generate a genetic database of cat breeds.

High discriminating potential for genetic individualization was observed in the sample data set for the forensic panel. The average probabilities of finding a matching genotype was estimated as $5.5 \times 10^{-7}$ (British short hair) to $3.25 \times 10^{-13}$ (Norwegian Forest cat), given observed allele frequencies in the 29 breeds. The allele frequency database for specific cat breeds provides a powerful analytical resource for assessing the statistical strength of genetic individualizations of cat specimens discovered at crime scenes.

## CONCLUSIONS AND FUTURE PROSPECTS

Advances in comparative genomics have transformed this discipline from a cottage industry to the framework for annotation of the human and mouse WGS and the basis for future species genome exploration. The feline genome project, now entering its third decade and armed with a broad array of advanced genomic resources, is positioning the domestic cat and its wild relative species to make substantive contributions to a number of scientific fields.

Over 258 hereditary pathologies have been reported in the domestic cat, largely due to intensive medical surveillance of cats in the veterinary profession (Table 1). To date, mutations have been characterized in nine hereditary disease genes. Though few in number, these feline models have been important in elucidating molecular pathogenesis and are playing a critical role in evaluating and optimizing therapeutic strategies prior to clinical trials in humans. With continued development of a high-resolution integrated map of the cat (Table 2), mapping and

**TABLE 5** Developed feline genome project resources (May 2002)

| Resource | Citation |
|---|---|
| I. Somatic cell hybrid panel framework physical map >100 Type I genes | (135, 138) |
| II. Interspecies Backcross (ISB) Genetic Linkage Map | (108) |
| III. Intra species Nestlé-Purina pedigree Genetic Linkage Map | (44) |
| IV. 5000-rad radiation hybrid panel and map | (119, 121) |
| V. Arrayed BAC and PAC libraries | (9) |
| VI. Flow sorted feline chromosome libraries: reciprocal chromosome paint map | (140, 152, 182) |
| VII. Tissue/cell line DNA repository of >10,000 exotic and domestic feline specimens | (84, 162) |
| VIII. Domestic cat breed forensic database 40 breeds, 11multiplexed, optimized STRs, ISTs | Unpublished |
| IX. Domestic cat Y chromosome cosmid library | Unpublished |
| X. Complete sequence | |
| a. mtDNA genome | (100) |
| b. Major histocompatibility complex | (193) |

characterization of many hereditary pathologies in the domestic cat are anticipated in the future.

The feline model shows continued promise for resolution, diagnostics, vaccine and treatment of human infectious disease. The identification of FIV in domestic cats offers a viable model for HIV pathogenesis as it provides the only known naturally occurring model for human AIDS pathogenesis. The revelation that strains of FIV in exotic felid species, such as lion and puma, show little immune depletion implicates naturally evolved adaptation to FIV in protecting wild cat species in the face of constant exposure to the virus (25). Future applications of available feline project resources (Table 5) to studying FIV in domestic and exotic felids hold the potential to unlock mechanisms behind this resilience.

The feline genome project has also contributed to the field of criminal justice. A Canadian homicide case involving the defendant's pet cat provided a strong legal precedent for the introduction of animal DNA as evidence in a criminal case (111). As a result of this precedent, the National Institute of Justice has endorsed the creation of a microsatellite-based forensic typing system and genetic database for the domestic cat that will contribute to the analysis of physical evidence of criminal investigations.

The domestic cat was the first nonhuman, nonrodent gene map developed that illustrated the strong degree of conserved synteny among mammalian orders (138). Further investigations using chromosome painting and ultimately, linkage and radiation hybrid mapping have begun to reveal a more dynamic view of comparative

genomic organization, where intrachromosomal change plays a fundamental role in reshaping modern genomes. Nonetheless, the overall slow rate of genomic change in the cat lineage provides an important opportunity for understanding the persistence of very large stretches of conserved gene order since the earliest divergence events in mammalian evolution. Through these studies, it is becoming increasingly clear that the reshaping of genomes is not random. Comparisons of the cat map with other species maps are revealing deserts and hotspots of genomic instability across mammalian orders and may ultimately contribute to characterizing fundamental processes involved with chromosomal rearrangement, and their potential contributions to speciation and to disease. At the sequence level, multispecies megabase sequence comparisons of the cat, human, and mouse MHC have begun to reveal the nature of gene loss and genomic adaptation (59, 193). As the Human Genomic Sequencing Consortium winds down on the drafts of the human, mouse, and rat genomes, other species are jumping in line to reap the benefits of WGS comparisons (136). The discussions laid forth here present a compelling case for considering the domestic cat as one of the next candidates for whole genome sequencing of mammalian species.

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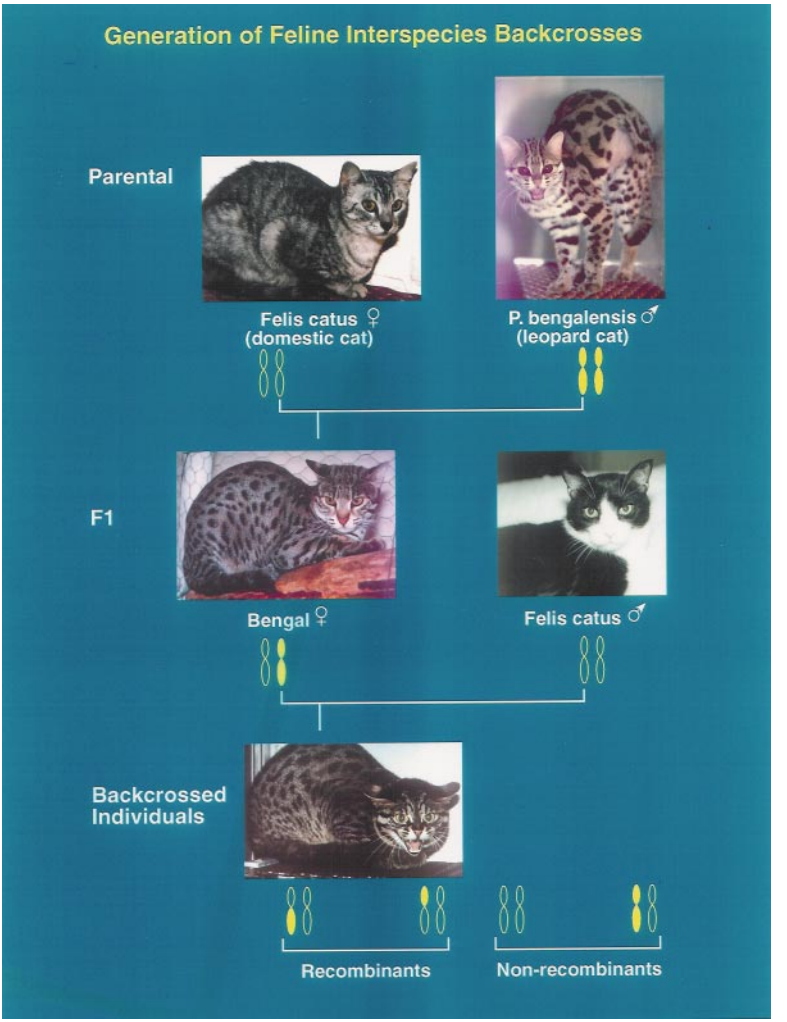


**Figure 1** Domestic and Asian leopard cat Interspecific Backcross Pedigree. Parental species used to generate $F_1$ individuals included domestic cat females and leopard cat males. Bengal: $F_1$ hybrid females; backcrossed individuals: progeny of $F_1$ hybrid females backcrossed to domestic cat males.

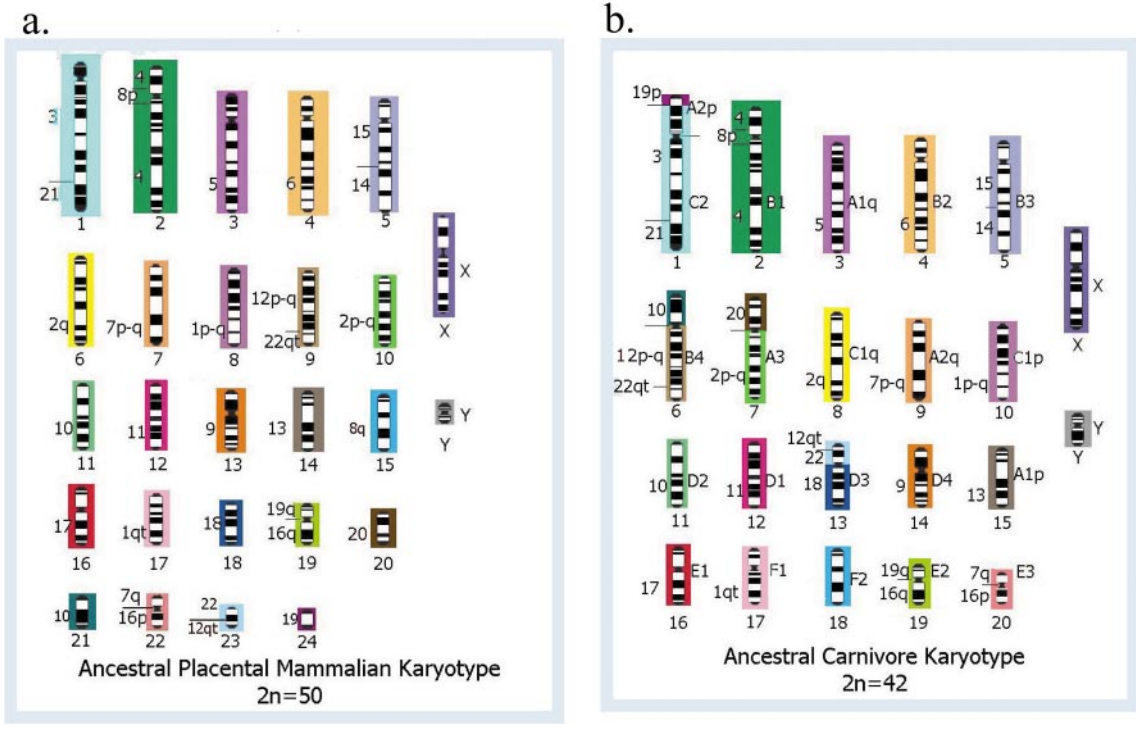


**Figure 3** Hypotheses for the chromosome content of the (*a*) ancestral placental mammal karyotype and (*b*) ancestral carnivore karyotype. Numbers to the left and right of each chromosome represent homologous regions of the human and cat genomes, respectively. Numbers below each chromosome are identifiers for the chromosome in each ancestral genome. The colors represent the chromosome in the ancestral mammalian genome from which each region originated. The G-banded karyotype depicted for the ancestral placental genome is imputed.

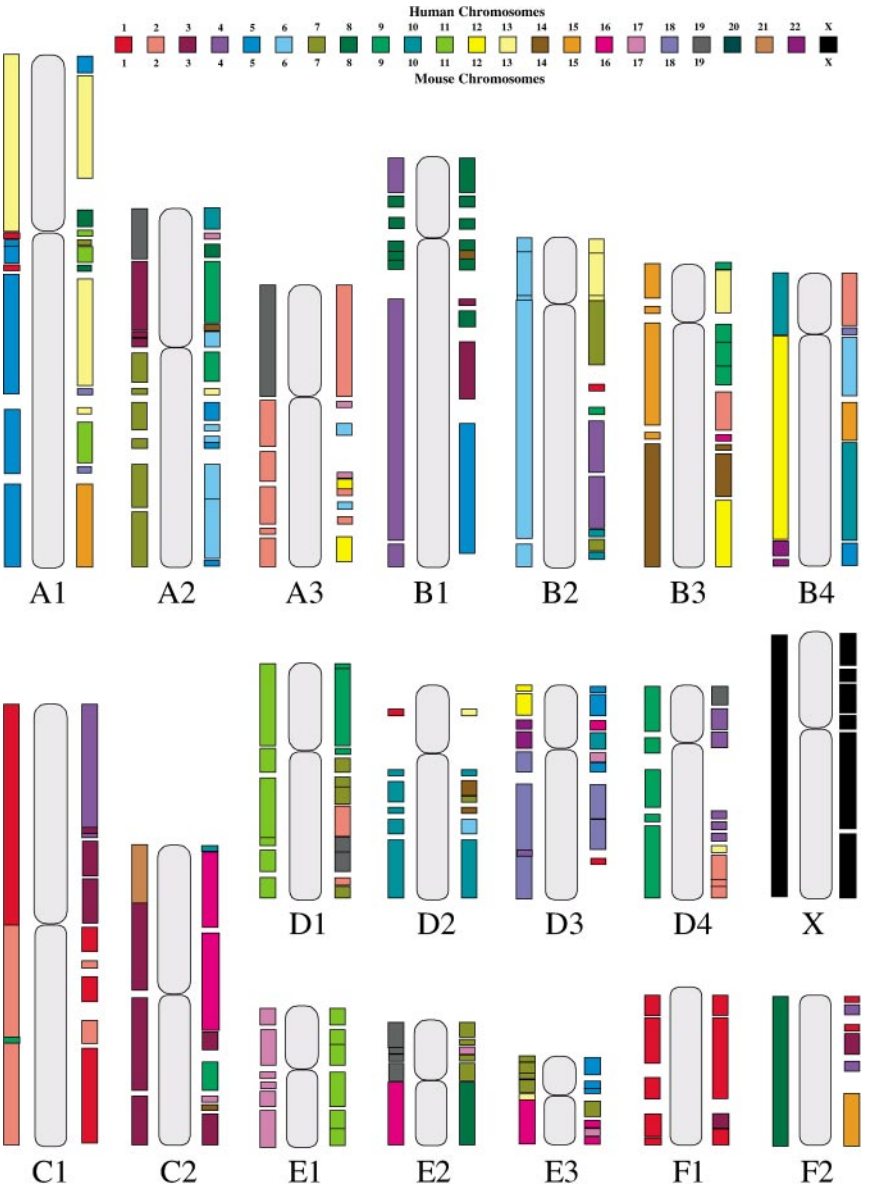


**Figure 4** Cat-human (*left*) and cat-mouse (*right*) homology maps showing conserved ordered segments between each genome, using the feline karyotype, drawn to scale, as an index genome. The Y chromosome is not included in these comparisons. The homology of human and mouse chromosome segments is indicated by the color key shown at the top of the figure. The human and mouse conserved ordered segments are drawn to their approximate position relative to the feline genome, assuming a physical scale proportional to the feline radiation hybrid maps. Gaps would indicate regions of homology not covered by Type I markers (microsatellites only) or inferred spans of ordered conserved segments.

*Annual Review of Genetics*
*Volume 36, 2002*

# CONTENTS

FRONTISPIECE—*R. C. Lewontin* xii

DIRECTIONS IN EVOLUTIONARY BIOLOGY, *R. C. Lewontin* 1

GENETIC MATING SYSTEMS AND REPRODUCTIVE NATURAL HISTORIES OF FISHES: LESSONS FOR ECOLOGY AND EVOLUTION, *John C. Avise, Adam G. Jones, DeEtte Walker, J. Andrew DeWoody, and collaborators* 19

GENETICS OF MOTILITY AND CHEMOTAXIS OF A FASCINATING GROUP OF BACTERIA: THE SPIROCHETES, *Nyles W. Charon and Stuart F. Goldstein* 47

RECOMBINATION IN EVOLUTIONARY GENOMICS, *David Posada, Keith A. Crandall, and Edward C. Holmes* 75

DEVELOPMENT AND FUNCTION OF THE ANGIOSPERM FEMALE GAMETOPHYTE, *Gary N. Drews and Ramin Yadegari* 99

PRIMORDIAL GENETICS: PHENOTYPE OF THE RIBOCYTE, *Michael Yarus* 125

STUDYING GENE FUNCTION IN EUKARYOTES BY CONDITIONAL GENE INACTIVATION, *Manfred Gossen and Hermann Bujard* 153

DNA TOPOLOGY-MEDIATED CONTROL OF GLOBAL GENE EXPRESSION IN *ESCHERICHIA COLI*, *G. Wesley Hatfield and Craig J. Benham* 175

MEIOTIC RECOMBINATION AND CHROMOSOME SEGREGATION IN DROSOPHILA FEMALES, *Kim S. McKim, Janet K. Jang, and Elizabeth A. Manheim* 205

*XIST* RNA and the Mechanism of X Chromosome Inactivation, *Kathrin Plath, Susanna Mlynarczyk-Evans, Dmitri A. Nusinow, and Barbara Panning* 233

ORIGINS OF SPONTANEOUS MUTATIONS: SPECIFICITY AND DIRECTIONALITY OF BASE-SUBSTITUTION, FRAMESHIFT, AND SEQUENCE-SUBSTITUTION MUTAGENESES, *Hisaji Maki* 279

GENETICS OF INFLUENZA VIRUSES, *David A. Steinhauer and John J. Skehel* 305

ALLOSTERIC CASCADE OF SPLICEOSOME ACTIVATION, *David A. Brow* 333

GENETIC ENGINEERING USING HOMOLOGOUS RECOMBINATION, *Donald L. Court, James A. Sawitzke, and Lynn C. Thomason* 361

CHROMOSOME REARRANGEMENTS AND TRANSPOSABLE ELEMENTS, *Wolf-Ekkehard Lönnig and Heinz Saedler* 389

GENETICS OF SENSORY MECHANOTRANSDUCTION, *Glen G. Ernstrom and Martin Chalfie* 411

UNDERSTANDING THE FUNCTION OF ACTIN-BINDING PROTEINS THROUGH GENETIC ANALYSIS OF *DROSOPHILA* OOGENESIS, *Andrew M. Hudson and Lynn Cooley* 455

THE GENETICS OF RNA SILENCING, *Marcel Tijsterman, René F. Ketting, and Ronald H. A. Plasterk* 489

TRANSVECTION EFFECTS IN DROSPHILA, *Ian W. Duncan* 521

GENETICS OF *CRYPTOCOCCUS NEOFORMANS*, *Christina M. Hull and Joseph Heitman* 557

TOWARD MAINTAINING THE GENOME: DNA DAMAGE AND REPLICATION CHECKPOINTS, *Kara A. Nyberg, Rhett J. Michelson, Charles W. Putnam, and Ted A. Weinert* 617

THE FELINE GENOME PROJECT, *Stephen J. O'Brien, Marilyn Menotti-Raymond, William J. Murphy, and Naoya Yuhki* 657

GENETIC APPROACHES TO MOLECULAR AND CELLULAR COGNITION: A FOCUS ON LTP AND LEARNING AND MEMORY, *Anna Matynia, Steven A. Kushner, and Alcino J. Silva* 687

ESTIMATING F-STATISTICS, *B. S. Weir and W. G. Hill* 721

INDEXES

Subject Index 751

Cumulative Index of Contributing Authors, Volumes 32–36 787

Cumulative Index of Chapter Titles, Volumes 32–36 790

ERRATA

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