Comparative genome organization of the major histocompatibility complex: lessons from the Felidae

Authors' address

Stephen J. O'Brien, Naoya Yuhki,
Laboratory of Genomic Diversity, National
Cancer Institute, Frederick, Maryland, USA.

Correspondence to:

Stephen J. O'Brien
Laboratory of Genomic Diversity
National Cancer Institute
Frederick MD 21702-1201
USA
Fax: 1 301 846 1686
e-mail: obrien@mail.ncifcrf.gov

Summary: The mammalian major histocompatibility complex (MHC) has taught both immunologists and evolutionary biologists a great deal about the patterns and processes that have led to immune defenses. Driven principally by human and mouse studies, comparative MHC projects among other mammalian species offer certain advantages in connecting MHC genome characters to natural situations. We have studied the MHC in the domestic cat and in several wild species of Felidae. Our observations affirm class I and class II homology with other mammalian orders, derivative gene duplications during the Felidae radiation, abundant persistent trans-species allele polymorphism, recombination-derived amino acid motifs, and inverted ratios of non-synonymous to silent substitutions in the MHC peptide-binding regions, consistent with overdominant selection in class I and II genes. MHC diversity as quantified in population studies is a powerful barometer of historic demographic reduction for several endangered species including cheetahs, Asiatic lions, Florida panthers and tigers. In two cases (Florida panther and cheetah), reduced MHC variation may be contributing to uniform population sensitivity to emerging infectious pathogens. The Felidae species, nearly all endangered and monitored for conservation concerns, have allowed a glimpse of species adaptation, mediated by MHC divergence, using comparative inferences drawn from human and mouse models.

Introduction

Within the genomes of living mammalian species are between 50,000 and 100,000 genes which by evolutionary adaptation have contributed to their survival. If nature's struggle for existence rewards species with propagation success, the genes we uncover in genome sequencing projects reveal the scripture as vestiges of ancient adaptive episodes. A half century of research into the biology and functioning of the group of genetically clustered loci called the major histocompatibility complex (MHC) has resolved a rich and intricate genomic system upon which immune defenses depend, exposing patterns and processes of immune co-adaptation by mammalian hosts to invading parasites. Given the critical influence that fatal infectious diseases have exerted both on demographic and adaptive events for virtually every living species (1-5), insight gained from MHC studies has traversed many biological disciplines including immunology, x-ray crystallography, molecular genetics, functional genomics, bioinformatics, molec-

ular anthropology, forensics, genetic epidemiology of human scourges (e.g. AIDS, malaria, diabetes, and hepatitis), mammal systematics, species conservation, and evolution.

To describe the contributions made by comparative studies of the feline MHC reviewed here, we first summarize general conclusions derived from human (primate) and mouse (rodent) MHC studies detailed elsewhere in this volume. The mammalian MHC encodes two categories of cell surface glycoproteins (class I and II) which transport and present peptides from invading parasites or microbes to the T-cell receptors of immune cells (6–8). Class I products, non-covalently associated with β_2 -microglobulin, are expressed on the surface of all somatic cells. They capture 8–10 amino acid-long peptides in the endoplasmic reticulum prior to presenting these on cell surfaces to CD8-positive, CD4-negative cytotoxic T lymphocytes. Class II molecules are heterodimers of α and β chains (i.e. products of *DRA* and *DRB* genes), expressed on antigen-presenting cells (APC) such as B cells, dendritic cells, and macrophages. They load 12–24 amino acid-long peptides from endosomes and present these to CD4-positive, CD8-negative T-helper cells, which respond by releasing cytokines and stimulating both humoral antibody production by B cells and cytotoxic T-cell proliferation.

Certain human class I and class II genes (class I: *HLA-A*, *B*, and *C*; II: *DRB*, *DQA*, *DQB*, and *DPB*) are highly polymorphic, far more so than would be anticipated from the prediction of the neutral theory for new mutations (6, 9, 10). The extensive allele variation produces a situation where no allele is common or wild type; rather, scores of alleles at multiple linked genes ensure that most individuals of outbred populations (like man) are heterozygous at each hypervariable MHC locus. Further, nucleotide sequences of single MHC alleles differ at multiple nucleotide positions (up to 100 substitutions between *HLA-B* alleles), rendering the MHC the most extremely polymorphic gene cluster described. A relatively slow spontaneous mutation rate plus the identification of patchwork display of amino acid motifs among MHC class I and II alleles lends support to the interpretation that gene conversion is an equivalent or greater source of novel alleles than is mutation for some MHC loci (10–13). The identification of near identical peptide motifs among related species (e.g. human, gorilla or chimpanzee) alleles has been interpreted as evidence that much of the variation present today originated in common ancestors of the great apes or earlier (10–17).

Any explanation for the persistence of multiple MHC loci, multiple alleles and retention of ancient diversity would consider their demonstrated function as facilitator of immune recognition (6–8) combined with the x-ray crystallographic structure of human class I and class II molecules bound to rec-

ognized peptides (18–21). Zinkernagel and Doherty suggested 25 years ago that MHC diversity may function as an intrinsic monitor of self-recognition (MHC restriction) in presenting parasitic peptides to T cells (7, 22, 23). Thus, individuals who could maximize their peptide recognition potential, itself limited by MHC allele recognition repertoire, would be more likely to survive outbreaks of lethal pathogens than those with fewer specificities. Strong support for that inference was the demonstration that nucleotide substitutions between MHC class I and between class II alleles were predominantly non-synonymous (codon altering) for residues in the peptide-binding region (PBR) (also called the antigen recognition site), while synonymous substitutions were far more common among the other regions of the molecule not involved in peptide recognition (24–27). These observations were interpreted as evidence for positive selective pressures favoring increased diversity (and antigen recognition) within the PBR, but purifying selection limiting amino acid-changing substitutions for the rest of the MHC molecule.

The concept of heterozygote advantage, also called the overdominant selection hypothesis, for the MHC diversity is appealing because it relates MHC antigen recognition with a non-neutral (i.e. natural selection-driven) pattern of PBR sequence divergence (28–33). It also would be consistent with maximizing allele variation by selective retention of new alleles containing increased diversity generated by allele recombination, gene conversion, and the persistence of ancient variable recognition motifs (10, 25, 34). The constant affliction of species with fatal infectious diseases is a likely candidate for the principal selective pressure simply by virtue of the variety of pathogens and fitness cost that are encountered. Proposed alternative selective pressures (e.g. maternal–fetal incompatibility, assortive mate choice mediated by olfaction genes, some of which are included within the MHC) (35–38) are difficult to reconcile with the selective drive to increased codon alteration in the PBR, and not in the other portion of class I and class II genes (24). Finally, a role for MHC allele-specific influence in pathogen sensitivity has been observed in a few cases (avian Marek's disease, human malaria, and bovine immunodeficiency virus) (39–42), and MHC homozygosity has been implicated in accelerating two human infectious diseases (hepatitis B and AIDS) (43, 43a). As many articles in this volume detail, the vertebrate MHC has enormous diversity, handed down from ancestral species adaptations that protected their progenitors from the ravages of historic epidemics, the footprints of which we encounter today in genomes of living species.

A case for cats

To extend these inferences to a group with considerable biological background and information, we began to study the MHC organization and allelic diversity in domestic cats (*Felis catus*) and their wild relatives. The domestic cat offers some special advantages for such an approach (44, 45). First, there are over 60 million cats in the U. S. (compared to 50 million dogs) and the veterinary community offers a vast resource for ascertainment of hereditary, immunological, and infectious diseases. Deadly viral pathogens have been described, including feline infectious peritonitis virus (FIPV), a coronavirus (46); feline leukemia/sarcoma virus (FeLV/FeSV), an oncogenic retrovirus (47); feline immunodeficiency virus (FIV), a lentivirus causing feline AIDS (48); and others for which diagnostic, immunologic, and therapeutic reagents are developed (3). Feral domestic cats are distributed throughout the world and have adapted to human environments as well as any carnivore except domestic dogs. Finally, a moderate resolution genetic map of the cat has been developed (including comparative anchor loci and hypervariable microsatellite markers) which by comparative mapping displays striking homology to the more "gene-dense" human and mouse gene maps (45, 49).

The domestic cat was derived from the African wildcat *Felis sylvestris libyca* within the past 4,000 years. The relatives of domestic cat within the family Felidae include 37 wild species, each both admired and feared by humankind for thousands of years. Nearly every species of Felidae is displayed in zoological collections and studied in depth by veterinarians for diseases, including transmissible agents (50). Studies on Felidae evolution, systematics and conservation have led to the assembly of skeletons, pelts, and blood and tissue cell lines from individuals of each species allowing an in-depth look at genomic divergence within a relatively recent evolutionary period (51–53). Because many wild cats are both charismatic and highly endangered, populations are monitored closely for ecological studies (54–56) and more recently for clinical health including infectious disease (50, 57–59). As such, the cat family offers an unusual opportunity to monitor MHC genomic patterns in the context of past and present disease outbreaks.

The Felidae radiations

The earliest mammals trace back to the small rodent-like miacid ancestors that co-existed with the dinosaurs through the Mesozoic era 63–150 million years before present (MYBP). Divergence to what are now the 20 modern mammalian orders began later, 60–80 MYBP, but was not really accelerated until

the dinosaurs disappeared abruptly at the Cretaceous/Tertiary boundary, about 63 MYBP. The carnivore order first appears in the fossil record in the Eocene, while the ancestors of modern true cats are more recent, occurring at mid-Miocene (~15–20 MYBP) and diverging into the 37 species of modern cats at 10–15 MYBP (60–63).

The phylogenetic hierarchy or pattern of Felidae divergence has been controversial, although the assemblage of molecular evolutionary data, collected most recently by students and fellows from our group, allows depiction of a consensus phylogeny of the Felidae presented in Fig. 1. Briefly, the modern Felidae can be considered as eight groups of phylogenetically related species. Monophyletic clustering of these species using mitochondrial DNA sequences, allozymes, albumin immunological distance, Y chromosome sequences, and other methods affirm these groupings (52, 53, 60). The first and oldest group includes eight South American cat species related to the ocelots and margays, species which migrated into South America when the Panama isthmus joined North and South Americas 2–5 MYBP. The second group, including Mediterranean small cat species closely related to the African/European wildcat, the domestic cat's closest ancestor, diverged 8–10 million years ago. The remaining groups of middle and large-sized cats began divergence 5–8 MYBP and led to the great cats, genus *Panthera*, which began its speciation events 2–5 MYBP. For scale consider that all 37 cat species emerged in a 12–15 million year period, the same time frame that separated the three African great ape species (gorilla, chimps, and man) from the single Asian great ape species, orang utan. It is in the context of the relatively recent, but effective, species development of modern felids that we discuss below the pattern of MHC evolution.

Feline MHC organization

The feline MHC was once perceived as inefficient or weak, since neither blood transfusion nor pregnancy regularly induces allogeneic antibodies, and because organ transplants, particularly bone marrow, are not immunologically rejected as vigorously as in other species (64, 65). Nonetheless, a controlled skin graft analysis of 59 domestic cats demonstrated consistent acute graft rejection, although only a quarter of these resulted in allogeneic antibody production (66). The antibody specificities define overlapping serospecificities in outbred cats allowing the recognition of 24 haplotypes. Feline MHC class I and II gene homologs were cloned and genetically mapped to feline chromosome B2q11, syntenic with some nine non-MHC genes whose homologs reside on human chromosome six adjacent to HLA (45, 67).

Fig. 1. An abridged phylogeny of Felidae species based on a consensus of molecular phylogenetic approaches (52, 53, 63) and including species discussed in this review of feline MHC genes.

The human MHC structure (68) includes six class I genes (A, B, C, E, F, and G), eight class II loci (DRA, DRB, DQA, DQB, DPA, DPB, DNA, DOB) plus the transporter associated with antigen processing (TAP) and low molecular mass polypeptide (LMP) genes within 4,000 kb of chromosome 6p21.3 (Fig. 2). Additional non-histocompatibility loci are found in both class I (β -tubulin, S, HSR, MOG, olfactory receptor gene homologs) and class II (DMA, DMB, RING3) regions. The class III regions, located between class I and II, include genes of the complement system and other genes as well. There appear to be at least two functional class I cat loci based on the presence of three or four distinct cDNA transcripts found in single individuals (69, 70). It is not possible to recognize whether feline class I genes are more closely related to HLA-A, -B or -C since, as for other mammal orders (71, 72), the MHC class I gene orthologs have become reorganized to obscure specific locus homology.

We have learned from comparative studies of MHC class II genes in several species that gene ortholog identification is generally preserved but that specific gene usage varies in different mammalian orders (73). For example, humans use DP, DQ, and DR, while mouse uses primarily the DQ (I-A) gene orthologs

(74). The mouse DP region includes mainly pseudogenes and the DR (I-E) region is non-functional in mouse strains due to a deletion of the promoter region, a premature termination codon, and a defect in RNA processing of Ea. Mole rats, also rodents, are reported to use DP class II genes exclusively (75). In domestic cats we can identify MHC contigs that include DPB, DPA, DNA, RING3, DMA, TAP1, LMP2, TAP2, LMP7, DOB, DRB and DRA homolog (Fig. 2). At least three active DRB genes occur in certain cats since up to six distinct DRB transcript alleles were found in several individuals (76). The gene order and haplotype distribution of class I and class II alleles is not described precisely in cats; however, the high resolution MHC genome sequence project, recently initiated for the cat, will reveal these associations.

Class I and II DNA sequence variation in cats affirm overdominant selection

Analyses of cDNA transcripts for domestic cat class I and II loci revealed extensive allele and sequence variation (67, 69, 70). Full-length sequence analysis of eight class I transcripts showed a max-

Fig. 2. An abridged map of HLA, the human major histocompatibility complex (67) indicating feline homologs that have been demonstrated (66, 67, 69, 70, 76). The gene order in the domestic cat is not yet determined and should not necessarily be thought to recapitulate the human order.

imum of 9% nucleotide and 13% amino acid sequence difference, or up to 100 nucleotide substitution differences between alleles.

For class II genes, limited DRA variation was observed as three cDNA transcripts shared 97% or greater nucleotide sequence identity (76). A single cat displayed three unique DRA transcripts, implying at least two functional DRA loci. A total of 61 distinct DRB alleles were detected from full sequences of eight DRB transcripts plus PCR amplification of β 2 domain, DRB exon 2 from 36 feral cats (76). Phylogenetic analysis of the 61 DRB variable regions revealed five monophyletic clusters or clades (Fig. 3). The five cluster lineage is itself monophyletic with respect to the canid DRB genes, indicating that cat MHC evolution has proceeded in a cumulative divergence since the separation of arctoid (dog-like) and feloid (cat-like) carnivores. In five cats, three alleles of a single phylogenetic lineage were observed, suggesting rather recent duplication of the DRB loci after the evolutionary establishment of monophyly. In general, clade monophyly and locus duplication might be expected to correlate, unless duplication is so recent (as noted here) that locus-specific divergence has not accumulated sufficient divergence to be apparent.

Analysis of the pattern of nucleotide variation in feline MHC genes (class I and II) in the context of their role in presenting peptides to T-cell receptors revealed strong parallels to observations in human and murine systems. X-ray crystallographic structure of both HLA-A2 and DR1 alleles led to identification of amino acid residues that mediate T-cell receptor interaction and react with common features for all antigens (Fig. 4). In human and mouse class I molecules, such residues are genetically invariant, while others which recognize variable antigen sites are highly polymorphic. The same is true for the cat, where the variant and invariant amino acid residues are generally in homologous amino acid sites in all three species. For example, of 32 invariant human class I residues, 31 are invariant in the cat and 27 of the 32 conserved residues have identical amino acids in both species (69).

The vast majority of amino acid substitutions between alleles occurs in the PBR of feline MHC genes (69, 70, 76). Consistent with the observation in the human and mouse comparisons, an analysis of the relative rate of synonymous vs non-synonymous nucleotide substitution within the PBR versus the residual residues of domestic cat cDNA transcripts show nearly double the incidence of non-synonymous (codon-altering) variants among the PBR residues (Fig. 5A). By contrast, synonymous (or silent) substitutions are more common in the non-PBR regions. The same discrepancy (i.e. an excess of non-synonymous substitution in the PBR) is observed when feline class II DRB alleles are compared. The domestic cat then pro-

vides the third mammalian species where the pattern is affirmed: $d_n \gg d_s$ in PBR, but $d_s \gg d_n$ in the remainder of the region. In Fig. 5, a consistent excess of non-synonymous substitutions within, but not outside, the PBR was apparent for comparisons within two other species (ocelot and margay) as well as between feline species MHC genes (class I and II). The simplest interpretation for the pattern would be that natural selection has acted to preserve PBR amino acid sequence variation in both MHC class I and class II genes, likely in response to ancient epidemics. Sites outside the PBR which confer structure, assembly, and recognition of common antigen features are constrained from amino acid changes by purifying selection, as is observed in most other genes, while synonymous mutations occur at a steady rate similar to selectively neutral regions. This overdominance hypothesis, which favors heterozygote advantage, has been presented and supported cogently based on similar data in human and mouse (10, 24–27).

MHC variation in non-domestic Felidae cat species

Although the recency of the Felidae radiation led to some uncertainties in the details of their phylogeny, it provides an advantage in dissecting genome evolutionary events such as posed by the MHC. A comparison of class I sequence diversity among three species (70) from separate evolutionary lineages of Felidae (Fig. 1) revealed that the extent of allele sequence diversity within outbred species was comparable to the extent of diversity among the species. Thus, nucleotide sequence similarity between class I gene transcripts was 93% for cheetah, 93–99% for ocelot, 92–100% for domestic cat, and 90–95% when compared between species. Further, only 13% of all Felidae nucleotide variation observed was species specific. The equivalence of the quantity of variation within and among cat species, plus the paucity of species-specific variation, suggests that the majority of variation preceded species origins and has been transmitted *en bloc* to modern species from their ancestors.

Even more compelling evidence for the ancestral origin and selective retention of MHC polymorphism comes from the identification of amino acid motifs (combinations of shared amino acid stretches) that are observed in feline class I and II molecules. A number of such motifs were identified in exon 1–2 class I sequences aligned from domestic cat, cheetah, and ocelots (69, 70). The motifs were polymorphic within cat species but present in two or three tested species. In addition, database searches with three conserved 20 nucleotide-long polymorphic motifs revealed strong homologies to human, orang utan and bovine class I sequences. Clearly such motifs are quite ancient, predating the divergence of the mammalian orders.

FELINE MHC CLASS II DRB GENE EXON 2 NUCLEOTIDE SEQUENCES

1 UNIT = 1% SEQUENCE DIVERGENCE

Fig. 3. Neighbor-joining tree using 238 bp nucleotide sequences of $\beta 1$ domain coding region in MHC class II DRB genes based on Kimura's two-parameter distance. Percentage sequence divergence is indicated above each branch; numbers in parentheses are bootstrap percentages (out of 100 iterations) in support of each node. MHC abbreviations used for species are indicated as follows: Feca, *Felis catus* (domestic cat); Onge, *Oncifelis geoffroyi* (Geoffroy's cat); Mair, *Mayailurus iriomotensis* (Iriomote cat); Leti, *Leopardus tigrina* (tiger cat); Cafa, *Canis familiaris* (domestic dog). H-2E β and HLA-DRB are used for murine and human class II β chain respectively.

Evidence for trans-species retention of site polymorphism and motif retention has also been observed in MHC genes of primates and rodents (10–17) as well as in class II DRB genes of the cats (76) (N. Yuhki, E. Eizirik, W. Johnson, S. J. O'Brien, manuscript submitted). When DRB sequences from five non-domestic Felidae species (Geoffroy's cat, Iriomote cat, tigrina, ocelot, margay) were analyzed phylogenetically, their sequences were never identical to domestic cat sequences but in most cases they clustered closely within one of the five monophyletic clades described by domestic cat DRB (Figs 3 & 6). This suggests that moderate mutation or recombination-mediated divergence has occurred since speciation, but in the framework of much older clade reorganizations, dating to the Miocene or Oligocene. An extensive phylogenetic analysis of DRB genes from sympatric ocelot and margay species sampled across their range in South and Central America revealed that both species retain the domestic cat DRB2, 3, and 5 clade variants, but lack the DRB1 and DRB4. Ocelots revealed 111 alleles (in 35 individuals, Table 1) and showed a clade (DRB7) while margay (69 alleles from 26 individuals) showed a new species-specific clade, DRB6 (N. Yuhki, E. Eizirik, W. Johnson, S. J. O'Brien, manuscript submitted). If we consider the time elapsed since these two species shared a common ancestor (about 4–6 MYBP), then a DRB clade has emerged every 4–6 million years, about the time estimated for two new carnivore species to emerge (77). If clade formation coincides roughly with gene duplication, such calculations are valuable in monitoring the selective forces which influence genome and MHC evolutionary processes.

MHC diversity as a window to natural history

The accumulated understanding of selective forces that promoted abundant allelic diversity and duplication redundancy of certain class I and II loci suggest the MHC as a sensitive monitor for the demographic history of vertebrate populations (78–82). Since allele variation is reinforced and maximized in outbred populations by periodic outbreaks of infectious diseases and perhaps other forces, the variation itself is a monitor of population contraction. Fifteen years ago, genetic unifor-

Fig. 4. Top view of the x-ray crystallographic structure of human MHC A) class I HLA-A2 and B) class II DR1 molecules indicating peptide inserted in binding groove. The amino acids involved in peptide recognition (termed peptide-binding region (PBR), formerly antigen recognition site) in class I and class II DRB antigens are indicated as solid circles (18–20).

mity of class I loci was discovered among free-ranging cheetahs, based upon immunological acceptance of skin allografts exchanged surgically between unrelated cheetahs (83). MHC monotony in cheetahs was affirmed by restriction fragment length polymorphism (RFLP) studies of class I probes (84) plus by a reduction in DRB allele diversity detected, 20-fold less than in outbred species such as domestic cats or ocelots (Table 1). The cheetah's MHC allelic homogenization was correlated with reduction in genome-wide allele variation in multiple non-MHC loci as well (81–83). The interpretation of such genomic uniformity indicates that population-wide MHC variation in the ancestors of modern cheetahs was lost through an historic population bottleneck or series of bottlenecks which caused shedding of endemic species diversity during a near extinction event. Modern cheetahs carry the risk today of species-wide hypersensitivity to emerging infectious disease. The risk was realized in multiple fatal outbreaks of FIPV (a virus with low morbidity in domestic cats) which decimated several

Fig. 5. A. Mean numbers of synonymous (d) and non-synonymous (d_n) nucleotide substitutions of class I and class II DRB antigens in cats. Non-synonymous and synonymous nucleotide substitution rates were calculated using p-distance within domestic cat (FCA), ocelot (LPA), and margay (LWI) MHC antigens. Seven and four class I sequences were used for FCA and LPA, respectively (69, 70). Sixty, 76, and 45 class II DRB sequences were used for FCA, LPA, and LWI, respectively (76) (N. Yuhki, E. Eizirik, W. Johnson, S. J. O'Brien, manuscript submitted).

captive cheetah colonies (83, 85). That free-ranging cheetahs survive at all today is probably due to their solitary territoriality, limiting the spread of lethal pathogens (55). Nevertheless, the cheetah's natural history revealed by relative monomorphism at the MHC is a chilling example of the perils of abrupt loss of population variation following a population bottleneck.

Additional examples of genomic homogenization among small threatened populations were also revealed by screens of MHC variation. A tiny relict population of Asiatic lions, living in the Gir Forest sanctuary in eastern India, descended from Asian subspecies that ranged widely from Pakistan to Saudi Arabia until human depredation reduced their numbers to fewer than 20 animals in the late nineteenth century (86). An RFLP screen of class I loci revealed extreme uniformity (84), which conformed to identical allozyme, mtDNA, and minisatellite DNA fingerprints in the population (86–88). The lion population displayed several morphological congenital defects that are most likely the consequence of inbreeding depression in recent history.

A dramatic scenario about the perils of a small endangered population suffering from close inbreeding involves the Florida panther (*Puma concolor coryi*). A relict subspecies of the mountain lion (also called puma or cougar), the Florida panther clings to survival in the inhospitable mosquito and alligator-infested cypress swamp lands of southwestern Florida (89). Following generations of close inbreeding reflected in MHC-RFLP and other genomic measures, the population displays frequent cryptorchidism, malformed sperm, congenital heart defects, and a large parasite load. In 1996, population managers supple-

B. Patterns of nucleotide substitutions of class I and class II DRB antigens between domestic cat and other felids. Non-synonymous and synonymous nucleotide substitution rates were calculated using p-distance between FCA and other cats. Seven FCA, four LPA, and one African cheetah sequence were used for class I genes. We examined 32 FCA, 40 LPA, and 24 LWI sequences for class II DRB genes.

mented the doomed population with outbred healthy Texas cougars (*Puma concolor stanleyi*), a neighboring subspecies, to ameliorate the demographic and genetic problems of the species. The conservation community is acutely aware of the cost of close inbreeding and its near certain guarantee of loss of fitness and potential for survival (81, 82, 90, 91). The value of MHC assessment in monitors of endangered species can be extremely informative, although one should be cautious about interpretation of limited descriptive data (78–82).

Conclusions

Our molecular genetic analysis of feline MHC genes in the context of elegant advances in human and mouse MHC organization has revealed several factors that contribute to the origin and sustenance of abundant allele and sequence diversity (69, 70, 76, 92) (N. Yuhki, E. Eizirik, W. Johnson, S. J. O'Brien, manuscript submitted). These include: 1) gradual mutational accumulation over time in regions of class I and class II molecules not involved in peptide recognition, but constrained by purifying selection; 2) reorganization by duplication, exchange, and extinction of class I loci within the Felidae to obscure orthologous gene identification with other mammalian orders; 3) positive balancing (overdominant) selection favoring the persistence of amino acid alterations and heterozygosity in the PBRs of polymorphic class I and II molecules; 4) common recombinational exchange within and between loci to produce a "patchwork" of amino acid motifs in modern transcripts, likely gener-

Fig. 6. Neighbor-joining tree of DRB sequences. DRB alleles of four individuals each from domestic cats, ocelots, and margays are indicated by pink, blue, and green lines, respectively.

Table 1. Number of DRB allele sequences in studied mammalian species

Species	No. of individuals studied	No. of DRB alleles	Reference
Human	251	126	94
Common chimpanzee	64	85	95
Gorilla	19	40	95
Cattle	Not known	64	34–39, S. Mikko, L. Anderson, personal communication,
Goat	25	21	96
Red deer	50	34	97
European moose	30	10	98
Felidae:			
Domestic cat	37	61	76
Ocelot	35	111	N. Yuhki, E. Eizirik, W. Johnson, S. J. O'Brien, manuscript submitted
Margay	26	69	N. Yuhki, E. Eizirik, W. Johnson, S. J. O'Brien, manuscript submitted
Lion	12	20	N. Yuhki, S. J. O'Brien, unpublished
Tiger	40	10	N. Yuhki, S. J. O'Brien, unpublished
African cheetah	6	4	N. Yuhki, S. J. O'Brien, unpublished

ated by gene conversion, intragenic recombination or both; 5) the trans-species persistence of both nucleotide site and peptide motif polymorphism among modern Felidae species implicating Miocene or earlier origin for the shared ancestral (symplesiomorphic) variation; and 6) cladogenesis of DRB gene families among and within feline species, allowing an estimate for new DRB1 cladogenesis every 4–6 million years. Each of these features can be interpreted as consistent with the pathogen-mediated overdominance (heterozygote advantage) hypothesis postulated to drive the allele and sequence diversity of the MHC.

An important consequence of the functional explanations of MHC variability is our ability to make predictions about its behavior in natural settings. Thus, we expect that populations that suffer severe demographic contractions would show a reduction in MHC variation relative to more outbred populations. This inference has been used to reveal historic population bottlenecks in several felids, particularly cheetahs, Asian lions, and Florida panthers. A similar reduction in DRB variation among five tiger subspecies using only “voucher” animal specimens born in the wild has also signaled historic population founder effects for that species (93). In at least one case, the cheetah, a viral outbreak revealed extreme sensitivity which may relate to MHC uniformity, although other explanations have not been vigorously excluded. Because of its sensitivity to

both demographic contractions and infectious disease, the MHC has been a valuable monitor of the genetic structure of endangered species.

The intersection of advances from multiple disciplinary perspectives makes the MHC a powerful paradigm for approaching the secrets for survival of modern mammals. The development of a moderate-resolution gene map of domestic cat including both microsatellite markers and comparative anchor loci that relate conserved genome segments to human or mouse maps offers the prospect of genetic analysis of epidemiologic heterogeneity to infectious disease. This means that in the near future MHC and other loci in cat species can be tested explicitly for association with disease resistance. Given the panoply of disease outbreaks known in cats (e.g. FIPV in cheetahs, FIV in several species, FeLV in domestic cats, canine distemper virus in lions and others), the genetic approaches are now approachable, due to new advances and tools in each of the areas. For the first time, we have the opportunity to test complex ecological hypotheses about population survival, an important step toward identifying the genetic adaptations that lead to species formation. Our hope is that the knowledge gained from studies on the world's cats will contribute in part to resolving the secrets encrypted in species' genomes that have assured their successful struggles for existence.

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