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Research Article

Genetic Differentiation Between Domestic Cats and Wildcats

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ABSTRACT

The process of commensal domestication of cats from their ancestor wildcats (*Felis silvestris*) is not well understood. To identify the genetic underpinnings of cat domestication, we analyzed 46 whole genome sequences (WGS) comprising 3 wildcat species and 16 cat breeds, documenting over 34 million single nucleotide polymorphisms (SNPs). We first showed clear evidence of genomic divergence of domestic cats and wildcats. A genomic comparison between domestic cats and wildcats revealed evidence of genetic selection of underlying neurological functions, nutrient metabolism, and coat patterns, mirroring their historical roles of domestic cats and morphological and behavioral differentiation from wild progenitors.

Key words: domestication, domestic cats, wildcats, positive selection, genome

Introduction

The domestication of cats (*Felis silvestris catus*), dating at least 3,600 BP, has led to the widespread adoption of cats initially to rid rodents and small animals in human settlements (Clutton-Brock 1990, Hu, Hu et al. 2014). More specifically, wildcat domestication occurred through a self-selective process in which behavioral reproductive isolation evolved as a correlated character of assortative mating coupled to habitat choice for urban environments. Eurasian wildcats gave rise to a domestic population and their evolutionary paths to companion animals was initially a process of natural selection rather than human-driven artificial selection over time driven during their sympatry with ancestral wildcats (Driscoll, Menotti-Raymond et al. 2007). Unlike many other domesticated mammals bred for specific occupations such as hunting and guarding, most of the modern domestic cat breeds originated recently within the past two decades, largely due to selection for aesthetic rather than functional traits (Montague, Li et al. 2014). The domestic cat has now become one of the most popular pet species, with over 600 million individuals across the world (Association 2007). *Felis silvestris (F. s.)*, from which domestic cats were derived, is classified as a polytypic wild species composed of at least four distinct subspecies: *F. s. silvestris* in Europe, *F. s. lybica* in Africa and the Near East, *F. s. ornata* in the Middle East and Central Asia, and the Chinese desert cat, *F. s. bieti* (Driscoll, Menotti-Raymond et al. 2007).

In this regard, we analyzed the whole genome sequence (WGS) data of 46 samples comprising wildcat (*F. s. silvestris*) and domestic cat (*F. s. catus*) populations to identify the signals of adaptive response to natural and human artificial selection. The genomic regions inferred to be positively selected likely provide a clue to the understanding of biological mechanisms underlying the historical roles of domestic cats.

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Materials and Methods

Genotype Data and Haplotype Sharing Analysis

Data from 46 individuals were obtained via the Short Read Archive (ncbi.nlm.nih.gov/sra) from previously published studies (Table S1). The pair-end sequence reads were then mapped against the FelCat 8.0 reference genome using BWA 0.7.17 (Li and Durbin 2009), sorted with SAMtools 1.9 (Li, Handsaker et al. 2009), and screened for putative PCR duplicate reads with PicardTools 1.119 (https://github.com/broadinstitute/picard). Genome Analysis Toolkit 4.1.4 (GATK) (McKenna, Hanna et al. 2010) was used to perform local realignment of reads to correct misalignments due to the presence of indels (Axelsson, Ratnakumar et al. 2013) as the training set. SNPs were called per-individual in gVCF mode of HaplotypeCaller (Auwera, Carneiro et al. 2013), with subsequent joint-calling across all individuals. GATK best practices and default parameters, together with the initial alignment training sets, were used for variant quality score recalibration of single nucleotide variants. A total of ~34 million autosomal SNVs that were polymorphic in the population and passed our quality control of maximum missing rate < 20% were used for subsequent analyses.

Population Structure Analysis

We used the genome-wide complex trait analysis (GCTA) tool for PCA (Yang, Lee et al. 2011) which implements EIGENSTRAT (Price, Patterson et al. 2006) to estimate eigenvectors, incorporating genotype data from all samples. We estimated the fixation index statistic (in windows of 100 kb) using VCFtools (v0.1.13) (Danecek, Auton et al. 2011). Finally, the distance matrix was estimated using PLINK (Chang, Chow et al. 2015) and the phylogenetic tree was constructed using Phylip software (Felsenstein 1993) .

Results & Discussion

Population Structure

We carried out whole-genome re-sequencing of 32 domestic cats (Birman, Burmese, Devon Rex, Domestic, Donskoy, Himalayan, Korat, Maine Coon, Maine Coon Cross, Munchkin, Oriental, Siamese, Siamese cross, Sphynx, Tonkinese, and Toybob; each breed of 2 individuals), and 14 wildcats (5 Felis silvestris, 6 Felis silvestris bieti, and 3 Felis silvestris ornata), comprising a total of 46 cats (Table S1). Genome alignment indicated an average of 24.1X depth of coverage and 94.4% mapping rate, providing a total of ~34 million high-quality autosomal SNPs over two cat populations.

To examine genetic relationships among three cat populations, we conducted principal component analysis (PCA) based on whole-genome SNPs. The first eigenvector (27.4%) and the second eigenvector (14.1%) identified three genetically independent clustering of wildcats and a distinct domestic cat population (Figure 1a). We note that a widely dispersed distribution of *F. s. bieti* individuals indicate a higher level of heterogeneity in this population compared to other wildcat populations. To further understand the degree of admixture in the populations, we used the Admixture program (Alexander, Novembre et al. 2009) on a randomly sampled subset of SNPs (~20,000 SNPs). We increased K from 1 to 4, where K is the assumed number of ancestral populations (Figure 1b). The analysis using K=4 reflected the divergence between domestic cats and wildcats and further subpopulation structures within each population. *F.s.bieti* again showed the level of heterogeneity within the wildcat population. In addition, a neighbor-joining tree (Figure 1c) separated each domestic cat breed into its own separate clade.

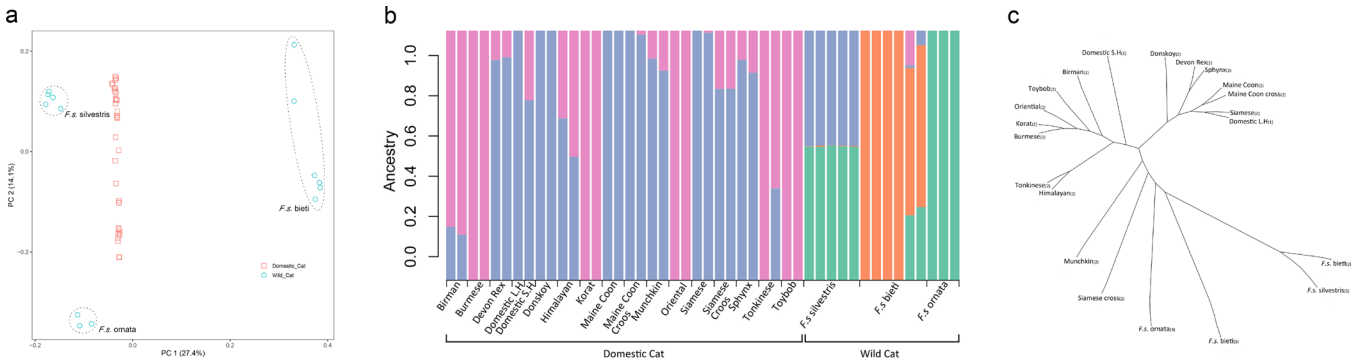

Figure 1. (A) Each individual samples (Domestic cats and Wildcat populations) are plotted along the principal component (PC) 1 and PC 2. (B) Proportions of ancestry for each sample assuming 4 ancestral populations (K=4). (C) Neighbor-joining tree of the genetic relationships between the populations and breed groups.

Genomic regions with allele frequency differentiation

We then scanned genomic regions of non-overlapping 100kb windows with extreme allele frequency differentiation between domestic cats against ancestral wildcats using the fixation index (F_{ST}) statistic (Figure 2). Using the top 0.1% of the empirical distribution among genomic regions, a total of 34 genes were identified as putative candidate regions of selective sweep (Table 1). In addition, in order to perform an enrichment analysis of significantly differentiated genes, we applied a relaxed genome-wide threshold (top 1%) from F_{ST} analysis and identified 229 suggestive genomic windows. A total of 22 pathways were significantly over-represented in our analysis (Table 2).

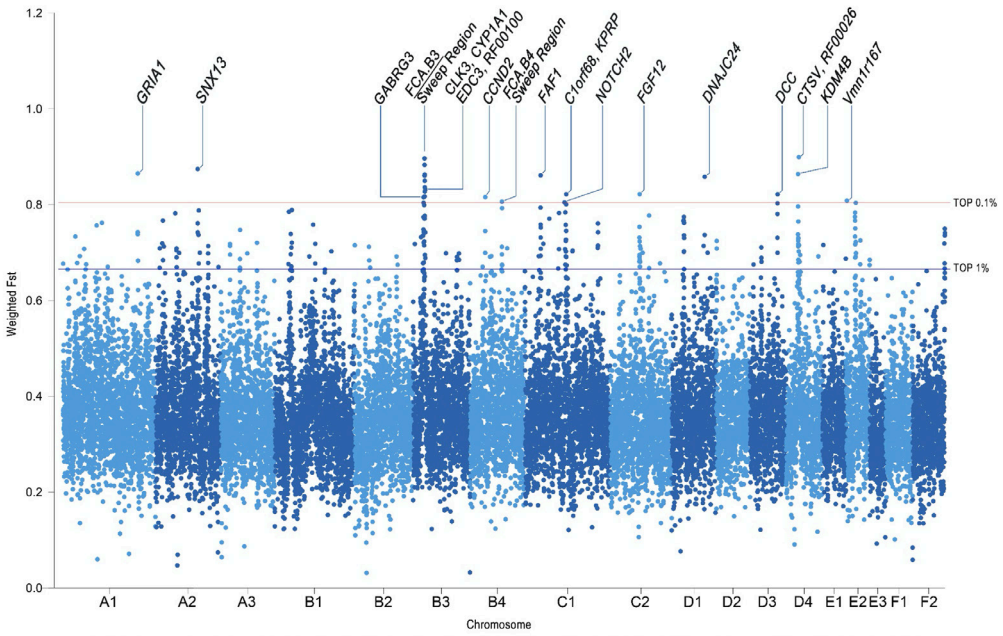

Figure 2. Genome-wide identification of genetic windows with significant allele frequency differentiation between domestic cat and wildcat populations.

Table 1. A list of significant windows from FST analysis conducted to compare the allele frequency differentiation over 100 kb genomic regions. Presented here in the table represent the top 0.1% genome-wide significant regions.

Postion	Fst (Weighted)	Gene
D4:32,900,001 – 33,000,000	0.899063	KDM4B
B3:29,500,001 – 30,500,000	0.896179	SCAPER, PSTPIP1, TSPAN3, RF00003, PEAK1, HMG20A, RCN2
A2:109,200,001 - 109,300,000	0.87411	SNX13
A1:193,900,001 – 194,000,000	0.864842	GRIA1
D4:31,000,001 – 31.100,000	0.86351	CTSV, RF00026
C1:40,700,001 – 40,800,000	0.860903	FAF1
D1:85,600,001 – 85,700,000	0.857841	DNAJC24
B3:31,600,001 – 31,700,000	0.831592	CLK3, CYP1A1, EDC3, RF00100
C2:76,000,001 – 76,100,000	0.821372	FGF12
D3:71,800,001 – 71,900,000	0.821344	DCC
C1:107,300,001 - 107,400,000	0.821311	C1orf68, KPRP9
B4:39,400,001 – 39,500,000	0.815331	CCND2
B3:27,000,001 – 27,100,000	0.815128	GABRG3
E2:2,400,001 – 2,500,000	0.808159	RFPL2, Vmn1r167
B4:82,700,001 – 82,800,000	0.805854	ESYT1, MYL6, MYL6B, NABP2, RNF41, SLC39A5, SMARCC2
C1:102,800,001 – 102,900,000	0.804371	NOTCH2

Table 2. The result of Gene Set Enhancement Analysis (GSEA) using significantly differentiated genes derived from FST analysis. The threshold to define significant enrichment was P-value of 5 x 10-4.

Term	P-value
Asthma or allergic disease (pleiotropy)	1.75E-08
Anorexia nervosa	3.74E-08
Allergic disease (asthma, hay fever or eczema)	6.78E-08
Red vs. brown/black hair color	2.53E-06
Educational attainment	3.55E-06
Regular attendance at a gym or sports club	8.35E-06
Vitiligo	1.56E-05
Caffeine metabolism plasma 1,7-dimethylxanthine (paraxanthine) to 1,3,7-trimethylxanthine (caffeine) ratio	1.75E-05
Age at first birth	2.61E-05
Blood metabolite levels	3.92E-05
Brown vs. black hair color	6.95E-05
Skin pigmentation traits	1.67E-04
Axial length	1.72E-04
Low tan response	1.95E-04
Craniofacial microsomia	2.72E-04
Regular attendance at a religious group	2.81E-04
Logical memory (immediate recall) in normal cognition	3.20E-04
Intelligence (MTAG)	3.60E-04
Serum metabolite levels	3.72E-04

Domestic cats clearly exhibit morphological and behavioral differences from wildcats, such as docility, gracility, and pigmentation (Montague, Li et al. 2014). The most distinguishable adaptation is the tolerance of living in human-dominated environments, a key attribute shared across any domesticated animal (Driscoll, Macdonald et al. 2009). Previously, Berteselli et al. reported that the most frequently performed behavior categories are different between cat populations with "vigilance" in wildcats and "resting" in domestic cats, respectively (Berteselli, Regaiolli et al. 2017). Among 34 significant genes, we found the *GRIA1* gene, and the behavioral consequences of the *GRIA1* knockout have been

extensively studied, including impulse and memory control (Kilonzo, Strahnen et al. 2022). Also supporting our hypothesis, pathways related to behavioral and neuronal functions were highly enriched ("educational attainment", "logical memory", and "intelligence") (Table 2). The significant genes and pathways together may play important roles in neural processes, notably pathways associated with synaptic circuitry that influence social behavior and contextual clues related to reward (Montague, Li et al. 2014).

As the case for all felids, wildcats belong to obligate carnivores, indicating they have a limited metabolic ability to digest nutrients except proteins (Bradshaw, Goodwin et al. 1996). Domestic cats, in contrast, have longer intestines than wildcats, a "less strictly carnivorous diet" trait as a result of feeding on kitchen scraps (Darwin 1868). Of the significant genes, *CYP1A* is responsible for the differential effect of polyunsaturated fatty acids (PUFA) diet on tissue fatty acid composition and the generation of cytochrome P450-dependent metabolites including linoleic acid, and ultimately body and organ weights (Agbor, Wiest et al. 2014).

Domestic cats have become polyestrous, and their coat colors and patterns often deviate wildly from the wildcat's striped mackerel tabby (Driscoll, Macdonald et al. 2009). These included new variations of the tabby coat, and the introduction of black, orange, and white colors (Kaelin, Xu et al. 2012). The significantly over-represented pathways ("Red vs. brown/black hair color", "Brown vs. black hair color", and "Skin pigmentation traits") indicate that genes associated with coat patterns explain the population differentiation between cat groups. Interestingly, we also found that the *SCAPER* gene was under selection pressure, whose mutation is related to syndromic autosomal recessive retinitis pigmentosa and has a role in the function and maintenance of photoreceptors. A previous study reported that densities of cone photoreceptors are higher in wildcat in the centralis, whereas rod densities are higher in domestic cats (Williams, Cavada et al. 1993). Compared to wildcats, researchers have found a reduction in brain size in the domestic lineage (Williams, Cavada et al. 1993). One explanation for the reduction in the size of the domestic cat's brain is that far fewer neurons are generated early in development (Williams et al., 1986). Coinciding with this observation, we found that the *DCC* gene showed significant differentiation between wildcats and domestic cats. Mice with homozygous null *DCC* mutations have severe defects of commissural development in the brain and spinal cord, with absent corpus callosum and decreased number and misrouting of commissural axons (Srour, Rivière et al. 2010).

In conclusion, cats are unique as a semi-domesticated species, and many populations are not completely separated from wildcats, and humans did not control their food supply or breeding (Cameron-Beaumont, Lowe et al. 2002). However, the characterization of the whole genome sequences of domestic cats and wildcats evidently indicates that genes under selection are involved in traits such as socialization, nutrient metabolism, and coat patterns. Our study is unique in creating a large catalog of genomic variation for cats and tracing back the evolutionary path to the divergence of domestic cats and their progenitors. These genes may reflect the genomic landscape of domestic cats in response to human selection and natural selection that have shaped traits specific to domestic cats.

Conflict of Interest

The authors declare that they have no conflicting interests.

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Table S1. List of individuals used in the analysis.

SRR ID	Population	Breed (Subspecies)
SRR11392477	Domestic Cat	Birman
SRR11392567	Domestic Cat	Birman
SRR11392468	Domestic Cat	Burmese
SRR11392531	Domestic Cat	Burmese
SRR11392588	Domestic Cat	Devon Rex
SRR11392633	Domestic Cat	Devon Rex
SRR11392635	Domestic Cat	Domestic Longhair
SRR8092630	Domestic Cat	Domestic shorthair
SRR11782894	Domestic Cat	Donskoy
SRR11782897	Domestic Cat	Donskoy
SRR5043257	Domestic Cat	Himalayan
SRR5366692	Domestic Cat	Himalayan
SRR11392472	Domestic Cat	Korat
SRR11392474	Domestic Cat	Korat
SRR11392457	Domestic Cat	Maine Coon
SRR11392632	Domestic Cat	Maine Coon
SRR11392615	Domestic Cat	Maine Coon Cross
SRR11392617	Domestic Cat	Maine Coon Cross
SRR8237422	Domestic Cat	Munchkin
SRR8237424	Domestic Cat	Munchkin
SRR11392520	Domestic Cat	Oriental
SRR11392564	Domestic Cat	Oriental
SRR11392454	Domestic Cat	Siamese
SRR11392455	Domestic Cat	Siamese
SRR5055390	Domestic Cat	Siamese cross
SRR5055406	Domestic Cat	Siamese cross
SRR11392636	Domestic Cat	Sphynx
SRR11392637	Domestic Cat	Sphynx
SRR5043261	Domestic Cat	Tonkinese
SRR5366725	Domestic Cat	Tonkinese
SRR11392563	Domestic Cat	Toybob
SRR11392584	Domestic Cat	Toybob
SRR066071	Wildcat	Felis silvestris silvestris
SRR066072	Wildcat	Felis silvestris silvestris
SRR066073	Wildcat	Felis silvestris silvestris
SRR066074	Wildcat	Felis silvestris silvestris
SRR066075	Wildcat	Felis silvestris silvestris
SRR7621226	Wildcat	Felis silvestris bieti
SRR7621227	Wildcat	Felis silvestris bieti
SRR7621229	Wildcat	Felis silvestris bieti
SRR7621235	Wildcat	Felis silvestris bieti
SRR7621236	Wildcat	Felis silvestris bieti
SRR7621239	Wildcat	Felis silvestris bieti
SRR7621238	Wildcat	Felis silvestris ornata
SRR15116525	Wildcat	Felis silvestris ornata
SRR15116526	Wildcat	Felis silvestris ornata