Defining and Mapping Mammalian Coat Pattern Genes: Multiple Genomic Regions Implicated in Domestic Cat Stripes and Spots

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ABSTRACT

Mammalian coat patterns (e.g., spots, stripes) are hypothesized to play important roles in camouflage and other relevant processes, yet the genetic and developmental bases for these phenotypes are completely unknown. The domestic cat, with its diversity of coat patterns, is an excellent model organism to investigate these phenomena. We have established three independent pedigrees to map the four recognized pattern variants classically considered to be specified by a single locus, Tabby; in order of dominance, these are the unpatterned agouti form called "Abyssinian" or "ticked" (T^a), followed by Spotted (T^s), Mackerel (T^M), and Blotched (t^b). We demonstrate that at least three different loci control the coat markings of the domestic cat. One locus, responsible for the Abyssinian form (herein termed the Ticked locus), maps to an ∼3.8-Mb region on cat chromosome B1. A second locus controls the Tabby alleles T^M and t^b, and maps to an ∼5-Mb genomic region on cat chromosome A1. One or more additional loci act as modifiers and create a spotted coat by altering mackerel stripes. On the basis of our results and associated observations, we hypothesize that mammalian patterned coats are formed by two distinct processes: a spatially oriented developmental mechanism that lays down a species-specific pattern of skin cell differentiation and a pigmentation-oriented mechanism that uses information from the preestablished pattern to regulate the synthesis of melanin profiles.

PATTERNED coats are typical of many mammalian groups, whose spots, stripes, and other markings have been hypothesized to play important adaptive roles in camouflage, predator evasion, and social communication (COTT 1940; SEARLE 1968; ORTOLANI and CARO 1996). Many mammals bear striped or spotted coats, and these phenotypes have historically drawn attention from many fields of human science and culture (e.g., the leopard's spots, or the stripes seen in tigers and zebras). Although several theoretical studies have proposed mathematical models that could underlie the developmental dynamics of coat pattern formation in mammals (MURRAY and OSTER 1984; OYEHAUG et al.

2002), no direct investigation of the genetic basis of these phenotypes has yet been performed, so that their mechanistic causes remain a mystery. Recent advances in genomics, molecular biology, and evolutionary developmental biology (Evo-Devo) have revealed genes and pathways involved in skin pattern formation in Drosophila (SCHUG et al. 1998; GOMPEL et al. 2005; PRUD'HOMME et al. 2006; PARCHEM et al. 2007), butterflies (JORON et al. 2006a,b), and zebrafish (IWASHITA et al. 2006; WATANABE et al. 2006; SVETIC et al. 2007). In contrast, despite the relevance of characterizing equivalent processes in mammals, little progress toward this goal has been accomplished, perhaps due to the lack of adequate mammalian models exhibiting variation in skin pattern and for which genetic and genomic tools were available.

The domestic cat is a very promising model in this regard, as it presents several coat pattern variants and a growing body of genetic and genomic tools suitable for gene identification (MENOTTI-RAYMOND et al. 2003; MURPHY et al. 2007; PONTIUS et al. 2007; PONTIUS and O'BRIEN 2007; DAVIS et al. 2009). Classic work on
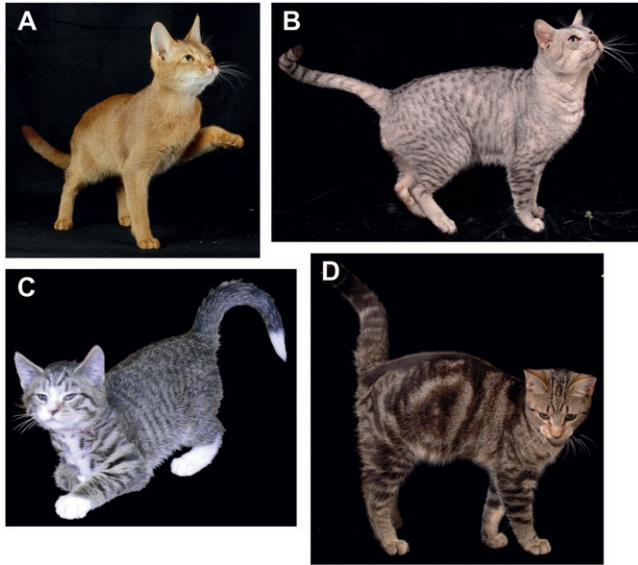
FIGURE 1.—Major coat pattern phenotypes of the domestic cat (*Felis silvestris catus*). A "hierarchy" of pelage patterns is observed in this species, with the absence of markings seen in Abyssinian cats (A) dominating over a spotted coat (B), which dominates over a "mackerel" (striped) coat (C), itself dominant over the blotched pattern (D). The classical, single-locus model for this phenotypic variation proposed the allelic series $T^a > T^s > T^M > t^b$ for these respective variants.

domestic cat coat color (ROBINSON 1958; LOMAX and ROBINSON 1988) has suggested that there is a monogenic allelic series of coat patterns in the domestic cat, controlled by the *Tabby* (T) locus: in order of dominance, the four recognized alleles would be Abyssinian or "ticked" (T^a), Spotted (T^s), Mackerel (T^M), and Blotched (t^b) (Figure 1). Although there has been little doubt among breeders that the "mackerel" and "blotched" forms segregate as a single autosomal locus, this may not be the case for the other two phenotypes (T^a and T^s), which so far have not been tested thoroughly for allelism relative to the more common *Tabby* variants T^M and t^b. Some breeding data have suggested that these variants may not be allelic with the main *Tabby* locus (LORIMER 1995), but further scrutiny is required to test this hypothesis. A recent genetic study (LYONS *et al.* 2006) considered the Abyssinian variant as an allele of *Tabby*, reflecting the prevalent perception that they are coded by the same locus. Testing this hypothesis, and identifying the implicated genomic region (or regions), is a first step in the process of dissecting the molecular and developmental basis for these pattern-formation phenotypes.

Aiming to investigate the genetic basis of pattern formation on the domestic cat pelage by genomic, positional methods, we established three separate pedigrees segregating for different combinations of coat pattern phenotypes. Our results demonstrate that at least three different loci underlie the striping and spotting patterns observed in domestic cats and identify the genomic location of two of them.
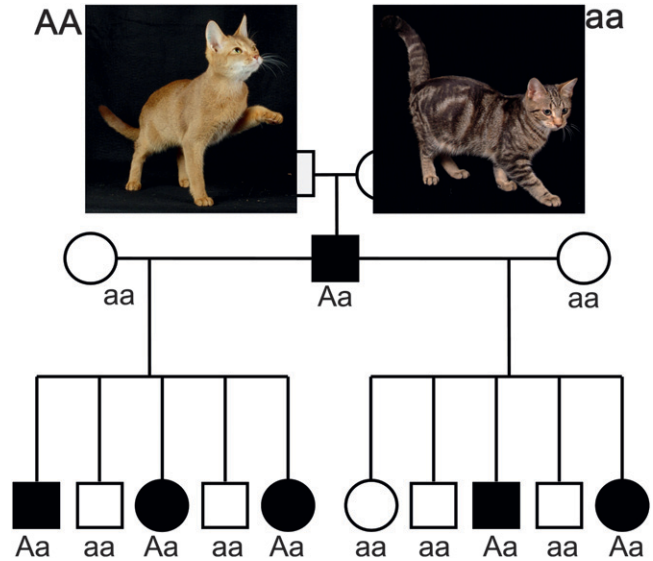

FIGURE 2.—Schematic of the mating strategy employed here to separately investigate the "spotted" and Abyssinian ("ticked") domestic cat pattern variants. In both cases, individuals known to be homozygous for the allele of interest (symbolized here by "A," and exemplified by ticked) were bred to tester animals known not to carry this allele, and thus to be homozygous for a recessive variant (symbolized by "a" and exemplified by "blotched"). F_1 individuals were necessarily heterozygous at the locus of interest and were subsequently backcrossed to additional, unrelated tester cats. Phenotypic segregation among third-generation offspring was then used to assess Mendelian inheritance, allelism, and genomic mapping of the implicated locus. The drawing is a simplified abstraction (see text and Figure S1 and Figure S2 for details), as the parental generation may be composed of one male mated to multiple females (pedigree 2), or different pairs (pedigree 3).

MATERIALS AND METHODS

Pedigrees and phenotyping: To avoid potential complications stemming from multiple loci affecting coat pattern formation phenotypes, we chose to analyze each variant separately, a strategy that could simultaneously test for allelism and identify the implicated genomic regions. We thus established three independent pedigrees, each of which segregated for a single "*Tabby*" variant, relative to a standard. Pedigree 1 was a multigenerational outbred (and nonbreed) domestic cat pedigree maintained by the Nestlé Purina PetCare Company for nutrition studies, which we have previously used to build a genetic linkage map of the domestic cat genome (MENOTTI-RAYMOND *et al.* 2009; SCHMIDT-KÜNTZEL *et al.* 2009) and also to identify genes involved in coat color and hair length (EIZIRIK *et al.* 2003; ISHIDA *et al.* 2006; KEHLER *et al.* 2007). It consisted of 287 individuals, 256 of which were genotyped. This pedigree showed segregation for the mackerel and blotched *Tabby* variants, providing a basis for their mapping using a genome scan.

Pedigrees 2 and 3 were specifically designed and developed for this study, in each case aiming to isolate the inheritance of a single *Tabby* variant. Both of these pedigrees were developed at the National Institutes of Health Animal Center (NIHAC), using a backcross mating design (Figure 2). Pedigree 2 focused on the inheritance of the "spotted" (T^s) variant and was founded with a pure-bred male cat of the Egyptian Mau breed, which was crossed with three unrelated blotched
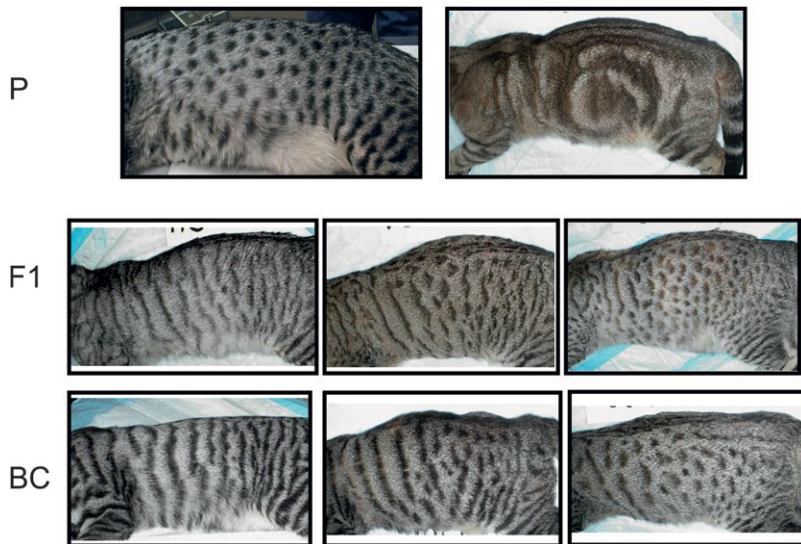
FIGURE 3.—Phenotypic variation observed in pedigree 2, designed to characterize the "spotted" coat variant. P, parental generation; F1, F_1 generation; BC, backcross generation. For the F_1 and BC generations, only examples of non-blotched patterns are shown to illustrate the range of markings observed in these animals (from spotted to fully striped phenotypes). Some individuals in the BC generation bore complete stripes, to the point that they could be fully categorized as a typical "mackerel" cat (bottom left).

females (supporting information, Figure S1). Egyptian Mau cats are fully spotted (Figure 3) and breed true for this trait, thus being homozygous for whatever alleles cause this coat pattern. Blotched was selected as the tester phenotype, since it is determined by a recessive allele relative to mackerel, so that these individuals can be confidently assumed to be homozygotes (t^b/t^b) at the *Tabby* locus. The founder crosses of this pedigree were therefore set up to be $T^sT^s \times t^bt^b$, considering the single-locus model of *Tabby* inheritance.

Pedigree 3 focused on the inheritance of the Abyssinian (T^a) variant (known as ticked in breeder terminology), also based on a backcross mating design (Figure 2 and Figure S2). The Abyssinian form is semidominant relative to any of the other variants, with homozygotes T^a/T^a bearing plain color (a homogeneous agouti coat, with banded individual hairs but no body markings) (Figure 1), and heterozygotes exhibiting faint, thin body stripes and banding on the legs and tail (LOMAX and ROBINSON 1988). The founders of this kindred were two males previously generated in an Abyssinian pedigree segregating for retinal atrophy which had been outcrossed to non-Abyssinian European shorthairs (MENOTTI-RAYMOND *et al.* 2007). Given their progenitors (both had different Abyssinian sires, each of which had been crossed to a blotched and a mackerel female, respectively), these two individuals were known to be heterozygotes carrying the T^A allele, and indeed displayed stripes on the legs and tail of an otherwise typical Abyssinian (*i.e.*, agouti or ticked) coat. These two F_1 individuals were used for backcrossing with 12 non-Abyssinian females (mackerel or blotched), and phenotypic segregation of ticked was analyzed in the resulting third-generation individuals.

For all three pedigrees, individuals were phenotyped by E. Eizirik and photographs recorded in a central database maintained at the Laboratory of Genomic Diversity, National Institutes of Health (NIH). All cats analyzed in this study were maintained in facilities inspected by the United States Department of Agriculture, under conditions established by the American Association of Laboratory Animal Care in compliance with the federal Animal Welfare Act.

DNA extraction and marker genotyping: Blood samples were obtained from all individuals in pedigrees 1–3. In addition, for pedigrees 2 and 3, fibroblast cell lines were established as a source of high-quality genomic DNA. DNA was extracted from whole blood or cell lines using a QIAamp DNA Blood Midi kit (QIAGEN). PCR amplification was performed with a touchdown PCR protocol as described previously (MENOTTI-RAYMOND *et al.* 2005). Sample electrophoresis and genotyping, as well as Mendelian inheritance checking, were carried out as previously described (ISHIDA *et al.* 2006). See the RESULTS section and Table S1 for the microsatellite loci typed in each pedigree.

Development of microsatellites for fine mapping of *Tabby* and *Ticked*: After linkage was established to a known region using previously published cat STR markers, additional microsatellites from candidate regions were mined from the cat 1.9X whole genome sequence (PONTIUS *et al.* 2007). Initially microsatellites were selected on the basis of their conserved syntenic position in the dog, following the method described by ISHIDA *et al.* (2006). Following the availability of a cat genome assembly (PONTIUS *et al.* 2007), microsatellite markers were selected on the basis of their location on cat chromosomes using the algorithm ABCC Retrieve STRs (ABCC STR-centric tools, http://www.abcc.ncifcrf.gov/Genomes/Cat/index.php) (PONTIUS and O'BRIEN 2007) (these include all loci used for *Ticked* mapping with prefix "chrB1," see below). Primers (see Table S1) were designed with Primer 3 (http://frodo.wi.mit.edu/cgi-bin/primer3/primer3_www.cgi (ROZEN and SKALETSKY 2000), including an M13 tail for fluorescent labeling of PCR products (BOUTIN-GANACHE *et al.* 2001).

Genetic linkage mapping: Single-marker LOD scores were computed using Superlink (FISHELSON and GEIGER 2002; FISHELSON and GEIGER 2004), as described in ISHIDA *et al.* (2006) and KEHLER *et al.* (2007). Recombination fractions are optimal to within 0.01. Multipoint analyses were performed to clarify which markers belong above or below the intervals of zero recombination. The traits were modeled as fully penetrant. For the LOD scores shown here, the trait-associated allele frequency was set to 0.25, but the LOD scores are insensitive to this value. Marker-allele frequencies were set all equal, but since most of the cats are genotyped, the marker allele frequencies have little effect on the LOD scores.

RESULTS

A detailed inspection of phenotype segregation in pedigree 1 corroborated the expected monogenic inheritance of the mackerel and blotched *Tabby* variants (Figure 1), with the latter being a fully penetrant,

autosomal recessive allele. In the absence of candidate genes for this phenotype, a genome scan was performed using 483 microsatellite markers. Among these established markers, significant linkage to the *Tabby* locus was observed in a region of chromosome A1 [markers FCA566 (LOD = 31.4, θ = 0.01), FCA90 (LOD = 10.7, θ = 0.05), and FCA1331 (LOD = 42.61, θ = 0)] (Table 1). Fine mapping of *Tabby* was accomplished by developing additional microsatellite markers for the region (see Table S1 for a list of all new primers), combined with the subsequent addition of meioses from pedigree 2 (see below). This joint approach defined a genomic region of ∼5 Mb for the *Tabby* locus, on the basis of conserved synteny with the human and dog genomes (Table 1). Although the domestic cat sequence assembly is not yet complete in this region, the current radiation hybrid (RH) map (DAVIS *et al.* 2009) shows that this genomic segment is well defined and exhibits conserved synteny to human chromosome 5 (113.0–118.6 Mb) and dog chromosome 11 (6.6–11.5 Mb).

The parental crosses of pedigree 2 (spotted *vs.* blotched phenotypes) yielded seven F_1 individuals, none of which were fully spotted, but rather exhibiting intermediate patterns between spotted and mackerel (*i.e.*, they would be considered to be "broken mackerel," due to a mixture of spots and broken stripes; Figure 3). These F_1 individuals were backcrossed to nine tester blotched cats, producing a third-generation progeny of 39 animals, 35 of which could be confidently phenotyped (Figure S1). These backcross offspring conformed to a 1:1 ratio of blotched ($n = 19$) to nonblotched ($n = 16$) pattern, supporting an allelic relationship at the *Tabby* locus. However, the nonblotched animals exhibited a full range from spotted to striped phenotypes, including individuals that could be fully categorized as mackerel (see Figure 3). Since the mackerel form was not present in any of the original crosses, this result demonstrates that it was represented by the spotted individuals, modified by the epistatic action of other genes. The segregation we observed was therefore between the two common alleles at the *Tabby* locus, T^M and t^b, with the former originally masked as the spotted phenotype by modifier loci. Given these findings, we added the meioses from this pedigree to the analysis of the *Tabby* locus (T^M and t^b), described above, to refine the mapping of its genomic position (Table 1).

In the case of pedigree 3 (Figure S2), the two male founders (F_1 individuals) were backcrossed to multiple non-Abyssinian females, producing a total of 85 offspring, 73 of which could be phenotyped with confidence (the remaining 12 were stillborn and did not allow for reliable recognition of coat color features). These third-generation individuals exhibited an almost perfect 1:1 ratio of T^a carriers ($n = 36$) *vs.* noncarriers (mackerel or blotched) ($n = 37$), in accordance to Mendelian expectations for a single locus. To verify whether this segregation in pedigree 3 occurred at the

same locus as the T^M and t^b alleles mentioned above, we initially genotyped the members of this kindred for six microsatellites linked to the *Tabby* locus (mapped to chromosome A1 using pedigrees 1 and 2, see Table 1), as well as three additional markers in the same region of chromosome A1. The results from this experiment excluded this chromosomal segment, refuting *Tabby* as the implicated locus in the T^a variant (Table S2).

This finding indicated that the Abyssinian phenotype is not coded by an allele of *Tabby*, but rather by a separate genetic locus, which we henceforth call *Ticked*. Before initiating a whole genome scan for this locus, we proceeded to genotype three microsatellites in a candidate region linked to "brindling" (the "K" locus) in dogs (G. S. BARSH, personal communication; CANDILLE *et al.* 2007; KERNS *et al.* 2007), as this canine variant might be homologous to some aspect of the cat pelage patterning. Microsatellites were selected for a region on cat chromosome B1 with conserved synteny to the brindling locus, at 34.8 Mb on dog chromosome 19 (CANDILLE *et al.* 2007; KERNS *et al.* 2007). Although *Ticked* demonstrated significant linkage to the K region [markers FCA522 (LOD = 11.4, θ = 0.09) and FCA519 (LOD = 11.6, θ = 0.05)], recombination between the markers and this trait was demonstrated within this genomic segment of cat chromosome B1 (Table 2). Although this result indicated that the dog brindling and cat ticked coat color variants were not caused by the same gene, this initial finding allowed us to restrict all further marker development and genotyping to chromosome B1. Additional markers were thus designed to identify a region of zero recombination with *Ticked* (see MATERIALS AND METHODS). The genomic region for *Ticked* was ultimately defined as a segment of ∼3.8 Mb, with conserved synteny to Hsa 8: 23–19.7, 43.1–42.9 Mb. This region is equivalent to that reported by LYONS *et al.* (2006) as linked to the *Tabby* locus. The defined segment occurs within a region showing an intrachromosomal break in synteny between cat and human, and an interchromosomal break between cat and dog (Table 2).

DISCUSSION

The results presented here indicate that there are at least three different loci determining the pattern of coat markings in the domestic cat: (1) the *Tabby* locus (for which we propose the symbol "*Ta*"), mapped to chromosome A1 and containing alleles Ta^M and ta^b (*mackerel/blotched*); (2) one or more modifier loci that create a spotted coat by altering the mackerel stripes and that possibly also influence variation in the blotched pattern; and (3) the *Ticked* locus (for which we propose the symbol "*Ti*"), mapping to chromosome B1 and containing alleles Ti^A (Abyssinian) and Ti^+ (non-Abyssinian). The Ti^A allele is semidominant and has an epistatic effect on the expression of *Tabby* and its shape-altering modifiers (Table 3).

TABLE 1

Linkage mapping of the domestic cat *Tabby* locus

Marker[a]	Cat chr.	GL map position[b]	RH map position[b]	Peak LOD[c]	Θ of peak[c]	Position in GARFIELD (start)[d]	Hsa36 (start)[e]	Cfa2 (start)[e]
FCA566	A1	144.8	755.7	31.36*	0.01*	Chr Un8: 85,018	Unknown	Unknown
FLT4	A1	—	—	28.73	0.03	Chr Un11: 349,316	Chr 5: 179,972,586	Chr 11: 4,173,747
ChrUn11_369202		—	—	24.99	0.05	Chr Un11: 369,202	Chr 5: 179,954,132	Chr 11: 4,190,117
Hsa5:179.47	A1	—	—	23.43*	0.02*	Chr Un11: 699,876	Chr 5: 179,470,809	Chr 11: 4,568,828
Hsa5:178.67	A1	—	—	20.19	0.02	Chr Un11: 1,234,398	Chr 5: 178,672,817	Chr 11: 5,312,931
Col23A1	A1	—	—	21.32	0.06	Chr A1: 124,220,448	Chr 5: 177,617,085	Chr 11: 6,306,204
YTHDC2	A1	—	—	37.78	0.01	Chr A1: 123,872,511	Chr 5: 112,958,230	Chr 11: 6,617,669
Cfa11:7.68	A1	—	—	30.27	0	Chr A1: 123,080,103	Chr 5: 113,730,035	Chr 11: 7,288,271
FCA1331	A1	147.3	—	42.61	0	Chr A1: 122,968,298	Chr 5: 113,814,901	Chr 11: 7,382,372
Hsa 5-116.83	A1	—	—	32.45	0	Chr Un11: 2,154,347	Chr 5: 116,836,837	Chr 11: 10,069,791
Hsa-5-118.59	A1	—	—	40.85	0.01	Chr Un11: 3,108,430	Chr 5: 118,596,950	Chr 11: 11,556,021
Hsa5-118.72	A1	—	—	34.57	0.01	Chr Un11: 3,208,206	Chr 5: 118,721,887	Chr 11: 11,656,633
Cfa11:11.26	A1	—	—	22.92*	0.01*	Chr Un11:253,059	Chr 5: 119,149,336	Chr 11: 13,698,383
Cfa11:12.55	A1	—	—	30.58*	0.01*	Chr Un11: 4,235,006	Chr 5: 120,543,823	Chr 11: 14,227,481
FCA90	A1	154.7	947.3	10.67*	0.05*	Unknown	Chr 5: 123,155,913	Chr 11: 16,596,908

[a] Markers are shown in genomic order along the domestic cat chromosome A1, on the basis of the most recent genetic linkage (GL) (MENOTTI-RAYMOND *et al.* 2009) and radiation hybrid (RH) (DAVIS *et al.* 2009) maps of the domestic cat. Markers with prefix other than FCA represent loci that were developed for fine mapping after multipoint linkage analysis demonstrated that FCA566 and FCA90 flanked the *Tabby* interval.

[b] Positions of markers with a blank in these columns were not determined in those versions of the RH or GL maps.

[c] Peak LOD score and estimated recombination fraction (θ) for linkage between each polymorphic marker and the *Tabby* locus. Asterisks indicate loci that were genotyped only in the Nestlé-Purina colony (pedigree 1); all other loci were additionally genotyped in pedigree 2 and reflect cumulative LOD scores from the two pedigrees.

[d] Position in the domestic cat whole genome sequence assembly as visualized in the GARFIELD browser (PONTIUS and O'BRIEN 2007) (http://lgd.abcc.ncifcrf.gov/cgi-bin/gbrowse/cat/).

[e] Columns 8 and 9 show the positions in the human (HSA build 36) (http://www.ncbi.nlm.nih.gov/projects/mapview/stats/BuildStats.cgi?taxid=9606&build=36&ver=3) and dog (CFA build 2) (http://www.ncbi.nlm.nih.gov/projects/mapview/stats/BuildStats.cgi?taxid=9615&build=2&ver=1) genome assemblies found by BLAT analysis to be orthologous to sequences flanking the respective domestic cat markers.

TABLE 2

Linkage mapping of the domestic cat *Ticked* locus

Marker[a]	Cat chr.	GL map position[b]	RH map position[b]	Peak LOD[c]	Θ of peak[c]	Position in GARFIELD (start)[d]	Hsa36 (start)[e]	Cfa2 (start)[e]
FCA522	B1	47.9	197.8	11.39	0.09	B1: 20,954,176	Chr 8: 12,886,556	Chr 16: 39,395,506
FCA519	B1	60.2	247.2	11.60	0.05	B1: 26,480,800	Chr 8: 32,537,942	Chr 16: 34,857,499
ChrB1_36137001	B1			14.36	0.05	B1: 36,137,001	Chr 8: 25,348,288	Chr 25: 35,009,943
ChrB1_38697912	B1			7.53	0.03	B1: 38,697,912	Chr 8: 23,191,720	Chr 25: 37,192,502
ChrB1_38976563	B1			20.32	0	B1: 38,976,563	Chr 8: 22,766,523	Chr 25: 37,435,625
ChrB1_39401113	B1			19.41	0	B1: 39,401,113	Chr 8: 22,400,723	Chr 25: 37,774,501
ChrB1_39665480	B1			12.34	0	B1: 39,665,480	Chr 8: 22,151,140	Chr 25: 38,010,313
ChrB1_39873803	B1			18.52	0	B1: 39,873,803	Chr 8: 21,963,551	Chr 25: 38,177,122
ChrB1_40183201	B1			11.53	0	B1: 40,183,201	Chr 8: 21,646,142	Chr 25: 38,463,167
ChrB1_41254204	B1			16.10	0	B1: 41,254,204	Chr 8: 20,606,466	Chr 25: 39,390,344
ChrB1_41701042	B1			16.40	0	B1: 41,701,042	Chr 8: 20,198,053	Chr 25: 39,762,212
ChrB1_41772882	B1			17.89	0	B1: 41,772,882	Chr 8: 20,127,674	Chr 25: 39,821,805
ChrB1_41799674	B1			10.02	0	B1: 41,799,674	Chr 8: 20,104,102	Chr 25: 39,846,768
ChrB1_41829103	B1			17.00	0	B1: 41,829,103	Chr 8: 20,085,589	Chr 25: 39,870,873
ChrB1_42060627	B1			2.86	0	B1: 42,060,627	Chr 8: 19,843,732	Chr 25: 40,092,520
FCA559	B1		381.0	17.33	0	unknown	Chr 8: 19,546,837	Chr 16: 25,388,799
ChrB1_53277193	B1			18.77	0	B1: 53,277,193	Chr 8: 19,709,004	Chr 16: 25,555,047
ChrB1_53355916	B1			13.86	0	B1: 53,355,916	Chr 8: 43,120,843	Chr 16: 25,623,485
ChrB1_53581031	B1			14.81	0.02	B1: 53,581,031	Chr 8: 42,881,429	Chr 16: 25,839,630
CFA16:29.97	B1			14.09	0.03	unknown	Chr 8: 38,458,010	Chr 16: 29,972,613
FCA23	B1	83.6	463.3	11.90	0.05	Un: 77,729,549	Chr 8: 31,307,618	Chr 16: 32,706,503
FCA809	B1	84.5	476.7	12.43	0.05	B1: 68,657,050	Chr 8: 27,646,753	Chr 25: 32,866,052
FCA811	B1	84.5	488.6	1.15	0.10	B1: 68,391,021	Chr 8: 27,433,306	Chr 25: 33,063,420
FCA700	B1	108.1	571.9	7.24	0.10	B1: 74,811,696	Chr 8: 174,967,845	Chr 25: 27,356,441

[a] Markers are shown in genomic order along the domestic cat chromosome B1, on the basis of the most recent genetic linkage (GL) (MENOTTI-RAYMOND *et al.* 2009) and radiation hybrid (RH) (DAVIS *et al.* 2009) maps. Markers with prefix "chrB1" represent loci that were developed for fine mapping after multipoint linkage analysis demonstrated that FCA23 and FCA519 flanked the *Ticked* interval.

[b] Positions of markers with a blank in these columns were not determined in those versions of the RH or GL maps.

[c] Peak LOD score and estimated recombination fraction (θ) for linkage between each polymorphic marker and the *Ticked* locus.

[d] Position in the domestic cat whole genome sequence assembly as visualized in the GARFIELD browser (PONTIUS and O'BRIEN 2007). "Un" refers to a sequence block unassigned to a chromosome.

[e] Columns 8 and 9 show the positions in the human (HSA build 36) (http://www.ncbi.nlm.nih.gov/projects/mapview/stats/BuildStats.cgi?taxid=9606&build=36&ver=3) and dog (CFA build 2) (http://www.ncbi.nlm.nih.gov/projects/mapview/stats/BuildStats.cgi?taxid=9615&build=2&ver=1) genome assemblies found by BLAT analysis to be orthologous to sequences flanking the respective domestic cat markers.

TABLE 3

Summary of dominance and epistasis relationships leading to genotype–phenotype correspondence at the domestic cat pattern-forming loci *Ticked* and *Tabby* (see text for details)

Composite genotypes at the *Ticked* (*Ti*) and *Tabby* (*Ta*) loci	Resulting phenotype
Ti^A/Ti^A; __/__	Abyssinian or "ticked" (plain agouti coat)
Ti^A/Ti^+; __/__	Banded legs and tail, along with faint body stripes on an otherwise plain agouti coat
Ti^+/Ti^+; $Ta^M/$__	Mackerel tabby (vertically striped pattern)[a]
Ti^+/Ti^+; ta^b/ta^b	Blotched tabby (circular markings and broad stripes)

[a] One or more modifier genes transform the "mackerel" striping into a "spotted" pattern (previously attributed to the T^s allele in the classical single-locus model).

Since our results seem to be discrepant with those presented by Lyons *et al.* (2006), who reported the mapping of *Tabby* to the same location that we have defined as the *Ticked* locus, we offer the following clarification. Lyons *et al.* (2006) mapped *Tabby* under the assumption that a single locus was responsible for all coat pattern phenotypes in the domestic cat. Their utilization of a single pedigree that segregated for all but four meiotic events for the ticked phenotype identified the B1 locus that we report here to be responsible for the alleles Ti^A (Abyssinian) and Ti^+ (non-Abyssinian), and have elected to call the *Ticked* locus. The results of the two studies are therefore congruent, but we show that two different genetic loci are implicated in this particular set of coat patterning variants. In this context, we point out that the name *Tabby* should be applied to the locus on A1, which specifies the mackerel and blotched alleles, as these phenotypes are classically identified with the *Tabby* locus.

In Table S3, we list all the human genes (human genome build 36.3) located in the syntenic segments corresponding to the *Tabby* and *Ticked* linkage intervals. For the case of *Tabby*, we noted that the interval includes the gene *AP3S1*, which may be a good candidate since the AP3 complex transports pigment and the gene *AP3B1*, encoding another protein in this complex, is mutated in the hypopigmentation-inducing Hermansky-Pudlak syndrome type 2 (Dell'Angelica *et al.* 1997). Another possible candidate gene for *Tabby*, *TYRP1*, is involved in dog coat color phenotypes (Schmutz *et al.* 2002; Cargill *et al.* 2005) and maps to dog chromosome 11, 20 Mb distal from the conserved syntenic canine interval identified in Table 1. However, in the domestic cat *TYRP1* has been mapped to chromosome D4, and is associated with *brown* and *cinnamon* coat color phenotypes (Lyons *et al.* 2005; Schmidt-Küntzel *et al.* 2005), and not to patterning traits such as *Tabby*. Even though the actual genes corresponding to *Tabby* and *Ticked* have not been identified at the molecular level, the clarification that multiple loci are involved and the delimitation of two implicated regions enable the design of detailed studies targeting the iden-

tification and characterization of these loci and their functions.

We hypothesize that mammalian patterned coats are formed by two distinct processes: (i) a spatially oriented developmental mechanism that lays down a species-specific pattern of skin cell differentiation; and (ii) a pigmentation-oriented mechanism that uses information from the preestablished pattern to regulate the synthesis of particular melanin profiles. Our results, in combination with phenotype-based observations, indicate that the *Tabby* locus is involved in establishing the shape of the pattern (process "i" above), and so are the modifier loci that produce a spotted coat. The *Ticked* locus may control process "ii" defined above, *i.e.*, the coupling between pigmentation pathways and the preexisting pattern laid down by *Tabby*, so that variants may exhibit differing amounts of pigmentation on the coat areas destined to be spots or stripes. As an alternative hypothesis, however, the *Ticked* locus may also be involved in process i, affecting the shape of markings, by leading to progressively thinner and more numerous stripes that lead them to "disappear" amid the agouti banding of individual hairs. This idea fits the observation of very thin but discernible flank stripes in heterozygous animals Ti^A/Ti^+.

The logic behind this two-step process stems from observations such as the following: (a) coat patterns are species specific, with instances of intraspecific polymorphism that also seem to be heritable; (b) many variants affect the spatial conformation of the pattern (process i), but do not seem to affect the mechanism that "reads" this pattern to promote differential pigmentation (process ii) (*e.g.*, the case of the domestic cat mackerel and blotched variants); (c) conversely, there are variants that affect pigmentation pathways (*e.g.*, X-linked *Orange* in domestic cats; Schmidt-Küntzel *et al.* 2009; melanism in several species) but do not change the underlying pattern, which remains constant and often visible in a different color; (d) the instructions to produce darker pigment on stripes/spots overrides the dorsal-ventral patterning often seen on mammalian coats (*e.g.*, the black stripes present on the whitish tiger

ventrum); and (e) two felids [lion (*Panthera leo*) and puma (*Puma concolor*)] exhibit a developmentally regulated fading of body markings, which are present in juveniles and essentially disappear in adults. The latter observation suggests that it is process ii that is developmentally regulated, gradually decoupling the pigmentation pathways from the underlying pattern (which was evidently formed in the juvenile). Many such comparisons are possible within and among the extant 37 felid species, highlighting the potential of this mammalian family as a model for investigating the evolutionary genetics of coat pattern formation. Although the understanding of the molecular basis of this phenomenon is still in its infancy, we propose that comparative genetic analyses of the domestic cat and its wild relatives hold promise for unraveling these complex and potentially revealing developmental pathways for mammals in general.

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GENETICS

Defining and Mapping Mammalian Coat Pattern Genes: Multiple Genomic Regions Implicated in Domestic Cat Stripes and Spots

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TABLE S1

Primers used for fine-mapping of the *Ticked* and *Tabby* loci

Ticked locus

Marker	Repeat*	Product Size (bp)	Forward Primer	Reverse Primer
chrB1_36137001	TG(18)	181	TAAACATGAGGGGTGCCAGT	CGGATTTTCAACTGCACAGA
chrB1_38697912	GATA(11)	243	AAACAAAGGAAAACATGAAACTG	GGGAGATCTGCAGCTACTTGA
chrB1_38976563	CA(22)	171	TACCTGGAATCATGCGTTGA	CCTGAATCCAGAGACGATGAG
chrB1_39401113	TC(21)	160	GGTTCATGAAATCAACCTCCA	GAGAAAGGGCTAAAGGGGATT
chrB1_39665480	GA(20)	176	CACCCAAACTTTTGATGTTCC	CCTGGAGCCTGTTTCAGATT
chrB1_39873803	GT(20)	248	GGCATGGTAGCTGTCAAGGT	GGGGGATGGTCTACTGGTCT
chrB1_40183201	GT(22)	202	TTGGCTGAATTCTCTCACCC	ACATAACCATCCTCCCCACA
chrB1_41254204	AC(20)	224	TGATCGAAGCATACAAGGCA	GTGGAACTGGTGGAGGTGTT
chrB1_41701042	AC(24)	320	CCAGTACCTGGAACAAAGGC	AATGCCATGGAGAGACCAAC
chrB1_41772882	AC(18)	176	AAAATTGTGATGGTCGCACA	GGTCATGCAGGAGAATGGTT
chrB1_41799674	GA(16)	169	GGTGGGGGTGAGGATCTAGT	GCCTGCTTTGGATTCTGTGT
chrB1_41829103	GA(19)	209	TCAGGATCTTGAGATGGAGGA	AGTTTCTTGGCTTGCAGCTC
chrB1_42060627	CA(13)	301	GGTGAAGAAATGGGCAAAAG	CCATTCGATTTGGTTTTTGG
chrB1_53277193	AAG(18)	309	TGTGCTGAGTATGGAGCCTG	TTTGGACCATCTTGGGTTGT
chrB1_53355916	AC(21)	234	ATGCCTGCCCTCTAAGGTTC	AGTCCCCGTACACACAGGAC
chrB1_53581031	AC(18)	224	AACAAATCCCTGCCTCTGTG	CAGTGAGGCCTTCTCTGACC
CFA16:29.97	AC(31)	233	TGAATCAACACCTAGGCCATT	GGACACCCCTAAGTGACACG

Tabby locus

Marker	Repeat	Product Size (bp)	Forward Primer	Reverse Primer
Hsa5:179.47	TG(14)	250	GCTTCCCCTTGGACACAGTA	TGGAGAAAAGATGCCTCAGC
FLT4	GA(15)	228	CTGTTGTCCTCTCTCTGCCC	TCAACACAGAGCCCTACACG
Hsa5:178.67	CA(15)	247	CAATGCATTCTCACACTTGC	TGGACTCAGGAACACAGTGC
Col23A1	AAAC(6)	226	TGGAGCCTGCTTGAAACTCT	TCATCCTGCCACCCTCTTAC
YTHDC2	CA(26)	208	AAAAAGCCACTGGGGAAACT	TCCTTGCGTTTTTGGATTTC
Cfa11:7.68	TTCT(14)	247	CCTGAAGCCCAGTGGAACTA	CCCCTGTGCTGAATTCTTTC
Hsa5- 116.83	CA(18)	195	CCACGTCAAGCTCTGCATT	GCCAGCCTGGACATAAACAT
Hsa-5- 118.59	GT(21)	250	TGTTCAGTTGGTTCTTTCTGGA	AAGCCCCCAGATTTTACTCA
Hsa5-118.72	CT(10)	231	TGGGCTCTGTGCTGACAGT	CGGCTCTGATCTCTGAGGAC
Cfa11:11.26	CA(22)	224	ATAACTGGTGCCCATTCCTG	TGCTGTAGAGGCTATCCAGATG
Cfa11:12.55	GT(18)	201	AAGCATGAAATCAACCTTTGTG	CTGTTGCCTGTTAGGTGAAAGA

* Indicates number of repeat units in the microsatellite that produces a PCR product specified in column 3.

TABLE S2

Exclusion mapping of the *Ticked* locus to the *Tabby* genomic region

Marker	Cat Chr.	Cat RH Map Position	Values of θ							
			0.05	0.1	0.15	0.2	0.25	0.3	0.35	0.4
FCA083	A1	1051.5	-24.55	-15.09	-9.93	-6.59	-4.27	-2.63	-1.48	-0.73
FCA1054	A1	954.8	-15.65	-9.07	-5.56	-3.33	-1.84	-0.84	-0.21	0.12
FCA566*	A1	755.7	-11.11	-5.81	-3.07	-1.43	-0.4	0.19	0.46	0.48
Hsa5:179.47*	A1		-12.09	-6.46	-3.53	-1.73	-0.58	0.12	0.48	0.57

* With demonstrated linkage to the *Tabby* locus (see Table 1)

Note: LOD scores obtained from genomic mapping of *Ticked* pedigree to markers with demonstrated linkage to the *Tabby* region (see Table 1). The marker with prefix "Hsa" was developed for fine mapping of the *Tabby* locus (Table 1) and was not mapped in the cat RH map [DAVIS *et al.* 2009]. LOD scores are presented for multiple values of recombination fraction (θ) with respect to the *Ticked* locus.

TABLE S3

Human genes from genome build 36.3 that may be located in the segments syntenic to the linkage intervals for the *Tabby* and

***Ticked* loci.**

Locus	Human Chr.	Gene Symbol	Gene Name
Tabby	5	*YTHDC2*	YTH domain containing 2
Tabby	5	*KCNN2*	potassium intermediate/small conductance calcium-activated channel, subfamily N, member 2
Tabby	5	*TRIM36*	tripartite motif-containing 36
Tabby	5	*PGGT1B*	protein geranylgeranyltransferase type I, beta subunit
Tabby	5	*CCDC112*	coiled-coil domain containing 112
Tabby	5	*FEM1C*	fem-1 homolog c
Tabby	5	*TICAM2*	toll-like receptor adaptor molecule 2
Tabby	5	*TMED7*	transmembrane emp24 protein transport domain containing 7
Tabby	5	*CDO1*	cysteine dioxygenase, type I
Tabby	5	*ATG12*	autophagy related 12 homolog
Tabby	5	*AP3S1*	adaptor-related protein complex 3, sigma 1 subunit
Tabby	5	*LVRN*	laeverin
Tabby	5	*COMMD10*	COMM domain containing 10
Tabby	5	*SEMA6A*	sema domain, transmembrane domain (TM), and cytoplasmic domain, (semaphorin) 6A
Tabby	5	*DTWD2*	DTW domain containing 2
Tabby	5	*DMXL1*	DMX-like 1
Ticked	8	*INTS10*	integrator complex subunit 10
Ticked	8	*LPL*	lipoprotein lipase
Ticked	8	*SLC18A1*	solute carrier family 18 (vesicular monoamine), member 1
Ticked	8	*ATP6V1B2*	ATPase, H+ transporting, lysosomal 56/58kDa, V1 subunit B2
Ticked	8	*LZTS1*	leucine zipper, putative tumor suppressor 1
Ticked	8	*GFRA2*	GDNF family receptor alpha 2
Ticked	8	*DOK2*	docking protein 2, 56kDa
Ticked	8	*XPO7*	exportin 7
Ticked	8	*NPM2*	nucleophosmin/nucleoplasmin, 2
Ticked	8	*FGF17*	fibroblast growth factor 17
Ticked	8	*EPB49*	erythrocyte membrane protein band 4.9 (dematin)
Ticked	8	*FAM160B2*	family with sequence similarity 160, member B2
Ticked	8	*NUDT18*	nudix (nucleoside diphosphate linked moiety X)-type motif 18
Ticked	8	*HR*	hairless homolog
Ticked	8	*REEP4*	receptor accessory protein 4
Ticked	8	*LGI3*	leucine-rich repeat LGI family, member 3
Ticked	8	*SFTPC*	surfactant protein C

Ticked	8	*BMP1*	bone morphogenetic protein 1
Ticked	8	*PHYHIP*	phytanoyl-CoA 2-hydroxylase interacting protein
Ticked	8	*MIRN320A*	microRNA 320a
Ticked	8	*POLR3D*	polymerase (RNA) III (DNA directed) polypeptide D, 44kDa
Ticked	8	*PIWIL2*	piwi-like 2
Ticked	8	*SLC39A14*	solute carrier family 39 (zinc transporter), member 14
Ticked	8	*PPP3CC*	protein phosphatase 3 (formerly 2B), catalytic subunit, gamma isoform
Ticked	8	*SORBS3*	sorbin and SH3 domain containing 3
Ticked	8	*PDLIM2*	PDZ and LIM domain 2 (mystique)
Ticked	8	*KIAA1967*	KIAA1967
Ticked	8	*BIN3*	bridging integrator 3
Ticked	8	*EGR3*	early growth response 3
Ticked	8	*PEBP4*	phosphatidylethanolamine-binding protein 4
Ticked	8	*RHOBTB2*	Rho-related BTB domain containing 2
Ticked	8	*TNFRSF10B*	tumor necrosis factor receptor superfamily, member 10b
Ticked	8	*TNFRSF10C*	tumor necrosis factor receptor superfamily, member 10c
Ticked	8	*TNFRSF10D*	tumor necrosis factor receptor superfamily, member 10d
Ticked	8	*TNFRSF10A*	tumor necrosis factor receptor superfamily, member 10a
Ticked	8	*CHMP7*	CHMP family, member 7
Ticked	8	*HOOK3*	hook homolog 3
Ticked	8	*FNTA*	farnesyltransferase, CAAX box, alpha
Ticked	8	*SGK196*	protein kinase-like protein SgK196
Ticked	8	*HGSNAT*	heparan-alpha-glucosaminide N-acetyltransferase

FIGURE S1.—Graphic depiction of Pedigree 2, developed for this study to map the domestic cat 'spotted' coat color variant. Dark symbols indicate spotted or mackerel phenotypes, while white symbols indicate blotched phenotypes. Individuals bearing both colors indicate inferred heterozygotes, all of which exhibited mackerel/spotted phenotypes. Numbers indicate the individual ID for each member of the pedigree.
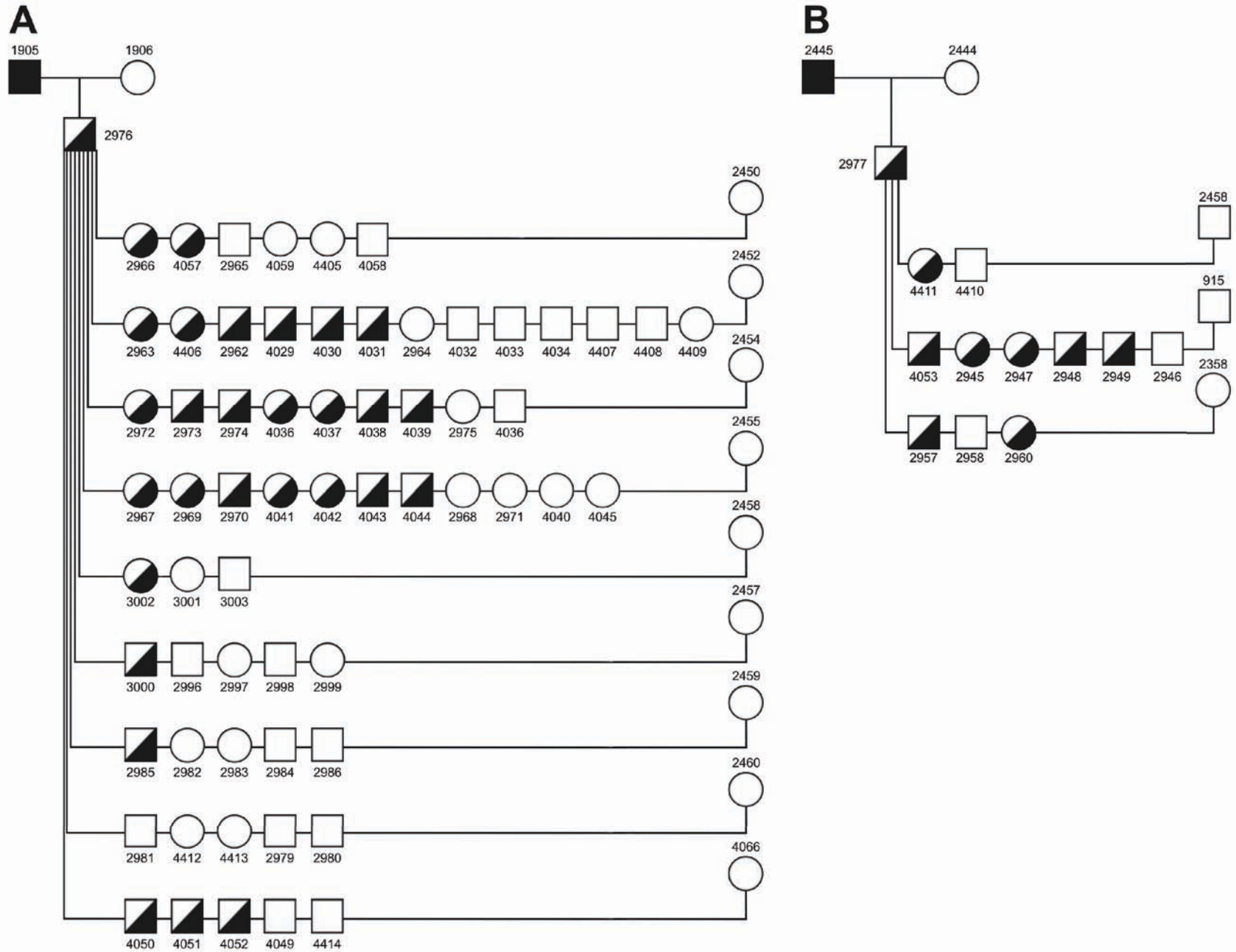
FIGURE S2.—Graphic depiction of Pedigree 3, developed for this study to map the domestic cat 'ticked' or 'Abyssinian" coat color variant. Dark areas in the symbols indicate the presence of an Abyssinian (Ti^A) allele, while fully white symbols indicate non-Abyssinian phenotypes (*i.e.* mackerel or blotched). Individuals bearing both colors indicate inferred heterozygotes, all of which exhibited a phenotypes denoting the presence of the semi-dominant Abyssinian allele (see text for details). Numbers indicate the individual ID for each member of the pedigree. Panels A and B represent two sub-pedigrees centered on two different F1 male individuals (2976 and 2977, respectively).