REPORT

RUSSIAN BLUE CAT GENETIC DIVERSITY PROJECT

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The Russian Blue cat breed is thought to trace its origin to the Russian port of Arkhangelsk on the White Sea from cats brought to England and other parts of Europe by sailors. Russian Blue cats are characterized by their short, dense silver-tipped blue fur, blue nose and paw pads, and vivid emerald-green eyes. Establishment of breed status dates to early 1900's which makes the Russian Blue an older breed, relative to many other modern cat breeds. With declining breeding population after World War II, Russian Blues were outcrossed to cats with similar phenotypes such as the British Shorthair, blue-point Siamese and even blue cats from Finland, with distinct lineages developing in England and Scandinavia. In the United States, lineages from England and Scandinavia were combined. Today's Russian Blues thus reflect some level of introgression from a few other breeds. From the outcrossing program, variations of Russian Blues have also been produced that are full-color (Russian Black) or all-white (Russian White). However, only cats that meet the breed standards for Russian Blues can be registered as such with most breed organizations.

Concern for the present and future genetic health of the breed prompted the Russian Blue Breeders Association from the UK to organize a genetic diversity study that sought to include cats representative of the different lineages within the breed. The objectives of the study were a) to analyse genetic variation for single nucleotide polymorphisms (SNPs) and microsatellites (STRs), phenotypic traits (color and hair length) and possible genetic defects that could be present in the breed (PKD, PK-Deficiency, PRA-CEP290), and b) to provide information and recommendations to improve the genetic health of the breed. A central issue concerns the impact of breed management versus outcrossing as a means to effect changes in the genetic make-up of the breed while maintaining phenotypic standards.

DNA sampling

DNA samples (n= 52) were obtained from buccal swabs provided by owners in the UK and submitted to the VGL for testing between June, 2015 and April, 2016. Four of these samples were Russian Whites and two were Russian Blacks. DNA was extracted from all samples according to standard protocols used by the VGL.

DNA Testing

DNA samples were assayed for 136 SNPs, 35 STRs, 6 coat color genes, hair length and 3 disease genes as detailed below (Table 2). The anonymous SNP and STR markers, which were validated in Dr. Leslie Lyons laboratory and the VGL, provided data for genetic diversity analysis. The two types of markers are different in that for SNPs only two variants can be present in each marker while each STR locus can have more than two variants. The use of both types of markers provide a more comprehensive view of cat genome. The phenotypic traits (coat color and hair length) and genetic disease testing are based on published causative mutations.

Table 2. Markers used for assessment of genetic diversity, phenotypic traits and genetic disease.

SNPs					
A1_10141047	A3_99507784	B4_149532846	D1_105498119	D4_42000379	F1_82068276
A1_133621071	B1_10420438	B4_21098349	D1_10789012	E1_130875919	F1_82716202
A1_151648701	B1_12214271	B4_255106	D1_11484008	E1_131587399	F1_91517402
A1_175780586	B1_195678303	B4_3093827	D1_117527468	E1_3912105	F2_26886470
A1_208054462	B1_199564532	B4_40319102	D1_125811329	E1_4114158	F2_38395360
A1_223501140	B1_202966562	B4_47638578	D1_126256993	E1_48228153	F2_46855978
A1_223506906	B1_54775572	C1_116355295	D1_126847301	E1_48700963	F2_68572596
A1_235579538	B1_80161671	C1_123164748	D1_15984279	E1_5453028	F2_74863327
A1_27523501	B1_88148379	C1_181852965	D1_16242433	E2_22632289	F2_78303221
A1_69424718	B2_138312489	C1_190502133	D1_18390852	E2_34027888	F2_79632602
A1_7429296	B2_146660650	C1_215441574	D1_18570323	E2_35914023	F2_8427817
A1_8742286	B2_41509834	C1_24148281	D1_66177762	E2_36986631	
A2_152258936	B2_45093345	C1_28702055	D2_1020904	E2_38860686	
A2_201526186	B2_6949528	C1_34981315	D2_105772916	E2_65436639	
A2_202225770	B3_104483970	C1_396397	D2_1752007	E2_7950477	
A2_44241149	B3_111000326	C1_44520932	D2_56777338	E2_8422942	
A2_554046	B3_13666494	C1_52456776	D2_717969	E3_36044809	
A3_101420069	B3_39203469	C2_147124460	D2_74293444	E3_55434272	
A3_11480952	B3_51317931	C2_150774106	D2_91989307	E3_67006512	
A3_12082294	B3_77094074	C2_156491175	D3_103840114	F1_20309325	
A3_130195244	B4_105706694	C2_187325	D3_122502120	F1_21799641	
A3_159537633	B4_142658074	C2_262401	D3_1810839	F1_26100599	
A3_162208567	B4_144693308	C2_5215469	D3_24565823	F1_27124984	
A3_38781591	B4_146486983	D1_101321498	D3_24823793	F1_38051725	
A3_75156179	B4_147206961	D1_104941557	D4_41078218	F1_565223	
STRs					
FCA005	FCA058	FCA096	FCA201	FCA262	FCA453
FCA008	FCA069	FCA097	FCA211	FCA290	FCA628
FCA023	FCA075	FCA105	FCA220	FCA293	FCA649
FCA026	FCA077	FCA123	FCA223	FCA310	FCA678
FCA035	FCA080B	FCA132	FCA224d	FCA391	FCA698
FCA043	FCA090	FCA149	FCA229	FCA441	
Color and Hair Length					
Agouti (ASIP)					
Brown					
Colorpoint					
Dilute					
MC1R					
Long Hair					
Genetic Disease					
Polycystic Kidney Disease (PKD)					
Pyruvate Kinase Deficiency, (PKDef)					
Progressive Retinal Atrophy, (PRA-CEP290)					

Genetic Variation Analysis

Genetic variation for SNPs and STRs was assessed according to overall presence of allelic variants among loci screened (**genetic diversity**), average number of alleles, population inbreeding (Fis) and proportion of polymorphic loci for each individual (**heterozygosity**). Among the 52 cats submitted for testing, there were 8 sets of half-siblings and 1 set of full-siblings, as well as the 6 non-Russian Blue cats. Therefore, for breed-level genetic diversity analyses, the non-Russian Blue and 1-3 cats from sibships were removed such that only declared Russian Blue cats that were assumed not to be first-degree relatives according to parentage records were considered (N= 34). In addition, 3 samples failed to yield adequate DNA quality for the SNP-panel testing and were not considered for these analyses.

At the individual level, heterozygosity values were obtained for all cats to provide owners with an estimate of the amount of genetic variation in each cat. Comparisons were made to published data from the study by Kurushima et al. 2012. Because of the nature of SNP and STR markers (maximum of 2 variants vs typically several variants), diversity estimates are numerically different (larger for STRs than for SNPs) but both convey the same information about genetic variation.

Variation of phenotypic traits and genetic disease were summarized by observed alleles and their frequencies.

Results

Genetic Diversity – SNPs and STRs

Overall genetic diversity, average number of alleles and population-level inbreeding (Fis) for SNP and STR markers for 34 Russian Blues are summarized in Table 2. Compared to Kurushima et al. (2012), the UK Russian Blues sampled showed more genetic diversity and average number of alleles for both SNP and STR markers. The level of population inbreeding (*Fis*) was lower for SNPs but similar for STR markers. Both *Fis* estimates were positive which suggests non-random breeding in the population, with increased homozygosity, albeit at low level.

Table 2: Overall genetic diversity and population-level inbreeding (Fis) of Russian Blue cats for SNPs and STRs.

Estimate	**Present study (n=34)**	**Published Russian Blue (n=17)**	**Published Other Breeds**
SNP Genetic Diversity (136 markers)	0.26 ± 0.04	0.18	0.17 – 0.30
STR Genetic Diversity (34 markers)	0.52 ± 0.07	0.34	0.42 – 0.70
Average # of alleles (SNP)	1.79 ± 0.41	1.75	1.50 – 1.92
Average # of alleles (STR)	4.09 ± 1.16	3.84	2.42 – 7.24
Fis (SNP)	0.03± 0.02	0.16	-0.06 – 0.16
Fis (STR)	0.07 ± 0.2	0.063	-0.06 – 0.16

For each cat tested in the study (n = 52), heterozygosity ranged from 18 to 41% for SNP markers and from 34-66% for STR markers. Individual heterozygosity values and a ranking based on SNP-panel (N=49) is provided in Appendix 1. The ranking is based on the SNP panel because of the higher coverage and representation of the cat genome. However, this ranking also captures cats that have good levels of diversity based on the STR

panel. Based on the cats tested, 55% (27/49) have average or above average levels of diversity and among these, 14% (7/49) are in the most diversity category of 32% and above. Forty-five percent (22/49) of the cats have diversity levels that are below average and among these, 8 cats are at the bottom of the low end of the diversity distribution.

Phenotypic Traits

Genetic variation for coat color and hair length genes was evaluated for all sampled cats (N=52) in order to capture all information available but for description of the Russian Blue breed, only 34 cats were considered. Allele frequencies of phenotypic traits are summarized in Table 3 below.

Table 3. Allele frequency of variants for coat color, hair length and genetic disease.

Color	**Allele Freq. (%)**
Agouti (ASIP)	
Agouti (A)	0
Non-Agouti (a)	100
Extension (MC1R)	
Wild-type	100
Amber	0
Colorpoint	
Burmese	0
Siamese	0
Full Color	100
Brown	
Full Color	100
Brown	0
Cinnamon	0
Dilute	
Full Color	0
Dilute	100
Hair Length	
Shorthair	100
Longhair	0
Disease	
PKD	
Normal	100
PKD	0
PKDef-A	
Normal	100
PKDef	0
PRA	
Normal	100
rdAC (CEP290)	0

With respect to phenotypic traits, Russian Blue cats conformed to genetic expectations. All cats were homozygous for the non-Agouti allele “a” and therefore were all self-colored, not-tabby. They were also fixed for the wild-type allele in the Extension (MC1R) gene and therefore are all black-pigmented as a base color. Colorpoint (Burmese, Siamese) or Brown (Brown, Cinnamon) mutations were not observed. As expected, all cats were homozygous for the Dilute mutation, which accounts for the diluted coat color typical of the breed. None of the 4 longhair mutations were observed among animals tested.

In contrast, 2 Russian Black cats and 1 Russian White differed from the typical Russian Blues in the Dilute gene in that they possessed one copy of the normal non-dilute allele in the Dilute locus. While full color is expected for the Russian Black cats, presence of the non-dilute allele in the Russian White is masked by presence of the Dominant White gene.

Recessive long-hair alleles LHM1, LHM2, LHM3 and LHM4 were not detected in any of the 52 cats.

Genetic Disease

All cats tested clear of disease alleles for the three genetic defects screened: PKD, PK-Def and PRA-CEP290. If present in the UK Russian Blue population, alleles for any of these diseases would occur in frequency less than 1.4% (or less than 1 in 5000 individuals expected to be affected one of these diseases). These genetic defects are thus not a concern but any outside cat coming into the UK breeding pool should be screened to ensure that these genetic defects are not introduced into the population.

Discussion and Recommendations

The estimates obtained in this study for SNP and STR DNA markers based on 34 unrelated Russian Blue cats indicate that the UK cats have overall a more diverse genetic status than their counterpart in the United States. This could be due to some level of outcrossing to rebuild the breed after World War II which resulted in a higher level of genetic variation observed, despite selection to fix the phenotypic traits typical of the breed. Other contributing factors related to breeding schemes and efforts towards inbreeding avoidance, could also have contributed to the observed genetic variation among UK Russian Blues but this cannot be assessed by this study.

Compared to other breeds, our results place UK Russian Blues closer to more diverse or outbred breeds (for example, Norwegian Forest Cat, Siberian Cat, Exotic Shorthair) than to breeds that show significant signs of genetic depletion (for example, Burmese, Birman, Singapura).

In order to preserve and likely improve the genetic diversity in the UK Russian Blue breed the following recommendations are offered:

1. Maintenance of accurate pedigree records employing parentage verification using DNA markers to verify parentage of registered cats. As for other domestic species, parentage testing program is highly beneficial to help record the genetic history of pedigreed animals. Accurate records ensure greater rate of success of a managed breeding program for the breed.

2. Avoidance of breeding between close relatives. This will help reduce chances of homozygosity for deleterious, recessive genes that may be present in the breed and that could lead to emergence of

other genetic defects, or result in other problems such as, for example, reduced fertility. While the risk for the 3 common diseases tested here (PKD, PK-Def, PRA-CEP290) is low, other genetic defects not yet recognized could emerge through inbreeding. Other recessive diseases, if present, can be managed through appropriate mate selection without removal of carriers from breeding pool, which could result in genetic loss for the breed.

3. Selection of mating pairs based on genetic information that maximizes differences among mates. Routine screening for genetic diversity markers, either SNP or STR panels, can be used for comparison of prospective mating pairs to select those that are genetically more distinct from each other and could produce litters with increase diversity. A database for Russian Blue cats could be set up and used by breeders for this purpose. Increased use of genetic information in mate selection will help improve the distribution of genetic diversity across the breed. If of interest, the VGL can assist the UK RBBA with this program.

These are common-sense solutions that are designed to improve overall levels of heterozygosity at the individual level, which will then help improve the genetic health of the population. Outcrossing is also a viable approach to increase diversity but most likely not necessary at this time for the UK Russian Blues, based on the levels of diversity observed in this study.

Reference

Kurushima JD, MJ Lipinski, B Gandolfi , L Froenicke , JC Grahn, RA Grahn, LA Lyons (2013). Variation of cats under domestication: genetic assignment of domestic cats to breeds and worldwide random-bred populations. Anim Genet. 44(3):311-324.