

Investigation of a Genetic Etiology for Multiple Ocular Coloboma in the Captive Snow Leopard Population

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Background / Description

- Multiple Ocular Colobomas (MOC) in snow leopards were first reported in the 1960s but first studied and documented in 1976¹
- Clinical presentations are often characterized by:
 - Eyelid aplasia of the upper eyelid and trichiasis
 - Uveal colobomas
 - Microphthalmia or anomalous orbit development
 - Persistent pupillary membranes
 - Congenital cataracts
 - Corneal leukoma
 - Retinal Cysts and dysplasia
- Lesions affect each cat to varying degrees. Often surgical intervention is required, including:
 - Surgical lip-to-lid transposition
 - General surgery of upper eyelid agenesis
 - Cryoablation
- Snow leopards are the only non-domesticated species in which this disease has regularly been reported
- Between 2000 – 2020, 49 cases of eyelid colobomas were reported in the Snow Leopard Species Survival Plan (SSP) population²
 - 18.3% of the current SSP population is affected with eyelid colobomas
 - 26.0% of the current SSP population is affected with a congenital eye defect
 - In 44 cases studied, 39 resulted in needing surgical intervention³
- The etiology remains undetermined but a heritable component is suspected



Figure 1: A snow leopard cub following surgical correction of eyelid coloboma⁴. Located in the upper outside corners of the eye are scars due to surgical correction.



Figure 2: A snow leopard seen with scarring due to a lip-to-lid transposition surgery⁵. Around the outer and upper eye can be seen the consequences of coloboma surgery.

Objective

- To identify DNA variants in the genome of the snow leopard associated with embryonic development of the eye and correlated with disease presentation
- Long-term goal of genotyping the DNA variant in the captive population of snow leopards to predict future affected animals leading to a reduction and potential elimination of disease

Results

- The whole genome sequencing of 14 snow leopard has been completed and analysis is in process
- A segregation analysis is in process to determine mode of inheritance
- The prevalence of this disease in snow leopards has led the Species Survival Plan members to be more proactive

Mating Type		Litter Size	Offspring Outcome								
M	F		A			N			U		
			M	F	U	M	F	U	M	F	U
1800	2101	2	1						1		
		2	1	1							
		2	1							1	
1800	1739	2									3
1800	1650	3							1	2	
		3									2
2818	2829	2				1			2		
		2									1
		3	3								
			A			N			U		
M	F		M	F	U	M	F	U	M	F	U
2573	2662	2	2								
		3	1	2							
2219	2368	2							1	1	
2602	2368	2							2	2	
2219	2170	2									2
2421	2170	1							1		
			A			N			U		
M	F		M	F	U	M	F	U	M	F	U
2659	2563	2									
		3	1	2					1		
2659	2730	2							1	1	
3014	2830	1									1
		1									
		1		1							
2660	3017	1									1
2765	2714	1		1							
2232	2222	2				1			1		

Table 1: Partial segregation analysis using the snow leopard pedigree from the 2018 studbook and collected data from ophthalmic exams. Individuals that are unaffected by disease are indicated by a "N", individuals that are affected are indicated by an "A", and individuals with unknown disease status are indicated by an "U".

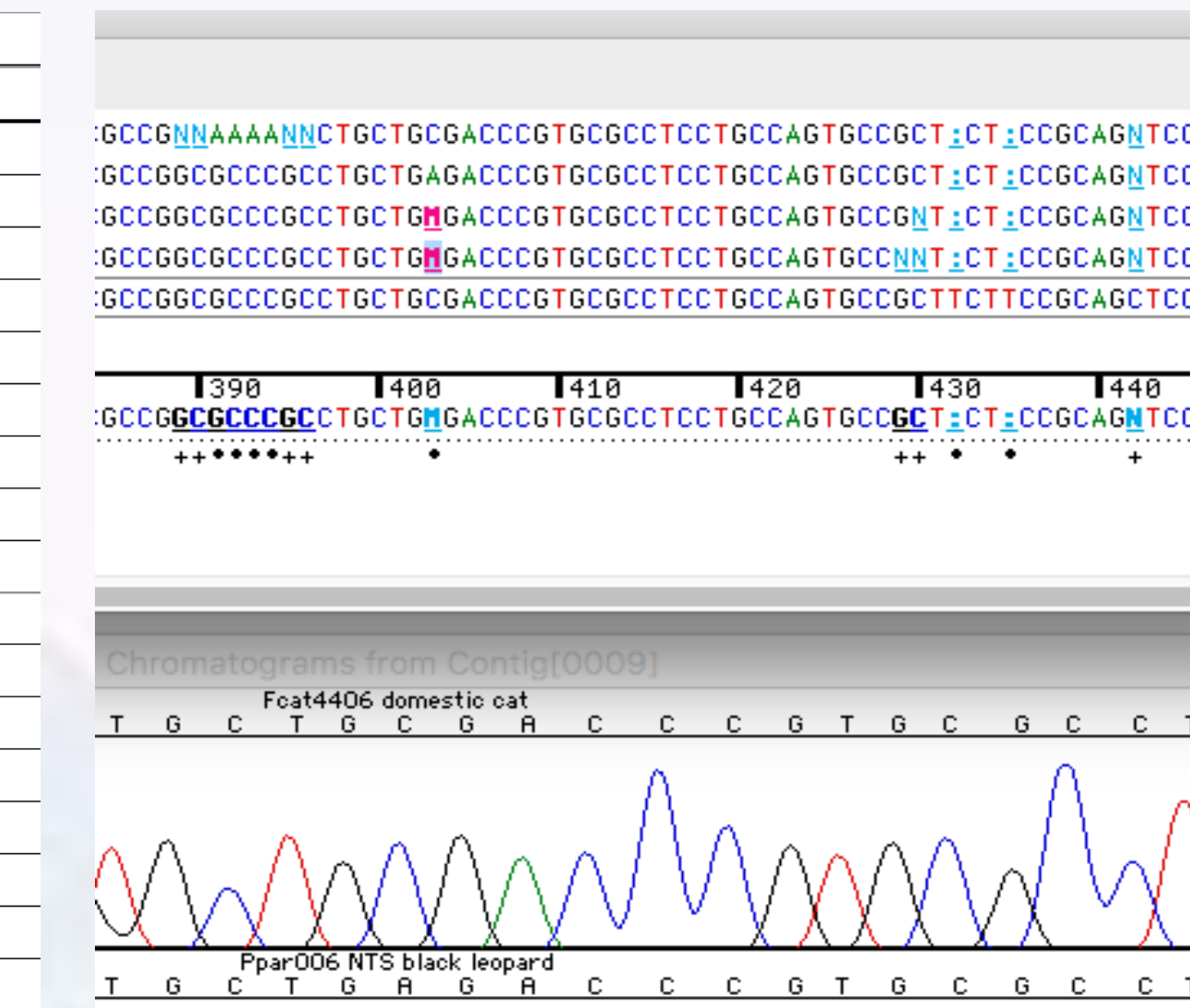
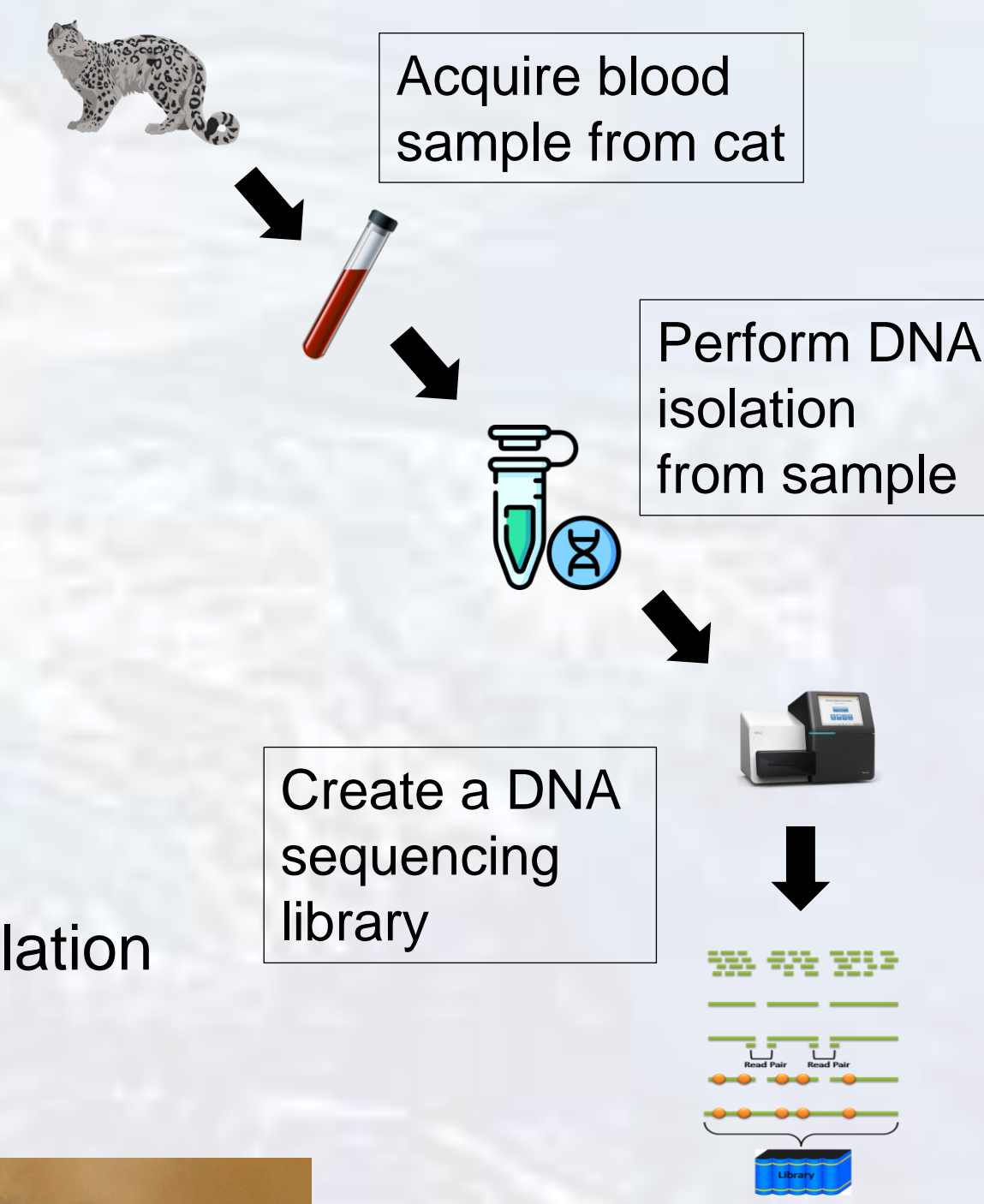


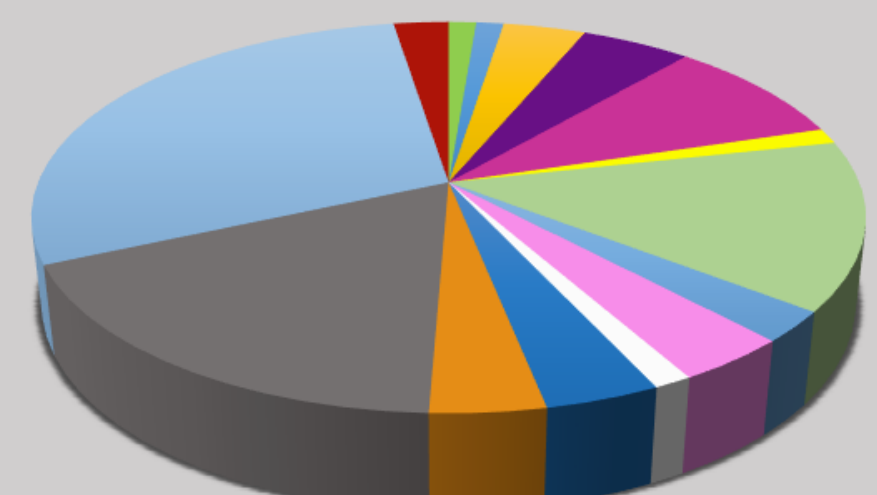
Figure 5: Sanger Sequencing of Amur Leopards. This image depicts the type of analysis used to validate candidate gene variants.

Materials and Methods

- Whole genome sequencing of affected and unaffected snow leopards
 - 14 snow leopard affected and unaffected
 - 65 additional wild felids
 - 404 domestic cats
- The processing for whole genome sequencing:
 - Blood sample from individual – DNA isolation
 - Create DNA sequencing library – conduct whole genome sequencing
 - Conduct sequence data processing using GATK best practices
 - Analyze variants for candidate causal mutation
- 37 eye exams performed on sampled snow leopards
- Studbooks and health records to determine possible mode of inheritance
 - Segregation Table
- Sanger sequencing to validate the genetic variance within the snow leopard population



Wild Felids Sample Demographic



Cheetah, Jungle Cat, Sand Cat, Black-Footed Cat, Fishing Cat, Eurasian Lynx, Iberian Lynx, Pallas' Cat, Lion, Jaguar, Leopard, Panther, Snow Leopard, Leopard Cat

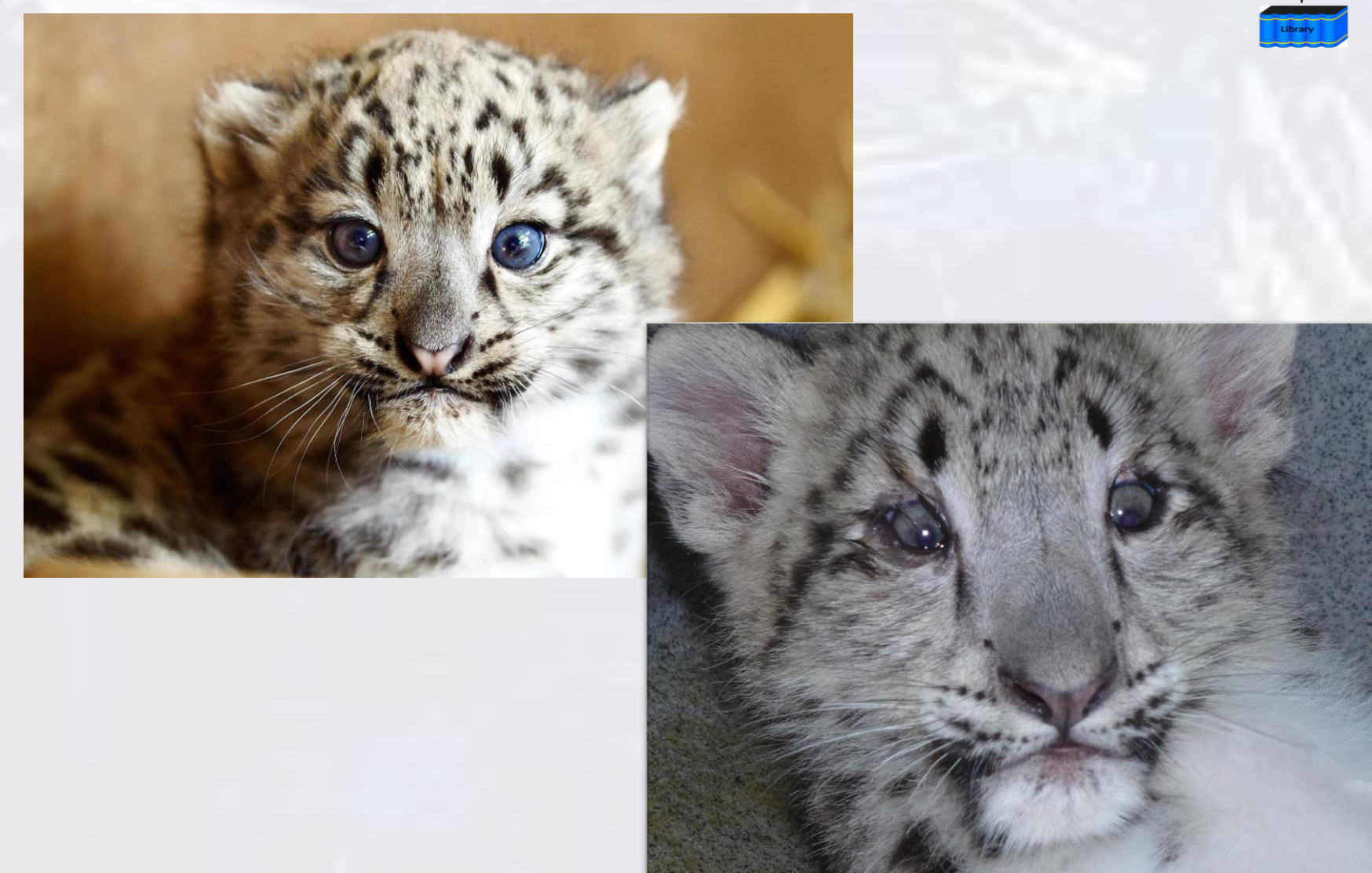


Figure 4: A comparison of snow leopard cubs without and with ocular coloboma of the upper eye lid^{4,6}. The cub on the left is a normal cub with complete closure of the upper eyelid and clear eyes. The cub on the right has ocular colobomas on the upper lid.

Conclusion / Future Goals

- The initial analyses of the whole genome sequencing data did not identify a simple Mendelian inheritance pattern for MOC
- What could this mean?
 - A genetic component is still considered likely
 - Multiple genes may be involved
 - Incomplete penetrance of the disease may be occurring
- Future goals:
 - In depth ophthalmology exams need to be performed on all newborn snow leopards
 - Breeding pairs that have been known to produce detrimentally affected offspring should not be bred together
- With the whole genome sequencing accomplished of 14 individuals, future analysis of other genetic variances in the snow leopard population will be conducted

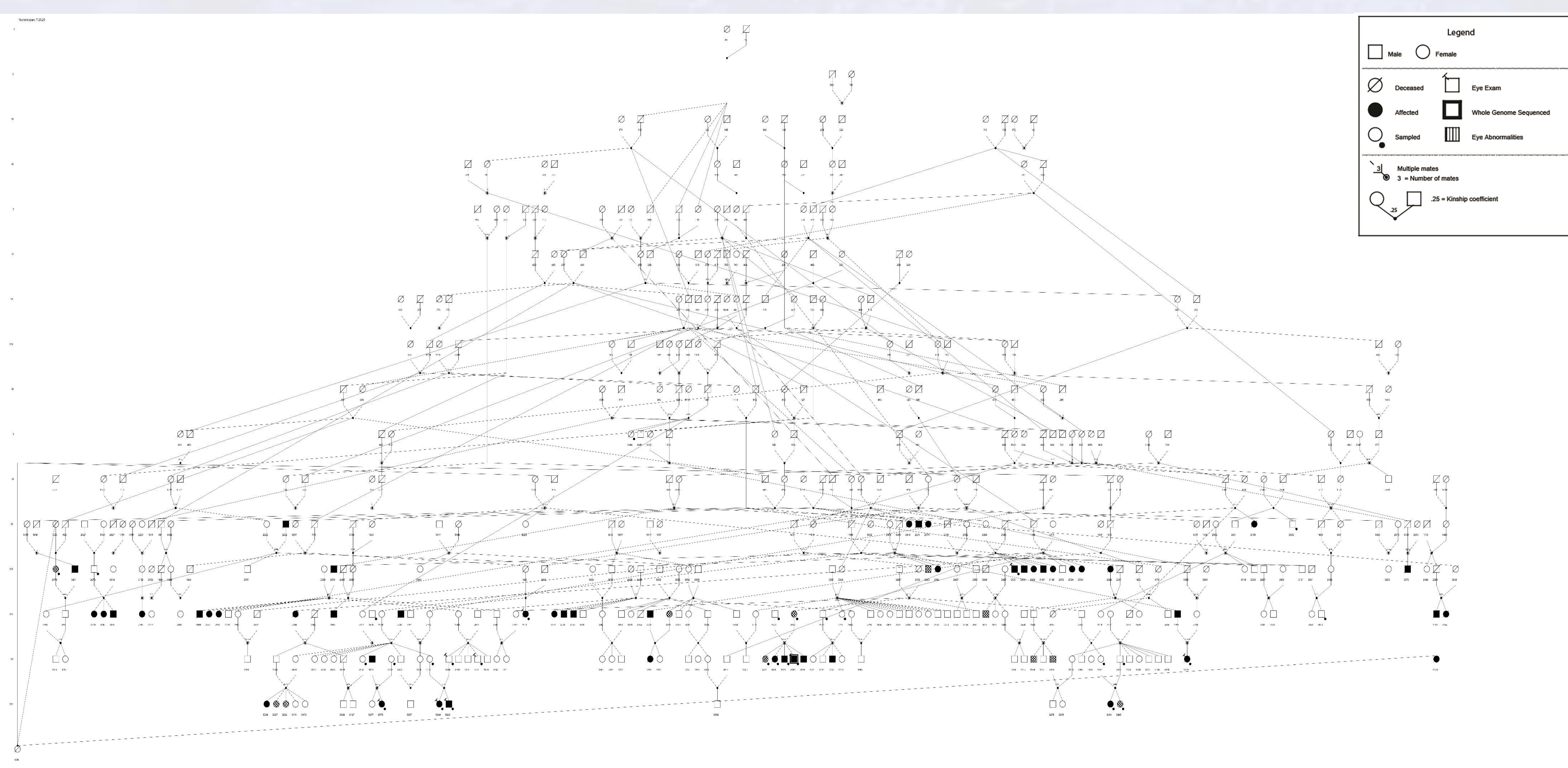


Figure 6: Snow leopard pedigree of all MOC affected individuals. Affected individuals of eyelid coloboma's are signified by a filled in circle/square. Individuals affected with other congenital eye abnormalities are indicated by slashed vertical lines. DNA samples are indicated by a small dot and deceased individuals are indicated by a single diagonal line. Individuals with a T-bar in the upper left corner have had an eye exam performed.

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