COLOR INHERITANCE AND INBREEDING IN AMERICAN MINK

by

Persia Carol Thapa

Submitted in partial fulfilment of the requirements

for the degree of Master of Science

at

Dalhousie University

Halifax, Nova Scotia

August 2023

This thesis is dedicated to my dearest husband,

Mr. Kalyan Mahat,

who fulfilled all my wishes, supported me and made me who I am today.

Also,

to the almighty Shree Pashupatinath for all the blessings.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

ABSTRACT

The intensive phenotypic selection commonly practiced in mink farms that is mostly color driven, might lead to the selection and mating of genetically similar individuals resulting in reduction of fitness traits. In this thesis, we analyzed color inheritance of four different color types, investigated the effects of inbreeding on reproductive traits, and estimated inbreeding coefficients using both pedigree (F_{PED}) and genomic information: runs of homozygosity (F_{ROH}) and excess of homozygosity (F_{HOM}). It was discovered that more than 75% of the time when the same color parents were mated, offspring of the same color was produced. The effect of F_{PED} on reproductive traits was negligible. Similarly, the average inbreeding was observed to be 0.28, -0.03, and 0.02 for F_{ROH}, F_{HOM}, and F_{PED} respectively. Short ROH segments were the highest in number and the longest segment was 88.58 Mb in length. These results indicated that inbreeding has been successfully avoided by the farm.

LIST OF ABBREVIATIONS AND SYMBOLS USED

%	Percent
A	Additive relationship matrix
AD	Aleutian disease
AWB	Average kit weight per litter at birth
AWW	Average kit weight per litter at weaning
BLUP	Best linear unbiased prediction
CCFAR	Canadian Centre for Fur Animal Research
CFC	Coancestry, inbreeding (F) and contribution
CV	Coefficient of variation
D	Dark
DE	Demi
DNA	Deoxyribonucleic acid
E	Expected homozygotes
EBV	Estimated breeding value
F	Inbreeding coefficient
F_{HOM}	Excess of homozygosity-based inbreeding coefficient
F_{PED}	Pedigree-based inbreeding coefficient
F_{ROH}	Run of Homozygosity-based inbreeding coefficient
g	Gram
GL	Gestation length
HOM	Excess of homozygosity
HWE	Hardy-Weinberg equilibrium
IBD	Identical-by-descent
Kb	Kilobase
Kg	Kilogram
LAUTO	Total length of the autosomal genome covered by SNPs
LB	Total number of kits alive after 24 hours of birth
LD	Linkage disequilibrium
LROH	Total length of an individual's ROH
LW	Total kits alive at weaning
M	Mahogany
Mb	Megabase
NAFA	North American Fur Auctions
O	Observed homozygotes
P	Pastel

ROH	Runs of homozygosity
SB	Survival rate at birth
SD	Standard deviation
SE	Standard error
SW	Survival rate at weaning
TB	Total number of kits born
WGS	Whole Genome Sequence
β	Regression coefficient
σ_a^2	Additive genetic variance
σ_e^2	Residual variance
σ_{pe}^2	Permanent environmental variance

ACKNOWLEDGEMENTS

I would like to express my heartfelt appreciation to my supervisor Dr. Younes Miar. Dr. Miar's support, guidance, and encouragement played a pivotal role in making my academic journey in Canada smooth and enjoyable. His belief and confidence towards me were crucial to keep me motivated. Dr. Miar's willingness to be available every time I had difficulty in my research is praiseworthy. His inputs and constructive criticisms were very valuable in my research and academic achievements. Overall, Dr. Miar has helped in strengthening my confidence and been a source of inspiration and motivation for my academic endeavors.

I would like to acknowledge the contribution of my supervisory committee Dr. Ghader Manafiazar and Dr. Sean Myles for their mentorship and valuable recommendations. Their views and opinions have been influential in my research journey. I am also extremely grateful to Dr. Duy Ngoc Do and Dr. Karim Karimi for their support and suggestions which were very valuable. Their willingness to assists me in every step of my research is admirable. Similarly, my lab members Guoyu Hu and Pourya Davoudi have helped me in so many aspects and have made my stay in Canada very smooth since the day of my arrival. I am grateful to their understanding and patience.

My dearest friend Mariana Ramapaul who I met unexpectedly and has been one of my greatest friends. Thank you for always being there for me, cheering me on during moments of doubt, and celebrating my achievements.

My dearest husband, my soulmate, who emotionally supported me and helped me in every possible way, I was and am forever grateful. My respected late grandparents, my

cheerleader my buwa Mr. Dadhiram Marasini and my food basket aama Mrs. Remakala Marasini, I miss you and I know you have been blessing me from heaven. My parents, Mr. Nigam Kumar Thapa and Mrs. Kalpana Marasini, who made me a strong woman I am today, there are no words to express my love and gratitude. My not so little brother Mr. Parishkrit Soyooj Thapa, I am proud to be your source of inspiration.

Finally, this research would not have been possible without the financial assistance of the funding agencies; Natural Sciences and Engineering Research Council (NSERC), Canada Mink Breeders Association, Nova Scotia Mink Breeders Association, Fur Commission USA, and Mink Veterinary Consulting and Research Service Ltd., and Mitacs. I would like to thank them and Dr. Miar for providing the funding to facilitate the exploration of new ideas through this research.

CHAPTER 1: GENERAL INTRODUCTION

1.1 INTRODUCTION

American mink (*Neogale vison*) is a semi-aquatic, carnivorous species belonging to the Mustelidae family and is bred primarily for fur (Milanović et al., 2013; Hansen, 2014). The fur produced by American mink is one of the most desirable furs due to the astonishing variation in color and high quality (Tamlin et al., 2009). The Canadian mink industry produced approximately 24 million pelts, which contributed nearly 1.23 billion dollars from 2010 to 2018 (Statistics Canada, 2018). Among the Canadian provinces, Nova Scotia has the highest number of mink farms and produced 259,400 pelts in 2020 (Statistics Canada, 2021). However, the decreasing trend of overall mink pelt production by 43% from 2018 to 2020 (Statistics Canada, 2021) and increasing demand for fur after COVID-19 pandemic, strongly suggests for development and improvement of the mink industry. This can be accomplished by implementing an optimal selection and mating strategy that involves mating of the parents as per the demand of coat color and minimizing the breeding of related individuals.

The improvement of fitness traits such as survival and reproduction are one of the major objectives of animal breeding as these traits are of economic importance and are intended to maximize the profit (Goddard, 2009). Similarly, boosting these traits are considered as a crucial aspect of mink production (Koivula et al., 2010; Cai et al., 2018; Karimi et al., 2018). Other than fitness traits, fur quality and coat color are also equally important in mink production because they influence the final economic value of fur (Valipour et al., 2022).

For instance, uniform coat colors such as black, white, and pastel are sold at higher prices compared to patterns due to its easily blending nature and can be dyed into any preferable colors as per the demand of the customer (Wang et al., 2022). In general, the mink industry is color-driven as the market is based on the demand for a specific color at a specific time.

The overall breeding objectives of the mink industry are fur quality, reproductive performance, body mass, and coat color (Cai et al., 2018; Valipour et al., 2022). Thus, selection practices such as line breeding and positive assortative mating are commonly used in the mink industry which can result in the depletion of reproductive performance and even negatively impact crucial traits stated above (Belliveau et al. 1999; Goddard, 2009; Thirstrup et al., 2014). This decline in reproductive performance might be due to inbreeding, which arises because of the mating between pair of individuals who are more related to each other than the average pairs within the population (Curik et al., 2014; Neaves et al., 2015). Inbreeding which increases homozygosity is not only responsible for the reduction of overall fitness traits (also known as inbreeding depression) but also loss of genetic diversity in the population (Falconer and Mackay, 1996). Genetic diversity is crucial in animal breeding as it allows genetic progress, survival, and adaptation (Spielman et al., 2004; Ellegren and Galtier, 2016).

Inbreeding and the effect of inbreeding on fitness traits can be estimated using pedigree information, commonly referred as F_{PED} and is considered as traditional method (Zhang et al., 2015). The estimation of inbreeding is based on the principle that two alleles present at a locus are Identical by Descent (IBD) and it refers to those homozygous alleles that are derived from the common ancestor (Zhang et al., 2015). The pedigree method suffers from drawbacks such as incomplete pedigree and assumption of unrelated founders which

frequently results into faulty inbreeding estimates (Lopes et al., 2013; Forutan et al., 2018; Alemu et al., 2021). However, the availability of genomic data has resulted into more accurate estimation of the genomic level inbreeding as it recognizes the relationship between animals in a population based on observed proportion of genome that is IBD (Lopes et al., 2013). Runs of homozygosity (ROH), is one of the estimates of genomic inbreeding where the inbreeding coefficient is estimated as F_{ROH} (Zhang et al., 2015). The estimation of inbreeding using ROH is commonly used in humans (Ceballos et al.,2018), cattle (Goszczynski et al., 2018), pigs (Xie et al., 2019), and horses (Santos et al., 2021).

This thesis begins by a detailed overview of coat color variation in American mink, inbreeding depression, and genomic inbreeding estimates followed by identifying the gaps in the knowledge in each study. These gaps were subsequently addressed in three different studies (chapters 3-5) with an aim to provide foundational understanding of the topics and offering basic knowledge on the subject matter in American mink. By analyzing real-world farm data, this thesis also aims to offer applicable and effective solutions that can be implemented by the farmers to address specific challenges and improve their operations. Finally, the conclusions and insights drawn from this study will probably influence future investigations eventually uplifting the overall mink industry.

1.2 OBJECTIVES

The objectives of this thesis were:

1) To investigate the crosses responsible for producing color types (Dark, Pastel, Demi, and Mahogany) and determine the allelic pair (homozygous or heterozygous) responsible for the color type. (**Chapter 3**).
2) To evaluate the effect of inbreeding (inbreeding depression) on reproductive traits: gestation length (GL), total born (TB), total kits alive after 24 hours of birth (LB), total kits alive at weaning (LW), survival rate at birth (SB), survival rate at weaning (SW), average kit weight per litter at birth (AWB) and average kit weight per litter at weaning (AWW). (**Chapter 4**).
3) To estimate the level of inbreeding using pedigree and genomic information (runs of homozygosity and excess of homozygosity). (**Chapter 5**).

CHAPTER 2: LITERATURE REVIEW

2.1 COAT COLOR VARIATION IN MINK

In the wild, American mink are commonly dark brown or black in color (Shackelford, 1948). The domestication of wild mink, which is considered to have happened approximately 120-150 years ago, is owed to the variations observed between wild and farm raised mink today (Kruska, 1996; Morris et al., 2020). The process of domestication is lengthy, which commonly involves selecting a few individuals and breeding them (Ujvari et al., 2018). Prasolova and Trapezov (2006) and Cieslak et al. (2011) suggested that when wild animals were domesticated for a prolonged period following artificial selection, their coat color also undergoes inevitable transformations, resulting in the emergence of new color variations. In general, with domestication, the variation in the phenotypes arises due to adaptation to the change in habitat, as well as varying human preferences (Voß et al., 2022).

The variation of coat color is one of the most important characteristics of American mink. Until now, more than 35 color variations and hundreds of color types after combination have been produced in American mink (Manakhov et al., 2019). Out of the total color variations seen in American mink, only eight have been studied using genomic information (Manakhov et al., 2022). The genes responsible for coat color variation in mink were differentiated into dominant and recessive mutant genes (Shackelford, 1949; Song et al., 2017), which might justify the variation of color observed after crossbreeding different color types. These coat color variations along with its quality plays an important role on the final economic value, and thus, considered as an important breeding objective

(Valipour et al., 2022). Furthermore, coat colors such as Black, Pastel, and Stardust are greatly valued and sold at higher prices because of the uniformity and unique natural color (Wang et al., 2022).

The understanding of color inheritance using both ancestral and genomic information is crucial for all animal breeding industries irrespective of the species. Coat color is considered as one of the primal indicators of breed composition in animals (Kuehn et al., 2018). The estimation of breed composition has been studied in dogs (Huson et al., 2010) and cattle (Li et al., 2020). The breed composition of an animal can be utilized to plan an improvement of the population (Gorbach et al. 2010). Especially in the case of American mink, breeders often aim to produce a specific color or color patterns. Thus, unraveling color inheritance can help the breeders to select the parents to produce the offspring with desired coat color.

2.2 INBREEDING

Inbreeding is defined as the mating of individuals who are more related to each other than the average population (Curik et al., 2014; Neaves et al., 2015). These related individuals share the same ancestral haplotype known as Identical by Descent (IBD), in the absence of recombination or mutation (Curik et al., 2014). The measure of inbreeding is the inbreeding coefficient (F) which is the probability that two alleles at a locus of an individual are IBD (Leutenegger et al., 2003). Using both pedigree and genomic information, inbreeding coefficient can be estimated for a population (Zhang et al., 2015).

2.3 MEASURES OF INBREEDING

2.3.1 PEDIGREE-BASED INBREEDING MEASURES

The pedigree-based inbreeding coefficient (F_{PED}) is based on the probability that the locus is IBD (Kardos et al., 2016). To estimate inbreeding based on pedigree information, either the path method or tabular method is used (Wang, 2011). The path method is preferred if the pedigree is simple (Wang, 2011) whereas, for an exceptionally large population with a complex pedigree, the tabular method is favored (Curik et al., 2014). In the tabular method, which is also known as the numerator relationship matrix or additive relationship matrix (A), the pedigree is converted into a covariance table that shows the coancestry (or kinship) between individuals (Cortés et al., 2010). Moreover, the diagonal element reveals the level of inbreeding of the individual, and the off-diagonal element shows the level of relationship between individuals (Cortés et al., 2010).

2.3.2 GENOMIC-BASED INBREEDING MEASURES

The use of genomic information allows for estimation of the realized proportion of the genome that the individuals share (Howard et al., 2017). When two segments of the genome that are identical from a common ancestor are inherited from each parent it is referred as autozygosity (Keller et al., 2012). Genomic measures of inbreeding are considered better estimates of autozygosity than pedigree method (Howard et al., 2017; Alemu et al., 2021).

A segment-based approach can be used to estimate genomic inbreeding. Inbreeding results in the long stretches of homozygous unbroken regions, known as Runs of Homozygosity (ROH) that are considered as good indicators of autozygosity (Baes et al., 2019). The

application of ROH was first implemented in the human population (McQuillan et al., 2008) and later in cattle population (Ferencakovic et al., 2011). Inbreeding calculated from ROH is widely used because it is considered as a powerful method to distinguish between recent and ancient inbreeding (Makanjuola et al., 2020). A short ROH and a long ROH justifies the distant and the recent inbreeding, respectively (Baes et al., 2019). The inbreeding coefficient estimated based on ROH (F_{ROH}) is calculated as (Zhang et al., 2015):

$$F_{ROH} = \frac{\sum LROH}{LAUTO},$$

where LROH is the sum of ROH lengths and LAUTO is the total length of the autosome genome. The use of ROH has been widely accepted in domestic animals where the studies are not limited to estimating F_{ROH} but also linking ROH to selection signatures, population history, understanding genetic architect of complex traits, and identifying candidate genes responsible for important traits (Curik et al., 2014; Dixit et al., 2020). This might be due to the characteristics of ROH such as: a) easy biological interpretation, b) can be differentiated for each chromosome, and c) no requirement for reference population (Curik et al., 2014).

Another genomic inbreeding coefficient can be calculated for each animal based on the excess number of observed homozygous genotypes (F_{HOM}) relative to the reference population (Addo et al., 2019). This measure of inbreeding is based on the excess of homozygosity where the reference population is expected to be a hypothetical ancestral population in the Hardy-Weinberg equilibrium (Carothers et al., 2006). The equilibrium maintained in the hypothetical ancestral population can be disrupted by factors such as selection, mutation, and inbreeding (Mayo, 2008). Inbreeding increases the proportion of

homozygotes at the expense of heterozygotes (Keller and Waller, 2002). Thus, the excess of homozygotes might have been produced due to inbreeding above the Hardy-Weinberg expectations in the population (Costantini et al., 2007). This genomic inbreeding (F_{HOM}) is calculated by using the following equation:

$$F_{HOM} = \frac{O - E}{1 - E}$$

where O and E are the observed and expected numbers of homozygous genotypes, respectively (Zhang et al., 2015).

2.3.3 ADVANTAGES AND LIMITATION OF DIFFERENT MEASURES OF INBREEDING

The traditional method used to estimate inbreeding using pedigree data suffers from limitations which are compensated by using genomic data. The pedigree data is often incomplete with errors, assumes that the founders are unrelated, and unable to capture the Mendelian sampling (Staples et al., 2014; Legarra et al., 2015), which might result in decreased accuracy of the inbreeding estimation (Leroy, 2014). The F_{PED} is based on the assumption that the founders are unrelated, and no inbreeding exists in the founder population (Hogg et al., 2019). However, this might not be the case as the founders might be inbred or related (Hedrick et al., 2016). The misidentification and missing animal in the pedigree record is not uncommon which might result in faulty inbreeding estimates. Hence, the true level of inbreeding can be underestimated (Forutan et al., 2018). However, some advantages of pedigree-based inbreeding are: 1) pedigree data is easily assessable, and 2)

pedigree-based inbreeding is simple, easy to understand, practical, and cost-effective measure for the farm.

Using molecular information can lead to more accurate estimation of the true relationship between individuals (Howard et al., 2017). Firstly, F_{PED} predicts the expected proportion of an individual's genome that is IBD, whereas genomic measures capture the Mendelian sampling variation, and hence, estimating the realized IBD (Nietlisbach et al., 2017). Secondly, recent and ancient inbreeding can be differentiated by the length of autozygous segments, which is estimated by one of the most widely used genomic inbreeding estimates; ROH (Doekes et al., 2019). Thirdly, the differentiation of local vs. genome-wide inbreeding (estimating different inbreeding levels for different chromosomes) is possible through the genomic inbreeding estimates (Keller et al., 2011; Ghoreishifar et al., 2020). Finally, the hidden relationship among individuals in a population, which is not shown in the pedigree, can be detected by genomic inbreeding estimates.

2.4 INBREEDING DEPRESSION

2.4.1 DEFINITION

The reduced fertility and survival of the descendants of closely related individuals is known as inbreeding depression (Charlesworth and Willis, 2009). Inbreeding might result in inbreeding depression where the mean performance of an animal is reduced compared to the offspring of randomly mated individuals (Hedrick and Kalinowski, 2000; Doekes et al., 2020). The decline in overall fitness might be due to two different phenomena. Firstly, the genetic mechanism of inbreeding is considered to be the increased offspring homozygosity

resulting in the expression of previously masked recessive deleterious alleles (Pekkala et al., 2014). Secondly, the increased homozygosity might negatively affect loci with heterozygote advantage (Charlesworth and Willis, 2009). However, it is difficult to differentiate the actual cause of inbreeding depression, but the expression of deleterious recessive allele is considered as the common cause for the decline of fertility and survival traits due to inbreeding (Charlesworth, 1990).

2.4.2 EFFECT OF INBREEDING ON TRAITS

The resultant effect of mating closely related individuals varies between population and traits (Wright et al., 2008). Small populations are vulnerable to inbreeding depression where the probability of mating between related individuals is high (Kardos et al., 2016). Inbreeding depression is often seen in reproductive traits, disease resistance, and survival of an animal (Crnokrak and Roff, 1999; Howard et al., 2017). Additionally, the age of inbreeding or when inbreeding occurred in the population might also impact the extent of effect on the traits. As per Doekes et al. (2019), not all inbreeding is equally detrimental, and recent inbreeding was observed to be more harmful than ancient inbreeding. It was suspected so because of the phenomenon known as purging, where the homozygous deleterious alleles (due to inbreeding) are actively selected against (Pekkala et al., 2014; Doekes et al., 2019). The presence of purging was experimentally proven by Hinrichs et al. (2015), where ancient inbreeding was determined to be less harmful than recent inbreeding in stillbirth, calving ease, and birthweight in German Holstein dairy cattle. Interestingly, in theory, purging can result in restoration of population fitness to, or even above, the original level (Theodorou and Couvet, 2006). Furthermore, the magnitude of

inbreeding depression is also dependent upon the environment the animal is raised in (Cheptou and Donohue, 2011). In one of the meta-analyses conducted by Fox and Reed (2011), it was revealed that inbreeding depression increased by 66% with the increased stressful environment in beetle *Callosobruchus maculatus*. Similarly, in Drosophila melanogaster environmental stress was directly correlated with severe inbreeding depression (Miller, 1994).

Inbreeding depression has been studied in many species with an aim to investigate the effect on several important traits. In Jersey cattle, with every 1% increase in inbreeding, milk yield decreased by 9.84 kg in first lactation (Miglior et al., 1992). Similarly in Holstein cattle, nearly 260kg less milk was produced when cows with more than 15% inbreeding coefficients were compared with the non-inbred ones during 305-day milk production (Silva et al., 2019). In beef cattle Brahman, 1% increase in inbreeding was associated with 0.51 kg decrease in body weight (Reverter et al., 2017). In Qira black sheep, body size traits were discovered to have negatively affected with inbreeding (Tao et al., 2021). Rodrigáñez et al. (1998) observed that with 10% increase in inbreeding, 0.27 less piglets were born in each litter of Large White pigs. Similarly, in Golden Retrievers, a statistically negative correlation was observed between inbreeding and litter size traits, suggesting that individuals with higher levels of inbreeding tends to have fewer puppies that were born and later on weaned (Chu et al., 2019).

2.5 INBREEDING AND INBREEDING DEPRESSION IN AMERICAN MINK

Even though inbreeding and its resultant inbreeding depression is well known among livestock framers, there are few studies conducted in American mink regarding these aspects. Demontis et al. (2011) studied inbreeding depression in the Danish farm of American mink using microsatellite markers and discovered negative correlation (-0.55) between inbreeding coefficient and litter size traits. This result led to the conclusion that increased inbreeding decreases the number of kits born. In a study conducted on six different mink farms of Norway, it was revealed that the reproductive trait (litter size at 3 weeks) was affected by inbreeding where inbred females produced 0.11 kits less than non-inbred females (Johannessen et al., 2004). Recent study using genotyping by sequencing data was performed in American mink where the average of the genomic estimates F_{ROH} and F_{HOM} was 0.13 and 0.15 respectively (Karimi et al., 2020). The study also revealed that short segments of ROH (500 kb to 1 Mb) were higher in numbers compared to long ones (>16 Mb) indicating that inbreeding might have happened in the studied population 50 generations ago. This corresponds to the approximate period of the beginning of domestication of wild mink (Karimi et al., 2021). Moreover, these are the few studies where inbreeding was the focus of the study in American mink. However, comparison between the studies should be performed very cautiously because the methods used for estimating the relatedness between the individuals and the studied population are different.

2.6 GAPS IN KNOWLEDGE

Color inheritance is one of the basic genetic studies that has been studied in wide range of species ranging from humans to rodents. However, studies to acknowledge the genetic mechanism involved in coat color variation in American mink is hardly few and most of

them were done couple of decades ago. Similarly, studies involving inbreeding estimation and inbreeding depression have also been infrequent in American mink. The negative effect of inbreeding is well documented in a wide range of species. However, very few studies performed to exhibit the effect of inbreeding on important traits of American mink is what this study hopes to address. Similarly, with the rapid increase in genomic data availability, the estimation of inbreeding has been more accurate and commonly used in livestock species. Therefore, this thesis aims to fill the gaps and put forward a foundation regarding all three aspects of American mink: coat color inheritance, the consequences of inbreeding on reproductive traits, and the genomic assessment of inbreeding levels.

CHAPTER 3: COAT COLOR INHERITANCE IN AMERICAN MINK[1]

3.1 INTRODUCTION

American mink (*Neogale vison*) is a semi-aquatic and carnivorous species belonging to the Mustelidae family, and a native species to North America (Macdonald and Harrington, 2003). The fur produced by American mink is one of the most desirable furs due to the astonishing variation in color and high quality, which resulted in its domestication in the late 1800s in Canada (Morris et al., 2020). The domestication and breeding of mink in captivity is primarily focused on the production of excellent-quality fur (Milanović et al., 2013; Bowman et al., 2017). Due to the increase in demand for fur, farmed mink has been bred intensively for selected traits such as fur color, size, and temperament (Belliveau et al. 1999; Kruska and Sidorovich 2003). This increase in demand in the past decades can be attributed to the color, shades, and texture of their fur (Amstislavsky and Ternovskaya, 2000). Thus, to meet the demand for fur, mink have been bred to produce an extensive range of colors.

Other than color variation, characteristics of pelt size, color purity, and fur quality also play significant roles in determining the price of a pelt (Kołodziejczyk and Socha, 2008; Zieliński et al., 2016, Valipour et al., 2022). It is clear and understandable that production is influenced by the market demand for a specific color or color combination. Consequently, some mink farmers may focus on producing a particular color of fur, whereas others may cross different color types (Karimi et al., 2021). Selective breeding of

[1] This chapter has been published in BMC Genomics Journal. Thapa et al., 2023. Coat color inheritance in American mink. BMC Genomics, 24(1), 1-9.

farmed mink has resulted in a broad range of colors commonly introduced as color phases (Shackelford, 1948).

The color phases have arisen due to genetic mutations and farming practices (Cirera et al., 2013; Manakhov et al., 2019). Presently, more than 35 color variations and their combinations have resulted in more than 100 color types in American mink (Manakhov et al., 2019). The immense interest in understanding the mechanism of color mutation led to several genomic studies (Anistoroaei et al., 2013; Cirera et al., 2013, 2016; Manakhov et al., 2019, 2020). Until now, out of the total color variations seen in American mink, only eight have been linked with specific DNA mutations (Manakhov et al., 2022). During the last decade, most studies have focused on identifying specific mutant genes rather than investigating the color inheritance using pedigree-based approach in American mink.

However, studying the genetic basis of color inheritance is challenging as an in-depth pedigree expanding up to several generations with the exact color identification and recording is required. One prominent study by Shackelford (1949) investigated six coat colors (Royal Silver, Colmira, Ebony, Aleutian, Green Eyed Pastel, and Goofus) in ranch-based American mink and differentiated dominant with recessive color types. Over the past decades, no research has been conducted using pedigree to understand the influence of crossbreeding on the inheritance of specific colors. The genotypes of nine colors of American mink including Pastel, raised in Poland were identified based on the similarity of colors in other mammalian species (Wacławik et al., 2020), yet the color inheritance in mink remains unclear.

This study aimed to investigate the inheritance of four colors (Dark, Pastel, Demi, and Mahogany) in ranch-based American mink (Figure 3.1). The objectives of this pedigree-

based study were to 1) investigate the crosses responsible for producing a specific color type under different scenarios, and 2) determine the allelic pair (homozygous or heterozygous) responsible for color type.

3.2 MATERIALS AND METHODS

3.2.1 ANIMAL CARE AND MANAGEMENT

The research work was approved by the Dalhousie University Animal Care and Use Committee (certification# 2018-009 and 2019-012). The mink used in this study were raised according to the Code of Practice for the Care and Handling of Farmed Mink guidelines published by the Canada Mink Breeders Association (https://www.nfacc.ca/pdfs/codes/mink_code_of_practice.pdf, accessed on 9 October 2022) at the Canadian Center for Fur Animal Research (CCFAR), Dalhousie University, Faculty of Agriculture (Truro, Nova Scotia, Canada). Mink were housed individually under standard farming conditions, and their diets were regulated according to the production cycle. Each annual production cycle (early March) started with mating between selected males and females. These males and females were selected (late November) based on criteria such as fur grade, disease history, weight, and litter size determining if animals were either used for pelting or breeding. The breeding males and females were selected from the same population and no new mink were introduced in the farm for the sole purpose of breeding. Ideally, two mink (a male and a female) were kept in a single cage with ad libitum feed. However, in the CCFAR, this was not practiced all the time. Some of them were housed separately for feeding measurements. After the birth of the kits (approximately 6-8 weeks), the kits were separated from the dam and housed either in pairs or multiples (mostly same litter).

3.2.2 COLOR RECORDING

Since mink fur quality is one of the main selection criteria, grading of fur quality in CCFAR was usually performed on live mink in November or early December each year. Grading requires a physical examination of fur where the guidelines provided by the North American Fur Auctions (NAFA) were followed by an experienced technician (NAFA, 2014). The characteristics such as texture, density, nap length, and color were assessed during grading. The color of a mink was recorded two times during its lifetime: at weaning (6–8-week-old) and based on live grading in November. The color recorded during grading was assigned as the final color type.

3.2.3 DATA COLLECTION

A pedigree containing 23,282 mink raised from 2003 to 2021 was used in this study. There were eleven different color types in the pedigree, however, only four of them were used in this study. The four colors were selected since a) they had the highest frequency (more than 3,000 animals per coat color) compared to other colors which were less than 500, and b) these four colors had complete information available regarding the color of ancestors until four generations.

Accordingly, 21,931 out of 23,282 mink were included in the study. There were 9,100 Dark, 5,161 Pastels, 4,312 Demi, and 3,358 Mahogany in the pedigree. These 21,931 minks were from 1,403 sires and 3,533 dams in the pedigree data, tracing back to 16 generations.

3.2.4 STATISTICAL METHODS

The pedigree data was inspected for all four colors extending up to four generations to investigate ancestral color. The animals were grouped into three categories to evaluate different types of mating strategies practiced at the farm. These categories were a) when the coat color of offspring is similar to the color of the sire, b) when the coat color of offspring is similar to the color of the dam, and c) when both of the parents (sire and dam) and offspring have the same color. While grouping animals based on three categories, those animals were not included if the color of either parent was unknown.

For the statistical analysis, the chi-squared test was used in R software version 4.2.0 using the function chisq - test (R Core Team, 2022). Assessment of ancestral background until four generations revealed that Dark and Pastel were the only color types with pure backgrounds (all ancestors of the same color). Thus, it was hypothesized that those color types were homozygous and produced only one colored phenotype when crossed based on Mendelian inheritance. On the other hand, the heterozygous pair of gene was tested using the following two Mendelian principles.

The two principles of Mendelian inheritance were considered to determine the allelic pairs responsible for colors. Firstly, genes in charge of the specific color were considered heterozygous (either sire or dam) and the other parent was considered recessive homozygous (i.e., Aa x aa). Secondly, when both parents were considered heterozygous (Aa × Aa). The expected 1:1 (from crossing Aa x aa) and 3:1 (from crossing Aa × Aa) among the offspring based on the Mendelian ratio show a cross between heterozygote with homozygote parent and crosses between two heterozygous parents, respectively (Sarfatti et al., 1991; Roy et al., 1992).

3.3 RESULTS

The overall parentage of Dark, Pastel, Demi, and Mahogany colored individuals in the pedigree is shown in Figures 3.2, 3.3, 3.4, and 3.5, respectively. The pie charts (Figures 3.2a, 3.3a, 3.4a, and 3.5a) depict the overall parentage of all four-color types under five different conditions. These conditions are: 1 = Sire is of the same color as offspring, 2 = Dam is of the same color as offspring, 3 = Both sire and dam of the same color as offspring, 4 = Neither sire nor dam is of the same color as offspring, and 5 = Color not identified in sire or dam or both. Dark, Pastel, and Mahogany colors were produced more frequently when both of the parents were of the same color. On the contrary, Demi colored offspring were produced more frequently (32.47 %) when Demi color dams were used (Figure 3.4a). The least number of offspring (0.59 % Dark and 2.23 % Pastel) were produced when none of the parents were of the same color. In the case of Demi coloration, the overall parentage suggests that few Demi-colored offspring (10.90 %) were produced when used as sires. When both parents of Mahogany color were mated, highest numbers of Mahogany colored offspring (37.94 %) were produced.

The mating of two parents of Dark, Pastel, Demi, and Mahogany color is shown in Figures 3.2b., 3.3b., 3.4b., and 3.5b. respectively. The offspring of two Dark parents resulted in the production of 99.38 % Dark, and 0.62 % of other colors (Figure 3.2b). The parentage of Pastel color individuals also followed a similar pattern as Dark color individuals, where 98.35 % of Pastel and 1.65 % of other colors were observed (Figure 3.3b). Mating two Demi parents resulted in the segregation of offspring, where 85.24 % of the offspring were Demi, 8.39 % were Mahogany, and 6.37 % were others (Figure 3.4b). Mating two

Mahogany parents resulted in the segregation of offspring where, 77.59 % Mahogany, followed by 19.55 % Demi and 2.86 % other color were observed (Figure 3.5b).

The production of variety of colored offspring when Dark, Pastel, Demi, and Mahogany were used as both sires and dams is shown in Figures c, and d. In the case of Dark coat color, 1.48 % and 1.16 % of Dark colored offspring were used when used as sires and dams of the same color respectively (Figure 3.2c, and 3.2d). Similarly, Pastel color when used as sires produced 4.19 % and when used as dams produced 5.3 % Pastel colored offspring (Figure 3.3c, and 3.3d). However, these numbers were higher when Demi and Mahogany were used as sires and dams alternatively. When used as sires Demi offspring produced 10.93 % and when used as dams produced 32.5 % of Demi offspring (Figure 3.4c, and 3.4d). Approximately similar number of offspring were recorded when Mahogany colored sires (18.16 %) and dams (18.19 %) were used (Figure 3.5c, and 3.5d).

Due to incomplete information about the color of either sire or dam and sometimes both, the color inheritance pattern of some individuals was not accurately identified. These individuals that were considered as “color not identified in sire or dam or both” group, accounted for 18.23 % of Dark color, 12.09 % of Pastel color, 13.82 % of Demi color, and 10.39 % of Mahogany color offspring.

When the same-colored parents were mated, the production of other colored offspring was found to be the highest for Demi and Mahogany. These findings suggest that among all four colors, at least two (Demi and Mahogany) were heterozygous. Similarly, the ancestral background also proposed that these colors had diverse colored ancestors until four generations. To verify this hypothesis, the breeding results of crossing (A× B) and reciprocal crossing (B × A) of Dark, Pastel, Demi, and Mahogany were performed, and the

results are shown in Table 3.1. The statistically significant results (P value less than 0.05) shown in Table 3.1 demonstrated that the genes responsible for Dark, Pastel, Demi, and Mahogany colors have met the expected ratios (1:1 and 3:1) and thus can be considered heterozygous.

The ancestral background revealed that the ancestors of all four colors' offspring were sometimes limited to a specific color and, most of the time, were diverse. Few individuals of Dark and Pastel were produced by mating sire and dam of the respective colors only (pure cross). On the other hand, Demi and Mahogany color mink ancestors were remarkably diverse in their background.

These results exhibiting the production of offspring from diverse colored ancestors demonstrated that the inheritance of coat color was considerably more than the involvement of a few sets of genes.

3.4 DISCUSSION

We found that mating sire and dam of the same color produced offspring of variety of colors. However, the probability of obtaining offspring of the same color as that of the parents was over 75 % for all four-color types. This study also showed that using one of the parents with the desired color in offspring will probably result in the production of that color. These findings were further verified by retracing the pedigree up to four generations.

The mating of same-color parents (Dark and Pastel) produced Dark offspring at 99.38 %, and Pastel offspring at 98.35 % of the time. A study on Pastel coat color inheritance in sables *(Martes zibellina)* also showed that the mating of both parents of Pastel color produced offspring with Pastel fur coats only (Manakhov et al., 2021). The breeding results of green-eyed Pastel individuals in Ontario, Wisconsin in 1941 showed that using the same color for sire and dam resulted in the production of only green-eyed Pastel phenotypes (Shackelford, 1949). On the same farm, when green-eyed Pastel was crossed with Dark individuals, both Dark and green-eyed Pastel color individuals were observed (Shackelford, 1949). Similarly, offspring produced by crossbreeding Pastel and black sables followed the 1:1 ratio corresponding to Mendelian inheritance (Manakhov et al., 2021). The heterozygous nature of alleles responsible for Pastel color was also reported by Eklund et al. (1968) and Shackelford (1949) while crossing Ebony and Pastel color sire and dam respectively. It was concluded that all Pastel-colored mink in the ranch in Wisconsin originated from the heterozygous pairs (Shackelford and Cole, 1947). Similarly, we also observed that crossing Pastel color individuals, irrespective of sex, with individuals of any other color followed both 1:1 and 3:1 ratios suggesting that Pastel color individuals produced by crossbreeding are indeed heterozygous.

Since the beginning of the domestication of wild mink, Dark color pelts have always been in high demand and are known as the standard color. These standard minks are almost Dark in color and is considered genetically a dominant color (Kidd et al., 2009; Bowman et al., 2017). This explained the highest number of Dark color offspring produced (99.31 %) when Dark color was characteristic of both sires and dams in our study. To the best of our knowledge, there is no report about the crosses determining the genetic component underlying Dark color. Other than pure crossing (sire and dam of the same color), crossbreeding of Dark color with other color types was frequently practiced in mink breeding to produce new color phases such as Mahogany (Shackelford, 1948). The heterozygous genes responsible for Dark color is demonstrated in our research where both 1:1 and 3:1 ratio was followed in the respective crosses analyzed.

In the case of Demi and Mahogany, the mating of sires and dams of the same color produced 14.76 % and 22.41 % of other color offspring rather than Demi and Mahogany respectively. Additionally, the production of Demi and Mahogany colored offspring in the pedigree when neither parent was of the same color revealed that both Demi and Mahogany colors might be produced as the result of crossbreeding and their heterozygous nature. The pedigree file analysis of the ancestors aided in the findings that the antecedents of the present Demi and Mahogany color in mink were from various colorations. The genomic studies in the same population using the whole genome sequence (WGS) data characterized Demi and Mahogany as highly admixed color types with observed heterozygosity of 31.12 % and 30.93 %, respectively (Karimi et al., 2021).

The origin of Mahogany color in mink is attributed to the crossbreeding of brown and black mink lines which justifies its higher heterozygosity level (Bowman et al., 2017). The exact

origin of Demi color mink is not mentioned in the literatures, but it is considered that crossbreeding might have resulted in coloration. Moreover, it has been evident that Demi and Mahogany color types have small genetic distances (Karimi et al., 2021). The small genetic distance can be attributed to the common ancestors in recent generations (Higgs and Derrida, 1992). To further investigate this, the comparison of Demi and Mahogany colors within themselves was conducted. It was shown that the crossing of Demi and Mahogany irrespective, of sex, produced the offspring of either color notably. Furthermore, mating sire and dam of Demi color produced Mahogany color offspring 8.39 % of the time. This scenario was also noted while Mahogany-colored sires and dams resulted in Demi-colored offspring 19.55 % of the time (Figure 3.4b, 3.5b). This might signify that the genes responsible for both of these colorations might be pleiotropic.

3.5 CONCLUSION

The overall crosses analyzed provided valuable insight into the color type of parents which could be selected to produce offspring with desired coat colors. In general, mating parents with the same color results in the production of offspring with the same color most of the time. Similarly, using either sire or dam with the same desired color in offspring may also be advisable. However, the production of a specific color in offspring when none of the parents are of the same color as the offspring demonstrated that the inheritance of coat color is complicated. Furthermore, heterozygous genes responsible for color type have assisted the understanding of color inheritance in American mink. Even though coat color is termed a qualitative trait (Nguluma et al., 2022), the mutation of genes responsible for coat color and pleiotropic effect on morphological and physiological traits has made the genomic study of color inheritance in American mink necessary.

Table 3.1 Crossing of sire and dam with alternate colors and reciprocal crossing along with the production of offspring.

The table also shows the P-value for the expected ratios of heterozygosity 1:1 and 3:1.

Sire color	**Dam color**	**Offspring color**		**P-value for 1:1**	**P-value for 3:1**
		D	**NON-D**		
D	NON-D	134	650		
NON-D	D	106	470	< 0.00002	< 0.00001
		P	**NON-P**		
P	NON-P	215	747		
NON-P	P	272	609	< 0.00002	< 0.00001
		DE	**NON-DE**		
DE	NON-DE	470	340		
NON-DE	DE	1400	860	< 0.00002	< 0.00001
		M	**NON-M**		
M	NON-M	609	844		
NON-M	M	610	499	<0.00002	< 0.00001

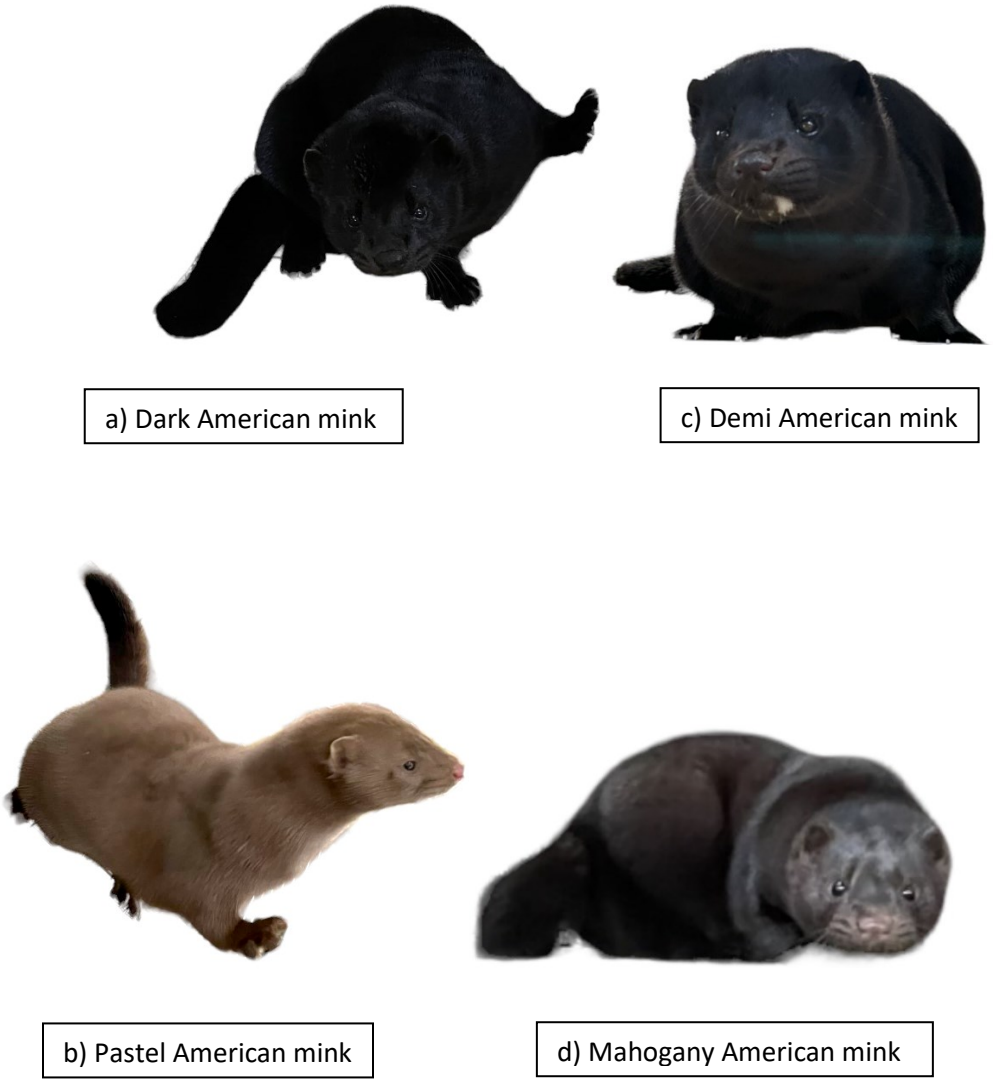


Figure 3.1 Photographs of American mink showing four types of coat colors.

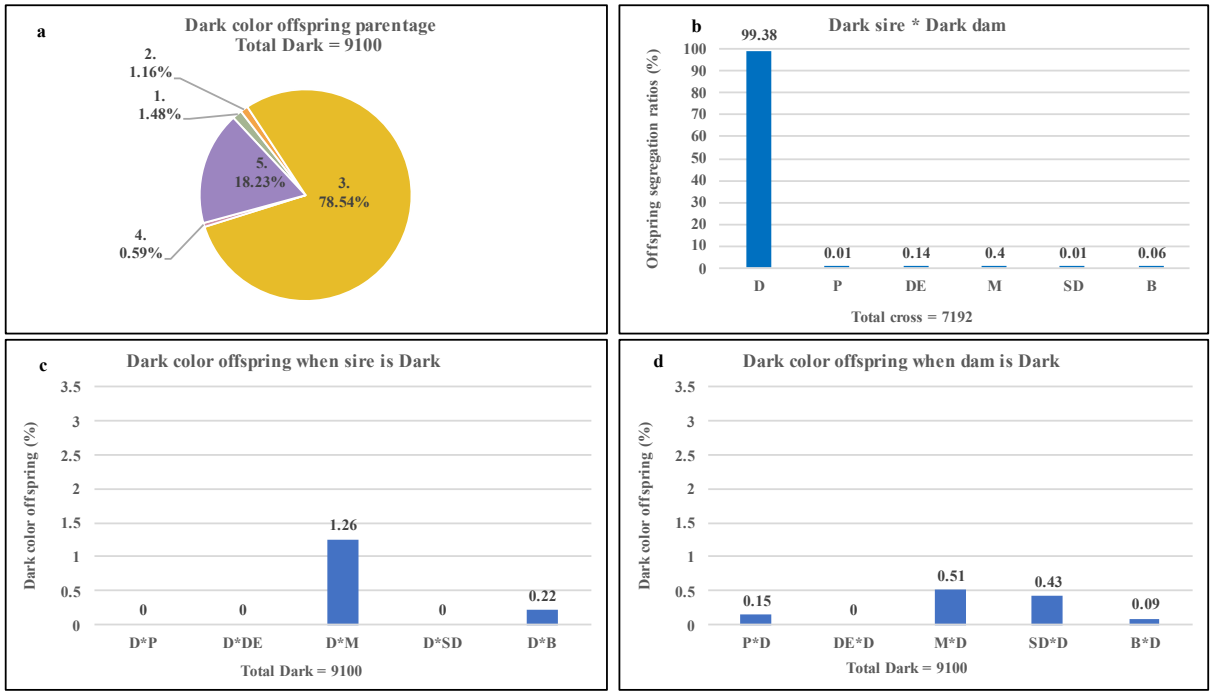


Figure 3.2 Types of crosses involved in the production of Dark-colored American mink.

a. The overall parentage of Dark-colored offspring where; 1 = Sire is Dark, 2 = Dam is Dark, 3 = Both sire and dam are Dark, 4 = Neither sire nor dam is Dark, and 5 = Color not identified in sire or dam or both. b. mating sire and dam of the Dark color in American mink. c. production of Dark color offspring when sire is Dark, and dam is of a different color. d. production of Dark color offspring when dam is Dark, and sire is of a different color. D = Dark, P = Pastel, DE = Demi, M = Mahogany, SD = Stardust, and B = Brown.

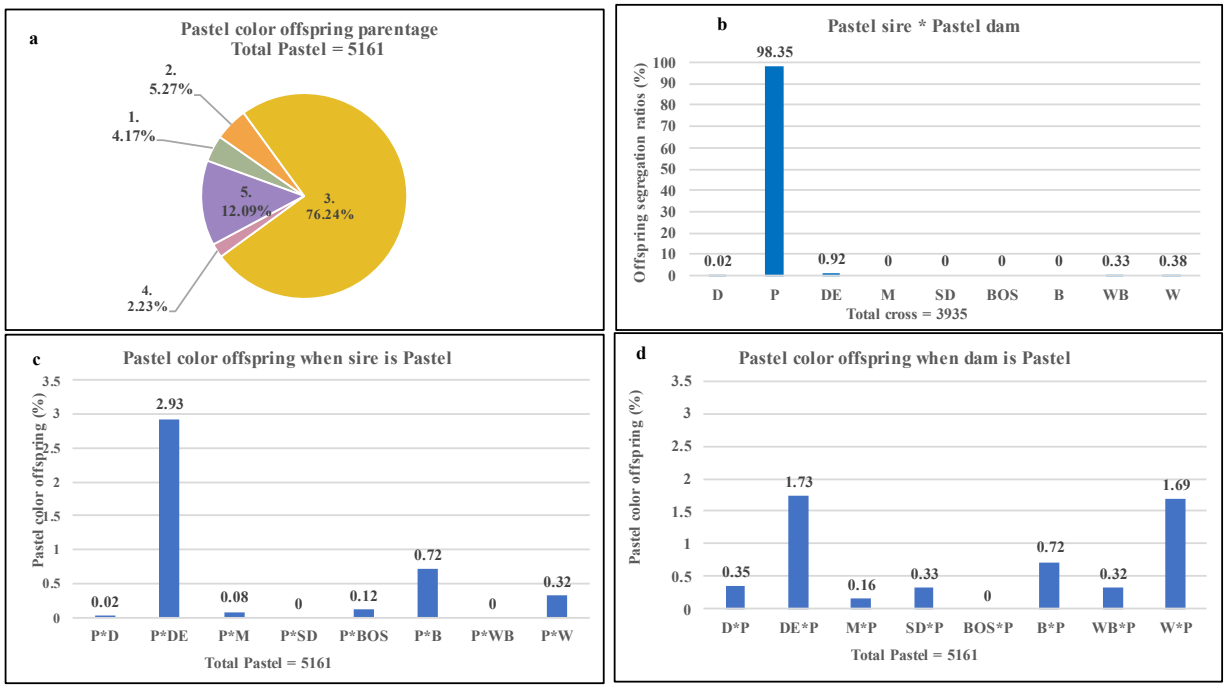


Figure 3.3 Types of crosses involved in the production of Pastel-colored American mink.

a. The overall parentage of Pastel-colored offspring where; 1 = Sire is Pastel, 2 = Dam is Pastel, 3 = Both sire and dam are Pastel, 4 = Neither sire nor dam is Pastel, and 5 = Color not identified in sire or dam or both. b. mating sire and dam of the Pastel color in American mink. c. production of Pastel color offspring when sire is Pastel, and dam is of a different color. d. production of Pastel color offspring when dam is Pastel, and sire is of a different color. D = Dark, P = Pastel, DE = Demi, M = Mahogany, SD = Stardust, BOS = Breath of Spring, B = Brown, WB = Winter Blue, and W = White.

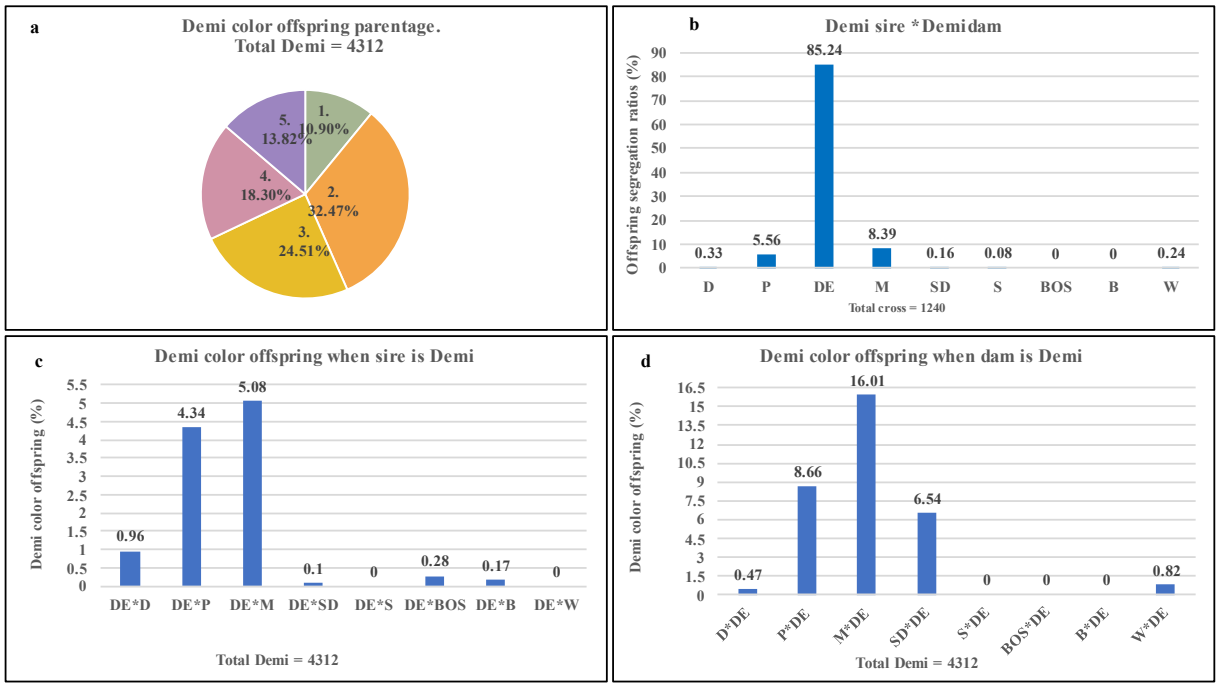


Figure 3.4 Types of crosses involved in the production of Demi-colored American mink.

a. The overall parentage of Demi-colored offspring where; 1 = Sire is Demi, 2 = Dam is Demi, 3 = Both sire and dam are Demi, 4 = Neither sire nor dam is Demi, and 5 = Color not identified in sire or dam or both. b. mating sire and dam of the Demi color in American mink. c. production of Demi color offspring when sire is Demi and dam is of a different color. d. production of Demi color offspring when dam is Demi and sire is of a different color. D = Dark, P = Pastel, DE = Demi, M = Mahogany, SD = Stardust, BOS = Breath of Spring, B = Brown, W = White, and S = Sapphire.

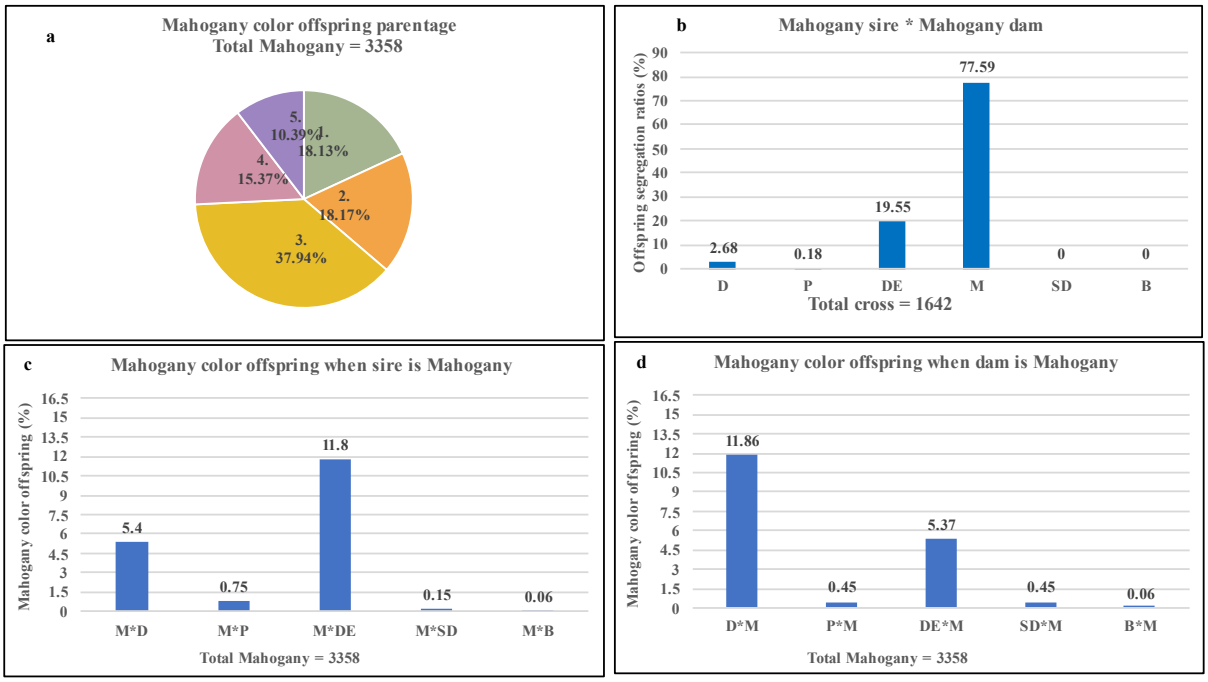


Figure 3.5 Types of crosses involved in the production of Mahogany-colored American mink.

a. The overall parentage of Mahogany-colored offspring where; 1 = Sire is Mahogany, 2 = Dam is Mahogany, 3 = Both sire and dam are Mahogany, 4 = Neither sire nor dam is Mahogany, and 5 = Color not identified in sire or dam or both. b. mating sire and dam of the Mahogany color in American mink. c. production of Mahogany color offspring when sire is Mahogany and dam is of a different color. d. production of Mahogany color offspring when dam is Mahogany and sire is of a different color. D = Dark, P = Pastel, DE = Demi, M = Mahogany, SD = Stardust, B = Brown.

CHAPTER 4: ESTIMATION OF INBREEIDNG DEPRESSION IN REPRODUCTIVE TRAITS OF AMERICAN MINK

4.1 INTRODUCTION

The breeding of pairs of individuals who have greater genetic affinity than the average pair of individuals in a population is known as inbreeding (Wright et al., 2008; Curik et al., 2014). The inbreeding coefficient (F) is defined as the likelihood that two alleles from a randomly sampled locus are identical by descent (Falconer and Mackay, 1996). Genetically, inbreeding is responsible for increased homozygosity for both desirable and undesirable genes (Baneh et al., 2019). Moreover, the increased homozygosity results in increased frequency of homozygous deleterious genotypes which were present previously as recessive allele and masked by the presence of dominant allele (Tanchev, 2016; Doekes et al., 2019; Sumreddee et al., 2021). This phenomenon is the genetic mechanism involved in inbreeding depression (Charlesworth, 1990; Kardos et al., 2016). Inbreeding depression, which has been observed in all livestock species, causes a decrease in the mean value of traits such as fitness and performance (Jarnecka et al., 2021).

Reproductive traits are considered important in all livestock species (Köster and Visser, 2021) since without acceptable reproductive performance, any progress made in production performance could be overshadowed. In mink production, the overall economic efficiency is determined by reproductive performance. Moreover, inbreeding depression is often seen in reproductive traits (Crnokrak and Roff, 1999; Howard et al., 2017). Despite the negative relationship between inbreeding and reproductive performance, we still know very little about inbreeding and its effects on reproductive traits of American mink. Among

the limited available reports, a study conducted in Norway where the average inbreeding coefficient was 1.27%, revealed that inbred American mink females produced 0.11 fewer kits compared to the non-inbred females (Johannessen et al., 2004). Similarly, decrease in the litter size at second counting was reported in Danish farmed mink with the increasing level of inbreeding coefficient (Demontis et al., 2011).

The study of inbreeding and inbreeding depression in livestock species is not limited to reproductive performance but has been studied with respect to numerous phenotypes. Traits such as milk production, fat, and protein yields were significantly reduced with 1% increase in inbreeding in Holstein and Jersey dairy cattle (Pryce et al., 2014). In swine, with every 10% increase of inbreeding coefficient, growth traits such as weight at 120 days and average daily gain decreased by 5.37% and 6.49%, respectively (Fernández et al., 2002). In addition, inbreeding is responsible for recessive genetic diseases such as hip dysplasia and congenital heart anomalies in dogs (Bell, 2011).

The understanding of inbreeding, its effects, and practices to avoid it is crucial in livestock breeding programs. Measuring inbreeding is also an essential component of research in ecology, evolution, and conservation biology due to the profound effects of inbreeding on population dynamics and individual fitness (Kardos et al., 2015). Similarly, to run a sustainable mink farm, farmers greatly depend upon high quality producing and reproducing individuals. Thus, the objectives of this study were to: (1) assess inbreeding in the American mink population using pedigree information, and (2) estimate the impact of inbreeding (inbreeding depression) on reproductive traits of American mink.

4.2 MATERIALS AND METHODS

The Dalhousie University's Animal Care and Use Committee (certification# 2018-009, and 2019-012) approved the proposed work. The Code of Practice for the Care and Handling of Farmed Mink requirements were followed while rearing the mink used in this study (Turner et al., 2013).

4.2.1 ANIMAL MANAGEMENT AND PEDIGREE

All mink used in this study were born from 2010 until 2020 at the Canadian Center for Fur Animal Research (CCFAR) at Dalhousie University, Faculty of Agriculture (Truro, Nova Scotia, Canada).

The annual production cycle (early March) starts based on phenotypic selection of mink as breeders. Selection criteria were based on their fur grades, disease history, weight, and litter sizes. Similarly, in November (or early December) mink were selected for either pelting or breeding for the next breeding season (Karimi et al., 2018). The male and female mink chosen for breeding were both selected from the existing population, without any new mink being brought in specifically for breeding purposes. A male and a female were kept in a single cage for breeding. Once the kits were born, typically around 6-8 weeks later, they were taken away from their mothers and placed in separate housing units, often with their littermates or in pairs.

Female mink were mated multiple times (maximum 3 times) to ensure conception. However, the same males were used if more than one mating was required 9 days after the first mating. The kits were born from late April to mid-May (whelping season).

Immediately after birth, the number of kits were recorded and weighted. Similarly, 24 hours after birth, the total number of kits alive was recorded. Finally, at the time of weaning (6 weeks) when kits were separated from their dams, the number of kits was counted and weighted (Karimi et al., 2018). A total of 18,372 individuals with pedigree information raised since 2010 were included in this study.

4.2.2 MEASURED AND DERIVED TRAITS

The information on reproductive traits were collected based on gestation length (GL), number of kits born (TB), number of kits alive after 24 hours of birth (LB), number of kits alive at weaning (LW). The gestation length is measured in the number of days from mating to parturition (Karimi et al., 2018). The two traits measured in percentage were survival rate at birth (SB) and weaning (SW). Both parameters (SB and SW) were calculated by dividing the number of kits alive at birth and number of kits alive at weaning by total number of kits born and total number of kits alive at birth times hundred, respectively. The weight of kits was recorded in grams (g) as average kit weight per litter at birth (AWB), and average kit weight per litter at weaning (AWW). The reproductive data set consists of 4,723 dams with reproductive records collected each year from 2010 to 2021.

4.2.3 MEASURES OF INBREEDING

The inbreeding coefficient was estimated for each individual. Pedigree information was analyzed, and inbreeding coefficients (F_{PED}) were estimated using the CFC (Coancestry, Inbreeding (F) and Contribution) 1.0 software (Sargolzaei et al., 2006). The individuals were categorized based on the level of inbreeding generated by the software into the

following groups: 0, $0 < F \leq 5$, $5 < F \leq 10$, $10 < F \leq 15$, $15 < F \leq 20$, $20 < F \leq 25$, $25 < F \leq 30$, and >30. The first category (F =0) included non-inbred individuals whereas the remaining categories included inbred individuals with the respective level of inbreeding coefficients.

4.2.4 ESTIMATION OF BREEDING VALUES

The breeding value of an individual is defined as the average effects of the genes that are passed on from its parents (Werf, 2015). The breeding value is used to rank individuals where the highest one is termed as "high genetic merit" and the process to identify these individuals is known as breeding value prediction (Groen, 2000). The breeding values for traits including reproductive traits were estimated for all individuals using individual's own and relative information based on the best linear unbiased prediction (BLUP) method fitting within a univariate animal model using ASReml 4.1 (Butler et al., 2017):

$$\boldsymbol{y} = \boldsymbol{Xb} + \boldsymbol{Za} + \mathbf{Wpe} + \boldsymbol{e},$$

where **y i**s the vector of phenotypes of interest (reproductive traits); **b** is the vector of fixed effects; X is design matrix for fixed effects; **Z** is the incidence matrix that relates additive genetic effect to the phenotypes; **a** is the vector of random additive genetic effects or their breeding values; **pe** and **W** are the vector of random permanent environmental effects and the incidence matrices relating the permanent environmental effects respectively; the vector of random residual effects was denoted by **e.** The random effects were presumed to be independent and normally distributed:

$$\boldsymbol{a} \sim N(0, \boldsymbol{A}\sigma_a^2),\ pe \sim N(0, \mathbf{I}\sigma_{pe}^2), \text{ and } \boldsymbol{e} \sim N(0, \boldsymbol{I}\sigma_e^2),$$

where σ_a^2 is the additive genetic variance, σ_{pe}^2 is the random permanent environmental variance, and σ_e^2 is the residual variance, **A** is the additive relationship matrix, and **I** is an identity matrix. Fixed effects used in this study were: a) age of dam (1, 2, and 3), b) color (11 distinct color types), c) number of mating (1-4 mating/breeding), d) origin of the dam (CCFAR, combination (both CCFAR and donated), and donated), and e) year (2010 to 2020). Moreover, only significant effects (P<0.05) were used.

4.2.5 ESTIMATION OF INBREEDING DEPRESSION

The effect of inbreeding on reproductive traits of American mink was estimated using two methods. Firstly, the effects of inbreeding on reproductive traits were calculated by regressing the phenotypic values on inbreeding coefficients as a standard approach for estimating inbreeding depression (Saura et al., 2015). Inbreeding depression was estimated separately for each trait using the following univariate model:

$$\boldsymbol{y} = \boldsymbol{Xb} + \boldsymbol{\beta F} + \boldsymbol{Za} + \boldsymbol{e},$$

where, **y** is the vector of phenotypes of interest in the mink population (reproductive traits); **b** and **a** are the vector of fixed effects and random additive genetic effect respectively; **β** is the regression coefficient on **F**. The vector of inbreeding coefficients (based on pedigree) is denoted by **F**. **X**, is the incidence matrix relating the phenotypic observations to fixed effects; and **Z**, is the incidence matrices linking phenotypic observations and random additive genetic effects. Additionally, **e** is the vector of random residual effects. Random

effects were assumed to be independent and follow normal distribution. $\boldsymbol{a} \sim N(0, \mathbf{A}\sigma_a^2)$, pe $\sim N(0, \mathbf{I}\sigma_{pe}^2)$ and $\boldsymbol{e} \sim N(0, \mathbf{I}\sigma_e^2)$.

The statistical analyses were performed using ASReml 4.1 software (Butler et al., 2017) and only significant effects ($P<0.05$) were used in the analyses. Secondly, the Pearson correlation coefficient was estimated to quantify the extent of relationship between F_{PED} and EBV of the studied traits. The correlation was calculated in R software version 4.2.0 (R Core Team, 2022).

4.3 RESULTS

4.3.1 DESCRIPTIVE STATISTICS

The descriptive statistic for each trait is presented in Table 4.1 showing the number of records, mean, standard deviation (SD), range, and coefficient variation (CV). The GL ranged from 32 to 69 days with an average of 46.04 for the period of ten years. The litter size traits of TB, LB, and LW had average values of 6.45, 5.67, and 4.25, respectively, showing a decrease in average number of kits from birth to weaning. The average weight of the kit during birth was 12.03 g. The final weight was calculated at weaning which was observed to be 353.78 g on average. The average weight of kits was observed to be a minimum of 5 g at birth and 586 g at weaning. The mean values for survival at birth and weaning were 86.89% and 90.03% respectively. Among all the traits, LW had the highest CV of 54.83 and GL had the lowest CV was of 9.17.

4.3.2 INBREEDING ESTIMATES OF THE POPULATION

As shown in Table 4.2, out of the total mink present in the CCFAR, 69.60% were inbred and 30.40% were non-inbred. Among the inbred, 65.41% of individuals' inbreeding levels did not exceed 5%. Similarly, fewer individuals (0.01%) had inbreeding level in the range of 20% to 25%. Additionally, 4.09 % of individuals had inbreeding level more than 5% and less than 20%. The maximum level of inbreeding (25% to 30%) was found in 16 individuals which accounts for 0.09% of the total population. Additionally, there were no individuals with inbreeding coefficient of greater than 30%.

The number of individuals with the highest F_{PED} is illustrated in Table 4.3. In comparison to the number of inbred individuals of the population (69.60%), only fewer had the highest F_{PED} in the respective years. The highest level was 28.51% in the year 2017 with only one individual. However, in the year 2011, nine individuals had an inbreeding level of 12.01% which was the highest number of individuals in ten years. The trend of average F_{PED} from 2010-2020 is shown in Figure 4.1. The mean F_{PED} were maximum in 2014 (2.8%), 2015 (2.6%), and 2016 (2.8%) compared to the other years (below 2.5%). Moreover, it is crucial to note that the average F_{PED} did not exceed 3% for ten years. Figure 4.2 shows the number of dams and their respective inbreeding levels. There were fewer dams with F_{PED} above 10%.

4.3.3 ESTIMATION OF INBREEDING DEPRESSION

The results yielded from the first method to estimate inbreeding depression is shown in Table 4.4 where the regression coefficients for all the traits were statistically non-significant ($P>0.05$) and had high standard errors. Secondly, Pearson's correlation coefficient was used to evaluate the direction and strength of the relationship between EBV

of the studied traits and F_{PED} (Table 4.5). The correlation values for each of the traits were very low ranging from –0.17 to 0.11 and all of them were statistically significant ($P<0.05$). The lowest correlation was observed for LB (-0.17) and the highest was for TB (0.11). The correlation coefficient values were –0.16 and 0.08 for weight traits AWB and AWW respectively. For both the survival rates at birth and at weaning the correlation vales were 0.02.

4.4 DISCUSSION

In the studied population, pedigree information was used to estimate inbreeding coefficient. The pedigree analysis gave an insight to the distribution of inbreeding in the studied population where the highest number of individuals (65.41%) had F_{PED} in the range of $0 < F \leq 5$ (Table 4.2) followed by the individuals that were the product of non-related mating (30.40%). Only 0.69% of mink had inbreeding level above 10%. This result is not uncommon as avoiding the mating of closely related individuals is one of the major breeding strategies of the CCFAR farm.

The average inbreeding coefficient (F_{PED}) was 0.02. It is important to consider that the average inbreeding has remained below 3% throughout the years in the CFFAR farm (Figure 4.1). The primary reason for this is the use of software called ZooEasy (version 13.05) for selecting compatible sires and dams according to the extent of their relationships. This software estimates inbreeding percentage for the offspring in advance, which is considered a useful tool for selecting possible mates in mink farms. Among other phenotypical selection criteria such as fur color, quality, purity, and disease resistance, the

coefficient of relationship between prospective parents below 3% was an additional requirement in this farm.

The average F_{PED} over the years did not fluctuate, however an increasing trend of mean F_{PED} was observed from 2013 to 2016, and this might be because of the outbreak of Aleutian disease (AD) in the time frame of 2014 (Karimi et al., 2018). As a result of the outbreak, only few individuals were phenotypically selected as breeders that were AD resistant. Moreover, inbreeding is more prone to small population where mating between relatives is recurrent (Keller and Waller, 2002). The sharp decline in F_{PED} after 2016, might be due to the introduction of new males in the population during the same year. The reason for this might be the reduced mating of closely related individuals, which eventually decreases inbreeding (Strandén and Peura, 2007; Pike et al., 2021). There have been very few studies on estimating inbreeding and inbreeding depression in American mink. For instance, Demontis et al. (2011) reported inbreeding rates in the range of 0.017 to 0.52 in Danish farmed mink. The inbreeding range in our studied population was lower (0.001 to 0.28) than the Danish study (Demontis et al. 2011). One important factor to consider is that the rate of inbreeding is greatly dependent upon effective population size, genetic drift, and the selection and mating strategy opted by the farm (Mátyás, 2004; Kokko and Ots, 2006; Duthie and Reid, 2016).

The standard procedure to estimate inbreeding depression in livestock species is to regress the phenotype on the individual pedigree inbreeding coefficients (Leroy, 2014). The regression coefficient is defined as the inbreeding depression experienced by the phenotype (Dorostkar et al., 2012; Leroy, 2014). In this study the regression coefficients were all statistically non-significant ($P>0.05$) with high standard errors. Similar observations were

also reported by Hermas et al. (1987) and Miglior et al. (2008) in reproductive performance of Guernsey cattle, and three different dairy cattle breeds (Ayrshire, Jersey, and Brown Swiss), respectively. The effect of inbreeding on sperm quality, feed efficiency, and carcass traits in beef cattle using regression analysis was found to be non-significant (Carolino and Gama, 2008; Dorado et al., 2017). The non-significant regression coefficient along with high standard errors observed in the above-mentioned studies were likely the result of sampling error associated with small sample sizes (Keller and Waller, 2002). In our population, there were very few individuals with high inbreeding coefficients, which explains the results obtained from the first method. High standard errors observed from the regression method indicated that little can be inferred from the regression coefficients (Hermas et al., 1987).

On the other hand, potential purging of harmful alleles might also explain the non-significant inbreeding depression observed in our study. Purging is a phenomenon where the frequencies of deleterious recessive alleles decrease significantly over time due to selection practices either natural or artificial (Hedrick and Dorado, 2016). Since mink, similar to other livestock species, are subjected to strong artificial selection based on phenotypes, those individuals that are fit and qualified as per the selection guideline are only selected.

The second method used to assess inbreeding depression was based on the correlation coefficient between EBV of the studied traits and F_{PED}. In contrast to the regression method, all correlation coefficients were statistically significant, but the values were low (-0.17 to 0.11). The results suggested that inbreeding might affect breeding value of the litter size traits (LB and LW) and both body weight traits (AWB and AWW), but the effects

were extremely small (Table 4.5). Moreover, these estimates were in agreement with the concept that inbreeding and resultant increased homozygosity might have negative effects on reproductive traits. In contrast to this study, a strong negative correlation (R = -0.53) between inbreeding coefficient and litter size at second counting was observed, suggesting that inbreeding decreases litter size at second counting in farmed mink (Demontis et al., 2011). However, the range of inbreeding in the studied population was higher (0.01 to 0.52) than ours (0.01 to 0.28).

The level of inbreeding is subjected to vary depending on the population and the consequences are also dependent upon the age of inbreeding where ancient inbreeding is considered to have lesser effect than recent inbreeding (Sumreddee et al., 2021). In our studied population, the average F_{PED} has always remained below 3% over a decade and avoiding mating of closely related individuals has been one of the crucial breeding objectives resulting in no recent inbreeding. Similarly, the level of inbreeding might also lead to variation of inbreeding depression exerted by the trait. It was discovered that pregnancy rate decreased, and dystocia rate increased significantly (-6.37 and 1.67%, respectively) when F was greater than 25% compared to 12% (Recio et al., 2007) in Holstein cattle. In contrast, our studied population had very few numbers (0.69% individuals had F_{PED} above 10%) of highly inbred individuals (Figure 4.2).

The two methods (regression and correlation of EBV with traits) suggests that there was no inbreeding depression on the farm. However, the statistically non-significant result does not necessarily mean that there was no inbreeding depression at all. This might be due to small sample size, environmental variation, and low level of inbreeding in the population. It is very important to interpret the results of this study based on the overall context and

limitations. The high standard errors observed in this study can be overcome by increasing sample size hence increasing the accuracy of the results.

In this study, we used pedigree information to estimate inbreeding and inbreeding depression on the traits. However, there are potential drawbacks of the pedigree method which can be compensated by using genomic information. With the availability of genomic information in livestock species, there have been remarkable studies focused on the genomic estimation of inbreeding. On the contrary, very few studies have been conducted on American mink using genomic information. Therefore, in the next chapter (Chapter 5), inbreeding will be estimated using genomic information that would accurately estimate the true level of inbreeding in the population.

4.5 CONCLUSION

The results suggest that inbreeding and the resultant inbreeding depression on reproductive traits does not appear to be a common problem in the CCFAR farm at present. It is highly likely that the current practices to avoid mating of closely related individuals are effective. Further investigation of inbreeding involving a new population with different management practices and including other traits is equally important in American mink. These findings prove that with simple management practices, the harmful effects of inbreeding that can drastically influence the farm's future can be mitigated.

Table 4.1 Descriptive statistics for reproductive performance traits in American mink.

Traits[1]	Number of records	Mean	SD	Range	CV (%)
GL (days)	3,491	46.04	4.22	32-69	9.17
TB	3,513	6.45	2.68	1-17	41.56
LB	3,513	5.67	2.61	0-14	46.04
LW	2,516	4.25	2.33	0-10	54.83
SB (%)	3,409	86.89	22.45	0-100	25.83
SW (%)	3,178	90.03	16.03	0-100	17.8
AWB (g)	1,224	12.03	2.45	5-23.86	20.36
AWW (g)	1,100	353.78	63.59	140-586	17.97

[1]GL = gestation length, TB = total number of kits born; LB = number of kits alive after 24 hours of birth; LW = number of kits alive at weaning, SB = survival rate at birth, SW = survival rate at weaning, AWB = average kit weight per litter at birth, and AWW = average kit weight per litter at weaning, SD = Standard deviation, CV = Coefficient of variation.

Table 4.2 Number of mink along with the percentage with different levels of F_{PED}.

Inbreeding coefficient (F_{PED}) in %	No. of individuals	Percentage of individuals
0	5,584	30.40
$0 < F \leq 5$	12,016	65.41
$5 < F \leq 10$	644	3.50
$10 < F \leq 15$	101	0.55
$15 < F \leq 20$	8	0.04
$20 < F \leq 25$	3	0.01
$25 < F \leq 30$	16	0.09
>30	0	0
Total	18,372	100
Total inbred	12,788	69.60

Table 4.3 Number of individuals with the highest level of inbreeding coefficient (F_{PED}) over the years.

Year	Maximum F_{PED}	Number
2010	15.24	7
2011	12.01	9
2012	10.87	6
2013	10.43	7
2014	13.09	6
2015	7.61	1
2016	26.51	3
2017	28.51	1
2018	25.62	5
2019	6.65	4
2020	26.67	3

Table 4.4 Regression coefficient (β) between F_{PED} and phenotypes along with their standard errors (SE).

Traits[1]	β (±SE)
GL (days)	7.25 (8.87)
TB	-1.4 (5.47)
LB	-1.17 (5.57)
LW	-1.17 (5.11)
SB (%)	-50.67 (26.34)
SW (%)	-22.67 (15.77)
AWB (g)	-0.67 (3.74)
AWW (g)	-213.35(120.87)

[1]GL = gestation length, TB = total number of kits born; LB = number of kits alive 24 hours of birth; LW = number of kits alive at weaning, SB = survival rate at birth, SW = survival rate at weaning, AWB = average kit weight per litter at birth, and AWW = average kit weight per litter at weaning.

Table 4.5 Correlation coefficients between F_{PED} and EBV of traits.

Traits[1]	Correlation coefficient (P-value)
GL (days)	0.02 (0.001) *
TB	0.11 (0.001) *
LB	-0.17 (0.001) *
LW	-0.08 (0.001) *
SB (%)	0.02 (0.01) *
SW (%)	0.02 (0.01) *
AWB (g)	-0.16 (0.0005) *
AWW (g)	-0.08(0.0005) *

*P<0.05 [1]GL = gestation length, TB = total number of kits born; LB = number of kits alive 24 hours of birth; LW = number of kits alive at weaning, SB = survival rate at birth, SW = survival rate at weaning, AWB = average kit weight per litter at birth, and AWW = average kit weight per litter at weaning.

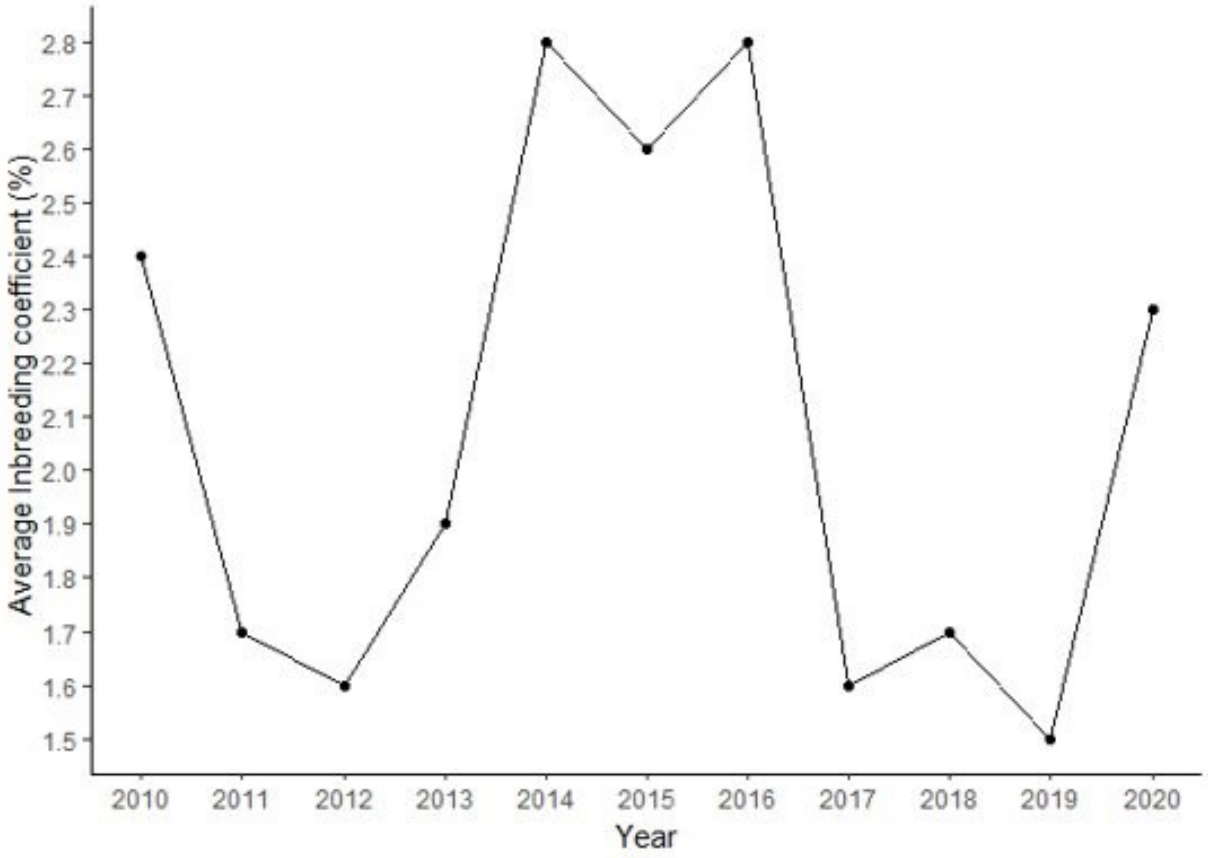


Figure 4.1 The average inbreeding in the farm in the respective years from 2010 to 2020.

The average was observed to be 0.02. The highest level of inbreeding was observed in the years 2014 and 2016 whereas the lowest was 1.5 in the year 2019.

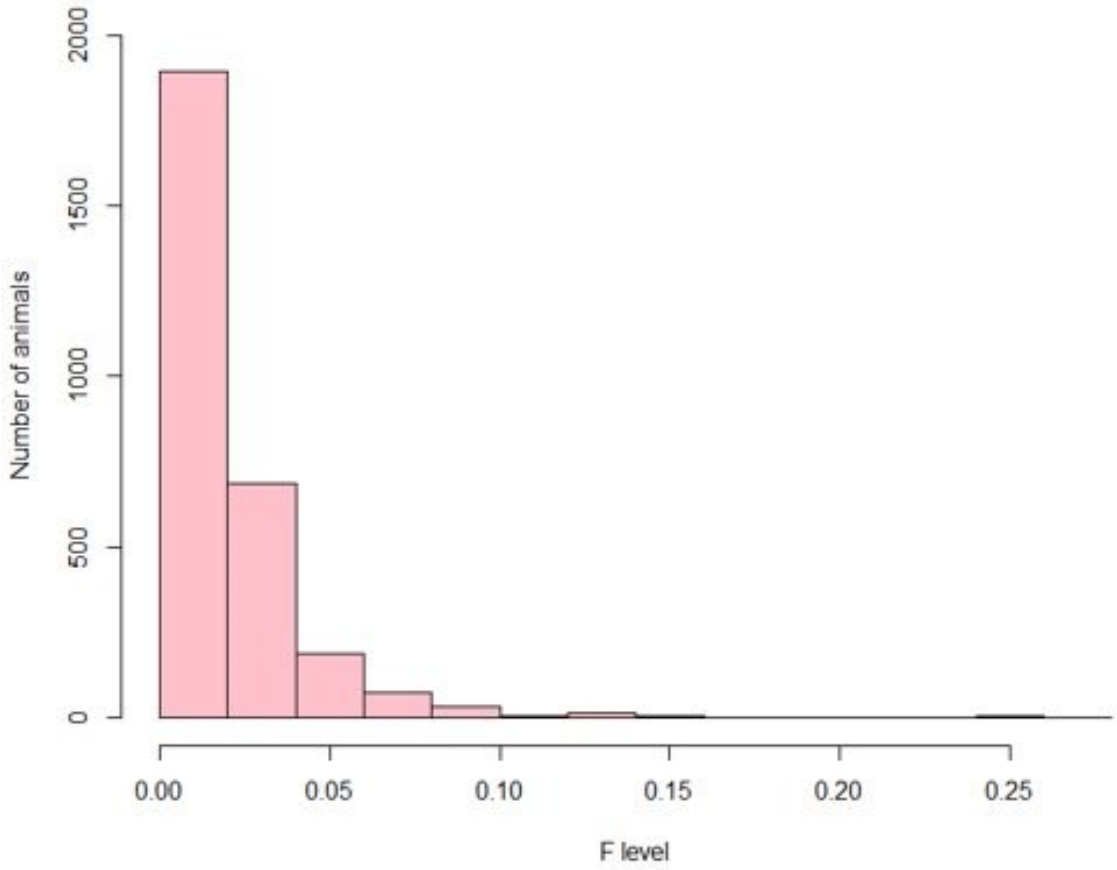


Figure 4.2 Number of female mink selected as dams and their coefficients of inbreeding.

CHAPTER 5: INBREEDING ESTIMATES IN AMERICAN MINK USING GENOTYPE DATA

5.1 INTRODUCTION

Avoiding mating of genetically related individuals is one of the major concerns of livestock production. The mating of individuals who share one or more recent ancestors is known as inbreeding (Curik et al., 2014). The increased homozygosity as the result of inbreeding has been associated to reduced individual performance commonly known as inbreeding depression and decreased population viability (Charlesworth and Willis, 2009). Therefore, estimating, monitoring, and managing inbreeding is one of the most important practices in operations of animal breeding.

The degree of inbreeding is measured by the inbreeding coefficient (F) and is defined as the probability of two alleles at any locus being identical by descent (IBD) (Falconer and Mackay 1996). The inbreeding coefficient is regarded as a principal parameter to realize the extent of relationship between individuals in a population (Raya et al., 2015). Traditionally, inbreeding was methodically computed using pedigree information (F_{PED}), and it is based on the probability of finding two identical copies of genes originating from the same ancestor at any locus via parental pathways (Hernández et al., 2021).

The availability of high-throughput single nucleotide polymorphism (SNP) genotyping tools that can examine numerous SNPs across the genome has created new prospects for directly calculating the level of inbreeding (Peripolli et al., 2017). Inbreeding coefficient based on the excess of homozygosity (F_{HOM}), which measures the population's departure from expected homozygosity, can be calculated using genomic data. (Nasner et al., 2021).

F_{HOM} has been used as one of the genomic estimators of inbreeding in American mink (Karimi et al., 2021), river buffalo (Ghoreishifar et al., 2020), cattle (Zhang et al., 2015), pig (Schiavo et al., 2021), and chicken (Yuan et al., 2022).

Runs of Homozygosity (ROH) are the long stretches of homozygous segments recognized first by Broman and Weber in 1999 in humans (Curik et al., 2014). These segments are considered to provide a better estimate of the identical chromosomal segments, known as autozygous inherited from a common ancestor (Broman and Webber, 1999; Yoshida et al., 2020). These long stretches of homozygous segments might have resulted from reduction in population size, mating between close relatives, and/or selection (Falconer and Mackay, 1996; Yuan et al., 2022). The identification and classification of ROH can provide valuable information about population demography, diversity, structure, and history (Peripolli et al., 2017; Yuan et al., 2022). One of the most prominent uses of the classification of ROH based on the length is to define the age of inbreeding. Long ROH may indicate recent inbreeding (Keller et al., 2011), whereas short ROH might indicate ancient inbreeding (Marras et al., 2015).

The inbreeding coefficient derived from ROH (F_{ROH}) is considered the most accurate estimator of individual inbreeding level (Purfield et al., 2012; Zhang et al., 2015; Hasler et al., 2017; Schäler et al., 2020; Zhang, et al., 2022). This might be the reason why F_{ROH} has been studied in a wide range of species such as humans (Kirin et al., 2010; Fatma et al., 2023), cattle (Marras et al., 2015; Mulim et al., 2022), pig (Schiavo et al., 2021; Zhang et al., 2022), and poultry (Marchesi et al., 2018; Heidaritabar et al., 2022). Similarly, inbreeding rates were estimated in American mink using ROH, however, the lack of availability of chromosome-scale genome assembly limited the exploration of longer ROH

(Karimi et al., 2021). The recent development of a chromosome-level genome assembly (Karimi et al., 2022) and very limited number of studies aimed at estimating F_{ROH} in American mink has been one of the major bases for this study. Therefore, the objectives of this study were to; 1) estimate inbreeding using genomic data, 2) evaluate the distribution of different lengths of ROH in all chromosomes, and 3) calculate the correlation between F_{ROH}, different lengths of ROH, F_{PED} and F_{HOM}.

5.2 MATERIALS AND METHODS

The proposed work was approved by the Dalhousie University Animal Care and Use Committee (certification# 2018-009, and 2019-012). The mink were cared for according to the Code of Practice for the Care and Handling of Farmed Mink guidelines published by the Canada Mink Breeders Association (Turner et al., 2013).

5.2.1 ANIMALS AND MANAGEMENT

The annual production cycle (early March) starts based on phenotypic selection of mink as breeders. Selection criteria were based on their fur grades, disease history, weight, and litter sizes. Similarly, in November (or early December) mink were selected for either pelting or breeding for next season (Karimi et al., 2018). The distinction between pelting and breeding was determined by the procedure commonly known as grading. Those mink selected for pelting were euthanized in November as winter fur starts developing in August. Mink used in this study were euthanized in November of 2021. In total, 1,413 mink were raised from 2018 to 2020 and used in this study. Similarly, pedigree information containing complete

ancestral information of 23,282 mink were also used. The pedigree depth was estimated using CFC software 1.0 (Sargolzaei et al., 2006).

5.2.2 SAMPLE COLLECTION AND GENOTYPING

DNA was isolated from the tongue tissue sample using DNeasy Blood and Tissue Kit (Qiagen, Hilden, Germany). To measure the quality and quantity of extracted DNA samples, ND-1000 spectrophotometer (NanoDrop Technologies Inc., Wilmington, DE) was used. The 280/260 ratio which measures the purity of DNA ranged from 1.7 to 2.0 for all the samples. High-quality DNA was normalized to the required concentration (50 ng/μL). Finally, Axiom Affymetrix Mink 70K panel (Neogen, Lincoln, Nebraska, USA) was used for genotyping of 1,413 individuals. All the procedures were carried out in accordance with the manufacturer's protocol.

A total of 62,375 SNPs were genotyped for all 1,413 individuals. Genotype quality control was performed in PLINK v1.09 (Purcell et al., 2007) using the parameters to exclude: individuals and SNPs with call rates <90%, SNPs with Hardy–Weinberg Equilibrium (HWE) ($P < 10^{-5}$) and remove sex chromosome. After quality control, a total of 50,902 SNPs on autosomal chromosomes of 1,413 individuals remained for subsequent analyses.

5.2.3 DEFINITION, DISTRIBUTION OF RUNS OF HOMOZYGOSITY AND INBREEDING (F_{ROH})

Runs of Homozygosity were examined in PLINK v1.09 (Purcell et al., 2007) using the sliding window approach and the function --*homozyg*. The sliding window approach

scanned through the genotype data to detect homozygous segments for each individual (Howrigan et al., 2011). The following parameters and thresholds were applied to define the ROH segment**:** (i) a minimum length of 1 Mb, (ii) a minimum of 50 SNPs, (iii) a minimum density of one SNP per 100 kb, (iv) a maximum of one heterozygous call within an ROH, (v) a maximum gap of 1000 kb between consecutive SNPs, and (vi) scanning window of 50 SNPs, with a maximum of one heterozygote per window. Obtained ROH were further classified into five categories based on their lengths: (i) 1-2 Mb, (ii) 2-4 Mb, (iii) 4-8 Mb, (iv) 8-16 Mb, and (v) > 16 Mb.

To analyze the overall distribution of ROH in the population, the mean number of ROH per individual, the mean total length of ROH per individual, and the F_{ROH} for each individual were estimated. F_{ROH} was calculated following the method proposed by McQuillan et al. (2008):

$$F_{ROH=} \frac{\sum LROH}{LAUTO},$$

where LROH is the total length of an individual's ROH and LAUTO is the total length of the autosomal genome covered by SNPs. Based on the distribution of five different lengths of ROH (i) 1- 2 Mb, (ii) 2 - 4 Mb, (iii) 4 - 8 Mb, (iv) 8 - 16 Mb, and (v) > 16 Mb), F_{ROH} was estimated ($F_{ROH\ 1\text{-}2\ Mb}$, $F_{ROH\ 2\text{-}4\ Mb}$, $F_{ROH\ 4\text{-}8\ Mb}$, $F_{ROH\ 8\text{-}16\ Mb}$, and $F_{ROH\ >\ 16\ Mb}$, respectively) for each individual.

5.2.4 GENOMIC INBREEDING BASED ON EXCESS OF HOMOZYGOSITY (F_{HOM})

The second method to estimate inbreeding was based on the genome-wide excess of homozygosity (F_{HOM}). F_{HOM} was calculated in PLINK v1.09 (Purcell et al., 2007) and is the

moment estimator based on the expected and observed individual homozygosity (Purcell et al., 2007; Alemu et al., 2021). It was calculated using the following formula:

$$F_{HOM} = \frac{O - E}{1 - E},$$

where, O, and E are the number of observed and expected homozygotes, respectively (Zhang et al., 2015).

5.2.5 PEDIGREE BASED INBREEDING MEASURE (F_{PED})

The CFC 1.0 (Coancestry, Inbreeding (F) and Contribution) software (Sargolzaei **e**t al., 2006) was used to estimate F_{PED} by analyzing the pedigree information. Pearson's correlation was used to assess the strength and direction of relationship between F_{ROH}, F_{HOM}, and F_{PED}. Additionally, the correlations between F_{HOM}, and F_{PED}, were calculated for five different lengths of F_{ROH}. The correlation coefficients were calculated in R software version 4.2.0 (R Core Team, 2022).

5.3. RESULTS

5.3.1 CHARACTERISTICS OF RUNS OF HOMOZYGOSITY

The number and average length of ROH per individual are one of the most important characteristics of the ROH structure. Irrespective of the length, ROH was identified in all individuals. The mean (±SD) number of ROH per individual was 149.61(±13.82). Similarly, on average (±SD), 4.74 (±4.63) Mb of ROH was present across individuals. The longest segment of ROH was 88.58 Mb. Among the five classes of ROH, (Table 5.1) the smallest segment (1-2 Mb), was found in 20.41% of the total ROH length and segments with the

length greater than 16 Mb, covered only 2.69% of the total ROH length. Similarly, ROH of the length 2- 4 Mb was the most abundant throughout the genome (40.30%). Shorter ROH fragments ($ROH_{1\text{-}2\ Mb}$ and $ROH_{2\text{-}4\ Mb}$) accounted for almost 60% and medium ROH ($ROH_{4\text{-}8\ Mb}$ and $ROH_{8\text{-}16\ Mb}$) covered approximately 35% (Table 5.1) of all ROH detected. Figure 5.1 displays the variation in the number of segments that made up the total length of the genomic regions covered by ROH in individuals (each dot represents an individual), despite these regions being the same length. As shown in Table 5.2, out of the total ROH (204,539), the highest number of ROH was found on chromosome 1 (16.63%), followed by chromosomes 3 (13.99%), 6 (13.34%), 4 (12.65%), and 2 (12.28%). However, the lowest number of ROH was found on chromosomes 8 and 9 (Table 5.2). The chromosome-wise distribution of ROH also revealed that all chromosomes had fewer percentages of long stretches (> 16 Mb) of homozygous regions compared to smaller segments ($ROH_{1\text{-}2\ Mb}$ and $ROH_{2\text{-}4\ Mb}$). The percentage of chromosomes covered by ROH is demonstrated in Figure 5.2 and 5.3, which shows an uneven distribution of ROH. The highest genomic coverage by ROH was found on chromosome 10 (9.12%), while the lowest coverage was on chromosome 7 (1.75%).

5.3.2 INBREEDING COEFFICIENTS

The distributions of three inbreeding coefficients (F_{ROH}, F_{HOM}, and F_{PED}) are shown in Figure 5.4. The average F_{ROH} was 0.28 and the values ranged from 0.12 to 0.57. Similarly, the average inbreeding coefficient of genomic estimates of excess of homozygosity (F_{HOM}) was -0.03 and ranged from -0.28 to 0.31. The pedigree-based estimates (F_{PED}) ranged from 0 to 0.39 with a mean value of 0.02. The descriptive statistics of inbreeding coefficients along

with the number of individuals based on five different lengths of F_{ROH}, F_{ROH}, F_{HOM}, and F_{PED} are summarized in Table 5.3.

5.3.3 PEARSON CORRELATIONS AMONG INBREEDING COEFFICIENTS

The pairwise correlations among F_{ROH}, F_{HOM}, F_{PED}, and different lengths of F_{ROH} are presented in Table 5.4. The highest correlation was observed between $F_{ROH,}$ and F_{HOM} (0.72). The lowest and insignificant correlation (P>0.05) was found between small-length F_{ROH} ($F_{ROH\ 1\text{-}2\ Mb}$, $F_{ROH\ 2\text{-}4\ Mb}$) with both F_{PED} and F_{HOM}. The correlation between different lengths of F_{ROH} with F_{PED} ranged from -0.08 to 0.35. Similarly, the correlation between different lengths of F_{ROH} with F_{HOM} ranged from -0.002 to 0.43. It was revealed that the correlation among all inbreeding measures increased with the increasing length of ROH. Interestingly, the correlation of F_{PED} with both genomic estimators of inbreeding was found to be 0.34.

5.4 DISCUSSION

ROH was identified in the genome of American mink and classified based on the length into five classes of 1-2 Mb, 2-4 Mb, 4-8 Mb, 8-16 Mb, and >16 Mb. We observed a higher number of shorter segments (1-4 Mb) in the genome, which accounted for 60.71% of all ROH. On the other hand, longer fragments greater than 16 Mb were fewer in number (2.69%). It is evident that short ROH reflects ancient parental relatedness (Szpiech et al., 2013; Liu et al., 2022). The highest prevalent of short ROH in the present study correlates with current working mechanism of the farm where mating between related individuals is seldom practiced resulting in considerably less related sires and dams in the population. Similarly, the more common the shorter fragments are, it is more likely that inbreeding

events happened in the population generations ago showcasing the demographic history of the given population (Goszczynski et al., 2018). Moreover, the decline in effective population size was suspected to have happened 100–200 years ago corresponding to the rough period where wild mink were domesticated (Hu et al., 2023).

Nevertheless, inbreeding in ancient parents might not be the sole reason for the highest number of small ROH. A great deal of shorter ROH detected in this study could also be explained by the high degree of LD (Ferenčaković et al., 2013; Marras et al., 2015). In American mink, high level of LD (average r^2 in the range of 0.37 to 0.40) was observed between adjacent markers (Hu et al., 2023). It is important to consider that short ROH (less than 4 Mb), must be interpreted cautiously as they might not be directly related to autozygosity (Ferenčaković et al., 2013). Interestingly, the increase in homozygosity among individuals can also be a result of selection (Forutan et al., 2018). Furthermore, demographic events such as population bottleneck, and genetic drift might also result in long stretches of homozygous segments in the genome (Gorssen et al., 2021).

The long stretches of homozygous segments were randomly distributed throughout the chromosome in this study. Among all 14 chromosomes, the highest ROH is found in chromosome 1. It is understandable as chromosome 1 is the longest chromosome (317.07 Mb) in American mink (Karimi et al., 2022). The distribution of ROH irrespective of the length was found to be highest in chromosomes 1, 2, 3, 4, 6, and 11. The LD for these chromosomes was reported to be more than 0.30 (Hu et al., 2023). This might be because frequent ROH are often due to high LD (Mamun et al., 2015) as the limited recombination results into extended regions of homozygosity (Bosse et al., 2012).

The genomic regions harboring high frequencies of ROH, are known as “ROH islands” (Curik et al., 2014; Beishova et al., 2022). These islands might foster genes that are associated with economically important traits that have been selected intensely during breeding (Ospina et al., 2022). Higher number of ROH on chromosomes 1, 2, 3, 4, 6, and 11 indicate that these regions might harbor important genes of American mink as human and livestock population studies have revealed the existence of principal genes in genomic regions with high ROH (Kim et al., 2013; Cardoso, et al., 2020; Beishova et al., 2022). However, the identification of candidate genes within ROH islands has yet to be performed in American mink.

Limited literature is available regarding inbreeding level in American mink population. In Danish farm mink, the pedigree-based inbreeding was estimated to be in the range of 0.01 to 0.52 (Demontis et al., 2011). Our observation for average F_{ROH}, F_{HOM}, and F_{PED} was 0.28 and -0.03, and 0.02 respectively. Karimi et al. (2021) discovered an average of 0.13 and 0.15 for F_{ROH} and F_{HOM} in the American mink population, respectively. These observations were contradicting to our findings which is likely explained by a difference in sample size, methodology to detect ROH, HOM, and the filtration used for genotyping. Most importantly, it is important to consider that inbreeding level is greatly dependent on the breeding programs opted for by the farm (selection and mating strategies) and population size (Kokko and Ots, 2006; Duthie and Reid, 2016). The negative value of F_{HOM} denotes that an individual’s average homozygosity is lower than the population's predicted homozygosity under HWE (Wang, 2014; Xu et al., 2021). Additionally, the negative value indicates that inbreeding has been successfully avoided by the farm (Curik et al., 2014; Karimi et al., 2020). This is true because the CFFAR's inbreeding rate has consistently

been less than 3% on average over the years. The main cause of this is the adoption of "ZOOEASY" software (version 13.05), which allows for the selection of appropriate sires and dams based on the degree of their relationships obtained from their pedigree information.

The relationship among inbreeding coefficients revealed that the correlation among genomic inbreeding coefficients (F_{ROH} and F_{HOM}) was higher (0.72) compared to the pedigree-based (F_{ROH} and F_{PED}, F_{HOM} and F_{PED}) estimator (0.34). Similar results were also observed by Karimi et al. (2021), where the correlation of F_{ROH} with F_{HOM} was 0.85, and 0.47 between F_{ROH} and F_{PED}. Moderate correlations between F_{PED} and genomic inbreeding coefficients (F_{PED} and F_{ROH}) observed in our study could be attributed to the immense difference in the distribution of inbreeding predicted by pedigree versus genomic data (Table 5.3). The low correlation in our study was in agreement with a similar correlation (0.30) observed between F_{PED} and F_{ROH} in cattle (Hernández et al., 2021) and 0.31 for purebred line C in turkey (Adams et al., 2021). In contrast, a high correlation of 0.82 was observed by Zhang et al. (2015). This might be due to the difference in population size, parameters used to define ROH, and depth of the pedigree. The highest pairwise correlation (0.72) among the different inbreeding coefficient estimates (F_{ROH} and F_{HOM}) was in accordance with other studies; 0.83 in sheep (Bérénos et al., 2016), and 0.95 in pig (Shi et al., 2020). This might be because, the actual fraction of the genome that is homozygous can be determined by directly measuring homozygosity (Shi et al., 2020; Karimi et al., 2021). Similarly, with the increasing length of ROH, more homozygous genotypes are observed. This might account for the increased correlation between F_{ROH} and F_{HOM} with the increasing length of ROH.

The increasing correlation from -0.08 to 0.35 and -0.002 to 0.43 for both F_{PED} and F_{HOM} respectively with the increasing lengths of ROH was observed in this study. Similar to our observations, Hernández et al. (2021) also observed a negative correlation of smaller F_{ROH} lengths ($F_{ROH\ 1\text{-}2\ Mb}$, $F_{ROH\ 2\text{-}4}$) with F_{PED} and F_{HOM} in the cattle population, attributing to the fact that short ROH might not reflect the proportion of the genome that is IBD. As longer ROH are considered to arise due to recent inbreeding, and F_{PED} are to give better of estimate of recent inbreeding the correlation between long F_{ROH} and F_{PED} seems to be increased (Doekes et al., 2019). In addition, we used pedigree information from 16 generations which aided in the increased correlation between F_{PED} and F_{ROH} of the longest length. This finding has also been reported in dairy cattle where the correlation between F_{PED} and F_{ROH} was increased with the increased information of the ancestors (Purfield et al., 2012; Marras et al., 2015).

Our main objective was to present genomic-based inbreeding work as a framework and guidance for future studies regarding ROH in mink. Future studies involving larger population would be highly valuable for advanced genomic studies in American mink. Thus, the study of ROH is very broad as it gives valuable information not only about the extent of the relationships among individuals but also about population history, demographics, and the identification of candidate genes responsible for economically important traits.

5.5 CONCLUSION

ROH of five different lengths was estimated and inbreeding coefficients based on these lengths were calculated in American mink population. Both long and short ROH lengths were detected, however short fragments were higher in frequency. In addition to the ROH-based inbreeding, the genomic inbreeding based on excess of homozygosity was estimated. The correlation among the genomic estimates was higher than between genomic and pedigree estimates. These results are the first to be found after the availability of chromosome scale of genome assembly in American mink, providing an insight to the genomic architecture of inbreeding.

Table 5.1 Different lengths of runs of homozygosity (ROH) along with the percentage covered of the total ROH length.

ROH length	%
1-2 Mb	20.41
2-4 Mb	40.30
4-8 Mb	26.43
8-16 Mb	10.17
>16 Mb	2.69

Table 5.2 Chromosome-wise distribution of runs of homozygosity (ROH).

Chromosome	Length (Mb)	ROH (%)
1	317.04	16.63
2	240.42	12.28
3	235.65	13.99
4	231.36	12.65
5	167.25	2.44
6	224.56	13.34
7	207.08	2.44
8	144.01	2.07
9	101.7	1.68
10	75.57	4.75
11	220.35	9.7
12	148.69	3.38
13	152.77	2.45
14	46.74	2.29
Total		100

Table 5.3 Descriptive statistics of inbreeding coefficients based on runs of homozygosity (F_{ROH}), excess of homozygosity (F_{HOM}), pedigree (F_{PED}) and different lengths of ROH

Inbreeding estimates	Mean	Median	Min	Max	Number
F_{ROH}	0.28	0.28	0.12	0.57	1,413
$F_{ROH\ 1\text{-}2\ Mb}$	0.02	0.02	0.01	0.03	1,410
$F_{ROH\ 2\text{-}4\ Mb}$	0.06	0.06	0.04	0.13	1,410
$F_{ROH4\text{-}8\ Mb}$	0.08	0.08	0.04	0.15	1,410
$F_{ROH\ 8\text{-}16\ Mb}$	0.06	0.06	0	0.14	1,409
$F_{ROH\ >16\ Mb}$	0.04	0.03	0.01	0.21	1,360
F_{HOM}	-0.03	-0.03	-0.28	0.31	1,413
F_{PED}	0.02	0.01	0	0.39	14,226

Table 5.4 Pearson's correlation coefficients among all inbreeding coefficients.

	F_{ROH}	$F_{ROH\ 1\text{-}2\ Mb}$	$F_{ROH\ 2–4\ Mb}$	$F_{ROH\ 4–8\ Mb}$	$F_{ROH\ 8–16\ Mb}$	$F_{ROH\ >16\ Mb}$	F_{PED}
F_{PED}	0.34*	-0.08	-0.02	0.14 *	0.27 *	0.35 *	
F_{HOM}	0.72*	-0.002	-0.13	0.06*	0.23*	0.43*	0.34*

*P<0.05

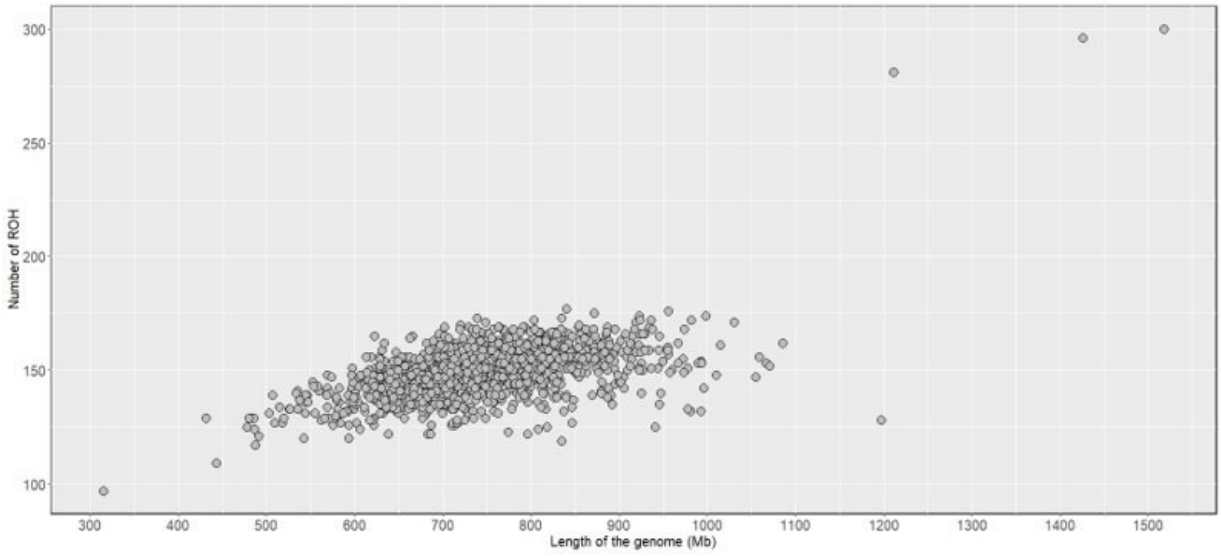


Figure 5.1 Relationship between the total number of runs of homozygosity (ROH) segments (y-axis) and the total length of ROH (Mb) covering the genome (x-axis) for all individuals.

Each dot represents an individual.

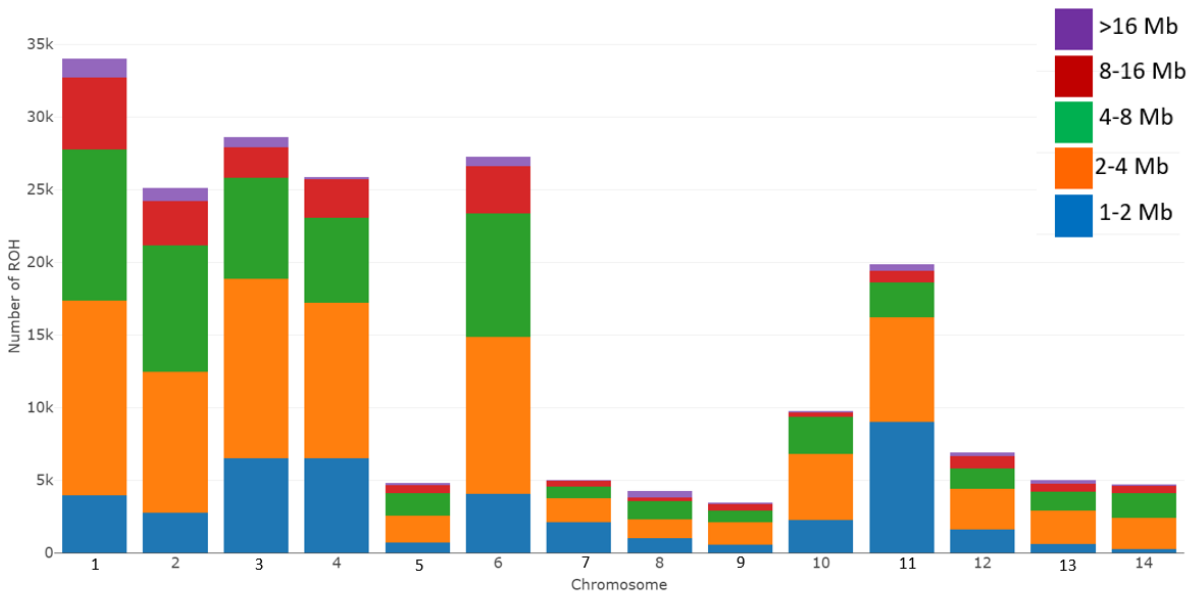


Figure 5.2 Chromosome wise distribution of ROH along with five different lengths of ROH.

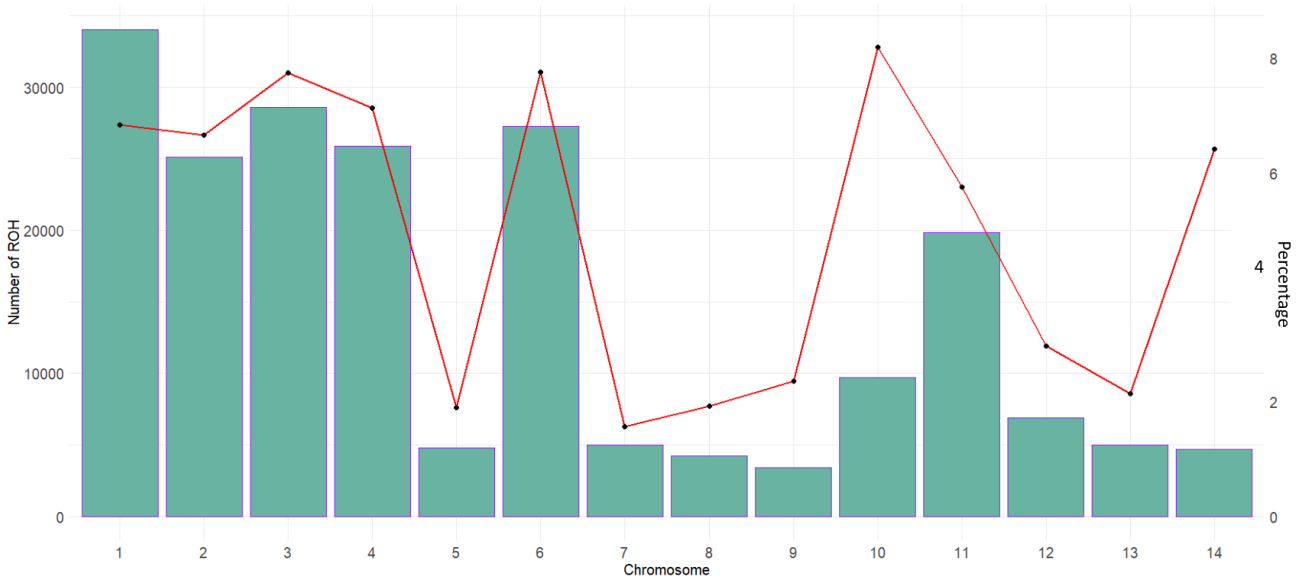
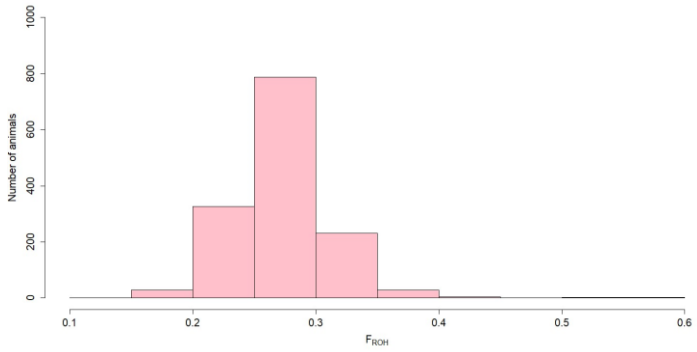


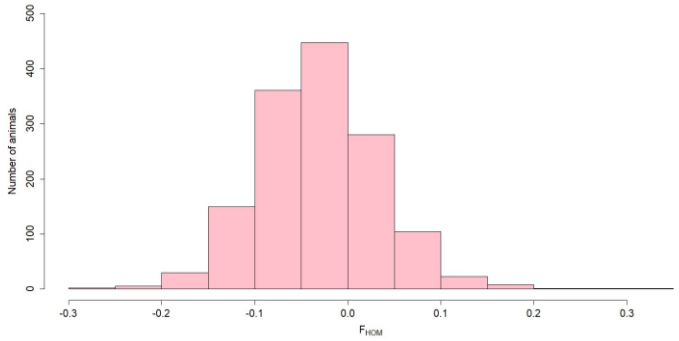
Figure 5.3 The percentage coverage of ROH per chromosome.

The bars show the total number of ROH per chromosome. The line shows the average percentage of ROH for each chromosome.

(a)



(b)



(c)

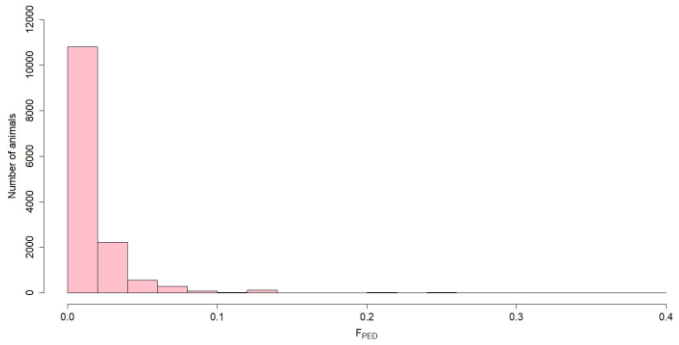


Figure 5.4 Histogram representing the distribution of three estimates of inbreeding coefficients (F) in American mink population.

a) Inbreeding coefficient based on runs of homozygosity (F_{ROH}), b) Inbreeding coefficient based on excess of homozygosity (F_{HOM}), and c) Pedigree-derived inbreeding coefficient (F_{PED}),

CHAPTER 6: GENERAL CONCLUSION, RECOMMENDATIONS AND LIMITATIONS

6.1 GENERAL CONCLUSION

The overall aesthetic appearance, wide range of color variation, and excellent features such as durability, softness, warmness, etc. is what makes the fur produced by American mink one of the most desirable furs in the world. Coat color is one of the most important characteristics of American mink that is why mink industry is highly driven by color. The market of mink is dependent upon the demand as per the customer's preference. Generally, uniform coat colors are preferred. However, patterns and sometimes mixed colors are also in demand. Moreover, breeders in the mink industry understand that color plays a crucial role in determining the final value of the pelt. Thus, selecting parents as per the desired coat color in offspring is given the utmost attention in mink breeding industries. If only there were multiple studies where many variations of coat colors were extensively studied to understand the color inheritance, it would have been immensely beneficial for the mink industry. Thus, this gap is what we wanted to address through the first study of this thesis. To understand color inheritance of four different color types (Dark, Pastel, Demi, and Mahogany), pedigree information of 23,282 individuals were used. The findings of the first study indicated that mating sire and dam of the same color yields offspring of the same color more than 75% of the time. This phenomenon was observed in all four-color types. Additionally, it was observed that to obtain the desired color in offspring, we can consider using either sire or dam of the same color. This implies that the likelihood of producing the expected coat color increases by selecting either of the parent with desired color type even

if same color sire and dam are not available. Similarly, the understanding of color inheritance also includes the determination of the allelic pair (homozygous or heterozygous) responsible for color type. To test if the genes were homozygous or heterozygous, two Mendelian ratios were tested. These ratios were 1:1 and 3:1, which are expected ratios when the genotypes of the parents were considered to be Aa × aa and Aa × Aa, respectively. The results shows that all four colors followed the expected 1:1 and 3:1 ratios suggesting that the color types were heterozygous and were produced as a result of crossbreeding. This finding was further verified by further looking back at the pedigree until four generation where the ancestors of almost all the color types were diverse and only few individuals belonging to the Dark and Pastel color types had the same coat color ancestors. The discovery of the results implies that color inheritance among the mink population is highly consistent and predictable. Farmers can utilize this knowledge and select sire and dam of particular color type as per the demand in the market and maintain the desired coat color within the population thus, maximizing the value and profitability of their mink production.

The importance of carefully crafted selection and mating strategy is not only limited to color but also greatly reduces the mating between related individuals. Mating between related individuals, commonly known as inbreeding, results in inbreeding depression where the overall biological fitness of an animal is reduced. The harmful consequence of inbreeding is largely known among all livestock producers. That is why avoiding inbreeding is one of the major aspects of animal breeding programs. In contrast to other livestock species, there is a notable scarcity on studies focused on investigating inbreeding depression on fitness traits of American mink. Additionally, there have been few studies

utilizing both pedigree and genomic information for estimating inbreeding in mink. Addressing this existing gap is the objective of the second and third study of this thesis.

The pedigree information of 23,282 and genomic information of 1,413 individuals were used to estimate pedigree-based inbreeding (F_{PED}) and two genomic based inbreeding (F_{ROH}, F_{HOM}) coefficients, respectively. Similarly, the reproductive records of 4,723 dams were used to investigate the effect of F_{PED} on the reproductive traits. The traits were, gestation length (GL), total born (TB), total kits alive after 24 hours of birth (LB), total kits alive at weaning (LW), survival rate at birth (SB), survival rate at weaning (SW), average kit weight per litter at birth (AWB) and average kit weight per litter at weaning (AWW). Two different methodologies were employed to estimate inbreeding depression on reproductive traits. Using the first method, where phenotypes were regressed on F_{PED}, it was discovered that the regression coefficients were non-significant ($P>0.05$) with high standard errors. Likewise, using the second method, it was observed that all the correlations between F_{PED} and estimated breeding value (EBV) of the traits were significant ($P<0.05$) but were small and ranged from -0.17 to 0.11. Furthermore, the average inbreeding estimates were calculated as 0.28, -0.03, 0.02 for F_{ROH}, F_{HOM}, and F_{PED}, respectively. It was revealed that ROH was randomly distributed, short ROH (1-4 Mb) were present in higher frequency (60.71%) throughout the genome, and chromosome 1 had the highest number of ROH. In total, 204,539 ROH were identified in the autosomal chromosomes where siding window approach was used.

Based on the findings; a) higher number of short ROH, b) negative average F_{HOM}, and c) lower average F_{PED}, we implied that mating between genetically related individuals has been successfully avoided by the farm. This is further backed up by the observations

regarding inbreeding depression, where both methods showed no inbreeding depression exists in the farm at the present.

Relying solely on phenotypes for selection and the absence of specific breeding program is what makes the mink breeding prone to mating between related individuals and consequently harmful effects on fitness traits. However, based on the observations of the chapters 4 and 5, it is safe to say that the 3% threshold that the farm has been using was effective where the mating between genetically related individuals is seldom practiced. This is exemplary considering the farm because the CCFAR is a small-scale non-commercial farm. The farm does not introduce new mink to the population frequently so that the selection of individuals for breeding is performed from the same population. In general, the current working mechanism of the farm is highly recommendable and can be employed by any other livestock farms without any challenges. Most importantly, no additional resources are required to adopt the practices. Therefore, by leveraging the observations derived from this thesis, other farms can imitate the methodologies to improve the production and achieve breeding goals while minimizing additional resource requirements.

Overall, studying color inheritance, estimating inbreeding, and inbreeding depression gave valuable and broader insights from transmission of coat color traits to understanding inbreeding and its effects on the traits. We hope that these results empower both farmers and future researchers to further expanse their efforts eventually leading to continuous improvement and advancement of breeding practices eventually benefiting their respective sectors.

6.2 RECOMMENDATIONS

a) The coat colors in American mink are very diverse and has many variations. Since only four-color types were studied in this thesis, further studies including diverse array of colors is necessary which will result into more comprehensive understanding of coat color inheritance in American mink.

b) Estimating inbreeding and inbreeding depression in this thesis was performed in a small population and conclusions were derived based on the findings. However, a large population or several populations with different management practices might also provide a divergent prospective regarding inbreeding in American mink population.

c) The focus of this thesis was on reproductive traits. Reproductive traits are indeed one of the most important traits but, it is also crucial to recognize the impact of inbreeding on traits beyond reproduction. Additionally, all the traits in this thesis were from the earlier stages of life. The extended scope to investigate inbreeding depression on later stage traits such as fur quality, growth rate, longevity, disease resistance, sperm quality, quantity, feed efficiency, environmental adaptation etc. can uncover more about the effects of inbreeding.

d) Finally, in this thesis pedigree derived inbreeding coefficient was used to estimate inbreeding depression. However, utilizing genomic information to assess the impact of inbreeding on principal traits of American mink could accurately determine the effects of inbreeding and identify the potential markers involved in the effect.

6.3 LIMITATIONS

a) In Chapter 3, we used pedigree records to categorize individuals based on color type. Since color is objective, it is necessary to acknowledge that the human's perception of color might introduce variations. This subjectivity might lead to inconsistency while categorizing an individual based on color. In contrast, using a spectrophotometer might account for this variation which might result in accurate identification of individuals depending on the color type.

b) In Chapter 4, avoiding mating of closely related individuals is one of the breeding objectives of the farm, which might have played an obvious role in the absence of large numbers of highly inbred individuals and the lack of inbreeding depression for reproductive performances. The strategic selection of prospective sires and dams based on the extent of relationship (not exceeding 3%) might have significantly reduced the negative impacts of inbreeding in the studied population.

c) In chapter 5, we had only 1,413 individuals with genotype information. The smaller sample size might not accurately represent the overall distribution of ROH in the population. A universal limitation across literatures where ROH is used is the lack of consistency in the parameters defining ROH segments which makes comparison between studies very difficult. Lastly, we genotyped 1,413 individuals using SNP chip which was derived from the WGS of 100 individuals. These SNPs were selected from few individuals so the resultant genotyped SNPs are not random SNPs. This phenomenon might lead to bias as a result of using already confirmed SNPs commonly known as "ascertainment bias".

REFERENCES

Adams, S. M., Derks, M. F., Makanjuola, B. O., Marras, G., Wood, B. J., & Baes, C. F. (2021). Investigating inbreeding in the turkey (Meleagris gallopavo) genome. *Poultry Science*, 100(11), 101366.

Addo, S., Klingel, S., Hinrichs, D., & Thaller, G. (2019). Runs of Homozygosity and NetView analyses provide new insight into the genome-wide diversity and admixture of three German cattle breeds. *PLoS One*, *14*(12), e0225847.

Alemu, S. W., Kadri, N. K., Harland, C., Faux, P., Charlier, C., Caballero, A., & Druet, T. (2021). An evaluation of inbreeding measures using a whole-genome sequenced cattle pedigree. *Heredity,* 126(3), 410-423.

Amstislavsky, S., & Ternovskaya, Y. (2000). Reproduction in mustelids. *Animal Reproduction Science*, 60, 571-581.

Anistoroaei, R., Krogh, A. K., & Christensen, K. (2013). A frameshift mutation in the LYST gene is responsible for the Aleutian color and the associated Chédiak–Higashi syndrome in American mink. *Animal Genetics*, *44*(2), 178-183.

Baes, C. F., Makanjuola, B. O., Miglior, F., Marras, G., Howard, J. T., Fleming, A., & Maltecca, C. (2019). Symposium review: The genomic architecture of inbreeding: How homozygosity effects health and performance. *Journal of Dairy Science*, *102*(3), 2807-2817.

Baneh, H., Ahmadpanah, J., & Mandal, A. (2019). Studies on inbreeding and its effects on growth traits of Iran-Black sheep. *Songklanakarin Journal of Science & Technology, 41(6).*

Beishova, I., Dossybayev, K., Shamshidin, A., Belaya, A., Bissembayev, A., Khamzin, K., Kovalchuk, A., & Nametov, A. (2022). Distribution of Homozygosity Regions in the Genome of Kazakh Cattle Breeds. *Diversity*, 14(4), 279.

Bell, J. S. (2011). *The Ins and Outs of Pedigree Analysis, Genetic Diversity, and Genetic Disease Control*. In:Tufts' Canine and Feline Breeding and Genetics Conference; North Graton, MA.

Belliveau, A. M., A. Farid, M. O'Connell. & J. M. Wright. (1999). Assessment of genetic variability in captive and wild American mink (*Mustela vison*) using microsatellite markers. *Canadian Journal of Animal Science,* 79:7-16.

Bérénos, C., Ellis, P. A., Pilkington, J. G., & Pemberton, J. M. (2016). Genomic analysis reveals depression due to both individual and maternal inbreeding in a free-living mammal population. *Molecular Ecology*, 25(13), 3152-3168.

Birchler, J. A., Yao, H., & Chudalayandi, S. (2006). Unraveling the genetic basis of hybrid vigor. *Proceedings of the National Academy of Sciences,* 103(35), 12957-12958.

Bosse, M., Megens, H. J., Madsen, O., Paudel, Y., Frantz, L. A., Schook, L. B., Crooijmans, R. P. M. A., & Groenen, M. A. (2012). Regions of homozygosity in the porcine genome: consequence of demography and the recombination landscape. *PLoS Genetics*, 8(11), e1003100.

Bowman, J., Beauclerc, K., Farid, A. H., Fenton, H., Klütsch, C. F., & Schulte-Hostedde, A. I. (2017). Hybridization of domestic mink with wild American mink (*Neovison vison*) in eastern Canada. *Canadian Journal of Zoology,* 95(6), 443-451.

Broman, K. W., & Weber, J. L. (1999). Long homozygous chromosomal segments in reference families from the centre d'Etude du polymorphisme humain. *The American Journal of Human Genetics,* 65(6), 1493-1500.

Butler, D. G., Cullis, B. R., Gilmour, A. R., Gogel, B. J., & Thompson, R. (2017). ASReml-R reference manual version 4. *ASReml-R Reference Man*.176.

Cai, Z., Villumsen, T. M., Asp, T., Guldbrandtsen, B., Sahana, G., & Lund, M. S. (2018). SNP markers associated with body size and pelt length in American mink (*Neovison vison*). *BMC Genetics,* 19(1), 1-10.

Cardoso, D. F., Fernandes Junior, G. A., Scalez, D. C. B., Alves, A. A. C., Magalhães, A. F. B., Bresolin, T., Ventura, R. V., Li, C., Oliveira, M. C. S., Porto-Neto, L. R., Carvalheiro, R., Oliveira, H. N., Tonhati, H., & Albuquerque, L. G. (2020). Uncovering sub-structure and genomic profiles in across-countries subpopulations of Angus cattle. *Scientific Reports,* 10(1), 8770.

Carolino, N., & Gama, L. T. (2008). Inbreeding depression on beef cattle traits: Estimates, linearity of effects and heterogeneity among sire-families. *Genetics Selection Evolution,* 40, 1-17.

Carothers, A. D., Rudan, I., Kolcic, I., Polasek, O., Hayward, C., Wright, A. F., & Weber, J. L. (2006). Estimating human inbreeding coefficients: comparison of genealogical and marker heterozygosity approaches. *Annals of Human Genetics*, *70*(5), 666-676.

Ceballos, F. C., Joshi, P. K., Clark, D. W., Ramsay, M., & Wilson, J. F. (2018). Runs of homozygosity: windows into population history and trait architecture. *Nature Reviews Genetics,* 19(4), 220-234.

Charlesworth, B. (1990). Mutation-selection balance and the evolutionary advantage of sex and recombination. *Genetics Research*, 55(3), 199-221.

Charlesworth, D., & Willis, J. H. (2009). The genetics of inbreeding depression. *Nature Reviews Genetics,* 10(11), 783-796.

Chen, Z. J. (2013). Genomic and epigenetic insights into the molecular bases of heterosis. *Nature Reviews Genetics,* 14(7), 471-482.

Cheptou, P. O., & Donohue, K. (2011). Environment-dependent inbreeding depression: its ecological and evolutionary significance. *New Phytologist,* 189(2), 395-407.

Chu, E. T., Simpson, M. J., Diehl, K., Page, R. L., Sams, A. J., & Boyko, A. R. (2019). Inbreeding depression causes reduced fecundity in Golden Retrievers. *Mammalian Genome*, 30(5-6), 166-172.

Cieslak, M., Reissmann, M., Hofreiter, M., & Ludwig, A. (2011). Colours of domestication. *Biological Reviews,* 86(4), 885-899.

Cirera, S., M. N. Markakis, K. Christensen, and R. Anistoroaei. (2013). New insights into the melanophilin (MLPH) gene controlling coat color phenotypes in American mink. *Gene,* 527:48–54.

Cirera, S., Markakis, M. N., Kristiansen, T., Vissenberg, K., Fredholm, M., Christensen, K., & Anistoroaei, R. (2016). A large insertion in intron 2 of the TYRP1 gene associated with American Palomino phenotype in American mink. *Mammalian Genome,* 27(3):135-43.

Cortés, G. L. A., Martínez-Ávila, J. C., & Toro, M. A. (2010). Fine decomposition of the inbreeding and the coancestry coefficients by using the tabular method. *Conservation Genetics*, *11*, 1945-1952.

Costantini, F., Fauvelot, C., & Abbiati, M. (2007). Fine-scale genetic structuring in Corallium rubrum: evidence of inbreeding and limited effective larval dispersal. *Marine Ecology Progress Series*, *340*, 109-119.

Crnokrak, P., & Roff. D. (1999). Inbreeding depression in the wild. *Heredity*. 83:260–270.

Curik, I., Ferenčaković, M., & Sölkner, J. (2014). Inbreeding and runs of homozygosity: A possible solution to an old problem. *Livestock Science,* 166, 26-34.

Demontis, D., Larsen, P. F., Bækgaard, H., Sønderup, M., Hansen, B. K., Nielsen, V. H., Loeschcke, V., Zalewski., H., & Pertoldi, C. (2011). Inbreeding affects fecundity of American mink (*Neovison vison*) in Danish farm mink. *Animal Genetics*, 42(4), 437-439.

Dixit, S. P., Singh, S., Ganguly, I., Bhatia, A. K., Sharma, A., Kumar, N. A., Dang, A. K., & Jayakumar, S. (2020). Genome-wide runs of homozygosity revealed selection signatures in Bos indicus. *Frontiers in Genetics,* 11, 92.

Doekes, H. P., Bijma, P., Veerkamp, R. F., de Jong, G., Wientjes, Y. C., & Windig, J. J. (2020). Inbreeding depression across the genome of Dutch Holstein Friesian dairy cattle. *Genetics Selection Evolution*, 52(1), 1-18.

Doekes, H. P., Veerkamp, R. F., Bijma, P., de Jong, G., Hiemstra, S. J., & Windig, J. J. (2019). Inbreeding depression due to recent and ancient inbreeding in Dutch Holstein–Friesian dairy cattle. *Genetics Selection Evolution*, 51, 1-16.

Dorado, J., Cid, R. M., Molina, A., Hidalgo, M., Ariza, J., Moreno-Millán, M., & Demyda-Peyrás, S. (2017). Effect of inbreeding depression on bull sperm quality and field fertility. *Reproduction, Fertility and Development,* 29(4), 712-720.

Dorostkar, M., Shodja, J., Rafat, S. A., Rokouei, M., & Esfandyari, H. (2012). Inbreeding and inbreeding depression in Iranian Moghani sheep breed. *Journal of Agricultural Science and Technology,* 14(3), 549-556.

Duthie, A. B., & Reid, J. M. (2016). Evolution of inbreeding avoidance and inbreeding preference through mate choice among interacting relatives. *The American Naturalist,* 188(6), 651-667.

Eklund, C. M., Hadlow, W. J., Kennedy, R. C., Boyle, C. C., & Jackson, T. A. (1968). Aleutian disease of mink: properties of the etiologic agent and the host responses. *The Journal of Infectious Diseases,* 118(5), 510-526.

Ellegren, H., & Galtier, N. (2016). Determinants of genetic diversity. *Nature Reviews Genetics,* 17(7), 422-433.

Falconer, D. S. & Mackay, T. F. C. (1996). *Introduction to Quantitative Genetics*; Pearson, P.H., 3rd Ed.; Longman: Harlow, UK.

Fatma, R., Chauhan, W., & Afzal, M. (2023). The coefficients of inbreeding revealed by ROH study among inbred individuals belonging to each type of the first cousin marriage: A preliminary report from North India. *Genes & Genomics*, 1-13.

Ferencakovic, M., Hamzic, E., Gredler, B., Curik, I., & Sölkner, J. (2011). Runs of homozygosity reveal genome-wide autozygosity in the Austrian Fleckvieh cattle. *Agriculturae Conspectus Scientificus*, *76*(4), 325-329.

Ferenčaković, M., Hamzić, E., Gredler, B., Solberg, T. R., Klemetsdal, G., Curik, I., & Sölkner, J. (2013). Estimates of autozygosity derived from runs of homozygosity: empirical evidence from selected cattle populations. *Journal of Animal Breeding and Genetics,* 130(4), 286-293.

Fernández, A., Rodrigáñez, J., Toro, M. A., Rodríguez, M. C., & Silió, L. (2002). Inbreeding effects on the parameters of the growth function in three strains of Iberian pigs. *Journal of Animal Science,* 80(9), 2267-2275.

Forutan, M., Ansari Mahyari, S., Baes, C., Melzer, N., Schenkel, F. S., & Sargolzaei, M. (2018). Inbreeding and runs of homozygosity before and after genomic selection in North American Holstein cattle. *BMC Genomics*, *19*, 1-12.

Fox, C. W., & Reed, D. H. (2011). Inbreeding depression increases with environmental stress: an experimental study and meta-analysis. *Evolution*, *65*(1), 246-258.

Getahun, D., Alemneh, T., Akeberegn, D., Getabalew, M., & Zewdie, D. (2019). Importance of hybrid vigor or heterosis for animal breeding. *Biochemistry and Biotechnology Research,* 7(1), 1-4.

Ghoreishifar, S. M., Moradi-Shahrbabak, H., Fallahi, M. H., Jalil Sarghale, A., Moradi-Shahrbabak, M., Abdollahi-Arpanahi, R., & Khansefid, M. (2020). Genomic measures of inbreeding coefficients and genome-wide scan for runs of homozygosity islands in Iranian river buffalo, Bubalus bubalis. *BMC Genetics,* 21(1), 1-12.

Goddard, M. (2009). Fitness traits in animal breeding programs. *Adaptation and Fitness in Animal populations*, 41-52.

Gorbach, D., M. Makgahlela, J. S. R. Oum., O. Mwai, K. Marshall, B. Murdoch, S. Moore, & M. Rothschild. (2010). Use of SNP genotyping to determine pedigree and breed composition of dairy cattle in Kenya. *Journal of Animal Breeding and Genetics,* 127: 348-351.

Gorssen, W., Meyermans, R., Janssens, S., & Buys, N. (2021). A publicly available repository of ROH islands reveals signatures of selection in different livestock and pet species. *Genetics Selection Evolution*, 53, 1-10.

Goszczynski, D., Molina, A., Terán, E., Morales-Durand, H., Ross, P., Cheng, H., Giovambattista, G., & Demyda-Peyrás, S. (2018). Runs of homozygosity in a selected cattle population with extremely inbred bulls: Descriptive and functional analyses revealed highly variable patterns. *PloS One,* 13(7), e0200069.

Groen, A. F. (2000). Breeding goal definition. Developing Breeding Strategies for Lower Input Animal Production Environments. *ICAR, Villa del Ragno, Via Nomentana,* 134, 00162.

Hansen, H. O. (2014). The global fur industry: Trends, Globalization and Specialization. *Journal of Agricultural Science and Technology,* 4(7A).

Hasler, H. S., Burren, A., Neuditschko, M., Frischknecht, M., Garrick, D., Stricker, C., Gredler, B., Bapst, B., & Flury, C. (2017). Population structure and genomic inbreeding in nine Swiss dairy cattle populations. *Genetics Selection Evolution,* 49, 1-13.

Hedrick, P. W., & Dorado, G. A. (2016). Understanding inbreeding depression, purging, and genetic rescue. *Trends in Ecology and Evolution*, 31(12), 940-952.

Hedrick, P. W., & Kalinowski, S. T. (2000). Inbreeding depression in conservation biology. *Annual Review of Ecology, Evolution, and Systematics*, 31:139–62.

Hedrick, P. W., Hoeck, P. E., Fleischer, R. C., Farabaugh, S., & Masuda, B. M. (2016). The influence of captive breeding management on founder representation and inbreeding in the ʻAlalā, the Hawaiian crow. *Conservation Genetics,* 17, 369-378.

Heidaritabar, M., Carney, V., Groenen, M. A., & Plastow, G. (2022). Assessing the genomic diversity and relatedness in 10 Canadian heritage chicken lines using whole-genome sequence data. *Journal of Animal Breeding and Genetics,* 139(5), 556-573.

Hermas, S. A., Young, C. W., & Rust, J. W. (1987). Effects of mild inbreeding on productive and reproductive performance of Guernsey cattle. *Journal of Dairy Science,* 70(3), 712-715.

Hernández, J. C., García-Ruiz, A., Vásquez-Peláez, C. G., & Ruiz-Lopez, F. D. J. (2021). Correlation of Genomic and Pedigree Inbreeding Coefficients in Small Cattle Populations. *Animals*, 11(11), 3234.

Higgs, P. G., & Derrida, B. (1992). Genetic distance and species formation in evolving populations. *Journal of Molecular Evolution,* 35, 454-465.

Hinrichs, D., Bennewitz, J., Wellmann, R., & Thaller, G. (2015). Estimation of ancestral inbreeding effects on stillbirth, calving ease and birthweight in German Holstein dairy cattle. *Journal of Animal Breeding and Genetics*, *132*(1), 59-67.

Hogg, C. J., Wright, B., Morris, K. M., Lee, A. V., Ivy, J. A., Grueber, C. E., & Belov, K. (2019). Founder relationships and conservation management: empirical kinships reveal the effect on breeding programmes when founders are assumed to be unrelated. *Animal Conservation,* 22(4), 348-361.

Howard, J. T., Pryce, J. E., Baes, C., & Maltecca, C. (2017). Invited review: Inbreeding in the genomics era: Inbreeding, inbreeding depression, and management of genomic variability. *Journal of Dairy Science*, 100(8), 6009-6024.

Howrigan, D. P., Simonson, M. A., & Keller, M. C. (2011). Detecting autozygosity through runs of homozygosity: a comparison of three autozygosity detection algorithms. *BMC Genomics,* 12(1), 1-15.

Hu, G., Do, D. N., Manafiazar, G., Kelvin, A. A., Sargolzaei, M., Plastow, G., Wang, Z., & Miar, Y. (2023). Population Genomics of American Mink Using Genotypes Data. *Frontiers in Genetics*, 14, 698.

Huson, H. J., Parker, H. G., Runstadler, J., & Ostrander, E. A. (2010). A genetic dissection of breed composition and performance enhancement in the Alaskan sled dog. *BMC Genetics*, 11(1), 1-14.

Isa, A. M., Sun, Y., Shi, L., Jiang, L., Li, Y., Fan, J., Wang, P., Ni, A., Huang, Z., Ma, H., Li, D., & Chen, J. (2020). Hybrids generated by crossing elite laying chickens exhibited heterosis for clutch and egg quality traits. *Poultry Science,* 99(12), 6332-6340.

Jarnecka, O., Bauer, E. A., & Jagusiak, W. (2021). Pedigree analysis in the Polish Red cattle population. *Animal,* 15(6), 100238.

Jaworski, Z., Kamiński, S., Hering, D. M., Zabolewicz, T., & Ruść, A. (2017). Assessment of genomic inbreeding in Polish Konik horses. *Polish Journal of Veterinary Sciences*, 3.

Johannessen, K. R., Børsting, E., & Kristiansen, H. (2004). *Inbreeding in a commercial fur animal breeding program.* In Proceedings of the VIII International Scientific Congress in Fur Animal Production. 201-205. The Netherlands: 's-Hertogenbosch.

Kardos, M., Luikart, G., & Allendorf, F. W. (2015). Measuring individual inbreeding in the age of genomics: marker-based measures are better than pedigrees. *Heredity,* 115(1), 63-72.

Kardos, M., Taylor, H. R., Ellegren, H., Luikart, G., & Allendorf, F. W. (2016). Genomics advances the study of inbreeding depression in the wild. *Evolutionary Applications*,9(10), 1205-1218.

Karimi, K., Do, D. N., Wang, J., Easley, J., Borzouie, S., Sargolzaei, M., Plastow, G., Wang, Z., & Miar, Y. (2022). A chromosome-level genome assembly reveals genomic characteristics of the American mink (*Neogale vison*). *Communications Biology,* 5(1), 1381.

Karimi, K., Farid, A. H., Sargolzaei, M., Myles, S., & Miar, Y. (2020). Linkage disequilibrium, effective population size and genomic inbreeding rates in American mink using genotyping-by-sequencing data. *Frontiers in Genetics*, 11, 223.

Karimi, K., Ngoc, Do. D, Sargolzaei, M., & Miar, Y. (2021). Population genomics of American mink using whole genome sequencing data. *Genes,*12(2):258-276.

Karimi, K., Sargolzaei, M., Plastow, G. S., Wang, Z., & Miar, Y. (2018). Genetic and phenotypic parameters for litter size, survival rate, gestation length, and litter weight traits in American mink. *Journal of Animal Science*, 96(7), 2596-2606.

Keller, L. F., & Waller, D. M. (2002). Inbreeding effects in wild populations. *Trends in Ecology & Evolution*, *17*(5), 230-241.

Keller, M. C., Simonson, M. A., Ripke, S., Neale, B. M., Gejman, P. V., Howrigan, D. P., Sang H. L., Lencz, T., Levinson, D. F., & Sullivan, P. F. (2012). Runs of homozygosity implicate autozygosity as a schizophrenia risk factor. *PLoS Genetics,* 8(4), e1002656.

Keller, M. C., Visscher, P. M., & Goddard, M. E. (2011). Quantification of inbreeding due to distant ancestors and its detection using dense single nucleotide polymorphism data. *Genetics,* 189(1), 237-249.

Kettunen, A., Daverdin, M., Helfjord, T., & Berg, P. (2017). Crossbreeding is inevitable to conserve the highly inbred population of puffin hunter: The Norwegian Lundehund. *PLoS One,* 12(1), e0170039.

Kidd, A. G., Bowman, J., Lesbarreres, D., & Schulte-Hostedde, A. I. (2009). Hybridization between escaped domestic and wild American mink (*Neovison vison*). *Molecular Ecology,* 18(6), 1175-1186.

Kim, E. S., Cole, J. B., Huson, H., Wiggans, G. R., Van Tassell, C. P., Crooker, B. A. C., Liu, G., Tad, Y. D., & Sonstegard, T. S. (2013). Effect of artificial selection on runs of homozygosity in US Holstein cattle. *PloS One,* 8(11), e80813.

Kirin, M., McQuillan, R., Franklin, C. S., Campbell, H., McKeigue, P. M., & Wilson, J. F. (2010). Genomic runs of homozygosity record population history and consanguinity. *PloS One,* 5(11), e13996.

Koivula, M., Strandén, I., & Mäntysaari, E. A. (2010). Genetic and phenotypic parameters of age at first mating, litter size and animal size in Finnish mink. *Animal,* 4(2), 183-188.

Kokko, H., & Ots, I. (2006). When not to avoid inbreeding. *Evolution,* 60(3), 467-475.

Kołodziejczyk, D., & Socha, S. (2008). Analysis of conformation traits in mink of standard and palomino colour types. *Scientifur. IX International Scientific Congress in Fur Animal Production*. Halifax, Nova Scotia, Canada, August 19-23;32(4):59-62.

Köster, E. V. M., & Visser, C. (2021). Unintended consequences of selection for increased production on the health and welfare of livestock. *Archives Animal Breeding,* 64(1), 177-185.

Kruska, D. (1996). The effect of domestication on brain size and composition in the mink (*Mustela vison*). *Journal of Zoology*, *239*(4), 645-661.

Kruska, D. C., & Sidorovich, V. E. (2003). Comparative allometric skull morphometrics in mink (*Mustela vison* Schreber, 1777) of Canadian and Belarus origin; taxonomic status. *Mammalian Biology,* 68(5), 257-276.

Kuehn, L. A., J. W. Keele, & R. M. Thallman. (2018). Using Breed Composition, Breed Differences, Selection Tools, and New Technologies to Optimize Commercial Cattle Production and Allocation of Beef Cattle in Research Programs. *Journal of Animal Science, 9,* 216-217.

Legarra, A., Christensen, O. F., Vitezica, Z. G., Aguilar, I., & Misztal, I. (2015). Ancestral relationships using metafounders: finite ancestral populations and across population relationships. *Genetics*, *200*(2), 455-468.

Leroy, G. (2014). Inbreeding depression in livestock species: review and meta-analysis. *Animal Genetics*, 45(5), 618-628.

Leutenegger, A. L., Prum, B., Génin, E., Verny, C., Lemainque, A., Clerget-Darpoux, F., & Thompson, E. A. (2003). Estimation of the inbreeding coefficient through use of genomic data. *The American Journal of Human Genetics*, 73(3), 516-523.

Li, Z., Wu, X. L., Guo, W., He, J., Li, H., Rosa, G. J. M., Gianola, D., Tait, Jr. R. G., Praham, J., Genho, J., Schultz, T., & Bauck, S. (2020). Estimation of genomic breed composition of individual animals in composite beef cattle. *Animal Genetics,* 51(3), 457-460.

Liu, S. H., Ma, X. Y., Hassan, F. U., Gao, T. Y., & Deng, T. X. (2022). Genome-wide analysis of runs of homozygosity in Italian Mediterranean buffalo. *Journal of Dairy Science,* 105(5), 4324-4334.

Lopes, M. S., Silva, F. F., Harlizius, B., Duijvesteijn, N., Lopes, P. S., Guimarães, S. E., & Knol, E. F. (2013). Improved estimation of inbreeding and kinship in pigs using optimized SNP panels. *BMC Genetics,* 14(1), 1-11.

Macdonald, D. W., & Harrington, L. A. (2003) The American mink: the triumph and tragedy of adaptation out of context. *New Zealand Journal of Zoology*, 30(4):421-441.

Makanjuola, B. O., Maltecca, C., Miglior, F., Schenkel, F. S., & Baes, C. F. (2020). Effect of recent and ancient inbreeding on production and fertility traits in Canadian Holsteins. *BMC Genomics,* 21, 1-15.

Mamun, H. A., Clark, S., Kwan, P., & Gondro, C. (2015). Genome-wide linkage disequilibrium and genetic diversity in five populations of Australian domestic sheep. *Genetics Selection Evolution,* 47(1), 1-14.

Manakhov, A. D., Andreeva, T. V., Trapezov, O. V., Kolchanov, N. A., & Rogaev, E. I. (2019). Genome analysis identifies the mutant genes for common industrial Silverblue and Hedlund white coat colours in American mink. *Scientific Reports*, 9(1), 4581.

Manakhov, A. D., Mintseva, M. Y., Andreeva, T. V., Filimonov, P. A., Onokhov, A. A., Chernova, I. E., Kashtanov, S. N., Rogaev, E. I. (2021). Genome analysis of sable fur color links a lightened pigmentation phenotype to a frameshift variant in the tyrosinase-related protein 1 gene. *Genes,* 12(2):157-165.

Manakhov, A. D., Mintseva, M. Y., Andreeva, T. V., Trapezov, O. V., & Rogaev, E. I. (2022). Shadow coat colour in American mink associated with a missense mutation in the KIT gene. *Animal Genetics,* 53(4):522-525.

Manakhov. A. D., Andreeva, T. V., Trapezov, O. V., Kolchanov, N. A., Rogaev, E. I. (2009). Genome analysis identifies the mutant genes for common industrial Silverblue and Hedlund white coat colours in American mink. *Scientific Reports,* 9:1-8.

Marchesi, J. A. P., Buzanskas, M. E., Cantão, M. E., Ibelli, A. M. G., Peixoto, J. O., Joaquim, L. B., Moreira, G. C. M., Godoy, T. F., Sbardella, A. P., Figueiredo, E. A. P., Coutinho, L. L., Maunari, D. P., & Ledur, M. C. (2018). Relationship of runs of homozygosity with adaptive and production traits in a paternal broiler line. *Animal,* 12(6), 1126-1134.

Marras, G., Gaspa, G., Sorbolini, S., Dimauro, C., Ajmone-Marsan, P., Valentini, A., Williams, J. L., & Macciotta, N. P. (2015). Analysis of runs of homozygosity and their relationship with inbreeding in five cattle breeds farmed in Italy. *Animal Genetics*, 46(2), 110-121.

Mátyás, C. S. (2004). Genetics and Genetic Resource| Population, Conservation and Ecological Genetics. Encyclopedia of Forest Sciences, *Elsevier,* 188-197.

Mayo, O. (2008). A century of Hardy–Weinberg equilibrium. *Twin Research and Human Genetics*, *11*(3), 249-256.

McQuillan, R., Leutenegger, A. L., Abdel-Rahman, R., Franklin, C. S., Pericic, M., Lauc, B. L., Narancic, S., Janicijevic, B., Polasek, O., Tenesa, A., Macleod, A. K., Farrington S. M., Rudan, P., Hayward, C., Rudan, V. V. I., Wild, S. H., Dunlop, M. G., Wright, A. F., Campbell, H., & Wilson, J. F. (2008). Runs of homozygosity in European populations. *The American Journal of Human Genetics*, *83*(3), 359-372.

Miglior, F., Szkotnicki, B., & Burnside, E. B. (1992). Analysis of levels of inbreeding and inbreeding depression in Jersey cattle. *Journal of Dairy Science*, 75(4), 1112-1118.

Miglior, F., Van Doormaal, B. J., & Kistemaker, G. (2008). Phenotypic analysis of inbreeding depression for traits measured in Canadian dairy cattle breeds. *Canadian Journal of Animal Science,* 89, 128.

Milanović, V., Mrvić, V., Teodorović, V., Milosavljević, P., Prokić, B., & Avdić, R. (2013). Morphology investigation of the mink's brain (*Mustela vison*). *Acta Veterinaria- Beograd*, 63(2-3), 337-345.

Miller, P. S. (1994). Is inbreeding depression more severe in a stressful environment? *Zoo Biology*, 13(3), 195-208.

Morris, K. Y., Bowman, J., Schulte-Hostedde, A., & Wilson, P. J. (2020). Functional genetic diversity of domestic and wild American mink (*Neovison vison*). *Evolutionary Applications,* 13(10), 2610-2629.

Mulim, H. A., Brito, L. F., Pinto, L. F. B., Ferraz, J. B. S., Grigoletto, L., Silva, M. R., & Pedrosa, V. B. (2022). Characterization of runs of homozygosity, heterozygosity-enriched regions, and population structure in cattle populations selected for different breeding goals. *BMC Genomics*, 23(1), 209.

Nasner, S. C., López-Herrera, A., González-Herrera, L. G., & Rincón, J. C. (2021). Diversity analysis runs of homozygosity and genomic inbreeding reveal recent selection in Blanco Orejinegro cattle. *Journal of Animal Breeding and Genetics,* 138(5), 613-627.

Neaves, L. E., Eales, J., Whitlock, R., Hollingsworth, P. M., Burke, T., & Pullin, A. S. (2015). The fitness consequences of inbreeding in natural populations and their implications for species conservation–a systematic map. *Environmental Evidence*, 4(1), 1-17.

Nest, M. A., Hlongwane, N., Hadebe, K., Chan, W. Y., Van der Merwe, N. A., De Vos, L., Greyling, B., Kooverjee, B, B., Soma, P., Dzomba, E, F., Bradfield, M., & Muchadeyi, F. C. (2021). Breed ancestry, divergence, admixture, and selection patterns of the Simbra crossbreed. *Frontiers in Genetics,* 1796.

NFACC. Code of practice for the care and handling of farmed mink. (2014). National Farm Animal Care Council, Lacombe, Alberta. Retrieved from https://www.nfacc.ca/pdfs/codes/mink_code_of_practice.pdf, Date accessed: Sept. 14th, 2022.

Nguluma, A., Kyallo, M., Tarekegn, G. M., Loina, R., Nziku, Z., Chenyambuga, S., & Pelle, R. (2022). Typology and characteristics of indigenous goats and production systems in different agro-ecological zones of Tanzania. *Tropical Animal Health and Production,* 54(1), 70.

Nietlisbach, P., Keller, L. F., Camenisch, G., Guillaume, F., Arcese, P., Reid, J. M., & Postma, E. (2017). Pedigree-based inbreeding coefficient explains more variation in fitness than heterozygosity at 160 microsatellites in a wild bird population. *Proceedings of the Royal Society B: Biological Sciences,* 284(1850), 20162763.

North American Fur Auctions (NAFA). (2014). Wild Fur Pelt Handling Manual. *In Wild Fur Pelt Handling Manual*; NAFA: Toronto, ON, Canada, 38. Available online on: https://paperzz.com/doc/8033272/ranch-mink. Date accessed: Sept. 14th, 2022.

Notter, D. R. (1999). The importance of genetic diversity in livestock populations of the future. *Journal of Animal Science,* 77(1), 61-69.

Ospina, A. M., Herrera Rios, A. C., Pimenta Schettini, G., Vallejo Aristizabal, V. H., Bizarria dos Santos, W., Zapata, C. A., & Ortiz Morea, E. G. (2022). Identification of Runs of Homozygosity Islands and Genomic Estimated Inbreeding Values in Caqueteño Creole Cattle (Colombia). *Genes,* 13(7), 1232.

Pekkala, N., Knott, K. E., Kotiaho, J. S., Nissinen, K., & Puurtinen, M. (2014). The effect of inbreeding rate on fitness, inbreeding depression and heterosis over a range of inbreeding coefficients. *Evolutionary Applications,* 7(9), 1107-1119.

Peripolli, E., Munari, D. P., Silva, M. V. G. B., Lima, A. L. F., Irgang, R., & Baldi, F. (2017). Runs of homozygosity: current knowledge and applications in livestock. *Animal Genetics,* 48(3), 255-271.

Pike, V. L., Cornwallis, C. K., & Griffin, A. S. (2021). Why don't all animals avoid inbreeding? *Proceedings of the Royal Society B,* 288(1956), 20211045.

Praharani, L., & Krisnan, R. (2016). A preliminary study on some reproductive traits and heterosis effects of Anglo Nubian and Etawah grade crossbred does. *International Seminar on Livestock Production and Veterinary Technology,* 252-260.

Prasolova, L. A., & Trapezov, O. V. (2006). Effect of Coat Color Genes on the Hair Pigmentation Morphology in the American Mink. *Russian Journal of Genetics,* 43:815–818.

Pryce, J. E., Haile-Mariam, M., Goddard, M. E., & Hayes, B. J. (2014). Identification of genomic regions associated with inbreeding depression in Holstein and Jersey dairy cattle. *Genetics Selection Evolution,* 46, 1-14.

Purcell, S., Neale, B., Todd-Brown, K., Thomas, L., Ferreira, M. A. R., Bender, D., Maller, J., Sklar, P., Bakker, P. I. W., Daly, M. L., & Sham, P. C. (2007). PLINK: A tool set for whole-genome association and population-based linkage analyses. *American Journal of Human Genetics,* 81, 559–575.

Purfield, D. C., Berry, D. P., McParland, S., & Bradley, D. G. (2012). Runs of homozygosity and population history in cattle. *BMC Genetics,* 13(1), 1-11.

Raya, L. G., Rodríguez, C., Barragán, C., & Silió, L. (2015). Genomic inbreeding coefficients based on the distribution of the length of runs of homozygosity in a closed line of Iberian pigs. *Genetics Selection Evolution,* 47, 1-15.

Recio, G. O., Maturana, E. L., & Gutiérrez, J. P. (2007). Inbreeding depression on female fertility and calving ease in Spanish dairy cattle. *Journal of Dairy Science,* 90, 12: 5744-5752.

Reverter, A., Porto-Neto, L. R., Fortes, M. R. S., Kasarapu, P., De Cara, M. A. R., Burrow, H. M., & Lehnert, S. A. (2017). Genomic inbreeding depression for climatic adaptation of tropical beef cattle. *Journal of Animal Science*, 95(9), 3809-3821.

Rodrigáñez, J., Toro, M. A., Rodriguez, M. C., & Silió, L. (1998). Effect of founder allele survival and inbreeding depression on litter size in a closed line of Large White pigs. *Animal Science,* 67(3), 573-582.

Roy, A., Frascaria, N., MacKay, J., & Bousquet, J. (1992). Segregating random amplified polymorphic DNAs (RAPDs) in Betula alleghaniensis. *Theoretical and Applied Genetics,* 85, 173-180.

R Core Team. (2022). R: A Language and Environment for Statistical Computing. *R Foundation for Statistical Computing.* Vienna, Austria.

Santos, W. B., Pimenta Schettini, G., Fonseca, M. G., Pereira, G. L., Loyola Chardulo, L. A., Neto, O. R. M., Baldassini, W. A., Oliveira, H. N., & Curi, R. A. (2021). Fine-scale estimation of inbreeding rates, runs of homozygosity and genome-wide heterozygosity levels in the Mangalarga Marchador horse breed. *Journal of Animal Breeding and Genetics,* 138(2), 161-173.

Sarfatti, M., Abu-Abied, M., Katan, J., & Zamir, D. (1991). RFLP mapping of I1, a new locus in tomato conferring resistance against *Fusarium oxysporum* f. sp. lycopersici race 1. *Theoretical and Applied Genetics*, 82, 22-26.

Sargolzaei, M., Iwaisaki, H., & Colleau, J. J. (2006). *CFC: A tool for monitoring genetic diversity.* In Proceedings of the 8th world congress on genetics applied to livestock production. Belo Horizonte, Brazil, 13-18.

Saura, M., Fernández, A., Varona, L., Fernández, A. I., de Cara, M. Á. R., Barragán, C., & Villanueva, B. (2015). Detecting inbreeding depression for reproductive traits in Iberian pigs using genome-wide data. *Genetics Selection Evolution,* 47(1), 1-9.

Schäler, J., Krüger, B., Thaller, G., & Hinrichs, D. (2020). Comparison of ancestral, partial, and genomic inbreeding in a local pig breed to achieve genetic diversity. *Conservation Genetics Resources,* 12, 77-86.

Schiavo, G., Bovo, S., Bertolini, F., Tinarelli, S., Dall'Olio, S., Costa, L. N., Gallo, M., & Fontanesi, L. (2021). Comparative evaluation of genomic inbreeding parameters in seven commercial and autochthonous pig breeds. *Animal,* 14(5), 910-920.

Shackelford, R. M. (1948). The nature of coat color differences in mink and foxes. *Genetics,* 33(3), 311.

Shackelford, R. M. (1949). Six mutations affecting coat color in ranch-bred mink. *American Naturalist,* 8:49-67.

Shackelford, R. M., Cole, L. J. (1947). "Screw neck" in the Pastel color phase of ranch-bred mink. *Journal of Heredity,*38(7):203-209.

Shi, L., Wang, L., Liu, J., Deng, T., Yan, H., Zhang, L., Liu, X., Gao, H., Hou, X., Wang, L., & Zhao, F. (2020). Estimation of inbreeding and identification of regions under heavy selection based on runs of homozygosity in a Large White pig population. *Journal of Animal Science and Biotechnology,* 11, 1-10.

Silva, M. H. M. A. D., Malhado, C. H. M., Kern, E. L., Daltro, D. D. S., Cobuci, J. A., & Carneiro, P. L. S. (2019). Inbreeding depression in Holstein cattle in Brazil. *Revista Brasileira de Zootecnia*, 48.

Song, X., Xu, C., Liu, Z., Yue, Z., Liu, L., Yang, T., Cong, B., & Yang, F. (2017). Comparative transcriptome analysis of mink (*Neovison vison*) skin reveals the key genes involved in the melanogenesis of black and white coat colour. *Scientific Reports,* 7(1), 1-11.

Spielman, D., Brook, B. W., Briscoe, D. A., & Frankham, R. (2004). Does inbreeding and loss of genetic diversity decrease disease resistance? *Conservation Genetics,* 5(4), 439-448.

Staples, J., Qiao, D., Cho, M. H., Silverman, E. K., Nickerson, D. A., & Below, J. E. (2014). PRIMUS: rapid reconstruction of pedigrees from genome-wide estimates of identity by descent. *The American Journal of Human Genetics*, *95*(5), 553-564.

Statistics Canada. (2018). Number and value of mink pelts sold, by colour type. Retrieved March 30, 2023, from https://www150.statcan.gc.ca/t1/tbl1/en/tv.action?pid=3210011501.

Statistics Canada. (2021). Supply and disposition of mink and fox on fur farms. Retrieved March 30, 2023, from https://www150.statcan.gc.ca/t1/tbl1/en/tv.action?pid=3210011601

Strandén, I., & Peura, J. (2007). Inbreeding and relationship coefficients in the Finnish blue fox population. *Agricultural and Food Science.* 16, 147-156.

Sumreddee, P., Toghiani, S., Roberts, A., Aggrey, S. E., & Rekaya, R. (2021). Grid search approach to discriminate between old and recent inbreeding using phenotypic, pedigree and genomic information. *BMC Genomics,* 22(1), 1-17.

Szpiech, Z. A., Xu, J., Pemberton, T. J., Peng, W., Zöllner, S., Rosenberg, N. A., & Li, J. Z. (2013). Long runs of homozygosity are enriched for deleterious variation. *The American Journal of Human Genetics,* 93(1), 90-102.

Tamlin, A. L., Bowman, J., & Hackett, D. F. (2009). Separating wild from domestic American mink *Neovison vison* based on skull morphometries. *Wildlife Biology*. 15(3):266-277.

Tanchev, S. (2016). Classical and modern concepts of inbreeding and effects of inbreeding depression in animals. *Agricultural Science and Technology,* 8(1), 3-13.

Tao, L., Liu, Y. F., Zhang, H., Li, H. Z., Zhao, F. P., Wang, F. Y., Zhang, R., & Chu, M. X. (2021). Genome-wide association study and inbreeding depression on body size traits in Qira black sheep (*Ovis aries*). *Animal Genetics*, 52(4), 560-564.

Theodorou, K., & Couvet, D. (2006). On the expected relationship between inbreeding, fitness, and extinction. *Genetics Selection Evolution,* 38(4), 371-387.

Thirstrup, J. P., Larsen, P. F., Pertoldi, C., & Jensen, J. (2014). Heterosis and genetic variation in the litter size of purebred and crossbred mink. *Journal of Animal Science*, 92(12), 5406-5416.

Turner, P., Buijs, S., Rommers, J. M., & Tessier, M. (2013). The Code of Practice for the Care and Handling of Farmed Mink; *The National Farm Animal Care Council*: Rexdale, ON, Canada,58.

Ujvari, B., Klaassen, M., Raven, N., Russell, T., Vittecoq, M., Hamede, R., Thomas, F., & Madsen, T. (2018). Genetic diversity, inbreeding and cancer. *Proceedings of the Royal Society B: Biological Sciences,* 285(1875), 20172589.

Valipour, S., Karimi, K., Do, D. N., Barrett, D., Sargolzaei, M., Plastow, G., Wang, Z & Miar, Y. (2022). Genome-Wide Detection of Selection Signatures for Pelt Quality Traits and Coat Color Using Whole-Genome Sequencing Data in American Mink. *Genes,* 13 (11), 1939.

Voß, K., Blaj, I., Tetens, J. L., Thaller, G., & Becker, D. (2022). Roan coat color in livestock. *Animal Genetics,* 53(5), 549-556.

Wacławik, P., Grabolus, D., Zatoń-Dobrowolska, M., & Kruszyński, W. (2021). Coat colour inheritance in American mink (*Neovison vison*): pedigree analysis. *Acta Scientiarum Polonorum Zootechnica,* 19(4), 33-38.

Wang, J. (2011). COANCESTRY: a program for simulating, estimating and analysing relatedness and inbreeding coefficients. *Molecular Ecology Resources*, *11*(1), 141-145.

Wang, J. (2014). Marker-based estimates of relatedness and inbreeding coefficients: an assessment of current methods. *Journal of Evolutionary Biology,* 27(3), 518-530.

Wang, L., Zhou, S., Liu, G., Lyu, T., Shi, L., Dong, Y., He, S., & Zhang, H. (2022). Comparative transcriptome reveals the mechanism of mink fur development and color formation. *Research Square.* Retrieved from: https://www.researchsquare.com/article/rs-1556708/v1, Date Assessed: 29th May 2023.

Werf, J. V. D. (2015). Principles of estimation of breeding values. *Genetic evaluation and breeding program design.* Armidale, Australia: University of New England, 1-17.

Wright, L. I., Tregenza, T., & Hosken, D. J. (2008). Inbreeding, inbreeding depression and extinction. *Conservation Genetics*, 9, 833-843.

Xie, R., Shi, L., Liu, J., Deng, T., Wang, L., Liu, Y., & Zhao, F. (2019). Genome-wide scan for runs of homozygosity identifies candidate genes in three pig breeds. *Animals,* 9(8), 518.

Xu, Z., Mei, S., Zhou, J., Zhang, Y., Qiao, M., Sun, H., Li, Z., Li, L., Dong, B., Oyelami, F. O., Wu, J., & Peng, X. (2021). Genome-wide assessment of runs of homozygosity and estimates of genomic inbreeding in a chinese composite pig breed. *Frontiers in Genetics,* 12, 720081.

Yoshida, G. M., Cáceres, P., Marín-Nahuelpi, R., Koop, B. F., & Yáñez, J. M. (2020). Estimates of autozygosity through runs of homozygosity in farmed coho salmon. *Genes*, 11(5), 490.

Yuan, J., Li, S., Sheng, Z., Zhang, M., Liu, X., Yuan, Z., Yang, N., & Chen, J. (2022). Genome-wide run of homozygosity analysis reveals candidate genomic regions associated with environmental adaptations of Tibetan native chickens. *BMC Genomics,* 23(1), 91.

Zhang, Q., Calus, M. P., Guldbrandtsen, B., Lund, M. S., & Sahana, G. (2015). Estimation of inbreeding using pedigree, 50k SNP chip genotypes and full sequence data in three cattle breeds. *BMC Genetics,* 16(1), 1-11.

Zhang, Y., Zhuo, Y., Ning, C., Zhou, L., & Liu, J. F. (2022). Estimate of inbreeding depression on growth and reproductive traits in a Large White pig population. *G3 Genes| Genomes| Genetics.* 12(7), 118.

Zieliński, D., Ślaska, B., Zoń, A., & Rozempolska-Rucińska, I. (2016). Comparative analysis of variation of conformation traits in different-colour minks. *Polish Journal of Natural Science*, 31(2), 191-197.

APPENDIX 1. STATUS OF MANUSCRIPTS PUBLISHED FROM THE MASTER'S THESIS (AS OF 18 JUNE 2023)

1. Based on **Chapter 3**

   Thapa, P. C., Do, D. N., Manafiazar, G., & Miar, Y. (2023). Coat color inheritance in American mink**. PUBLISHED in BMC Genomics**, 24(1), 1-9.