Genomic Studies of Reproductive Performance and Fur Quality Traits in American Mink

by

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Dalhousie University is located in Mi'kma'ki, the
ancestral and unceded territory of the Mi'kmaq.
We are all Treaty people.

Table of Contents

List of tables

List of figures

Abstract

This thesis aimed to enhance reproductive performance and pelt quality traits in American mink (*Neogale vison*) using genomics approaches. Chapter 3 involved estimation of genetic and phenotypic parameters for pelt quality traits. Low-to-moderate heritabilities (±SE), ranging from 0.12±0.04 to 0.44±0.047, were estimated for dried pelt, live grading, body weight and length traits indicating these traits can be improved by genetic/genomic selection. The estimated genetic correlations demonstrated body weight and length measured in November of the first year of life was a good indicator for pelt size without negative influence on overall quality of dried pelt. The moderate positive genetic correlations between body length in November and harvest with overall quality of dried pelt suggested their utility as indicators to select for increased size and overall quality of dried pelt. Chapter 4 analyzed whole-genome data from 100 mink to detect selection signatures in the genome influencing pelt quality traits and coat color. Selection signatures were detected through three methods of fixation index (Fst), cross population extended haplotype homozygosity (XP-EHH), and nucleotide diversity (θπ). Overlapping top 1% of Fst and XP-EHH contained 376 genes for pelt quality and coat color. Overlapping top 1% of Fst, XP-EHH and θπ revealed 19 selection signature regions on chromosomes 3, 4, 5, 6, 7, 8, 9, and 10, including *APCDD1* gene with important roles on hair follicular process. In Chapter 5, genome-wide association studies performed to identify markers associated with eight reproductive traits and five pelt quality traits. The most significant associations were found on chromosomes 1, 2, and 4 for gestation length and on chromosome 6 for dried pelt size. Several candidate genes with important roles in reproduction were detected, along with novel genes related to pelt quality and size. In Chapter 6, prediction performance of three genomic evaluation approaches of genomic best liner unbiased prediction (GBLUP), BayesCπ, and single-step Bayesian multiple marker regression (SSBR) were compared for reproductive and pelt quality traits. SSBR consistently yielded higher predictive accuracy for all traits compared to both GBLUP and BayesCπ. The findings of these studies suggest that genomic approaches hold promise for improving these economically important traits.

List of Abbreviations Used

AWB	Average kit weight per litter at birth
AWW	Average kit weight per litter at weaning
BAM	Binary alignment map
BMP	Bone morphogenic protein
BLUP	Best linear unbiased prediction
BWA	Burrows-Wheeler Aligner
CCFAR	Canadian Centre for Fur Animal Research
COX-2	Cyclooxygenase-2
CV	Coefficient of variation
dEBV	De-regressed estimated breeding value
DNA	Deoxyribonucleic acid
DNAP	Dried pelt nap size
DPS	Dried pelt size
DQU	Overall quality of dried pelt
EBV	Estimated breeding value
FDR	False discovery rate
FSH	Follicle-stimulating hormone
Fst	Fixation index
g	Gram
GATK	Genome analysis toolkit
GBS	Genotyping-by-sequencing
GBLUP	Genomic best liner unbiased prediction
GEBV	Genomic estimated breeding value
GL	Gestation length
GnRH	Gonadotropin-releasing hormone
GS	Genomic selection
GWAS	Genome-wide association studies
HL	Harvest length
HW	Harvest weight
Kg	Kilogram
LB	Total number of kits alive after 24 hours of birth
LH	Luteinizing hormone
LIF	Leukemia Inhibitory Factor
LNAP	Live grading nap size
LQU	Live grading pelt quality
LD	Linkage disequilibrium
LW	Total kits alive at weaning
LW2	Litter size at two weeks after birth
LW3	Litter size at three weeks after birth
Mb	Mega base pair
Msx	Muscle segment homeobox
NAFA	North American Fur Auctions
Nov_BL	November body length
Nov_BW	November body weight

ODC1	Ornithine decarboxylase 1
PCR	Polymerase Chain Reaction
QTL	Quantitative trait locus
RNA	Ribonucleic acid
SB	Survival rate at birth
SW	Survival rate at weaning
SD	Standard deviation
SNP	Single-nucleotide polymorphisms
SSBR	Single-step Bayesian multiple marker regression
TB	Total number of kits born
TYR	Tyrosinase
VEGF	Vascular endothelial growth factor
WGS	Whole-genome Sequence
XP-EHH	Cross population extended haplotype homozygosity
$\theta\pi$	Nucleotide diversity

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Chapter 1: Introduction

1.1 Introduction

American mink (*Neogale vison*) is a semi-aquatic species of mustelid belong to the order of carnivorous and native to North America. There are two extant species commonly referred to as "mink": the American mink and the European mink (*Mustela lutreola*). Despite sharing similar names, the genetic studies reveal that the European mink is more closely related to the European polecat and Siberian weasel than to the American mink (Davison et al., 2000; Marmi et al., 2004). Taxonomically, the American mink was previously classified within genus, *Neovison*, but recently reclassified into the genus *Neogale* (Patterson et al., 2021). American mink has become the most used animals in the fur industry because of its luxurious appearance and high-quality fur, as a result, mink farming has become widespread across North America, where the species originates, as well as in Europe, where mink were introduced for fur farming purposes during the 1920s (Kauhala, 1996). Increasing the profitability of mink production requires applying methods for reducing production costs and increase production efficiency. Reproductive performance and pelt quality are two components of production efficiency in mink, as both can have synergistic effects on profitability. The reproductive performance of a female mink can be defined as the total lifetime production of kits that can successfully reach the weaning stage. This metric is determined by a combination of factors including total number of kits born, mortality at birth, kits survival from birth to weaning, pre-weaning weight and gestation length (Hansen et al., 2010; Karimi et al., 2018). Another main component of improving profitability within the mink industry is quality of pelt. Since the final price of skin is determined by the pelt size and fur quality (Lagerkvist and Lundeheim, 1990; Lagerkvist, 1997). Improving reproductive performance of female mink will reduce the production cost per unit of skin by increasing the number of offspring per breeding

female (Lagerkvist, 1997). Therefore, when coupled with efforts to enhance fur quality, this means more mink with superior fur characteristics, such as higher density of hair, short nap size, silky and healthy appearance of guard hair, and clear underfur color. This results in a larger quantity of high-quality pelts available for sale, increasing the overall revenue potential.

In mink, numerous studies have been undertaken to explore reproduction and pelt quality traits, ranging from estimation of heritability and genetic parameters (Lagerkvist et al., 1994; Hansen et al., 2010; Thirstrup et al., 2017; Karimi et al., 2018), estimation of genomic breeding values (Villumsen et al., 2021) or genome comparative analysis (Cai et al., 2018; Manakhov et al., 2019; Karimi et al., 2021a). Using linkage analysis some quantitative trait loci (QTLs) were successfully identified related to pelt quality traits in mink (Thirstrup et al., 2014). Although linkage mapping is a useful tool for identifying markers associated with a phenotype, the low mapping resolution has limited the use of linkage analysis for genes in domesticated species (Hayes et al., 2003; Goddard and Hayes, 2009; Kim et al., 2009).

In the field of animal breeding, genome-wide association studies (GWAS) are widely used for identification of genetic markers and genes underlying economically important traits. Unlike linkage mapping, GWAS offers significantly higher mapping resolution (Goddard and Hayes, 2009; Myles et al., 2009). The application of genomic selection has become widespread in animal breeding programs, particularly for traits with low heritability (less than 0.2), for example, reproduction traits or traits which are measured after slaughter such as pelt quality traits. Genomic selection enhances genetic gain by increasing accuracy of breeding value prediction, increase intensity of selection and by allowing to select animals early in life (Boitard et al., 2016).

This thesis aimed to improve reproductive performance and pelt quality traits in mink using genomic methods. To achieve this goal first a comprehensive literature review of the physiological

aspects of mink reproduction and pelting cycle, the different measurements of reproductive performance and pelt quality, and the current genomic methods that could use to improve reproduction and pelt quality traits in mink is presented in Chapter 2. The subsequent four study chapters of this thesis are centered on estimation of genetic and phenotypic parameter for pelt quality traits to determine heritability and genetic correlations among these traits. In chapters 4 and 5, a selection signature detection study and GWAS were performed to identified genomic regions influencing variation of these traits. Finally, the prediction accuracy and bias of prediction of genomic breeding values for reproduction and pelt quality traits were compared using different statistical models. This is important to noted that this thesis follows a publication format structure, with chapters 3 and 4 have been successfully published in peer-reviewed journals. Additionally, chapters 5 and 6 are currently undergoing the submission process for publication.

1.2 Objectives

The objectives of this thesis were to:

1) Estimate the genetic parameters for pelt quality traits and investigate the genetic and phenotypic correlations between these traits;

2) Identify signatures of selection for pelt quality properties and coat color;

3) Identify genetic variants associated with reproductive performance and pelt quality traits through GWAS; and

4) Estimate the accuracy of breeding values prediction for reproduction and pelt quality traits using different statistical methods.

Chapter 2: Literature review

2.1 Introduction

Improving animal production performance is the primary objective in the field of animal breeding. This endeavor is driven not only by the need to align with consumer preferences and confront new disease outbreaks but also to boost overall profitability and sustainability in production. Attaining these objectives requires the incorporation of new technologies into animal production systems. Genomics, a scientific field dedicated to mapping, sequencing, and analyzing DNA information at the genomic level, has enabled the identification of polymorphic markers distributed across the genome, offering valuable opportunities for detection of genomic regions underlying various traits and phenotypes. Using these genetic markers and genetic maps for major livestock species such cattle (Kappes et al., 1997; Ihara et al., 2004), pig (Rohrer et al., 1996), and chicken (Groenen et al., 2000) facilitated the investigation of genome regions harboring genes that influence the performance of economically important traits. Later on, availability of a whole-genome assembly for cattle (Zimin et al., 2009) followed by construction of reference genome for other major livestock species provided new opportunities for identification of quantitative trait loci (QTL), single nucleotide polymorphisms (SNPs), and genes underlying variation in phenotypic performance. The commercial SNP genotyping panels provided a cost-effective approach to genotype a large number of SNPs through the genome to significantly speed up the availability of genotyped marker information for genomic research in major agricultural species. All these advancements facilitated the detection of many SNP and candidate genes through genome-wide association studies which is reflected in the steady data increase in the QTLdb (https://www.animalgenome.org/cgi-bin/QTLdb/index). Another major advancement in animal breeding was the invention of genomic selection, which uses both genotype marker information

and phenotype within the training population to predict performance of individuals only based on their genotype information and regardless of their phenotypes (Meuwissen et al., 2001). Genomic selection has the potential to increase the rate of genetic improvement for economically important traits, especially for traits that can only be measured in one sex, after death or late in life, or measuring the trait is expensive (Goddard, 1996).

American mink pelt stands as a primary global source for the fur industry. Canada emerging as a major producer of mink pelt. In 2018 alone, Canadian mink farms produced over 1.7 million mink pelts, generating a substantial contribution of 44 million dollars to the Canadian economy. With approximately 98 active farms engaged in mink production across the country (Mink Statistical Briefer, 2021). Despite the recent progress in the field of mink genomics, the application of genomic breeding strategies in mink is still limited. Among the traits targeted for breeding in mink, reproduction performance and pelt quality are of major economic importance for the fur industry. To formulate a comprehensive and enduring breeding strategy, it is crucial to synthesize current advancements in mink genetic improvement related to these traits. Unfortunately, there is no existing literature that has put these advancements in perspective.

This review provides a comprehensive analysis of the key physiological aspects of mink reproduction and the pelting cycle, present various measurements related to reproductive performance and pelt quality and explores contemporary genomic methods that could contribute to improving these traits in mink.

2.2 Mink reproductive cycles

2.2.1 Reproductive seasonality

Mink exhibit seasonal reproductive activity, which is regulated by changes in photoperiod (Murphy et al., 1993; Amstislavsky and Ternovskaya, 2000). In the Northern hemisphere, the breeding period for mink occurs between late February and early March. Sexual maturity in mink kits is typically attained during the first spring of their lives, around ten months of age (Enders, 1952). Following puberty, mink display a seasonal annual reproduction cycle. The practical considerations dictate that dams are often removed from the herd after their third reproductive season, as fertility tends to decline in subsequent breeding seasons. During each breeding period, four or more waves of follicles mature at approximately 8-day intervals (Sundqvist et al., 1989)

2.2.2 Folliculogenesis and estrus

The estrous cycle of mink involves a series of morphological, biochemical, and physiological changes in the ovaries, leading to ovulation. Follicular growth occurs in wave-like manner, characterized by the sequence of three gonadotropin-dependent events: recruitment, selection, and dominance. The release of gonadotropin-releasing hormone (GnRH) from the hypothalamus stimulates the pituitary gland to secrete follicle-stimulating hormone (FSH) and luteinizing hormone (LH). FSH initiates waves of follicular growth in the ovary, in each follicular wave about 20 follicles start growing, some of them regress and around half of this number reach the stage of ovulation (Hansson, 1947). Once the dominant follicle matures, ovulation is induced by luteinizing hormone (LH) (Murphy, 1979; Sundqvist et al., 1988). Mink exhibit induced ovulation, with copulation triggering ovulation within approximately 48 hours (Hansson, 1947; Pilbeam et al., 1979; Sundqvist et al., 1989). During the estrous cycle, estrogen secretion from antrum follicles

plays a crucial role in initiating estrus (Klotchkov and Eryuchenkov, 2003). Vaginal smear patterns and vulval swelling can provide partial indicators of the estrous cycle in mink (Travis et al., 1978; Sundqvist et al., 1988).

2.2.3 Fertilization and delay implantation

After mating and release of oocytes from the ovarian follicles, one or more eggs enter to the oviduct tube. Ovulation is stimulated by LH, and ovulated follicles convert to corpora lutea in the cortex of ovary (Hansson, 1947; Enders, 1952). In mammals, the corpora lutea is responsible for production of a relatively high level of progesterone and moderate levels of estradiol and inhibin. In mink, corpora lutea does not immediately suppress development of primary follicles and this feature leads to continuous development and ovulation of new sets of follicles (Sundqvist et al., 1988). In delayed implantation, which is also called embryonic diapause, the blastocyst created from fertilized oocytes does not immediately implant in the uterus. The blastocysts regularly remain in the state of dormancy for approximately 1-2 weeks (Fenelon et al., 2017); however, it was reported that the blastocyst stage might last up to 49 days *post coitum* (Enders, 1952). Fertilization of eggs and early embryonic development occurs in the oviduct and the mink embryo develops from the zygote into a blastocyst by the 8th day *post coitum*. During embryonic diapause, the trophoblast provides nutrients for the blastocyst. The diapause period is ended by an increase in release of prolactin from pituitary, which will induces a rapid increases in progesterone synthesis (Murphy and Moger, 1977; Murphy et al., 1993). In addition, other factors such as polyamines of the uterus can influence embryonic diapause (Murphy, 2012).

2.2.4 Implantation of embryo, gestation, parturition and lactation

The elevation in blood prolactin titres leads to the activation of corpora lutea, the end of embryo dormancy, and the blastocyst is implanted in the endometrium (Enders, 1957). Gestation length is calculated as the number of days from last mating to parturition and can range from 39 to 75 days owing to the delayed implantation (Enders, 1952). The pregnancy length can vary depending on color type, photoperiod, age of female, mating frequency, and ambient temperature (Sundqvist et al., 1989). Dams give birth from the last week in April to the middle of May and the litter size varies from one to 17 (Hansen et al., 2010; Karimi et al., 2018; Sundqvist et al., 1989). The maternal nursing behaviour is vital during the first days after birth for survival of kits, as new born kits weight only 5 to 15 g with low liver glycogen reserves (Tauson, 1994), and a poorly developed thermoregulation system (Rouvinen-Watt and Harri, 2000). The lactation period is about 6-7 weeks (Hunter and Lemieux, 1996) and the total number of activated teats may vary from one to ten. There is a positive relationship between litter size and the number of active teats in female mink. The number of activate teats for litter sizes ≥ seven is around seven to ten. The number of active teats declines after week 4 post-partum (Korhonen, 1992).

2.3 Seasonal fur growth cycle

Mink exhibit two hair growth cycles each year regulated by the photoperiodic cues (Bissonnette and Wilson, 1939; Duby and Travis, 1972). In the vernal transition, the increasing photoperiod during spring triggers the initiation of the summer fur growth, accompanied by the concurrent molting of the thicker winter coat. Conversely, decreasing photoperiod in the fall initiates the onset

of dense winter fur growth and the shedding of the summer pelt (Rose et al., 1985; Johnston and Rose, 1999). The regulation of these growth phases is mediated, in part, by the modulation of pituitary hormone prolactin and the influence of melatonin sourced from the pineal gland (Rust et al., 1965; Rose et al., 1985, 1998; Johnston and Rose, 1999). The summer coat is "flat" due to its low density of hair. Moreover, it has a reddish-brown tint, which makes it unsuitable for commercial purposes due to its nonconforming attributes. The summer coat is maintained from mid-July through early August when the fall molting begins. The maturation of the dense winter fur takes place during September, October, and early November, culminating in what is recognized as the "prime" coat, characterized by its optimal quality and suitability for commercialization (Bassett and Llewellyn, 1949; Rust et al., 1965; Kaszowski et al., 1970). The mink farming calendar aligns with these natural transitions, with the period spanning mid-November to mid-December typically earmarked for pelt harvesting, and subsequently their presentation at auction houses for sale.

2.4 Genetic aspects of reproduction and pelt quality traits

Heritability is defined as the proportion of phenotypic variance explained by underlying genetic factors (Falconer and Mackay, 1996). The estimation of heritability can help predict genetic gain from selection (Boitard et al., 2016). The estimation of heritability for reproductive traits and their genetic correlations with other important production traits is necessary for designing effective selection program in mink. In this section, most important measurements of reproductive performance and pelt quality are reviewed. Table 2.1 presents the list of literature on genetic parameters for reproduction performance and pelt quality measurements in mink.

2.4.1 Reproductive measurements and their heritabilities

2.4.1.1 Litter Size

The most important reproductive traits in mink are the litter size traits including total number of kits born (TB), and number of live kits at birth (LB), number of live kits at two weeks after birth (LW2), number of live kits at three weeks after birth (LW3), and number of live kits at weaning (LW). Increasing litter size has been identified as the most profitable strategy in mink farming (Lagerkvist, 1997). The heritability of TB ranges from 0.02±0.03 to 0.09±0.05 (Lagerkvist et al., 1994; Hansen et al., 2010; Karimi et al., 2018). LB heritability was estimated to be in the range of 0.01 to 0.07 (Hansen et al., 2010; Karimi et al., 2018). LW2 heritability is estimated to be in a range between 0.13±0.01 and 0.15±0.02 (Koivula et al., 2010). Moreover, the heritabilities ranging from 0.06±0.06 to 0.14±0.09 were estimated for LW3 (Lagerkvist et al., 1993). The heritability of LW also has been estimated to be between 0.03±0.03 to 0.09±0.04 (Hansen et al., 2010; Karimi et al., 2018). The genetic correlations among litter size traits are strongly positive, ranging from 0.56 to 0.92 (Hansen et al., 2010; Karimi et al., 2018). These high genetic correlations suggest that litter size traits are likely to be under the control of common genes or genetic mechanisms.

2.4.1.2 Survival rate

Survival rate is important reproduction traits in the mink farms since a large number of kits at birth might not lead to higher number of kits at weaning due to mortalities. The average kit mortality rate from birth to weaning (7-8 weeks) ranges from 10 to 30% (Einarsson and Elofson, 1988; Martino and Villar, 1990; Schneider and Hunter, 1993; Malmkvist et al., 1997). Survival rate is the proportion of live born kits to total number born in each litter. The heritability of survival rate

was estimated to be from 0.13 to 0.18 for survival rate at birth (SB), and 0.07 to 0.10 for survival at weaning (SW) (Hansen et al., 2010; Karimi et al., 2018).

Several studies have estimated the genetic correlation between litter size traits and survival rate in mink. Lagerkvist et al., (1994) reported a non-significant genetic correlation between TB and number of still born kits (-0.40 ± 0.33), and kits mortality at age 3 weeks (0.14 ± 0.34). Similarly, Hansen et al. (2010) reported a significant positive genetic correlation between LB and survival at seven days after birth (0.42 ± 0.18), and a non-significant genetic correlation between LB with survival at twenty-eight days after birth (0.06 ± 0.23), LB with SW (-0.04 ± 0.24). In another study by Karimi et al. (2018), no significant genetic correlation was identified between TB with SB (-0.13 ± 0.18) and SW (-0.29 ± 0.23).

2.4.1.3 Gestation length

Gestation length (GL) in mink is influenced by delay implantation. Gestation length was defined as the number of days between the dates of last mating and whelping (Karimi et al., 2018). The optimal gestation length (45–60 days) results in/is associated with larger litters owing to reduction in kit mortality (Święcicka, 2013). The heritability of gestation length was estimated to be ranged from 0.17 to 0.29 (Kołodziejczyk and Socha, 2011; Karimi et al., 2018). Inconsistency of heritability estimations among various studies can be attributed to several factors including statistical models, population structure, pedigree completeness, sample size, and trait definition (Miar et al. 2014a,b). Another investigation by Karimi et al. (2018) revealed that GL had moderate negative genetic correlations with SB (-0.43 ± 0.14) and SW (-0.37 ± 0.15).

2.4.1.4 Pre-weaning weight

High birth weight is important for survival and lifetime performance of animals (Milligan et al., 2002; Snowder and Fogarty, 2009). Despite the critical role of this trait in the performance of farmed animals, only a few studies are considered the average weight of kits as a reproductive trait. The heritability of average birth weight, weight at week 3 and weight at weaning was 0.28 ± 0.05, 0.19 ± 0.04 and 0.10 ± 0.04, respectively (Karimi et al. 2018). In addition, Hansen & Berg (1997) estimated the direct heritability of 0.19 ± 0.05, 0.15 ± 0.03 and 0.31 ± 0.04 for weight at birth, two weeks after birth and weaning, respectively. Body weight is the best-known trait affected by maternal abilities (Willham, 1972). The heritability of maternal effects was high for kits body weight at birth (0.31± 0.05) and it decreased slowly afterward to weaning 0.09 ± 0.04, which implied on the importance of maternal performance during suckling period (Hansen and Berg, 1997).

2.4.2 Pelt quality measurements and their heritabilities

In mink farming, the comprehensive evaluation of fur characteristics constitutes a pivotal aspect, which is usually performed on both live animals and dried skins. The live assessment of pelage quality is conducted on-farm by certified technicians, directly assessing the fur attributes of the live animals. The analysis of dried pelt characteristics, by contrast, is undertaken at auction houses, using automated machinery and fur specialists, before the pelts are sent for sale. The most commonly used pelt quality measurements are overall pelt quality, guard hair length, underfur density, nap size (NAP), and the size of the dried pelt (DPS). Overall pelt quality can be defined as general appearance of pelt such as smooth appearances and silky textures (Lagerkvist et al., 1994; Thirstrup et al., 2014; Thirstrup et al., 2017; Hu et al., 2021). Nap size is defined as the

proportion of guard hair (long stiff hair) that protrudes out of the underfur (thin-short hairs) (Thirstrup et al., 2017). DPS highly influences the final price of pelt, and is measured as the length from the tip of the nose to the base of the tail (Lagerkvist et al., 1994; Thirstrup et al., 2017).

Table 2.1 summarizes the estimated heritabilities of pelt quality traits in mink. Underfur density heritability range from 0.06±0.02 to 0.35±0.05 on live animals (Lagerkvist et al., 1993; Lagerkvist et al., 1994; Thirstrup et al., 2017). Several studies have reported heritability of guard hair length and its closely related trait of nap size in mink (Table 2.1). Thirstrup et al., (2017) estimated the heritability of 0.14±0.02 for guard hair length evaluated on live animals, while Liu et al., (2017) estimated a higher heritability of 0.53 for this trait. Additionally, the heritability of 0.50±0.06 was reported for nap size evaluated on live animals (Hu et al., 2021). Two studies also estimated heritability for dried pelt size, and reported heritabilities ranging from 0.45±0.05 to 0.57±0.09 for this trait (Lagerkvist et al., 1994; Thirstrup et al., 2017).

Section 2.4 provided a thorough review of previous literature on the heritability of reproduction and pelt quality traits in mink. Published studies implied that these traits have a low to moderate heritabilities, suggesting these traits have a promising potential for improvement by selection. However, it is crucial to recognize that heritability is a population-specific parameter, and that can vary across different populations, and it can change over time. Moreover, the choice of statistical model, along with the inclusion of fixed and random effects in the model, can significantly influence the estimation of heritability.

Furthermore, factors such as the accuracy of trait measurement, the meticulous documentation of data, completeness of pedigree, the size of the population under consideration are directly affects the reliability of the heritability estimates. Therefore, it is essential to estimate heritability within

the specific population targeted for selection. This ensures that essential information is obtained to guide for choosing appropriate selection methods suitable to each trait.

2.5 Candidate genes and molecular mechanisms involved in female mink reproduction

Investigation of the expression patterns of transcription factors in the reproductive organs of mink can serve as a valuable approach for identifying genes that play an important role in regulating the reproductive performance of female mink. The quantitative PCR technique is suitable for analyzing a relatively small number of transcripts in a set of samples. Using this technology, several candidate genes were identified in the embryo or reproductive tissues of mink. This section reviews some of candidate genes related to reproductive traits in mink.

Leukemia Inhibitory Factor (*LIF*) is one of genes that may be involved in the embryonic implantation process in mink. The *LIF* is essential for embryo implantation in mice and rodents (Stewart et al., 1992; Stewart, 1994). The *LIF* cDNA cloned and sequenced in mink, and RT-PCR analysis indicated that this gene is expressed in the mink uterus when the embryo escapes diapause and early post-implantation. Immunohistochemistry revealed the LIF protein is present in uterine glands just prior to and shortly following the embryo implantation, suggesting it is involved in the implantation process, and may be a maternal signal which terminates obligate embryonic diapause (Song et al., 1998a).

Cyclooxygenase-2 (*COX-2*) plays an important role in facilitating the synthesis of prostaglandin that is essential for embryo implantation in several mammalian species including humans (Han et al., 1996), ovines (Charpigny et al., 1997) and bovines (Asselin et al., 1997). Knocking out the *COX-2* had profound negative consequences on reproduction including interference with the

ovulatory process and failure of embryo implantation in mice (Lim et al., 1997). In mink, COX-2 is locally expressed at sites of embryo invasion, particularly in the necks of the uterine glands during early implantation. The abundance of *COX-2* messenger RNA reached a peak on days 3–5 of post-implantation and gradually decreased through day 9 and was not present after that. The coincidence of COX-2 expression in uterus with embryo implantation, decidualization, and placenta formation suggests that locally produced COX-2 may play a role in implantation and placentation in mink (Song et al., 1998b).

Vascular endothelial growth factor (*VEGF*) is another gene that may contribute to the process of embryo implantation in mink. Three VEGF isoforms protein as well as the VEGF receptors Kinase insert domain region (KDR) and fms-like tyrosine kinase (Flt-1) were upregulated during the peri-implantation period at the glandular epithelium of uterus in mink (Lopes et al., 2003). Moreover, *VEGF* was detected in endometrial stroma, luminal and glandular epithelium in implanted uteri. The presence of the embryo appears to regulate expression of the VEGF receptors. However, upregulation of *VEGF* during the implantation process is dependent on maternal factors, presumably gonadal steroids (Lopes et al., 2003).

Muscle segment homeobox (*MSX)* gene is critical for the initiation and maintenance of embryonic diapause, and uterine readiness to confer blastocyst reactivation and implantation in mammals e.g., mouse and Australian tammar wallabies (*Notamacropus eugenii*). In mink, *Msx1* is highly expressed in the uterine epithelium during embryonic diapause and becomes undetectable with the initiation of implantation (Cha et al., 2013).

The ornithine decarboxylase 1 (ODC1) protein is required for biosynthesis of polyamines. The uterine polyamines had an essential role in embryo reactivation and consequent embryo development (Lefèvre et al., 2011). It was indicated that inhibiting ODC-1 using α-

difluoromethylornithine treatment inhibited polyamine synthesis. This process led to a reversible arrest of embryo development, which returned the mink embryo to diapause and induced a second delay in embryo implantation (Lefèvre et al., 2011). Furthermore, Fenelon et al. (2016) examined expression of the prolactin receptor, progesterone receptor, and estrogen receptor 1 in the uterus to determine the regulatory role of prolactin in *ODC1* expression in the uterus, which subsequently regulates the uterine polyamine levels. Prolactin upregulated *ODC1* expression in the mink uterine epithelial cells. Moreover, prolactin was a regulator of *ODC1* in the mink uterus through the Jak-Stat pathway and mechanistic target of rapamycin (mTOR) proteins (Fenelon et al. 2016).

2.6 Mapping QTLs and candidate genes associated with reproduction and pelt quality traits

Mapping of QTLs and candidate genes responsible for the phenotypic variations is vital for improving economically important traits. In mink, linkage maps (also referred to as genetic maps) were preliminary tools for identification of genetic markers associated with a phenotype. Anistoroaei et al. (2007) established the first-ever linkage map of mink consists of 85 microsatellite markers. Subsequently, this genetic map was further extended by including additional markers or using homology with dog and human genome, which enriching the map's resolution and coverage and allowed for more precise localization of genetic variants within the mink genome (Anistoroaei et al., 2009; 2012). This genetic map has allowed researchers to identify and locate a nonsense mutation on exon 1 of *tyrosinase (TYR)* gene on chromosome 7 using fluorescent *in situ* hybridization method, associated with the albino phenotype (also known as Regal White) in mink (Anistoroaei et al., 2008). In the following years, Thirstrup et al. (2014) used 104 microsatellite markers covering all 14 autosomal chromosomes to improve the previous published mink linkage map (Anistoroaei et al., 2012), and to detect QTLs associated with pelt quality traits

and pelt length. In their study, 11 QTLs associated with guard hair thickness, guard hair length, wool density, wool quality, and skin length were identified on nine autosomal chromosomes (Thirstrup et al., 2014). Although QTL mapping is a useful method to determine the QTL regions associated with the traits of interest, it is unlikely to pinpoint causal variants and potential candidate genes associated with traits, due to low resolution of QTLs detected by this method. The reason is that the experimental designs adopted to identify phenotype-genotype relationships through linkage mapping were often established using full-sibling/half-sibling families where limited recombination events have occurred leading to extended linkage disequilibrium (LD) and low resolution of QTL detection (Goddard and Hayes, 2009). For example, in the Thirstrup et al. (2014) study, the QTL intervals were ranging from 15 to 100 Mb.

GWAS has the power of detecting variants with small effects with a much higher mapping resolution compared to traditional QTL mapping methods. GWAS has emerged as a powerful tool in the field of animal genetics, enabling the identification of genetic variants associated with various traits (Tam et al., 2019). The advancement of whole-genome sequencing technologies has revolutionized genetic research in various species, including mink. These efforts have enabled researchers to establish a comprehensive reference genome for mink. The availability of a reference genome for mink opened new ways for exploring the genetic basis of complex traits. The first draft of the mink genome assembly was published by Center for Quantitative Genetics and Genomics, Denmark (Cai et al., 2017), this initial draft was notably fragmented, consisted of 1,175 scaffolds. Eventually, this foundational work was further augmented by the comprehensive efforts of Karimi et al. (2022) with construction of a contiguous chromosome-level genome assembly. With the establishment of the higher quality mink reference genome, researchers now have a powerful tool to investigate genotype-phenotype associations in this species using GWAS.

A few GWAS were performed in mink. Cai et al. (2018) used the genotype-by-sequencing method to extract SNP markers to conduct GWAS for fur quality and body size traits. They identified *WWC3*, *MAP2K4*, *SLC7A1* and *USP22* as candidate genes for body weight and pelt length in mink. In another study, three distinct color types of mink were genotyped using whole-genome sequencing and a causative SNP was identified on *MLPH* gene which was responsible for silver-blue coat color in mink (Manakhov et al., 2019). The availability of a commercial SNP genotyping array for mink would provide more opportunities for further investigation on genotype-phenotype association of traits of interest in mink.

2.7 Genomic selection

Genomic selection has transformed livestock breeding practices, allowing for early prediction of an animal's genetic potential. The advantages of genomic selection include the increase in the rate of genetic gain, increase accuracy of breeding value prediction, increase selection intensity, and reducing the generation interval (Miar et al., 2015; Boichard et al., 2016). The impact of genomic selection can vary depending on the specific species and industry. In mink, the impact of genomic selection is to increase the accuracy of selection for traits with low heritability. This means that using genomic information allows breeders to more accurately identify mink with desirable traits, which can lead to better breeding decisions and faster genetic improvement. Additionally, genomic selection in mink has enabled the selection of animals for traits that are measured postmortem, such as dried pelt quality traits, which is not possible with traditional selection methods. Villumsen et al. (2021) reported that the genomic estimated breeding value (GEBV) improved prediction accuracy for pelt quality traits in mink compared with the pedigree-based method. They used a single-step method (commonly known as ssGBLUP) for estimation of GEBV, which is a model

that can simultaneously implement both pedigree and genomic information. Genomic selection is a new procedure. Currently, various statistical algorithms have been developed for genomic prediction, including Genomic Best Linear Unbiased Prediction (GBLUP), Bayesian methods (BayesA, BayesB, BayesCπ, and Bayesian LASSO). These methods are widely employed for predicting breeding values in many livestock and plant species. In the context of mink breeding, the performance of different statistical models has not been thoroughly investigated. Therefore, additional studies employing diverse statistical approaches are essential to increase our understanding of the most suitable methods for applying genomic selection to different traits in mink.

2.8 Conclusion

This chapter provides a comprehensive overview of the physiological aspects of mink reproduction and the pelting cycle. It explored various measurements of reproductive performance and pelt quality, shedding light on critical factors pivotal for successful mink breeding programs. The chapter emphasized the importance of assessing genetic parameters for traits within a population targeted for genetic or genomic selection programs. Furthermore, this literature review highlighted the importance of employing genomic techniques for identifying genetic variants and genomic regions underling phenotypic variation of traits. The potential of GWAS for detecting genetic variants associated with reproductive performance and pelt quality traits, was explored. Lastly, the chapter discussed the benefits of implementing genomic selection for increasing genetic gain for economical important traits in mink industry.

Table 2.1 The literature on estimation of heritability (h^2± SE) of reproduction and pelt quality traits in mink.

Traits	Reference	Population	h^2±SE[1]
Total Number of kits born	Lagerkvist et al., 1994	Standard dark mink	0.09±0.05
	Hansen et al., 2010	Standard dark mink	0.02±0.03
	Karimi et al., 2018	11 color types	0.07±0.3
Number of kits alive at birth	Hansen et al., 2010	Standard mink	0.06±0.03-0.08±0.04
	Karimi et al., 2018	11 color types	0.07±0.02
Number of kits alive at two weeks after whelping	Koivula et al., 2010	Standard mink	0.13±0.01-0.15±0.02
Number of kits alive at three weeks after whelping	Lagerkvist et al., 1993	Standard mink	0.06±0.06-0.14±0.09
Number of kits alive at weaning	Hansen et al., 2010	Standard mink	0.03±0.03-0.06±0.03
	Karimi et al., 2018	11 color types	0.09±0.04
Survival rate at birth	Hansen et al., 2010	Standard mink	0.14±0.4-0.18±0.05
	Karimi et al., 2018		0.13±0.03
Stillbirth	Lagerkvist et al., 1994	Standard mink	0.07±0.04
Survival rate at weaning	Hansen et al., 2010	Standard mink	0.07±0.03-0.10±0.05
	Karimi et al., 2018		0.10±0.02
Mortality at weaning	Lagerkvist et al., 1994	Standard mink	0.14±0.06
Age at first mating	Koivula et al., 2010	_	0.10±0.01
Gestation length	Karimi et al., 2018	11 color types	0.29±0.03
Average body weight at birth	Karimi et al., 2018	11 color types	0.28±0.05
Average body weight at three weeks after birth	Karimi et al., 2018	11 color types	0.19±0.04
Average body weight at weaning	Karimi et al., 2018	11 color types	0.10±0.04
Overall quality of pelt on live animals	Thirstrup et al., 2017	Standard mink	0.17±0.02

Traits	Reference	Population	h^2±SE[1]
	Hu et al., 2021	11 color types	0.34±0.06
Underfur density on live animals	Lagerkvist et al., 1994	Standard mink	0.35±0.05
	Thirstrup et al., 2017	Standard mink	0.06±0.02
	Lagerkvist et al., 1993	Standard mink	0.21±0.06
Guard hair length on live animals	Thirstrup et al., 2017	Standard mink	0.14±0.02
	Liu et al., 2017	silver blue mink	0.53
Nap size on live animals	Liu et al., 2017	silver blue mink	0.52
	Hu et al., 2021	11 color types	0.50±0.06
Overall quality of pelt on dried pelt	Lagerkvist et al., 1994	Standard mink	0.38±0.08
	Thirstrup et al., 2017	Standard mink	0.30±0.03
Dried pelt size	Thirstrup et al., 2017	Standard mink	0.45±0.05
	Lagerkvist et al., 1994	Standard mink	0.57±0.09

[1] SE = standard error

Chapter 3: Genetic and phenotypic parameters for pelt quality, and body length and weight traits in American mink[1]

3.1 Introduction

American mink (*Neogale vison*) is the most favoured fur-bearing animal being raised under an intensive production system. Mink farming was initiated in Canada in 1866 (Bowness, 1996), and since then, due to its importance for fur industries, mink farming is extensively practiced in North America, Europe, and Asia (Anistoroaei et al., 2009; Thirstrup et al., 2015). The quality of fur and pelt size are the main factors determining the final price of pelt and subsequently the profitability of mink producers (Lagerkvist, 1997). Therefore, there is an increasing interest in breeding mink with more desirable fur characteristics. Pelts with larger size, higher density of hair, and healthy appearance of guard hair gain the highest economic value (Thirstrup et al., 2017; Cai et al., 2018).

Pelt quality is a composite trait that includes pelt nap size, underfur density, silky appearance of fur, guard hair thickness and purity of color of underfur (Thirstrup et al., 2014). In current commercial evaluation system of mink pelt, pelt nap size (NAP) and overall quality of fur (QU) are the most used objective traits for genetic improvement of pelt quality. The proportion of guard hair (long stiff hairs) that protrudes out of underfur (thin-short hairs) is referred to as the nap causing the wavy and shiny appearance of fur, and clothing industries and consumers demand short nap fur (Thirstrup et al., 2014; De Reviziis, 2018; Wang et al., 2022).

The evaluation of fur characteristics can be performed on both live mink (grading traits) and dried skins (pelt traits). Live grading is subjective and performed by the certified technicians on the farm

[1] A version of this chapter has been published. Valipour et al., 2022. Genetic and Phenotypic Parameters for Pelt Quality and Body Length and Weight Traits in American Mink. Animals, 12(22), p.3184. https://doi.org/10.3390/ani12223184.

while evaluation of fur characteristics is performed on dried pelt using sorting machines by certified fur grading specialists in auction houses. Although the price of pelts is highly determined based on the pelt quality traits, these traits are not available until post-harvest. Alternatively, live grading, and body weight and length measurements can be used as potential indicators of dried pelt characteristics (Lagerkvist et al., 1994; Møller, 1999). Therefore, evaluating animals for both live grading and dried pelt traits are important for selection of mink with better quality of fur. Moreover, assessing the correlations between live grading and dried pelt quality traits should be considered for designing a successful breeding program.

Canada is a major producer and exporter of mink pelt in the world with a production record of 1.76 million pelts in 2018 (Statista, 2022); however, a comprehensive genetic breeding program has not been implemented by the Canadian mink industry. Farmers select animals phenotypically with higher litter size and better fur characteristics as parents of the next generation. However, phenotypic selection is not an effective method to improve performance of animals because a large portion of phenotypic variation of traits are explained by environmental and non-additive genetic effects that are not transmissible to the next generation (Wellmann and Bennewitz, 2019). On the other hand, genetic selection for pelt quality traits requires estimating the heritabilities as well as the phenotypic and genetic correlations among these traits. Several studies have estimated the genetic parameters and heritabilities for live grading traits in mink populations (Kenttämies and Vilva, 1988; Lagerkvist and Lundeheim, 1990; Socha et al., 2008; Kołodziejczyk and Socha, 2012; Liu et al., 2017; Thirstrup et al., 2017; Hu et al., 2021). However, heritabilities for dried pelt quality traits and their genetic correlations with live grading traits and body weight and length traits have rarely been reported (Lagerkvist et al., 1994; Thirstrup et al., 2017). To our knowledge, no study has estimated the genetic and phenotypic parameters for pelt quality traits on dried pelt and their

genetic and phenotypic correlations with live grading traits in Canadian mink populations. Therefore, the objectives of this study were as follows: 1) to estimate heritabilities for three dried pelt quality traits (dried pelt size, dried pelt nap size, and overall quality of dried pelt), 2) to estimate genetic and phenotypic correlations between dried pelt quality traits and live grading traits (live grading nap size , and live grading overall quality), 3) to estimate genetic and phenotypic correlations between pelt quality traits and four body size measurements, including November body weight, November body length, harvest weight and harvest length in mink.

3.2 Materials and Methods

The present study was approved by the Dalhousie University Animal Care and Use Committee (certification#: 2018-009, and 2019-012). Moreover, mink used in this research work were cared for following the Code of Practice for the Care and Handling of Farmed Mink guidelines (https://www.nfacc.ca/pdfs/codes/mink_code_of_practice.pdf).

3.2.1 Animals and management

Phenotypic records used in this study were collected from animals at the Canadian Centre for Fur Animal Research (CCFAR) at Dalhousie University, Faculty of Agriculture (Truro, Nova Scotia, Canada) and Millbank Fur farm (Rockwood, Ontario, Canada). Animals consisted of five color types including dark, demi, mahogany, pastel, and stardust. All mink were raised under standard farming conditions and had ad libitum access to food and water. The diets were formulated based on the nutrient requirements of animals in each production period. No formal breeding program was used in CCFAR or Millbank Fur Farm. Before each breeding season, weak and infertile

animals were culled from the herd in December, and those with an adequate score for live grading, disease history, and reproductive records were kept for breeding. The pedigree file included 25,688 animals (1,155 founder and 24,533 non-founder individuals) and was traced through 16 generations.

3.2.2 Evaluation of fur characteristics on live animals and dried pelts

Pelt quality traits were assessed on live animals (n=1608) for mink in CCFAR at the end of November in 2018, 2019, and 2021 when the coat was in prime condition. Live quality grading of pelage was performed on these mink using the North American Fur Auctions (NAFA) live animal grading procedure by their certified technician. Live grading traits included the overall quality of fur (**LQU**) and nap size (**LNAP**). The LQU was scored into three categories from 1 (poor) to 3 (best). The LNAP was measured as the length of guard hair protruding from the underfur and scored into five categories from 1 (long) to 5 (short).

Mink were euthanized in December 2018 and 2019 (n=1,195), for mink in Millbank, pelting was carried out in the pelting facility located in Millbank Fur Farm (Rockwood, ON, Canada) and mink from CCFAR were sent to the custom pelting facilities (Arcadia, NS, Canada). The dried raw pelts were shipped to the North American Fur Auctions - NAFA (Toronto, ON, Canada) and Saga Furs (Vantaa, Finland) auction houses for fur quality evaluation and sale. Dried pelt quality traits included dried pelt size (**DPS**), dried pelt nap size (**DNAP**), and overall quality of dried pelt (**DQU**). Evaluation of DPS and DNAP was performed using sorting machines on dried skins. All skins were stretched with differential weights to adjust skin lengths into categories. The DPS was measured from the tip of nose to the base of tail. The DPS was classified into nine categories of

47.1-53 cm (category 1), 53.1-59 cm (category 2), 59.1-65 cm (category 3), 65.1-71 cm (category 4), 71.1-77 cm (category 5), 77.1-83 cm (category 6), 83.1-89 cm (category 7), 89.1-95 cm (category 8), and 95.1-101 cm (category 9). The DNAP was scored into eight categories: 8 (extra short nap), 7 (short nap), 6 (short-medium open), 5 (short-medium nap), 4 (short-medium to medium nap), 3 (medium nap), 2 (medium nap to medium-long nap), and 1 (medium-long nap). The DQU was performed by professional fur grading experts in auction houses and classified into four categories:

1) Bronze: weakest grade assigned to the pelts, no damages, but weakest in terms of underfur, guard hair, or general appearance. These pelts were very flat, coarse, weak, and loose;

2) Silver: these pelts were complete and prime and had silky appearances. However, they had weaker underfur and poorer or uneven coverage or coarser guard hair;

3) Silver to golden: higher quality than silver pelts but not as good golden quality furs; and

4) Golden: these pelts had very high-quality with good and even guard hair coverage and dense underfur. These pelts were fully prime and had smooth appearances and silky textures.

3.2.3 Body weight and length measurement

November body weight (Nov_BW) and November body length (Nov_BL) were measured on live animals at approximately seven-month-old (n=1,734) in mid-November 2018 and 2019. The Nov_BL was measured from snout to tail base of each mink. Following euthanasia in December 2018 and 2019, harvest body weight (HW) and harvest body length (HL) were collected from 2,162 mink. HW was obtained by measuring the weight of a whole body of animals and HL was the length of body measured from snout to base of tail on a whole body of mink. In this study body

weight and length traits have been measured on both male and female animals. It is noteworthy that male animals typically have larger body sizes, both in weight and height, compared to females of similar age (Do and Miar, 2019). Thus, the fixed effect of sex has been incorporated into models for estimation of the heritability of these traits.

3.2.4 Statistical analyses

The significant influence ($P < 0.05$) of non-genetic factors including fixed effects of farm (CCFAR and Millbank Fur farm), year (2018, 2019, and 2021), sex (female and male), color type (dark, demi, mahogany, pastel, and stardust), and age (1 and 2 years), and random effects of additive genetic, maternal genetic, and common litter were tested for studied traits using univariate models implemented in ASReml 4.1 (Gilmour et al., 2018). Only significant ($P < 0.05$) effects included in subsequent mixed model analyses. The following general univariate model was used:

$$\mathbf{y} = \boldsymbol{X}\mathbf{b} + \boldsymbol{Z}\mathbf{a} + \boldsymbol{G}\mathbf{m} + \boldsymbol{W}\mathbf{c} + \mathbf{e},$$

where $\boldsymbol{y}$ was the vector of phenotypic observations; $\boldsymbol{b}$ was the vector of fixed effects; $\boldsymbol{a}$ was the vector of random additive genetic effects; $\boldsymbol{m}$ was the vector of random maternal genetic effects; $\boldsymbol{c}$ was the vector of common litter effects; and $\boldsymbol{e}$ was the vector of residual effects; and $\boldsymbol{X}$, $\boldsymbol{Z}$, $\boldsymbol{G}$, and $\boldsymbol{W}$ were the incidence matrices relating the phenotypic observations to fixed, random additive genetic, maternal genetic, and common litter effects, respectively. It was assumed that random effects are independent and normally distributed:

$$\boldsymbol{a} \sim \mathrm{N}(0, \boldsymbol{A}\sigma_{\mathrm{a}}^2), \boldsymbol{m} \sim N(0, \boldsymbol{A}\sigma_{m}^2), \boldsymbol{c} \sim N(0, \boldsymbol{I}\sigma_{\mathrm{c}}^2), \text{and } \mathbf{e} \sim N(0, \boldsymbol{I}\sigma_{\mathrm{e}}^2),$$

where $\boldsymbol{A}$ was the numerator relationship matrix; $\boldsymbol{I}$ was an identity matrix; σ_a^2, σ_m^2, σ_c^2, and σ_e^2 were the variances of random additive genetic, maternal genetic, common litter, and residual effects. Bivariate models were used to estimate the genetic and phenotypic correlations between traits using ASReml 4.1 software (Gilmour et al., 2018).

The likelihood ratio test was used to determine the significance of different random terms in the mixed model analyses using ASReml 4.1 (Gilmour et al., 2018). By comparing the difference in logarithmic likelihoods between full and reduced models using the following statistics:

$$-2(\log L_{\text{reduced model}} - \log L_{\text{full model}}) \sim \chi^2_{\text{df(full model)}-\text{df(reduced model)}}$$

Relevant significant fixed and random effects were included in bivariate analyses for each trait (Table 3.1). Generally, the following bivariate model was used to analyze the traits:

$$\begin{bmatrix} \boldsymbol{y_1} \\ \boldsymbol{y_2} \end{bmatrix} = \begin{bmatrix} \boldsymbol{X_1} & \boldsymbol{0} \\ \boldsymbol{0} & \boldsymbol{X_2} \end{bmatrix} \begin{bmatrix} \boldsymbol{b_1} \\ \boldsymbol{b_2} \end{bmatrix} + \begin{bmatrix} \mathbf{Z_1} & \boldsymbol{0} \\ \boldsymbol{0} & \mathbf{Z_2} \end{bmatrix} \begin{bmatrix} \boldsymbol{a_1} \\ \boldsymbol{a_2} \end{bmatrix} + \begin{bmatrix} \boldsymbol{G_1} & \boldsymbol{0} \\ \boldsymbol{0} & \boldsymbol{G_2} \end{bmatrix} \begin{bmatrix} \boldsymbol{m_1} \\ \boldsymbol{m_2} \end{bmatrix} + \begin{bmatrix} \boldsymbol{W_1} & \boldsymbol{0} \\ \boldsymbol{0} & \boldsymbol{W_2} \end{bmatrix} \begin{bmatrix} \boldsymbol{c_1} \\ \boldsymbol{c_2} \end{bmatrix} + \begin{bmatrix} \boldsymbol{e_1} \\ \boldsymbol{e_2} \end{bmatrix},$$

where $\boldsymbol{y_1}$ and $\boldsymbol{y_2}$ were the vectors of phenotypic observations for trait 1 and trait 2, respectively; $\boldsymbol{b_1}$, $\boldsymbol{b_2}$, $\boldsymbol{a_1}$, $\boldsymbol{a_2}$, $\boldsymbol{m_1}$, $\boldsymbol{m_2}$, $\boldsymbol{c_1}$, $\boldsymbol{c_2}$, $\boldsymbol{e_1}$, and $\boldsymbol{e_2}$ were the vectors of fixed, additive genetic, maternal genetic, common litter, and residual effects for trait 1 and trait 2, respectively; and $\boldsymbol{X_1}, \boldsymbol{X_2}, \mathbf{Z_1}, \mathbf{Z_2}, \boldsymbol{G_1}, \boldsymbol{G_2}, \boldsymbol{W_1}, \boldsymbol{W_2}$, were the incidence matrices relating phenotypic observations to fixed, random additive genetic, maternal genetic, and common litter effects for traits 1 and 2, respectively. It was assumed that random effects were normally distributed:

$$\begin{bmatrix} \boldsymbol{a_1} \\ \boldsymbol{a_2} \end{bmatrix} \sim \boldsymbol{N}\left(\boldsymbol{0}, \boldsymbol{A} \otimes \begin{bmatrix} \boldsymbol{\sigma}^2_{\boldsymbol{a_1}} & \boldsymbol{\sigma}_{\boldsymbol{a_1 a_2}} \\ \boldsymbol{\sigma}_{\boldsymbol{a_1 a_2}} & \boldsymbol{\sigma}^2_{\boldsymbol{a_2}} \end{bmatrix}\right),$$

$$\begin{bmatrix} \boldsymbol{m_1} \\ \boldsymbol{m_2} \end{bmatrix} \sim \boldsymbol{N}\left(\boldsymbol{0}, \boldsymbol{A} \otimes \begin{bmatrix} \boldsymbol{\sigma^2_{m_1}} & \boldsymbol{\sigma_{m_1 m_2}} \\ \boldsymbol{\sigma_{m_1 m_2}} & \boldsymbol{\sigma^2_{m_2}} \end{bmatrix}\right),$$

$$\begin{bmatrix} \boldsymbol{c_1} \\ \boldsymbol{c_2} \end{bmatrix} \sim \boldsymbol{N}\left(\boldsymbol{0}, \boldsymbol{I} \otimes \begin{bmatrix} \boldsymbol{\sigma^2_{c_1}} & \boldsymbol{\sigma_{c_1 c_2}} \\ \boldsymbol{\sigma_{c_1 c_2}} & \boldsymbol{\sigma^2_{c_2}} \end{bmatrix}\right), \text{ and}$$

$$\begin{bmatrix} \boldsymbol{e_1} \\ \boldsymbol{e_2} \end{bmatrix} \sim \boldsymbol{N}\left(\boldsymbol{0}, \boldsymbol{I} \otimes \begin{bmatrix} \boldsymbol{\sigma^2_{e_1}} & \boldsymbol{\sigma_{e_1 e_2}} \\ \boldsymbol{\sigma_{e_1 e_2}} & \boldsymbol{\sigma^2_{e_2}} \end{bmatrix}\right),$$

where $\boldsymbol{A}$ was the numerator relationship matrix; $\boldsymbol{I}$ was an identity matrix; $\sigma^2_{a_1}$, $\sigma^2_{a_2}$, $\sigma^2_{m_1}$, $\sigma^2_{m_2}$, $\sigma^2_{c_1}$, $\sigma^2_{c_2}$, $\sigma^2_{e_1}$, and $\sigma^2_{e_2}$ were the variances of random additive genetic, maternal genetic, common litter, and residual effects for traits 1 and 2, respectively; $\sigma_{a_1 a_2}$, $\sigma_{m_1 m_2}$, $\sigma_{c_1 c_2}$ and $\sigma_{e_1 e_2}$ were the covariances of random additive genetic, maternal genetic, common litter, and residual effects between traits 1 and 2, respectively. Phenotypic variance was calculated as $\sigma^2_P = \sigma^2_a + \sigma^2_c + \sigma^2_e$ for HL, $\sigma^2_P = \sigma^2_a + \sigma^2_m + \sigma^2_e$ for DNAP, LNAP, Nov_BW, and Nov_BL and $\sigma^2_P = \sigma^2_a + \sigma^2_e$ for other traits. Heritability of direct additive genetic effects (h^2_a), heritability of maternal genetic effects (h^2_m) and proportion of common litter variance (c^2) were defined as follows:

$$h^2_a = \frac{\sigma^2_a}{\sigma^2_P},$$

$$h^2_m = \frac{\sigma^2_m}{\sigma^2_P}, \text{ and}$$

$$c^2 = \frac{\sigma^2_c}{\sigma^2_P}.$$

Phenotypic and genetic correlations among traits were calculated using (co)variance components estimated by bivariate models.

3.3 Results and Discussion

3.3.1 Descriptive statistics

Genetic and phenotypic parameters for dried pelt, live grading, and body weight and length traits were estimated using animal models. The number of records, mean, range, standard deviation and coefficient of variation are presented in Table 3.2. The LQU, HW, and Nov_BL had the highest coefficient of variation (CV) among the studied traits (35.14%, 33.48%, and 31.19%, respectively). The presence of these variations indicated that there might be potential to improve them through genetic/genomic selection. In the present study estimated CVs for DQU, LNAP and LQU were 22.87%, 30.94%, and 35.14%, respectively (Table 3.2). Thirstrup et al. (2017) reported a higher CV of 34.53% for DQU and lower CVs of 27.43% and 25.73% for LNAP and LQU, respectively. The population used in our study consisted of five color types while the mink used by Thirstrup et al. (2017) were all standard brown color mink, this difference in population structure might be the reason for higher genetic variation in our study relative to their study.

3.3.2 Fixed and random effects

To determine the influence of non-genetic factors on phenotypic variation of the traits, the significance of fixed effects and non-genetic environmental effects have been examined using univariate animal models. The fixed effects of farm and year were significant ($P < 0.05$) for all pelt, and body weight and length traits (Table 3.1). The fixed effect of sex was significant ($P < 0.05$) for DPS, Nov_BW, Nov_BL, HW, and HL. This is in agreement with the previous investigation that reported the difference in mature body weight and body length between female and male mink (Do and Miar, 2019). The effect of color type was significant for DNAP, LNAP, LQU, Nov_BL, and HL (Table 3.1). The difference in body weight of mink at maturity among

different color types has been previously reported in the study of the growth pattern of mink (Liu et al., 2011).

Estimated variance components for each trait obtained from univariate models are presented in Table 3.2. The maternal genetic effects (±SE) were significant ($P < 0.05$) for DNAP, LNAP, Nov_BW, and Nov_BL (Table 3.1) and explained 0.15±0.06, 0.070.03, 0.19±0.04, and 0.15±0.04 of the phenotypic variation for these traits, respectively. These results revealed that maternal genetic effects might be an important determinant of phenotypic variation for nap size, body weight, and body length. Selection of animals for maternal ability by culling out dams with weak maternal ability on these traits will then have positive effects both on body weight and length and pelt quality. There were no previous reports on the significance of random maternal genetic effects on pelt traits and body weight and body length traits in mink for comparison. However, in Alpine Merino sheep (*Ovis aries*), maternal genetic effects were significant ($P < 0.05$) for yearling staple length (0.03) and yearling body weight (0.18) (Li et al., 2022).

Random common litter effect was only significant ($P < 0.05$) for HL (0.09±0.03). The proportion of common litter effects was not tested for live grading and pelt traits since there were no common dams in different years of data collection for these traits. However, previous studies reported a minor contribution of common litter effects on live grading and pelt traits in mink, ranging from 0.01 to 0.12 (Lagerkvist and Lundeheim, 1990; Thirstrup et al., 2017).

3.3.3 Heritability estimations

Heritability estimations for all traits obtained from bivariate models are presented in Table 3.4 (diagonal elements). Average value of bivariate estimations of heritabilities were similar to those

obtained by univariate analyses. Minor differences between these estimates can be due to the differences in the number of records available for some traits.

The estimated heritabilities (±SE) were 0.41±0.06 for DPS, 0.29±0.10 for Nov_BW, 0.28±0.09 for Nov_BL, 0.41±0.07 for HW, and 0.31±0.06 for HL (Table 3.4). The moderate heritability of these traits indicated that selection for higher body weight and length, and larger skin size might be possible through genetic selection. Thirstrup et al. (2017) estimated the heritability of 0.45 for both male and female skin size which was similar to the heritability (±SE) obtained for skin size in our study (0.41±0.06). However, Lagerkvist et al. (1993) reported higher heritability for skin size (0.57). Lagerkvist et al. (1993) only used the records collected from one sex (male) that have been under selection for five generations to estimate the genetic parameters. Moreover, the selection for males body weight might influence the genetic variation of this trait due to the allele frequencies changes. Therefore, the statistical models and population characteristics might be the potential reasons leading to these discrepancies.

Heritabilities (±SE) estimated for DNAP, DQU, LNAP, and LQU were equal to 0.23±0.10, 0.12±0.04, 0.44±0.07, and 0.28±0.06, respectively (Table 3.4). Low to moderate heritabilities for these traits revealed that there is a potential to improve these fur characters using genetic and genomic selection. Among silver blue mink in China, the estimated heritability for LNAP was 0.52 (Liu et al., 2017), which was higher than our result (0.44±0.07). In blue fox (*Alopex lagopus*), the heritability of LNAP was estimated at 0.19 (Peura et al., 2005), which was lower than our estimated heritability for this trait. Berg (1993) estimated the heritability for mink pelt guard hair length ranged from 0.22 to 0.34 which is comparable with the result of our study (0.23±0.10). The heritability for DNAP in blue fox was estimated to be 0.36 (Kempe et al., 2013), which was higher than our estimate (0.23±0.10). Difference in genetic background and environmental factors

between the two species might be the potential reasons for difference in estimated heritability. The heritability reported for LQU ranged from 0.19 to 0.35 in the previous studies of mink (Kenttämies and Vilva, 1988; Lagerkvist and Lundeheim, 1990; Lagerkvist et al., 1993; Koivula et al., 2008; Thirstrup et al., 2017), which were comparable with our result (0.28±0.06). Thirstrup et al. (2017) estimated a heritability of 0.30 for DQU which is higher than our estimate of 0.12± 0.04 for this trait. In our study, we had four categories while Thirstrup et al. (2017) defined 12 categories for this trait. Additionally, DQU evaluation is being subjectively performed by fur evaluators and there is no universal definition for DQU available in the literature. Therefore, it is possible that slightly different criteria used by Thirstrup et al. (2017) for definition of DQU compared with our study.

3.3.4 Genetic and phenotypic correlations between dried pelt, and body weight and length traits

Phenotypic and genetic correlations between body weight, body length, and pelt traits are shown in Table 3.4. High genetic correlations were estimated between DPS with Nov_BW (0.89±0.10), Nov_BL (0.81±0.07), HW (0.85±0.05), and HL (0.85±0.06). These results indicated that both body weight and length traits could be used as reliable measurements to predict the dried pelt size for the market. Considering the strong genetic correlations of Nov_BW with HW (0.99±0.01) and Nov_BL with HL (0.86±0.05), selection based on body weight and body length measured in November of the first year of life could be used as good indicators for indirect selection of final skin size of mink. This can be particularly beneficial for mink farmers because selection of breeders for the next breeding season is usually performed in November. This result suggested that selection based on body weight and length in November of the first year of life would be reliable

indicators for market pelt size, which is determinant of the final price of skin and subsequently the profitability of mink producers.

All phenotypic correlations between DQU with body weights and lengths in November and harvest time were not significant ($P > 0.05$). However, DQU had positive genetic correlations with Nov_BL (0.55±0.24) and HL (0.46±0.20), suggesting that body length traits might be good indicator traits to improve both dried pelt size and overall quality. On the other hand, the genetic correlations of DQU with Nov_BW (0.25±0.25) and HW (0.06±0.20) were not significant ($P > 0.05$). However, Thirstrup et al. (2017) reported significant negative genetic correlations between HW and DQU (ranged from -0.38 to -0.52). This discrepancy might be due to the differences in the definition of traits and scores applied to measure this trait in these studies. In our study, DQU had four categories while Thirstrup et al. (2017) considered 12 different categories for DQU that might be based on different criteria compared with the categories applied in the current study.

No significant ($P > 0.05$) phenotypic or genetic correlations were estimated between DNAP with body weight and length traits, which ranged from 0.29±0.19 to 0.48±0.32 for body weight and -0.01±0.30 to -0.02±0.19 for body length traits. These results showed that independent genes might be involved in controlling pelt quality, and body weight and length traits suggesting that selection for larger body weight and length would not have a negative impact on dried pelt nap size.

3.3.5 Genetic and phenotypic correlation between live grading and body weight and length traits

Phenotypic and genetic correlations between live grading, and body weight and length traits are shown in Table 3.4. The Nov_BW had low positive phenotypic correlation with LNAP (0.11±0.04) and LQU (0.10±0.04). However, genetic correlations of LNAP and LQU with all body weight and

length traits were not significantly different from zero ($P > 0.05$). These results suggested that selection for larger body size in November of the first year of life would not negatively affect live grading traits in mink. Similar to our results, Thirstrup et al. (2017) reported non-significant genetic correlations between body weight and LQU (-0.13 to 0.18), and between body weight and LNAP (-0.01 to 0.07).

3.3.6 Genetics and phenotypic correlations between dried pelt and live fur grading traits

A strong positive genetic correlation (0.82±0.22) was observed between LNAP and DNAP (Table 3.4). This suggested that selection for shorter DNAP based on the corresponding live grading trait could be effective in breeding programs. To our knowledge, this is the first report on estimation of genetic correlation between DNAP and LNAP in mink, therefore no previous study was available for comparison. There was a moderate genetic correlation between LNAP and LQU (0.45±0.12), which was lower than the value (0.86) estimated previously in the literature (Thirstrup et al., 2017). Differences in scoring scales, statistical models and population structure might be responsible for this difference. LNAP and LQU were scored in five categories in Thirstrup et al. (2017) study, however, LNAP had five and LQU had three categories in our study. Moreover, the fixed effect of color type was significant for nap size traits in the present study. However, all mink used in Thirstrup et al. (2017) were from the same color type (i.e., standard dark brown), so no fixed effect of the color type needed to be specified in their statistical model for estimation of genetic correlations. Genetic correlation of the LQU and DQU (0.08±0.45) was not significant in the present study. Lagerkvist et al. (1994) reported a non-significant genetic correlation of 0.15±0.16 between underfur density on live mink and dried pelt, which was in accordance with our result. In our study, the density of underfur was the main criteria for classification of pelts in

different quality categories. Kaszowski et al. (1970) found the average underfur density for live mink to be approximately 34 percent less than underfur density in the dried pelts, which might be due to shrinkage of pelts during drying process leading to a change in the density of hairs relative to the pelt surface on the skins.

Indirect selection for pelt traits based on live grading is in demand by mink breeders since they can be measured on selection candidates. In the present study, high positive genetic correlation was estimated between LNAP and its corresponding dried pelt trait (0.82±0.22), and between body weight and length traits with DPS ranged from 0.81±0.07 to 0.89± 0.10. However, the genetic and phenotypic correlations between LQU and DQU were poor and non-significant. Therefore, selection solely based on live grading may not lead to the maximum improvement for dried pelt characters in mink. Peura et al. (2005) suggested using a multi-trait selection approach to estimate the breeding values for selection candidates by combining information from live grading and pelt traits recorded on relatives of selection candidates for improving pelt traits in blue foxes. However, a multi-trait selection may not be an effective method for pelt traits in our mink population. The first reason is the non-significant genetic correlation between LQU and DQU (0.08±0.45) estimated in our study. Moreover, when information from relatives is used for the estimation of breeding value, the selection accuracy will be considerably lower than the accuracy of the breeding value estimated based on animals' own performance which would lead to lower selection response and genetic improvement (Bourdon, 2000). An alternative method can be selecting animals based on their genomic breeding values directly estimated from genotype information obtained for pelt traits. Genomic selection has proved to be particularly beneficial to select for traits that are measured post-mortem (Miar et al., 2014a; Miar et al., 2014c; Meuwissen et al., 2016) The correlated traits evaluated in the current study such as body weight and body length with DPS,

body length in November with DQU, and live grading nap size with dried pelt nap size along with molecular information can be used to obtain more accurate estimates of breeding value for pelt quality traits using multi-trait genomic prediction.

Our study provides insights into the proportion of genetic and environmental sources of phenotypic variation in dried pelt, live grading, and body weight and body length traits in mink. The absence of live grading records for Millbank farm restricted the number of available records for these traits, which might impact the genetic correlations between live grading traits and pelt traits in our study. The estimated genetic parameters in this study provided the basic knowledge for designing the genetic selection programs for fur quality in Canadian mink populations.

3.4 Conclusions

Genetic selection for fur quality and skin size can increase the production efficiency and consequently the economic profits of mink farmers. The present study was the first estimation of genetic parameters for fur quality traits in Canadian mink populations. Estimated moderate heritabilities for pelt, live grading, and body weight and length traits suggested that improvement of these traits is possible using genetic/genomic selection. The estimated genetic parameters showed the potential of Nov_BW and Nov_BL as good indicators of DPS without negative effects on DQU and DNAP. Presence of moderate positive genetic correlations between body length in November and harvest with DQU, makes these traits suitable traits to select for increase DPS and DQU. In addition, live grading nap size is a reliable indicator of dried pelt nap size. The results established a foundation for a more efficient selective breeding method for the mink industry that can be incorporated into multi-trait genetic or genomic selection program.

Table 3.1 Significance of fixed and random effects included in the models for dried pelt, live grading, and body weight and length traits in mink.

Traits[1]	Fixed effects					Random effects	
	Farm	Sex	Color type	Year	Age	Common litter	Maternal genetic
DPS	*	*	NS	*	NT	NT	NS
DNAP	*	NS	*	*	NT	NT	*
DQU	*	NS	NS	*	NT	NT	NS
LNAP	NT	NS	*	*	*	NT	*
LQU	NT	NS	*	*	*	NT	NS
Nov_BW	*	*	NS	*	NT	NT	*
Nov_BL	*	*	*	*	NT	NT	*
HW	*	*	NS	*	NT	NS	NS
HL	*	*	*	*	NT	*	NS

[1] DPS = dried pelt size; DNAP = Dried pelt nap size; DQU = overall quality of dried pelt; LNAP= live grading nap size; LQU = live grading overall quality of fur; Nov_BW = November body weight; Nov_BL= November body length; HW = Harvest body weight; HL = Harvest body length, NT: Not tested, NS; Not significant.

* $P < 0.05$.

Table 3.2 Descriptive statistics for dried pelt, live grading, and body weight and length traits in mink.

Traits[1]	Number of records	Mean	Standard deviation	Range	Coefficient of variation (%)
DPS	1,195	5.71	1.70	1 – 9	29.82
DNAP	1,125	6.17	1.40	1 – 8	22.69
DQU	1,191	3.40	0.78	1 – 4	22.87
LNAP	1,608	3.07	0.95	1 – 5	30.94
LQU	1,607	2.02	0.71	1 – 3	35.14
Nov_BW (kg)	1,734	2.18	0.68	0.92 – 3.86	31.19
Nov_BL (cm)	1,734	39.57	4.68	31 – 51	11.82
HW (kg)	2,162	2.24	0.75	0.79 – 4.1	33.48
HL (cm)	2,162	45.38	5.03	35.5 – 59	11.08

[1] DPS = dried pelt size; DNAP = Dried pelt nap size; DQU = overall quality of dried pelt; LNAP= live grading nap size; LQU = live grading overall quality of fur; Nov_BW = November body weight; Nov_BL= November body length; HW = Harvest body weight; HL = Harvest body length

Table 3.3. Variance components and heritabilities (±SE) estimated using univariate models for dried pelt traits, live grading, and body weight and length traits in mink.

	Variance components[2]				Genetic parameters[3]		
Traits[1]	$\boldsymbol{\sigma^2_a \pm SE}$	$\boldsymbol{\sigma^2_c \pm SE}$	$\boldsymbol{\sigma^2_m \pm SE}$	$\boldsymbol{\sigma^2_e \pm SE}$	$\boldsymbol{h^2_a}$	$\boldsymbol{c^2}$	$\boldsymbol{h^2_m}$
DPS	0.24 ±0.04	NT	NS	0.35±0.03	0.41 ±0.06	NA	NA
DNAP	0.21±0.10	NT	0.14 ±0.05	0.59 ±0.06	0.22 ±0.10	NA	0.15 ±0.06
DQU	0.07±0.03	NT	NS	0.51±0.03	0.12±0.04	NA	NA
LNAP	0.26±0.05	NT	0.04±0.02	0.32 ±0.03	0.42 ±0.06	NA	0.07±0.03
LQU	0.11 ±0.02	NT	NS	0.37±0.02	0.23 ±0.5	NA	NA
Nov_BW	0.016±0.73E-02	NT	0.015±0.37E-02	0.047±0.42E-02	0.21±0.09	NA	0.19 ±0.04
Nov_BL	0.92±0.33	NT	0.51±0.16	2.02±0.19	0.27 ±0.09	NA	0.15±0.04
HW	0.04±0.57E-02	NS	NS	0.05±0.41E-02	0.44 ±0.09	NA	NA
HL	1.32±0.34	0.45±0.16	NS	3.29±0.22	0.26±0.06	0.09 ±0.03	NA

[1] DPS = dried pelt size; DNAP = Dried pelt nap size; DQU = overall quality of dried pelt; LNAP= live grading nap size; LQU = live grading overall quality of fur; Nov_BW = November body weight; Nov_BL= November body length; HW = Harvest body weight; HL = Harvest body length.

[2] σ^2_a= additive genetic variance; σ^2_m= maternal genetic variance; σ^2_c= common litter variance; σ^2_e= residual variance.

[3] h^2_a= heritability from univariate models; c^2= proportion of phenotypic variance explained by common litter effects; h^2_m= proportion of phenotypic variance explained by maternal genetic effects.

NS: not significant (P > 0.05); NA: not applicable; NT: not tested.

Table 3.4. Estimated heritabilities (±SE) (diagonal), genetic correlations (above diagonal), and phenotypic correlations (below diagonal) for dried pelt, live grading, and body weight and length traits in mink.

Traits[1]	DPS	DNAP	DQU	LNAP	LQU	Nov_BW	Nov_BL	HW	HL
DPS	**0.41±0.06**	0.26±0.20	0.26±0.21	0.04±0.20	-0.14±0.32	**0.89±0.10**	**0.81±0.07**	**0.85±0.05**	**0.85±0.06**
DNAP	0.01±0.03	**0.23±0.10**	0.13±0.25	**0.82±0.22**	0.42±0.34	0.48±0.32	-0.01±0.30	0.29±0.19	-0.02±0.19
DQU	0.01±0.03	**0.14±0.03**	**0.12±0.04**	0.15±0.30	0.08±0.45	0.25±0.25	**0.55±0.24**	0.06±0.20	**0.46±0.20**
LNAP	0.01±0.07	**0.45±0.06**	0.06±0.06	**0.44±0.07**	**0.45±0.12**	0.20±0.19	0.12±0.25	0.09±0.21	0.15±0.26
LQU	-0.07±0.07	**0.29±0.06**	0.01±0.06	**0.23±0.02**	**0.28±0.06**	-0.04±0.20	-0.15±0.22	-0.36±0.21	-0.28±0.25
Nov_BW	**0.64±0.01**	0.06±0.04	-0.01±0.03	**0.11±0.04**	**0.10±0.04**	**0.29±0.10**	**0.79±0.12**	**0.99±0.01**	**0.53±0.02**
Nov_BL	**0.50±0.03**	-0.06±0.04	0.03±0.03	-0.08±0.04	-0.04±0.04	**0.55±0.02**	**0.28±0.09**	**0.90±0.05**	**0.86±0.05**
HW	**0.69±0.01**	0.07±0.03	-0.02±0.03	0.04±0.06	0.01±0.06	**0.83±0.01**	**0.52±0.02**	**0.41±0.07**	**0.83±0.05**
HL	**0.49±0.02**	0.005±0.3	0.01±0.03	0.04±0.06	-0.02±0.05	**0.53±0.02**	**0.51±0.02**	**0.53±0.01**	**0.31±0.06**

[1] DPS = dried pelt size; DNAP = Dried pelt nap size; DQU = overall quality of dried pelt; LNAP= live grading nap size; LQU = live grading overall quality of fur; Nov_BW = November body weight; Nov_BL= November body length; HW = Harvest body weight; HL = Harvest body length.

[2] The significant heritabilities and correlations are highlighted in bold ($P < 0.05$).

Chapter 4: Genome-wide detection of selection signatures for pelt quality traits and coat color using whole-genome sequencing data in American mink[2]

4.1 Introduction

American mink (*Neogale vison*) is a semi-aquatic species of the carnivorous native to north America and is the most important fur-bearing species used in the fur industry worldwide. The mink was initially bred in captivity in 1866 in Canada (Bowness, 1996). Since then, due to its importance for fur industries, mink farming is extensively practiced in North America, Europe, and Asia. Farmed mink are selectively bred for improved litter size, pelt quality, disease resistance, body growth, and behavioral traits (Hansen, 1996). Evidence indicates that artificial selection during the last 150 years has driven the differentiation between farmed mink and wild populations (Kruska, 1996; Tamlin et al., 2009; Morris et al., 2020). Pelt quality and coat color are important breeding objectives because of their effects on the final economic value of fur. Short-haired large pelts with dense hair coverage and healthy guard hair hold the highest economic value in the fur industry. Black mink coats are the most used color in fur fashion industry as it can be worn with all other colors of clothing, and colors such as pastel and stardust are sold at high price because of their unique natural color that can meet consumer preferences for natural products (Fur Commission USA, 2011; Wang et al., 2022).

In mink, a few attempts have been made to pinpoint genes associated with pelt quality traits, using genome-wide association studies (GWAS) (Cai et al., 2018) or linkage mapping (Thirstrup et al., 2014). However, only a limited number of significant quantitative trait loci (QTL) with small effects on their genetic variations have been identified (Thirstrup et al., 2014; Cai et al., 2018).

[2] This chapter has been published. Valipour et al., 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), p.1939. https://doi.org/10.3390/genes13111939.

Similarly, a few candidate genes potentially involved in pigmentation have been identified in mink such as the *MLPH*, *LYST*, *TYR*, *MITF* and *TYRP1* genes (Anistoroaei et al., 2008; Anistoroaei et al., 2013; Cirera et al., 2013; Markakis et al., 2014; Cirera et al., 2016). In mice, more than 170 genes involved in pelage pigmentation have been detected (Bennett and Lamoreux, 2003). Moreover, 15 genes with known roles in canine coat color has been reported (Brancalion et al., 2022). Therefore, understanding molecular genetic mechanisms underlying pelt quality and coat color regulation in mink required further investigation.

Wild mink was originally dark brown which is commonly known as standard dark brown or black mink (Shackelford, 1948; Song et al., 2017; Manakhov et al., 2019). Appearance of mutant colors was documented as early as 1929 in ranch-raised mink (Shackelford, 1948). It has been suggested that restriction of free mating and increased inbreeding during high-intensity artificial selection in commercial farms led to increased homozygosity of natural recessive coat color mutations that have been accumulated in genome of individuals have led to the appearance of mutant color types (Trapezov, 1997; Trapezov and Trapezova, 2016). For instance, appearance of Black Crystal, Himalayan coat colors and intensification of expressivity of the white piebalds after multiple generations of selection for tame behaviour have been reported in farmed mink (Trapezov, 1997; Trapezov and Trapezova, 2016). During the last century due to the economic profit offered by producing mutant coat colors, mink breeders have selectively bred mink for different coat colors which led to the creation of great diversity (rainbow of colors) of color types in farmed mink. From the view of population genetics, the effect of artificial selection for pelt quality and coat color would leave detectable selection signatures within the mink genome (Qanbari and Simianer, 2014; Ma et al., 2015). Therefore, identifying the selection signatures underlying these traits would

provide opportunity to characterize the genomic regions contributing to the pelt quality and coat color traits in domesticated mink.

Availability of whole-genome sequencing (WGS) made it possible to discover sequence variants such as single nucleotide polymorphisms (SNPs) at a population scale that can facilitate the mapping of selection signatures at higher resolution than SNP microarrays or genotyping-by-sequencing data (Boitard et al., 2016). Multiple approaches have been developed to detect the patterns of selection signatures in the genome, based on different features of selective sweeps (Vitti et al., 2013). For instance, nucleotide diversity ($\theta\pi$), which is the average number of pairwise nucleotide differences between sequences in a sample, cross population extended haplotype homozygosity (XP-EHH) that is based on the extend of linkage disequilibrium within the populations, and fixation index (Fst), which is the measure of real allele frequency differences between individuals in a population (Ma et al., 2015). Selection signatures can be identified by the changes in the allele frequency spectrum, increase in homozygous genotypes, and extended linkage disequilibrium levels i.e., long haplotypes exist with high frequency (Ma et al., 2015). Therefore, using a combination of multiple statistics to detect the targets of selection is often a good option.

Scanning the genome for evidence of selection signatures has been extensively used for identification of genes and genomic regions related to disease phenotypes in humans (Harris and Meyer, 2006), or economic traits in crops (Wang et al., 2018), and livestock species (Gouveia et al., 2014). In mink, selection signature approaches were used to reveal the putative regions for response to Aleutian mink disease virus infection (Karimi et al., 2021a). However, to our knowledge, no study investigated the selection signatures for pelt quality, pelt size and coat color traits in farmed mink. Therefore, the objectives of this study were to, 1) identify the selection

signatures for pelt quality and coat color traits in mink genome using WGS data, and 2) identify the candidate genes related to pelt quality, pelt size and coat color traits.

4.2 Materials and Methods

4.2.1 Animals and sampling

Phenotypic records were collected from animals born and raised in 2018 at the Canadian Centre for Fur Animal Research (CCFAR) at Dalhousie University (Truro, NS, Canada) and Millbank Fur Farm (Rockwood, ON, Canada). Animal management and sampling procedures were performed in accordance with the standards of the Canadian Council on Animal Care (Turner et al., 2013) after approval by the Dalhousie University Animal Care and Use Committee (certification#: 2018-009). In December 2018, mink were euthanized using the approved method of carbon monoxide gas to provide a quick and humane death. Tongue tissues were collected from 100 animals, obtained by removing a section from the tip and body regions of the tongue using sterile surgical tools. Subsequently, the excised tissues were carefully transferred into storage tubes, then tubes were stored at –80°C until they underwent further procedures for the isolation of DNA.

4.2.2 Animal grouping

Mink used in this study were euthanized in December of 2018 and were shipped to the custom pelting facilities (Arcadia, NS, Canada). Dried pelts were shipped to the North American Fur Auction (NAFA) house (Toronto, ON, Canada) where the evaluation of dried pelts was performed by certified technicians. Three pelt quality traits including nap size, overall quality of fur, and pelt size; and three color types including black, stardust and pastel were used to divide animals into

subgroups based on their phenotypic information. Nap size is defined as the length of guard hair protruding out of underfur (Thirstrup et al., 2017). It was scored into eight categories: ranging from extra short nap (category 1) to medium-long nap (category 8) (North American Fur Auctions, 2014). For genomic analysis, we grouped the animals with extra short nap and short nap into subgroups of short nap (n = 14) and animals with medium nap to medium-long nap and medium-long nap into the long nap subgroup (n =15). The overall quality of fur describes the general appearance of fur in terms of density of underfur, healthy appearance of guard hairs and underfur, and smooth and silky textures of fur. For overall quality of fur, animals were classified into high fur quality (n = 16), i.e., pelt of very high quality, fully prime, dense, and resilient underfur with good and even guard hair coverage and super silky textures, and low fur quality (n = 11), i.e., weakest pelts in terms of underfur, guard hair, uneven coverage of guard hairs and underfur, coarser guard hair, and weak general appearance. Pelt size is the length of dried pelt measured from the tip of nose to the base of tail. Since pelt size is influenced with sex, we only used pelts from female mink. Male pelts size was not included in this study as our sequenced population did not contain a sufficient number of males with small pelt size. Therefore, female pelts larger than 77 cm were assigned to large pelt size subgroup (n = 10) and pelts smaller than 59 cm were categorized as small pelt size (n = 25). In addition, since black or dark color is considered the mink wild color type for mink (Song et al., 2017), we also examined the signatures of selection for coat color by comparing the stardust (n = 7) and pastel (n = 10) color types versus black color type (n = 31).

4.2.3 Whole-genome sequencing, reads alignment and variant calling

DNA was isolated from tongue samples using the Qiagen DNeasy Blood and Tissue Kit (Qiagen, Hilden, Germany). Sequencing and generation of paired-end libraries (100 bp pair-end reads) was performed using the BGISEQ-500 platform at Beijing Genomics Institute with an average coverage of 40X per sample (BGI, Guangdong, China). After sequencing, sequencing adapters and low-quality reads were removed using SOAPnuke software (Chen et al., 2018). The filtered reads were aligned against the recent American mink reference genome (https://www.ncbi.nlm.nih.gov/assembly/GCF_020171115.1/) using Burrows-Wheeler Aligner (BWA) 0.7.17 (Li and Durbin, 2009). The aligned files were converted to binary alignment map (BAM) format and sorted using SAMtools 1.11 (Li et al., 2009) and the potential PCR duplicates were then removed using the MarkDuplicates command tool of Picard (Picard toolkit, 2019). The BAM files were then indexed by SAMtools 1.11. Finally, variant calling was performed with SAMtools 1.11 and Genome Analysis Toolkit 4.1.9.0 (GATK) pipeline using haplotypecaller (McKenna et al., 2010). Quality control of variants was performed using VCFtools (Danecek et al., 2011). Variants with minor allele frequency (MAF) < 0.05; maximum missing rate <1.0; deviating from Hardy–Weinberg equilibrium ($P < 10^{-6}$) were removed. Moreover, only bi-allelic variants on autosomal chromosomes were kept. After quality control, 9,922,758 bi-allelic variants from 100 individuals remained for further analysis.

4.2.4 Detection of selection signatures

4.2.4.1 Pairwise fixation index (Fst)

The Fst values were calculated for each SNP according to Weir and Cockerham (Weir and Cockerham, 1984) for all pairwise subgroups using VCFtools (Danecek et al., 2011). The Fst

measures the real allele frequency differences between two groups. Fst values ranges from 0 (i.e., no differentiation) to 1 that represent the complete divergence between two groups at a given locus. The negative Fst values were converted to zero as there was no biological interpretation for negative values (Akey et al., 2002). The Fst values were plotted relative to their physical position within each autosomal chromosomes and visualized using the 'qqman' package in R (Turner, 2018). The top 1% of genome-wide Fst values were considered as the potential selection candidates (Qanbari et al., 2014; Qanbari and Simianer, 2014).

4.2.4.2 Cross-population extended haplotype homozygosity (XP-EHH)

The profiles of EHH were compared between each pair group by calculating XP-EHH statistics using Selscan v2 software (Szpiech, 2021) with the --max-gap set to 200 kb based on the default program (Szpiech, 2021). The XP-EHH statistics can be used to detect selective sweeps in which the selected allele has approached or achieved fixation in one group but remained polymorphic in the other group through comparison of EHH scores of two groups (Sabeti et al., 2007). In the current study, long nap, small pelt size, low fur quality and black mink were considered as control subgroups, which were compared to individuals in the test subgroups including short nap, large pelt size, high fur quality and non-black mink (pastel and stardust), respectively. Finally, the XP-EHH values were normalized by subtracting the mean XP-EHH and dividing by the standard deviation using 'Norm' software (Szpiech, 2021). Those SNPs with XP-EHH values within the top 1% of positive normalized genome-wide values were considered as selection candidates in each group. Finally, the overlapped SNPs located in the top 1% of both Fst and XP-EHH values were identified (Qanbari et al., 2014; Qanbari and Simianer, 2014). Gene annotations were then carried out on the 5-kb flanking region around each SNP (5 kbp downstream and upstream of the given SNP).

4.2.4.3 Nucleotide diversity (θπ)

Nucleotide diversity was calculated for each group separately using the VCFtools (Danecek et al., 2011) –site-pi option. The θπ ratios were computed as θπ-(long nap, small pelt size, low fur quality)/θπ-(short nap, large pelt size, high fur quality) for pelt quality traits and θπ-(black mink)/θπ-(pastel, and stardust) for coat color traits. For all pairs of groups and were then log2-transformed (log2 (θπ ratios)). Finally, SNPs in the top 1% of log2 (θπ ratios) values were overlapped with the highest 1% of both Fst and XP-EHH values. For each overlapped SNP, a window of the 5-kb flanking region was considered for gene annotations.

4.2.5 Gene ontology and functional analysis

BEDtools (Quinlan, 2014) were used to find the gene IDs overlapped with the candidate regions using general feature format of recent genome assembly of *Neogale vison* (https://www.ncbi.nlm.nih.gov/genome/16995?genome_assembly_id=1704888). The biological process, molecular function and cellular component terms were assessed for all genes using PANTHER 14.1 (Thomas et al., 2003). Benjamini-Hochberg False Discovery Rate (FDR) correction was used for both multiple testing and overrepresentation test. Moreover, Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway analyses (Kanehisa and Goto, 2000) was carried out using the g:Profiler (Raudvere et al., 2019). These genes were further investigated by reviewing relevant literature in relation to the phenotypes or pathways of interest in different groups.

4.3 Results

4.3.1 Selection signatures based on XP-EHH and Fst

There were 4,469, 6,960, 3,880, 5,776, and 2,804 SNPs with values within the top 1% of both test statistics for Nap size, overall fur quality, pelt size, Pastel_Black, and Stardust_Black groups, respectively (Supplementary Dataset S1a-e). The distribution of Fst and XP-EHH statistics on different chromosomes showing potential signatures of selection in different groups are presented in Figure 4.1 and Figure 4.2, respectively. Moreover, a complete list of candidate regions along with their positions was provided in Supplementary S1a-e. The total number of candidate regions and their associated genes for each phenotypic group are presented in Table 4.1.

Figure 4.3 presents the Panther pie chart of molecular functions for candidate genes in the putatively selected regions (Supplementary Table S3). These results indicated that significant proportions of genes were involved in binding (37.60%), and catalytic activities (29.80%). Based on the overlaps of these tests, 110 genes for nap size, 163 genes for overall fur quality, 98 genes for pelt size, 123 for pastel and 71 for stardust groups were identified to be putatively under selection (Supplementary Table S1a-e). The gene ontology analysis resulted in 988, 129, and 261 overrepresented ($P < 0.05$) GO enrichment terms related to different biological processes, molecular function, and cellular components, respectively (Supplementary Dataset S4 a-c). Top ten significant GO terms enriched in candidate regions are presented in Figure 4.4. In addition, the KEGG pathway analysis revealed two significantly enriched pathways including axon guidance (KEGG:04360) and small cell lung cancer (KEGG:05222) (Supplementary Dataset S4-d). Gene ontology revealed the biological roles of several genes related to follicular hair functions including hair cycle process (GO:0022405) and molting cycle process (GO:0022404) *APCDD1, BCL2, TSPEAR, FGFR2,* and *LRP4*; epidermis development (GO:0008544) *HOXB13, SLITRK6, UGCG,*

COL7A1, FGFR2, MST1, APCDD1, OPN3, LAMB3, LIPK, BCL2, and *LRP4*; and the Wnt signaling pathway (GO:0016055) *TIAM1, MCC, WNT5B, APCDD1, NR4A2, NLK, PSMB3, CTNND2, RHOA, CCND1, SIAH2, DRAXIN* and *LRP4*. Moreover, we obtained two GO terms with important biological processes related to growth performance of animals including Wnt signaling pathway (GO:0016055) and regulation of striated muscle tissue development (GO:0016202) *SHOX2, MTPN, ACVR1,* and *BCL2*.

4.3.2 Differentiation of individuals within each group based on θπ ratios

We used the θπ ratios statistics to put an upper limit to the signatures of selection detected by overlaps of Fst and XP-EHH methods. We filtered top 1% of empirical distribution of $\log_2$ ($\theta_{\pi\ ratios}$) in different groups and then only considered the overlapping regions between top 1% of $\log_2$ ($\theta_{\pi\ ratios}$) with significant candidate regions identified by previous approaches (XP-EHH and Fst). There were 152, 16, 14, 324, and 7 SNPs within the top 1% of all three methods in nap size, overall fur quality, pelt size, Pastel_Black, and Stardust_Black groups, respectively. Supplementary Dataset S2a-e presents the overlap of top 1% values of all three approaches including Fst, XP-EHH and $\log_2$ ($\theta_{\pi\ ratios}$) in different groups. The list of overlapping candidate regions along with the genes involved in those regions are presented in Table 4.2. The nap size group had the highest number of overlapping regions (12), distributed across the chromosomes 3, 5, 6, and 8.

4.4 Discussion

We reported the first genome-wide analysis of putative signatures of selection for pelt quality and coat color traits using WGS data in mink. Previous study of signatures of selection in mink was

performed using 47,800 SNPs generated by genotyping-by-sequencing (GBS) technique (Karimi et al., 2021a), however; the application of high-depth WGS was suggested to improve the accuracy of selective sweep detection (Boitard et al., 2016). This is because GBS uses the restriction enzymes to reduce the complexity of genome for sequencing, and therefore; only a reduced subset of genome is sequenced (Gurgul et al., 2019). Additionally, the current study used the variants called from a chromosome-level reference genome (ASM_NN_V1) which is more comprehensive compared to the previous studies (Cai et al., 2018; Karimi et al., 2021a) that used variants derived from the scaffold-based reference genome (Cai et al., 2017).

The primary purpose of mink farming is producing a high-quality fur. During the last 100 years of mink farming, ranchers continuously bred mink for more desirable pelt characteristics. The fur's guard hairs are responsible for its shine and color (Ward, 2016). Farmers select mink for shorter length of guard hair (nap size) because short-haired furs are more fashionable, while long-haired furs are used for trim (Ward, 2016). In addition to nap, hair density and healthy and silky appearance of fur influence the price of pelt. We identified 8 key genes (*APCDD1, HOXB13, TSPEAR, TIAM1, OPN3, BCL2, ACVR1, and LRP4*) related to hair follicle function, which might play important roles in regulating the guard hair length and density of hair follicles in mink. We obtained several biological terms related with hair growth. The Wnt pathway (GO:0016055) was significant in nap size group, which is the biological pathway considered to be the key regulator of hair follicle morphogenesis (Rishikaysh et al., 2014). The *APCDD1* gene was detected at chr3: 182,662,938-182,685,570 bp by integrated analysis of Fst and XP-EHH in nap size group. The *APCDD1* product is a membrane-bound glycoprotein that is abundantly expressed in human hair follicles and can interact in vitro with *WNT3A* and *LRP5,* which are the two essential components of Wnt signalling to regulate the hair growth (Shimomura et al., 2010). *HOXB13* (chr5:

46,761,410- 46,773,568 bp, nap size group) is involved in the regulation of human hair keratin gene expression. *HOXB13* is a member of the HOX multigene family that has an important role in regulation of fetal hair formation (Kömüves et al., 2003). *TSPEAR* gene (chr6: 1,764,452-1,774,452 bp in stardust group) plays a critical role in human hair follicle morphogenesis through regulation of the Notch signaling pathway. It was shown that silencing *TSPEAR* in mouse hair follicles caused apoptosis in hair follicular epithelial cells, leading to a decline in hair bulb diameter (Peled et al., 2016). *LRP4* (chr7: 189,857,928-189,874,416, stardust group) mutation can cause defects in hair follicle development (Ahn et al., 2013). *TIAM1* (chr6: 13,118,844-13,264,781 bp, nap size group) was identified to be essential regulator gene in keratinocytes. The phenotype of keratinocytes with a targeted inactivation of the *TIAM1* gene can cause severe defects in hair follicle morphogenesis, including greatly reduced follicle numbers, failure to progress beyond very early developmental stages, and pronounced defects in follicular keratinocyte proliferation (Nakrieko et al., 2008). *OPN3* (chr10: 38,151,971- 38,202,287 bp in stardust group) was detected in anagen hair follicles and it was shown that blue light (453 nm), which corresponded to the absorption spectra of OPN3, prolonged the anagen hair growth phase (Buscone et al., 2017). In the current study, *BCL2* gene (chr3: 136,814,007-136,824,892 bp in pastel group) enriched in GO term related to pigmentation. This gene was shown to be related with normal function of the melanocyte stem cell. The *BCL2* null mice displayed the loss of pigmentation after entering the first hair cycle (Mak et al., 2006). Moreover, we found *RAB27B* on chr3: 143746006- 143797790 bp with top XP-EHH values of 2.74 and top Fst value of 0.48. *RAB27B* is a small GTPase that shows 71% homology to *RAB27A,* which is involved in melanosome transport and biogenesis. Deletion of *RAB27A* is associated with Griscelli-Pruniéras syndrome type II in human with an unusual silvery-grey hypopigmented color of hair (Westbroek et al., 2004). There is evidence that

up-regulation of *RAB27B* in melanocytes of the Griscelli-Pruniéras patient can partially acquire the function of *RAB27A*, which can cause an evenly pigmented hair in the absence of *RAB27A* (Westbroek et al., 2004).

In addition, selection for larger pelt size and body size is one of the top priorities for mink breeders and has been a key target during mink farming and breeding. We found several genes related to body growth e.g., *NR4A2, ACVR1, RB1, POPDC2, FGFR2, TBX5, and TBX3. NR4A2* (chr3: 54,184,456-54,201,558 bp, in pelt size group) is a member of orphan *NR4A* subgroup, that is involved in the regulation of metabolic function and energy homeostasis (Pearen and Muscat, 2010; Pérez-Sieira et al., 2014). Mutations in *ACVR1* are associated with fibrodysplasia ossificans progressive (i.e., abnormal formation of bone in areas of the body such as the ligaments, tendons, and skeletal muscles) (Shore et al., 2006). In addition, *ACVR1* was identified as a candidate gene for growth traits in Chinese beef cattle (Cheng et al., 2019). *RB1* is essential for skeletal myogenesis and development and has an important role in muscular hypertrophy (Huh et al., 2004; Go et al., 2020). *POPDC2* has an important role in skeletal muscle development, and knockdown of this gene resulted in abnormal development of skeletal muscle (Kirchmaier et al., 2012). FGFR2 is a member of fibroblast growth factors family and is the most commonly distributed growth factor receptors in mammalian species. It has been demonstrated that FGFR2 is important component of miR-327–FGF10–FGFR2-mediated autocrine signaling mechanism that is involved in the control of adipocytes metabolism (Fischer et al., 2017). In human, methylation of *FGFR2* gene was associated with high birth weight centile (Haworth et al., 2014). *TBX5* and *TBX3* are required for formation and normal development of forelimbs; mutation in these genes is associated with Holt-Oram syndrome (Hasson et al., 2010).

In the present study, we implemented three complementary tests (Fst, XP-EHH and θπ) to identify the candidate regions of positive selection of pelt quality and coat color in mink. Importantly, *APCDD1* in nap size group with important function in hair follicles was validated by all three methods, indicating that it can be considered as reliable candidate of selective sweeps in mink. Furthermore, the *BRINP1* gene within the chromosomal region chr9: 14771596-14857094 bp, was identified as another candidate gene within the pastel group. This gene was annotated within the significant region identified by overlapping the top 1% of all three test statistics. Previous study on human hair indicated that *BRINP1* was associated with hair loss and hair greying phenotype in human (Pośpiech et al., 2020). Another gene was *EPHA6* on chr6: 45162190-45182915 bp in the pastel group. Down-regulation of *EPHA6* expression was associated with low wool density in rabbit (Liu et al., 2016). *EPHA3* gene is a member of ephrins which was suggested to act as a hair development promoter (Midorikawa et al., 2004) and had a potential role in the wool structure of sheep (Kang et al., 2013).

Selection for pelt quality and body size in mink is certainly a feasible approach to increase the profitability of the mink farms (Thirstrup et al., 2017). Genomic selection can be applied as a useful breeding strategy to improve the economically important traits in the mink industry (Villumsen et al., 2021). In this study, numerous loci were detected for pelt quality, pelt size and coat color. Incorporating these loci into current 62 K SNP-chip for mink can be used to improve increase in the accuracy of prediction of genomic estimated breeding values for these traits.

4.5 Conclusions

This study was the first scan for signatures of putative selection for pelt quality and coat color in mink genome using WGS data. Our results demonstrated that mink genome contained multiple

regions likely subjected to selection, some of which appeared to be related to pelt quality, coat color and also body size traits. One strongly selected gene was detected for nap size (*APCDD1*) which was related to hair follicular process. However, more investigation might be required to confirm the roles of these genes in controlling hair follicles in mink. These results provide a foundation to study the genetic diversity driven by domestication and selection mechanisms in mink.

Supplementary files are available through https://doi.org/10.3390/genes13111939.

Table 4.1 Number of candidate regions and genes detected by overlapping Fst and XP-EHH in differential phenotypic groups of fur quality and coat color in mink.

Group	Number of candidate regions	Number of genes
Nap size	177	110
Overall fur quality	261	163
Pelt size	204	98
Pastel_Black	201	123
Stardust_Black	103	71

Table 4.2 Overlapping candidate regions and annotated genes identified by three methods (θπ ratios, Fst, and XP-EHH) for fur quality and coat color in mink.

Chromosome	Position (bp)	Group	Genes
3	182130062-182140062	Nap size	*RAB31*
3	182662938-182672938	Nap size	*APCDD1*
3	182736504-183045067	Nap size	*NAPG, PIEZO2*
3	211123039-211140367	Nap size	*LDLRAD4*
5	2940988-2952647	Nap size	*RBFOX3*
5	7488192-7513312	Nap size	*CDC42EP4, SDK2*
5	7547720-7557720	Nap size	*CPSF4L, C5H17orf80*
6	27565214-27575214	Nap size	*USP25*
6	29333509-30616460	Nap size	*ROBO2*
8	26111849-26121849	Nap size	*CCM2L*
5	28909887-28931171	Fur quality	*TMEM199, SARM1*
5	28921171-28931171	Fur quality	*POLDIP2*
5	29000969-29010969	Fur quality	*NLK*
3	2371161-2435977	Pelt size	*RPS6KA2*
3	54202833-54212833	Pelt size	*GPD2*
4	4730111-4750052	Pelt size	*FAM135B*
3	127226629-127236629	Pastel_Black	*ZADH2, TSHZ1*
3	127816455- 127839967	Pastel_Black	*CNDP1, CNDP2*
6	27436943-27589421	Pastel_Black	*USP25*
6	32412680- 32782053	Pastel_Black	*ROBO1*
6	41725705- 41738492	Pastel_Black	*EPHA3*
6	45162190-45182915	Pastel_Black	*EPHA6*
6	47936381-47946381	Pastel_Black	*COL8A1*
9	14771596- 14857094	Pastel_Black	*BRINP1*
10	62452956- 62473337	Pastel_Black	*KCNH1*
7	5300435- 5439094	Stardust_Black	*CDH13*
10	37647562-37657562	Stardust_Black	*RGS7*

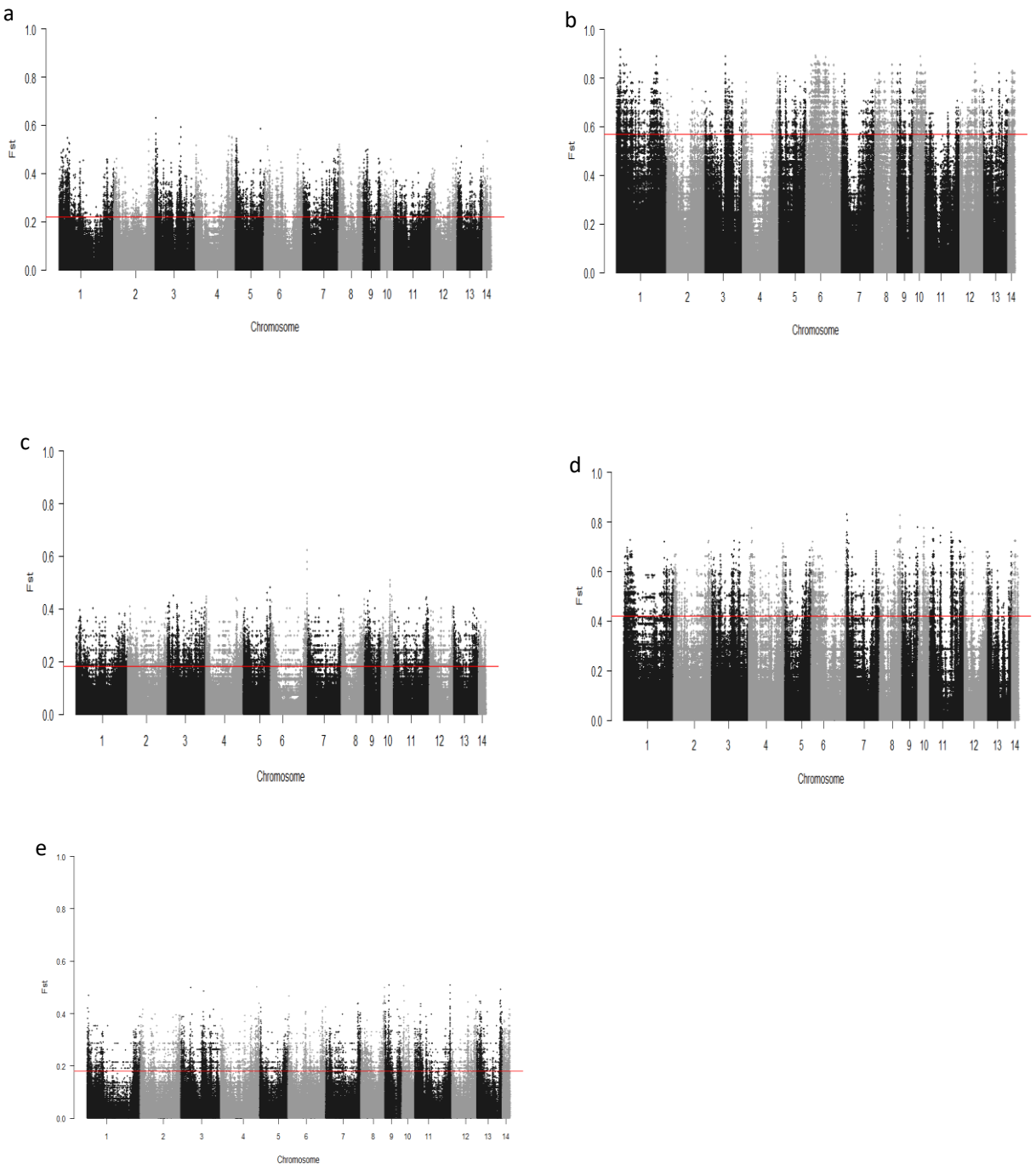

Figure 4.1 Genome-wide distribution of Fst across chromosomes in different groups of fur quality and coat color in mink. The horizontal lines indicate the top 1% of values across the entire genome: Nap size (**a**), Overall fur quality (**b**), Skin size (**c**), Pastel_Balck (**d**), and Stardust_Black (**e**).

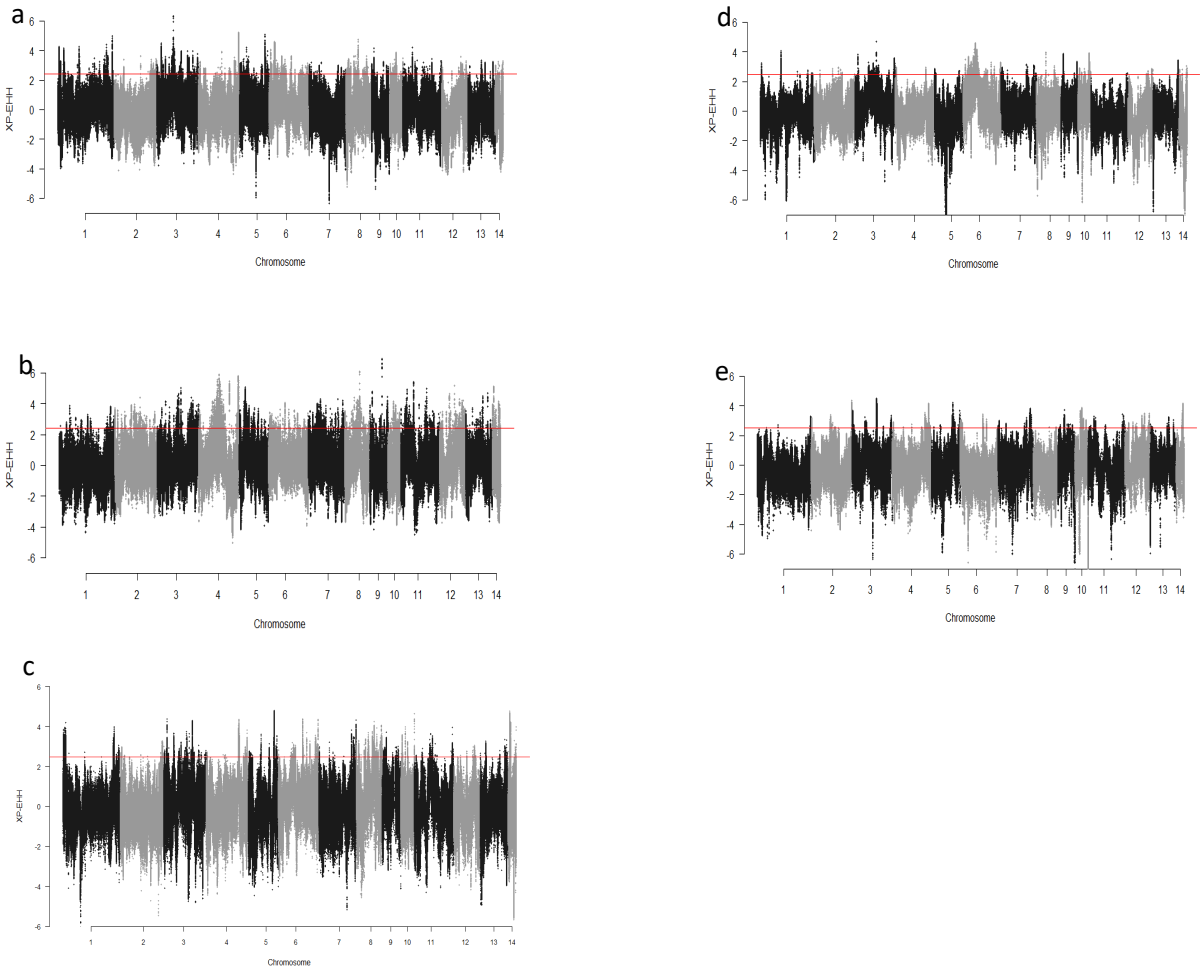

Figure 4.2. Genome-wide distribution of XP-EHH across chromosome regions in different groups of fur quality and coat color in mink. The horizontal lines indicate the top 1% of values for each test across the entire genome. High positive values indicate the selection in short nap size (a), high overall fur quality (b), large skin size (c), pastel coat color (d), and stardust coat color (e).

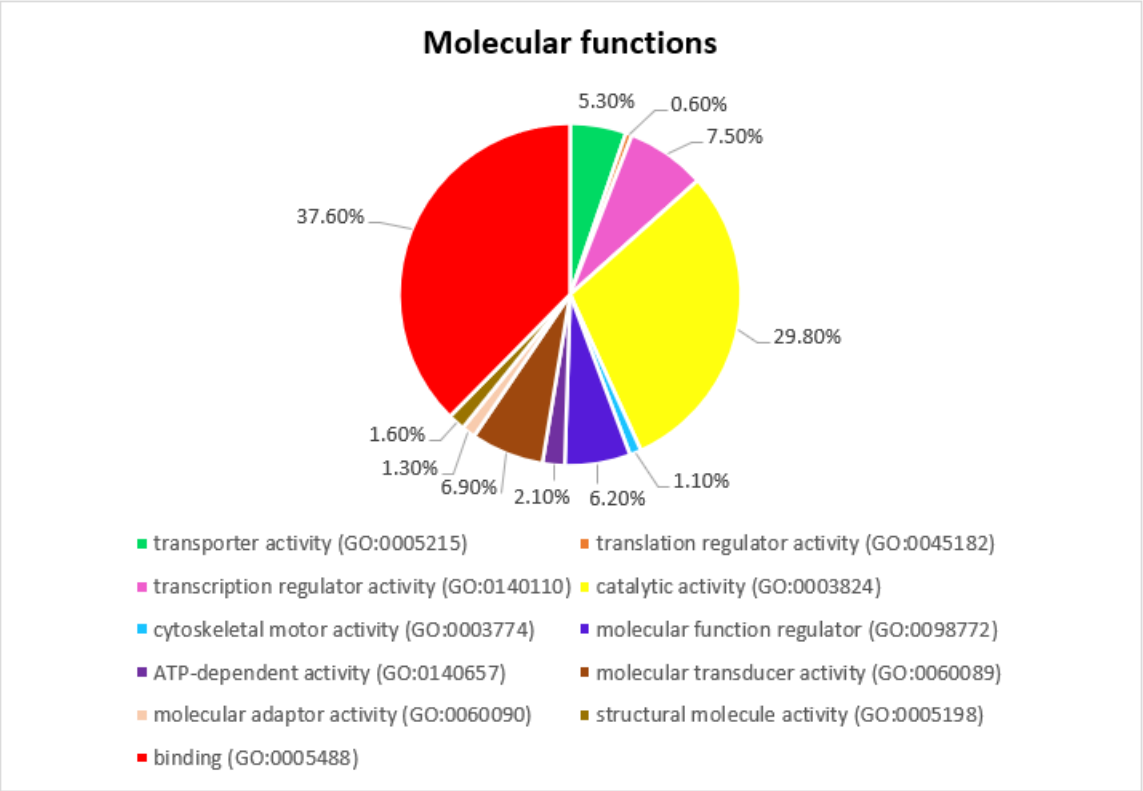

Figure 4.3 The pie chart of molecular functions attributed to candidate genes detected by overlapping selective signals of FST and XP-EHH.

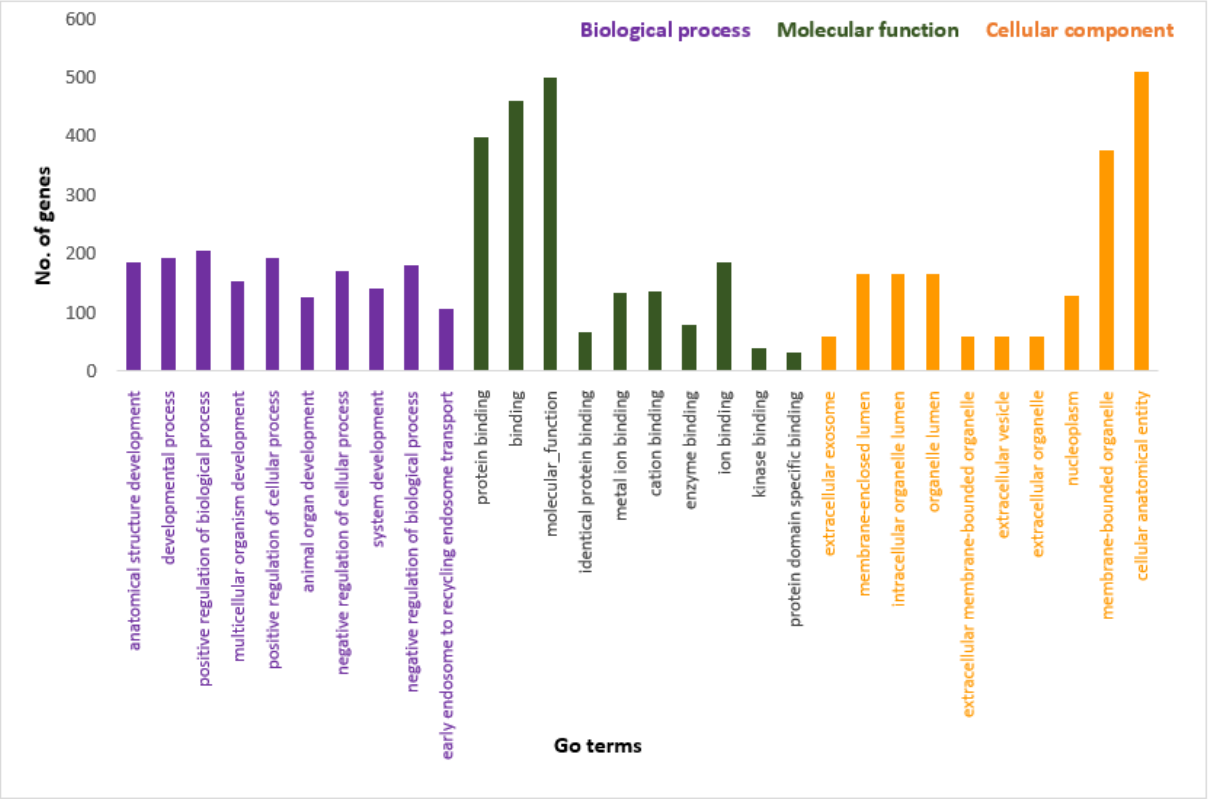

Figure 4.4 Top ten significant gene ontology terms (GO terms) enriched in overlapping selective signals of Fst and XP-EHH.

Chapter 5: Genome-wide association studies for reproductive performance and fur quality traits in American mink[3]

5.1 Introduction

In mink production systems the most economically important traits are reproductive performance, pelt quality, and pelt size (Lagerkvist et al., 1994; Valipour et al., 2022a). Across various species, these traits generally demonstrate a polygenic nature, affected by many genes and variants, each with small effects on the observed phenotype. This has been reported in studies focusing on reproduction traits in pigs and cattle (Ding et al., 2021; Chen et al., 2022) as well as in GWAS concerning pelt quality traits in mink (Cai et al., 2018). In mink, reproductive traits, especially litter size traits have low heritabilities (less than 0.2) and this makes the genetic improvements of these traits difficult (Karimi et al., 2018). Genetic improvement of low heritable traits using traditional breeding strategies often has slow progress (Cassell, 2009). Moreover, pelt quality traits such as length of guard hair (nap size) and overall fur quality have been found to have non-significant genetic correlation with reproduction traits (Lagerkvist et al., 1994). Therefore, the advantage of identifying mink with high pelt quality values is to improve pelt quality traits without adversely affecting the female's fertility. However, a negative correlation between pelt size with quality traits and reproductive performance in mink is evident (Lagerkvist et al., 1994; Thirstrup et al., 2017). These undoubtedly encouraged to advance understandings of the hidden genetic architecture of these traits using GWAS.

The limited knowledge of quantitative trait loci (QTL) associated with reproductive traits in mink indicates the critical need for further investigation. A few attempts to pinpoint genes that are the

[3] A version of this chapter will be submitted to the Frontiers in Genetics.

sources of variation in body size, pelt quality traits and pelt size have been made either by means of QTL mapping (Thirstrup et al., 2014), GWAS (Cai et al., 2018) or whole-genome comparative analysis (Davoudi et al., 2022; Valipour et al., 2022b). The complexity of reproductive and pelt quality regulation in mink demands additional exploration to identify variants and molecular genetic mechanisms underlying regulation of these traits in mink.

The success of GWAS for detection of sequence variation affecting complex traits in humans has prompted interest in the use of large-scale high-density single nucleotide polymorphism (SNP) genotyping for identification of QTLs and marker-assisted selection/genomic selection (GS) in agricultural species. Genomic selection has outstanding advantages for traits with low heritability (Klápště et al., 2020). Incorporation of functional mutations into statistical models used for genomic prediction can increase GS accuracy across populations (Kadarmideen, 2014; Gebreyesus et al., 2019; Zhang et al., 2023).

Currently an efficient custom genotyping assay (70K SNP) to support GWAS in mink is available through collaboration between Dalhousie University (Dr. Miar lab), Canada Mink Breeders Association and Fur Commission USA (Do et al., 2024). This marker density is a practical starting point for GWAS in mink as a set of approximately 7,700 to 60,000 variable SNPs would be sufficient to represent the 2.7 billion bases of sequence in mink genome at an average linkage disequilibrium of $r^2<0.2$ (Karimi et al., 2020; Karimi et al., 2021b; Hu et al., 2023). Moreover, a complete contiguous chromosome-level genome assembly of mink was published recently (Karimi et al., 2022).

In the context of mink research, GWAS serves as a powerful method for identifying the genomic regions that underlie genetic variation in traits. The identified target regions can then be used for identification of candidate genes. The objectives of the current study were to identify SNPs,

candidate genes and biological pathways involved in the regulation of reproduction and pelt quality traits via GWAS and pathway enrichment.

5.2 Materials and Methods

5.2.1 Ethics statement

All procedures related to animals in this study were performed strictly in accordance with the standards of the Guide to the care and use of experimental animals (CCAC, 1993) after approval by the Dalhousie University Animal Care and Use Committee.

5.2.2 Animals and sampling

Animals used in this research were raised under standard farming conditions at the Canadian Centre for Fur Animal Research (CCFAR) at Dalhousie University, Faculty of Agriculture (Truro, Nova Scotia, Canada) from 2006 to 2021. The 5,824 study animals were the progeny of 1,051 sires and 2,097 dams. Pedigree information of 17 generations comprising of 26,575 individuals was used. In December of each year, mink were euthanized using the approved method of carbon monoxide gas. Tongue tissues were collected from all animals by excising a standardized section from the tip and body region of the tongue using sterile surgical instruments. Following excision, the tissues were transferred to the storage tubes. The tubes were then stored at –80°C until further processing for DNA isolation.

5.2.3 Female reproductive performance traits

Reproductive outcomes were recorded for each of the 16 reproductive cycles from 2006 through 2021. Detailed procedures for reproduction management and data collection are documented by Karimi et al. (2018) and Do et al. (2021). Briefly, mink were raised in standard farming settings, with fed diets composed of byproducts from human food production (chicken and fish factories), tailored to meet their specific needs during each production phase. Do and Miar (2019) reported a comprehensive detail of the feed ingredients of the diets throughout various periods. In early March, the annual reproductive cycle of mink was initiated. Technicians paired male and female for the purpose of mating, by transferring female mink to male mink pens (one male typically mates with approximately five females). In instances where it was necessary that females were mated multiple times (up to three occasions), the same males were utilized for subsequent matings. The second mating occurred approximately nine days after the initial one. The birth of kits occurred from late April to the middle of May. Individual female reproductive measurements were gestation length (GL), the total number of kits born (TB), the number of kits alive at 24h after birth (LB), the number of kits alive at weaning (LW), survival rate at 24h after birth (SB), survival rate at weaning (SW), average weight of kits per litter at birth (AWB), and average weight of kits per litter at weaning (AWW).

5.2.4 Evaluation of fur characteristics on live animals and dried pelts

Procedures for assessment pelt quality on live animals and on dried pelts have been described by Valipour et al. (2022). In brief, live grading traits were measured on 1,608 animals and were included the overall quality of fur (LQU) and nap size (LNAP). LQU categorized into three scores from 1 (poor) to 3 (best), and LNAP, length of guard hair protruding from the underfur, categorized

into five categories from 1 (long) to 5 (short). Dried pelt quality was evaluated on 1,195 mink. Dried pelt size (DPS) was classified into nine categories (1=small; 9=large), dried pelt nap size (DNAP) was scored into eight categories 1 (long) to 8 (short). Overall quality of dried pelt (DQU) scored into four categories from 1 (poor) to 4 (best).

5.2.5 Estimation of breeding values

Breeding values were estimated for all studied traits using the following univariate animal model implemented in ASReml 4.1 (Gilmour et al., 2018):

$$\mathbf{y} = \boldsymbol{X}\mathbf{b} + \boldsymbol{Z}\mathbf{a} + \boldsymbol{G}\mathbf{m} + \boldsymbol{W_1}\mathbf{pe} + \boldsymbol{W_2}\boldsymbol{c} + \mathbf{e},$$

where ***y*** was the vector of phenotypic observations; ***b*** was the vector of fixed effects; ***a*** was the vector of random additive genetic effects; ***m*** was the vector of random maternal genetic effects; ***Pe*** was the vector of permanent environmental effects; ***c*** was the vector of common litter effects; and ***e*** was the vector of residual effects; and ***X***, ***Z***, ***G***, ***W₁*** and ***W₂*** were the incidence matrices relating the phenotypic observations to the fixed, random additive genetic, maternal genetic, permanent environmental, and common litter effects, respectively. It was assumed that random effects are independent and normally distributed:

$$\boldsymbol{a}\sim \mathrm{N}(0, \boldsymbol{A}\sigma_{\mathrm{a}}^2), \boldsymbol{m}\sim N(0, \boldsymbol{A}\sigma_m^2), \boldsymbol{Pe}\sim N(0, \boldsymbol{I}\sigma_{\mathrm{Pe}}^2), \boldsymbol{c}\sim N(0, \boldsymbol{I}\sigma_{\mathrm{c}}^2) \text{ and } \mathbf{e}\sim N(0, \boldsymbol{I}\sigma_{\mathrm{e}}^2),$$

where ***A*** was the numerator relationship matrix; ***I*** was an identity matrix; σ_a^2, σ_m^2, σ_{pe}^2, σ_c^2 and σ_e^2 were the variances of random additive genetic, maternal genetic, permanent environmental, common litter, and residual effects. Fixed effects were sex (male and female), year (2006 to 2021),

and color type (brown, breath of spring, dark, demi, gray, mahogany, pastel, sapphire, stardust, white, and white-blue), number of matings (1 to 3 times) and age of the dam (1 to 5 years).

The significance of fixed effects and covariates was tested using Wald statistics in the REML procedure of ASReml 4.1 (Gilmour et al., 2018), and only significant ($P < 0.05$) effects were kept in the mixed model analyses for each trait. The significance of different random effects for each trait was determined by comparing the full model and the reduced model using the following statistic:

$$-2(\log L_{\text{reduced model}} - \log L_{\text{full model}}) \sim \chi^2_{\text{df(full model)}-\text{df(reduced model)}}$$

Finally, breeding values were estimated using the REML procedure in ASREML 4.1 (Gilmour et al., 2018).

5.2.6 Calculation of de-regressed breeding values

We used the de-regressed EBVs (dEBVs) of animals instead of their phenotypes. For each trait, reliability of EBVs was calculated according to the approach by Tier and Meyer (2004) using the following equation:

$$Reliability\ of\ EBVs = 1 - \frac{PEV_i}{\sigma_a^2},$$

where PEV_i is the prediction error variance of the breeding value for the ith animal, and σ_a^2 is the direct additive genetic variance from the respective model. The prediction error variance for a breeding value was the square of the posterior standard deviation of the respective breeding value. Finally, the dEBVs for reproduction and pelt quality traits were calculated according to Garrick et

al. (2009) to remove the parent average effects. De-regressed EBVs were used in the GWAS if the corresponding EBV reliability was ≥ 0.1.

5.2.7 Genotypes

Quality control of genotypes of individuals genotyped by the 70K MINK SNP panel was performed using the PLINK software (Chang et al., 2015). Variants with minor allele frequency (MAF) < 0.05; maximum missing rate <90%; individual call rate < 90% and deviating from Hardy–Weinberg equilibrium ($P < 10^{-6}$) were removed. Moreover, only bi-allelic variants on autosomal chromosomes were kept. After quality control, 26,930 bi-allelic variants on autosomal chromosomes of 1,321 individuals were remained for further analyses.

5.2.8 Genome-wide association studies

The single-marker GWAS was performed using the linear mixed model association method as implemented in the EMMAX software (Kang et al., 2010). After calculating the genomic relationship matrix constructed with identity-by-state used in the single-marker association analyses, the genomic relationship matrix was fitted in the model to correct for population stratification. The following model used for associated analysis:

$$\boldsymbol{y} = \boldsymbol{Xb} + \mu + \boldsymbol{e},$$

where **y** was the vector of dEBVs, $\boldsymbol{X}$ was the vector of genotypes, **b** was the allele substitution effect, μ was the vector of the background polygenic effects with $\mu \sim N(0, \boldsymbol{G}\sigma_{\mu}^{2})$ in which **G** was the genomic relationship matrix built based on the SNP genotypes using EMMAX software, and

$\boldsymbol{e}$ was the vector of random residuals assumed to have a normal distribution: $\boldsymbol{e} \sim N(0, \boldsymbol{I}\sigma_e^2)$, where $\boldsymbol{I}$ was an identity matrix, σ_μ^2 was the additive genetic variance and σ_e^2 was the residual variance. A Bonferroni correction was applied to account for multiple testing in the GWAS. Accordingly, the Bonferroni-corrected genome-wise significance threshold was defined as 0.05/N, and chromosome-wise significant threshold was defined as 1/N, where N was the total number of SNPs (Lander and Kruglyak, 1995; Yang et al., 2005).

5.2.9 Functional enrichment analyses

The positional candidate genes located within a flanking distance of 0.5 Mb from significant and suggestive SNPs were selected using general feature format of recent genome assembly of *Neogale vison* (Karimi et al., 2022), and gene IDs overlapped with the candidate regions determined using BEDtools (Quinlan, 2014). The biological process, molecular function and cellular component terms were assessed for all genes using PANTHER 14.1 (Thomas et al., 2003). Benjamini-Hochberg False Discovery Rate (FDR) correction was used for both multiple testing and overrepresentation test. These genes were further investigated through a comprehensive review of the relevant literature in relation to the phenotypes or pathways of interest.

5.3 Results

5.3.1 Description of phenotypes and estimated heritabilities

The de-regressed breeding values for reproduction and pelt quality traits were estimated. Number of records, standard deviations (SD) range of dEBVs, and heritabilities for each trait are presented in Table 5.1. The number of animals with de-regressed proofs ranged from 1,321 for GL and TB

to 1,148 for DQU. The estimated heritabilities (±SE) for reproduction traits were 0.23±0.03 for GL, 0.07±0.01 for TB, 0.07±0.02 for LB, 0.06±0.02 for LW, 0.13±0.03 for SB, 0.08±0.02 for SW, 0.14±0.04 for AWB, and 0.16±0.04 for AWW. For pelt quality traits, heritability (±SE) estimates were 0.41±0.06 for DPS, 0.22±0.10 for DNAP, 0.12±0.04 for DQU, 0.23±0.05 for LQU, 0.42±0.06 for LNAP.

5.3.2 Detection of SNPs associated with reproductive traits

Thirty-nine SNPs, including three genome-wide significant, and 36 chromosome-wide suggestive SNPs were identified for the reproduction traits. The association patterns of SNPs with GL, TB, LB, LW, SB SW, AWB, and AWW are presented in Figure 5.1a-h. Moreover, a complete list of candidate regions along with their positions and associated genes for each phenotypic group is presented in Table 5.2 and Additional file 1. The most significant signal was detected for GL at genome-wide threshold of P < 2E-06 with the SNPs located on the chromosomes 1, 2 and 4. Five chromosome-wise significant level (P < 4E-05) SNPs were identified for GL in which four of these suggestively associated SNPs were located on Chr 5:3,820,750 bp to 3,865,375 bp. An additional suggestively associated SNP was identified on Chr 2 at position 228,006,636 bp. In total, 11 chromosome-wise suggestive significant SNPs (P < 4E-05) were associated with litter size traits, including 7 SNPs (Chr 1, 3, 6, and 11) with TB, 2 SNPs (Chr 4, and 11) with LB, and 2 SNPs (Chr 2, and 6) with LW (Table 5.2). AX-647660520 SNP on chromosome 11 was the common SNP showing significant associations with both TB and LB (Table 5.2). Three SNPs on Chr 3, 10, and 11 for AWB, and two SNPs on Chr 1 and 6 for AWW passed the chromosome-wide significant threshold. For SB, the majority of associated SNPs (6 out of 9) were located on Chr 6, and two

SNPs were identified on Chr 11 and one on Chr 3. Moreover, four SNPs on Chr 1 and one SNP on Chr3 were significant for SW at the threshold (P < 4E-05).

In total, 294 positional candidate genes (93 for GL, 43 for TB, 22 for LB, 2 for LW, 49 for SB, 25 for SW, 24 AWB, and 35 for AWW) were annotated at 0.5 Mb flanking regions of each SNP (significant and suggestive) for reproduction traits (Additional file 1). Moreover, a total of 84 candidate genes were shared between at least two reproduction traits (Additional file 1). The gene ontology analysis resulted in 114, 12, and 63 overrepresented (P < 0.05) GO enrichment terms related to different biological processes, molecular function, and cellular components, respectively (Additional file 2 a-c). The top ten significant GO terms enriched within the candidate regions are presented in Figure 5.2. Gene ontology revealed the biological roles of several genes related to the reproductive performance including placenta development (GO:0001890) *PKD2, STOX2, SPP1,* and *SOCS3*; glycosaminoglycan biosynthetic process (GO:0006024) *HS3ST5, GCNT2*, and *DSE*; chordate embryonic development (GO:0043009) *PKD2, MARCKS, SOCS3, TFAP2A, CASP3,* and *HS3ST5*; embryonic morphogenesis (GO:0048598) *PKD2, FN1, RNF2, SOCS3, TFAP2A, CASP3,* and *HDAC2*; and embryo development (GO:0009790) *PKD2, STOX2, FN1, RNF2, MARCKS, SOCS3, TFAP2A, CASP3, PGAP1, HS3ST5, HDAC2,* and *HUS1*.

5.3.3 Detection of SNPs associated with pelt quality traits

GWAS revealed one SNP was significantly associated with DPS at the genome-wide threshold of P< 2E-06 (Table 5.3, Figure 5.3). Moreover, 11 SNPs were suggestively associated with other pelt quality traits at the threshold of P < 4E-05 (Table 5.3). Additional file 3 presents the significant SNPs associated with pelt quality traits along with the candidate genes located in 0.5 Mb flanking

regions around these SNPs. In total, 93 candidate genes were identified for different pelt quality traits (six for LQU, five for LNAP, 28 for DPS, 48 for DNAP, six for DQU, and). The GO enrichment analysis indicated that selected regions were involved in 88, 16, and 36 GO terms related to biological processes, molecular functions, and cellular components, respectively (Additional file 4). The summary of top ten significant GO terms pathways is presented in Table 5.4. Gene ontology revealed the biological roles of several genes related to follicular hair functions and growth performance including developmental process (GO:0032502) *P2RY1*; macromolecule metabolic process (GO:0010604) *PRSS53* and *PRSS8*; animal organ development (GO:0048513) *STC1* and *CER1*.

5.4 Discussion

Genome-wide association studies stands a highly effective method for genetic investigation, facilitating the pinpointing of genetic markers and genes linked to important traits in livestock animals. In the context of mink, GWAS can assist in developing genomic breeding strategies, thus enhancing the efficiency and precision of selective breeding programs. By identifying specific genetic variations associated with desirable traits such as fur quality, and reproductive performance, GWAS enables breeders to make informed decisions in selecting breeding stock, ultimately leading to the accelerated improvement of mink populations for commercial purposes. The current study provides the first GWAS for reproduction and pelt quality traits in mink.

The primary purpose of mink farming is producing high quality fur preferably from mink with high reproductive performance. In total, 39 SNPs, including three genome-wide significant, and 36 chromosome-wide suggestive SNPs were identified for reproduction traits. The three genome-wide significant SNPs were detected for GL. In mink, the ideal gestation period, within the range

of 45 to 60 days, is important in achieving larger litters by minimizing the mortality rate among the kits (Święcicka, 2013). For GL, one of the most significant SNPs (AX-647624369) is located close to *GRIK2* gene at 29,082,126 bp on Chr 1. This gene has an important role in behavioural response to fear in mice (Iida et al., 2021). *GRIK2* was associated with several behavioral disorders in humans (Shaltiel et al., 2008). In mink, it has been shown that fearfulness have negative impacts on mating behavior and fearful mink have weaker reproductive performance comparing with timid mink (Korhonen et al., 2002; Andersen, 2013). We identified 10 key genes (*SPP1, STOX2, SOCS3*, *PKD2, MARCKS, CASP3*, *FN1, RNF2, TFAP2A,* and *HDAC2*) related to fertility with important roles in regulating biological processes related to the female reproductive performance. *SPP1* on Chr 11 has been located on 0.5 Mb of two suggestive significant SNPs (AX-647660520 and AX-647660520) associated with TB and LB, respectively. *SPP1* is one of the important genes involved in blastocyst implantation and maintaining pregnancy in several species including mice, humans, pigs, sheep, rabbits, cattle, and goats (Kramer et al., 2021). Expression of *SPP1* mRNA was detected within the endometrial tissue of mice. Disruption of the *SPP1* gene in mice led to the increased early pregnancy loss, and these kits were significantly smaller than kits born from the wild-type counterparts (Weintraub et al., 2004). *STOX2* is located on chr 11 close to suggestively significant SNP (AX-647664582) associated with AWB. STOX2 is a transcription factor that is highly expressed in pre-eclampsia pluripotent cells of the inner cell mass of a developing embryo and has an important role in differentiation and invasion of trophoblast (Harrison et al., 1997; Van Dijk et al., 2005; Oudejans et al., 2016). Lower expression of STOX2 in patients with pre-eclampsia has been reported in humans. Pre-eclampsia is a serious complication of pregnancy and a major cause of preterm intervention by Caesarean section (Fenstad et al., 2010). Severe pre-eclampsia not only affects the mother, but it also significantly impacts the fetus, leading to

complications such as intrauterine growth restriction, altered fetal movement patterns, and a reduction in amniotic fluid levels (oligohydramnios) (Gifford et al., 2000). *SOCS3* on Chr 5 located within 0.5 Mb flanking regions of four suggestively significant SNPs (AX-647743710, AX-647743712, AX-647743699 and AX-647743689) associated with GL. SOCS3 is an important negative regulator of leukemia inhibitory factor receptor signaling with an essential role in placenta development. Disruption of *SOCS3* resulted in embryonic lethality due to the placental defect (Takahashi et al., 2006). *PKD2* on Chr 11 located within 0.5 Mb flanking regions of two suggestive SNPs (AX-647660520 and AX-647660520) associated with TB and LB, respectively. PKD2 is essential for normal development of the placenta and deletion of this gene is associated with lethality in mutant embryos murine models (Garcia-Gonzalez et al., 2010). *MARCKS* on Chr 3 located within 0.5 Mb flanking regions of two suggestive SNPs (AX-647710483 and AX-647623152) is associated with SW. Myristoylated alanine-rich C kinase substrate (MARCKS) is an actin-binding, membrane-associated protein translated from *MARCKS* gene with high expression during *Xenopus* embryogenesis (Iioka et al., 2004; El Amri et al., 2018). This protein has an essential role in transformation of blastula or blastocyst to the gastrula during embryo development. Blocking the expression of this gene in chicken embryos lead to impaired morphogenetic movements, including convergent extension (Iioka et al., 2004). Convergent extension is a pivotal morphogenetic process essential for shaping the elongated vertebrate body plan from its initially radially symmetrical embryo form. Moreover, it governs the specific shape changes observed in numerous individual tissues during development (Sutherland et al., 2020). *TFAP2A* on Chr 1 located within 0.5 Mb flanking regions of three suggestive SNPs (AX-647633370, AX-647633372, and AX-647633386) is associated with TB. *TFAP2A* is essential for normal embryonic development in mammal species. Knockout of this gene in mice resulted in

craniofacial malformations and embryonic lethality. In human, missense mutations in the *TFAP2A* gene result in branchio-oculo-facial syndrome, a developmental deficiency characterized by abnormalities on the neck and face (Rothstein and Simoes-Costa, 2020). *HDAC2* on Chr 1 located within 0.5 Mb flanking regions of two suggestively significant SNPs (AX-647623185 and AX-647623152) associated with SW. *HDAC2* is the major histone deacetylase involved in oocyte development by regulating histone acetylation, gene transcription, and DNA methylation. It has a critical role for preimplantation development by regulating histone acetylation, cell cycle progression and the development of a transcriptionally repressive state that initiates in 2-cell embryos (Ma and Schultz, 2016). *CASP3* on Chr 11, within 0.5 Mb flanking of AX-647664582, is suggestively associated with AWB. Similarly, *FN1* on Chr 3, within 0.5 Mb flanking of AX-647711903, is suggestively associated with TB. Additionally, *RNF2* on Chr 10, within 0.5 Mb flanking regions of AX-647648273, is suggestively associated with AWB. Gene ontology (GO) analysis supports their involvement in biological processes such as chordate embryonic development (GO:0043009) for *CASP3* and embryonic morphogenesis (GO:0048598) for *FN1* and *RNF2*. However, a direct link between the functions of these genes and the observed phenotypes remains unestablished in the published literature.

Identification of genes and genetic markers underlying pelt quality traits is the other main focus of mink genomic improvement project. GWAS revealed 12 SNPs including one genome-wide significant and 11 suggestive significant SNPs for pelt quality traits. Among genes that have been annotated in proximity with these SNPs, three candidate genes (*P2RY1*, *PRSS53,* and *PRSS8*) related to hair follicle function were identified. Purinergic receptor P2Y (*P2RY1*) on Chr 6 located within 0.5 Mb flanking regions of AX-647762037, which is suggestively associated with LNAP. P2RY1 is a member of the family of *purinergic* G protein-coupled receptors, that has been detected

in the basal layer of normal epidermis of mice and humans, and is associated with a signalling system for proliferation and differentiation in distinct cell lineages in human anagen hair follicles (Greig et al., 2008). Protease serine S1 family member 53 (*PRSS53*) on Chr 14 located within 0.5 flanking regions of AX-647691922 is suggestively associated with LNAP. GWAS showed that *PRSS53* was associated with human hair shape and highly expressed in the follicle inner root sheath (Adhikari et al., 2016). *PRSS8* has been detected in epidermal tissue and it is essential for normal formation of hair follicles and pelage in mice. *PRSS8* on Chr 14 located within 0.5 flanking regions of AX-647691922 is suggestively associated with LNAP. *PRSS8* is required for barrier acquisition of the interfollicular epidermis and for normal hair follicle development, the prostasin null mice die shortly after birth (Friis et al., 2016). The present study also identified two genes with important functions related to growth performance, STC1 and CER1. *STC1* is a glycoprotein expressed in bone tissue in humans, rats, and mice. It has a regulatory role in mammalian bone growth (Jiang et al., 2000; Stasko and Wagner, 2001). Overexpression of *STC1* in transgenic mice resulted in significant reduction in birth weight and lower adult body size (Filvaroff et al., 2002; Varghese et al., 2002). The *CER1* gene is one of the Cerberus-related cytokines and belong to the group of bone morphogenic protein (BMP) antagonists, these cytokines can bind directly to BMP and inhibit its activity (Katoh and Katoh, 2006). BMP signalling has an important role in differentiation of mesenchymal cells into osteoblasts and therefore is essential for normal bone development (Blaščáková et al., 2021).

5.5 Conclusions

This study was the first GWAS for reproduction traits in mink. This investigation yielded compelling results, with the strongest associations observed for SNPs located on chromosomes 1,

2, and 4 in the context of GL, and on chromosome 6 for DPS. Notably, the study uncovered several candidate genes (*STOX2, SPP1, SOCS3, PKD2, MARCKS, TFAP2A, CASP3, FN1, RNF2,* and *HDAC2*) that play pivotal roles in the regulation of reproduction. Moreover, the research identified a set of novel candidate genes (*P2RY1, Prss53PRSS53, PRSS8, STC1,* and *CER1*) associated with pelt quality traits and pelt size. This comprehensive analysis offers valuable insights into the genomics of reproduction and pelt quality traits in mink. This not only enhanced our understanding of the genetic underpinnings of reproduction and pelt quality traits but also moved us a step closer to employing targeted breeding strategies such as gene editing aimed at improving these traits in mink. As a result, the findings of this study can serve as a crucial reference point for future research endeavors, aiding in the identification and confirmation of causal mutations responsible for these traits in the mink population.

Supplementary Materials

Additional file 1: List of positional candidate genes annotated at 0.5 Mb flanking regions of each significant SNPs for reproduction traits. **Additional file 2 a–c:** List of GO enrichment terms related to biological processes, molecular function and cellular components for candidate genes associated with reproduction traits. **Additional file 3:** List of positional candidate genes annotated at 0.5 Mb flanking regions of each significant SNPs for pelt quality traits. **Additional file 4 a–c:** List of GO enrichment terms for candidate genes related to biological processes, molecular function and cellular components annotated to significant SNPs for pelt quality traits.

Table 5.1 Descriptive statistics of estimated de-regressed EBVs for the female reproduction and pelt quality traits in mink.

Traits	Abbreviation	Number of observations	Mean	Standard deviation	Range (Min. – Max.)	Heritability
Gestation length	GL	1321	-0.44	2.74	-29.01 – 25.43	0.23±0.03
Total number of kits born	TB	1321	0.25	1.37	-6.51 – 11.10	0.07±0.01
Number of kits alive at birth	LB	1319	0.35	1.26	-5.15 – 9.14	0.07±0.02
Number of kits alive at weaning	LW	1314	-0.04	1.20	-8.51 – 7.39	0.06±0.02
Survival rate at birth	SB	1169	2.39	7.77	-61.69 – 46.51	0.13±0.03
Survival rate at weaning	SW	1166	-0.27	14.26	-86.29 – 51.27	0.08±0.02
Average kit weight per Litter at birth	AWB	1168	0.01	0.83	-4.90 – 5.98	0.14±0.04
Average kit weight per Litter at weaning	AWW	1167	0.95	23.02	-168.37 – 132.25	0.16±0.04
Live grading overall quality of fur	LQU	1260	0.05	0.56	-3.96 – 3.79	0.23±0.05
Live grading nap size	LNAP	1260	0.14	0.79	-3.84 – 3.60	0.42±0.06
Dried pelt size	DPS	1169	0.02	0.70	-7.20 – 8.24	0.41±0.06
Dried pelt nap size	DNAP	1159	0.17	1.10	-4.91 – 4.94	0.22±0.10
Overall quality of dried pelt	DQU	1148	-0.01	0.56	-3.92 – 2.17	0.12±0.04

Table 5.2 List of genome-wide and chromosome-wide significant SNPs using single-SNP regression mixed linear model for reproduction traits in mink.

Traits[1]	Chromosome	Position (bp)	SNP	Regression Beta	SE	P-Value	Candidate Genes
GL	2	159455840	AX-647703700	5.76	0.93	9.94E-10	*LOC122900624, LOC122900625, LOC122900626*
GL	1	29082126	AX-647624369	4.85	0.80	4.53E-07	*LOC122897569, GRIK2*
GL	4	135965137	AX-647738774	4.29	0.81	2.33E-06	*ABCA13, UPP1, C4H7orf57, SUN3, HUS1, PKD1L1, TNS3*
GL	5	3861814	AX-647743710	0.60	0.13	2.35E-05	*CYTH1, LOC122908097, DNAH17, PGS1, SOCS3, LOC122907519, LOC122905935, TMEM235, BIRC5, LOC122907520, AFMID, TK1, SYNGR2, C5H17orf99, TMC8, TMC6, TNRC6C, LOC122907522, SEPTIN9, LOC122905944, SEC14L1*
GL	5	3865375	AX-647743712	0.58	0.13	2.94E-05	*CYTH1, LOC122908097, DNAH17, PGS1, SOCS3, LOC122907519, LOC122905935, TMEM235, BIRC5, LOC122907520, AFMID, TK1, SYNGR2, C5H17orf99, TMC8, TMC6, TNRC6C, LOC122907522, SEPTIN9, LOC122905944, SEC14L1*
GL	5	3838675	AX-647743699	0.56	0.13	4.02E-05	*CYTH1, LOC122908097, DNAH17, PGS1, SOCS3, LOC122907519, LOC122905935, TMEM235, BIRC5, LOC122907520, AFMID, TK1, SYNGR2, C5H17orf99, TMC8, TMC6, TNRC6C, LOC122907522, SEPTIN9, LOC122905944*
GL	5	3820750	AX-647743689	0.50	0.12	0.000104524	*CYTH1, LOC122908097, DNAH17, PGS1, SOCS3, LOC122907519, LOC122905935, TMEM235, BIRC5, LOC122907520, AFMID, TK1, SYNGR2, C5H17orf99, TMC8, TMC6, TNRC6C, LOC122907522, SEPTIN9, LOC122905944*
GL	2	228006636	AX-647708696	0.90	0.22	0.000107779	
TB	6	26648995	AX-647758271	-0.46	0.10	4.47E-06	*LOC122908374, LOC122910358*
TB	6	26692765	AX-647758275	-0.44	0.10	1.12E-05	*LOC122908374, LOC122910358*

Traits[1]	Chromosome	Position (bp)	SNP	Regression Beta	SE	P-Value	Candidate Genes
TB	11	119404538	AX-647660520	0.64	0.15	1.59E-05	*PPM1K, LOC122889425, LOC122889426, ABCG2, PKD2, SPP1, MEPE, LOC122889433, DMP1, LOC122889431, LOC122890709, LOC122889434, SPARCL1, NUDT9, HSD17B11, HSD17B13, LOC122890569*
TB	1	133204590	AX-647633370	0.55	0.13	2.40E-05	*LOC122908058, TFAP2A, GCNT2*
TB	1	133214228	AX-647633372	0.54	0.13	4.17E-05	*LOC122908058, TFAP2A, GCNT2*
TB	1	133403246	AX-647633386	0.54	0.13	4.19E-05	*LOC122908058, TFAP2A, GCNT2, MAK, LOC122912729, PAK1IP1, C1H6orf52*
TB	3	26440043	AX-647711903	0.52	0.13	4.40E-05	*FN1, MREG, LOC122902281, LOC122902282, LOC122902283, LOC122902284, TMEM169, XRCC5, MARCHF4*
LB	4	11621301	AX-647727904	-0.47	0.10	2.83E-06	*LOC122904295, LOC122905775, LOC122905696, LOC122905698, GSDMC*
LB	11	119404538	AX-647660520	0.67	0.14	7.21E-06	*PPM1K, LOC122889425, LOC122889426, ABCG2, PKD2, SPP1, MEPE, LOC122889433, DMP1, LOC122889431, LOC122890709, LOC122889434, SPARCL1, NUDT9, HSD17B11, HSD17B13, LOC122890569*
LW	6	96541355	AX-647763241	0.94	0.21	9.52E-06	*LOC122908374, LOC122910358*
LW	2	230564175	AX-647708975	0.83	0.20	4.50E-05	-
SB	11	202350460	AX-647665123	0.81	0.18	5.84E-06	*LOC122889158, LOC122890806, LOC122890601, LOC122889162*
SB	11	202028676	AX-647665081	0.69	0.15	8.34E-06	*LOC122890009, LOC122889158, LOC122890806, LOC122890601, LOC122889162*
SB	6	176506451	AX-647768171	2.64	0.61	1.61E-05	–
SB	6	176962800	AX-647768208	2.64	0.61	1.61E-05	*LOC122910625, EDEM1, ARL8B, LOC122909250*
SB	6	177705921	AX-647768289	2.64	0.61	1.61E-05	*ITPR1, SUMF1, LOC122909254, LOC122910626, EDEM1, ARL8B, LOC122909250*
SB	6	177712750	AX-647768291	2.64	0.61	1.61E-05	*ITPR1, SUMF1, LOC122909254, LOC122910626, EDEM1, ARL8B, LOC122909250*
SB	6	177032271	AX-647768227	2.62	0.61	1.98E-05	*LOC122910625, EDEM1, ARL8B, LOC122909250*

Traits[1]	Chromosome	Position (bp)	SNP	Regression Beta	SE	P-Value	Candidate Genes
SB	6	178216434	AX-647768324	2.80	0.65	2.07E-05	*ITPR1, SUMF1, LOC122909254, LOC122910626, LRRN1, LOC122910628*
SB	3	8870113	AX-647710409	0.83	0.19	2.10E-05	*DNAH7, STK17B, LOC122902144, ECW2, LOC122903916, CCDC150, LOC122903886, GTF3C3, C3H2orf66, PGAP1, ANKRD44*
SW	1	19172531	AX-647623185	4.66	1.01	4.18E-06	*HS3ST5, HDAC2, MARCKS, LOC122898354*
SW	3	9720984	AX-647710483	4.87	1.10	1.15E-05	*ANKRD44, LOC122903767, LOC122903783, LOC122902150, LOC122902151, COQ10B, HSPD1, LOC122902155, RFTN2, LOC122902157, MARS2, BOLL, LOC122902161*
SW	1	19699203	AX-647623239	4.36	1.02	2.20E-05	*LOC122898354*
SW	1	19864650	AX-647623262	4.27	1.02	3.00E-05	*LOC122898354, RFPL4B, LAMA4*
SW	1	18952749	AX-647623152	4.20	1.00	3.14E-05	*HS3ST5, HDAC2, MARCKS, LOC122898354*
AWB	1	59453486	AX-647626409	-0.28	0.06	3.94E-06	*FUCA2, PEX3, ADAT2, AIG1, HIVEP2*
AWB	11	197859049	AX-647664582	0.21	0.05	1.47E-05	*CASP3, LOC122889140, LOC122889141, IRF2, ENPP6, STOX2, LOC122889144, TRAPPC11, RWDD4, ING2, LOC122890000*
AWB	10	52059897	AX-647648273	0.27	0.06	1.98E-05	*RNF2, TRMT1L, SWT1, LOC122918625, LOC122917981, LOC122918627, LOC122918628, HMCN1*
AWW	1	16752230	AX-647622983	11.74	2.77	2.41E-05	*PPP1R14C, LOC122898126, LOC122899149, LOC122898230, RAET1E, LOC122900474, LOC122901067, LOC122898158, LOC122902644, CALHM5, TRAPPC3L, CALHM6, DSE, TSPYL1, TSPYL4, LOC122903379, NT5DC1, COL10A1, LOC122903395, LOC122903395, FRK*
AWW	1	5228501	AX-647622004	9.02	2.14	2.72E-05	*QKI, LOC122902708, LOC122902715, PACRG, LOC122902728*
AWW	6	110404078	AX-647764056	10.64	2.56	3.36E-05	*SST, RTP2, BCL6, LOC122908795, LOC122910513, LOC122910514 LOC122908796, LOC122908798, LOC122908799*

[1] GL = gestation length; TB = total number of kits born; LB = number of kits alive at 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; AWW = average kit weight per litter at weaning.

Table 5.3 List of genome-wide and chromosome-wide significant SNPs using single-SNP regression mixed linear model for pelt quality traits in mink.

Traits[1]	Chromosome	Position (bp)	SNP	Regression Beta	SE	P-Value	Candidate Genes
LQU	9	17882544	AX-647797565	-0.11	0.03	5.13E-05	*ASTN2, PAPPA*
LQU	3	222397250	AX-647725944	-0.21	0.05	1.08 E-0405	*SEZ6L, MYO18B*
LQU	3	222429371	AX-647725949	-0.21	0.05	1.08 E-0405	*SEZ6L, MYO18B*
LNAP	6	78125738	AX-647762037	0.25	0.06	1.86E-05	*SUCNR1, LOC122908613, MBNL1, LOC122908616, P2RY1*
DPS	6	77192086	AX-647761986	-0.32	0.06	5.41E-07	*MINDY4B, CLRN1, MED12L*
DPS	9	53181800	AX-647800462	0.16	0.04	1.82E-05	*CCDC171, LOC122917928, PSIP1, SNAPC3, LOC122916943, TTC39B, LOC122917591, LOC122917593, LOC122917592, LOC122917866, CER1, ZDHHC21*
DPS	6	72955123	AX-647761834	-0.30	0.07	4.08E-05	*PLOD2, LOC122910438, PLSCR4, LOC122908582, PLSCR1, PLSCR5*
DPS	11	162864156	AX-647661433	-0.20	0.05	4.60E-05	*ADAM7, LOC122890342, ADAMDEC1, ADAM28, STC1, LOC122890743, NKX2-6*
DNAP	14	40392262	AX-647691922	0.41	0.10	4.66E-05	*FBXL19, LOC122895607, LOC122896122, LOC122896123, LOC122895288, LOC122896125, LOC122896126, LOC122895286, STX4, ZNF668, ZNF646, PRSS53, VKORC1, BCKDK, KAT8, PRSS8, PRSS36, FUS, LOC122896237, LOC122895549, TRIM72, ITGAM, ITGAX, ITGAD, LOC122895723, LOC122895433, ARMC5, TGFB1I1, SLC5A2, RUSF1, LOC122895780, LOC122895860*
DNAP	13	14031167	AX-647678777	0.24	0.06	5.39E-05	*DPF3, DCAF4, ZFYVE1, RBM25, LOC122894773, PSEN1, PAPLN, NUMB, HEATR4, RIOX1, LOC122892989, LOC122893762, LOC122894728, LOC122893820, ACOT6, DNAL1*
DQU	4	211090536	AX-647741458	0.17	0.04	2.16E-05	*PLXNA4*

DQU	2	40384318	AX-647694986	0.17	0.04	2.72E-05	*ELAVL4, DMRTA2, FAF1, LOC122899957, LOC122901490*

[1] LQU = live grading overall quality of pelt; LNAP = live grading nap size; DPS = dried pelt size; DNAP = dried pelt nap size; DQU = overall quality of dried pelt.

Table 5.4 Top ten significant gene ontology (GO) terms enriched for potential candidate genes of pelt quality traits.

GO terms[1]	Description	Number of genes	P-value
GO_BP	animal organ development	18	4.26E-10
GO_BP	tissue development	13	1.03E-08
GO_BP	forebrain development	7	1.53E-08
GO_BP	anatomical structure development	23	3.34E-08
GO_BP	developmental process	24	3.88E-08
GO_BP	multicellular organismal process	27	8.31E-08
GO_BP	multicellular organism development	20	1.17E-07
GO_BP	system development	19	1.19E-07
GO_BP	positive regulation of biological process	24	1.71E-07
GO_BP	negative regulation of biological process	22	6.19E-07
GO_MF	protein binding	47	7.19E-25
GO_MF	binding	51	1.65E-10
GO_MF	molecular_function	58	5.68E-08
GO_MF	metal ion binding	22	1.32E-07
GO_MF	cation binding	22	2.70E-07
GO_MF	ion binding	25	4.42E-04
GO_MF	endopeptidase activity	9	1.06E-03
GO_MF	phospholipid scramblase activity	3	1.37E-03
GO_MF	peptidase activity	10	2.63E-03
GO_MF	catalytic activity, acting on a protein	17	1.64E-02
GO_CC	extracellular exosome	7	4.68E-11
GO_CC	extracellular organelle	7	2.26E-10
GO_CC	extracellular vesicle	7	2.26E-10
GO_CC	extracellular membrane-bounded organelle	7	2.26E-10
GO_CC	membrane-bounded organelle	42	1.02E-06
GO_CC	nucleoplasm	13	4.22E-06
GO_CC	organelle lumen	16	1.36E-05
GO_CC	intracellular organelle lumen	16	1.36E-05

GO_CC	membrane-enclosed lumen	16	1.36E-05
GO_CC	nuclear lumen	14	5.39E-05

[1] GO_BP: Biological processes gene ontology term, GO_MF: Molecular function gene ontology term, and GO_CC: Cellular component gene ontology term.

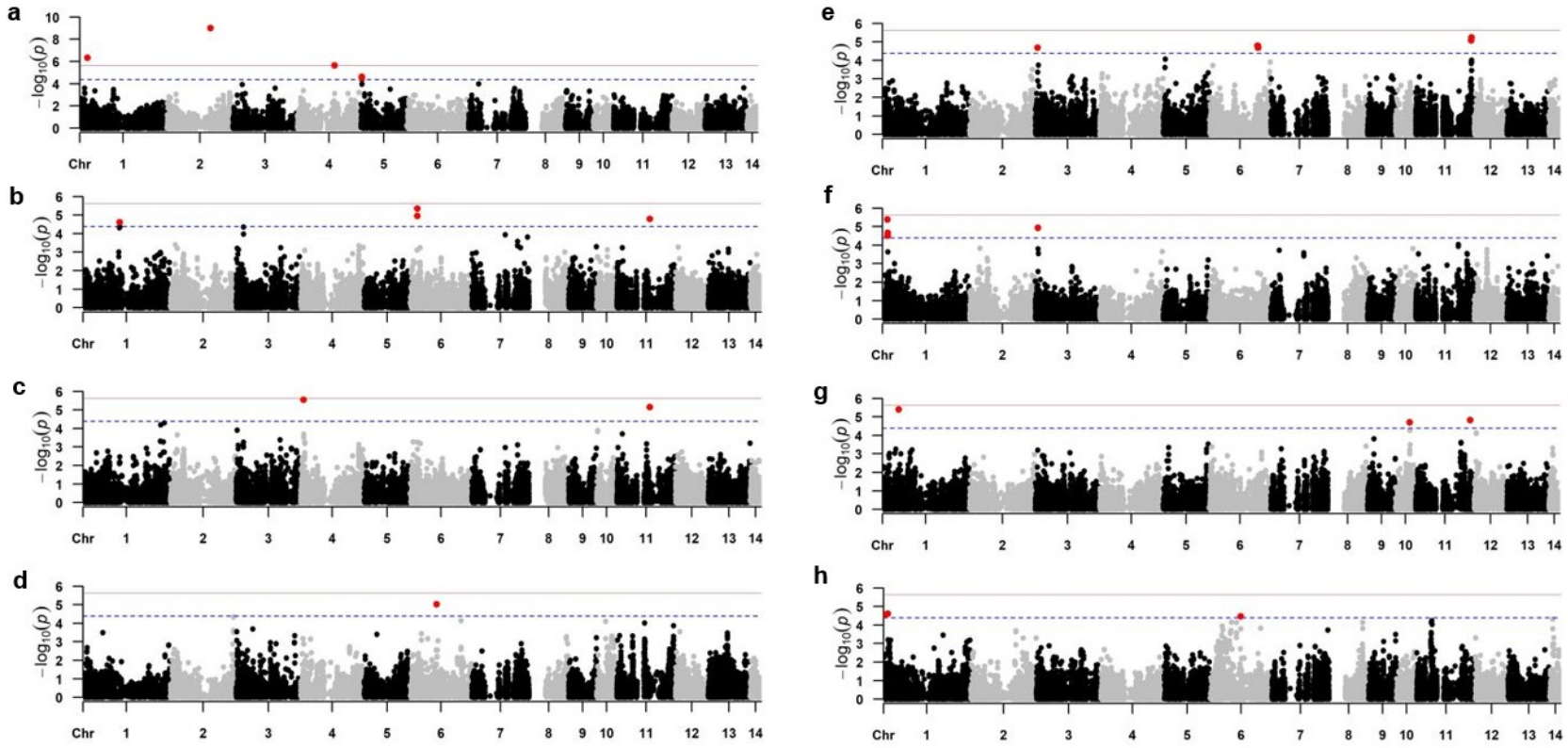

Figure 5.1 The Manhattan plots of genome-wide association studies for reproduction traits in mink: (a) gestation length; (b) total number of kits born; (c) number of kits alive at birth; (d) number of kits alive at weaning; (e) survival rate at birth; (f) survival rate at weaning; (g) average kit weight per litter at birth; and (h) average kit weight per litter at weaning. The horizontal solid line depicts the genome-wide significance level (-log10 (P-values) = 5.69) and the horizontal dashed line depicts the suggestive significance level (-log10 (P-values) = 4.39).

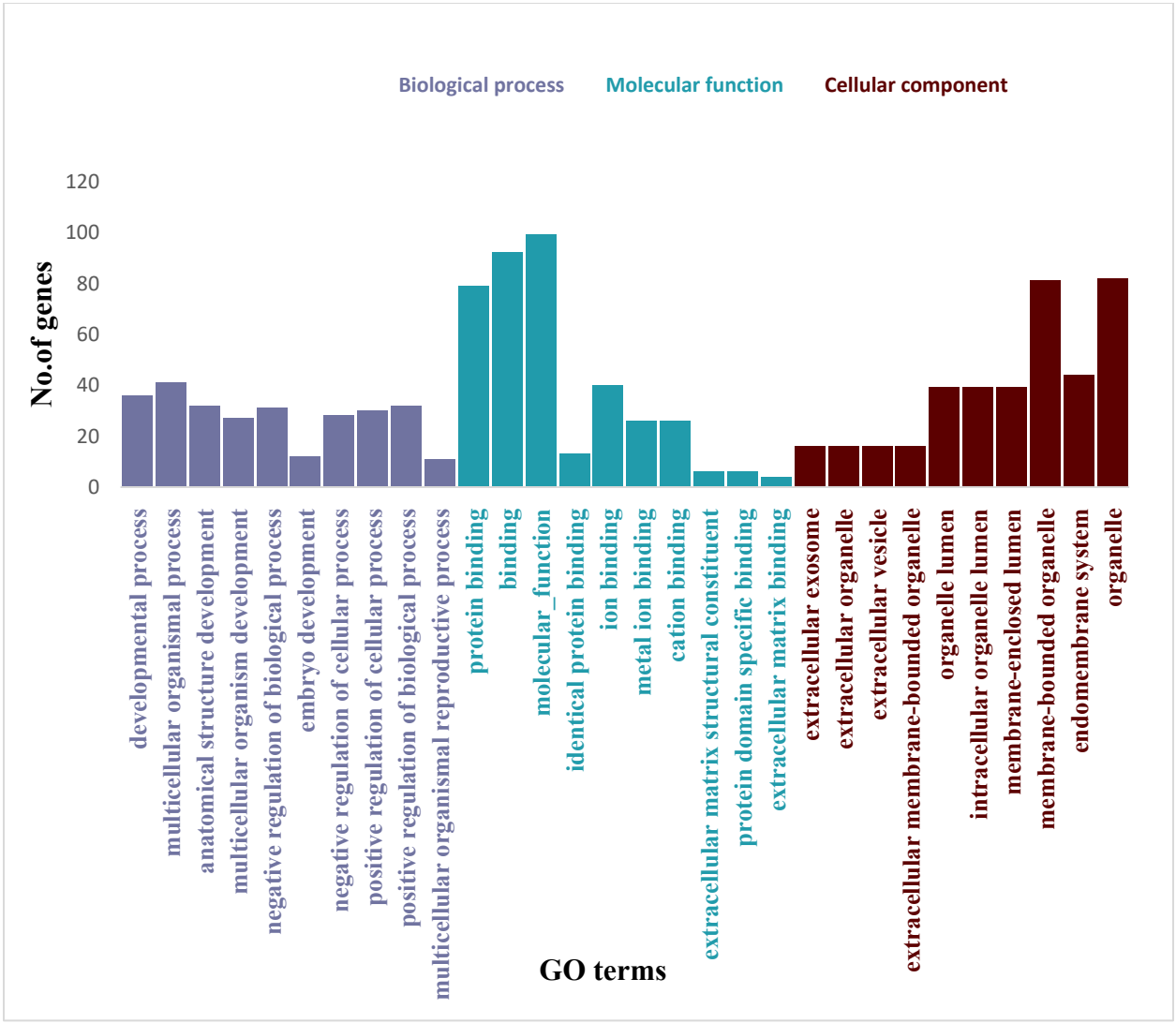

Figure 5.2 Top ten significant gene ontology (GO) terms enriched for potential candidate genes of reproduction traits.

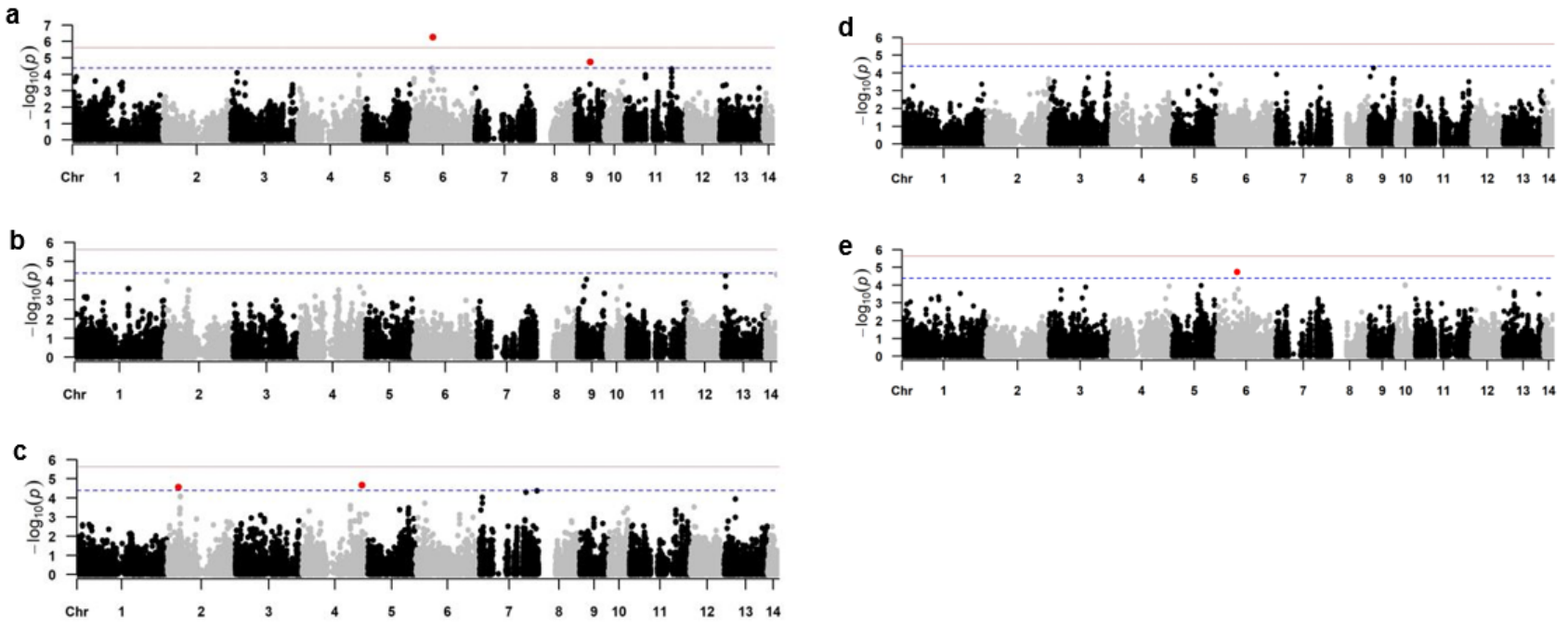

Figure 5.3 The Manhattan plots of genome-wide association studies for fur quality traits in mink: (a) dried pelt size; (b) dried pelt nap size; (c) overall quality of dried pelt; (d) live grading pelt quality; and (e) live grading nap size. The horizontal solid line depicts the genome-wide significance level (-log10 (P-values) = 5.69) and the horizontal dashed line depicts the suggestive significance level (-log10 (P-values) = 4.39).

Chapter 6: Comparison of genomic prediction approaches for reproduction and pelt quality traits in American mink

6.1 Introduction

The main goal in animal breeding is to increase genetic gain for economically important traits by selecting individuals with the highest genetic merit as parents of the next generation. Since the advent of breeding technologies that use genome-wide single nucleotide polymorphism (SNP), genomic selection was rapidly adopted for major livestock species and has replaced the traditionally used pedigree-based best linear unbiased prediction. Genomic selection refers to a genetic evaluation method that utilizes phenotypic data and genotypes information to estimate the effects of genetic markers from a population consists of individuals with both genotypes and phenotypes (training population) and subsequently to predict the genetic values of selection candidates only based on their genotypes (Meuwissen et al., 2001). In genomic selection, quantitative trait loci (QTL) are presumed to be in linkage disequilibrium (LD) with at least one of the genotyped markers and the markers are used to estimate the level of genetic similarity between individuals (Goddard and Hayes, 2007).

Genomic prediction for American mink (*Neogale vison*) is attractive because many traits that affect the profitability of production, such as reproduction and pelt quality traits, are difficult to select for because they have low heritabilities or the measures are not available for selection candidates (Villumsen et al., 2021). Most reproduction traits in mink have low heritabilities ($h^2 < 0.2$) (Hansen et al., 2010; Karimi et al., 2018), which led to the decreased accuracy of breeding value prediction and selection response (Cassell, 2009). Dried pelt quality traits can only be measured on dried skins, so these records are not available on selection candidates. Therefore, genetic evaluation of selection candidates relies on using body weight and fur quality measurements recorded on live

animals as indicator traits for dried pelt size and quality (Thirstrup et al., 2017; Villumsen et al., 2021; Valipour et al., 2022a). Therefore, accurate genomic estimated breeding values would lead to greater genetic gain for these traits (Karimi et al., 2019).

Several statistical approaches have been proposed for genomic prediction of breeding values, among which genomic best linear unbiased prediction (GBLUP), Bayesian statistics have been extensively employed in many agricultural species. GBLUP is based on infinitesimal model which assume that effects of all SNPs are drawn from the same normal distribution and therefore, all SNPs have small and equal amount of variance (Meuwissen et al., 2001). On the other hand, certain Bayesian methods, such as BayesCπ, operate under the assumption that genetic variation is explained by a limited number of genetic loci. In BayesCπ model proposed by Habier et al., (2011), in this model all SNPs have a common variance and the proportion of SNPs with no effect (π) has a uniform prior distribution that is estimated during the analysis. The starting value for π determines the scale parameter that is being used for estimation of prior distribution of variances of SNPs effects. In other words, scale parameter can affect to what extent π is used to shrink SNP effects, hence the estimate of π (Habier et al., 2011).

Typically, in most commercial or research breeding units, only a small subset of the population with both pedigree and phenotypic information are genotyped. Therefore, single-step approaches can be used to take advantage of all pedigree, phenotypic and genomic information simultaneously. Fernando et al. (2014) proposed a class of single-step Bayesian regression methods (SSBR) to extend the single-step GBLUP (ssGBLUP) to incorporate Bayesian statistics assumption regarding priors for variance of SNP effects as well as including information from non-genotyped animals to the prediction model.

Several investigations have undertaken comparisons between performance of statistical approaches used for genomic selection. These studies consistently highlighted that genomic assessment tends to outperform traditional genetic evaluation. This trend is evident across various livestock species, including dairy cattle (Gao et al., 2012; Chen et al., 2014), beef cattle (Lee et al., 2017; Mehrban et al., 2017), pig (Salek Ardestani et al., 2021), and poultry (Ni et al., 2017). However, only a single study has thus far explored the performance of genomic selection in the context of mink (Villumsen et al., 2021). Currently, BLUP based models (i.e., GBLUP and ridge regression BLUP) have become popular approaches for practical genomic prediction, as they are simple and have low computational requirements. However, several studies have shown that Bayesian approaches may produce higher accuracies than linear models when traits are influenced by genes with large effects (Hayes et al., 2010; Clark et al., 2011; Rolf et al., 2015; Mehrban et al., 2017), which means that genetic architecture of these traits does not follow the infinitesimal model.

The objective of this study was to compare predication accuracy of different genomic evaluation methods including GBLUP, BayesCπ and SSBR-Cπ for reproduction and pelt quality traits in mink population.

6.2 Materials and Methods

6.2.1 Ethics statement

All procedures related to the animals in this study were performed strictly in accordance with the standards of the Guide to the Care and Use of Experimental Animals (CCAC, 1993) after approval by the Dalhousie University Animal Care and Use Committee.

6.2.2 Animals

The animals were bred in accordance with standard farming conditions at the Canadian Centre for Fur Animal Research (CCFAR) within Dalhousie University's Faculty of Agriculture in Truro, Nova Scotia, Canada, during the years 2006 to 2021. The 5,824-mink used in this study were the progeny of 1,051 sires and 2,097 dams. Pedigree information of 17 generations comprising 26,575 individuals was used. In December of each year, mink were euthanized using the approved method of carbon monoxide gas.

6.2.3 Female reproductive performance traits

Phenotypic data for female reproductive performance was collected for each annual reproduction cycle from 2006 to 2021. The detailed procedure for reproduction management in CCFAR and data collection process has been previously described in Karimi et al. (2018), Do et al. (2021) and Hu et al. (2021). Briefly, female reproductive measurements included the total number of kits born (TB), gestation length (GL), average weight of kits per litter at birth (AWB), average weight of kits per litter at weaning (AWW).

6.2.4 Evaluation of fur characteristics on live animals and dried pelts

The detailed procedure of pelt quality assessment on live animals and dried pelts have been described by Valipour et al., (2022a). In brief, dried pelt quality measures were including, dried pelt size (DPS), dried pelt nap size (DNAP), and overall quality of dried pelt (DQU), and live grading traits were including the overall quality of fur (LQU) and nap size (LNAP). Pelt quality assessment for live mink was conducted by a certified technician, following the North American

Fur Auctions (NAFA) live animal grading procedure. Evaluation of dried pelt quality traits was carried out on dried pelts of 1,195 mink at North American Fur Auctions-NAFA in Toronto, Canada, and Saga Furs in Vantaa, Finland.

6.2.5 Estimation of breeding values

Breeding values were estimated using the following univariate animal model implemented in ASReml 4.1 (Gilmour et al., 2018):

$$\mathbf{y} = \boldsymbol{X}\mathbf{b} + \boldsymbol{Z}\mathbf{a} + \boldsymbol{G}\mathbf{m} + \boldsymbol{W_1}\mathbf{pe} + \boldsymbol{W_2}\boldsymbol{c} + \mathbf{e},$$

where ***y*** was the vector of phenotypic observations; ***b*** was the vector of fixed effects; ***a*** was the vector of random additive genetic effects; ***m*** was the vector of random maternal genetic effects; ***Pe*** was the vector of permanent environmental effects; ***c*** was the vector of common litter effects; and ***e*** was the vector of residual effects; and ***X***, ***Z***, ***G***, ***W1*** and ***W2*** were the incidence matrices relating the phenotypic observations to fixed, random additive genetic, maternal genetic, permanent environmental, and common litter effects, respectively. It was assumed that random effects are independent and normally distributed:

$$\boldsymbol{a}\sim \mathrm{N}(0, \boldsymbol{A}\sigma_{\mathrm{a}}^2), \boldsymbol{m}\sim N(0, \boldsymbol{A}\sigma_m^2), \boldsymbol{Pe}\sim N(0, \boldsymbol{I}\sigma_{\mathrm{Pe}}^2), \boldsymbol{c}\sim N(0, \boldsymbol{I}\sigma_{\mathrm{c}}^2), \text{and } \mathbf{e}\sim N(0, \boldsymbol{I}\sigma_{\mathrm{e}}^2),$$

where ***A*** was the numerator relationship matrix; ***I*** was an identity matrix; σ_a^2, σ_m^2, σ_{pe}^2, σ_c^2 and σ_e^2 were the variances of random additive genetic, maternal genetic, permanent environmental effects, common litter, and residual effects. Fixed effects were sex (male and female), year (2006 to 2021), and color type (brown, breath of spring, dark, demi, gray, mahogany, pastel, sapphire, stardust, white, and white-blue), number of matings (1 to 3 times) and age of the dam (1 to 5 years).

The significance of fixed effects and covariates was tested using Wald statistics in the REML procedure of ASReml 4.1 (Gilmour et al., 2018), and only significant ($P < 0.05$) effects were kept in the mixed model analyses for each trait. The significance of different random effects for each trait was determined by comparing the full model and reduced model using the following statistic:

$$-2(\log L_{\text{reduced model}} - \log L_{\text{full model}}) \sim \chi^2_{\text{df(full model)}-\text{df(reduced model)}}$$

Finally, breeding values were estimated using the REML procedure in ASREML 4.1 (Gilmour et al., 2018).

6.2.6 Calculation of de-regressed breeding values

The de-regressed estimated breeding values (dEBV) for all traits were calculated according to Garrick et al. (2009) to remove the parent average effects and these dEBVs were used instead of the actual phenotypes in genomic models. By accounting for offspring and parents information, it is expected that dEBV can give more reliable results for genomic evaluation (Ostersen et al., 2011).

6.2.7 Genotypes

Genotypes were available for 1,321 individuals. All animals were genotyped with the 70K SNP chip. Quality control of variants was performed using PLINK Software (Chang et al., 2015). Variants with minor allele frequency (MAF) < 0.05; maximum missing rate <90%; individual call rate < 90% and deviating from Hardy-Weinberg equilibrium ($P < 10^{-6}$) were removed. After quality control, 27,516 variants were remained for further analysis.

6.2.8 Genomic prediction

6.2.8.1 Genomic best linear unbiased prediction model

The GBLUP method was performed using the following model:

$$\boldsymbol{y_c} = \mathbf{1}\mu + \boldsymbol{Zg} + \boldsymbol{e},$$

where $\boldsymbol{y_c}$ was the vector of dEBVs (reference population) as pseudo-phenotypes; 1 was the vector of ones; μ was the overall mean; $\boldsymbol{Z}$ was the incidence matrix of direct genomic breeding values (GEBV), and $\boldsymbol{g}$ was the vector of GEBVs and assumed to follow a normal distribution $g \sim N(0, \boldsymbol{G}\sigma_g^2)$, where $\boldsymbol{G}$ was the genomic relationship matrix and σ_g^2 was the genetic variance captured by the markers; $\boldsymbol{e}$ was the vector of random residual effects and assumed to follow a normal distribution $\boldsymbol{e} \sim N(0, \boldsymbol{I}\sigma_e^2)$, where $\boldsymbol{I}$ was an identity matrix; and σ_e^2 was the residual variance. The G-matrix was built using the information from genome-wide dense SNPs (VanRaden, 2008) with the default options in JWAS, the Julia package of the Gensel program (Habier et al., 2011; Cheng et al., 2018).

6.2.8.2 Bayesian methods

BayesCπ method was applied using pseudo-phenotypes from genotyped individuals according to the following model:

$$\boldsymbol{y_c} = \mathbf{1}\boldsymbol{\mu} + \boldsymbol{M_g a} + \boldsymbol{e},$$

where $\boldsymbol{y_c}$ was the vector of dEBVs (reference population); **1** was a vector of ones; $\boldsymbol{\mu}$ was the overall mean, $\boldsymbol{M_g}$ was the matrix of SNP covariates, and $\boldsymbol{\alpha}$ was a random vector of allele substitution effects, and $\boldsymbol{e}$ was the vector of random residuals effects. The prior for $\boldsymbol{e}$ was $\boldsymbol{e}|\sigma_e^2 \sim \mathrm{N}(0, \boldsymbol{I}\sigma_e^2)$, with $(\sigma_e^2|v_e, S_e^2) \sim v_e S_e^2 \chi_{v_e}^2$. Priors for SNP effects were a mixture of a point mass at zero and a normal distribution conditional on a common variance of SNP effects in BayesCπ method (Garrick et al., 2014). Accuracies in BayesC were compared using various π values i.e. 0.9999, 0.999, 0.995, 0.99, 0.98 and then, in steps from 0.95 to 0.6 decreasing by 0.05.

6.2.8.3 Single-step Bayesian regression method

For single-step Bayesian regression analysis, de-regressed breeding values from both genotyped and non-genotyped individuals were modeled as follows:

$$\boldsymbol{y_c} = \boldsymbol{X\beta} + \boldsymbol{ZM\alpha} + \boldsymbol{Z_n\varepsilon} + \boldsymbol{e},$$

where $\boldsymbol{y_c}$ was the vector of dEBVs for both genotyped and non-genotyped individuals, $X = \begin{bmatrix} \mathbf{1} & -\boldsymbol{Z_n A_{ng} A_{gg}^{-1}} \mathbf{1} \\ \mathbf{1} & -\boldsymbol{Z_g}\mathbf{1} \end{bmatrix}$, $\boldsymbol{\beta} = \begin{bmatrix} \boldsymbol{\mu} \\ \boldsymbol{\mu_g} \end{bmatrix}$, where $\boldsymbol{A_{gg}}$ was the numerator relationship matrix that corresponds to genotyped animals, $\boldsymbol{A_{ng}}$ was the numerator relationship matrix that corresponds to non-genotyped animals, $\boldsymbol{Z_n}$ and $\boldsymbol{Z_g}$ were the design matrices relating records to breeding values of non-genotyped animals and genotyped animals, $\boldsymbol{\mu}$ was the overall mean, and $\boldsymbol{\mu_g}$ represented the difference in breeding values between genotyped and non-genotyped animals, $\boldsymbol{Z} = \begin{bmatrix} \boldsymbol{Z_n} & \mathbf{0} \\ \mathbf{0} & \boldsymbol{Z_g} \end{bmatrix}$, was the design matrix and, $\boldsymbol{M} = \begin{bmatrix} \widehat{\boldsymbol{M_n}} \\ \boldsymbol{M_g} \end{bmatrix}$, where $\boldsymbol{M_g}$ was the matrix of SNP covariates for genotyped animals and $\widehat{\boldsymbol{M_n}} = \boldsymbol{A_{ng} A_{gg}^{-1} M_g}$, representing imputed SNP covariates for non-

genotyped animals derived from genotyped relatives, $\boldsymbol{\varepsilon}$ was the imputation residual. The prior for $\boldsymbol{e}$ was $\boldsymbol{e}|\sigma_e^2 \sim \mathrm{N}(0, \boldsymbol{I}\sigma_e^2)$, with $(\sigma_e^2|v_e, S_e^2) \sim v_e S_e^2 \chi_{v_e}^2$. The prior for $\boldsymbol{\varepsilon}$ was $\boldsymbol{\varepsilon}|\sigma_g^2 \sim \mathrm{N}(0, (\boldsymbol{A_{nn}} - \boldsymbol{A_{ng}A_{gg}^{-1}A_{gn}})\sigma_g^2)$ with $(\sigma_g^2|v_g, S_g^2) \sim v_g S_g^2 \chi_{v_g}^2$. The same priors for SNP effects in BayesCπ were used in single-step Bayesian regression method refer to as SSBR-Cπ. For SSBR-Cπ method, the analysis was performed in JWAS, the Julia package for whole-genome analyses (Cheng et al., 2018). The π values in SSBR-Cπ were chosen such that they provided the highest accuracies from 5-fold cross-validation in BayesCπ.

6.2.9 Cross-validation and prediction accuracy

For each validation set, the accuracy and biasedness of prediction were obtained through 5-fold cross-validation (CV), where the dataset was randomly split into five approximately equal subsets. In each round of CV, phenotypes from one subset (validation population) were removed from the dataset, and the remaining four subsets (reference population) were used to predict the future performance of animals in the validation population. This 5-fold CV was replicated ten times, and the results are presented as the mean for the ten replicates in GBLUP and BayesCπ method. For SSBR-Cπ method, a 5-fold cross-validation was applied where in each round the phenotypic records of 20% of the genotyped animals were set to missing and the remaining 80% of the genotyped animals plus 100% of the animals with only phenotypes were used as training dataset. Prediction accuracies from these 5-fold cross-validation sets were pooled to obtain a single accuracy as mean and standard deviation. The accuracy of genomic prediction was evaluated as the correlation between GEBVs and de-regressed breeding values in the validation population. In addition, the regressions coefficient of de-regressed breeding values on GEBVs were calculated to

assess the bias of prediction. Finally, the "Improvement of accuracy" was measured to provide a numerical value (%) to evaluate how much better or worse one method performs compared to another in terms of accuracy. The "Improvement of accuracy" was calculated by taking the percentage difference between the accuracy of the first method (GEBV) and the accuracy of the second method (GEBV), relative to the accuracy of the second method.

6.3 Results

6.3.1 Descriptive statistics of phenotypes

De-regressed breeding values for reproduction and pelt quality traits were used as pseudo-phenotypes for genomic prediction. The descriptive statistics of dEBVs including number of records, standard deviations (SD) and range of dEBVs for each trait are presented in Table 6.1. The number of animals with de-regressed proofs ranged from 1,900 for AWW to 4,538 for TB for reproduction traits and from 1,240 for DNAP to 2,248 for LNAP for pelt quality traits. Moreover, the number of genotyped individuals for reproduction traits from 1,167 for AWW to 1,321 for TB and GL and for pelt quality traits ranged from 1,148 for DQU to 1,260 for LNAP and LQU (Table 6.1). The estimated heritabilities (±SE) for reproduction traits were 0.07±0.01 for TB, 0.23±0.03 for GL, 0.14±0.04 for AWB, and 0.16±0.04 for AWW. For pelt quality traits, heritability (±SE) estimates were 0.41±0.06 for DPS, 0.22±0.10 for DNAP, 0.12±0.04 for DQU, 0.42±0.06 for LNAP, and 0.23±0.05 for LQU.

6.3.2 Comparison of methods

Accuracy and bias of prediction for a range of π from 0.60 to 0.9999 for each trait are presented in Tables 6.2 and 6.3, respectively. For all reproduction and pelt quality traits, prediction accuracies were decreased with the values of π larger than 0.995 where fewer markers were considered with non-zero effects (Table 6.2). The regression coefficient of GEBVs on dEBVs for validation animals has remained stable across different values of π for GL, AWB, DPS, DNAP, DQU, LNAP, and LQU. However, higher biases were estimated for smaller values of π for TB and AWW. The π values that showed the highest prediction accuracy were 0.65 for TB, 0.98 for GL, 0.90 for AWB, 0.95 for AWW, 0.99 for DPS, 0.90 for DNAP, 0.99 for DQU, 0.95 for LNAP and 0.60 for LQU, and these π values were implemented in SSBR-Cπ.

The accuracy of GEBV obtained using GBLUP, BayseCπ, and SSBR-Cπ are presented in table 6.4. The prediction performance of GBLUP and BayesCπ were similar for TB, GL, AWB, and DNAP. However, BayesCπ had slightly higher prediction accuracies relative to GBLUP and they were increased by 8.1% for AWW, 1.43% for DPS, 1.14% for DQU, 1.14% for LNAP, and 4.65% for LQU. We also compared the prediction performance of BayesCπ and GBLUP methods with SSBR-Cπ that uses both genotyped and non-genotyped animals for prediction of genomic breeding values. For all reproduction and pelt quality traits, SSBR-Cπ outperformed GBLUP and BayesCπ with improvement in accuracy of prediction ranged from 6.83-6.99 for DNAP to 68.96-82.70 for AWW (Table 6.4).

The inflation or deflation of prediction of genomic breeding values was measured by the regression coefficient of GEBVs on dEBVs. Regression coefficient of dEBV on predicted breeding values obtained from different methods for reproduction and pelt quality traits are presented on Table 6.5. The lowest regression coefficient was observed for AWB (0.47 for SSBR-Cπ) and TB (0.57 for

SSBR-Cπ). The highest regression coefficient was observed for LQU (1.13 SSBR-Cπ). The prediction bias was close to 1 for GL, DPS, DNAP, DQU, LNAP, and LQU. However, based on the results of t-test, the coefficient of regression significantly lower than 1 obtained for TB (0.83±0.23 for GBLUP, 0.73±0.19 for BayesCπ, and 0.54±0.12 for SSBR-Cπ), AWB (0.47±0.15 for SSBR-Cπ), and AWW (0.79±0.25 for GBLUP, 0.72±0.23 for BayesCπ, and 0.82±0.38 for SSBR-Cπ) suggesting that the genomic predictions for these traits are biased upwards.

6.4 Discussion

This study was the first investigation of genomic prediction of breeding values for pelt quality and reproduction traits in mink using genotype data. The present study assessed the accuracy of genomic prediction for reproduction and pelt quality traits in mink populations. This study was the first assessment of genomic prediction of breeding values using a SNP genotyping panel in mink. The custom genotyping 70K Mink SNP panel has provided sufficient SNPs density for performing genomic evaluation in this species (Karimi et al., 2020; Karimi et al., 2021b; Hu et al., 2023). The commercial SNP genotyping assays have been extensively used for genomic prediction of breeding values in many major agricultural species such as cattle (Abo-Ismail et al., 2017), pig (Tiezzi et al., 2020), and chicken (Groenen et al., 2011; Liu et al., 2014).

In the current study, the de-regressed breeding values were used as pseudo-phenotypes for genomic prediction of breeding vales using GBLUP, BayesCπ, and SSBR-Cπ methods. The use of dEBVs for prediction of genomic breeding values using solely genotyped animals and single-step approaches can be exemplified by studies of genomic prediction in Nordic Red Cattle (Su et al., 2012) and Danish Jersey populations (P. Ma et al., 2015).

In this study, we estimated prediction accuracy based on a range of π values from 0.60 to 0.9999 to determine the most accurate π for the BayesC method for each trait. Interestingly, all reproduction and pelt quality traits showed lower prediction accuracies for the large values of π (i.e., π = 0.9999, π = 0.999, and π = 0.995). These results suggest that these traits are controlled by many genes each with small effects on phenotypic variation of the trait. A similar pattern has been reported by Mehrban et al. (2017) and Lee et al. (2017) in Hanwoo beef cattle when their studied traits were influenced with many quantitative traits loci with small effects. Moreover, the prediction accuracies obtained with GBLUP and BayesCπ were similar suggesting that the genetic architecture of these traits follow an infinitesimal model (Meuwissen et al., 2001; VanRaden et al., 2009; Salek Ardestani et al., 2021).

The SSBR-Cπ method improved prediction accuracies compared to GBLUP and BayesCπ. These results confirmed that the inclusion of information from non-genotyped individuals in a single-step approach produced more accurate estimation of breeding values for the genotyped animals. The increased accuracies for nine traits in this study ranged from 6.83 to 82.70 using the SSBR-Cπ method. The superiority of single-step approaches compared with pedigree-based BLUP or models for estimation of EBV using only genotyped information has been reported by many previous studies in different livestock species. Villumsen et al. (2021) compared prediction accuracies of breeding values estimated for seven pelt quality traits in mink using ssGBLUP and a pedigree-based BLUP and indicated that ssGBLUP was more accurate than BLUP method. In another study, Lee et al., (2017) compared prediction performance of SSBR-C and SSBR-B with the conventional BayesCπ and BayesB, and reported improvement in prediction accuracy of GEBVs using SSBR methods compared with BayesCπ, and BayesB for three carcass traits in Hanwoo beef cattle.

The bias of prediction of genomic evaluation methods is important to be considered for designing a long-term breeding program (Vitezica et al., 2011). In this study the regression coefficient of GEBVs on dEBVs used to measure bias of prediction for different methods. For all pelt quality traits and GL, the regression coefficient was very close to one indicating no bias of prediction. However, the regression coefficient of GEBVs on dEBVs for TB, AWB, and AWW were lower than one, suggesting genomic predictions are biased upwards. Many factors might have contributed to this biasness of prediction. One reason might be associated with low heritability of these traits ($h^2 < 0.2$). It has been reported that increase in heritability would lead to less bias of prediction in genomic evaluation (Gowane et al., 2018; Karimi et al., 2019). Previous studies have reported prediction bias significantly different from one for reproduction traits, for instance, Esfandyari et al. (2016) reported prediction bias between 0.44 to 1.36 for litter size traits in purebred pig population. Another reason could be that markers were not in complete linkage disequilibrium with causal QTLs for these particular traits, and therefore could not fully account for the total genetic variance (Ma et al., 2015; Tsuruta et al., 2021).

6.5 Conclusions

This study was the first investigation of genomic prediction of breeding values for pelt quality and reproduction traits in mink genome using a SNP genotyping assay. Our results revealed that BayesCπ and GBLUP have relatively similar prediction performance and BayesCπ could provide higher prediction accuracy when the correct π value is used in the model. Moreover, the single-step method gives higher prediction accuracies compared to the methods using only genotyped individuals. The results of this study provide a valuable resource for implementation of different genomic evaluation models in mink.

Table 6.1 Descriptive statistics of estimated de-regressed EBVs for female reproduction and pelt quality traits in mink.

Traits[1]	Number of observations	Number of genotyped individuals	Mean	Standard deviation	Range (Min. – Max.)
TB	4538	1321	0.02	2.76	-90.76 – 45.03
GL	3917	1321	-0.42	3.22	-29.00 – 25.42
AWB	2007	1168	-0.01	1.54	-6.27 – 11.82
AWW	1900	1167	0.22	41.03	-630.63 – 205.31
DPS	1261	1169	0.02	0.70	-7.20 – 8.24
DNAP	1240	1159	0.17	1.20	-15.55 – 7.12
DQU	1253	1148	0.01	0.59	-3.92 – 4.40
LNAP	2249	1260	0.16	0.78	-3.84 – 3.60
LQU	2248	1260	0.08	0.59	-3.96 – 3.78

[1] TB = total number of kits born; GL = gestation length; AWB = average kit weight per litter at birth; AWW = average kit weight per litter at weaning; DPS = dried pelt size; DNAP = Dried pelt nap size; DQU = overall quality of dried pelt; LNAP= live grading nap size; LQU = live grading overall quality of fur.

Table 6.2 The prediction accuracies (%) and their standard deviation (%) obtained from BayesCπ using different values of π for reproduction and pelt quality traits in mink.

Traits[1]	Prediction accuracy by π												
	0.9999	0.999	0.995	0.99	0.98	0.95	0.90	0.85	0.80	0.75	0.70	0.65	0.60
TB	19.11 (6.5)	20.51 (6)	20.85 (5.7)	20.94 (4.9)	20.16 (5.8)	20.9 (5.6)	19.64 (5.2)	20.89 (6)	20.28 (5.5)	21.21 (5.7)	21.53 (5.7)	22.22 (5.9)	19.57 (4.8)
GL	29.54 (7.2)	38.45 (6.7)	40.49 (5.6)	40.43 (6.7)	40.66 (7.2)	39.87 (6)	39.47 (6.1)	38.37 (6.2)	39.68 (6.4)	40.39 (7)	39.19 (7.4)	40.05 (5.3)	39.95 (5.8)
AWB	39.93 (5.2)	41.95 (5.2)	42.94 (5.8)	42.76 (4.6)	43.14 (6.6)	43.29 (5.7)	43.88 (4.8)	42.38 (6.3)	42.57 (6.8)	43.35 (5.2)	43.71 (5)	43.27 (6.8)	42.47 (5)
AWW	22.51 (9.3)	23.52 (9.4)	24.22 (8.1)	24.15 (7.5)	24.66 (9.5)	25.26 (7.9)	24.54 (8.4)	24.26 (8.5)	24.62 (6.2)	25.09 (8.7)	24.18 (6.7)	23.55 (7.9)	24.40 (8.3)
DPS	41.89 (7.6)	41.50 (8.5)	43.95 (8.1)	44.01 (7.8)	43.73 (8.2)	43.62 (9)	43.63 (8.6)	43.88 (8.6)	43.06 (8.1)	43.43 (9.9)	44.99 (8.7)	42.80 (8)	43.00 (7.7)
DNAP	41.38 (6.2)	42.10 (5.6)	43.66 (4.8)	43.58 (6.4)	43.32 (5.5)	43.95 (5.4)	44.58 (6.3)	44.41 (6.4)	43.86 (6.5)	44.40 (5.8)	43.99 (6.2)	43.88 (5.2)	44.03 (6)
DQU	26.82 (6.3)	28.09 (6.5)	28.31 (5.2)	29.54 (4.8)	28.96 (5)	27.44 (5.8)	28.44 (5.9)	29.01 (5)	28.81 (5.7)	28.70 (5.2)	27.28 (5.7)	28.43 (5.2)	28.23 (5.7)
LNAP	42.90 (5.5)	44.43 (4.8)	49.48 (4.8)	49.43 (4.4)	49.58 (4.7)	50.27 (3.8)	50.22 (3.7)	49.99 (4)	50.06 (4.3)	49.09 (3.6)	49.70 (3.8)	50.15 (3.8)	49.97 (4.2)
LQU	40.10 (6.3)	41.04 (5.8)	43.48 (3.9)	44.04 (4.7)	44.15 (4.7)	44.74 (5.2)	44.36 (4.5)	44.83 (5.6)	44.57 (5.3)	44.80 (4.5)	44.11 (3.6)	44.13 (3.7)	45.18 (4.3)

[1] TB = total number of kits born; GL = gestation length; AWB = average kit weight per litter at birth; AWW = average kit weight per litter at weaning; DPS = dried pelt size; DNAP = Dried pelt nap size; DQU = overall quality of dried pelt; LNAP= live grading nap size; LQU = live grading overall quality of fur.

Table 6.3 Regression coefficient of deregressed EBV (dEBV) on predicted breeding values obtained from different models (standard deviation given in brackets)

Traits[1]	Prediction bias by π												
	0.9999	0.999	0.995	0.99	0.98	0.95	0.90	0.85	0.80	0.75	0.70	0.65	0.60
TB	0.96 (0.36)	0.81 (0.39)	0.86 (0.29)	0.82 (0.30)	0.83 (0.25)	0.79 (0.25)	0.77 (0.21)	0.71 (0.21)	0.72 (0.26)	0.76 (0.33)	0.70 (0.22)	0.73 (0.19)	0.69 (0.24)
GL	0.86 (0.27)	1.07 (0.25)	1.09 (0.22)	1.07 (0.30)	1.05 (0.26)	1.05 (0.24)	1.03 (0.24)	1.01 (0.23)	1.02 (0.24)	1.04 (0.26)	0.99 (0.19)	0.99 (0.21)	0.93 (0.17)
AWB	1.02 (0.30)	0.99 (0.20)	0.99 (0.20)	0.99 (0.25)	0.99 (0.24)	0.95 (0.20)	0.95 (0.18)	0.93 (0.16)	0.94 (0.19)	0.95 (0.22)	0.93 (0.19)	0.92 (0.20)	0.93 (0.18)
AWW	0.82 (0.34)	0.77 (0.25)	0.79 (0.27)	0.74 (0.25)	0.85 (0.23)	0.72 (0.25)	0.71 (0.23)	0.68 (0.19)	0.67 (0.23)	0.67 (0.18)	0.65 (0.20)	0.66 (0.24)	0.66 (0.20)
DPS	1.16 (0.26)	1.04 (0.20)	0.99 (0.22)	1.02 (0.20)	0.99 (0.18)	1.00 (0.21)	0.95 (0.21)	0.99 (0.19)	0.98 (0.20)	0.98 (0.20)	0.96 (0.20)	0.97 (0.19)	0.99 (0.19)
DNAP	1.05 (0.16)	1.05 (0.16)	1.03 (0.17)	1.04 (0.20)	1.03 (0.21)	1.02 (0.20)	0.99 (0.16)	1.01 (0.17)	1.00 (0.17)	1.01 (0.16)	1.01 (0.19)	1.02 (0.14)	1.01 (0.15)
DQU	1.21 (0.34)	1.04 (0.24)	0.98 (0.28)	0.99 (0.25)	0.97 (0.30)	0.94 (0.18)	0.90 (0.19)	0.90 (0.17)	0.86 (0.21)	0.89 (0.18)	0.89 (0.20)	0.83 (0.19)	0.87 (0.20)
LNAP	1.13 (0.20)	1.06 (0.14)	1.01 (0.11)	0.99 (0.11)	1.01 (0.15)	1.02 (0.13)	1.04 (0.15)	1.07 (0.15)	1.09 (0.13)	1.09 (0.16)	1.08 (0.13)	1.08 (0.11)	1.10 (0.12)
LQU	1.10 (0.21)	1.05 (0.18)	0.97 (0.15)	0.98 (0.15)	0.97 (0.15)	0.97 (0.13)	0.98 (0.14)	0.99 (0.15)	1.01 (0.13)	0.98±0.13	1.01 (0.17)	1.00 (0.14)	1.01 (0.13)

[1] GL = gestation length; TB = total number of kits born; AWB = average kit weight per litter at birth; AWW = average kit weight per litter at weaning; DPS = dried pelt size; DNAP = Dried pelt nap size; DQU = overall quality of dried pelt; LNAP= live grading nap size; LQU = live grading overall quality of fur.

Table 6.4 The prediction accuracies (%), their standard errors and improvement (%) in accuracy of GEBV from SSBR-Cπ method compared to the GBLUP and BayesCπ methods for reproduction and pelt quality traits in mink

Traits[1]	Method	Prediction accuracy (%)	Improvement in accuracy (%)
TB	GBLUP	22.13 (5.3)	30.04
	BayesC (π = 0.65)	22.22 (5.9)	29.52
	SSBR-C (π = 0.65)	28.78 (6.4)	NA
GL	GBLUP	40.66 (6.8)	9.61
	BayesC (π = 0.98)	40.66 (7.2)	9.61
	SSBR-C (π = 0.98)	44.57 (8)	NA
AWB	GBLUP	43.93 (5)	33.37
	BayesC (π = 0.90)	43.88 (4.8)	33.52
	SSBR-C (π = 0.90)	58.59 (29)	NA
AWW	GBLUP	23.36 (8.3)	82.70
	BayesC (π = 0.95)	25.26 (7.9)	68.96
	SSBR-C (π = 0.95)	42.68 (17)	NA
DPS	GBLUP	43.38 (8.2)	9.68
	BayesC (π = 0.99)	44.01 (7.8)	8.11
	SSBR-C (π = 0.99)	47.58 (5.2)	NA
DNAP	GBLUP	44.65 (5.6)	6.83
	BayesC (π = 0.90)	44.58 (6.3)	6.99
	SSBR-C (π = 0.90)	47.70 (8.3)	NA
DQU	GBLUP	27.63 (6.6)	18.75
	BayesC (π = 0.99)	29.54 (4.8)	15.13
	SSBR-C (π = 0.99)	34.01 (2.8)	NA
LNAP	GBLUP	49.70 (4.7)	11.12
	BayesC (π = 0.95)	50.27 (3.8)	9.86
	SSBR-C (π = 0.95)	55.23 (6.2)	NA
LQU	GBLUP	43.17 (5.3)	43.53
	BayesC (π = 0.60)	45.18 (4.3)	28.55
	SSBR-C (π = 0.60)	58.08 (6.2)	NA

[1] TB = total number of kits born; GL = gestation length; AWB = average kit weight per litter at birth; AWW = average kit weight per litter at weaning; DPS = dried pelt size; DNAP = Dried pelt nap size; DQU = overall quality of dried pelt; LNAP= live grading nap size; LQU = live grading overall quality of fur.

Table 6.5 Regression coefficient of de-regressed EBV (dEBV) on predicted breeding values obtained from different methods for reproduction and pelt quality traits in mink (standard deviation given in brackets).

Traits[1]	Method	Bias of prediction
TB	GBLUP	**0.83 (0.23)**
	BayesC (π = 0.65)	**0.73 (0.19)**
	SSBR-C (π = 0.65)	**0.54 (0.12)**
GL	GBLUP	1.05 (0.22)
	BayesC (π = 0.98)	1.06 (0.26)
	SSBR-C (π = 0.98)	0.98 (0.11)
AWB	GBLUP	1.02 (0.22)
	BayesC (π = 0.90)	0.96 (0.18)
	SSBR-C (π = 0.90)	**0.47 (0.15)**
AWW	GBLUP	**0.79 (0.25)**
	BayesC (π = 0.95)	**0.72 (0.23)**
	SSBR-C (π = 0.95)	**0.82 (0.38)**
DPS	GBLUP	1.03 (0.20)
	BayesC (π = 0.99)	1.01 (0.20)
	SSBR-C (π = 0.99)	0.94 (0.40)
DNAP	GBLUP	1.02 (0.15)
	BayesC (π = 0.90)	0.99 (0.16)
	SSBR-C (π = 0.90)	0.97 (0.22)
DQU	GBLUP	**0.92 (0.21)**
	BayesC (π = 0.99)	0.99 (0.25)
	SSBR-C (π = 0.99)	1.03 (0.31)
LNAP	GBLUP	1.03 (0.12)
	BayesC (π = 0.95)	1.02 (0.13)
	SSBR-C (π = 0.95)	1.10 (0.15)
LQU	GBLUP	0.98 (0.15)
	BayesC (π = 0.60)	1.01 (0.13)
	SSBR-C (π = 0.60)	1.13 (0.24)

[1] GL = gestation length; TB = total number of kits born; AWB = average kit weight per litter at birth; AWW = average kit weight per litter at weaning; DPS = dried pelt size; DNAP = Dried pelt nap size; DQU = overall quality of dried pelt; LNAP= live grading nap size; LQU = live grading overall quality of fur. Significantly different from 1 are highlighted in bold ($P < 0.05$).

Chapter 7: Conclusions

7.1 Summary of findings

The overall objective of this thesis was to develop and apply genomic breeding approaches for improving efficiency and profitability of mink production system. To design an effective breeding program, prior knowledge is needed about genetic components of the traits in the population. Therefore, in this thesis, the first study (Chapter 3) estimated the genetic and phenotypic parameters for pelt quality traits. This study sought to provide essential information related to the heritability of the traits, as well as outlining genetic and phenotypic correlations among them. In the second study (Chapters 4), detection of selection signatures was conducted to find genomic regions involved in the phenotypic variation of the traits. In the third study (Chapter 5) GWAS was performed for reproduction and pelt quality traits to find genetic markers associated with these traits in mink. In the final study of this thesis (Chapter 6), the prediction accuracy and bias of prediction for reproduction and pelt quality traits using different statistical models to apply genomic selection in mink were compared.

In Chapter 3, the heritabilities, genetic and phenotypic correlations were estimated using a series of univariates and bivariate BLUP models for various pelt quality and body size traits in mink. Moderate heritabilities were estimated, suggesting the potential for improving animal's performance through genetic/genomic selection. Nov_BW and Nov_BL were reliable indicators of DPS. Importantly, using Nov_BW and Nov_BL to select for DPS will not compromise the quality of the pelts in any adverse manner as Nov_BW has non-significant genetic correlation with DQU, and Nov_BL has a positive genetic correlation with DQU. Moreover, Nov_BL and HL had moderate positive genetic correlations with DQU, suggesting the attractiveness of these traits as indicators for selection of increased pelt size. A strong positive genetic correlation was evident

between LNAP and DNAP, emphasizing that measuring nap size on live animals serves as a suitable indicator for predicting DNAP. On the other hand, a low and non-significant genetic correlation was observed between LQU and DQU, indicating that relying on live grading for indirect selection may not substantially enhance the DQU. These findings provide crucial insights for subsequent GWAS and genomic selection studies (Chapters 4, 5, and 6) and offer valuable information for estimating genetic gains from trait selection.

In Chapter 4, I posit that farmed mink has been undergone selection for over 100 years to improve characteristics such as increased pelt size, better fur quality, and coat color. Therefore, it hypothesised that the effect of human-mediated selection for these traits could leave detectable selection signatures within the mink genome. The WGS data from 100 mink—Fst, XP-EHH, and θπ—were employed to detect selection signatures. The SNPs that passed the top 1% of genome-wide values were considered potential selection candidates. The overlapping top 1% SNPs of Fst and XP-EHH analyses revealed 376 candidate genes. Gene Ontology analysis highlighted biological processes such as hair cycle, epidermis development, Wnt signaling, and muscle development. Notably, eight key genes associated with hair follicle function and four related to growth performance were identified. Integrating Fst, XP-EHH, and θπ tests unveiled 19 strongly selected regions on chromosomes 3, 4, 5, 6, 7, 8, 9, and 10 that contained 33 candidate genes. Among these, *APCDD1*, crucial for hair follicle function, was identified in the nap size group. This study provides a comprehensive insight into potential selection signals linked to pelt quality and coat color traits in mink.

The objective of chapter 5 was to perform GWAS for reproduction and pelt quality traits. Association analysis was performed using 26,930 SNPs for eight reproductive traits and five pelt quality traits. For this study, dEBVs have been used as pseudo-phenotypes and the number of

individuals with de-regress proof varied between 1,148 for DQU to 1,321 for TB and GL. GWAS identified three genome-wide significant SNPs for GL on chromosomes 1, 2, and 4, and one genome-wide significant SNP on chromosome 6 for DPS. Moreover, 36 SNPs were significant for reproduction traits at chromosome-wide suggestive ($P < 4E-05$) level and 11 SNPs were associated with pelt quality traits at chromosome-wide suggestive ($P < 4E-05$) level. Moreover, ten candidate genes with important functions in reproduction were detected. Five novel candidate genes for pelt quality traits and pelt size were identified. This research was the first GWAS for reproduction traits in mink and provided a useful resource about genetic architecture of reproduction and pelt quality traits and it is a good reference for conducting future GWAS for these traits.

Chapter 6, aimed to compare the performance of three genomic prediction models GBLUP, BayesCπ, and SSBR across four reproductive and five pelt quality traits. GBLUP and BayesCπ differ in their assumptions regarding SNP effect distribution: GBLUP assumes that all SNPs contribute equally to the overall variance of a trait, while BayesCπ assumes that a few genetic loci predominantly influence trait variance. This characteristic proves advantageous for traits deviating from the infinitesimal model. SSBR incorporates information from genotyped and non-genotyped animals, has a potential to increasing accuracy of GEBV prediction. For BayesCπ, prediction accuracy was assessed across values of π (0.60 to 0.9999). For all traits prediction accuracies were decreased with the values of $\pi > 0.995$, indicating that these traits are controlled by many genes each with small effects. GBLUP and BayesCπ showed similar prediction accuracies, suggesting that the genetic structure of these traits follow the infinitesimal model. The SSBR-Cπ model outperformed both GBLUP and BayesCπ in terms of prediction accuracy. These outcomes serve as confirmation that integrating data from individuals without genotypic information into a single-step approach yielded more precise estimations of breeding values for the genotyped animals.

Future directions

This thesis conducted a comprehensive analysis to estimate heritability, genetic and phenotypic correlations for pelt quality traits in mink. Heritability, a critical factor in breeding program planning for livestock, is a population-specific parameter, and influenced by factors such as allele frequencies, the impact of gene variants, and environmental variations (Visscher et al., 2008; Rosa, 2022). The numerical value of a heritability estimate can fluctuate, due to changes in any of its underlying components, such as shifts in allele frequencies caused by selection or inbreeding (Massey and Vogt, 2018). Timely, update of heritability estimation is important to ensure it accurately reflect the genetic variance of traits, especially in cases of diseases outbreak in which a large portion of individuals should be replaced with animals from unrelated herds. Chapter 1 calculated heritabilities using data from over 1000 individuals representing five color types, providing a valuable genetic pool for the assessment of genetic parameters. Nonetheless, increasing the sample size to encompass a broader spectrum of individuals would enhance the accuracy of estimation, enabling better understanding of the existing genetic variation within the mink population.

Within this thesis several novel genetic markers, genomic regions and candidate genes were identified using GWAS and selection signatures scan related to reproductive performance and pelt quality measurements in mink. For GWAS, I used SNP markers genotyped using 70K MINK SNP panel. Though it provided the sufficient number of markers for genotype-phenotype association analysis, this opportunity still exists to enhance the power of GWAS by employing techniques such as imputation to the whole-genome sequence data. Discovery of causative mutations is an important goal for accelerating genetic improvement of traits. Uncovering these mutations offer

the potential to utilize gene editing techniques in mink breeding. This can be used to create mink that harbor specific mutant genes, thus introducing genetic diversity to the population.

Chapter 6, compared three statistical models of GBLUP, BayesCπ, and SSBR for genomic prediction of breeding values. Results showed that SSBR incorporating information from both genotyped and non-genotyped individuals outperformed GBLUP and BayesCπ in terms of prediction accuracy for all traits. This study utilized a relatively small number of genotyped animals. To enhance prediction accuracy, future research should prioritize using a larger training population (Karimi et al., 2019). The field of developing novel statistical models for predicting genomic breeding values is advancing rapidly, offering researchers new algorithms. Kadarmideen (2014) introduced an extension to GBLUP, called Systems Genomic BLUP, that incorporates two sets of SNPs one set with known biological functions and another with unknown functional roles as random effects within the GBLUP framework. Statistical tools such as genomic feature model (GFBLUP) (Sørensen et al., 2014) uses the same approach for prediction of breeding value. An example of their success can be seen in dairy cattle breeding, where the GFBLUP model has increased the accuracy of genomic prediction for mastitis susceptibility and milk production (Fang et al., 2017).

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APPENDIX 1. STATUS OF MANUSCRIPTS SUBMITTED FROM THE PhD THESIS

(AS OF 14 November 2023)

1- Based on **Chapter 3:**

Valipour, S., Karimi, K., Barrett, D., Do, D. N., Hu, G., Sargolzaei, M., Wang, Z., & Miar, Y. (2022). Genetic and Phenotypic Parameters for Pelt Quality and Body Length and Weight Traits in American Mink. Animals, 12(22), 3184.

2- Based on **Chapter 4:**

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.

Conference Presentations (Presenting speaker)

Valipour, S., Karimi, K., & Miar, Y. (2021). Genomic Studies of Reproductive Performance in American Mink. Journal of Animal Science, 99(Supplement_3), 26-27**.**