Functional Genetic Diversity in American Mink (*Neovison vison*)

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ABSTRACT

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The release of domestic organisms to the wild is considered a threat to biodiversity because the introduction of domestic genes through interbreeding can negatively impact wild conspecifics via outbreeding and local extinction. In North America, captive American mink (*Neovison vison*) are frequently escaping into the wild, yet the impact of these events on the functional genetic diversity of wild mink populations is unclear. I characterized domestic and wild mink in Ontario at 17 trinucleotide microsatellites located in functional genes thought to be associated with traits affected by domestication. I found low functional genetic diversity, as only 4 of 17 genes were variable and of those four there was little evidence of allele frequency differences between domestic and wild mink. Using redundancy analysis and a spatial analysis of principal components on the four variable loci (AR, ATN1, IGF-1, and TOB1) I found no evidence to suggest domestic release events are affecting functional genetic diversity of free-ranging mink at the set of markers assessed.

Keywords: domestication, introgression, functional gene, American mink, *Neovison vison*.

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INTRODUCTION

Domestication is a sustained multigenerational relationship in which humans influence the reproduction and care of another organism in order to more predictably secure a resource (Zeder 2015). The process involves changes in morphology, physiology, and behaviour of a lineage in order to adapt to the captive environment (Price 1984; Trut 1999). Domestication in a variety of fish, mammals, and plants has been well studied, and while each domestic species has undergone a unique process, all have experienced similar phenotypic changes such as an increase in size, change in colour, and shifts in the timing of reproductive cycles (Zeuner 1954; Wayne 1993; Morey 1994). Both pets and farmed animals are typically selectively bred for specific temperament and morphological characteristics (Price 1984; Rauw et al. 1998; Lynch and O'Hely 2001).

In Canada, domestication of American mink (*Neovison vison*) began in 1866 as the fur industry expanded from its origins in Ontario. Faced with a spike in the demand for fur, entrepreneurs saw value in farming mink because if executed successfully the practice resulted in a larger number of high quality pelts to sell than most trappers would procure in the same season (Travis and Schaible 1914). Given that it was common practice for ranchers to depend on locally-trapped mink to build their herds, contemporary captive mink in Ontario have undergone 150 years, which is 150 generations, of intentional breeding that has genetically altered them from their wild ancestors (American Fur Breeder 1959; Belliveau et al. 1999; Kidd et al. 2009). Artificial selection has been so successful, in fact, that farmed mink are both phenotypically and behaviourally different than their wild counterparts: farmed mink produce thicker pelts, are less aggressive, and are approximately twice the size of free-ranging mink, making

the farm and wild type easily distinguishable from one other (Belliveau et al. 1999; Malmkvist and Hansen 2002). Particularly easy to distinguish is pelt colour: wild mink are usually dark brown in colour, but farmed mink have a wide range of whites, browns, blacks, and spotted pelts (American Fur Breeder 1959; Joergensen 1985). Comparison between captive and wild mink in Ontario may provide new insight on the genetic effects of domestication, namely artificial selection and adaption to captivity.

Genetic changes will occur in populations undergoing domestication as a result of random genetic drift and the shift in selection pressures that accompany the transition from nature to captivity (Price 1984). The effects of domestication vary depending on the selection pressure placed on different traits and species (Price 1984). For example, natural and artificial selection, inbreeding, and genetic drift are thought to reduce genetic variability, while relaxed natural selection, introgression, and the production of new alleles (as a result of mutation) are thought to increase genetic variability (Price 1984). Therefore, the amount of genetic variability in farmed mink relative to wild mink differs by specific trait and the conditions under which that trait is maintained in each individual farm.

Several generations of genetic alterations are required for a species to become domesticated, and the process is only complete when the fitness of the population has reached a maximum, that is, no further genetic or environmental changes will have significant positive effects on the population (Price 1984). There is no universal agreement to what extent a population needs to adapt to a captive environment in order to be labelled domestic, however, it is generally agreed upon that a population is domesticated once they have a predisposition for tameness (Hediger 1964; Hale 1969). Contemporary captive American mink are still aggressive towards humans and therefore,

by the above definition, are still undergoing the domestication process. The main genetic mechanisms influencing the domestication of mink are predicted to be: artificial selection, natural selection, relaxation of natural selection, inbreeding, and genetic drift. Individually or in combination, these processes may influence trait intensity and/or direction.

According to the 2010 Canadian Fur Statistics, approximately 2.2-2.6 million mink pelts are produced each pelting season, contributing almost $200 million annually to the Canadian economy (Statistics Canada 2010). With such a high economic value it is important to both ranchers and consumers that mink pelts are of the highest quality, with fur that is thick, soft, and glossy (Obbard 1987). As a result, the domestication of American mink has produced captive mink that are phenotypically and genetically different than their free-ranging counterparts. Not only are captive mink larger, less aggressive, and have higher quality pelts than wild mink, they have experienced a reduction in sexual dimorphism and brain size (Lynch and Hayden 1995; Kruska 1996; Malmkvist and Hansen 2002; Kruska and Sidorovich 2003). While large size, lower aggression level, and high quality fur are a result of intense selection, reduced sexual dimorphism and brain size may be due genetic drift or to linkage with genes causing traits directly under selection (Lynch and Hayden 1995, Belliveau et al. 1999, Hansen et al. 2000).

In order to assess genetic differences between groups of American mink under predicted differential selective pressures, we sought to analyze loci found in coding regions of the genome and in promoter regions that are non-coding but may be linked to functionally important genes. Sequence polymorphisms in coding regions are responsible for the variation in traits exhibited among individuals – variation that can be targeted by

artificial selection and other genetic mechanisms (Borštnik and Pumpernik 2002). Areas under artificial selection are especially interesting as we expect to see the greatest genetic difference between wild and domestic populations at these loci. Previous studies comparing functional loci between wild and domestic species have used microsatellites and/or single nucleotide polymorphisms (Vigouroux et al. 2002; Renaut and Rieseberg 2015). Both markers have unique advantages and disadvantages depending on their intended use; microsatellites are highly polymorphic, informative, and robust, whereas SNPs are more commonly found in the genome, produce no stutter, and can amplify extremely degraded samples (Butler 2010). DNA slippage, unequal crossing over, and mutations can all elongate or shorten repeating units to create microsatellite polymorphisms, whereas SNPs arise due to point mutations (Borštnik and Pumpernik 2002, Butler 2010). Microsatellite loci mutate at a higher rate than SNPs, consequently making these markers our first choice to screen for signatures of domestication in American mink (Haasl and Payseur 2011).

Microsatellites, also known as simple sequence repeats or short tandem repeats, are tandem repeating units of 1-6 base pairs, named according to the number of nucleotides in the repeat unit (Lai and Sun 2003). We chose to use trinucleotide markers, as they are commonly found throughout the entire genome and are more stable than dinucleotides (Borštnik and Pumpernik 2002). Variation in microsatellites can be seen in: (1) the size of the repeat unit (eg. "AA" versus "AAA"), (2) the number of repeats present (eg. "AA AA" versus "AA AA AA"), (3) nucleotide sequence (eg. "ACA ACA ACA" versus "ATA ATA ATA", and (4) how accurately each repeat conforms to the nucleotide pattern in the repeat unit "ATA ATA ATA" versus "ATA ACA ATA") (Butler 2010). Variation in DNA sequence is usually the cause of a point mutation, a nucleotide change

that can occur in either of the first two bases in a codon (non-synonymous) or in the third base of a codon (synonymous); a synonymous mutation will not change the amino acid produced by the codon and therefore not impact the protein produced, but a non-synonymous mutation does change the amino acid, which may affect the protein product and consequently the function it performs (Borštnik and Pumpernik 2002).

Though microsatellites can vary in size, repeat sequence, and repeat conformity, traditional STR genotyping distinguishes alleles solely on the number of repeats present (Butler 2010). Modern genotyping incorporates the sequence of the alleles along with their length, allowing for differentiation of alleles of identical size but different sequence, resulting in a much higher resolution than traditional size-based analysis (Butler 2010). With data from multiple farm and wild sites across Ontario, our first objective was to determine if American mink alleles of identical size differed in sequence, which we accomplished by sequencing trinucleotide microsatellite markers. We hypothesized that wild mink display a higher frequency of imperfect repeats than captive mink because, while DNA slippage occurs in both the farm and in the wild, selection against new mutations causing amino acid repeat length change may be less constrained in captivity therefore making signatures of slippage more prevalent. And, as DNA slippage is likely to remove imperfections, we expected to see a higher number of perfect repeats in captive mink than in wild mink.

Secondly, we sought to assess how domestication affected functional allele frequencies in American mink. Studies have established that domestication lowers genetic diversity in neutral loci within lines (Belliveau *et al.* 1999, Kidd *et al.* 2009), however, little is known about how it affects genetic diversity of functional genes. Natural selection may eliminate maladaptive alleles created by DNA slippage and with the removal of this

selective pressure we expect these alleles to remain in the population. If loci are solely influenced by relaxed natural selection, we expect to see a larger variety of alleles in captive American mink than in the wild. However, intense artificial selection can increase the frequency of an allele until it is fixed in the population, and considering American mink undergo intense artificial selection (e.g. for large size), it is likely the alleles associated with these traits are nearing fixation.

Our third objective was to determine how functional genetic diversity of wild mink populations is affected when domestic mink are introduced. Captive American mink are continuously being released and escaping to the wild (Joergensen 1985). Kidd *et al.* (2009) estimated that 38% of mink trapped per province per year from wild populations in Canada were of domestic origin and found that escapees are persisting within wild mink populations, with one wild population containing only 22% wild individuals. Kidd *et al.* (2009) also found evidence of backcrossing in both directions, indicating that domestic alleles at neutral loci are being introgressed into wild mink populations. We hypothesized that the phenotypes unique to captive mink are maladaptive to life in the wild, therefore alleles common in domestic mink are selected against in the wild and consequently occur in wild mink populations at relatively low frequencies. Domestic alleles confer low fitness in wild mink, and therefore will only occur in close proximity to mink farms as a result of recent introgression. We sought to quantify this by testing genetic variation against mink farm density per township using redundancy analysis.

METHODS

Sample Collection and DNA Preparation

Free-ranging American mink were sampled in locations across Ontario during 2005-2015. The 144 samples originated from Bruce County, Durham, Essex County, Grey County, Huron County, Kirkland Lake, Leeds, Niagara, Nippissing, Perth, Peterborough County, Wellington County, and York (Table 1, Figure 1). Domestic mink (n=143) were donated by three farms in Ontario and a pelting service in Nova Scotia. Two subspecies of American mink occur in Ontario; *N. v. lacustris* inhabiting the western side of the province up to the western shores of Hudson Bay; *N. v. vison*, inhabiting southern shores of Hudson Bay and the eastern portion of the province (Hollister 1965). It is thought that *N. v. vison*, *N. v. melampeplus*, and *N. v. ingens* were most heavily sampled to create the founding domestic herds in Ontario (American Fur Breeder 1959). However, most contemporary mink herds are unidentifiable as belonging to a particular subspecies due to cross-breeding between farms and the continuous introduction of wild mink into domestic populations (American Fur Breeder 1959). DNA was extracted from mink spleen and liver samples using a DNeasy Blood & Tissue Kit (Qiagen). Extracted DNA samples were quantified using a Quant-iT Picogreen® dsDNA Assay Kit (Invitrogen TM) then diluted to a concentration of 2.5ng/µL.

Control Validation

Extracted DNA samples selected to become positive controls were quantified using a Nanodrop 8000 UV-Vis Spectrophotometer (Nanodrop) then diluted to 100, 25, 5, 2.5, 1.25, 0.5, 0.25, 0.125, 0.05, and 0.025ng/µL using TE buffer. Each dilution was amplified in 8µL reactions containing 1mM PCR buffer (10x), 1.5mM $MgCl_2$, 0.2mM

dNTPs, 0.25mg/mL BSA, 0.2µM LGL331, 0.2µM LGL335, 0.1U Taq, and 5ng genomic DNA. Thermal-cycling parameters consisted of: 94ºC for 5 min, 55ºC for 30 s, and 72ºC for 30 s; 29 cycles of 94ºC for 30 s, 55ºC for 30 s, and 72ºC for 30 s, followed by 94ºC for 30 s, 55ºC for 30 s, 72ºC for 10 min, and an 8ºC hold. Amplicons were visualized with the QIAxcel system (Qiagen) using a DNA Screening Kit.

Gene Selection

Genes were selected for their putative adaptive potential. Genes related to functions such as behaviour, development, immunity, and stress, were targeted because they can be artificially selected for by mink ranchers and may therefore show variation between domestic and wild mink. We downloaded DNA sequences from the National Center for Biotechnology Information (NCBI), searched for regions with six or more repeat units and designed primers using Geneious 9.0.5 (Biomatter Ltd.), and ordered primers from Integrated DNA Technologies (Weber and May 1989, Collins et al. 2003). Primers that successfully amplified polymorphic loci in other mammal species were also used in our study.

Primer Optimization

We screened each primer against the KM20 control. Each 8µL reaction contained 1mM PCR buffer (10x), 2mM $MgCl_2$, 0.2mM dNTPs, 0.1mg/mL BSA, 0.2µM forward primer, 0.2µM reverse primer, 0.04U Taq, and 5ng genomic DNA. Thermal-cycling parameters consisted of: 95ºC for 10 min, 94ºC for 30 s, 55ºC for 1 min, and 72ºC for 1 min; 30 cycles of 94ºC for 30 s, 50-65ºC for 1 min, and 72ºC for 1 min, followed by 65ºC

for 15 min, and an 4°C hold. Amplicons were visualized with a QIAxcel capillary electrophoresis system (Qiagen) using a DNA Screening Kit.

Microsatellite Genotyping

Primers that successfully amplified their target were then tested for polymorphisms using a subset of samples that was comprised of wild and multiple lines of domestic American mink, both males and females, in order to represent the complete sample set and encompass multiple levels of variation (between sex, colour, and origin (domestic v. wild)). The same PCR cocktail was prepared, this time containing fluorescently labelled forward primer, and amplified with an annealing temperature of 55-61 ^{o}C depending on the locus (Table 2). New primers were fluorescently labelled with either HEX or 6-FAM (Integrated DNA Technologies, Inc.) or NED (Applied Biosystems). Genotyping products were suspended in HiDi formamide and the GeneScan size standard LIZ500 (Applied Biosystems) and visualized on an ABI3730 DNA Analyzer (Applied Biosystems). Genotypes were scored using GeneMarker v1.70 (SoftGenetics) software. If polymorphic, the primer was used on the complete sample set.

Microsatellite Genotyping Analysis

American mink were separated into five groups: Nova Scotia domestic black (NSB), Ontario domestic black (ONB), Ontario domestic brown (OND), Ontario wild (Wild), and Ontario mink of mixed origin that can not be classified as either fully domestic or fully wild (Hybrid) based on Bayesian assignment tests carried out in a previous study; individuals with a mean membership coefficient $q \geq 0.8$ were assigned as wild, those with $q \leq 0.2$ were domestic, and the remaining ($0.2 > q < 0.8$) were considered

hybrid though they may have been backcrossed to domestic or wild groups (Kidd et al. 2009). We included microsatellite data from 15 neutral markers characterized in the same individuals in a previous study in our population genetic analyses (Appendix A, Beauclerc et al. 2013).

We tested for Hardy-Weinberg Equilibrium (HWE) and for linkage disequilibrium (LE) with GENEPOP 4.5.1 using Markov chain parameters of 5000 iterations with 10 repetitions (Rousset 2008). Effective number of alleles was determined using an allelic richness test in Fstat 2.9.3.2 based on a minimum sample size of 8 individuals (Goudet 1995). Population-specific variations in allele distributions were tested with a Principal Components Analysis (PCA) in RStudio 3.3.0 using the adegenet package (Jombart 2008).

Apportioning genetic variation in free-ranging mink to environmental variables

We evaluated anthropogenic and spatial variables that may explain genetic variation in free-ranging mink: mink farm density, road density, latitude, and longitude. We used mink farm density to test our hypothesis that free-ranging mink found in areas that have a high density of mink farms exhibit different allele frequencies from mink in areas of low density of mink farms. Road density was used as a proxy for human population density to test if allele frequencies of American mink varied according to changes in human population levels. Latitude and longitude were included as a measure of location, and because latitude has previously shown to be a good predictor of a free-ranging mink's probability of being classified as domestic based on neutral markers (Beauclerc et al. 2013).

To obtain mink farm density values we used ArcMap 10.1 to determine the township each sample was associated with, then divided the number of mink farms in that township by the township area (km^2) (Statistics Canada 2006). Similarly, road density values were obtained by dividing the sum of the lengths of all the roads (km) in the township by the township area (km^2). To determine if these variables explained the genetic variation seen in free-ranging mink, redundancy analysis (RDA) was performed for each locus on 159 individuals in RStudio 3.3.0 using the vegan package (Oksanen *et al.* 2016). An additional RDA was performed on the same individuals using the entire neutral data set, as opposed to the locus-by-locus method employed with the functional markers.

Additionally, we evaluated the principal components of each functional locus and neutral data set over geographic space to determine if allele frequencies varied by location. This was done using spatial analysis of principal components (sPCA) performed in RStudio 3.3.0 using the adegenet package (Jombart 2008).

Microsatellite Sequencing

We tested for sequence polymorphisms in AR and ATN1 alleles by sequencing multiple alleles of the same size. Due to the troublesome nature of dinucleotide sequencing, IGF1 was not tested, and TOB1 was not sequenced due to limited amplification success. Homozygous individuals were selected for Sanger sequencing. Heterozygous individuals could not be used due to the program being unable to distinguish a nucleotide as belonging to one allele or the other, thereby producing unclear results. Amplified products were purified with ExoSap (New England Biolabs) and sequenced using a BigDye Terminator v3.1 Cycle Sequencing Kit (Applied Biosystems).

Sequencing reaction products were ethanol precipitated and suspended in HiDi formamide then sequenced on an ABI3730 DNA Analyzer (Applied Biosystems). Sequences were edited and aligned with Molecular Evolutionary Genetics Analysis v6.06 (MEGA) software (Tamura *et al.* 2011).

RESULTS

Control Validation

LGL331 and LGL335 successfully amplified the mink control samples. The intensity of the bands gradually decreased as the DNA template dilution increased, and there was no smearing present, indicating the samples were non-degraded and free from the presence of inhibitors, and thus approved to use as controls in this study.

Primer Optimization

Of the 38 primer pairs tested, 17 amplified a single amplicon at the size expected, with no evidence of non-specific amplification or smearing (Table 3). Of these 17 loci, only four displayed length polymorphisms: Androgen Receptor (AR), Atrophin 1 (ATN1), Insulin-Like Growth Factor 1 (IGF1), and Transducer of ERBB2 (TOB1). The remaining 13 loci did not show variation within or among mink types.

Microsatellite Analysis

A total of 287 American mink were profiled at AR, ATN1, IGF1, and TOB1. All markers were polymorphic, with a total of 19 alleles and a mean of 4 alleles per locus (range 2-8) (Table 4).

Androgen Receptor

We profiled the AR trinucleotide repeat in 135 females and 145 males and found six alleles in total (range 289-304). Only domestic female American mink possessed allele 289; it was not found in wild female mink or in male mink of either type. Allele 292 was found only in wild mink. Population structure was assessed at AR using female

mink only, as this gene is located on the X chromosome. The frequency of allele 298 was significantly different between female populations at Bonferroni-corrected significance thresholds; only 40% of wild mink displayed AR298 whereas it appeared to be approaching fixation in domesticated black mink (Figure 2). This allele frequency disproportion between populations was supported by PCA, where only 25% of the ONB ellipse overlapped with wild mink (Figure 6, Table 5). We found no evidence for deviation from HWE at Bonferroni-corrected significance thresholds for each domestic population, however, for wild mink, AR deviated from HWE expectations (Appendix B). There was no evidence of deviation from LE for each population.

Atrophin 1

Profiling the ATN1 trinucleotide repeat in 282 individuals revealed four alleles in total (range 226-235). Accounting for sample size, wild mink displayed the highest number of alleles (2.79), followed by OND (2.00) and NSB (1.73), then ONB (1.27). Alleles were shared between both wild and domestic American mink, with the exception of 226 found only in black domesticated American mink, and 229 found only in wild American mink. Allele 226 was found at low frequencies (NSB 0.7%, ONB 1.8%) and may have gone undetected in other mink types. Allele 229 was also found at a low frequency (0.094%) in wild mink and therefore may have gone undetected in domestic mink. Significant frequency differences among populations were found only for allele 235 using Bonferroni-corrected significance thresholds (Figure 3). However, principal components of ATN1 gave little support for significant allele frequency differences among populations, as ellipses for each population displayed a minimum 63% overlap with wild mink (Figure 6, Table 5). We found little evidence for deviation from LE nor

from HWE at Bonferroni-corrected significance thresholds for each population: ATN1 appeared to be in linkage disequilibrium with the neutral locus Mvi072 in domesticated black mink in Nova Scotia, these two loci are not linked in any other population, nor is ATN1 linked with any other locus (Table 6).

Insulin-Like Growth Factor 1

We characterised the IGF-1 dinucleotide repeat in 285 individuals and found eight alleles (range 97-111). Wild mink had the highest number of alleles (3.46), followed by OND (2.46), then NSB (2.30) and ONB (2.04) after correcting for sample size. Alleles were shared between both wild and domestic American mink, with the exception of 97, 99, 101, and 109 found only in wild American mink. These four alleles were at very low frequencies (<0.035) and may have gone undetected in domestic mink. Frequency of allele 103 was significantly different between populations at Bonferroni-corrected significance thresholds, yet principal components of IGF-1 revealed high similarity of allele frequencies between domestic and wild populations (91-94% ellipse overlap, Figure 4, Table 5). We found no evidence for deviation from LE nor from HWE at Bonferroni-corrected significance thresholds for each population.

Transducer of ERBB2

We profiled the TOB1 trinucleotide repeat in 220 individuals and found two alleles (242 and 245). Frequency of allele 245 was significantly different between populations at Bonferroni-corrected significance thresholds. Allele 245 was more commonly found than 242 in all mink types except for domesticated black mink in Ontario, where both alleles were found to occur in equal frequency (Figure 5). Principal

components of TOB1 suggest moderate allele frequency similarity between all populations except for between OND and wild mink (Figure 6, Table 5). We found no evidence for deviation from LE nor from HWE at Bonferroni-corrected significance thresholds for each population.

Neutral Microsatellites

American mink (n=280) were profiled at 10-15 polymorphic neutral markers, which produced a total of 163 alleles and a mean of 11 alleles per locus (range 5-20) (Appendix A). We found minimal evidence for deviation from HWE at Bonferroni-corrected significance thresholds: Mvi1302 deviated from HWE in domesticated black mink in Ontario, Mvi1321 and Mvi2243 deviated from HWE in domesticated brown mink in Ontario, and Mvi3102, Mvi099, Mvi2243, Mvi075, Mvi072, and Mvi1342 deviated from HWE expectations in wild mink. Loci seemed to be in linkage equilibrium in domesticated brown mink in Ontario, however several pairs of neutral loci are in linkage disequilibrium in the remaining populations (Table 6).

Apportioning genetic variation to environmental variables

Only a small proportion of genetic variation in each functional locus was explained by the environmental variables (AR R^2_{adj}=0.0235, ATN1 R^2_{adj}=0.0116, IGF1 R^2_{adj}=0.0271, and TOB1 R^2_{adj}=0.0297) (Table 7). Of the variables tested, longitude significantly explained genetic variation in ATN1 (R^2 =0.0211) and road density was significant for TOB1 (R^2 =0.0425). Similar to the functional loci, the variables all

produced small effect sizes in neutral loci (R^2_{adj}=0.0411); here, latitude, longitude, and road density all significantly explained genetic variation (R^2 =0.0205) (Table 7).

None of the principal components seemed to display a particular spatial pattern (Figure 7). At neutral loci, individuals appeared panmictic across Ontario, whereas at functional loci slight admixture was observed (Figure 7). There was no evidence of clustering in farm locations at either neutral or functional loci.

Microsatellite Sequencing Analysis

We sequenced AR in 71 American mink. All alleles were sequenced except 289, due to an absence of homozygous mink for this allele in our sample set. Each sequence consisted of three repeat sections: a perfect repeat ('GCA'), followed by an imperfect repeat ('GGA GAC CAG TTC TCG'), and a second perfect repeat ('GCA'). Allele variation was due to the number of repeats present in the first perfect ('GCA') repeat section. There were no non-synonymous sequence differences between alleles of the same size.

ATN1 was sequenced in 133 individuals. All alleles were sequenced except 226, due to an absence of homozygous mink for this allele in out sample set. We observed that for ATN1, a compound microsatellite containing ('CAG') as well as ('CAA') repeats, the variation between alleles was due to the number of ('CAG') repeats present. There were no non-synonymous sequence differences between alleles of the same size.

DISCUSSION

Impact of domestication on functional trinucleotide microsatellites

Of 17 amplified loci, only four displayed length polymorphisms: AR, ATN1, IGF1, and TOB1; the remaining 13 loci did not show variation within or between mink types. This low proportion of polymorphic loci is not unlike other mustelids, a study on functional genes in fisher (*Pekania pennanti*) reported 20% polymorphic loci (Greenhorn 2016). American mink displayed an average of four alleles per locus, while allelic diversity in fisher was three, showing an overall low variation in functional genes for both species. Furthermore, PCA revealed high similarity of functional allele frequency among mink populations at individual loci, regardless of which principal component was used, thus providing further evidence to the general trend of low variation in functional genes for American mink.

Androgen Receptor (AR) encodes a protein involved in the regulation of androgen-responsive genes (Bolton et al. 2007). AR is involved in male sexual development and has been linked to aggressive behaviour (Hurd et al. 2010; Butovskaya et al. 2015). While AR may influence various phenotypes, we speculate that artificial selection for mink with lower aggression levels may have caused the difference in AR allele frequency we observe between wild and domestic mink; for example, allele AR298 was more widespread in domestic populations than in the wild, and since domestic mink are becoming increasingly more docile, this allele may be associated with lower aggression level. Varying levels of aggression have been reported in domestic mink among different breeding lines, however these studies focus on aggression level change related to change in pelt colour and do not factor total number of generations in captivity

into their study (Trapezov 2000, Kulikov et al. 2016). Further work genotyping AR in mink of varying aggression levels between domestic mink with a known number of generations in captivity and wild mink can help to determine if allele AR298 is associated with lower aggression level.

Insulin-Like Growth Factor 1 (IGF-1) encodes a hormone similar in structure to insulin, and is involved stimulating cell growth and inhibiting cell death in almost every cell in the body (Murray et al. 2003; Davison et al. 2011). Mutations of IGF-1 can cause abnormalities in metabolism, stature, and hearing (Riguelme et al. 2010; Aguirre et al. 2016). Eight IGF-1 alleles of lengths ranging from 97-111 were found in wild mink, while domestic mink in Ontario exhibit only the three mid-sized IGF-1 alleles (103, 105, and 107). In fact, frequency of allele 103 was significantly different between wild and domestic mink. The shift towards allele 103 and other alleles in the mid-section of the IGF-1 allele length range seen in domestic mink may be a result of artificial selection for large size, though this is purely speculative.

Atrophin 1 (ATN1) encodes a protein thought to be involved in kinase binding, toxin receptor binding, and transcription co-repressor activity (Wood et al. 2000). The exact function of ATN1 is unknown, but an expansion of the trinucleotide repeat within this gene is responsible for dentatorubral pallidoluysian atrophy (DRPLA), a neurodegenerative disorder similar to Huntington's disease (Wood et al. 1998). Transducer of ERBB2 (TOB1) encodes a protein thought to function as a tumor suppressor (Zhang et al. 2016). Allele frequency differences between populations at either ATN1 or TOB1 can not be attributed a specific function, making interpretations of our results challenging.

Sequencing results from AR and ATN1 showed no non-synonymous change in repeat structure between alleles of the same size nor between alleles of varying size, providing no evidence for our hypothesis that wild mink have more imperfect repeats than domestic mink. This may be because captivity is not protecting mink from selection against mutations causing amino acid length change at these loci, contrary to our prediction.

Overall, wild American mink displayed a higher number of alleles, at both neutral and functional loci, than domestic American mink. In addition to artificial selection, this loss of variation in domestic mink, and the difference in allele frequencies between wild and domestic mink may be a result of other genetic mechanisms influencing domestication: natural selection, relaxed natural selection, inbreeding, and genetic drift. Natural selection accompanies artificial selection in captive populations, usually in the form of reproductive failure or increased infant mortality rate due to inbreeding depression (American Fur Breeder 1959; Price 1984; Belliveau et al. 1999). Reproductive failure has been linked to both genetics and environmental factors such as dietary deficiencies, parasitism, and disease (Hediger 1964; Belliveau et al. 1999). Inbreeding depression in mink, as resulting from breeding related individuals to produce a specific trait, has generated a reduction in fitness in offspring; for example, breeding mink for recessive colours has resulted in animals with a multitude of physical disorders (mainly skeletal and sight related) that are also easily susceptible to disease (American Fur Breeder 1959; Joergensen 1985; Belliveau et al. 1999). Also, American mink possess many traits that are thought to impede the domestication process: separated group structure, altricial young, extreme wariness to man, and extreme agility (Hale 1969). These behavioural characteristics are not advantageous to life in captivity, therefore it is

very likely that the first mink herds experienced intense natural selection in the first few generations following captivity. Today, any natural selection captive American mink may be experiencing is likely at a much lower intensity.

Captive mink do not compete for resources, avoid predation, or compete for mates. These behaviours important for survival in the wild lose their significance in captivity, and as a result, genetic and phenotypic variability for these traits are likely to increase (Price 1984). Relaxed natural selection may result in an increase in the level of deleterious alleles normally selected against in nature (Price 1984; Snyder et al. 1996; Lynch and O'Hely 2001). These deleterious alleles may have no effect in a farm environment but could have negative impacts on mink survival in the wild (Ryhmer and Simberloff 1996). Allele AR289 was found in only in domestic mink populations, and while it is possible the trait associated with this allele is the target of artificial selection, the fact that it was found in each domestic population regardless of breeding line suggests captivity has relaxed the natural selection normally acting on this trait in the wild. However, it is not known whether AR289 originated in captivity or existed in the wild before mink were sampled for this study.

In captive populations inbreeding can result in reduced genetic variability and fitness, the latter typically caused by the expression of deleterious alleles normally eliminated by natural selection (Price 1984). Breeding related individuals can increase mortality rates in captive populations, and in the case of domesticated animals, has been attributed to decreased egg hatchability, milk yield, and litter size (Falconer 1960; Ralls et al. 1979). Inbreeding is usually a chance phenomenon, however mink breeders may choose to breed related individuals in an attempt to obtain or maintain a particular characteristic (Price 1984; Belliveau et al. 1999). For instance, many pelt colours, known

as colour phases, are recessive to the standard brown and must be line bred, however this breeding strategy can result in inbreeding at loci controlling fur quality traits as well as at traits linked with such loci (Gregorius 1980; Belliveau et al. 1999).

Mink breeders may choose to breed related individuals in attempt to avoid introducing disease to the herd. Of particular concern is Aleutian disease, a highly pathogenic parvovirus known to cause overproduction of plasma, inflame kidneys, reduce fertility, increase mortality, and decrease immune function in mink (Nituch et al. 2011). According to a national survey, 32% of Canadian mink ranchers reported cases of Aleutian disease in their herds; in Ontario, Aleutian disease occurrence ranged from 14-60% between 1986 and 2006 (Nituch et al. 2011). However it should be noted that in both the national and provincial survey the participation rates were low and estimates might be biased (Nituch et al. 2011). Regardless, Aleutian disease is of high concern and mink ranchers often find the consequence of breeding related individuals less costly than the consequence of introducing disease via outbreeding.

Genetic drift is the change in frequency of an allele in a population due to random sampling. The phenomenon is unpredictable and tends to reduce genetic variability within populations while increasing genetic variability between populations (Dobzhansky and Pavlovsky 1957). Considering the colour-phase strategy in which mink are bred, genetic drift may affect mink differently according to their line and farm. Genetic drift may reduce variability within lines, but increase variability between lines. Similarly, drift may reduce genetic variability within a ranch, but increase variability between ranches. As a consequence of repeatedly reducing genetic variability, alleles of equal selective value may become fixed in a population. Genetic drift is a common occurrence in captive groups of animals founded by small isolated populations, therefore, due to the founding

strategy of the first captive mink herds previously discussed, captive American mink are highly susceptible to this genetic mechanism (American Fur Breeder 1959; Price 1984)

Domestic introgression into wild mink populations

Despite previous finding of high levels of mink introgression from farm into wild, we found no evidence to suggest that captive American mink are introgressing into wild populations at functional markers. In order to quantify introgression of functional genes from domestic mink into the wild, I used two analyses: first, a redundancy analysis (RDA) to examine how much of the variation in mink farm density explained the variation in allele frequency in free-ranging mink, and second, a spatial analysis of principal components (sPCA) to visualize genetic differentiation in free-ranging mink between locations near farms and untouched by farms. The RDA showed that mink farm density was not significant in accounting for the genetic variation seen in each functional locus, indicating that the allele frequency of wild mink populations were not affected by the presence of mink farms. The sPCA showed no clustering around farms, where we would expect to see the greatest level of differentiation between mink types, assuming that allele frequency of the mink escaping the farms is in proportion to the allele frequencies of the herd from which they originate.

Contrary to our hypothesis that free-ranging mink found in areas that have a high density of mink farms should exhibit different allele frequencies from mink in areas of low density of mink farms, these two analyses on AR, ATN1, IGF1, and TOB1 provide little evidence of mink farms impacting the allele frequencies of surrounding wild mink populations. Considering these markers are not highly differentiated between wild and domestic mink to begin with, this result is not entirely surprising. However, potential

introgression from farm into the wild should not be dismissed as studies done in mink using neutral markers show clear signs of introgression (Kidd et al. 2009). It is possible that the functional markers used in this study were not linked to the morphological traits that wild and domestic mink so plainly differ in, and had we used markers that influence domestic traits such as large size and non-brown colour, stronger evidence of introgression would be seen.

It is also possible that uneven sampling produced these results; according to Bayesian Clustering Analysis using neutral markers, of the 159 free-ranging mink used in the RDA only 9 were non-wild (6 domestic, 3 hybrid). This number of non-wild type mink may have been too low for an RDA or sPCA to register a signature of introgression. If 9/159 (5.7%) represents the true proportion of domestic American mink in the wild then sampling would not be an issue here, however, regarding that previous studies in Ontario report free-ranging populations composed of up to 78% domestic individuals it is likely that 5.7% underrepresents the true proportion of domestic mink in the wild (Kidd et al. 2009). With a sample set comprised of an accurate proportion of non wild-type free-ranging mink it is possible that the RDA and sPCA could have distinguished genetic variation based on mink farm density.

The study of domestication has been approached in two ways, first by comparing present day wild and domestic populations; and second, by observing phenotypic changes in a population of wild animals over generations of captive breeding (Price 1984; Trut 1999). The comparative approach assumes the present day wild population is representative of the wild ancestors of the domestic population, meaning that: (1) the contemporary wild population derived from the same group as the ancestors of the domestic population; and (2) the wild type has not undergone significant evolutionary

change since the ancestors of the domestic population were sampled (Price 1984). In order to meet the first assumption the ranches assessed in this study need to have been stocked with mink of Ontario ancestry, however it is unknown whether these captive herds are descendants of wild Ontario mink or of mink from other geographic areas. Regardless, the second assumption cannot be confirmed, as it is unknown if contemporary wild mink have undergone significant evolutionary changes since 1866. As such, the comparative approach is only a means of identifying differences between specific wild and domestic populations at a single point in time.

CONCLUSIONS

Changes to morphology, behaviour, and declines in genetic diversity commonly result when a species undergoes the process of domestication (Price 1984; Hansen 2002). American mink, though fairly new to captivity, are already exhibiting many of these qualities: domestic mink display a reduction in brain size and sexual dimorphism, and are less aggressive than their wild counterparts (Kruska 1996; Malmkvist and Hansen 2002; Kruska and Sidorovich 2003). Selection and other genetic mechanisms in captivity are different than those in a natural environment, which may result in animals with lowered fitness when introduced to the wild (Price 1984; Lynch and O'Hely 2001; Hansen 2002). If large numbers of domestic individuals escape repeatedly, as reported in Ontario with domestic mink, and interbreed with native populations, this could result in the introgression of maladaptive alleles into the wild which may lower the fitness of the native population (Hindar et al. 1991; Hansen 2002; Bowman et al. 2007). Our study on functional trinucleotide markers implies domestic American mink populations are not impacting native populations, though this result is limited by the fact that only four variable markers were discovered and these markers are not concretely connected to functions impacted by domestication. We report declines in both neutral and functional genetic diversity in domestic mink. Despite these declines, there is little evidence for significant allele frequency differences between domestic and wild mink in Ontario. As well, trinucleotide repeat structure does not differ between domestic and wild mink. Overall, we found little evidence to suggest significant functional genetic diversity between wild and domestic American mink in Ontario.

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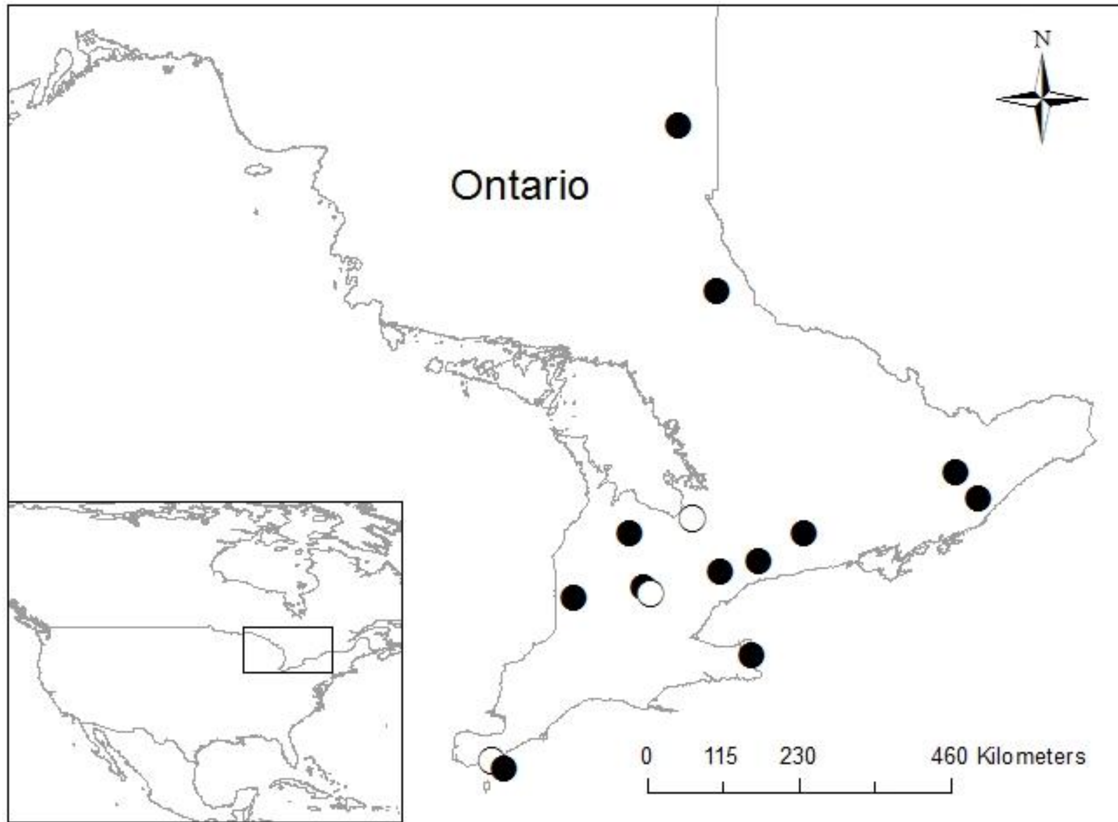

Figure 1. American mink samples were collected from 13 sites in Ontario from 2006-2015. Sites ranged from Kirkland Lake to Point Pelee Provincial Park. Black symbols represent the centroid of the sampling area and white symbols represent the centroid of the township the farm is located within.

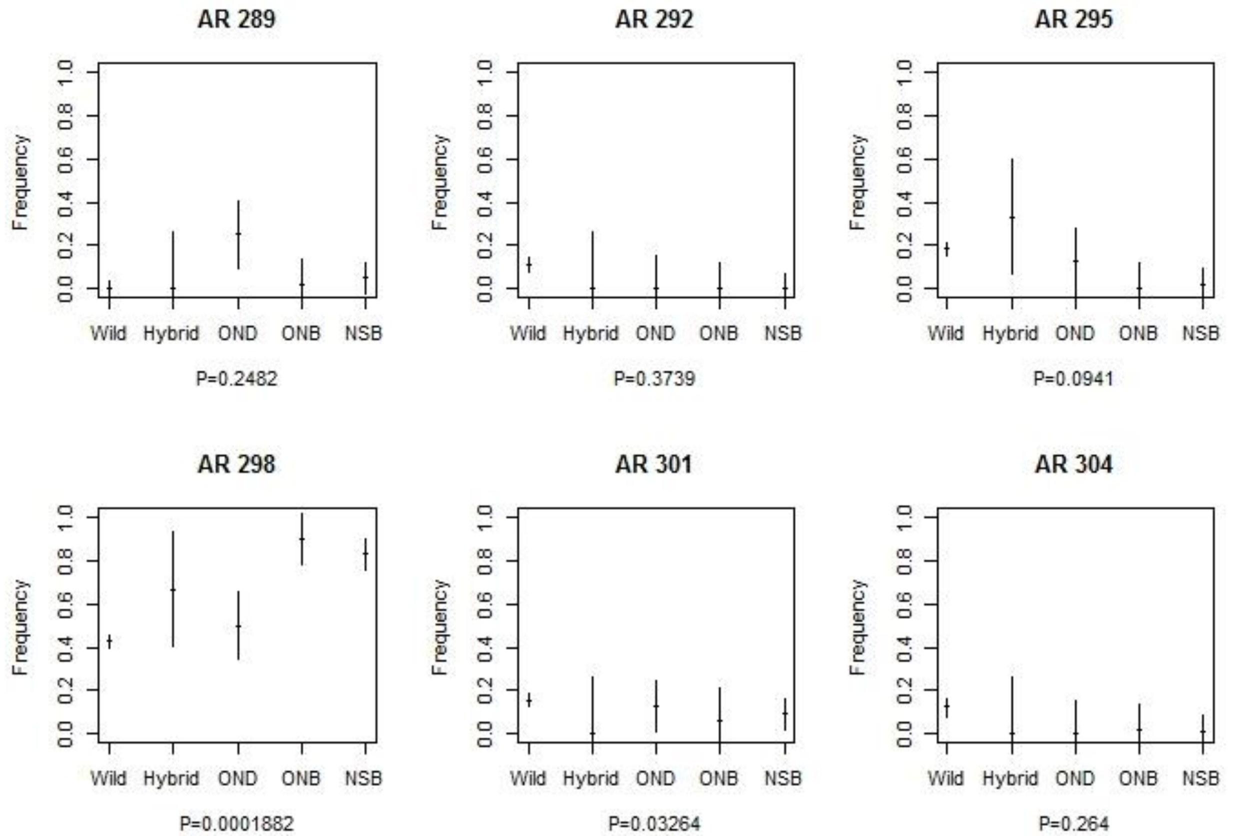

Figure 2. AR allele frequency distributions among wild, hybrid, domestic brown mink in Ontario (OND), domestic black mink in Ontario (ONB), and domestic black mink from Nova Scotia (NSB). Probability values below 0.0026 indicate significant difference among populations.

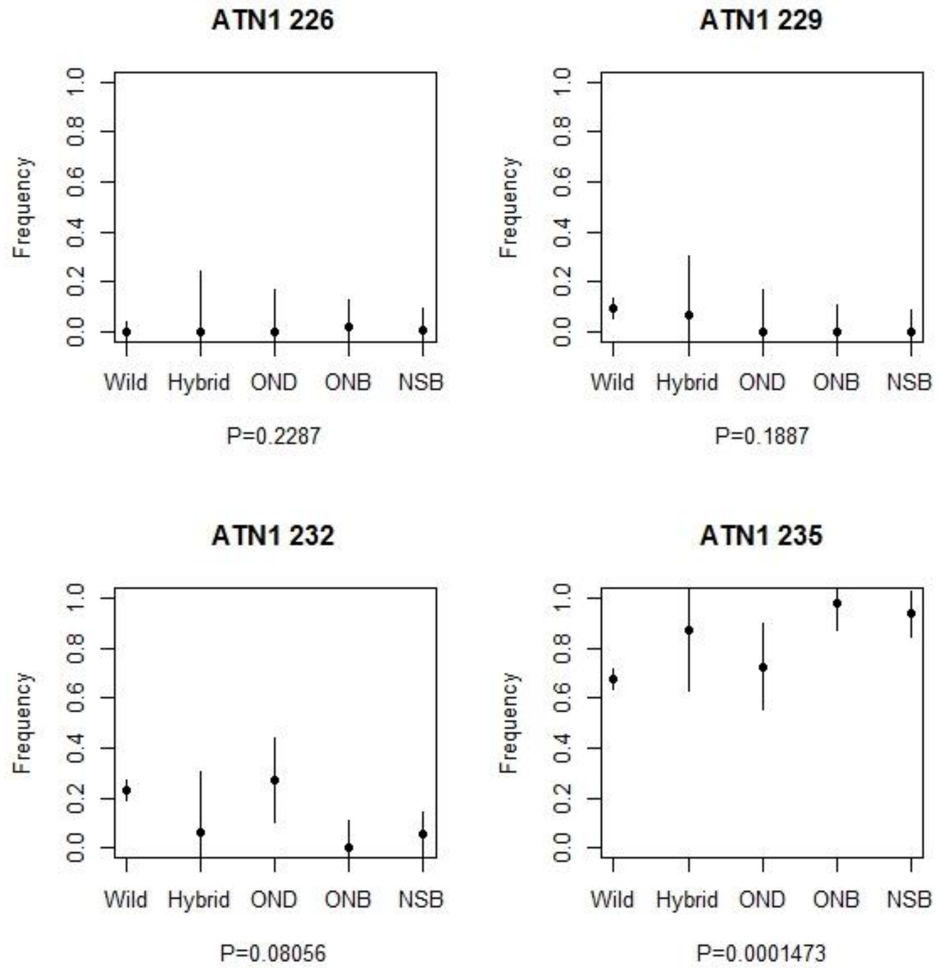

Figure 3. ATN1 allele frequency distributions among wild, hybrid, domestic brown mink in Ontario (OND), domestic black mink in Ontario (ONB), and domestic black mink from Nova Scotia (NSB). Probability values below 0.0026 indicate significant difference among populations.

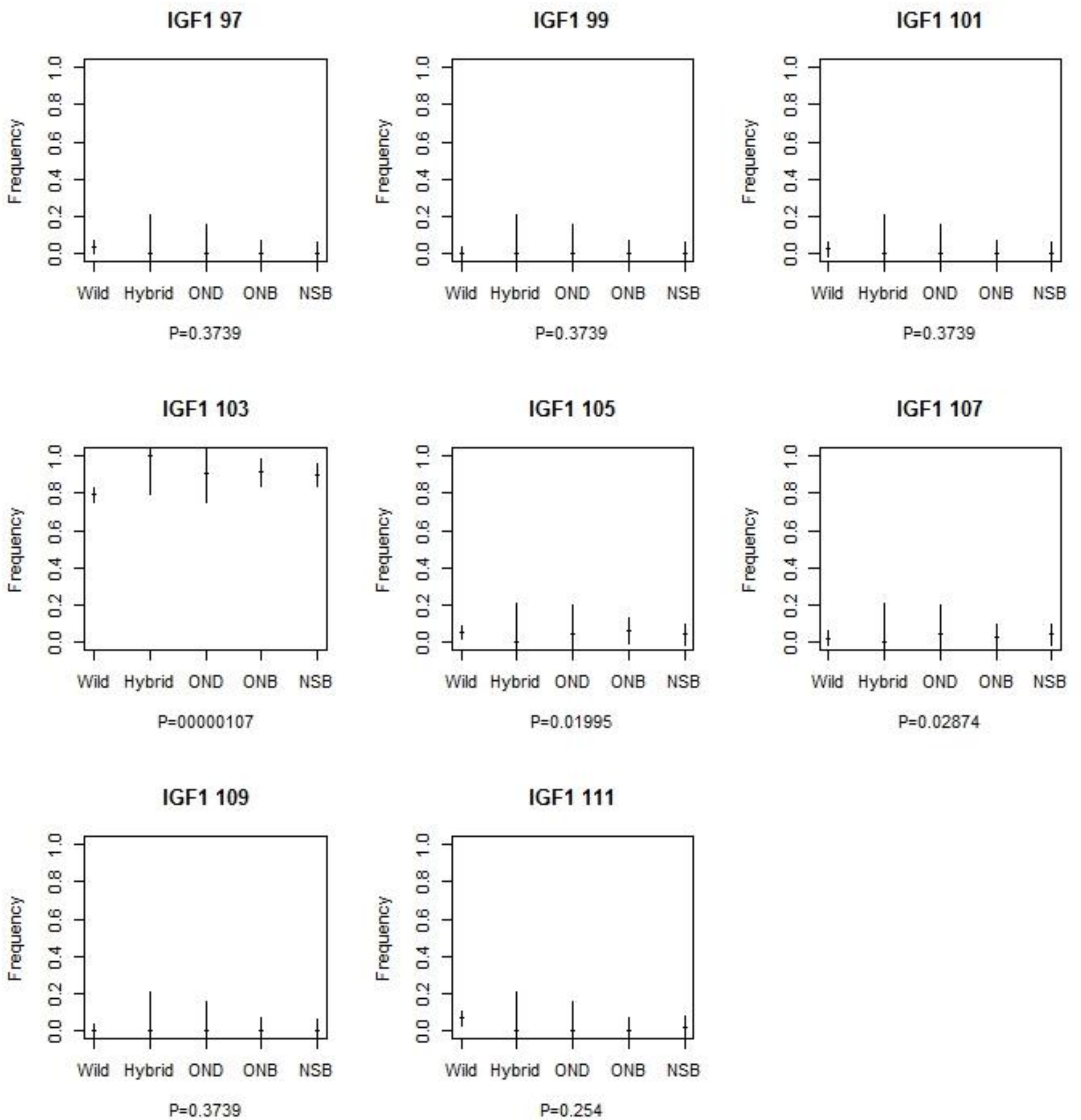

Figure 4. IGF-1 allele frequency distributions among wild, hybrid, domestic brown mink in Ontario (OND), domestic black mink in Ontario (ONB), and domestic black mink from Nova Scotia (NSB). Probability values below 0.0026 indicate significant difference among populations.

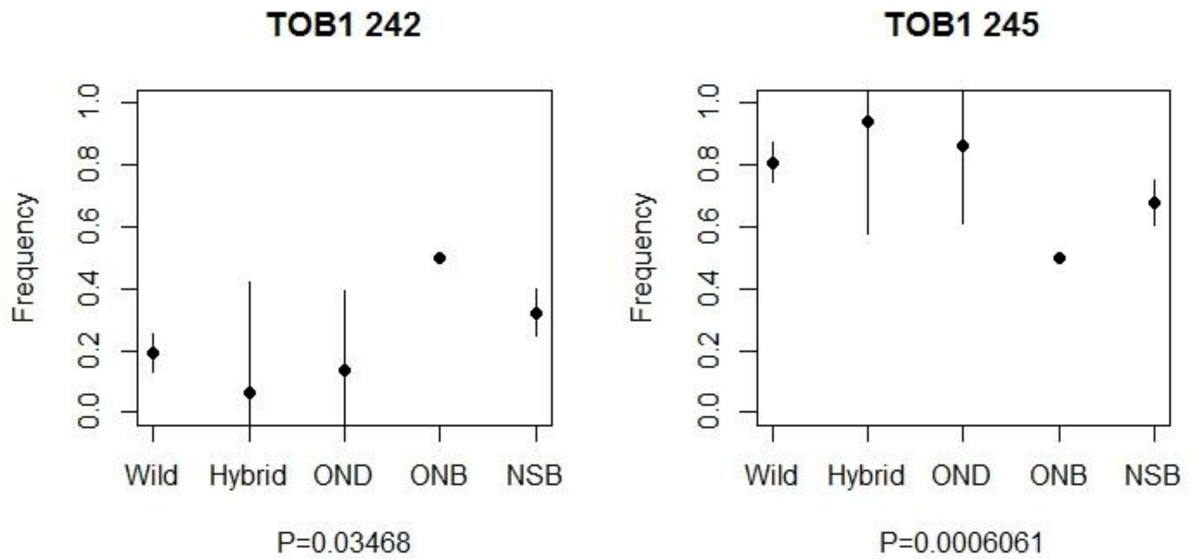

Figure 5. TOB1 allele frequency distributions among wild, hybrid, domestic brown mink in Ontario (OND), domestic black mink in Ontario (ONB), and domestic black mink from Nova Scotia (NSB). Probability values below 0.0026 indicate significant difference among populations.

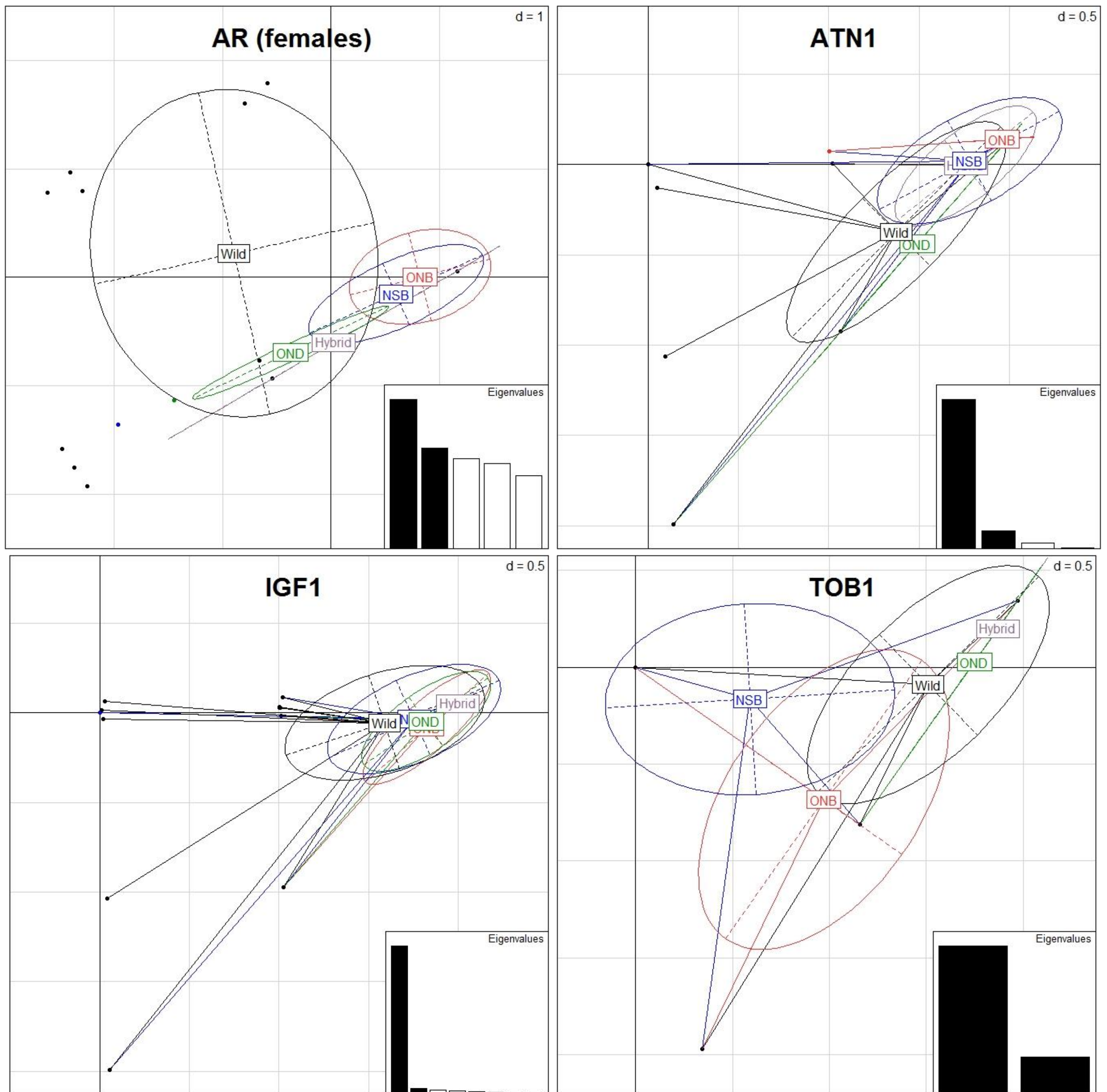

Figure 6. PCA (PC1 v. PC2) of AR (females only), ATN1, IGF1, and TOB1 allele frequencies by population. Non-overlapping ellipses indicate populations are genetically differentiated from one another.

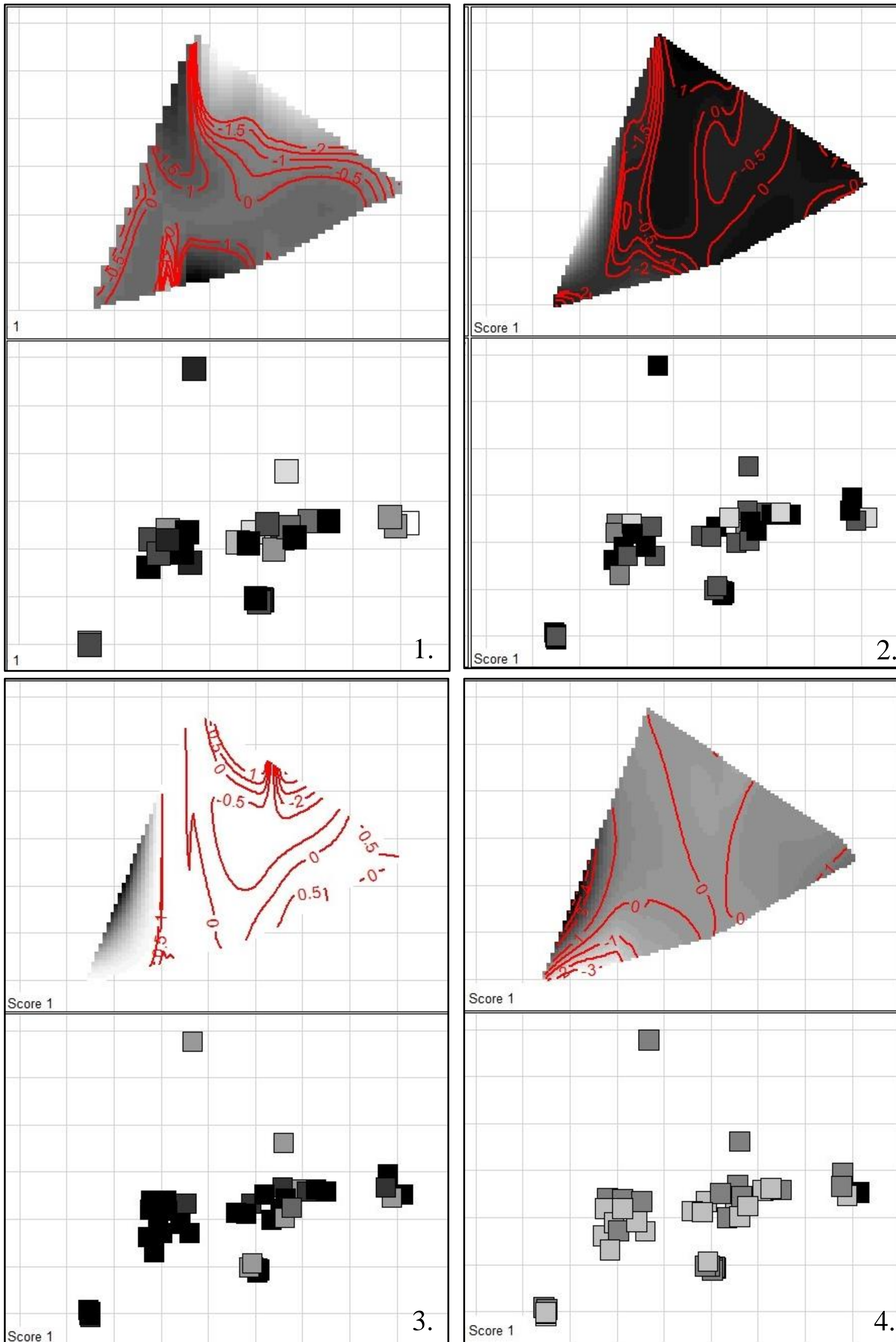

Figure 7. Spatial analysis of principal components of (1) AR (females only), (2) ATN1, (3) IGF-1, (4) Neutral loci, performed on free-ranging mink in Ontario. Genetic differentiation is steepest where contour lines are closest (top) and between large white squares and large black squares (bottom).

Table 1. Number of American mink retrieved from wild sampling locations and per domestic colour line.

Sites	County	Number of Mink
Wild	Bruce	5
	Durham	5
	Essex	6
	Grey	26
	Huron	6
	Kirkland Lake	47
	Leeds	5
	Niagara	7
	Nippissing	1
	Perth	5
	Peterborough	18
	Wellington	4
	York	9
Domestic Line	Nova Scotia (Black)	76
	Ontario (Black)	56
	Ontario (Brown)	11

Table 2. Primers, labels, and annealing temperature (T_a) used to amplify and genotype functional loci

Gene	Forward Primer	Reverse Primer	Label	T_a (°C)
ACTP	5'-ATG GGC GGG CCA GGG TTT TG-3'	5'-CAG AAA GGC CTC ATA GCG GTT CC-3'	6-Fam	52.4
APBB1	5'-ATC TGG CCT GAT CAT CAG C-3'	5'-TGG CAC TCT TGC TGT GAT CTC G-3'	6-Fam	63.8
AR	5'-GCT GAC TGT TGT TGG GAA GGC-3'	5'-GCC ATC CAA GAC CTA TCG-3'	Hex	61
ATN1	5'-TCT TAG CCA ACA GCA ATG C-3'	5'-GAA TGG TGG GAG CTA CTG CTC T-3'	Ned	55
DRD5	5'-GCC CTT CCG CTA TGA ACG C-3'	5'-CGA AGA CCC CCA TGA TCA CCG-3'	Hex	63.8
GRIN2B	5'-CGG ATA TCT ACA AGG AGC-3'	5'-CGA ACG TGT CGT ACG AGT GC-3'	Hex	56.4
HSPB7	5'-CTC CTC CAC CTT CAG AGC -3'	5'-GCT GCC AAA ATC CTC G-3'	6-Fam	50.4
HTT	5'-CCT CAA ATT GTA GAA ATG AAG GGC-3'	5'-GCC ACC ATC TTC AGA AGC-3'	6-Fam	63.8
IBSP	5'-CAT TAC CAT TTT CTG CCT CTG TGC-3'	5'-GCC TTA GTA TAG CCT GAG TTG-3'	Hex	61
IGF-1	5'-GGG TAT TGC TAG CCA GCT GGT-3'	5'-CAT ATT TTT CTG CAT AAC TTG AAC CT-3'	6-Fam	54.2
NEUR0D1	5'-CTC AGT TCT CAG GAC GAG GAG C-3'	5'-GCG CCT TAG CTT AAA ACG C-3'	Ned	61
NFE2L1	5'-CGA AGC CAT GCT GGA CGA GAT CAG C-3'	5'-GCA GAA CTT GGA GTA TTC GGG C-3'	Hex	63.8
NR1D1	5'-GTG TCA TCA CCT ACA TTG GC-3'	5'-GCT GGC AAT TTA CGC ACT GG-3'	6-Fam	63.8
PPP1R1B	5'-GCT ATG AAC TGG GAG GGG TGC-3'	5'-GCA TTG CTG AGT CGC ACC TGC-3'	Ned	63.8
RORC	5'-TGT CAA GTT TGG CCG CAT GTC-3'	5'-TCA GGC AGG TCA GGT GAA GAG-3'	Hex	61
TIMELESS	5'-AGC AGG GGC CAG AGG AAC AAG-3'	5'-AGG CTC GCA CAA CAG TTG AGC-3'	Ned	61
TNR	5'-GCT TTC TGG GCA GCA GTT GC-3'	5'-CGG TGG TCC TGA AGA ACA TGC-3'	Hex	63.8
TOB1	5'-GAG AGG ACT GAG GTT TAG GGG GC-3'	5'-GAG AGG ACT GAG GTT TAG GGG GC-3'	6-Fam	61

Table 3. Amplification success, annealing temperature, and observed alleles at 38 candidate trinucleotide loci.

Gene	Clean Amplification	Annealing temp (C)	Observed alleles (bp)			
			NSB	ONB	OND	Wild
ATN1	Yes	55	226, 232, 235	226, 235	232, 235	229, 232, 235
AR	Yes	61	289, 295, 298, 301, 304	289, 298, 301, 304	289, 295, 298, 301	292, 295, 298, 301, 304
IGF-1	Yes	54.2	103, 105, 107, 111	103, 105, 107	103, 105, 107	97, 99, 101, 103, 105, 109, 111
TOB1	Yes	61	242, 245	242, 245	242, 245	242, 245
ACTP	Yes	50	115	115	115	115
DRD5	Yes	58.9	467	467	467	467
GRIN2B	Yes	56.4	460	460	460	460
HSPB7	Yes	50.4	156	156	156	156
HTT	Yes	63.8	259	259	259	259
IBSP	Yes	61	247	247	247	247
NEUR0D1	Yes	62.7	229	229	229	229
NFE2L1	Yes	62.8	343	343	343	343
NR1D1	Yes	63.8	347	347	347	347
PPP1R1B	Yes	63.8	271	271	271	271
RORC	Yes	62.7	200	200	200	200
TIMELESS	Yes	64.7	262	262	262	262
TNR	Yes	63.8	465	465	465	465
ADAMTS1	No	NA	NA	NA	NA	NA
AKAP2	No	NA	NA	NA	NA	NA
APBB1	No	NA	NA	NA	NA	NA
ASCL1	No	NA	NA	NA	NA	NA
CHERP	No	NA	NA	NA	NA	NA
CLOCK	No	NA	NA	NA	NA	NA
DYRK1A	No	NA	NA	NA	NA	NA
HSP90AA1	No	NA	NA	NA	NA	NA
LCORL	No	NA	NA	NA	NA	NA
MECP2	No	NA	NA	NA	NA	NA
MLL2	No	NA	NA	NA	NA	NA
MTNR1B	No	NA	NA	NA	NA	NA
OXTR	No	NA	NA	NA	NA	NA
PAXIP1	No	NA	NA	NA	NA	NA
PER1	No	NA	NA	NA	NA	NA
PGC1B	No	NA	NA	NA	NA	NA
PPPARGC1B	No	NA	NA	NA	NA	NA
RXRB	No	NA	NA	NA	NA	NA
SLC6A4	No	NA	NA	NA	NA	NA
TRPC6	No	NA	NA	NA	NA	NA
ZNF804A	No	NA	NA	NA	NA	NA

Table 4. Allele frequencies for functional microsatellite loci in wild and domestic mink from three lines: Nova Scotia black (NSB), Ontario black (ONB), Ontario brown (OND).

Locus	Allele	Allele Frequency			
		NSB	ONB	OND	Wild
AR (females)	289	0.050	0.020	0.250	0.000
	292	0.000	0.000	0.000	0.109
	295	0.020	0.000	0.125	0.182
	298	0.830	0.900	0.500	0.427
	301	0.090	0.060	0.125	0.155
	304	0.010	0.020	0.000	0.127
ATN1	226	0.007	0.018	0.000	0.000
	229	0.000	0.000	0.000	0.094
	232	0.056	0.000	0.273	0.231
	235	0.938	0.982	0.727	0.675
IGF-1	97	0.000	0.000	0.000	0.035
	99	0.000	0.000	0.000	0.003
	101	0.000	0.000	0.000	0.024
	103	0.899	0.911	0.909	0.792
	105	0.041	0.063	0.045	0.052
	107	0.041	0.027	0.045	0.021
	109	0.000	0.000	0.000	0.003
	111	0.020	0.000	0.000	0.069
TOB1	242	0.323	0.500	0.136	0.191
	245	0.677	0.500	0.864	0.809

Table 5. Percentage of overlap between pairs of population PCA ellipses. Values represent the area (percentage) of Population A that is overlapped by Population B.

Locus	Population A	Population B				
		Wild	OND	ONB	NSB	Hybrid
AR (females)	Wild	-	6.46	3.34	5.66	0
	OND	82.88	-	19.99	40.37	0
	ONB	22.96	10.71	-	68.94	0
	NSB	41.7	23.21	74.02	-	0
	Hybrid	37.55	28.31	24.75	32.76	-
ATN1	Wild	-	0	0	43.09	40.49
	OND	100	-	100	100	100
	ONB	100	100	-	100	NA
	NSB	63.2	0	0	-	77.2
	Hybrid	75.2	0	0	95.32	-
IGF1	Wild	-	48.97	36.59	70.5	97.82
	OND	93.37	-	72.47	98.76	83.06
	ONB	91.15	94.7	-	93.29	92.54
	NSB	91.14	66.97	48.41	-	
	Hybrid	63.99	61.56	91.22		-
TOB1	Wild	-	0	53.07	25.03	0
	OND	96.92	-	33.89	0	0
	ONB	34.55	0	-	31.03	0
	NSB	19.39	0	36.93	-	0
	Hybrid	100	100	100	NA	-

Table 6. Microsatellite loci pairs under linkage disequilibrium.

Population	Loci		P
NSB	ATN1	Mvi072	< 0.001
	Mvi099	Mvi072	< 0.001
ONB	Mvi111	Mvi114	< 0.01
Wild	Mvi111	Mvi1272	< 0.001
	Mvi1003	Mvi4001	< 0.001
	Mvi1302	Mvi1321	< 0.001
	Mvi111	Mvi1016	< 0.001
	Mvi4001	Mvi2243	< 0.001
	Mvi1272	Mvi075	< 0.001
	Mvi4001	Mvi075	< 0.001
	Mvi1006	Mvi1354	< 0.001
	Mvi1302	Mvi1354	< 0.001
	Mvi1006	Mvi072	< 0.001
	Mvi1302	Mvi1342	< 0.001
	Mvi1321	Mvi1342	< 0.001

Table 7. Proportion of genetic variance of each locus explained by environmental variables (Farm Density, Road Density, Latitude, and Longitude) using redundancy analysis on free-ranging mink in Ontario. (*) Indicates variable is significant in explaining genetic variance.

Locus	Full (R^2_{adj})	Farm Density (R^2)	Road Density (R^2)	Latitude (R^2)	Longitude (R^2)
AR (females)	0.0235	0.0282	0.0183	0.0074	0.0413
ATN1	0.0116	0.0084	0.0045	0.0026	0.0211*
IGF-1	0.0271	0.0019	0.0050	0.0170	0.0088
TOB1	0.0297	0.0083	0.0425*	0.0214	0.0027
Neutrals	0.0411*	0.0066	0.0135*	0.0205*	0.0014*

APPENDIX A

Table A1. Allele frequencies for neutral microsatellite loci Mvi111, Mvi1272, Mvi1302, Mvi1016, Mvi114, Mvi4001, Mvi2243 and Mvi0072 in wild and domestic mink from three lines: Nova Scotia black (NSB), Ontario black (ONB), Ontario brown (OND).

Locus	Allele	Allele Frequency			
		NSB	ONB	OND	Wild
Mvi111	84	0.336	0.260	0.318	0.535
	88	0.000	0.010	0.000	0.004
	94	0.000	0.000	0.000	0.014
	96	0.022	0.130	0.045	0.106
	98	0.112	0.200	0.455	0.071
	100	0.358	0.230	0.136	0.071
	102	0.134	0.150	0.045	0.106
	104	0.000	0.000	0.000	0.025
	106	0.037	0.020	0.000	0.060
	108	0.000	0.000	0.000	0.007
Mvi1272	163	0.000	0.009	0.000	0.025
	165	0.014	0.000	0.000	0.000
	167	0.049	0.009	0.125	0.105
	169	0.141	0.151	0.188	0.091
	171	0.141	0.170	0.188	0.076
	173	0.246	0.236	0.188	0.362
	175	0.338	0.302	0.313	0.264
	177	0.035	0.057	0.000	0.040
	179	0.035	0.066	0.000	0.007
	181	0.000	0.000	0.000	0.014
	183	0.000	0.000	0.000	0.014
Mvi1302	207	0.198	0.177	0.182	0.018
	209	0.000	0.052	0.045	0.156
	211	0.009	0.000	0.000	0.018
	213	0.019	0.000	0.091	0.225
	215	0.255	0.135	0.182	0.283
	217	0.500	0.604	0.409	0.225
	219	0.000	0.010	0.091	0.043
	221	0.019	0.021	0.000	0.029
	223	0.000	0.000	0.000	0.004
Mvi1016	220	0.000	0.000	0.000	0.050
	222	0.118	0.214	0.273	0.340
	224	0.007	0.036	0.045	0.124
	226	0.000	0.000	0.000	0.032
	228	0.201	0.152	0.045	0.099
	230	0.361	0.339	0.364	0.113
	232	0.056	0.107	0.091	0.113
	234	0.236	0.152	0.000	0.128
	236	0.021	0.000	0.182	0.000
Mvi114	62	0.008	0.029	0.000	0.115
	68	0.371	0.337	0.227	0.216
	70	0.040	0.000	0.045	0.216
	72	0.000	0.000	0.000	0.011
	74	0.024	0.067	0.091	0.076
	76	0.331	0.385	0.591	0.115
	78	0.153	0.154	0.045	0.144
	80	0.065	0.029	0.000	0.065
	82	0.000	0.000	0.000	0.043
	84	0.008	0.000	0.000	0.000
Mvi4001	223	0.632	0.651	0.545	0.086
	225	0.044	0.028	0.091	0.032
	227	0.235	0.264	0.273	0.795
	229	0.081	0.057	0.091	0.011
	231	0.000	0.000	0.000	0.036
	233	0.007	0.000	0.000	0.040
Mvi2243	123	0.021	0.018	0.091	0.000
	127	0.000	0.000	0.000	0.004
	129	0.274	0.393	0.273	0.365
	131	0.000	0.000	0.000	0.022
	139	0.000	0.000	0.000	0.004
	141	0.000	0.000	0.000	0.007
	145	0.000	0.000	0.045	0.029
	147	0.021	0.027	0.182	0.113
	149	0.630	0.491	0.364	0.442
	151	0.000	0.000	0.045	0.000
	153	0.007	0.000	0.000	0.004
	155	0.048	0.071	0.000	0.004
	157	0.000	0.000	0.000	0.007
Mvi072	257	0.000	0.000	0.000	0.007
	259	0.000	0.000	0.000	0.014
	261	0.787	0.714	0.455	0.430
	263	0.096	0.188	0.136	0.113
	265	0.037	0.000	0.091	0.261
	267	0.074	0.080	0.318	0.106
	269	0.007	0.018	0.000	0.067
	271	0.000	0.000	0.000	0.004

Table A2. Allele frequencies for neutral microsatellite loci Mvi1321, Mvi099, Mvi1006, Mvi075, Mvi 1354, Mvi1342, and Mvi002 in wild and domestic mink from three lines: Nova Scotia black (NSB), Ontario black (ONB), Ontario brown (OND).

Locus	Allele	Allele Frequency			
		NSB	ONB	OND	Wild
Mvi1321	90	0.000	0.009	0.045	0.035
	92	0.048	0.064	0.000	0.052
	94	0.123	0.109	0.227	0.269
	96	0.438	0.445	0.409	0.339
	98	0.027	0.055	0.045	0.147
	100	0.075	0.036	0.000	0.073
	102	0.027	0.082	0.091	0.031
	104	0.212	0.200	0.182	0.042
	106	0.000	0.000	0.000	0.004
	108	0.048	0.000	0.000	0.007
Mvi099	320	0.000	0.000	0.000	0.004
	324	0.007	0.083	0.091	0.007
	326	0.014	0.000	0.000	0.011
	328	0.000	0.102	0.182	0.000
	330	0.079	0.056	0.227	0.000
	332	0.007	0.009	0.000	0.043
	334	0.000	0.000	0.000	0.004
	336	0.007	0.000	0.000	0.000
	338	0.236	0.287	0.091	0.193
	340	0.000	0.000	0.000	0.029
	342	0.086	0.093	0.091	0.386
	344	0.014	0.000	0.000	0.007
	346	0.086	0.074	0.182	0.018
	348	0.464	0.269	0.136	0.250
	350	0.000	0.000	0.000	0.004
	352	0.000	0.000	0.000	0.011
	354	0.000	0.009	0.000	0.021
	356	0.000	0.000	0.000	0.007
	358	0.000	0.019	0.000	0.000
	360	0.000	0.000	0.000	0.007
Mvi1342	138	0.246	0.313	0.182	0.405
	140	0.000	0.000	0.000	0.004
	142	0.000	0.009	0.000	0.015
	144	0.200	0.143	0.318	0.113
	146	0.115	0.054	0.227	0.135
	148	0.000	0.036	0.091	0.000
	150	0.408	0.420	0.136	0.047
	152	0.008	0.027	0.000	0.077
	156	0.008	0.000	0.000	0.164
	158	0.000	0.000	0.000	0.037
	160	0.000	0.000	0.000	0.004
	162	0.000	0.000	0.045	0.000
	164	0.008	0.000	0.000	0.000
	168	0.008	0.000	0.000	0.000

Locus	Allele	Allele Frequency			
		NSB	ONB	OND	Wild
Mvi075	105	0.000	0.000	0.000	0.003
	111	0.014	0.063	0.000	0.056
	113	0.000	0.000	0.000	0.003
	115	0.007	0.000	0.000	0.076
	117	0.336	0.384	0.364	0.122
	119	0.157	0.134	0.136	0.250
	121	0.057	0.036	0.136	0.083
	123	0.271	0.214	0.091	0.073
	125	0.043	0.116	0.136	0.038
	127	0.114	0.054	0.091	0.163
	129	0.000	0.000	0.045	0.080
	131	0.000	0.000	0.000	0.045
	137	0.000	0.000	0.000	0.007
Mvi1354	172	0.000	0.000	0.000	0.007
	176	0.000	0.000	0.000	0.209
	180	0.000	0.000	0.000	0.022
	182	0.007	0.000	0.000	0.212
	184	0.051	0.009	0.000	0.047
	186	0.232	0.393	0.227	0.007
	188	0.014	0.000	0.000	0.032
	190	0.072	0.080	0.000	0.155
	192	0.000	0.000	0.091	0.115
	194	0.543	0.393	0.545	0.112
	196	0.080	0.107	0.136	0.079
	198	0.000	0.018	0.000	0.004
Mvi1006	136	0.000	0.000	0.091	0.000
	144	0.000	0.000	0.000	0.004
	146	0.000	0.000	0.000	0.004
	150	0.324	0.264	0.182	0.077
	152	0.183	0.155	0.136	0.106
	154	0.303	0.418	0.545	0.278
	156	0.070	0.045	0.000	0.173
	158	0.021	0.009	0.000	0.099
	160	0.007	0.000	0.000	0.088
	162	0.007	0.018	0.045	0.092
	164	0.000	0.000	0.000	0.032
	166	0.021	0.082	0.000	0.039
	168	0.063	0.009	0.000	0.011
Mvi002	180	0.000	0.000	0.000	0.004
	182	0.014	0.000	0.000	0.017
	184	0.000	0.000	0.000	0.042
	186	0.978	1.000	0.909	0.909
	188	0.007	0.000	0.091	0.028

APPENDIX B

Table B. Number of alleles (Na), allelic richness (Ar), expected heterozygosity (He), and observed heterozygosity (Ho) for functional loci in wild and domestic mink from three lines: Nova Scotia black (NSB), Ontario black (ONB), Ontario brown (OND).

Population		NSB	ONB	OND	Wild
AR (females)	Na	5	4	4	5
	Ar	1.63	1.39	2.77	2.75
	He	0.300	0.186	0.656	0.732
	Ho	0.320	0.200	0.500	0.582
ATN1	Na	3	2	2	3
	Ar	1.73	1.27	2.00	1.79
	He	0.118	0.035	0.397	0.482
	Ho	0.097	0.036	0.364	0.469
IGF-1	Na	4	3	3	8
	Ar	2.30	2.04	2.46	3.46
	He	0.189	0.166	0.169	0.363
	Ho	0.176	0.179	0.182	0.347
TOB1	Na	2	2	2	2
	Ar	2.00	2.00	1.99	1.97
	He	0.437	0.500	0.236	0.309
	Ho	0.452	0.489	0.273	0.275