THE ASSESSMENT OF GENETIC VARIABILITY AND GENETIC DISTANCE IN NORTH AMERICAN MINK USING MICROSATELLITES

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ABSTRACT

Knowledge of genetic variability is helpful in designing proper breeding strategies for the mink industry. The genetic variability of 212 black mink from four breeding ranches in Nova Scotia, 20 from each of pastel and brown (wild-type) from one ranch in Prince Edward Island, and wild mink trapped in New Brunswick were assessed using seven microsatellite loci. Three of the black mink ranches (M1, M2 and M4) were chosen because of their long history of breeding (20 to 30 years), large herd size (> 1300 breeding females during the last 10 years), and limited gene flow between them. The fourth ranch (M3) was established in 1986 with a group of mink originated from 14 ranches in Nova Scotia, and has been maintained with approximately 100 breeding females. All the black mink ranches have been open to outside stock, and have used the Jetblack mink. The pastel and brown herds have been closed for 15 and 7 years, respectively. The average number of alleles per locus was 6.57 in the entire sample, and ranged between 4.4 in the wild mink and 5.1 in M1 and M2. The mean expected heterozygosity (H_E) was 0.63 over all populations and loci. The estimates of H_E were comparable among the black mink herds (0.53 to 0.61). The wild mink had the smallest H_E among populations (0.50), and the differences between M1 and brown mink were significant. These results indicate a considerable level of genetic variability within black mink, despite high levels of uniformity that have been achieved in fur quality traits as a result of many years of intense selection. The high level of genetic variability could be the result of continuous gene flow from outside sources, and the fact that ranched mink are a mixture of at least three subspecies of American wild mink. The brown mink had a significantly higher H_E (0.65) than that in M2 and M4, which could be the cause or the effect of higher vigour and reproductive performance of the brown compared to the black mink. All the populations showed a higher level of homozygosity than expected from the Hardy-Weinberg proportions at several loci, indicated by the significantly higher H_E than observed heterozygosity, and positive F_{IS} values. Linebreeding and positive assortative mating have possibly caused this phenomenon. Excess of homozygosity in the wild mink may indicate breeding between related individuals occupying adjacent territories, and a limited movement of mink in the wild. Three methods of assessing interpopulation genetic variability; the χ^2 tests of homogeneity of allele frequency distributions at each locus in each population, Cavalli-Sforza and Nei's genetic distances, and phylogenetic analysis, provided a similar profile of population divergence. The black mink herds were closely related to each other, as were pastel and brown. Gene flow from common sources to all the herds and infusion of the Jetblack allele during the last 20 years into all the ranches, were likely the causes of relatedness of the black mink herds. Contrary to the historical evidence on the time of divergence, the black mink herds were more closely related to the wild mink than to the colored mink. This panel of microsatellites correctly classified black and non-black mink into their respective populations in at least 92% of the individuals. The black mink were correctly assigned into their herd of origin with 66 to 82% precision.

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1.INTRODUCTION

Mink ranching began approximately 140 years ago when wild mink were trapped and bred in captivity (Nes *et al.* 1988). Today, Nova Scotia's mink industry is the second largest in Canada, after Ontario's (Fur Institute of Canada ND), with farm gate sales of $10 to $18 million between 1993 and 1996 (Johnson 1993, 1994, 1996, Johnson 1997 personal communication). Approximately 80% of Nova Scotia's mink production is located in Digby County, where other forms of agriculture are not feasible (Johnson 1994). In this county, mink production is a considerable source of income and employment (Mullen 1991c). Furthermore, approximately 85-90% of the mink diet comprises of non-competitive feed such as fish and slaughter waste (Johnson 1994).

Most of the mink raised in Nova Scotia are black, and have been intensely selected over many generations for fur quality traits using linebreeding and inbreeding (Prime 1991). The mink industry continuously tries to use mutations to produce new fur traits to satisfy the fashion industry's need for new material. One example is the Jetblack allele, a mutation which was discovered on Wallace and Edsel Mullen's farm in Nova Scotia in the 1960's and causes an intense black color favored by the fashion industry (Mullen 1991e). The Jetblack allele has been infused into a large proportion of mink populations world wide, which would reduce the genetic distance among many breeding farms. Intense selection, along with more uniform environmental and nutritional conditions compared with those in their natural habitat, may have led to reduction in genetic variability in farmed mink (Newman 1994).

Reduced genetic variability decreases response to selection and can lead to

reduced reproductive fitness (Falconer 1981). There is evidence that reproductive performance, particularly litter size, has generally shown a decline in Nova Scotia mink farms in the past two decades (Johnson 1997 persononal communication). In the wild, kits are born in litters of 5-8 (Collins 1981), and in farmed mink in Europe, the average is about 5 kits (Nes *et al.* 1988), while the average number of kits at weaning in Nova Scotia is 4 (Johnson 1997 personal communication). In addition, keeping mink under a rather constant environmental conditions and nutritional regimes may result in a decline in adaptability to changes in environmental conditions (Allard *et al.* 1968) which is important when mink are transported between farms.

When selection objectives are changing rapidly as a result of variable demands from the fashion industry, breeders do not have time to change fur quality by selection, and thus obtain the desirable breeding stock from other mink breeders whose mink pelts were sold at a high price at an auction. The consequences of gene flow from a few breeders is establishment of genetic links among the farms, reduced genetic distance, and a short term increase in genetic variability within farms purchasing the stock. Therefore, the selection schemes and breeding structure of the mink industry in Nova Scotia are very complex. There is limited published information on the genetic variability of farmed mink (Farid *et al.*1994).

Knowledge of baseline genetic information would help mink breeders to design breeding schemes and selection programs to accelerate genetic improvement of reproductive performance and fur quality traits. Lack of long term pedigree information in the mink industry makes it difficult to estimate genetic parameters using classical

approaches. DNA based methods are useful in estimating genetic parameters, as they are more accurate than analysing pedigree information. In this study, microsatellites were chosen because they are highy polymorphic, are scattered evenly throughout the genome, and are not directly selected for (Tautz *et al.* 1986; Levinson and Gutman 1987). In addition, the high mutation rates of microsatellite loci allow detection of genetic divergence among recently separated populations, as is the case in mink farming.

The objective of this study was to use microsatellites to estimate the genetic variability within and the genetic distance among four black ranched mink herds from Nova Scotia. It is difficult to enterpret the estimated levels of genetic variability and genetic distance of the ranched mink without having a reference population. Pastel and brown (wild type) mink from one ranch in Prince Edward Island, which have been separated from black mink for several decades, and wild mink trapped in New Brunswick, were used as reference populations. The wild mink were also used to determine the genetic effects of artificial selection and adaptation to captivity.

2. LITERATURE REVIEW

2.1. Effects of Selection and Mating Systems on the Genetic Variability of Ranched Mink

2.1.a. The American Wild Mink

The American wild mink are distributed thoughout Canada and most of the United States (Linscombe *et al.* 1982). Fifteen different subspecies have been identified in North America, based on differences in traits such as size, coat color and fur quality. This classification is somewhat arbritary and has not yet been confirmed by DNA analysis (Dunstone 1993). The original North American farmed mink were descended from three subspecies of *Mustela vison* (American mink); *M. vison vison* (found in Eastern Canada), *M. vison melampeplus* (Alaska) and *M. vison ingens* (Yukon, Alaska). Three other subspecies may have been raised in captivity and possibly contributed to the modern ranched mink. The American mink is an entirely different species than the European wild mink (*M. lutreola*) (Dunstone 1993).

The American wild mink have a variable coat color, ranging from light to dark brown with a light brown underfur and white markings under the chin, down the neck and on the belly. The weight of adult male and female wild mink range from 0.9-1.6 and 0.7-1.1 kg, respectively (Linscombe *et al.* 1982).

Mink reach puberty at nine months of age. They are seasonal breeders whose breeding season is controlled primarily by photoperiod through the release of melatonin from the pineal gland (Cochrane and Shackelford 1991). Mink breeding season starts at the beginning of March (Ashbrook 1928; Cochrane and Shackelford 1991; Mullen

1991a; Mullen 1991b; Rietveld 1991). The females are induced ovulators and ovulate 36 to 66 hours after mating (Madsen 1985). Matings last approximately 15 minutes, although shorter matings sometimes result in conception (Cochrane and Shackelford 1991; Dunstone 1993). Mink will usually return to estrus six to ten days after the first mating and sometimes again nine to ten days after the second mating (Hansen *et al.* 1985; Cochrane and Shackelford 1991). Females continue to ovulate after fertilization (superfecundation). Kits arising from the first, second and sometimes third ovulation periods may all be born in the same litter. If the female is mated to different males at the different estrous periods, the kits in a litter will likely be sired by more than one male, a phenomenon called superfetation (Cochrane and Shackelford 1991, Dunstone 1993).

In their natural habitat, males and females occupy their own territory, which ranges on average between 1-5 km^2 for males and 0.3-2 km^2 for females, and will only join together for breeding. Males search for females during breeding season, and often several males compete for a female. Each female is usually bred by more than one male (Dunstone 1993).

The duration of pregnancy in mink is from 39-92 days with the average being 49 days (Madsen 1985). This variation in the gestation length is due to the variation in the time of ova implantation. The fertilized ova of the mink from the different mating periods undergo embryonic diapause at the blastocyst stage before they implant all at the same time in the uterus around March 21st. This is called delayed implantation and is controlled by photoperiod (Cochrane and Shackelford 1991). Kits are born from the end of April until the beginning of May (Mullen 1991a; Rietveld 1991).

2.1.b. Breeding Practices in the Farmed Mink

Estrus can only be determined by the female's willingness to accept the male and she will display sexual receptivity for twelve to forty-eight hours after mating. Ranched mink are usually remated nine or ten days after the first mating (Mullen 1991a, 1991b). Some ranchers mate the females two days in a row in the first cycle and two days in a row in the second cycle (Rietveld 1991) although some feel this is not a very prudent practice because ova which have just been fertilized can be destroyed (Madsen 1985). Some breeders use a different male for the second mating to improve conception rate in the female. It is assumed that the progeny are sired by the second male. This is based on the notion that if the female is mated the day after the first mating, 75% of the progeny are sired by the second male, and when the female is mated 7-10 days after the first mating, approximately 90% of the progeny arise from the second male (Madsen 1985). In such cases, kits born in a litter could be sired by different males (Madsen 1985) and the pedigree information is not accurate for population genetic studies and genetic evaluation purposes. Female to male ratio in the ranched mink is usually 5:1 (Hansen *et al.* 1985; Mullen 1991b), which could potentially result in a lower rate of inbreeding per generation than that in other livestock species, such as cattle, sheep, pigs, etc., in which male to female ratio is much higher.

2.1.c. Selection and Mating Systems

North Americans were the first to capture and farm wild mink in the 1800s (Nes et al. 1988; Bowness 1996). The original mink trapped from the wild were small and had

variable fur characteristics, while the fashion industry, the ultimate market for fur, required uniform pelts of high quality. The most important traits were size, clarity, shade, nap length, silkiness of the guard hair, and texture and elasticity of individual fibers. In black mink, darkness of the fur is an additional trait with considerable importance (Hilleman 1985; Mullen 1991a). Mink breeders over the years have tried to improve the above traits to satisfy the market demands by following many different strategies (Prime 1991; Frye 1991; Rietvald 1991). The two major approaches to achieve this goal in Nova Scotia seems to be selection within a closed herd and/or the purchase of breeding stock from other sources.

Selection within a closed herd, at least for several generations, has been practised by some breeders in Nova Scotia (Prime 1991; Table 3.2). Body size and general appearance have higher heritability (0.08 to 0.18 and 0.2 to 0.43 in black males, respectively) (Kenttamies and Vilva 1988) than reproductive traits (0.05 to 0.16) (Einarsson 1988), and thus are expected to show a higher selection response and a lower inbreeding depression compared with reproductive traits (Einarsson 1988; Kenttamies 1988; Borsting 1988). This, along with the fact that fur quality traits have a considerable effect on sale price, caused many breeders to intensely select for fur characteristics while ignoring reproductive performance, especially during the early days of fur farming (Jones 1913; Ashbrook 1928; Hodgson 1937; Einarsson 1992). Linebreeding has been recommended in the past, and is still being practised, as a mean of improving size and fur quality traits in mink and foxes while maintaining high levels of uniformity within lines (Smith 1931, 1935; Gunn 1941; Lange 1983; Ellis et al. 1992; Leonard 1966;

Hansen *et al.* 1985; Prime 1991). Positive assortative mating has also been recommended while outbreeding was frowned upon because of the loss of uniformity in the kits (Hodgson 1937). Intense selection, linebreeding, and positive assortative mating are expected to increase inbreeding and reduce the genetic variability within closed herds (Mather 1973; Falconer 1981; Rumball *et al.* 1994; Roden 1995). The rate of inbreeding is inversely related to the population size (Allard *et al.* 1968) since there is a greater chance that relatives mate. With selection, the inbreeding effect is accentuated (Pirchner 1969) by the fact that a small number of superior animals, which are often related to each other, will be used for mating and are more likely to produce offspring which will also be used for breeding in the next generation (Robertson 1964). This is especially true in the mink industry where fur quality traits are heavily selected for and positive assortative mating has been practised.

One of the consequences of closing breeding herds is the creation of allele frequency differences, which can be expressed as genetic distance. Allele frequency differences are brought about by the founder effect, random genetic drift, selection and mutation. Founder effect would be large when a mink herd is established with a small number of animals (Falconer 1981), which seems to be the case in most of the breeding farms in Nova Scotia (Mullen 1991d). Fur breeders are unable to simultaneously select for the large array of economically important traits. Placing different selection pressure on various traits will result in differences in allele frequecies of the genes controling those characteristics. Mutation is another force which could result in allele frequency differences between closed herds, but its effect is small unless herds are kept closed for a

long period of time or when the mutation rate of a locus is very high, such as with microsatellites (Roy *et al.* 1994).

The second strategy for improving fur quality is based on the purchase of breeding stock. One of the main goals of a mink breeder is to keep up with the demands for particular types of furs at a particular time. The breeders do not have time to establish new lines and to wait for selection to result in a considerable change in a particular trait, and they must be able to respond rapidly to such a fluctuating market. Purchasing breeding stock from those breeders whose mink fur is favored by the fashion industry in a particular year is often practised (Pedersen 1985). One example is the Jetblack allele, which was discovered on the Mullen's farm in Nova Scotia in the 1960's and causes an intense black color favoured by the fashion industry (Cochrane and Shackelford 1991; Mullen 1991a). This gene has been infused into a large number of black mink populations in North America and other countries (Mullen 1991e). The process of gene flow from a few breeding herds to many other fur ranches may have increased genetic variability within and reduced genetic distance among mink farms in the short term.

Therefore, the effects of these two breeding strategies on genetic variabiltiy within herds and genetic distance between herds are opposite. The level of genetic variability of the mink populations in Nova Scotia, and in Canada as a whole, has not been studied in detail. The only published information indicates a considerable level of genetic variability within and a high level of genetic relatedness between two breeding herds in Nova Scotia (Farid *et al.* 1994).

The result of approximately 100 years of selection is approximately 30 different

coat colors, and a uniform, high quality fur (Nes *at al.* 1988). Ranched mink also have a heavier body weight, which is caused mainly by diet, with males weighing between 1.9-2.1 kg and females weighing 0.9-1.1kg, (Rouvinen *et al.* 1996; Rouvinen *et al.* 1997). Keeping mink in captivity and intense selection have brought about other changes. For example, the skulls of ranched mink are larger, have a shorter palate and have a narrower postorbital constriction compared to the wild mink, and the difference between sexes for the skull size in the ranched mink is smaller than that in the wild mink (Lynch and Hayden 1995). It was speculated that the reduced sexual dimorphism in the ranched mink may be the consequence of relaxed sexual selection due to lack of competition between males for mates, lack of competition for resources, and selective breeding for large body size (Lynch and Hayden 1995). It has also been shown that the ranched mink has a 20% smaller brain than the wild (Kruska 1996), possibly due to differences in environment and adaptation to captivity.

2.2. The Effects of Genetic Variability on Fitness, Response to Selection, and Adaptability

2.2.a. Reproductive Fitness

The fitness of an individual is the contribution of genes that it makes to the next generation, or the number of its progeny represented in the next generation (Falconer 1981). In general, as inbreeding increases over generations and the genetic variability decreases, homozygotes appear less reproductively fit than heterozygotes (inbreeding depression) (Gruneberg 1954; Pirchner 1969; Mackay 1979; Rumball *et al.* 1994).

There is limited published information on the effects of inbreeding on economically important traits in fur bearing animals. Estimates of heritability for reproductive traits in mink and foxes are low, ranging between 0.05 and 0.16 (Einarsson 1988; Lagerkvist 1992), indicating that the inbreeding depression is expected to be high (Falconer 1981). Berg (1994) found a small effect of direct inbreeding on the number of stillborn kits and early survival. However, Berg (1996) reported a significant decline in litter size (total born, kits born alive, kits at 3 weeks) due to maternal and direct inbreeding. Many strains of dark or black mink have been so intensively inbred in an attempt to increase darkness and fur quality that they have increased fertility problems, possibly due to male sterility and reduced ovulation rate (Hansen *et al.* 1985). It is known that many mutant color genes in mink and foxes reduce fertility and viability when in the homozygous state, including very dark black, very lustrous and shadow genes (Sundqvist *et al.* 1989). In blue foxes, another species used for fur production, Nordrom (1994) found that maternal and fetal inbreeding impaired reproductive performance.

Although reproductive efficiency, measured as the number of kits at pelting time per breeding females housed, would have a large impact on the output of any fur farm (Kenttamies and Vilva 1988; Ellis *et al.* 1992; Berg 1994), it has only recently been considered as a trait that must be improved (Einarsson 1992). Scientists working with fur animal species have recently begun to caution producers that inbreeding would result in increased incidence of mortality due to recessive lethal genes, decline in vigour, reduced litter size, slow early growth and reduced lactation capacity (King 1989; Ellis *et al.*

1992). When the pelt market is depressed, improving production efficiency becomes particularly vital to any rancher, and reproductive efficiency becomes one of the most important traits to be considered for genetic improvement. Genetic progress in reproductive performance will be hampered if mink herds have limited genetic variability and high levels of inbreeding.

2.2.b. Response to Selection

The response to selection (R) is a function of heritability (h^2), intensity of selection (i), and additive genetic standard deviation (σ_A), i.e. $R=hi\ \sigma_A$. Genetic uniformity will reduce the response to selection by diminishing the σ_A (Beardmore and Levine 1963; Falconer 1981). Roden (1995) found that in an open flock of sheep, the maintenance of genetic variability contributed to a higher selection differential ($i\sigma_P$, where σ_P is the phenotypic standard deviation) and therefore an improved selection response over a closed flock. In an extreme case, there is no response to selection in a pure line because there is no genetic variability (Spiess 1989). If the genetic variability within mink herds becomes very limited, response to selection will decrease.

2.2.d. Adaptability to Variable Environments

It has been speculated that loss of genetic variability due to intense selection or inbreeding, in a constant environment, allows populations to achieve adaptedness to their immediate environment. Such populations, however, lose flexibility to adapt to other environments (Allard *et al.* 1968). Individuals of populations exposed to variable

environments are more fit with respect to adaptability than those in uniform environments (MacKay 1979). Since ranched mink are kept under more uniform environmental and nutritional conditions compared to the wild mink, it is possible that the former have lost the ability to adapt to a different and/or variable environments. This may be a matter of concern when transporting breeding stock to other locations with different environmental conditions.

2.3. Means of Assessment of Genetic Variability

2.3.a. Phenotype

Phenotypic variability is a function of genetic variability as can be noted in the classic formula $\sigma^2_P = \sigma^2_G + \sigma^2_E + \sigma^2_{GE}$, where σ^2_P, σ^2_G, σ^2_E and σ^2_{GE} are the phenotypic, genetopic, environmental, and genotype-environmental variances (Falconer 1981). As the genetic variability increases, so does phenotypic variability if the environment remains constant. Phenotypic variance, however, is not a very accurate measure of the true genetic variability of a population unless the environmental variance is removed (Falconer 1981). Genetic variability of a trait can be estimated if large amounts of performance and pedigree information are available.

2.3.b. Allozymes

Allozymes are different forms of the same enzyme which have been used for years to assess genetic variability and genetic distance (Queller *et al.* 1993). A homogenized tissue is electrophoresed through a gel and an enzyme-specific reaction

highlights one locus whose alleles may migrate to different distances due to charge differences (Queller *et al.* 1993). Allozymes have been used for paternity identification and assessing genetic variability, but their usefulness is limited due to low variability. This is especially the case in large mammals, who typically possess limited protein variation (Bancroft *et al.* 1995). Low variability of allozymes is the result of low mutation rate of the functional genes, reduced zygote viability of some mutations, the redundancy of the genetic code, and the fact that not all amino acid substitutions result in different electric charges (Scribner *et al.* 1994). Allozyme analysis also requires fresh or carefully frozen tissue, which is a difficult requirement when samples are collected from remote areas (Bentzen *et al.* 1991). The variability among mink ranches for allozymes of esterase have been reported (Simonsen *et al.* 1992). Allozymes of blood plasma proteins have also been used to determine genetic variability in ranched foxes (Niini *et al.* 1992).

2.3.c. Multi-locus DNA Fingerprinting

2.3.c.i. Overview

There are two multi-locus fingerprinting tools, in which alleles from many loci are simultaneously identified in each sample. The large number of bands of complex patterns limits the usefulness of multi-locus DNA fingerprinting techniques when information is desired on the mode of inheritance and variability of each allele (Bentzen *et al.* 1991). Also, specific bands cannot be associated with specific loci (Lynch 1988; Scribner *et al.* 1994) and fragments with low molecular weights will usually go undetected (Lynch 1991). Multi-locus fingerprints reveal many bands of varying

intensities and similarly sized alleles from different loci which cannot be distinguished from each other (Queller *et al.* 1993; Lynch 1988).

2.3.c.ii. Random Amplification of Polymorphic DNA - Polymerase Chain Reaction

One genetic marker which is easy to generate is RAPD (random amplification of polymorphic DNA). This is the amplification of DNA loci by the polymerase chain reaction (PCR) with a single, short, random-sequence oligonucleotide primer. The PCR products are separated on agarose or polyacrylamide gels and visualized with ethidium bromide or silver staining, respectively (Rafalski and Tingley 1993). Problems associated with RAPDs are the presence of many bands of varying intensities, faint bands, and low reproducibility, i.e. slight variations in the experimental procedure can produce erratic results which can lead to incorrect decisions about band identities (Queller *et al.* 1993). In addition, RAPD markers are inherited as dominant alleles (presence or absence of a band), which makes it impossible to differentiate between homozygotes and heterozygotes (Queller *et al.* 1993; Lynch 1988). RAPD markers have been used for paternity indentification in multiple-sired mink litters (Xiong *et al.* 1992).

2.3.c.iii. Minisatellites

Minisatellites are a class of VNTRs (variable number tandem repeats), in which the tandem repeats are typically 9-65 base pairs long. Variability arises from the differences in the size of repeats (Queller *et al.* 1993; Scribner *et al.* 1994). Genomic DNA is cut with restriction enzymes, electrophoresed, blotted to membranes and probed

with radiolabelled DNA that hybridizes to the minisatellite sequence (Queller *et al.* 1993). Minisatellites are not distributed randomly in the genome and so are not very appropriate for certain genetic techniques such as gene mapping (Goodfellow 1993).

2.3.d. Single-Locus DNA Fingerprinting

2.3.d.i. Overview

In single-locus DNA fingerprinting, only one (homozygote) or two (heterozygote) bands are observed for each sample. Therefore, the inheritance of the alleles at a particular locus can easily be studied and the information obtained is more useful than that of multi-locus fingerprints (Queller *et al.* 1993). Also, single locus fingerprints allows the heterozygosity to be estimated for each locus, whereas only average heterozygosity over all loci can be estimated with multilocus fingerprints (Stephens *et al.* 1992).

2.3.d.ii. Restriction Fragment Length Polymorphisms

RFLP's (restriction fragment length polymorphisms) remain useful in research applications since they provide genetic information at a single locus (Goodfellow 1993; Rafalski and Tingey 1993). These are produced from the cutting of genomic DNA by restriction endonucleases that recognize and cleave specific sequences. There are usually hundreds or thousands of recognition sites throughout the genome for each restriction endonuclease. Therefore, many fragments of varying lengths are produced which can be separated by electrophoresis. A labelled probe based on cloned DNA specific for a

particular sequence of the genome is then hybridized to the digested DNA to identify different fragments (Kennedy *et al.* 1990). However, a relatively large amount of DNA is needed, and polymorphism is low because the majority of RFLP markers are caused by the loss or gain of a specific restriction enzyme site and have only two forms (Goodfellow 1993; Rafalski and Tingey 1993). DNA can be amplified by PCR, and then digested with restriction enzymes (RFLP-PCR). This technique eliminates the need for Southern blot hybrisization which involves the use of radioactive materials. Furthermore, it is fast, and small amounts of DNA can be used.

An ideal marker should be highly polymorphic and exist in many different alleles in order to maximize the chance of recognizing segregation in any particular family. RFLP's produce a large number of monomorphic alleles, and even when alleles are polymorphic, their frequencies are low. Also, RFLPs are not distributed evenly across the genome (Kennedy *et al.* 1990).

2.3.d.iii. Microsatellites

These are short repetitive DNA sequences of mono- di- tri- or tetra nucleotide repeats, flanked by unique DNA sequences that serve as primers for PCR (Ellegren 1992; Seriwaka *et al.* 1992). The tandem arrays are composed of repeats of 10 to more than 100 base pairs long. Each set of repeats is an allele, and differences between alleles are generated by variation in the number of repeating units (Crawford *et al.* 1991; Seriwaka *et al.* 1992; Buchanan *et al.* 1994). There are limitations on the number of repeats in polymorphic alleles, and the higher the number of repeats, the more unstable

the microsatellite becomes (Valdes *et al.* 1993; Holmes 1994).

Microsatellites have several attributes which make them ideal as genetic markers for population and quantitative genetic studies. First, they show extensive allelic variation and high levels of heterozygosity (Montagutelli *et al.* 1991; Scribner *et al.* 1994) which approaches 90 % (Ellegren 1992), as a result of high mutation rates (Estoup *et al.* 1995). Secondly, they are present in high copy numbers in the genome and are distributed evenly in the genome at approximately every 10 Kb (as opposed to minisatellites, which are located in subtelometric regions of the chromosome (Estoup *et al.* 1993). Third, they are inherited in a Mendelian fashion as codominant markers (Bentzen *et al.* 1991; Cohen *et al.* 1992). Therefore, each animal will show either one band (homozygous) or two bands (heterozygous) for that particular allele on the gel (Choudhary *et al.* 1993; Buchanan *et al.* 1994) and alleles usually differ in size by integer multiples of the repeat unit (Choudhary *et al.* 1993). Since all different forms of the allele can be detected, it is easy to compute allelic frequency, genetic variability, genetic distance and test for Hardy-Weinberg equilibrium (Buchanan *et al.* 1994). Fourth, microsatellites are usually selectively neutral, although they might be linked with functional genes. Some human diseases, such as myotonic dystrophy, spino-bulbo-muscular dystrophy, and fragile-X mental retardation syndrome, are the result of individuals possessing microsatellite alleles which are much larger than the strict upper limit in the number of repeat units which normal individuals have. The mechanism for instability of repeats is presently unknown (Valdes *et al.* 1993). Since microsatellites are amplified by PCR, highly degraded DNA can be used, including dried or alcohol preserved specimens, and

archived tissues (Bentzen *et al.* 1991; Bruford and Wayne 1993). As well, far less tissue sample is required than with other methods (Choudhary *et al.* 1993).

Microsatellites are easy to compare across gels. A sequencing ladder run on the same gel can provide a reproducible standard at every base position, and even without this standard, alleles can be identified using the bands of several reference individuals (Bruford and Wayne 1993). PCR primers can be chosen to ensure that different microsatellite loci produce PCR products of varying sizes so that they will run to distinct areas of the same gel (Bruford and Wayne 1993). The development of primers is time consuming and costly as the DNA sequence flanking the microsatellite must be known (Rafalski and Tingey 1993; Thomas and Scott 1994). However, when the sequences of primers specific for a given microsatellite marker are published, the technology is made available to the whole scientific community without any need to distribute clones or other materials (Rafalski and Tingey 1993).

A drawback of using dinucleotide repeats is the shadow or spurious bands observed when PCR products are resolved on a gel (Choudhary *et al.* 1993; Steffens *et al.* 1993). These shadow bands may arise from slippage during replication in the PCR reaction (Choudhary *et al.* 1993). However, this problem does generally not interfere with accurate reading of results, and can be eliminated by optimizing the conditions for amplification for each individual microsatellite, and then carrying out the correct number of amplification cycles at the proper annealing temperature (Steffens *et al.* 1993). Another problem which may be encountered is the presence of null alleles. If there is any mutation in the region complementary to one of the primers, the annealing will be

inhibited, resulting in the amplification of only one or no allele. This may lead to mistyping of heterozygotes as homozygotes, and underestimation of observed heterozygotes (Bruford and Wayne 1993; Callen *et al.* 1993). The problem can be overcome by the synthesis of new primers (Callen *et al.* 1993).

High levels of polymorphism makes microsatellites highly suited for genome mapping, paternity testing, and population genetic studies (Estoup *et al.* 1995). Microsatellites are especially important in species which are characterized by low levels of genetic variation resulting from small populations (Paetkau and Strobeck 1994). Genetic variability, genetic distance and other genetic parameters have been assessed using microsatellites in a very diverse range of species such as wolves (Roy *et al.* 1994; García-Moreno *et al.* 1996), sheep (Buchanan *et al.* 1994), cows and goats (Pepin *et al.* 1995), coyotes (Roy *et al.* 1994), humans (Bruford and Wayne 1993), snails (Jarne *et al.* 1994), black bears (Paetkau and Strobeck 1994), polar bears (Paetkau *et al.* 1995), toads (Scribner *et al.* 1994), wasps (Choudhary *et al.* 1993), wombats (Taylor *et al.* 1994), and cod (Ruzzante *et al.* 1996) to name a few. At the present time, there is no published information on using microsatellite loci in mink.

2.4. Formation of New Microsatellites Alleles

Two mechanisms have been proposed for the formation of new alleles: unequal sister chromatid exchange and replication strand slippage (Walsh 1987; Tachida 1993; Valdes *et al.* 1993; Stephan and Cho 1994).

2.4.a. Unequal Sister Chromatid Exchange

Unequal sister chromatid exchange (USCE) implies a mutational process of recombination between sister chromatids during meiosis or mitosis (Harding *et al.* 1992; Harding *et al.* 1993). USCE can only be successful if the invading strand finds a region of homology on the target strand (Stephan and Cho 1994). The following diagram explains USCE according to Smith (1976):

1. Two identical tandem arrays on sister chromatids pair with each other out of phase:

   CAA CAA CAA CAA
   CAA CAA CAA CAA

2. Homologous recombination occurs between the out-of-phase arrays:

   CAA CAA CAA CAA
   X
   CAA CAA CAA CAA

3. One of the repeats is lost from the shorter array and is added to the other sister chromatid:

   CAA CAA CAA
   CAA CAA CAA CAA CAA

4. Further cycles of USCE can either expand the short tandem array to its original length or continue to expand it.

USCE produces an allele with a different number of repeats than the parental allele (Tachida 1993). Therefore, the model which has been used to explain the formation of microsatellite alleles by USCE is the infinite allele model (IAM), which implies that an allele can mutate to any number of repeats irrespective of the state of the parental allele. The theory of the IAM states that every new allele is unique because it is unlikely

that an allele will mutate to one represented already in the population (Harding *et al.* 1993; Estoup *et al.* 1995). However, it has been suggested that USCE is not a major force in microsatellite evolution (Harding *et al.* 1993; Stephan and Cho 1994). Harding *et al.* (1993), tested the IAM through computer simulation on microsatellite loci, and found the diversity below expectation of the infinite allele model, i.e. a lower number of alleles was observed than expected.

Another reason for the disproval of the IAM is that the microsatellite repetitive sequences have to be longer than 100 base pairs for unequal SCE to work. This is due to the fact that an attempted recombination event can only be successful if the invading strand finds a region of homology on the target strand. Microsatellite runs are usually less than 100 base pairs long with often several runs found at one locus and short interrupting sequences between runs (Weber 1990; Stephan and Cho 1994).

2.4.b. Replication Slippage

Replication slippage implies the displacement of the strands of an unwound DNA fragment followed by mispairing of complementary bases at the site of an existing short repeat sequence. The simplest consequences of this mispairing, when followed by replication or repair, are the insertion or deletion of one or several of these repeats (Levinson and Gutman 1987). This is thought to be the major mechanism by which microsatellites are propagated (Tautz *et al.* 1986; Walsh 1987; Harding *et al.* 1992; Schlötterer and Tautz 1992; Stephan and Cho 1994; Fumagalli *et al.* 1996). The following diagram explains replication slippage according to Levinson and Gutman

(1987):

1. A parental DNA strand contains a GAA/CTT repeat.

GAAGAAGAAGAA
CT TCT TCTT CTT

2. A slippage event occurs in the parental or progeny strand during replication, creating a transient bulge that can move through the whole DNA strand. On the left, one of the CTT repeats of the parental strand loops out, leaving only 3 repeats for which the growing progeny to pair with, while on the right, one of the GAA repeats of the progeny strand loops out, allowing 5 GAA repeats to be inserted instead of 4.

Parental strand loops out
GAAGAAGAA
CTTCTT CTT
CT
T

A
Progeny strand loops out GA
GAAGAAGAAGAA
CTTC TTCT TCTT

3. After replication, on the left, a one repeat deletion has occurred while on the right, a one repeat insertion has occurred.

GAAGAAGAA
CTT CTT CTT

GAAGAAGAAGAAGAA
CTTCT TCTT CTT CTT

As repeats gain more units they provide a more efficient substrate for slippage and therefore for further expansion (Levinson and Gutman 1987). Two models are considered for the formulation of new alleles at microsatellite loci by replication slippage: the linear rate model and the stepwise mutation (Tachida 1993).

The linear rate model assumes that the rate of replication slippage increases linearly as the number of repeats increases because the probability of mispairing increases (Walsh 1987; Tachida and Iizuka 1992). However, Valdes *et al.* (1993) found that this was not the case with microsatellites and that the rate of replication slippage is

independent of the number of repeats.

The stepwise mutation model (SMM) implies that an allele mutates only by losing or gaining a single repeat and therefore possibly towards an allele already present in the population (Roy *et al.* 1994; Slatkin 1995; Estoup *et al.* 1995). In this model, the rate of replication slippage does not depend on the number of repeats in the parental gene (Tachida 1993). The SMM seems to be the model that most scientists agree to be the cause of microsatellite formation (Schlotterer and Tautz 1992
; Harding *et al.* 1993; Shriver *et al.* 1993, Valdes *et al.* 1993; Goldstein *et al.* 1995; Zhivotovsky and Feldman 1995). Computer simulations of replication slippage produced microsatellite diversity levels expected from the SMM (Harding *et al.* 1993). Valdes *et al.* (1993) found that their observations of allele frequencies at 108 human microsatellite loci was consistent with the stepwise mutation model at those loci in a population of constant size. Results of a study by Zhivotovsky and Feldman (1995) where they analysed between-locus variation in 86 human microsatellite loci also agreed with the SMM. However, Estoup *et al.* (1995) tested both the infinite allele model and the stepwise mutation model with honey bees and their findings were in agreement with the infinite allele model but not with the stepwise mutation model since some of their microsatellites had core sequences composed of repeats of two and even three different lengths. Although the exact mechanism of microsatellite formation is not known, it does not undermine their characteristics as valuable DNA markers for population genetic studies.

2.5. Rate of Microsatellite Mutation

As was mentioned previously, one of the many reasons microsatellites make good genetic markers is because of their high variability which is caused by their high mutation rate. Several studies have attempted to calculate the rate of microsatellite mutations:

Levinson and Gutman (1987) found the mutation rate to be 10^{-4} by observing directly the relative frequencies of insertions versus deletions during slipped-strand mispairing of a 40 base pair poly-CA tract in the bacteriophage M13.

Dallas (1992) estimated the mutation rate to be between $2x10^{-3}$ and $4.7x10^{-4}$ in three microsatellites in 9 strains of recombinant inbred mice and their parental inbred strains. The mice were analysed for the presence of mutant alleles (non-parental length variants) by PCR amplification of parental-offspring DNA. Mutation rate was calculated as the number of mutation events divided by the length of the lineage (in generations) from the start of the strain until the generation being considered.

Weber and Wong (1993) estimated the average mutation rate in humans to be $1.2x10^{-3}$/locus/gamete/generation by directly counting the mutation events uncovered through large scale genotyping of 40 reference families, giving a total of almost 20,000 parent-offspring transfers of alleles. Fifteen dinucleotide microsatellites, 12 tetranucleotide sequences, and one trinucleotide sequence were analysed, and 25 mutations were detected. The dinucleotide microsatellites by themselves had a mutation rate of $5.6x10^{-4}$.

Goldstein et al. (1995) estimated the mutation rate to be half the slope of the best

fitting line on a regression graph of microsatellite genetic distances on dates of intercontinental human migration. They found it to be 7.96×10^{-4}, which is close to the estimates of Levinson and Gutman (1987).

Ellegren (1995) estimated the mutation rate in pigs to be 7×10^{-5} for dinucleotide and 3×10^{-3} for tetra-pentanucleotide repeats. Using 236 pigs from a three-generation pedigree, sixty-two microsatellites (42 di-, 20 tetra-pentanucleotide) were used, and two mutations were observed on the 24,414 gametes screened (1 dinucleotide mutation in 17,514 gametes, 1 tetra-pentanucleotide mutation in 657 gametes), giving an overall mean of 8×10^{-5} per generation.

3. MATERIALS AND METHODS

3.1. Source of Tissues

Although the main purpose of this study was to compare the genetic variability of ranched black mink in Nova Scotia, colored mink (brown and pastel), which have been separated from black mink since at least the 1950's, were included in the study to provide a basis for comparison. In addition, wild mink were used to examine the genetic changes that could have occurred as a result of adaptation to captivity and intense selection for production traits.

3.1.a. Black Mink

Mink tissues were obtained from three breeding ranches in Nova Scotia, which were selected because of their large size of operation, long history of mink breeding, and limited exchange of animals among them. In addition, mink tissues from the Nova Scotia Agricultural College (NSAC) farm were used.

Farm 1: This is a large open farm located near Windsor in the Eastern Annapolis Valley in Nova Scotia. The farm was established in 1975 when 30 breeding females and 6 males were purchased from a mink producer in Nova Scotia who has been keeping mink for many years. The number of breeding females increased from 54 in 1978 to 1300 in 1985, and fluctuated between 1000 and 1400 between 1986 and 1993. Breeding stock, mainly pregnant females, were purchased from farms in both Nova Scotia and the United States almost every year during this period (Table 3.1). Cage cards are used for record keeping and are destroyed when the mink are pelted. Until 1990, selection was

based on fur quality traits (density, silky texture, clarity of color, and short nape lengths) among kits which were born in a litter of size 3 or larger. More attention has been paid to litter size since 1991, when replacement males and females were selected from litters of at least 5 and 4, respectively. Line-breeding is not practiced in this farm.

Farm 2: This is a large open farm in Digby County in North-Western Nova Scotia. The farm was established in 1966 with 15 pregnant females purchased from another farmer in Digby County. The number of breeding females has increased from 45 in 1967 to 2003 in 1980, and fluctuated between 2202 and 4434 between 1981 and 1993. Additional breeding stock was imported from the United States (Table 3.2). Cage cards are used for record keeping and are destroyed when the mink are pelted. Selection has been based primarily on fur quality traits prior to 1992, when emphasis was placed on litter size. There are 6 different lines on this farm, reflecting distinct color shading and fur characteristics, such as guard hair length and density.

Farm 3 (NSAC): The farm was established in 1986, with 47 females and 11 males originating from 14 ranches in Nova Scotia (including Farms 1 and 2). Breeding stock from various farms in the province has been acquired over the years (including Farms 1 and 2). This farm seems to have had the widest original genetic base among the populations studied. The number of breeding females ranged between 40 and 101 in this farm between 1987 and 1995 (Table 3.3). Selection has been based primarily on fur quality and reproductive traits including litter size, weight of kits, and the number of kits alive at 3 weeks. Cage cards are used for record keeping and may be destroyed when the mink are pelted, but pedigree information is kept on permanent record.

Farm 4: This is a large open farm in Digby County, North-Western Nova Scotia. The farm was established in 1971 with 20 pregnant females bought from another rancher in the region. This farm was kept closed until 1976, when 4 males were bought from Farm 2. Other males from various farms in Nova Scotia and the United States were purchased (Table 3.4). The number of breeding females has risen from 400 between 1971-1975 to 1200 between 1979-1983 and then to 2000 from 1984-1992. Line-breeding is practised in this ranch, and selection is based primarily on fur quality traits and litter size. There are 8 different lines on this farm, representing the farms they originate from.

Animals within these 4 farms are thus related to each other through purchase of breeding stock from the same sources, or through movement of animals among the farms. Male:female ratio in these farms is close to 1:4.5, each male is bred to 4 or 5 females. With the exception of farm 3, each female is sometimes bred to 2 different males, and the second male is considered the sire of the progeny. Matings between close relatives (brother-sister, parent-offspring) is avoided, and breeding is always between males and females of the best fur quality (positive assortative mating).

Table 3.1: Number of breeding females and number of breeding stock purchased in Farm 1*.

Year	# females kept	# males purchased	#females purchased	farm of origin
1976	30	6	30	I.S., Nova Scotia
1978	54	—	—	-----
1979	125	7	—	E.T., Nova Scotia
1980	250	—	50	J.M., Nova Scotia
1981	625	—	—	-----
1982	625	—	40	L.F., Illinois, U.S.[1]
1983	700	—	—	L.F., Illinois, U.S.[1]
1984	1100	—	10	L.F., Illinois, U.S.[1]
1985	1300	—	10	L.F., Illinois, U.S.[1]
1986	1200	—	12	L.F., Illinois, U.S.[1]
1987	1400	—	12	L.F., Illinois, U.S.[1]
1988	1300	—	12	L.F., Illinois, U.S.[1]
1989	1250	—	12	L.F., Illinois, U.S.[1]
1990	1000	—	12	L.F., Illinois, U.S.[1]
1991	1300	—	1	L.F., Illinois, U.S.[1]
1992	1400	—	2	L.F., Illinois, U.S.[1]
1993	1200	—	—	-----

[1] Pregnant females. Most of the male and female progeny were kept for breeding.

* Tissue samples were taken from this farm in 1994.

Table 3.2: Number of breeding females and number of breeding stock purchased in Farm 2*

Year	# females kept	# males purchased	# females purchased	farm of origin
1966	15	---	15	D.M., Nova Scotia[1]
1967	45	---	---	-----
1968	90	---	---	-----
1969	168	---	---	-----
1970	235	---	---	-----
1971	316	---	---	-----
1972	429	---	---	-----
1973	522	---	---	-----
1974	638	---	---	-----
1975	644	---	---	-----
1976	793	---	---	-----
1977	1069	---	---	-----
1978	1284	---	---	-----
1979	1565	---	---	-----
1980	2003	---	---	-----
1981	2353	---	---	-----
1982	2202	---	---	-----
1983	2570	---	---	-----
1984	2464	---	---	-----
1985	2367	---	---	-----
1986	2640	---	---	-----
1987	3434	9	24	D.F., L.F., Illinois, U.S.[1]
1988	4426	5	5	D.F., L.F., Illinois, U.S.[1]
1989	4434	---	---	-----
1990	3434	---	12	L.F., Illinois, U.S.[1]
1991	2965	5	88	L. F., H.S., Illinois, Utah, U.S.[1]
1992	3374	---	---	-----
1993	3836	---	---	-----

[1] Pregnant females

*Tissue samples were taken from this farm in 1994

Table 3.3: Number of breeding females and number of breeding stock purchased in Farm 3*

Year	# females kept	# males purchased	# females purchased	farm of origin
1986	47	11	47	Various (see text)
1987	87	---	---	-----
1988	71	---	---	-----
1989	81	5	---	P.C., Farms 1, 2
1990	80	---	---	-----
1991	61	---	---	-----
1992	101	15	63	P.C., Farm 1
1993	105	---	---	-----
1994	47	18	60	-----
1995	40	---	---	Farms 1, 2 and 4[1]

[1] None of the animals from Farms 1, 2 or 4 or their progeny was sampled from this study.

*Tissue samples were taken from this farm in 1993 and 1994.

Table 3.4: Number of breeding females and number of breeding stock purchased in Farm 4*

Year	# females kept	# males purchased	# females purchased	farm of origin
1971	20	---	20	H.H, Nova Scotia[1]
1976	400-500	4	---	Farm 2, Nova Scotia
1979	1200	10	---	A.LB., Nova Scotia
1982	1200	2	---	H.S., Utah, U.S.
1983	1200	8	---	L.F., Illinois; H.S., Utah, U.S.
1984	2000	---	10	C.V., Illinois, U.S.[1]
1985	2000	4	6	L.F., Illinois, U.S.
1986	2000	4	---	Farm 2, Nova Scotia
1987	2000	2	10	Farm 2, Nova Scotia
1988	2000	6	---	L.F., D.F., Illinois, U.S.
1989	2000	---	4	L.F., Illinois, U.S.[1]
1990	2000	---	10	H.S., Utah, U.S.[1]
1991	2000	---	---	-----
1992	2000	8	---	Farm2, Nova Scotia; L.F., P.C., U.S.
1993	2000	---	---	-----

[1] Pregnant females.

*Tissue samples were taken from this farm in 1994.

3.1.b. Colored Mink

Brown (wild-type) and pastel mink were from one farm in Prince Edward Island. The ranch has kept pastel mink since it started in 1962. These originated in Ontario and a few additional mink were purchased prior to 1980. Fifty female and 20 male brown mink were bought in 1989 from another ranch in Prince Edward Island. This population originated in France. The number of females gradually increased to 750 in 1995. This herd has been closed during this period. In contrast to the open breeding farms that constitute the four black mink farms in Nova Scotia, the brown and pastel mink have been kept in isolation with the exception of 175 female and 15 male pastels in 1995 which were imported from Quebec, but not used for this study. Table 3.5 shows the number of breeding animals each year since the 1980s.

Table 3.5: Number of breeding females and number of breeding stock purchased in PEI farm

Brown Mink			Pastel Mink		
Year	# females used	# males used	Year	# females used[1]	# males used
-	---	---	1980s	500-600	---
1989	50	20	1989	275	20
1990	200	50	1990	350	25-30
1991	350	75	1991	350	25-30
1992	450	80	1992	350	25-30
1993	500	100	1993	350	25-30
1994	600	110	1994	350	25-30
1995	750	150	1995	350	32

1. Some of the pastel females are bred to males of other colors.
*Tissue samples were taken from this farm in 1995

3.1.c. Wild Mink

Wild mink were trapped in northern New Brunswick in an area covering approximately 40 km^2 in October and November of 1995. The area was chosen because it was far from any mink ranch, and thus the chance of trapping crosses between wild and escaped ranch mink was slim. The mink were brown in color which also indicates that these were true wild mink. The following table (Table 3.6) shows where all the wild mink were trapped.

Table 3.6: Areas in New Brunswick where wild mink were trapped

Number of mink	County	Region	Location
3	Northumberland	West Branch Six Mile Brook	Wildlife Management Zone
2	Albert	Pleasantvale	Coverdale River
4	Albert	Parish Elgin	Campbell Brook
2	Albert	Ro Parish, Port Elgin	Coverdale River
1	Albert	Berryton	Beaver Brook
2	Albert	---	Prosser Brook
1	Albert	New Ireland	45 River
1	Albert	Salisbury Back Road	Duncan River
1	Westmorland	Junction Babcock Brook	Poller River
3	Westmorland	Nixon, Parish Salisbury	Coverdale River

3.2. Collection of Mink Tissue Samples and Selection of Animals for this Study

3.2.a. Black Mink

The tongue and/or liver samples were collected immediately after the mink were killed by cervical dislocation (except for farm 3, where they were killed with CO_2). Using the forceps and scalpel, approximately 3/4 of the tongue was removed, placed in the pre-labelled cryovial, making sure that the animal ID was recorded on the data sheet next to the cryovial number. The vials were quickly placed on a clip and submerged into liquid nitrogen.

Following pelting, a transverse incision was made across the abdomen and the liver was removed. This was placed on a plastic bag, cut into 3-4 pieces of approximately 2 g each, put in cryovials, and quick-frozen in liquid nitrogen. After each mink was sampled, the plastic bag, scalpel blade and gloves were changed to avoid cross-contamination. Samples were stored in a cryo-freezer at -80°C, and a sample inventory was established. Between March 1993 and December 1994, 276, 313, 331, and 74 mink were sampled from Farm 1, 2, 3, and 4 respectively.

Cage cards were obtained for each mink and information such as year of birth, mink ID, parents information, and line, was entered in a database file (dBase IV). This information was used to select one mink per sire family to be used in the project. The number of individuals used in this study was 78, 44, 50, and 40 from Farms 1, 2, 3, and 4 respectively.

3.2.b. Wild, Pastel and Brown Mink.

Frozen hind legs of 20 ranched pastel and 20 brown mink were shipped on ice to the NSAC in plastic bags along with their cage cards in the winter of 1995. Upon arrival, muscle tissue was taken from the legs with scissors, scalpel and forceps. The muscle pieces were placed in labelled cryovials (3-4/mink) and stored in the cryo-freezer at -80°C. These animals were unrelated to each other and chosen by the farmer.

Whole carcasses of 20 trapped wild mink were frozen in individual plastic bags with information on the location where each mink was trapped and were shipped to the NSAC. The same procedure for tissue collection was then used as the pastel and brown. Muscle tissue was taken from the legs, head and back as explained in the case of the brown and pastel mink.

3.3. Laboratory Procedures

3.3.a. DNA Extraction

DNA was extracted from mink liver, tongue or muscle using the two following methods. In the first method, DNA was extracted from approximately 150 mg of tissue using 2X lysis buffer (Applied Biosystems) to release the DNA and proteinase K (16 μg/μl, ICN) to dissolve the proteins surrounding the DNA. A phenol/choloform extraction with ethanol precipitation was then carried out. The resulting DNA was suspended in 50 μl 1X TE buffer. The DNA was quantified with a TKO 100 DNA fluorometer (Hoeffer Scientific Instruments). In the second method, approximately 0.05g of tissue was treated with proteinase K (16 μg/μl, ICN) and precipitated with ethanol. The DNA was then suspended in 100 μl 1X TE buffer. The DNA concentration was not

determined. Although the second method was much quicker, the DNA obtained was not of very high quality and so it is only recommended for PCR analysis when only small pieces of DNA, such as microsatellites, are to be amplified. Detailed procedures are explained in Appendix A.

3.3.b. M13 Size Marker

The M13 size marker was made with the ^{T7}Sequencing^{TM} kit (Pharmacia Biotech) according to the manufacturers instructions for sequencing reactions. However, 25 μl of Stop Solution (0.3 % each of bromophenol blue and xylene cyanol FF; 10mM EDTA and 97.5% deionized formamide) was added instead of 5 μl for termination of the reactions in order to extend the shelf life of the Stop solution and get more use out of it. The samples were denatured at 80°C prior to loading on a polyacrylamide gel.

3.3.c. PCR Amplification of Microsatellites

3.3.c.i. End-labelling Primer With γ-P^{32}

In a labelled microtube, the following materials were combined (Table 3.7), which was enough for 20 reactions (0.5 μl/reaction):

Table 3.7: Materials, volumes and final concentrations used in the end-labelling reaction

		Final Concentrations
4.5 μl	dH_2O	----
0.5 μl	T4 PNK (polynucleotide kinase)(10 U/μl, NE Biolabs)	0.5 U/μl
1.0 μl	10X T4 PNK buffer (New England Biolabs)	1X
1.0 μl	B primer (10 μM)	1 μM
3.0 μl	γ-P^{32}ATP (10 mCi/ml,ICN)	3 mCi/ml
10 μl	total volume	

The mixture was incubated at 37°C for at least 30 minutes, but the incubation time was sometimes extended up to 18 hours, after that the reaction tends to degrade. The kinase was inactivated by heating at 65°C for 15 minutes.

3.3.c.ii. Preparation of PCR Master Mix

For DNA which was extracted by the first protocol and for which the concentration was known, in a labelled PCR tube, between 1 and 3 μl of DNA was added. For the samples extracted by the second method, 3 μl of DNA were added to each PCR tube.

The following PCR master mix (Table 3.8), which is enough for 20 reactions (7.5 μl/reaction), was prepared in a labelled microtubes.

Table 3.8: Materials, volumes, and final concentrations in the PCR master mix

		Final Concentrations
20 μl	1% Tween® 20 (Fisher Scientific)	0.13%
74 μl	dH_2O	---
20 μl	10X PCR buffer (Boehringer Mannheim)	1.33 X
16 μl	dNTP (2.5 mM)	0.27 mM
10 μl	A primer (10 μM)	0.67 μM
9 μl	B primer (10 μM)	0.60 μM
1 μl	Taq DNA Polymerase (5 U/μl) (Boehringer Mannheim)	0.03 U
150 μl	Total volume	

The tube was spun for 1 second in a microcentrifuge, and the end-labelled primer was added to the PCR master mix. Of this master mix, 8 μl was added to each PCR tube containing the sample DNA. A drop of mineral oil was layered on each mixture and placed in a PTC-100™ Programmable Thermal Controller (MJ Research, Inc.). PCR

conditions were as follows (O'Connell et.al.1996):

Five cycles of a 94°C denaturation for 20 seconds, X°C for 20 seconds ((where X is the annealing temperature (Table 3.9), and depends on the primer)), and 72°C extension for 20 seconds, followed by 40 cycles of 90°C denaturation for 20 seconds, X°C annealing (Table 3.9) for 20 seconds, and 72°C extension for 20 seconds.

Table 3.9: Primers, annealing temperatures, and primer sequences used in this study

Primer	X (°C)	No. of repeats	Primer Sequence
Mvi24	56	$(GT)_{12}$	CTTGCCTCTATCAGTTCTCC / TATTCTGGGTCTTTTCTATCC
Mvi54	56	$(CA)_{13}$	AGAGTCTGTATACCTCCACC / CCCTCCTTGGCTCCGCAC
Mvi87	56	$(GT)_{13}$	ACAATAGTAGTGGCAGCAGC / TCTGTGAAACACTGCAAAGC
Mvi57	56	$(GT)_{17}$	GAACAGGACCAGCCCTGC / GTTGGAAATGAGGATCTCAC
Mvi111	57	$(GT)_{15}$	GTGGGCATAGAATTTAGAGG / TTATCAAAGACAATGTGCGAG
Mvi219	51	$(GT)_{13}$	GGGTGCGGCTCTCACTGC / GGAGTATTGTCCTCACCTGC
Mvi232	58	$(TG)_{14}$	GACGATTCACAAACCTATACC / TCACCAGGGACCAACAGGT

Following PCR amplification, 10 μl Stop Solution was added to each tube. The PCR products were either subjected to polyacrylamide gel electrophoresis on the same day or stored at 4°C until the following day.

3.4. Polyacrylamide Gel Electrophoresis

Wedge gels of 0.4-1 mm (8% acrylamide) were used to run the microsatellites on a Bio-Rad Sequi-Gen® II Nucleic Acid Sequencing Cell. The apparatus was assembled and the acrylamide gel poured according to the manufacturers instructions. The gel was pre-run for approximately 45 minutes at 1800-2000V, 70-95 mA until the temperature of the gel reached 55°C. The products were heated to 95°C for at least 5 minutes before they

were loaded on the gel

Between 2-2.5 μl of PCR reaction was loaded on the gel and 4-4.5 μl of marker was loaded on both sides of the gel and run at 55°C (1800-2000V, 70-95 mA) for 2-3 hours, until the fast dye (xylene cyanol) had reached the bottom of the gel. Following electrophoresis, the gels were exposed to Kodak X-Omat™ XK-1 film for 4-14 hours, depending on the age of the isotope. For each day the isotope aged, the exposure time was increased by approximately one hour. The film was developed using an AFP Imaging mini-Med/90 X-ray film processor. Gels were scored using the M13mp DNA size template, by comparing it to the size of the alleles on the gel. All stock solutions used for extraction, PCR and polyacrylamide gel electrophoresis are found in Appendix B.

3.5. Data analysis

3.5.a. Intrapopulation Genetic Variability

3.5.a.i. Polymorphism and Test for Hardy-Weinberg equilibrium

Allele frequencies were computed at each locus for each population using the FREQ procedure of SAS (SAS Institute 1996), and genetic polymorphism for each population was measured as the mean number of alleles per locus. Conformation of genotype frequencies to Hardy-Weinberg equilibrium was tested using the GENEPOP computer package, version 1.2 (Raymond and Rousset 1995a) using the default options (1000 dememorisation, 50 batches and 1000 iterations). The program uses the exact Hardy-Weinberg test (Louis and Dempster 1987) when there are fewer than five alleles per locus, and uses a Markov chain method to estimate the exact Hardy-Weinberg

probability without bias (Guo and Thompson 1992) when there are five or more of alleles. The probability of rejecting H_o, i.e. genotype frequencies are in Hardy-Weinburg equilibrium, and the standard error of this estimate were computed. When standard errors were larger than 0.01, the data were reanalyzed using a larger number of batches (100). This program does not perform any test when a locus is monomorphic or quasi monomorphic (two alleles, but one is represented only once). The allele frequency distributions in such cases did not obviously conform with Hardy-Weinburg proportions. Deviations from Hardy-Weinburg proportions for each population over all loci, and for each locus over all populations, were also tested by GENEPOP, which combines the results of individual tests on each single locus, using Fisher's method. This procedure is valid only if the loci are independent.

Prior to this test, alleles with frequency of less than 5% over all populations were considered as rare, and were pooled together, or were added to the allele class with the lowest frequency when there was only one rare allele in a locus.

3.5.a.ii. Heterozygosity

The observed heterozygosity (h_o) was computed for each population at each locus, and for each locus over all populations by direct count.

$h_o=\Sigma^{k}_{i,j=1,i<j} Q_{ij}$, where $Q_{ij}=n_{AiAj}/n$, $i\neq j$, and N_{AiAj} is the number of AiAj genotypes.

This procedure is valid for any number of alleles, and does not require assumption of Hardy-Weinburg equilibrium. The sampling distribution of heterozygosity in a locus is

binomial regardless of the number of alleles (heterozygous genotypes vs homozygous genotypes), i.e. $V(h_o)=h_o(1-h_o)/n$, where n is the sample size.

The average observed heterozygosity of each population (H_o) was computed by taking the weighted arithmetic mean of the heterozygosity at each locus. Weights were the number of individuals genotyped at each locus.

Expected unbiased heterozygosity of the m^{th} population at the k^{th} locus (h_{mk}) was computed as:

$$h_{mk}=2n(1-\Sigma x_{mi}^2)/(2n-1)$$

Where x_{mi} is the frequency of the i^{th} allele in the m^{th} population and n is the number of observations (Nei and Roychoudhury 1974b; Nei 1978). The sampling variance of this estimate is (Nei 1978):

$$V(h_{mk})=[1/n(2n-1)][\Sigma^k_{i=1}x_{mi}^2-(\Sigma^k_{i=1}x_{mi}^2)^2+4(n-1)\{\Sigma^k_{i=1}x_{mi}^3-(\Sigma^k_{i=1}x_{mi}^2)^2\}]$$

When sample size is large, [2n/(2n-1)] approaches unity and

$$h_{mk}=1-\Sigma^k_{i=1}x_{mi}^2$$

The sampling variance of this estimate is somewhat smaller than that for the previous estimate (Nei and Roychoudhury 1974b):

$V(h_{mk})=[(2n-1)/4n^3][(3-4n)(\Sigma^k_{i=1}x_{mi}^2)^2+4(n-1)(\Sigma^k_{i=1}x_{mi}^3+\Sigma^k_{i=1}x_{mi}^2]$, where n is the sample size.

Heterozygosity of the k^{th} locus over all populations was computed as $h_k=2n(1-\Sigma x_i^2)/(2n-1)$, where x_i is the frequency of the i^{th} allele in the entire sample. The arithmetic mean of h_{mk} estimates, weighted for the number of observations, was used as the average heterozygosity of the m^{th} population over all loci (H_m), and will be denoted as H_E.

Pairwise tests of homogeneity of expected heterozygosity of each locus in different populations were performed using the G-test (Sokal and Rohlf 1981). The hypothesis was that estimates of expected heterozygosity at each locus were the same in any two populations. The observed numbers of heterozygotes and homozygotes were tested against expected numbers using a χ^2 goodness-of-fit test. This test also examines excess of homozygotes compared with the Hardy-Weinburg values. All the above computations were performed by programs written in SAS (SAS Institute 1996).

3.5.b.Interpopulation Genetic Variability

3.5.b.i. Population Differentiation

Pairwise tests for homogeneity of allele distributions were performed using the GENEPOP computer package after pooling the rare alleles, which follows the Raymond and Rousset (1995b) method. The hypothesis tested is that allele distributions are independent of populations (no allele differences). An unbiased estimate of the Fisher's exact test on contingency tables is performed using a Markov chain method (1000 dememorisation, 50 batches and 1000 iterations). The program computes the probability of being wrong when H_o (allele frequency distributions are independent of populations) is rejected.

3.5.b.ii. Genetic Distance

Nei's genetic distance (Nei 1972) and Cavalli-Sforza's chord measure (Cavalli-Sforza and Edwards 1967) were computed using the allele frequencies from all the

microsatellite loci and the GENDIST option of the PHYLIP (Phylogeny Inference Package) computer program, version 3.57 (Felsenstein 1995).

Nei's standard genetic distance is defined as $D=-\ln(I)$, where $I=J_{XY}/\sqrt{J_{XX}J_{YY}}$. The term $J_{XY}=\Sigma^{k}_{i=1}p_iq_i$ is the probability that two alleles one taken at random from population X and one from population Y are identical, $J_{XX}=\Sigma^{k}_{i=1}p_i^2$ is the probability that two alleles chosen at random from populations X are identical, and $J_{YY}=\Sigma^{k}_{i=1}q_i^2$ is the same quantity for population Y. I is Nei's normalized identity for this gene, which is the ratio of the proportion of genes that are alike between and within populations (Nei 1972, 1977, 1978).

Cavalli-Sforza's chord measure is defined as $\sqrt{(2-2\cos\theta)}$, where $\cos\theta=\Sigma^{k}_{i=1}\sqrt{p_iq_i}$. This is the geometric distance between two points P and Q, with coordinates $P(\sqrt{p_1},\sqrt{p_2})$ and $Q(\sqrt{q_1},\sqrt{q_2})$, on the surface of a hypersphere with radius 1. p_i and q_i are the allele frequencies in populations X and Y, respectively.

3.5.b.iii. Phylogenetic Analysis

There are several methods of constructing phylogeny trees: One of the most common ones when dealing with allozymes and microsatellite loci is the distance matrix method, which is based on the set of distances calculated between populations. In this method, the genetic distances between all pairs of "operational taxonomic units" (OTU's) are calculated. These genetic distances are used to cluster OTU's. The following three methods were used to construct unrooted phylogenetic trees by the PHYLIP computer program. A rooted tree conveys the notion of temporal ordering of the species or

populations on a tree, while an unrooted tree merely reflects distances between units with no notion of which was ancestral to which. No outgroup was assumed in this study.

UPGMA (unweighted pair-group method using an arithmetic average) defines the intercluster distance as the average of all the pairwise distances for members of two clusters. In the Fitch-Margoliash method (Fitch and Margoliash 1967), missing OTU's are introduced as common ancestors of later OTU's, and fits branch lengths to groups of three OTU's at a time. Different trees are compared on the basis of a measure of goodness of fit, called the percentage standard deviation (s) (Fitch and Margoliash 1967).

$$s = 100\{\Sigma_{i,j}[(d_{ij}-e_{ij})/d_{ij}]^2/[n(n-1)\}^{1/2}$$

where d_{ij} is the observed distance pair, i and j, n is the number of OTU's, and e_{ij} is the sum of the branch lengths between them on the tree. The best tree will have the smallest percent standard deviation. It is possible to adjust branch lengths in the fitted tree to reduce the standard deviation. Other trees can be chosen for examination by selecting a different initial pair of OTU's. The tree with the smallest standard deviation is considered to be the best, and this criterion is the basis on which the Fitch-Margoliash algorithm operates.

The Neighbor-joining method for reconstructing phylogenetic trees from evolutionary distance data was developed by Saitou and Nei (1978). This method is based on finding OTUs that minimize the total branch length at each stage of clustering of OTUs starting with a starlike tree. Neighbor is defined as a pair of OTUs connected through a single interior node in an unrooted, bifurcating tree. Neighbor-joining produces tree without assumption of a clock.

3.5.b.iv. Assignment Test:

The value of this panel of microsatellite loci in correctly identifying the population from which individuals were sampled from was determined by an assignment test. This test is an indication of population differentiation, because the larger the genetic differences among populations, the lower the likelihood that an individual is assigned to a population other than the one it was sampled from.

The expected frequency of each individual's genotype in each of the seven populations was calculated. The product of these expected genotype frequencies at each of the seven loci, based on the observed allele distributions, was computed for each individual, and the individual was assigned to the population where its expected genotype frequency was highest.

The following example demonstrates this procedure for a simple case. Assume that allele frequency distributions of two populations ($i=1,2$) at two loci ($k=1,2$) are available; $[(p_{111}, p_{112}\cdots p_{11j}),(p_{121}, p_{122}\cdots p_{12j})]$ for population 1 and $[(p_{211}, p_{212}\cdots p_{21j}),(p_{221}, p_{222}\cdots p_{22j})]$ for population 2. The probability that an individual "m" with allele frequencies $f=[(p_{1j},p_{1j'}),(p_{2j},p_{2j'})]$ has come from each population is:

Population 1: $(p_{11j} \times p_{11j'})\times(p_{12j} \times p_{12j'})$

Population 2: $(p_{21j} \times p_{21j'})\times(p_{22j} \times p_{22j'})$

Locus	Allele (j)	Pop.1	Pop.2	Genotype of "m"
k=1	A1	0.25	.30	+
	A2	0.20	.30	+
	A3	0.10	.20	
	A4	0.45	.20	
k=2	B1	0.40	.30	+
	B2	0.60	.70	+

Individual m has alleles A1 and A2 at locus 1 and alleles B1 and B2 at locus 2, i.e. f=[(1,2),(1,2)]. If random mating and linkage equilibrium within each population could be assumed:

p(Population 1/((1,2),(1,2)))=(.25*.20)*(.40*.60)=(.50)*(0.24)=0.12

p(Population 2/((1,2),(1,2)))=(.30*.30)*(.30*.70)=(0.09)*(0.21)=.019

The likelihoods that individual m has come from populations 1 and 2 are 0.12 and 0.019, respectively, and thus it is more likely to have come from population 1.

3.5.c. F-statistics

F-statistics have been proposed by Wright (1943) and have been widely used in population genetic studies. When a population is subdivided into several subpopulations (farms, lines or families), only individuals within a subpopulation can breed with each other. Since each subpopulation has a smaller number of individuals compared with the population as a whole, the chance of mating between relatives will increase, resulting in an excess of homozygosity and the accumulation of inbreeding. A three tier hierarchical structure was defined by Wright (1943, 1978): individuals within a subpopulation, subpopulations within the whole population, and the population as a whole.

Each individual has a certain level of observed heterozygosity (H_I), which may be different than if that individual would have come from a randombred subpopulation of a similar size (H_S) or a randombred population with the same size as the whole population (H_T). The following heterozygosities are defined:

$H_I=\Sigma^k_{i=1} H_i/k$, where H_i is heterozygosity in subpopulation i and k is the number of subpopulations. H_I is therefore the average of heterozygosity of all the genes in an individual or the probability of heterozygosity of any one gene.

$H_S=1-\Sigma^k_{i=1}p_{i.s}^2$, where $p_{i.s}$ is the frequency of the i^{th} allele in subpopulation "s", and H_S is the heterozygosity that would be expected in a randombred subpopulation "s", i.e. a population under Hardy-Weinburg equilibrium. H'_S is the average of H_S taken over subpopulations.

$H_T=1-\Sigma^k_{i=1} p_i^2$, where p_i is the frequency of the i^{th} allele averaged over the subpopulations. H_T is the heterozygosity that would be expected in a randombred population consisting of all the subpopulations pooled and bred at random.

Since mating among individuals in a small population results in loss of heterozygosity and accumulation of inbreeding, three measures of inbreeding has been defined for population subdivision (Wright 1943, 1978; Nei 1973; Hartl and Clark 1989). These include:

$$F_{IS}=(H'_S-H_I)/H'_S$$

$$F_{ST}=(H_T-H'_S)/H_T$$

$$F_{IT}=(H_T-H_I)/H_T$$

F_{IS} is a measure of inbreeding coefficient of individuals in a subdivided population

due to nonrandom mating, or inbreeding of an individual relative to the subpopulation to which it belongs. When mating is at random in a subpopulation, F_{IS} is equal to zero, and consequently $F_{ST}=F_{IT}$. Positive F_{IS} values indicate within subpopulation inbreeding (more homozygosity than expected) due to mating between relatives. Negative F_{IS} values shows less homozygosity than expected from a population at Hardy-Weinberg equilibrium. Nei (1973) used G_{ST} for multiallelic loci.

F_{ST} is the effect of population subdivision on the reduction of heterozygosity due to random genetic drift, also called the fixation index. This is inbreeding in subpopulations relative to the total population of which they are a part. F_{ST} is equal to zero if all subpopulations are in Hardy-Weinburg equilibrium, otherwise it is larger than zero. Significant F_{ST} values indicate large genetic separation among subpopulations. F_{ST}, as a measure of population subdivision, is related to different measures of genetic distance.

F_{IT} is the overall inbreeding coefficient of an individual which includes contributions due to actual nonrandom mating within subpopulations (F_{IS}) and due to the subdivision itself (F_{ST}). This is a measure of inbreeding of an individual relative to the population as a whole, and is the most inclusive measure of inbreeding in that it takes into account both the effects on nonrandom mating within subpopulation and the effects of population subdivision. F_{IS} statistics, as measures of inbreeding, in each of the seven subpopulations were computed by GENEPOP.

4. RESULTS

4.1. Intrapopulation Genetic Variability

4.1.a. Allele Frequency Distribution and Polymorphism

The seven microsatellite primers: Mvi24, Mvi232, Mvi54, Mvi87, Mvi219, Mvi111 and Mvi57, generated 3, 6, 6, 4, 9, 8 and 10 alleles, respectively (Table 4.3). All the seven loci were polymorphic in every population, except Mvi24, the least polymorphic locus with only three alleles, which was monomorphic in pastel and wild mink. The average number of alleles per locus was 6.57 in the entire sample, and ranged between 4.14 on ranch 4 (M4) and 5.14 on ranches 1 and 2 (M1 and M2) (Table 4.3), indicating a large degree of genetic variability within populations. Seventeen of the 46 alleles were rare, i.e. with frequencies of less than 0.05 over all populations. Except for locus Mvi219, there was at least one allele in each locus with frequencies larger than 0.05 in every population ($Mvi24^{141}$, $Mvi232^{151}$, $Mvi54^{97,128,130}$, $Mvi87^{78,80}$, $Mvi111^{88}$ and $Mvi57^{98,104}$).

4.1.b. Hardy-Weinberg Equilibrium

Allele frequencies did not conform with Hardy-Weinberg values ($P < 0.05$) in 22 of the 49 population-locus subclasses (Table 4.4). Pooling the rare alleles did not change the results. Black mink from farms 1, 2, 3, and 4 were in Hardy-Weinberg equilibrium at 4, 5, 3 and 5 loci, respectively, while the pastel, brown and wild mink populations were in Hardy-Weinberg equilibrium at 2, 3 and 2 loci, respectively. There were differences among loci for the number of populations at Hardy-Weinberg equilibrium. Six of the

populations were in equilibrium at locus Mvi232 and none at locus Mvi87, indicating that forces which caused deviation of allele frequencies from Hardy-Weinberg equilibrium had dissimilar effects on various loci.

Significant departures from Hardy-Weinberg equilibrium were always associated with an excess of homozygous individuals, as shown by positive F_{IS} values (Table 4.4).

4.1.c. Heterozygosity

Estimates of expected unbiased heterozygosity (H_E) of each population at every locus, of individual loci over all populations, and averaged over all loci for each population are shown in Table 4.5, and the observed heterozygosities are shown in Table 4.13. Averages of H_E and H_O over all populations and loci were 0.633 and 0.417, respectively.

Heterozygosity of populations at each locus: H_E varied substantially among loci in each population (at least by 0.45 points). H_E of individual loci within populations ranged from zero (locus Mvi24 which was monomorphic in pastel and wild mink) to 0.841 (Mvi219 in brown). There was a positive relationship between H_E and the number of alleles segregating at each locus (Fig. 4.1, 4.2).

Pairwise comparisons between populations within each locus showed that black mink herds had comparable H_E at all loci, except for M2 which had a significantly smaller H_E (0.178) than both M1 (0.463) and M3 (0.413) at locus Mvi232, and M4 which had a significantly smaller H_E (0.318) than M1 (0.597), M2 (0.626) and M3 (0.640) at locus Mvi57 (Table 4.6). Small H_E estimates of M2 at locus Mvi232 and M4 at locus Mvi57

were the results of one allele with exceptionally high frequency in each of these populations (0.905 in Mvi232^{151} and 0.820 in Mvi57^{104}).

Pairwise comparisons of black mink with wild and coloured mink for H_E are shown in Table 4.7, 4.8 and 4.9. All the black mink populations had significantly larger heterozygosities (0.276 to 0.447) than those in wild, pastel and brown mink (0.0 to 0.097) at locus Mvi24, which was the result of the latter populations being almost monomorphic at this locus (Table 4.3). Significant differences ($P<0.05$) were also observed among these populations at locus Mvi232, except between M2 and wild mink which had comparable H_E due to a high frequency of allele 151 (0.905 and 0.975). Black mink had smaller H_E (0.178 to 0.463) than coloured mink (0.766 and 0.754) at this locus, as a result of a smaller number of alleles (3 or 4) in the former than in the latter populations (5 alleles). Locus Mvi232, on the other hand, was almost monomorphic in wild mink, with a very small H_E (0.050). The only other locus in which black mink herds had significantly lower heterozygosities (0.318 to 0.640) than coloured and wild mink (0.785 to 0.824) was Mvi57, again due to smaller number of alleles in black (4 to 8) compared with that in wild and coloured mink (6 to 8).

Wild, brown and pastel mink had comparable heterozygosities at all loci (Table 4.10). The only difference ($P<0.01$) was an exceptionally low H_E (0.050) in wild mink compared with that in pastel (0.766) and brown (0.754) at locus Mvi232. Only two of the six alleles at this locus were present in wild mink, one (Mvi232^{151}) with very high frequency (0.975).

Estimates of observed heterozygosity (H_O) were smaller in magnitude than H_E in

36 of 49 comparisons (Table 4.5 and 4.13 and Fig. 4.5), and the differences were significant in 18 of these cases (Table 4.14). The extent of differences between H_E and H_O was the attribute of the loci rather than the characteristic of the populations. H_E was larger than H_O at two (M2 and wild mink) or three loci in every population. Mvi87 was the only locus in which estimates of H_E were significantly larger than H_O in every population, while estimates of H_E and H_O were not different at locus Mvi232 in any of the populations. All the four alleles at locus Mvi87 were segregating in all the populations with comparable frequencies, except in M4 and wild mink, whereas there was an allele with a rather high frequency at locus Mvi232, and the other five alleles either were absent or had low frequencies at this locus in most populations (Table 4.3).

Locus heterozygosity: H_E of the seven loci, using pooled data over populations, ranged from 0.313 at locus Mvi24, to 0.822 at locus Mvi219 (Table 4.5). The former locus had the smallest number of alleles (3) and had the smallest H_E in most populations (0.0 to 0.447), and the latter, with 9 alleles, had generally the largest estimates in all populations (0.637 to 0.841). Only five of the pairwise comparisons; Mvi87 and Mvi54 (0.705 and 0.698), Mvi87 and Mvi57 (0.705 and 0.711), Mvi54 and Mvi57 (0.698 and 0.711), Mvi219 and Mvi111 (0.822 and 0.790), and Mvi111 and Mvi57 (0.790 and 0.711), were nonsignificant (Table 4.11).

H_O of different loci, pooled over populations, were smaller than the corresponding H_E estimates (Fig.4.3), ranging from 0.157 at locus Mvi87 to 0.711 at locus Mvi111 (Table 4.13). The ranking order of the loci based on H_O was different from that based on H_E. The main difference between H_E and H_O was in the case of locus Mvi87 with the

smallest H_O (0.157) and a larger than average H_E (0.705).

Population heterozygosity: Average H_E of each population over all loci ranged from 0.498 in wild mink to 0.651 in brown mink, and the black mink herds had intermediate values (Table 4.5). Average H_E of the four black mink herds were not different from each other (Table 4.12), although M1 tended ($P<.10$) to have a higher H_E (0.608) than M2 (0.543) and M4 (0.531). Average H_E of the wild mink was significantly smaller than that of M1 (0.608) and brown (0.651), and tended ($P<0.10$) to be smaller than that of M3 and pastel. H_E of the pastel population was comparable with those in other populations, while H_E of the brown mink, the most heterogeneous of all populations, was larger ($P<0.05$) than those in M2, M4 and wild mink.

Estimates of observed heterozygosity of the populations, averaged over all loci, ranged between 0.370 in wild mink and 0.504 in brown (Table 4.13). H_E was larger than H_O in every population (Fig.4.4), and the ranking of the populations was the same based on both estimates.

4.2. Interpopulation Genetic Variability

4.2.a. Allelic Differentiation

There were considerable differences in allele frequency distributions among the populations (Table 4.3). Although there was no allele with a high frequency in one population and absent in others, which could be used as a marker for population identification, there were many alleles with frequencies larger than 0.05 in one or more populations while absent in others. These alleles were summarized in the following table

(Table 4.1). The contrasts were, as expected, largely between black mink with wild and coloured mink, i.e. alleles were present in all black mink herds but absent in one of the other populations, or vice versa. The most notable observations were the allele $Mvi24^{143}$ which was present at frequencies of 0.163 to 0.329 in black mink herds but was absent in pastel, brown and wild mink; allele $Mvi111^{110}$ with frequency 0.150 in wild mink but was absent in other populations; and allele $Mvi219^{178}$ with frequencies of 0.198 to 0.554 in black mink samples, 0.15 and 0.325 in brown and wild mink, but absent in pastel.

Table 4.1: Alleles with frequencies larger than 0.05 in some populations while absent in others

Comparison	Locus	Allele	M1	M2	M3	M4	PAS	BRO	WIL
Black *vs* others	Mvi24	143	.230	.300	.163	.329	.0	.0	.0
Wild *vs* others	Mvi232	147	.205	.071	.173	.103	.265	.275	.0
	Mvi87	82	.273	.070	.143	.158	.200	.375	.0
	Mvi219	168	.066	.189	.058	.125	.063	.125	.0
	Mvi111	110	.0	.0	.0	.0	.0	.0	.150
	Mvi111	112	.0	.0	.0	.0	.0	.0	.050
Brown *vs* others	Mvi111	106	.195	.167	.141	.132	.053	.0	.100
Pastel *vs* others	Mvi219	172	.057	.162	.349	.139	.0	.050	.100
	Mvi219	178	.287	.554	.198	.500	.0	.150	.325
Coloured *vs* others	Mvi232	163	.0	.0	.0	.0	.059	.125	.0
Wild & pastel *vs* others	Mvi219	166	.082	.041	.047	.111	.0	.025	.0
Wild & brown *vs* others	Mvi57	106	.0	.0	.0	.0	.0	.025	.147

The results of χ^2 tests for pairwise comparisons between black mink herds at every locus are shown in Table 4.15. Pairwise comparisons of black mink herds showed that allele frequencies were different ($P<0.05$) in 22 of the 42 cases. The herds had different allele frequencies at 2 (M2 vs M4), 3 (M1 vs M2 and M4), 4 (M1 vs M3), or 5 (M3 vs M2 and M4) loci. Mvi87 was the only locus at which all the comparisons among black mink herds showed significant differences.

Most of the non-rare alleles were either present or absent in all the four black herds, except $Mvi232^{155}$, $Mvi87^{84}$, $Mvi219^{174}$ and $Mvi57^{96}$ that had frequencies larger than 0.05 in at least one of the black mink herds but were absent in another herd (Table 4.3).

All black mink herds had significantly different allele frequencies than wild, brown and pastel at every locus. The exceptions were wild mink and M1 at locus Mvi219, wild mink and M2 at locus Mvi232, and brown and M1 at loci Mvi87 and Mvi219 (Tables 4.16, 4.17 and 4.18). Differences between wild mink and M4 at locus Mvi111, brown and M3 at locus Mvi24, and pastel and M2 at locus Mvi87 approached significance ($P<0.10$). The results signify large differences between wild, brown and pastel mink with the black mink herds.

Brown and pastel mink were different ($P<0.05$) for allele frequencies at three loci (Mvi87, Mvi111 and Mvi57), as were brown and wild mink (Mvi232, Mvi111 and Mvi57) (Table 4.19). Pastel and wild mink showed significant allele frequency differences at four loci (Mvi87, Mvi232, Mvi219 and Mvi111). These two populations were monomorphic at locus Mvi24. Brown, pastel and wild mink populations had similar

allele frequencies at loci Mvi24 and Mvi54, while all these three populations were different for allele frequencies at locus Mvi111.

In summary, judging by the number of significant differences among populations for allele frequency distributions at various loci, the populations could be classified into two groups; black mink and non-black mink (wild, brown, and pastel). There is smaller number of significant differences in allele frequencies within each category (less than 60% of comparisons were significant), and larger number of significant differences between categories (more than 85%), as shown in the following summary table (Table 4.2).

This classification is sensitive to probability level, and altered to some extent when the probability level was set at 0.01 rather than 0.05. The number of significant differences did not change in some sets of comparisons, such as in black *vs* pastel, and pastel *vs* wild mink, while it changed substantially in other comparisons, such as in brown *vs* pastel. This resulted in a higher resolution of the differentiation among populations. Comparing the populations based on the number of significant differences in allele frequencies at 1% probability level resulted in three categories; coloured mink (no significant difference between pastel and brown), black herds (33% of the comparisons were significant), and wild mink (see the following summary table). The largest differences were observed between black and coloured mink (96 and 82% sig. differences), followed by black and wild (75% sig. differences), and coloured and wild mink (29 and 57%).

Table 4.2: Percentage and number of significant differences in pairwise comparison of allele frequency differences at $P<0.05$ and $P<0.01$

Population	Total number of comparisons	% and number of significant differences at $P<0.05$	% and number of significant differences at $P<0.01$
Black herds	42	52% (22)	33% (14)
Black *vs* wild	28	89% (25)	75% (21)
Black *vs* brown	28	86% (24)	82% (23)
Black *vs* pastel	28	96% (27)	96% (27)
Brown *vs* pastel	7	43% (3)	0% (0)
Brown *vs* wild	7	43% (3)	29% (2)
Pastel *vs* wild	7	57% (4)	57% (4)

4.2.b. Genetic Distance

Estimates of Nei's genetic distance between black mink populations ranged from 0.074 (M1 and M3) to 0.132 (M2 and M4) (Table 4.20). M1 had the smallest genetic distances with every other black mink herd. The largest genetic distances were observed between pastel and black mink populations, ranging from 0.652 (M4) to 0.343 (M1). Genetic distances between black mink populations and brown were generally larger than those between black mink and wild mink. M4 had the largest genetic distances with non-black mink populations, followed by M2. Genetic distance between wild mink and pastel (0.380) was larger than that between wild mink and brown (0.271). Brown and pastel had a small genetic distance (0.096).

The overall picture that can be deduced from the matrix of Nei's genetic distances is that black mink herds were closely related to each other, as were coloured mink

populations, and these two categories were genetically remote from each other. Wild mink was more closely related to the black mink herds than to brown and pastel. This was the same conclusion that was reached by comparing allele frequency distribution of the populations.

Estimates of genetic distance based on Cavalli-Sforza's chord measure were all smaller than the corresponding estimates based on Nei's formula (Table 4.21), but the populations ranked similarly in the majority of cases based on these two methods.

4.2.c. Phylogenic Tree

The phylogenetic tree based on UPGMA method and Nei's genetic distance is shown in Fig. 4.6. The four black mink herds were clustered together and formed a branch. Mink from M1 and M3 were the closest of the four herds forming the core of the branch, which was expanded when M4 and M2 appended this core. The wild mink joined the black mink cluster, while brown and pastel formed an entirely separate branch. This tree topology indicates that wild mink is genetically closer to black mink than to brown and pastel.

The Neighbor-joining (Fig. 4.7) and Fitch-Margoliash (Fig. 4.8) algorithms produced comparable tree topologies. The results indicate that the four black mink herds diverged from each other more recently, followed by wild and brown mink. Pastel was more remotely related to black mink than to brown and wild mink. Mink from M3 were the closest of the black mink herds to wild mink according to both Neighbor-joining and Fitch-Margoliash methods, while it was the most diverged herd of black mink from the

wild mink using UPGMA method. The results of these three methods were basically the same, and consistent with the findings of genetic distance and allele frequency distributions.

4.2.d. Assignment Test

Between 66.2% (M1) and 82.5% (M2) of black mink individuals were correctly assigned to the population in which they were sampled (Table 4.22). Between zero and 13% of black mink from various herds were assigned to other black mink herds, and smaller proportions were classified into non-black mink populations. None of the individuals from M4 were assigned to any of the non-black mink populations. Only 4.4% of M3 individuals were classified as wild mink and none to the other two populations, and 7.5% of M2 individuals were assigned to brown and none to other non-black populations. Small proportions of M1 individuals were assigned to brown (1.3%), pastel (2.6%) and wild mink (1.3%).

High proportions of brown (95%), wild (95%) and pastel (80%) individuals were correctly classified into their respective populations, and none to any of the black mink herds. None of the coloured mink was classified as wild mink, and 5% of wild mink individuals were assigned to pastel. The pastel individuals had the highest overlap (20%) with brown mink.

The results suggested a close relationship between pastel and brown populations, and a large difference between these two populations with black mink herds and wild mink. There was a considerable degree of overlap between black mink herds as well.

Table 4.3: Allelic size and frequencies at each locus in each population.

Locus	Allele	Size	M1	M2	M3	M4	PAS	BRO	WM	Pooled
Mvi24	1	139	0	0	0	0	0	0.050	0	0.004
	2	141	0.770	0.700	0.837	0.671	1.00	0.950	1.00	0.808
	3	143	0.230	0.300	0.163	0.329	0	0	0	0.188
# animal			63	40	46	38	20	20	20	
Mvi232	1	145	0	0.012	0	0.044	0	0	0.025	0.001
	2	147	0.205	0.071	0.173	0.103	0.265	0.275	0	0.158
	3	151	0.699	0.905	0.745	0.824	0.265	0.375	0.975	0.725
	4	153	0	0	0	0.029	0.088	0.050	0	0.013
	5	155	0.096	0.012	0.082	0	0.324	0.175	0	0.081
	6	163	0	0	0	0	0.059	0.125	0	0.013
# animals			78	44	49	34	17	20	20	
Mvi54	1	97	0.562	0.538	0.613	0.466	0.139	0.300	0.175	0.465
	2	126	0.046	0	0.038	0	0	0	0.075	0.026
	3	128	0.223	0.256	0.188	0.155	0.250	0.075	0.150	0.197
	4	130	0.100	0.141	0.138	0.259	0.250	0.325	0.375	0.188
	5	132	0.062	0.038	0.025	0.103	0.278	0.300	0.200	0.106
	6	134	0.008	0.026	0	0.017	0.083	0	0.025	0.017
# animals			65	39	40	29	18	20	20	
Mvi87	1	78	0.234	0.047	0.469	0.645	0.050	0.175	0.725	0.324
	2	80	0.455	0.547	0.214	0.197	0.575	0.425	0.275	0.382
	3	82	0.273	0.070	0.143	0.158	0.200	0.375	0	0.182
	4	84	0.039	0.337	0.173	0	0.175	0.025	0	0.112
# animals			77	43	49	38	20	20	20	
Mvi219	1	164	0.025	0.014	0.035	0	0	0	0	0.015
	2	166	0.082	0.041	0.047	0.111	0	0.025	0	0.056
	3	168	0.066	0.189	0.058	0.125	0.063	0.125	0	0.092
	4	170	0.025	0	0.047	0	0.031	0.050	0.075	0.028
	5	172	0.057	0.162	0.349	0.139	0	0.050	0.100	0.139
	6	174	0.148	0	0.081	0	0.281	0.125	0.225	0.103
	7	176	0.262	0.014	0.163	0.042	0.406	0.300	0.150	0.174
	8	178	0.287	0.554	0.198	0.500	0	0.150	0.325	0.318
	9	180	0.049	0.027	0.023	0.083	0.219	0.175	0.125	0.075
# animals			61	37	44	36	16	13	20	
Mvi111	1	88	0.161	0.278	0.346	0.158	0.211	0.289	0.350	0.244
	2	94	0.008	0.014	0	0	0.053	0.026	0	0.012
	3	100	0.161	0.056	0.064	0.237	0.158	0.026	0.200	0.119
	4	102	0.102	0.167	0.128	0.158	0.500	0.421	0	0.187
	5	104	0.373	0.319	0.321	0.316	0.026	0.237	0.150	0.291
	6	106	0.195	0.167	0.141	0.132	0.053	0	0.100	0.137
	7	110	0	0	0	0	0	0	0.150	0.007
	8	112	0	0	0	0	0	0	0.050	0.002
# animals			59	36	39	19	19	19	10	

Table 4.3.(cont.): Allelic size and frequencies at each locus in each population.

Locus	Allele	Size	M1	M2	M3	M4	PAS	BRO	WM	Pooled
Mvi57	1	90	0.028	0.014	0.011	0.040	0	0.025	0	0.019
	2	94	0.148	0.157	0.057	0.020	0.237	0.175	0.088	0.123
	3	96	0.042	0.043	0.080	0	0.026	0	0.088	0.043
	4	98	0.155	0.171	0.375	0.120	0.263	0.150	0.353	0.219
	5	100	0.028	0.014	0.011	0	0.289	0.075	0.059	0.048
	6	102	0	0.014	0	0	0.158	0.400	0.176	0.063
	7	104	0.599	0.571	0.466	0.820	0.026	0.150	0.059	0.468
	8	106	0	0	0	0	0	0.025	0.147	0.013
	9	108	0	0.014	0	0	0	0	0	0.002
	10	110	0	0	0	0	0	0	0.029	0.002
# animals			71	34	43	25	19	20	12	
Total # of alleles			36	36	34	29	32	35	31	46
Avg. # of alleles/locus			5.14	5.14	4.86	4.14	4.57	5.00	4.43	6.57

Table 4.4: Probability that the populations are in Hardy-Weinberg equilibrium for each locus and F_{IS} values with original data and pooled rare alleles.

		Unpooled		Pooled	
Locus	Pop.	Probability	Fis	Probability	Fis
Mvi24	M1	0.0716	+0.246	0.0716	+0.246
	M2	0.0014	+0.533	0.0014	+0.533
	M3	0.0091	+0.451	0.0091	+0.451
	M4	0.0004	+0.591	0.0004	+0.591
	PAS[1]	—	—	—	—
	BRO	1.0000	-0.027	1.0000	-0.027
	WM[1]	—	—	—	—
Overall		0.0000		0.0000	
Mvi54	M1	0.6288	+0.063	0.6233	+0.062
	M2	0.1255	+0.188	0.1143	+0.188
	M3	0.1912	+0.221	0.1915	+0.221
	M4	0.6929	+0.056	0.6872	+0.056
	PAS	0.0187	+0.164	0.0175	+0.164
	BRO	0.0060	+0.456	0.0060	+0.456
	WM	0.7908	+0.039	0.7198	+0.034
Overall		0.0152		0.0129	
Mvi219	M1	0.0000	+0.500	0.0000	+0.499
	M2	0.7142	+0.110	0.7063	+0.110
	M3	0.0000	+0.368	0.0000	+0.365
	M4	0.2251	+0.173	0.2194	+0.173
	PAS	0.0006	+0.405	0.0001	+0.405
	BRO	0.0043	+0.292	0.0052	+0.292
	WM	0.0021	+0.389	0.0016	+0.389
Overall		0.0000		0.0000	
Mvi57	M1	0.6227	+0.056	0.4977	+0.052
	M2	0.2219	-0.005	0.0800	+0.039
	M3	0.1905	+0.113	0.2038	+0.110
	M4	1.0000	-0.137	1.0000	-0.137
	PAS	0.0000	+0.604	0.0000	+0.604
	BRO	0.0178	+0.362	0.0413	+0.359
	WM	0.0093	+0.220	0.0143	+0.189
Overall		0.0000		0.0000	
Mvi87	M1	0.0000	+0.845	0.0000	+0.845
	M2	0.0000	+0.961	0.0000	+0.961
	M3	0.0000	+0.618	0.0000	+0.618
	M4	0.0000	+0.654	0.0000	+0.654
	PAS	0.0006	+0.597	0.0006	+0.597
	BRO	0.0187	+0.479	0.0187	+0.479
	WM	0.0002	+0.881	0.0002	+0.881
Overall		0.0000		0.0000	

Table 4.4.(cont.): Probability that the populations are in Hardy-Weinberg equilibrium for each locus and F_{IS} values with original data and pooled rare alleles.

		Unpooled		Pooled	
Locus	Pop.	Probability	Fis	Probability	Fis
Mvi232	M1	0.7549	+0.032	0.7549	+0.032
	M2	1.0000	-0.070	1.0000	-0.070
	M3	0.6232	-0.039	0.6232	-0.039
	M4	0.1472	+0.061	0.1757	+0.053
	PAS	0.0587	+0.238	0.0791	+0.306
	BRO	0.3815	+0.005	0.8565	-0.012
	WM[2]	—	—	—	—
Overall		0.3736		0.5835	
Mvi111	M1	0.0201	+0.095	0.0212	+0.095
	M2	0.4017	-0.080	0.4055	- 0.080
	M3	0.0139	+0.178	0.0139	+0.178
	M4	0.0654	+0.213	0.0621	+0.213
	PAS	0.2544	+0.168	0.2490	+0.168
	BRO	0.8174	-0.210	0.8225	-0.210
	WM	0.9914	-0.102	0.9724	-0.125
Overall		0.0205		0.0201	

[1] Population was monomorphic at this locus

[2] Population was quasi-monomorphic at this locus

Table 4.5: Expected unbiased heterozygosity in each population at each locus.[1]

Locus	M1	M2	M3	M4	PAS	BRO	WM	Pooled
Mvi24	0.357	0.425	0.276	0.447	0.000	0.097	0.000	0.313
	(0.165)	(0.174)	(0.271)	(0.143)	(0.000)	(0.386)	(0.000)	(0.049)
Mvi87	0.667	0.587	0.690	0.527	0.612	0.664	0.409	0.705
	(0.036)	(0.127)	(0.090)	(0.267)	(0.426)	(0.133)	(0.424)	(0.007)
Mvi54	0.624	0.630	0.576	0.693	0.794	0.727	0.779	0.698
	(0.138)	(0.176)	(0.270)	(0.159)	(0.069)	(0.065)	(0.143	(0.021)
Mvi232	0.463	0.178	0.413	0.313	0.766	0.754	0.050	0.443
	(0.154)	(0.296)	(0.284)	(0.481)	(0.106)	(0.130)	(0.220)	(0.058)
Mvi219	0.816	0.637	0.806	0.704	0.726	0.841	0.810	0.822
	(0.033)	(0.238)	(0.069)	(0.217)	(0.186)	(0.092)	(0.103)	(0.009)
Mvi111	0.767	0.773	0.747	0.798	0.693	0.699	0.821	0.790
	(0.041)	(0.044)	(0.059)	(0.078)	(0.366)	(0.141)	(0.286)	(0.005)
Mvi57	0.597	0.626	0.640	0.318	0.785	0.777	0.824	0.711
	(0.158)	(0.284)	(0.094)	(0.634)	(0.070)	(0.165)	(0.190)	(0.027)
Average[2]	0.608	0.543	0.586	0.531	0.614	0.651	0.498	0.633

[1] Variance of heterozygosities (x100) are shown in brackets

[2] Weighted arithmetic mean over all loci

Table 4.6: Pairwise comparison of expected heterozygosity between black mink herds at each locus (χ^2 values and probabilities)

	M1 vs M2		M1 vs M3		M1 vs M4		M2 vs M3		M2 vs M4		M3 vs M4	
Locus	Value	Prob.	Value	Prob.	Value	Prob.	Value	Prob.	Value	Prob.	Value	Prob.
Mvi24	0.479	0.489	0.805	0.370	0.813	0.367	2.108	0.147	0.040	0.842	2.681	0.102
Mvi87	0.757	0.384	0.073	0.787	2.093	0.148	1.055	0.304	0.295	0.587	2.408	0.121
Mvi54	0.004	0.947	0.242	0.623	0.400	0.527	0.249	0.618	0.273	0.601	0.967	0.325
Mvi232	10.244	0.001	0.317	0.573	2.266	0.132	6.064	0.014	1.863	0.172	0.869	0.351
Mvi219	3.819	0.051	0.015	0.901	1.566	0.211	2.875	0.090	0.378	0.539	1.101	0.294
Mvi111	0.003	0.954	0.057	0.812	0.038	0.845	0.070	0.791	0.019	0.889	0.129	0.720
Mvi57	0.075	0.785	0.209	0.648	5.939	0.015	0.019	0.890	5.659	0.017	6.806	0.009

Table 4.7: Pairwise comparison of expected heterozygosity between black and wild mink herds at each locus (χ^2 values and probabilities)

	M1 vs. WM		M2 vs. WM		M3 vs. WM		M4 vs. WM	
Locus	Value	Prob.	Value	Prob.	Value	Prob.	Value	Prob.
Mvi24	14.854	0.001	16.972	0.001	10.417	0.001	17.904	0.001
Mvi87	4.359	0.037	1.746	0.186	4.651	0.031	0.736	0.391
Mvi54	1.760	0.185	1.420	0.233	2.550	0.110	0.483	0.487
Mvi232	14.412	0.001	2.189	0.139	10.789	0.001	6.075	0.014
Mvi219	0.002	0.967	1.969	0.161	0.003	0.958	0.796	0.372
Mvi111	0.059	0.808	0.039	0.843	0.138	0.711	0.007	0.935
Mvi57	3.241	0.072	2.172	0.141	2.004	0.157	10.962	0.001

Table 4.8: Pairwise comparison of expected heterozygosity between black and brown mink herds at each locus (χ^2 values and probabilities)

	M1 vs. BRO		M2 vs. BRO		M3 vs. BRO		M4 vs. BRO	
Locus	Value	Prob.	Value	Prob.	Value	Prob.	Value	Prob.
Mvi24	5.712	0.017	7.509	0.006	2.881	0.090	8.251	0.004
Mvi87	0.001	0.980	0.343	0.558	0.044	0.833	1.018	0.313
Mvi54	0.746	0.388	0.572	0.449	1.357	0.244	0.077	0.781
Mvi232	5.618	0.018	19.621	0.001	6.876	0.009	10.183	0.001
Mvi219	0.074	0.785	2.829	0.093	0.123	0.726	1.380	0.240
Mvi111	0.331	0.565	0.333	0.564	0.133	0.715	0.388	0.533
Mvi57	2.328	0.127	1.415	0.234	1.255	0.263	9.916	0.002

Table 4.9: Pairwise comparison of expected heterozygosity between black and pastel mink herds at each locus (χ^2 values and probabilities)

	M1 vs. PAS		M2 vs. PAS		M3 vs. PAS		M4 vs. PAS	
Locus	Value	Prob.	Value	Prob.	Value	Prob.	Value	Prob.
Mvi24	9.781	0.002	11.854	0.001	10.417	0.001	17.904	0.001
Mvi87	0.214	0.643	0.033	0.855	0.390	0.532	0.380	0.538
Mvi54	1.895	0.169	1.555	0.212	2.678	0.102	0.590	0.443
Mvi232	5.256	0.022	18.183	0.001	6.456	0.011	9.569	0.002
Mvi219	0.640	0.424	0.374	0.541	0.461	0.497	0.018	0.894
Mvi111	0.403	0.526	0.399	0.528	0.176	0.675	0.450	0.503
Mvi57	2.431	0.119	1.511	0.219	1.350	0.245	9.959	0.002

Table 4.10: Pairwise comparison of expected heterozygosity between brown, pastel and wild mink herds at each locus (χ^2 values and probabilities)

	BRO vs PAS		BRO vs. WM		PAS vs. WM	
Locus	Value	Prob.	Value	Prob.	Value	Prob.
Mvi24	2.801	0.094	2.801	0.094	0.000	1.000
Mvi87	0.120	0.729	2.648	0.104	1.653	0.198
Mvi54	0.215	0.643	0.149	0.700	0.008	0.929
Mvi232	0.004	0.948	23.619	0.001	22.495	0.001
Mvi219	0.760	0.383	0.066	0.797	0.397	0.528
Mvi111	0.002	0.962	0.354	0.552	0.401	0.526
Mvi57	0.003	0.957	0.105	0.746	0.072	0.792

Table 4.11: Comparison of expected heterozygosity of different loci pooled over all populations (probability levels)

Locus	Mvi87	Mvi54	Mvi232	Mvi219	Mvi111	Mvi57
Mvi24	0.001	0.001	0.002	0.001	0.001	0.001
Mvi87	—	0.877	0.001	0.002	0.036	0.874
Mvi54	—	—	0.001	0.002	0.030	0.762
Mvi232	—	—	—	0.001	0.001	0.001
Mvi219	—	—	—	—	0.392	0.005
Mvi111	—	—	—	—	—	0.060

Table 4.12: Comparison of expected heterozygosity of different populations averaged over all loci (probability levels)

Pop.	M2	M3	M4	BRO	PAS	WM
M1	0.084	0.550	0.057	0.350	0.889	0.026
M2	—	0.292	0.791	0.034	0.176	0.400
M3	—	—	0.206	0.191	0.584	0.091
M4	—	—	—	0.024	0.128	0.552
BRO	—	—	—	—	0.532	0.011
PAS	—	—	—	—	—	0.060

Table 4.13: Observed heterozygosity of each population at each locus

Locus	M1	M2	M3	M4	PAS	BRO	WM	Pooled
Mvi24	0.270	0.200	0.152	0.184	0.000	0.100	0.000	0.166
Mvi87	0.104	0.023	0.265	0.184	0.250	0.350	0.050	0.157
Mvi54	0.585	0.513	0.450	0.655	0.667	0.400	0.750	0.563
Mvi232	0.449	0.190	0.429	0.294	0.588	0.750	0.050	0.385
Mvi219	0.409	0.568	0.511	0.583	0.438	0.600	0.500	0.506
Mvi111	0.695	0.833	0.615	0.632	0.579	0.842	0.900	0.711
Mvi57	0.563	0.629	0.568	0.360	0.316	0.500	0.647	0.532
Average[1]	0.430	0.404	0.419	0.388	0.395	0.504	0.370	0.417

[1] Weighted arithmetic mean over all loci

Table 4.14: Comparison between observed and expected heterozygosity at each locus in each population (G-values)

Locus	M1	M2	M3	M4	PAS	BRO	WM
Mvi24	2.188	9.098**	3.975*	11.724**	0.000	0.001	0.000
Mvi87	106.925**	65.930**	37.336**	19.117**	10.791**	8.197**	13.832**
Mvi54	0.419	2.238	2.559	0.194	1.570	9.335**	0.098
Mvi232	0.068	0.043	0.052	0.057	2.647	0.002	0.000
Mvi219	49.440**	0.749	18.781**	2.354	5.847*	6.659**	9.724**
Mvi111	1.620	0.809	3.240+	2.801	1.093	2.067	0.489
Mvi57	0.324	0.001	0.954	0.203	19.194**	7.327**	3.012+

\+ $p<0.1$
* $p<0.05$
** $p<0.01$

Table 4.15: Pairwise comparisons of allele frequencies between black mink populations at each locus[1, 2]

	M1 vs M2		M1 vs M3		M1 vs M4		M2 vs M3		M2 vs M4		M3 vs M4	
Locus	Prob	SE	Prob	SE	Prob	SE	Prob	SE	Prob	SE	Prob	SE
Mvi24	0.331	0.007	0.232	0.007	0.145	0.006	0.047	0.003	0.732	0.005	0.016	0.002
Mvi87	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
Mvi54	0.517	0.012	0.477	0.011	0.050	0.004	0.742	0.008	0.156	0.008	0.141	0.009
Mvi232	0.000	0.000	0.738	0.007	0.129	0.006	0.018	0.002	0.281	0.007	0.433	0.009
Mvi219	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.342	0.010	0.000	0.000
Mvi111	0.091	0.007	0.015	0.003	0.669	0.009	0.891	0.005	0.157	0.008	0.042	0.003
Mvi57	0.757	0.009	0.001	0.001	0.014	0.002	0.015	0.003	0.019	0.002	0.000	0.000

[1] In this and the following four tables, the probability of sample homogeneity and its standard error are shown for each comparison.

[2] In this and the following four tables, the rare alleles were pooled before the analysis.

Table 4.16: Pairwise comparisons of allele frequencies between black and wild mink populations at each locus

	M1 vs. WM		M2 vs. WM		M3 vs. WM		M4 vs. WM	
Locus	Prob.	SE	Prob.	SE	Prob.	SE	Prob.	SE
Mvi24	0.000	0.000	0.000	0.000	0.005	0.001	0.000	0.000
Mvi87	0.000	0.000	0.000	0.000	0.000	0.000	0.012	0.002
Mvi54	0.000	0.000	0.000	0.000	0.000	0.000	0.012	0.002
Mvi232	0.000	0.000	0.194	0.005	0.002	0.000	0.047	0.003
Mvi219	0.136	0.007	0.000	0.000	0.001	0.000	0.000	0.000
Mvi111	0.010	0.002	0.002	0.001	0.002	0.001	0.097	0.004
Mvi57	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000

Table 4.17: Pairwise comparisons of allele frequencies between black and brown mink populations at each locus

	M1 vs. BRO		M2 vs. BRO		M3 vs. BRO		M4 vs. BRO	
Locus	Prob.	SE	Prob.	SE	Prob.	SE	Prob.	SE
Mvi24	0.012	0.001	0.002	0.000	0.090	0.003	0.001	0.000
Mvi87	0.644	0.008	0.000	0.000	0.000	0.000	0.000	0.000
Mvi54	0.000	0.000	0.000	0.000	0.000	0.000	0.066	0.004
Mvi232	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
Mvi219	0.120	0.008	0.000	0.000	0.001	0.001	0.000	0.000
Mvi111	0.000	0.000	0.005	0.001	0.002	0.001	0.001	0.000
Mvi57	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000

Table 4.18: Pairwise comparisons of allele frequencies between black and pastel mink populations at each locus

	M1 vs. PAS		M2 vs. PAS		M3 vs. PAS		M4 vs. PAS	
Locus	Prob.	SE	Prob.	SE	Prob.	SE	Prob.	SE
Mvi24	0.000	0.000	0.000	0.000	0.005	0.001	0.000	0.000
Mvi87	0.001	0.000	0.073	0.005	0.000	0.000	0.000	0.000
Mvi54	0.000	0.000	0.000	0.000	0.000	0.000	0.002	0.000
Mvi232	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
Mvi219	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
Mvi111	0.000	0.000	0.000	0.000	0.000	0.000	0.001	0.000
Mvi57	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000

Table 4.19: Pairwise comparisons of allele frequencies between pastel, brown and wild mink populations at each locus

	BRO vs PAS		BRO vs. WM		PAS vs. WM	
Locus	Prob.	SE	Prob.	SE	Prob.	SE
Mvi24	0.492	0.002	0.496	0.002	1.000	—
Mvi87	0.015	0.002	0.500	0.000	0.000	0.000
Mvi54	0.093	0.005	0.199	0.009	0.514	0.008
Mvi232	0.510	0.006	0.000	0.000	0.000	0.000
Mvi219	0.082	0.007	0.069	0.006	0.000	0.000
Mvi111	0.013	0.002	0.000	0.000	0.000	0.000
Mvi57	0.013	0.001	0.018	0.002	0.540	0.008

Table 4.20: Nei's genetic distances among populations

	M2	M3	M4	BRO	PAS	WM
M1	0.081	0.074	0.095	0.218	0.343	0.251
M2	—	0.120	0.132	0.338	0.479	0.300
M3	—	—	0.104	0.256	0.408	0.164
M4	—	—	—	0.386	0.652	0.226
BRO	—	—	—	—	0.096	0.271
PAS	—	—	—	—	—	0.380

Table 4.21: Cavalli-Sforza's Chord distance among populations

	M2	M3	M4	BRO	PAS	WM
M1	0.051	0.027	0.054	0.102	0.140	0.138
M2	—	0.052	0.063	0.144	0.192	0.173
M3	—	—	0.061	0.117	0.166	0.124
M4	—	—	—	0.149	0.237	0.150
BRO	—	—	—	—	0.061	0.153
PAS	—	—	—	—	—	0.188

Table 4.22: The percentage of animals from each population (row headings) that were assigned to each of the 7 populations (column headings)

	M1	M2	M3	M4	BRO	PAS	WM	# animals
M1	66.2	7.8	7.8	13.0	1.3	2.6	1.3	77
M2	2.5	82.5	0	7.5	7.5	0	0	40
M3	8.9	6.7	71.1	8.9	0	0	4.4	45
M4	3.0	12.1	9.1	75.8	0	0	0	33
BR0	0	0	0	0	95.0	5.0	0	20
PAS	0	0	0	0	20.0	80.0	0	20
WM	0	0	0	0	0	5.0	95.0	20

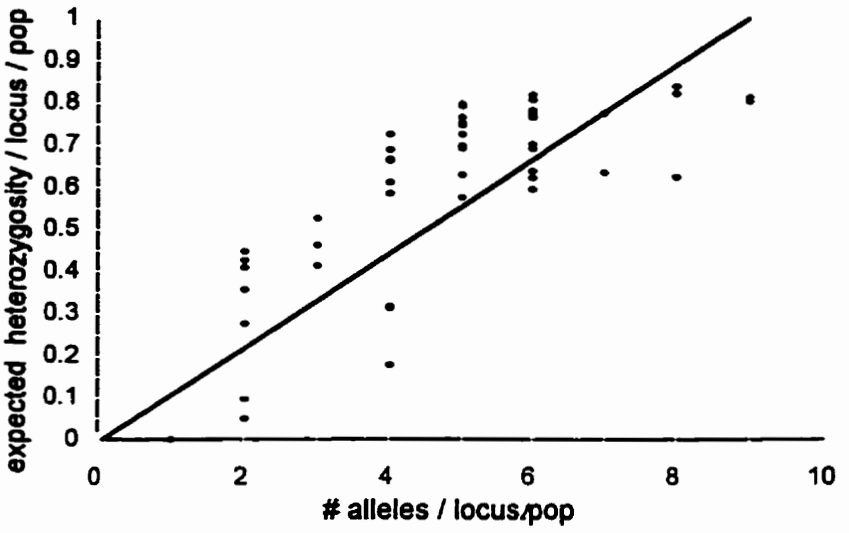

Figure 4.1. Scatterplot showing the expected heterozygosity/locus/population vs the number of alleles/locus/population (the line indicates the diagonal of the plot).

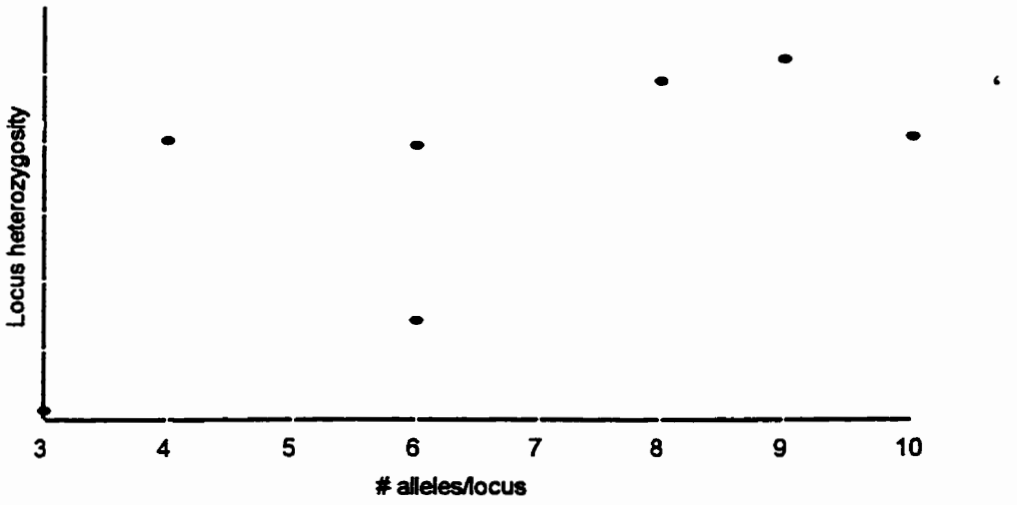

Figure 4.2. Scatterplot showing the expected locus heterozygosity vs the number of alleles/locus

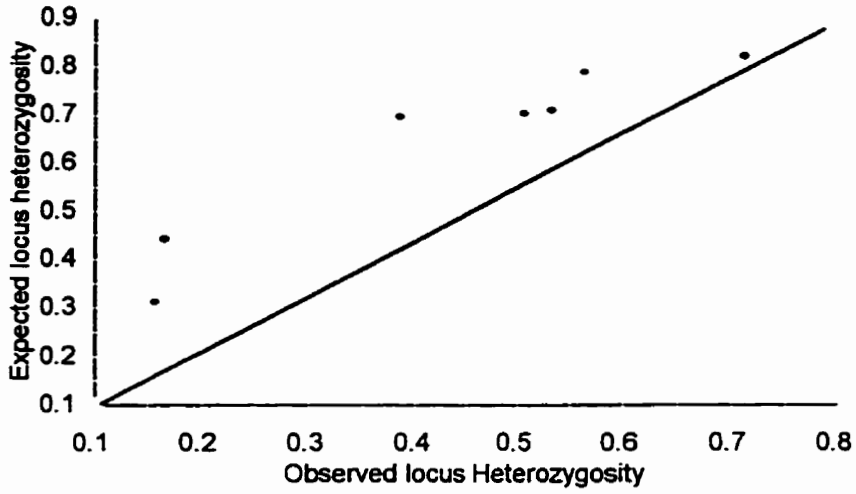

Figure 4.3. Scatterplot showing the expected locus heterozygosity vs the observed locus heterozygosity (the line indicates the diagonal of the plot).

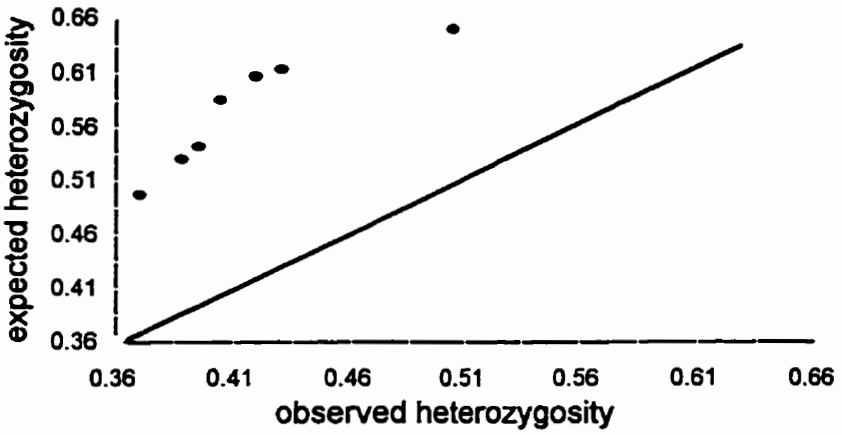

Figure 4.4. Scatterplot showing the expected population heterozygosity vs observed population heterozygosity (the line indicates the diagonal of the plot).

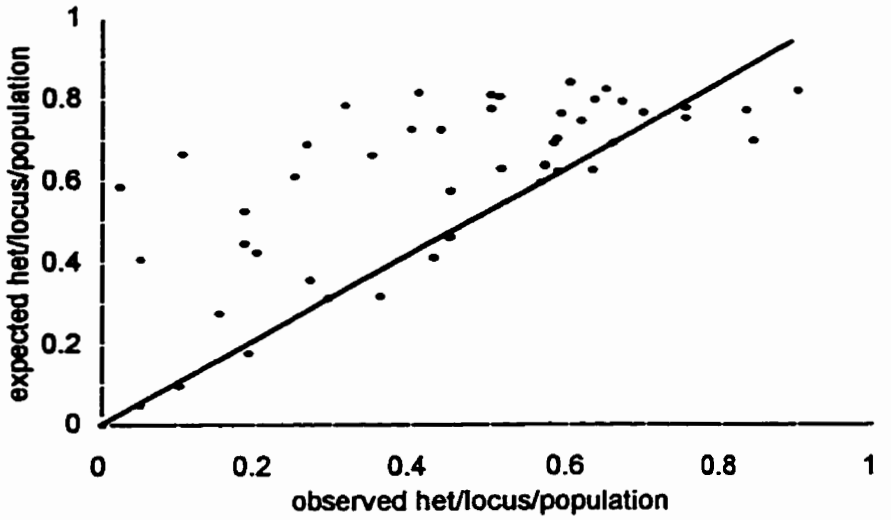

Figure 4.5. Scatterplot showing the expected heterozygosity/locus/population vs observed heterozygosity/locus/population (the line indicates the diagonal of the plot).

Figure 4.6. UPGMA dendogram based on Nei's genetic distance. The length of branches is shown (x1000)

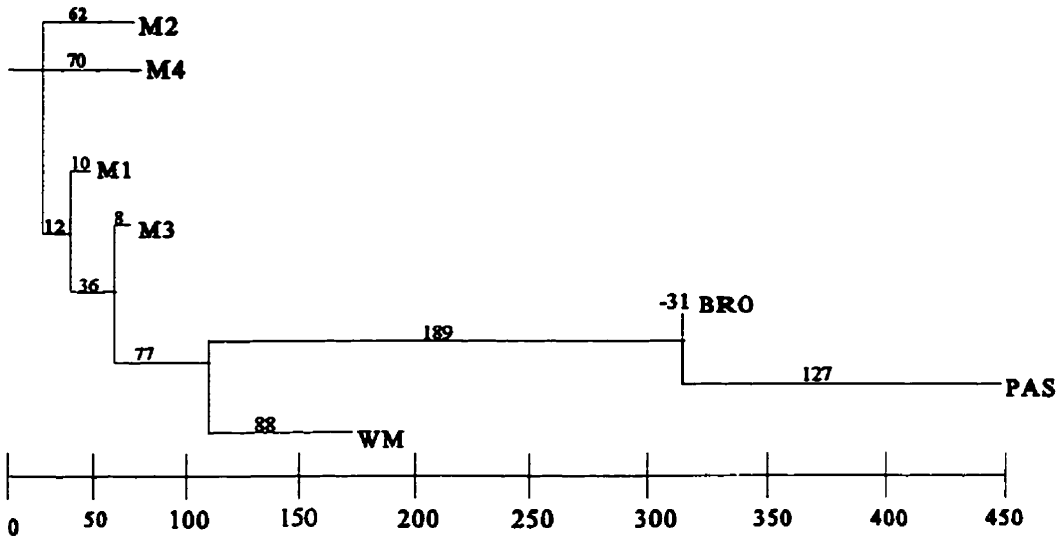

Figure 4.7. Neighbor-joining dendogram derived from Nei's genetic distance. The length of branches are shown (x1000).

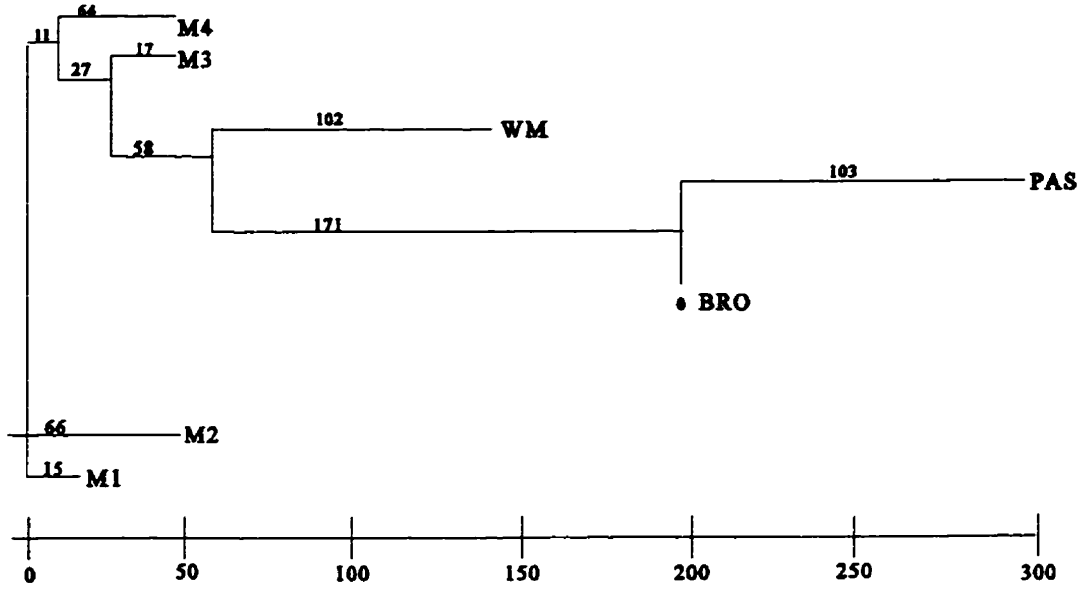

Figure 4.8. Fitch-Margoliash dendigram based on Nei's genetic distance. The length of branches are shown (x1000).

5. DISCUSSION

5.1. Intrapopulation Genetic Variability

Genetic variability within populations was measured as the level of polymorphism at microsatellite loci and heterozygosity. Test of Hardy-Weinberg equilibrium, along with estimates of F_{IS} and the differences between observed and expected heterozygosities were used to explore the breeding structure of the wild mink and genetic consequences of the mating systems employed by the mink ranchers.

5.1.a. Allele Frequency Distribution and Polymorphism

The number of alleles generated at each locus (from 3 to 10) and the average number of alleles per locus (6.57) indicates considerable levels of polymorphism in the populations studied (Table 4.3). The number of alleles at microsatellite loci and the average number of alleles per locus, as measures of variation within populations, is biased upward, because only polymorphic loci were used. Genetic variability of the structural genes, particularly those loci controlling fur quality traits, is certainly smaller than those for microsatellites, as a result of intense selection. Polymorphism at microsatellite loci is, however, an indication of the level of genetic variability of the populations studied.

Table 5.1. Number of loci examined and average number of alleles per locus in various studies.

Species	Number of loci tested	Average No. alleles/locus	Reference
Sheep	9	3.6	Bancroft et al. 1995
Pigs	376	5.8	Rohrer et al. 1994
Black bears	4	6.3	Paetkau & Strobeck, 1994
Polar bears	8	6.5	Paetkau et al. 1995
Canine	20	5.0	Ostrander et al. 1995
Gray wolves (7 groups)	10	3.4-6.4	Roy et al. 1994
Coyotes (7 groups)	10	4.9-6.9	" "
A red wolves	10	5.3	" "
Golden jackals	10	4.8	" "
Atlantic Cod	5	32.6	Ruzzante et al. 1996
Cattle	17	10.6	Ciampolini et al. 1995

There is no published information on the level of polymorphism at microsatellite loci in mink with which to compare these results. Although the level of polymorphism and maximum number of alleles per locus is the characteristic of individual microsatellite loci, they are expected to decline as inbreeding accumulates in a population and genetic variability is diminished. The number of alleles generated and the average number of alleles per locus in this study was, however, within the range of values reported for microsatellite loci in other species (Table 5.1), indicating that the genetic variability has not been exhausted in the ranched mink. This conclusion is further supported by the fact that the average number of alleles per locus in the ranched mink populations (4.14 to 5.14) was generally larger than that in the sample of wild mink (4.43, Table 4.3). In fact, while the wild mink was monomorphic at Mvi232, two alleles were segregating at high frequencies in the black mink herds at this locus (Table 4.3). Likewise, there were only two alleles at loci Mvi232 and Mvi87 in wild mink, but three to four alleles were

found in black mink herds at these loci.

The high levels of polymorphism in ranched mink populations can be attributed to the fact that they originated from 3 to 5 subspecies of wild mink (Dunstone 1993), and there has been continuous gene flow from other ranches. The average number of alleles per locus in M1 and M2 ranches was large, perhaps due to the fact that 13 males and 203 pregnant females were imported from one United States (US) ranch to M1 during 17 years, and 19 males and 129 pregnant females from three breeding ranches in the US were imported to M2 between 1987 and 1993. The M4 ranch purchased a smaller number of breeding animals, only 44 males and 40 pregnant females between 1982 and 1992, although they originated from 4 ranches in the US and from M2. In addition, microsatellite loci have high mutation rates (Levinson and Gutman 1987; Harding *et al.* 1993), and polymorphism is not expected to rapidly decline by selection because they are predominantly unlinked with structural genes.

5.1.b. Heterozygosity

The amount of heterozygosity is the most commonly used measure of genetic variation in a population. Heterozygosity represents a biologically useful quantity, because individuals in a diploid species are either heterozygous or homozygous at a given locus.

An average expected heterozygosity (H_E) of 0.633 over all populations and loci indicates a rather large level of genetic variability within these populations. Again, there is no published information on the level of polymorphism at microsatellite loci in mink

for comparison, but this estimate falls within the range of values (0.21 to 0.87) reported for microsatellite loci in various livestock species and natural populations (Table 5.2). These findings are in agreement with the report of Farid *et al.* (1994) who used minisatellite DNA fingerprinting and reported the heterozygosities of two mink ranches in Nova Scotia to be 0.51 and 0.53, and concluded that there is a considerable level of heterozygosity in the black mink herds in this province.

Table 5.2. Number of loci examined, expected (H_E) and observed heterozygosity (H_O) in various studies

Species	No. loci	H_E	H_O	Reference
Wasp	5	0.40	0.38	Choudhary *et al.* 1993
Barn swallows	2	0.66	-	Ellegren 1992
Pied flycatchers	2	0.63	-	Ellegren 1992
Brown trout	3	0.21-.59	-	Estoup *et al.* 1993
Toad	1	-	0.51-.83	Scribner *et al.* 1994
Gray wolves (7 groups)	10	0.57-.74	0.42-.61	Roy *et al.* 1994
Coyotes (7 groups)	10	0.63-.71	0.50-.65	Roy *et al.* 1994
Red wolves	10	0.55	0.51	Roy *et al.* 1994
Jackals	10	0.52	0.41	Roy *et al.* 1994
Bears (Nfld)	4	0.36	-	Paetkau and Strobeck 1994
Bears (continental)	4	0.80	-	Paetkau and Strobeck 1994
Polar bears	8	0.61-.64	-	Paetkau *et al.* 1995
Honey bees	12	0.29-87	-	Estoup *et al.* 1995
SNH wombat	16	0.65-.67	0.60-.67	Taylor *et al.* 1994
NHN wombat	16	0.27	0.28	Taylor *et al.* 1994
Atlantic cod	5	0.84-.89	0.82-95	Ruzzante *et al.* 1996

Heterozygosity of populations:

Despite many years of intense selection for fur quality traits, linebreeding, and positive assortative mating, the black mink herds have maintained rather high levels of

heterozygosity, as demonstrated by the high H_E estimates, ranging between 0.531 in M4 and 0.608 in M1 (Table 4.5). The rather high levels of heterozygosity of black mink could be the result of their diverse source, originating from 3 to 5 subspecies (Dunstone 1993), and of gene flow from other ranches with different allele frequency distributions. These estimates were not different between ranches (Table 4.12), perhaps as a result of comparable breeding strategies, and hybridization with animals from the same sources. The somewhat higher H_E of M1 compared to that in M2 and M4 ($P<0.10$, Table 4.12) could be due to the substantially larger number of breeding stock imported from a ranch in the US, which took place almost every year between 1982 and 1992. The absence of linebreeding in this ranch could also have contributed to its higher heterozygosity.

The numerical value of H_E in the sample of wild mink was the smallest among all the populations studied (0.498, Table 4.5), although significantly different only from that in M1 and the brown mink (Table 4.12). One possible explanation for the small H_E in the wild mink is the difference in sampling methods between the wild and ranched mink. Although the area where the wild mink were trapped was rather large (40 km^2), it could have been occupied by only 14 males, since the territory of each male can extend up to 2.79 km^2 (Dustone 1993). The mink occupying this area might have been related to each other, and thus the sample may not have been representative of the wild mink population in eastern Canada. The ranched mink, on the other hand, were unrelated to each other for at least one generation.

Heterozygosity in pastel and brown mink was expected to be smaller than that in the black mink herds, because these colored mink herds have been closed for

approximately 15 and 7 years, respectively, and had been kept in smaller herd sizes than those in M1, M2 and M4. The significantly higher H_E in brown mink than that in M2, M4 and wild mink could be explained by the fact that brown mink are sometimes produced by crossing brown with other color phases to produce new fur colors (Hansen *et al.* 1985), and perhaps to expand their genetic base and avoid inbreeding. Although this method had not been practiced with the mink samples used in this study, the high genetic variability could have possibly carried over from the previous crossings. Brown and pastel pelts are usually sold at a lower price than black mink pelts and one of the main reasons that ranchers maintain these strains of mink is their vigor and higher reproductive performance compared with black mink (Nes *et al.* 1988). The higher reproductive performance of the brown and pastel could be a reflection of their higher genetic variability. Conversely, the higher genetic variability of the colored mink could be the result of their higher reproductive performance, which results in less intense selection pressure and lower drift.

Heterozygosity, like any other estimate of population parameters, is affected by sampling error. A small sample may not be the representative of the allele count in a population. Sampling error was smaller in black mink than that in the colored and wild mink because of the larger sample sizes taken from the former herds.

There is no indication that heterozygosities were overestimated in this study. If anything, these could be somewhat underestimated because of several reasons:

i. Misidentification of the allelic states: Slipped strand mispairing of short tandem repeats (Tautz *et al.* 1986; Luty *et al.* 1990) and terminal transferase activity of *Taq*

DNA polymerase (Clark 1988) during PCR amplification results in a series of bands in some loci. These bands appear as stutter on a gel, and may result in misidentification of the alleles. The tendency is usually toward scoring a heterozygote as a homozygote because the second allele might be concealed by stutters, particularly if the two alleles differ by a few base pairs.

ii. Presence of the 'null' alleles: Null alleles can be produced by poor amplification of one of the alleles due to nucleotide changes at one of the primer sites and amplification of only one allele (Callen *et al.* 1993), resulting in an underestimation of heterozygotes.

iii. Selection: Although microsatellites are mostly selectively neutral, possible linkage between a microsatellite locus and a gene of economic importance in ranched mink or a gene that controls adaptation and survival in wild mink, could decrease heterozygosity in some of the microsatellite loci.

Locus heterozygosity:

Large and significant differences between loci within each population for H_E (by at least 0.45, Table 4.5), and between loci when pooled data over populations were used (0.313 to 0.822, Table 4.5), which were significantly different in most cases (Table 4.11), imply that the forces that create and retain genetic variability have distinctive effects on different loci. H_E is the function of the number of alleles and their relative frequencies, and its value increases as the number of alleles in each locus increases, as was the case in the present work (Figures 4.1 and 4.2).

The maximum value of H_E for a locus with any number of alleles is attained when alleles have equal frequencies. In a locus with two alleles (x and 1-x) in a population at Hardy-Weinburg equilibrium, heterozygosity is $H_E=2x(1-x)$. Setting the derivative of this equation equal to zero and solving for x:

$$d[2x(1-x)]/dx=d[2x-2x^2)]/dx=d(2x)/dx-d(2x^2)/dx=2-2(2x)=2-4x=0$$

$$4x=2 \text{ and } x=0.5$$

Likewise, maximum H_E in a locus with k alleles occurs when $x_1=x_2=..=x_k$, and thus $x_i=1/k$. $H_E=(1-\Sigma^k_{i=1}x_i^2)=1-(x^2+x^2+..+x^2)=1-k(x^2)=1-k(1/k)^2=1-(1/k)=(k-1)/k$. The following table (Table 5.3) shows the maximum values of H_E in a population at Hardy-Weinburg equilibrium at loci with different number of alleles (k). This table indicates that heterozygosities of more than 90% can be attained in a population at Hardy-Weinburg equilibrium when a locus has 10 or more alleles.

Table 5.3. Number of alleles per locus, allele frequency and maximum values of expected heterozygosity

Number of alleles (k)	Allele frequency (x)	Maximum H_E
2	0.50	0.50
3	0.333	0.667
4	0.25	0.75
5	0.20	0.80
6	0.167	0.83
10	0.10	0.90
20	0.05	0.95

Differences in the number of alleles and their relative frequencies among loci were the cause of deviations in H_E among populations at each locus, and among loci in each population. In the present study, for example, there were two loci each with six

alleles, Mvi232 and Mvi54 (Table 4.3), but the former locus had a significantly lower H_E (0.443) than the latter (0.698, Tables 4.5 and 4.11). The difference in H_E between these two loci was the result of their differences in relative allele frequencies. Mvi232 had one allele with very high frequency ($Mvi232^{151}$=0.725) while other alleles had small frequencies (0.158 to 0.001). In contrast, allele frequencies at locus Mvi54 were more uniform, ranging from 0.465 to .017, which resulted in a higher H_E at Mvi54 than that at Mvi232. Likewise, locus Mvi87 had a higher H_E than expected from its number of alleles (4) as shown in Figure 4.2, because of small differences among allele frequencies at this locus (0.32 to 0.112, Table 4.3). The number of alleles and allele frequency, in turn, depends on several factors:

i-Mutation rate: It has been shown that microsatellite loci have different mutation rates. Mutation rates in three mouse microsatellite loci were $2x10^{-3}$, $1.2x10^{-4}$ and $4.7x10^{-4}$ (Dallas 1992). Weber and Wong (1993) reported mutation rates in 28 microsatellites in human to range from $8x10^{-4}$ to $1.7x10^{-3}$. Although there is evidence that large stretches of DNA repeats are less stable than shorter ones (Valdes *et al.* 1993), and it has been postulated that longer repeats are prone to have higher mutation rates, no relationship between mutation rates and number of repeats has so far been reported (Valdes *et al.* 1993). The smallest allele sizes in the present study were observed at locus Mvi87, ranging between 78 and 84 bp, with only four alleles, while locus Mvi219 had the largest allele sizes (164 to 180) with nine alleles, indicating a positive relationship between the allele size and the number of alleles. This relationship was not, however, observed in other loci. The allele size at locus Mvi57 with the largest number of alleles (10) ranged

between 90 and 110, while the size of alleles at locus Mvi24 with only three alleles was between 139 and 143. Plaschke *et al.* (1995) found a small correlation (r=0.55) between the number of alleles and the average number of repeats in 23 wheat microsatellites. It should be noted that the allele sizes are not exactly the same as repeat numbers. There must be other mechanisms involved in causing differential rates of mutation among various microsatellite loci. Estoup *et al.* (1993) studied microsatellite loci in honey bees and concluded that mutations followed the infinite allele model. A newly formed allele would have a small frequency, and thus those loci that are prone to have a higher rate of mutation are expected to have large number of alleles, many with low frequencies.

ii-Selection: The presence of a linkage between a microsatellite locus and a gene under natural or artificial selection would result in a smaller number of alleles each with a high frequency, and thus a reduction in heterozygosity.

iii-Drift and population subdivision: Subdividing a population into lines and families could result in a few alleles each with high frequencies in some loci, due to drift and fixation. Using a small number of males would have a similar effect on the number of alleles and allele frequencies, causing differences among loci.

iv. Inaccuracies in genotyping: This could be a result of stutters on a gel, which may cause misidentification of the alleles. The degree of stuttering varied from locus to locus in this experiment, but did not obstruct accurate scoring of the gels. The only exception was Mvi57, which was somewhat difficult to score as a result of stutters. Although this locus had the largest number of alleles (10), estimates of H_E of this locus in different populations were within the range of values for other loci.

v. Sampling error: Statistical sampling, i.e., individuals that were included in the sample, is not expected to cause differences in H_E among loci, because genotypes of the same animals were determined in all loci under investigation. Mendelian sampling, however, could have played a role in differences in allele frequencies and H_E among loci. Mendelian sampling could change allele frequencies from parental to the filial generation, and its effect is more pronounced when population size is small or when it is subdivided into lines.

Observed vs. expected heterozygosity

The estimate of H_O over all populations and loci was 0.417 (Table 4.13), indicating that a randomly selected microsatellite locus in a randomly selected individual has a 41.7% chance of being heterozygous. This estimate is within the range of values reported in the literature for various species (Table 5.2). The H_O of populations averaged over all loci ranged between 0.370 in wild mink to 0.504 in brown mink (Table 4.13). The H_O in wild mink is close to that reported in wasps (0.38, Choudhary *et al.* 1993, Table 5.2), but is smaller than those reported in other species.

Estimates of observed and expected heterozygosities were positively correlated (Figures 4.3, 4.4 and 4.5). The observation that H_E was larger than H_O in the majority of comparisons, and significantly different in 18 of the 49 pairwise comparisons (Table 4.14), suggests an excess of homozygous individuals compared with Hardy-Weinburg proportions, i.e., individual animals were inbred to some degree (Scribner *et al.* 1994). These findings conformed with the results of tests for Hardy-Weinburg equilibrium and

F_{IS} values (Table 4.4). In cases where populations significantly deviated from Hardy-Weinburg equilibrium at a locus, it was always associated with a positive F_{IS}, which also indicated an excess of homozygous individuals and some degree of inbreeding (Wright 1978).

Two of the ranches (M1 and M4) have been practicing linebreeding which results in phenotypic and genetic uniformity, reduction of genetic variability within lines, and the accumulation of inbreeding. An increase in inbreeding and genetic uniformity within lines is associated with an increase in divergence among lines due to random genetic drift and differences in direction of selection, causing a shift in total genetic variability from within lines to among lines (Nei 1965; Falconer 1981). Positive assortative mating (breeding best-to-best) has been practiced on all the farms. This mating scheme results in the creation of distinct families, and the accumulation of inbreeding within families, because mates are likely to have descended from one or a few "superior" individuals. Although all the ranchers have avoided mating between closely related individuals (parent-offspring, half and full-sibs), matings between more distant relatives could not have been avoided because only one or two generations of relationships were taken into consideration, and thus the accumulation of inbreeding under positive assortative mating is unavoidable (Pirchner 1969).

It is well established that the avoidance of mating among closely related individuals has a minor effect on the level of heterozygosity and inbreeding, and that variance of family size and male to female ratio play more important roles in the rate of accumulation of inbreeding and homozygosity (Falconer 1981). For a given population

size and male to female ratio, keeping the variance of family size (V_k) equal to zero, i.e., selecting one male and one female from each dam and sire family, has the highest effect on effective population size (N_e) and rate of inbreeding (ΔF). In such cases, $\Delta F=(3/32N_m)+(1/32N_f)$, where N_m and N_f are the number of males and females respectively. When males and females are selected at random, (randomly selected pairs, $V_k=2$), and $\Delta F=(1/8N_m)+(1/8N_f)$ (Falconer 1981). In the mink industry, male to female ratio is approximately 1:4.5, and there is little variation among mink ranches for this ratio due to economic considerations (keeping as few males as possible to cut the production cost) and biological reasons (one male cannot breed with more than 10 to 15 females without jeopardizing the conception rate). Therefore, the expected rate of inbreeding for a mink herd with N_f females, and thus $N_m=N_f/4.5$ males, is:

$$\Delta F=(1/8N_m)+(1/8N_f)=(4.5/8N_f)+(1/8N_f)=5.5/8N_f \quad \text{(randomly selected pairs, } V_k=2)$$

$$\Delta F=(3/32N_m)+(1/32N_f)=(13.5/32N_f)+(1/32N_f)=14.5/32N_f \quad (V_k=0)$$

ΔF for a herd of 100 females, for example, would be 0.00687 and 0.00453 for randomly selected animals or when the variance of family size is zero, respectively.

Putting a great emphasis on fur quality traits with moderate to high heritabilities (Kenttamies and Vilva 1988), implies that many of the potential parents of the next generation would be related to a few animals with superior fur quality traits, which inflates the variance of family size much more than 2, which is expected under random selection of males and females, and would result in a reduction of N_e and an inflation in ΔF of more than $5.5/8N_f$. Positive assortative mating further increases ΔF, depending on

the genetic relationships between mates. Consequently, inbreeding is unavoidable in the mink industry under the existing selection and mating systems.

The existence of both large amounts of heterozygosity and an excess of homozygosity sounds inconsistent. The reason for these apparently contradictory results is attributable to linebreeding and positive assortative mating employed by the fur industry. Continuous flow of genetic materials from outside sources has increased heterozygosity by introducing new alleles into the herds. Imported individuals have been used in one of the two ways, depending on the farm: First, the imported mink were used in a closed line to evaluate their potential, such as in Farms 1 and 4. Lines were kept as long as their performances were up to the breeders' expectations. Second, the imported mink were bred with a random sample of mink. If descendants of these animals had high quality fur, they were selected and eventually bred to each other, which could happen when positive assortative mating is practiced. The consequences of these mating systems are the creation of distinct lines or families. Pastel, brown and wild mink had an excess of homozygosity at 4, 4 and 3 loci, respectively. Inbreeding as a result of closing these herds, and positive assortative mating could be the reasons for the excess of homozygosity in colored mink. Homozygosity in the wild mink could be the result of breeding between related males and females occupying an area.

When a population is divided into several subpopulations (lines), allele frequency distributions may differ among lines after a few generations solely because of random genetic drift. When samples are taken from k distinct lines (or subpopulations) each in Hardy-Weinburg equilibrium, but with different allele frequencies, the mean

heterozygosity of the pooled sample at a locus with two alleles (H) decreases by $2\sigma^2$, where σ^2 is the variance of the gene frequencies of the lines, i.e., $H=2p'q'[1-(\sigma^2/p'q')]$, which is known as Wahlund's formula (Cavalli-Sforza and Bodmer 1971). $\sigma^2= (\Sigma p_i^2/k)-p'^2$, where p' is the average gene frequency in the pooled data ($p'=\Sigma p_i/k$), $q'=1-p'$ and p_i is the allele frequency of the i^{th} line. When there are more than two alleles in a locus, the decline in heterozygosity is no longer equal to $2\sigma^2$ (Nei 1965). The deficiency of heterozygotes, compared with that expected from Hardy-Weinburg proportion, could thus be the result of the differences in allele frequencies between the lines caused by random genetic drift alone. In addition to random genetic drift, intense selection and positive assortative mating would accelerate the accumulation of homozygosity and inbreeding. Differences among lines or families gradually increase, and while populations as a whole possess high levels of genetic variability, most of that resides among lines or families.

More emphasis was put on H_E than H_O in this study because the former is a more accurate measure of a population heterozygosity than the latter due to several reasons (Nei and Roychoudhury 1974b):

i. H_E has a smaller variance than H_O.

ii. The genotype frequencies may deviate from Hardy-Weinburg proportions due to sampling error at the time of fertilization (Mendelian sampling). This deviation could be considerable when population size is small. Generally, gene frequencies are more stable than genotype frequencies in a finite population.

iii. Some genotypes, particularly in the homozygous state, may have a strong

effect on the survival rates of individuals during the embryonic stage and early period of development, prior to sampling. The proportion of heterozygotes would be distorted at the loci that are under such selection pressures, and at those linked with such loci. Consequently, the observed genotypic proportions would no longer correspond to the theoretical expectation of heterozygosity.

iv. If a population is divided into subpopulations (lines, families), inbreeding will accumulate within subpopulations, and the proportion of heterozygotes is a poor measure of the genetic heterozygosity of a population as a whole. The breeding strategies used by the mink industry, which creates subpopulations, was the main reason for using H_E in this study.

In most random breeding natural populations, the magnitude of H_o and H_E are expected to be comparable. Population subdivision and inbreeding (Wahlund's effect), however, would result in a considerable difference between the two estimates. Larger values of H_E than H_O have been reported in 16 groups of gray wolves, coyotes, red wolves and jackals (Roy *et al.* 1994), and wasps (Choudhary *et al.* 1993), as summarized in Table 5.2.

The equation for H_E has been derived under the assumption that genotype frequencies are at Hardy-Weinburg equilibrium, which was not a valid assumption in this study, at least for some loci (Table 4.4). Because the objective of this study was to estimate the genetic variability of populations irrespective of how selection, mating system and other factors influenced allelic frequencies, H_E is superior over H_O, although the populations were not always in Hardy-Weinburg equilibrium (Nei and Roychoudhury

1974b). H_E in such a case is the probability of non-identity of two randomly chosen alleles, and has been called heterozygosity index or gene diversity (Nei and Roychoudhury 1974b).

Population *vs* locus heterozygosity

The heterozygosity of a population is the average heterozygosity of all individuals in a population (assumed to be infinite) over all loci (assumed to be infinite). A random sample of individuals and a random number of loci are tested to estimate the heterozygosity of each population. Therefore, heterozygosity in a population has two components (Nei and Roychoudhury 1974a,b; Nei 1978):

i-heterozygosity of all individuals in a population at each locus

ii-heterozygosity of a specific individual over all loci

With regard to one locus, each individual is either homozygous or heterozygous. When k loci are considered, each individual could be heterozygous for 0, 1, 2, ...k loci, and average heterozygosity of each individual over k loci takes the values 0, 1/k, 2/k, .. k/k. When n individuals are sampled, locus heterozygosity takes the values 0, 1/n, 2/n, .., n/n.

	Locus				Individual
Individual	1	2	... j ...	k	Heterozygosity
1	H_{11}	H_{12}	... H_{1j} ...	H_{1k}	H_1
2	H_{21}	H_{22}	... H_{2j} ...	H_{2k}	H_2
:	:	:	::: : :::	:	:
i	H_{i1}	H_{i2}	... H_{ij} ...	H_{ik}	H_i
:	:	:	::: : :::	:	:
n	H_{n1}	H_{n2}	::: H_{nj} ...	H_{nk}	H_n
Locus heter.	$H_{.1}$	$H_{.2}$	... $H_{.j}$ ...	$H_{.k}$	H

In a sample of size n, heterozygosity of the jth locus in the ith individual is H_{ij}. The estimate of mean heterozygosity in a population over k loci is then:

$$H=(1/nk)\Sigma^{n}_{i=1}\Sigma^{k}_{j=1} H_{ij}=(1/k)\Sigma^{k}_{j=1} H_{.j}$$

Where $H_{.j}$ is the estimate of heterozygosity of the j^{th} locus. The sampling variance of heterozygosity over k loci is $V(H)=V(H_{.j})/k=H_{ij}(1-H_{ij})/nk$, assuming that heterozygosity at different loci are not correlated, which is a valid assumption unless loci are at linkage disequilibrium (Nei and Roychoudhury 1974a).

The variance, like the mean heterozygosity, has two components, that due to variation in heterozygosity among individuals in each locus, and that due to variation in heterozygosity of each individual among loci. The distributions of these two components are quite different. Individual heterozygosity has a fairly normal distribution, taking the values 0/k, 1/k, 2/k, ...k/k, where k is the number of loci. Locus heterozygosity (H_j) has a reverse J-shaped distribution when all loci in an individual are considered (Nei and Roychoudhury 1974b). The reverse J-shaped distribution is the result of many monomorphic and a relatively small number of highly polymorphic loci. In the case of microsatellites, however, where only polymorphic loci are considered, the observed distribution is no longer reverse J-shaped. The mean heterozygosity for both of these distributions is the same, but the variance among individual heterozygosities is usually much smaller than that for locus heterozygosities, because the heterozygosity differences among loci are large, particularly when monomorphic loci are included in the analysis (Nei and Roychoudhury 1974a; Nei 1978).

Using individuals with complete genotypes at all the 7 loci, the percentages of

individuals heterozygous at 0 to 7 loci in this study were 0.022, 0.118, 0.368, 0.324, 0.140, 0.029, 0.0 and 0.0, respectively, in the entire data set. This distribution is symmetric when the zero to 5 heterozygous loci are considered. This distribution suggests that the chance that a randomly selected individual will be heterozygous at 2 or 3 (or homozygous at 5 or 4) of the 7 loci is 69.2%. The distribution of individual heterozygosity is in agreement with the finding that some degree of inbreeding existed in these populations.

The variance of heterozygosity among loci is useful when one is interested in evaluating the reliability of heterozygosity estimates on these loci as a sample from all loci in the genome. The variance of heterozygosity of individuals is useful when one is interested in the estimate of heterozygosity of the total population (Nei and Roychoudhury 1974a; Nei 1978). Because variation among loci is generally greater than the variation among individuals, Nei (1978) suggested that when an accurate estimate of heterozygosity of a population is desired, with a constant number of determinations, it is best to examine more loci in fewer individuals, rather than few loci and larger number of individuals. In the case of zoo animals and rare breeds, where only a few individuals are available, a fairly good estimate of heterozygosity could be obtained by examining a large number of loci. Large differences among loci in H_E in the present work, which substantiate Nei (1978)'s suggestion implies that using a large number of loci would improve the accuracy of heterozygosity (or inbreeding) estimates of a population. Only seven loci were genotyped in this study, because the primer sequences of only nine mink microsatellite loci had been determined at the time (O'Connell *et al.* 1996). Two of these

loci did not amplify properly, producing stutters which interfered with the accurate scoring of the alleles, and were subsequently dropped.

5.1.c. Hardy-Weinberg Equilibrium

Almost half of the population-locus subclasses deviated from Hardy-Weinberg proportions (HWP), and excess homozygosity (positive F_{IS}) was the reason in every case (Table 4.4). Deviation from HWP could be due to several factors:

i. Sampling error: When the sample size is small, it is possible that the individuals in the samples are not representative of the allele counts in the populations. In order to sample as many alleles as possible, individuals that were unrelated for one generation were used in the black and colored mink. These individuals, however, might have been related to each other if the pedigree had been traced back farther. The sample sizes of black mink populations were large, ranging between 40 in M4 and 78 in M1. Therefore, it is unlikely that sampling error played a significant role in the deviation of black mink herds from HWP. Wild and colored mink had smaller sample sizes, which could have contributed to the observed deviation from HWP.

If sampling error was the major cause for deviation from HWP, it is expected that all loci in a population would be in disequilibrium, and it is unlikely that sampling error causes only certain loci to be in disequilibrium in most populations. All populations deviated from HWP at locus Mvi87 while all conformed with HWP at locus Mvi232. It seems that deviation from HWP is a characteristic of a locus rather than the attribute of a population and its breeding structure. When sampling error is the cause of

disequilibrium, loci with a higher number of alleles would be more likely to be in disequilibrium than loci with a lower number of alleles. This was not observed in our data either, as locus Mvi87, with 4 alleles, deviated from HWP in all populations, while locus Mvi232, with six alleles, conformed with HWP in all the populations. In addition, most populations deviated from HWP at locus Mvi24, with 3 alleles, while only four populations conformed with HWP at locus Mvi57, with 10 alleles. It should be noted that the fulfilment of HWP for a locus does not imply unbiased sampling procedures.

ii. The technical problems associated with generating microsatellites: i.e., stutters and null alleles, which influence allele frequency and heterozygosity, also affect HWP. Both of these factors result in the overestimation of homozygosity, and could cause departure from HWP. The effect of gel stutters on departures from HWP can be dismissed because Mvi57 with the highest level of stutters deviated from HWP in only two populations.

iii. Linkage: Linkage between a microsatellite and genes controlling traits of economic importance or fitness will increase homozygosity for the microsatellite locus, but this is not expected to be an important force for microsatellites.

iv. Positive assortative mating and linebreeding: Positive assortative mating, which has been practiced in all the ranches, and linebreeding, which has been practiced in two of the farms, could be the cause of excess of homozygosity and deviation from HWP. This seems to be the most admissible explanation, supported by positive F_{IS} values in all the loci that significantly deviated from HWP, and by the significantly larger H_E than H_O (Table 4.12) in almost every case where HWP was distorted. Factors that affect

excess of homozygosity, however, do not explain the reasons for the observed differences among loci for conformation with the expected HWP.

v. Statistical artifact: A total of 49 χ^2 tests were performed on the data, and thus the overall α (probability of rejecting a true hypothesis) was much larger than 0.05. To keep the overall α at 0.05, the Bonferroni correction of $p=\alpha/n=.05/49=0.00102$ (Miller 1981) could be considered, which would result in only 16, rather than 28 of the polymorphic loci to deviate from HWP.

Tests of Hardy-Weinburg equilibrium, both Fisher's exact test and Monte Carlo simulation procedure employed by GENEPOP, are designed to more accurately test for the differences between observed and expected frequencies when sample size is small. Nevertheless, very small cell numbers, resulting from rare alleles, would cause the rejection of a true hypothesis. To overcome this problem, rare alleles were pooled. This pooling, however, was based on the definition of a rare allele, i.e., when the frequency of an allele was less than 0.05 in the entire sample. There were several cases where the overall allele frequencies were larger than 0.05, but an allele had a frequency of smaller than 0.05 in a population, which could have influenced the results.

More than half of the 17 microsatellite loci in European cattle did not conform with HWP (Ciampolini *et al.* 1995), and sampling error was dismissed as a source of the disequilibrium. Roy *et al.* (1994) reported significant deviations from HWP in most of 16 groups of gray wolves, coyotes, red wolves and golden jackals. Up to 5 of the 10 microsatellite loci used in their study deviated from HWP. Significant deviations from HWP have also been reported in wombats (Taylor *et al.* 1994).

5.2. Interpopulation Genetic Variability

5.2.a. Genetic Diversity Among Populations

Genetic differences among populations were assessed using the χ^2 tests for allelic differentiation, genetic distances, phylogenetic analyses and the assignment test. These methods only provide relative measures of population differentiation, which is difficult to interpret without having a basis for comparison. The wild and colored mink were used as reference populations to facilitate interpretation of the degree of differentiation of the black mink herds.

The number of significant differences in allele frequency distributions between populations, estimates of genetic distance and phylogenetic analysis provided the same profile of population differentiation. The populations were classified into three categories; black mink herds, colored mink, and wild mink, with a large degree of genetic differences among these categories, and smaller levels of genetic differentiation between the populations within black and colored mink categories. The wild mink was found to be more closely related to black than colored mink, and the largest level of differentiation was detected between black and colored mink. Since the discriminating power of the assignment test is positively related to the degree of genetic differences between populations, the results of the assignment tests substantiated the above classification to a large degree.

Black mink herds: The observed levels of similarities (or differences) among black mink herds were the net result of two sets of opposing forces; one set pushing the herds apart,

and the other bringing them closer to each other.

i. Forces that create genetic similarity between herds:

Origin of the black mink herds: All the four black mink ranches were established by purchasing breeding stock from local farms in Nova Scotia, which might have originated from the same source or might have been related to each other.

Use of Jetblack: The Jetblack allele has been infused into all the four black herds used in this study. The Jetblack originated from a single litter discovered in Digby county in Nova Scotia (Mullen 1991e). Mink in these ranches have thus been genetically related to each other in the past through a few common ancestors.

Gene flow among ranches: Mink in M3 originated from several ranches in Nova Scotia in 1986, including M1, M2 and M4. Additional breeding stock were purchased from M1 and M2 in 1989 and from M1 in 1992. There has also been limited exchange of genetic material between M2 and M4 during the recent years.

Gene flow from common sources: A continuous gene flow from the same ranches in the US to three of the ranches in this study (M1, M2 and M4) has perhaps been the most important factor that has created genetic similarities among black mink herds during the recent years. Breeding stock from one farm in the US (L.F, Illinois) were imported into M1 almost every year from 1982 until 1992 (Table 3.1). Breeding stock from the same source were imported to M2 in 1987, 1988, 1990 and 1991 (Table 3.2), and to M4 in 1983, 1985, 1988, 1989 and 1992 (Table 3.4). Breeding stock from another ranch in the US (D.F., Illinois) were imported to M2 (1987, 1988) and M4 (1988). Although the number of imported stock has been a small fraction of the herd size in each of the

ranches, they may have been used quite extensively for breeding, and might have had a significant effect on genetic status of the herds.

Selection: When strong selection forces are applied to different populations in the same direction for a long time, the frequencies of the alleles directly under selection, and those that are linked with selected genes increase, resulting in comparable allele frequency distributions at such loci. All the black mink ranchers have followed somewhat similar selection schemes over the years, namely selecting for size and fur quality traits, with some attention being paid to litter size during recent years. Any linkage between microsatellite loci used in this study and genes that have been under selection would have increased genetic similarity. This is not expected, however, to have been a strong force in creating genetic similarity for microsatellite loci which are predominantly neutral.

ii- Forces that create genetic differences between herds:

Founder effect: Genetic drift may happen at several stages in a population's lifespan, one being at the time when a population is established (founder effect). If each of several populations is established with a few founder individuals, their genetic constitution may be drastically different, depending on the number of founder individuals and their genotype frequency distributions. The black mink herds used in this study were all started with a group of individuals of moderate size (Tables 3.1, 2, 3, 4, 5), and the founder effect is expected to be small.

Breeding structure: Allele frequency distributions of a population may change if it passes through a tight bottleneck (Chakraborty and Nei 1977), or when a small number of breeding individuals, particularly males, are used. When N_m males and N_f females are

selected and bred at random, the effective population size (N_e) is $N_e=4N_mN_f/(N_m+N_f)$ (Falconer 1982). Assuming the ration of one male to 4.5 females, $N_e=4(1)(4.5)/(1+4.5)=18/5.5=3.27$. Drift will increase by $5.5/3.27=1.68$ fold. This value would be much larger when intense selection pressure is applied to traits with moderate to high heritabilities, such as body size and fur quality. Many of the selected animals may be descendants of one or a few males with superior fur quality traits, causing a bottleneck, which could have escalated genetic drift and genetic differentiation between herds.

Population subdivision: When a population is divided into several subpopulations, each with a small number of individuals, genetic drift is expected to be large, and would result in genetic differentiation among subpopulations (Allard *et al.* 1968; Chakraborty and Nei 1977). Positive assortative mating, which has been practiced in all the mink ranches studied, would result in the creation of distinct families. This sort of population subdivision influences the genetic array of mink in a herd as a whole, because of a limited number of lines or families that are normally kept on each ranch.

Mutation: Mutation could create new alleles in a population. This is an important source of genetic differentiation in the case of microsatellite loci which have high mutation rates. Mutation, however, requires time to occur and for its frequency to reach a detectable level. The black mink herds used in this study were separated from each other for less than 30 generations (since 1966), and mutation is not expected to be a major source for genetic differentiation during this period of time, although its effect cannot be overlooked.

Sampling error: Two sampling processes were involved in this study; sampling of animals within populations, and sampling of loci within animals. Both of these sampling processes could influence the genetic arrays. It has been suggested that approximately 30 individuals per population and 30 loci per individual should be surveyed to obtain accurate estimates of genetic variability within and between populations (Nei 1975, 1976). The number of animals sampled from each of the black herds in this study was adequate, ranging between 40 in M4 and 78 in M1. The major effect of a small sample size on allele frequency distributions is on the probability of detecting rare alleles, which are common in microsatellites due to their high mutation rates. Seventeen of the 46 alleles detected in the entire sample in this study had frequencies smaller than 0.05, and there was a chance that these rare alleles may not have fallen into the samples. The probability (α) that an allele with a frequency of γ or less in a population falls into a sample of size n (i.e., 2n alleles) is $log(1-\alpha)=2n\ log(1-\gamma)$. Setting n=40 (the smallest sample size taken from black mink herds), and $\gamma=0.05$, gives $\alpha=0.98$, i.e., there was at least 98% probability that an allele with frequency of 0.05 in the black herds was included in the samples.

The net effects of the above forces have made the four black mink herds more similar than different, as shown by the small proportion of significant differences in pairwise comparisons among black mink herds for allele frequencies (33%), which was also revealed in the small genetic distances. Nevertheless, there was still sufficient genetic differences amongst these herds to allow correct assignment of 66% to 82% of black mink into their farm of origin.

Coloured mink: The smallest level of genetic differentiation was observed between pastel and brown, which could be due to two reasons:

i- The exact origin and time of divergence of these coloured mink has not been documented, but most of the published information on the early days of mink ranching make reference only to black (standard black) mink (Ashbrook 1928; Jones 1913), leaving the impression that black mink were kept in captivity earlier than the coloured mink. Pastel, a recessive mutation (bb), has been observed among standard black over years, but had been considered undesirable and discarded. Commercial production of pastel started in 1951 (Nes et al. 1988), but the exact time of divergence of the progenitors of pastel is not quite clear. Standard brown, also called wild-type or Mogul, are basically light coloured standard black (without Jetblack allele) that are maintained because of their vigour and high fertility (Nes et al. 1988). Detailed information on the history of divergence of brown from standard black has not been documented. The small level of genetic differentiation between pastel and brown could be due to their close time of divergence from the captive standard black.

ii-Pastel is sometimes crossed with other coloured mink including (standard) brown. Such deliberate crosses have been carried out to produce new color types (Hansen *et al.* 1985) and perhaps to widen the genetic base of pastel and brown mink. As mentioned before, such crossings might have occurred with the mink used in this study prior to their transfer to the current ranch.

Forces such as drift (founder effect, bottleneck), mutation, and sampling error, as explained in the case of black mink, have possibly caused the observed level of

differentiation between brown and pastel. Sampling error has probably played a more significant role in the case of colored mink compared with black mink herds in this study due to the smaller sample sizes taken from the former populations (20).

Wild vs. black and colored mink: It seems logical to assume that there has been no gene flow between black, colored and wild mink since they diverged. This assumption is based on the fact that economically important traits in black mink, such as color, size, hair length, darkness, shade of color, silkiness, and texture are controlled by a large number of genes. Modern black mink are the result of many generations of careful selection for all these traits. Crossing black mink with colored or wild mink results in the deterioration of many of these traits, and has not been practiced by the mink breeders.

Published information on the history of mink ranching indicates that the standard black diverged from wild mink between 1850s and 1920s, and commercial production of pastel started in 1951 (Nes et al. 1988). The progenitors of pastel must have diverged from standard black before 1951. The Jetblack mutation appeared in the mid 1960s and made up the backbone of the black mink industry in Nova Scotia, perhaps by the early 1970s. Based on the time of divergence of these populations, one would expect a closer genetic relationship between wild and colored mink than between wild and black mink.

Keeping mink in captivity has brought about some considerable morphological changes, even in the traits that have not been directly under selection. It has been shown that ranched mink have larger skulls, shorter palates, narrower postorbital constrictions, smaller size and skull-shape sexual dimorphism (Lynch and Hayden 1995) and smaller

brains (Kruska 1996) compared with the wild mink. Such differences could be examples of many morphological and physiological changes that have taken place solely as a result of keeping mink in captivity. Again, one would expect a greater divergence between wild and ranched mink due to such extensive changes that have taken place due to keeping mink under relatively uniform environmental and nutritional conditions.

This study, however, showed the greatest level of divergence between colored and black mink, not between wild and ranched mink, indicating that either the assumptions regarding the time of divergence and the effects of keeping mink in captivity were not correct, or factors such as genetic drift (founder effect and bottleneck) and sampling error were responsible for the creation of the observed pattern of population differentiation (Chakraborty and Nei 1977). It has been shown that if the population size increases over a long period of time, there will be a decrease in the rate of gene substitution by drift and the estimated genetic distance will be less than the true time of divergence (Nei 1976; Chakraborty and Nei 1977). Since the black mink population in North America has been expanding for many years, its genetic distance from the wild mink was perhaps underestimated, and it was positioned closer to the wild than the colored mink which had a smaller rate of population expansion.

5.2.b. Comparison Between Different Methods of Estimating Genetic Divergence

Although all the methods used in the calculation of population differentiation provided comparable results in this study, they have been developed using different algorithms based on different assumptions, which may not always produce the same

results. The degree of genetic divergence among black mink herds, for example, differed to some extent depending on the method used. M2 and M4 were the most diverged herds based on Nei's genetic distance, but the number of significant differences in allele frequencies among black mink herds put these two closer to each other than any other herd. Some comments on these methods are provided below:

i-Allelic differentiation

The levels of divergence of the populations were assessed by comparing allele frequency distributions in 147 χ^2 tests. The χ^2 test of similarity of allele frequency distributions provides an objective tool for assessing population divergence. The major problem with this method is the large number of tests that must be performed, which increases rapidly as the number of populations and loci increase. A total of $k(k-1)/2$ χ^2 tests should be performed for each locus when k populations are compared. The large number of tests creates two problems:

First, when a large number of χ^2 tests are performed, the overall α is much larger than 0.05 or 0.01 probability levels. The Bonferroni correction of $p=\alpha/n$, where n is the number of tests is recommended to control the overall probability of rejecting a true hypothesis (Miller 1981). In this study, the Bonferroni correction on all the 147 χ^2 tests performed (Tables 4.13 to 4.17) gives $p=\alpha/n= 0.05/147= 0.00034$, and some of the comparisons become nonsignificant. Therefore, the conclusions depend on the number of tests performed and the level of α.

Second, rare alleles, which are common in microsatellites, create a problem in the statistical handling of the data. Small cell frequencies may result in false rejection of the

hypothesis. Although rare alleles were pooled, it did not result in all cell frequencies to be larger than 5, which is recommended for an accurate χ^2 test. For example, allele frequencies of at least 0.25 are needed to have at least 5 observations in each cell when a sample of size 20 is taken, which was not certainly the case for many loci in the present data. Therefore, some of the significant differences observed when comparing allele frequency distributions of populations might have been due to the presence of rare alleles.

ii Genetic distance

Genetic distances are another measure of the degree of divergence between populations. These measures are particularly useful in uncovering the general relationships among the groups when there are a large number of populations and many loci, because they help to consolidate the data into a smaller set which facilitates the interpretation of the results compared with allele frequency distributions. Although some information is lost by reducing arrays of allele frequencies to a smaller set of numbers, existing patterns among populations obscured by the mass of numbers may become apparent by summarizing them into genetic distances. The major problem with genetic distance estimates is difficulties associated with their interpretation. Although Nei's standard genetic distance varies between zero and unity, it is not easy to accurately explain the meaning a particular value.

A number of genetic distances (or similarities) have been proposed to evaluate the amount of variation shared among the samples. Two of the most common methods of computing genetic distances were used in this study; Nei's standard genetic distance (Nei

1972) and Cavalli-Sforza's chord measure (Cavalli-Sforza and Edwards 1967). Both methods estimate the same quantity, but different assumptions are associated with them.

Nei's genetic distance (D) is interpreted as a measure of average number of codon or nucleotide substitutions per locus that have accumulated since the two populations separated from a common ancestral one (Nei 1972) . It has a linear relationship with time of divergence (t), i.e., $D=2\alpha t$, where α is the rate of codon change (gene substitution) per locus per generation. In the case of microsatellite loci, mutation is defined as the change in repeat number rather than codon substitution. Nei's genetic distance was developed under the following assumptions:

First, it is assumed that new mutations occur by way of the infinite allele model, i.e., mutations are neutral and each mutant result in a completely new allele. The assumption of neutrality is generally valid for microsatellite loci, but formation of new alleles by mutation does not seem to be a reasonable assumption, as microsatellite alleles may mutate to the one already existed in the population (Tautz *et al.* 1986; Walsh 1987; Harding *et al.* 1992; Schlotterer and Tautz 1992; Stephan and Cho 1994; Fumagalli *et al.* 1996).

Second, it is assumed that all loci have the same rate of neutral mutation. This assumption may not hold true for any loci, including microsatellite loci which have been shown to have different mutation rates (Dallas 1992; Weber and Wong 1993).

Third, mutations within a locus occur independently from each other.

Fourth, the number of allelic substitutions per locus follows a Poisson distribution.

Fifth, the genetic variability initially in the population is at equilibrium between mutation and genetic drift. There is no evidence that microsatellite loci may deviate from the above three assumptions more or less than any other loci.

Sixth, the effective size of each population remains constant. Genetic distance is escalated if the population passes through a tight bottleneck, but this requires extremely small breeding numbers; e.g. 2 to 10 for several generations (Chakraborty and Nei 1977). As mentioned before, if over a long period of time the population size increases, there will be a decrease in the rate of gene substitution by drift and the estimated genetic distance will be less than the true time of divergence (Nei 1976). It has been shown, however, that Nei's genetic distance is quite robust to founder effect and changes in population size (Nei 1975, 1976).

Cavalli-Sforza's chord measure assumes that there is no mutation, and that all gene frequency changes have been caused by genetic drift alone (pure drift model). No assumption is made about the constancy of population size over time or among populations.

Nei's genetic distance seems to be a better estimate than Cavalli-Sforza's chord measure, because absence of mutation may not be a valid assumption when using microsatellite loci, and has been extensively used to analyze microsatellite data in various species, including ovine (Buchanan *et al.* 1994), polar bears (Paetkau *et al.* 1995), wombats (Taylor *et al.* 1994), Atlantic salmon (McConnell *et al.* 1995), toads (Scribner *et al.* 1994), and wolflike canines (Roy *et al.* 1994). It is interesting to note that microsatellites provided much more accurate estimates of the time of divergence of five

sheep breeds compared with protein data (Buchanan *et al.* 1994). Although Nei's genetic distance was developed long before the discovery of microsatellites, its assumptions can be directly applied to microsatellite data.

iii. Phylogenetic analysis

Phylogenetic analysis is a step further than genetic distances in summarizing genetic data. Arrays of genetic distances are used to cluster populations according to their degree of similarities. Phylogenetic analysis has two primary uses. One is to trace evolutionary histories among populations. Often this involves confirming histories or assessing relationships already established by other methods, such as in established breeds with a known history. The second is to compare groups of unknown affinities to other known groups or populations. Although our knowledge of the history of the ranched mink is somewhat blurry, the approximate time of divergence of the populations used in this study are known.

The results of the three phylogenetic analyses; UPGMA, Neighbour-joining and Fitch-Margoliash, were similar and comparable to those obtained from comparing allele frequency distributions and genetic distances regarding wild, black and colored mink populations. There were minor differences between the methods in the order by which black mink herds were joined to the branch, indicating that these methods produce different results when genetic differentiation is small.

The UPGMA method assumes a constant rate of change along all branches in a tree (equal mutation rates). Nei *et al.* (1983) compared different methods of constructing trees using simulation, and concluded that UPGMA generally performed well when the

mutation rates were the same along all branches of the trees. When mutation rates are equal, a "molecular clock" is said to be operating. A constant rate of change along all the branches may be a permissible assumption when microsatellites are used as a result of their selection neutrality. UPGMA has been widely used with microsatellite data (Taylor *et al.* 1994; McConnell *et al.* 1995; Plaschke *et al.* 1995), perhaps because it is easy to interpret the tree.

The assumption of a constant rate of change along all branches (assumption of a clock), which is fundamental for UPGMA, is no longer needed in the Fitch-Margoliash method (Fitch and Margoliash 1967) or Neighbour-joining (Saitou and Nei 1987). For this reason, some researchers favor Fitch-Margoliash (Buchanan *et al.* 1994) and Neighbour-joining methods (Roy *et al.* 1994; Estoup *et al.* 1995; Tsumura *et al.* 1995) over UPGMA. The Fitch-Margoliash method, however, may not result in a correct topology, and it is recommended that other topologies be examined (Fitch and Margoliash 1967). Fitch-Margoliash method uses weighted least-squares, whereas UPGMA gives least-squares estimates of branch lengths. These two methods are very similar when there is a clock (equal mutation rates along all the branches).

The Fitch-Margoliash method can only produce an unrooted tree, unless assuming the same rates of change along all branches. Additional information from an "out-group" can be used to produce a rooted tree. Wild mink was not used as an out-group, because of its closer relationship with black mink.

It is certainly difficult to investigate the validity of the assumptions made in developing the algorithms of different phylogenetic trees. It may be logical to assume

that more confidence can be placed on the data and the results when similar trees are obtained from different methods. The patterns of differentiation between black mink herds and colored mink categories, and the wild mink were the same based on all the three methods, which may indicated a true divergence profile regardless of the assumptions associated with these methods. The pattern of differentiation of the black mink herds varies between the methods, and the results should be interpreted with cautious.

5.2.c. Assignment Test

The assignment test using the panel of 7 microsatellites discriminated between black and non-black mink with at least 83% accuracy, but assigning a black mink to its farm of origin had accuracies as low as 66.2%. The accuracy of the assignment test depends on the degree of differentiation among groups, i.e. the difference in allele frequency distributions. The addition of more microsatellite loci to this panel improves the accuracy of assigning an individual to its herd of origin, or even to a line. The practical application of the assignment test is to identify the farm of origin or line of individual animals in case of escape from a cage or release to the wild. This test can also be used to identify wild mink that have possibly bred with escaped ranched mink.

Buchanan *et al.* (1994) used Bayes' Theorem with uniform prior to identify breed of individual sheep. The Bayes' Theorem with uniform prior provides exactly the same result as the algorithm used in this study. Microsatellite data have been analyzed with slightly modified forms of this assignment test to differentiate between four polar bear

subpopulations (Paetkau *et al.* 1995), and to identify individual, sex and species of seal (harbour and gray) using DNA extracted from their feces (Reed *et al.* 1997).

6. CONCLUSIONS

i) Despite very uniform fur characteristics of the black compared with wild mink, the genetic variability of black mink herds in Nova Scotia was comparable with that observed in the wild mink. This considerable level of genetic variability is very likely the result of frequent importation of breeding stock from ranches outside the province, and the fact that ranched mink originated from at least three different subspecies.

ii) A considerable proportion of the genetic variability which exists in each ranch most probably resides among lines or families. This may be the result of linebreeding which has been followed in two of the ranches, and positive assortative mating, which has been practiced in all the ranches, which have resulted in creation of families. The consequence of these systems of mating has been an increase in phenotypic and genetic uniformity, reduced genetic variability, and accumulation of inbreeding within lines and families. At the same time, genetic variability among lines and families has increased, causing a considerable proportion of total genetic variability on any ranch to reside among lines and families.

iii) Although matings between close relatives, such as parent-offspring and brother-sister, have been avoided in all the ranches studied, their effects on the accumulation of inbreeding under linebreeding and positive assortative mating seems to be small, and black and coloured mink have become inbred to some extent. Some degree of inbreeding was also observed in the wild mink, indicating that probably related

individuals occupied adjacent territories, and that mink have a limited movement in the wild.

iv) Brown mink had the highest level of genetic variability among the populations studied, perhaps as a result of crosses between different colour phases during the past. Brown mink are known to be more vigourous and have higher fertility than black mink. This may be the cause or the effect of larger genetic variability. If large genetic variability in brown mink is the reason for their higher vigour and fertility, avoiding linebreeding may improve reproductive performance of the black mink as well. Linkage between genes controlling extreme black coloration and low fertility could not be disregarded. More studies are needed before definitive suggestions could be made.

v) The four black mink herds were genetically close to each other, most likely as a result of flow of breeding stock from common sources to all the herds, and due to the exchange of stock that has occurred, although on a limited basis, among the ranches. This may imply that a substantial expansion of the genetic base of the local stock cannot be achieved if these breeders exchange stock in the future.

vi) Despite similarities between ranched mink (black and coloured) in nutrition, environmental conditions and selection pressure compared with the wild mink, and given that historical evidence indicating a close time of divergence of black and coloured mink from each other, the results indicated that black mink was genetically closer to the wild

mink than to the coloured mink. The largest divergence was found between pastel and black mink.

In conclusion, the current breeding strategies in the mink industry may not diminish the present level of genetic variabilty in the near future. In order to reduce the level of inbreeding , a large number of lines with similar fur characteristics should be established within each ranch, and these lines should be frequently crossed.

APPENDIX A

The following protocols were used in this study for the extraction of DNA from mink tissue.

Protocol A

1. Approximately 150 mg of sample was placed into a 1.5 ml tube.

2. 30 μl of preheated 2X lysis buffer (Applied Biosystems) was added to the tube, and homogenized with the homemade homogenizer for a few seconds. This step was repeated once more.

3. 340 μl of the 2X lysis buffer was added and homogenized again. The homogenate was incubated at 65°C for 30 minutes.

4. 10 μl of proteinase K (16 μg/μl, ICN) was added, mixed, and incubated at 50°C overnight.

5. 400 μl of chloroform/phenol/water (34:7:9) was added to the tube, inverted first by hand until the mixture was milky, then on the chemistry mixer for 5 minutes.

6. The tube was centrifuged at 14 K rpm for 5 minutes, the top aqueous layer t transferred to a new, labelled microtube.

7. Steps 5 and 6 were repeated once more.

8. The tube was spun down for 2-3 minutes in order to get the protein to sink to the bottom.The aqueous layer was saved into a new labelled tube.

9. 200 μl of chloroform/isoamyl alcohol (24:1) was added to the tube, inverted for 5 minutes using the chemistry mixer, then centrifuged at 14 K rpm for 5 minutes.

10. The top aqueous layer was saved into a new tube and 2 volumes of 100% cold ethanol was added to precipitate the DNA.

11. The tube was inverted by hand and centrifuged at 7 K rpm for 2 minutes. The ethanol was poured off gently so as not to lose the pellet of DNA.

12. The DNA was washed with 500 μl of 70% ethanol for 10 minutes or longer using the chemistry mixer. The last of the ethanol was removed using the SpeedVac for 4 minutes on medium heat, or by leaving open tubes at room temperature for 15 minutes.

13. The DNA was re-suspended with 50 μl of 1X TE buffer and the tubes put at 4°C overnight

Protocol B

1. 0.05 g tissue was suspended in 500 μl 2X lysis buffer (Applied Biosystems).

2. 30 μl proteinase K (16 μg/μl, ICN) was added and then incubated at 50°C for 30 minutes (or until tissue dissolved).

3. 500 μl cold 100% ethanol was added, inverted by hand and centrifuged at 7 K rpm for 2 minutes.

4. The ethanol was poured off gently and 500 μl 70% ethanol added. 5. The tube was inverted on the chemistry mixer for 10 minutes or longer. It was then centrifuged at 5 K rpm for 1 minute, the ethanol poured off, and the remaining ethanol removed using the SpeedVac for 4 minutes on medium heat, or by leaving open tubes at room temperature for 15 minutes.

5. The tubes were suspended overnight in 100 μl 1X TE buffer.

APPENDIX B

Stock Solutions

The following stock solutions were used for extraction, PCR and polyacrylamide gel electrophoresis.

1M Tris-HCl stock solution (pH 8.0)
121.1 g Tris base crystals
42 ml HCl (12 N) to adjust pH
Dissolve Tris base crystals in about 750 ml dH_2O. Adjust the pH to 8.0 using HCl. Transfer to 1 L volumetric flask, allowing to cool before adjusting volume to 1 L with dH_2O. Mix well.
Store at room temperature.

0.5M EDTA (pH 8.0)
186.1 g $Na_2EDTA\ 2H_2O$
20 g NaOH
Dissolve $Na_2EDTA\ 2H_2O$ in about 750 ml dH_2O. Adjust the pH to 8.0 using NaOH crystals. Transfer to a 1 L volumetric flask, allowing to cool before adjusting volume to 1 L with dH_2O. Mix well.
Store at room temperature.

10X TE buffer (pH 8.0)
100 ml 1M Tris-HCl, pH 8.0
20 ml 0.5M EDTA, pH 8.0
Add together in a 1 L volumetric flask. Bring to 1 L with dH_2O. Mix well.
Store at room temperature.

1X TE buffer (pH 8.0)
10 ml 10X TE buffer (pH 8.0)
90 ml dH_2O
Store at room temperature.

Proteinase K (16 $\mu g/\mu l$)
Add 1 μl of 1X TE buffer (pH 8.0) to 16 μg Proteinase K.
Mix well.
Store at 4°C.

Chloroform/Isoamyl alcohol (24:1)
24 ml 100% chloroform
1 ml 100% isoamyl alcohol
Store in fume hood in amber bottle.

Chloroform/Phenol/ Water (34:7:9)
68 ml 100% chloroform
14 ml 100% phenol
18 ml dH_2O.
Store at 4°C in amber bottle.

70% Ethanol
70 ml 100% ethanol
30 ml dH_2O
Store at room temperature.

10X TBE buffer
108 g Tris base
55 g boric acid
9.3 g $Na_2EDTA \cdot H_2O$
Mix with dH_2O volumetric flask to 1 L.
Store at room temperature.

1X TBE buffer
200 ml 10X TBE buffer
1.8 L dH_2O
Store at room temperature.

10% Ammonium Persulfate (APS)
1 g APS
10 ml dH_2O
Store at 4°C.

25% Ammonium Persulfate (APS)
2.5 g APS
10 ml dH_2O
Store at 4°C.

1% Tween 20
10 μl Tween 20
990 μl dH_2O
Store at room temperature.

Primer Stock Dilutions

Primer	Stock(mM)	Stock(μl)
Mvi24a	50.96	19.62
Mvi24b	51.36	19.47
Mvi87a	57.54	17.38
Mvi87b	54.49	18.35
Mvi54a	295.86	3.38
Mvi54b	324.68	3.08
Mvi111a	55.37	18.06
Mvi111b	57.54	17.38
Mvi219a	350.88	2.85
Mvi219b	268.82	3.72
Mvi232a	50.30	19.88
Mvi232b	54.29	18.42
Mvi57a	296.74	3.37
Mvi57b	322.58	3.10

Water was added to 100 μl.
*The final concentration of each working primer was 10 μM.

40% Acrylamide Gel
400 g acrylamide:bis (19:1)
dH_2O to 600 ml
Store at room temperature in amber bottle.

8% Acrylamide gel
50 ml of 40% acrylamide stock
25 ml 10X TBE
117.5 g urea
add dH_2O to 250 ml

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IMAGE EVALUATION
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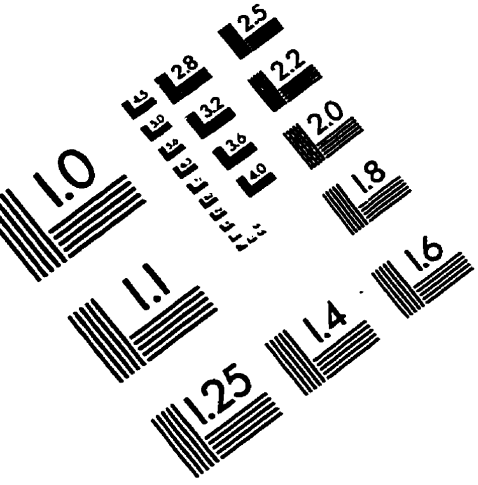

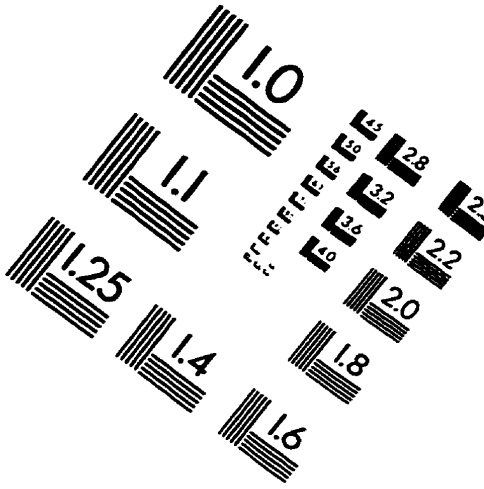

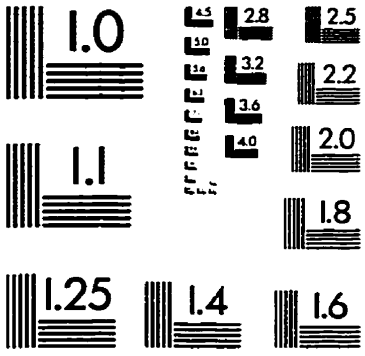

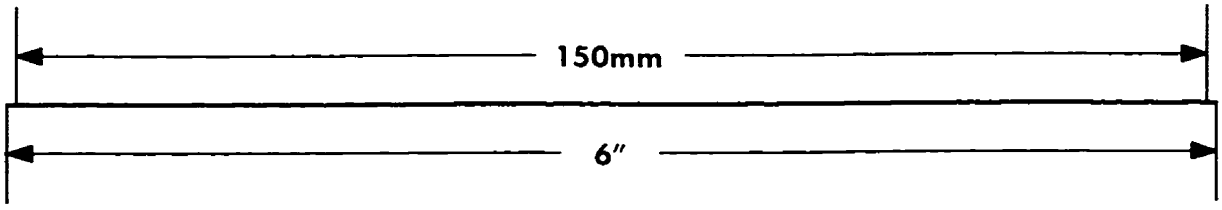

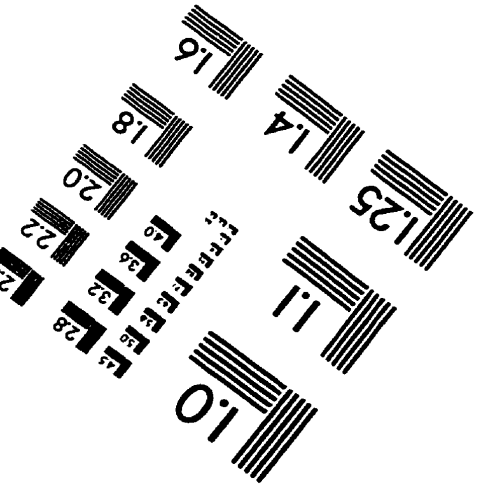

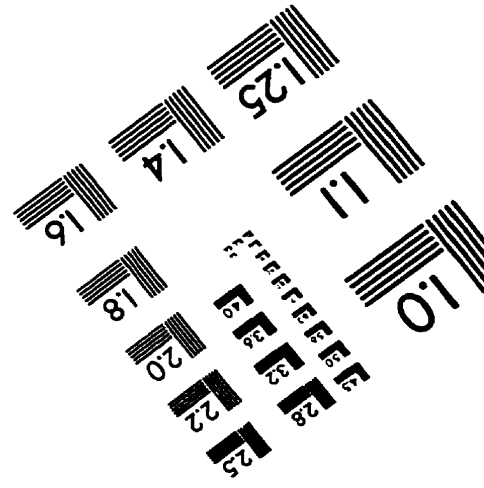

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