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Hybridization of domestic mink with wild American mink (*Neovison vison*) in eastern Canada

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Abstract: Farmed American mink (*Neovison vison* (Schreber, 1777)) pose a risk to biodiversity owing to escape and release from farms. Feral mink may affect native species in locations where American mink are not endemic, such as Europe. In contrast, escaping domestic mink may hybridize with wild mink in North America, leading to introgression of domestic traits via hybrid-mediated gene flow. We tested this idea in eastern Canada, which has a history of mink farming. We sampled known domestic and free-ranging mink, and profiled 508 individuals at 15 microsatellite loci. We found that 33% of free-ranging mink were either escaped domestic individuals, domestic–wild hybrids, or were introgressed to domestic or wild parental groups. The greatest prevalence of free-ranging domestic, hybrid, or introgressed mink (59%) occurred in Nova Scotia, which also had the most mink farms. Historic (1980s or earlier) mink sampled from museums had higher allelic richness and private allelic richness than contemporary wild mink. Domestic mink are artificially selected for traits desired by farmers, and as such, introgression with wild mink may lead to a loss of local adaptation. Our findings demonstrate that continued escape and release of mink could pose risks to the maintenance of genetic integrity in wild mink.

Key words: American mink, domestication, hybridization, insular, introgression, *Neovison vison*.

Résumé : Les visons d'Amérique (*Neovison vison* (Schreber, 1777)) d'élevage posent un risque pour la biodiversité découlant de leur évasion et de leur libération de fermes. Les visons férals peuvent avoir une incidence sur des espèces indigènes là où le vison d'Amérique n'est pas endémique, comme en Europe. En revanche, des visons domestiques évadés peuvent s'hybrider avec des visons sauvages en Amérique du Nord, menant à une introgression de caractères domestiques par l'entremise du flux génétique médié par des hybrides. Nous avons vérifié cette idée dans l'est du Canada, où l'élevage du vison est pratiqué. Nous avons échantillonné des visons domestiques et en liberté et profilé 508 individus en 15 sites de microsatellite. Nous avons constaté que 33 % des visons en liberté étaient soit des individus domestiques évadés, des hybrides domestiques–sauvages ou des individus introgressés à des groupes parentaux domestiques ou sauvages. La plus grande prévalence de visons en liberté domestiques, hybrides ou introgressés (59 %) se trouvait en Nouvelle-Écosse, province présentant le plus grand nombre de fermes d'élevage de visons. Des visons historiques (années 1980 ou avant) de musées échantillonnés présentaient de plus fortes richesse allélique et richesse allélique privée que les visons sauvages actuels. Les visons domestiques sont le fruit d'une sélection artificielle pour des caractères prisés par les éleveurs, de sorte que l'introgression avec des visons sauvages peut mener à une baisse de l'adaptation locale. Nos constatations démontrent que la poursuite de l'évasion et de la libération de visons peut poser des risques pour le maintien de l'intégrité génétique des visons sauvages. [Traduit par la Rédaction]

Mots-clés : vison d'Amérique, domestication, hybridation, insulaire, introgression, *Neovison vison*.

Introduction

Domestication is the genetic alteration of a species that leads to an inherited predisposition toward humans (Price 1984). Domesticated animals have genomes that differ from wild genomes due to artificial selection for certain traits, relaxed selection for other traits, and natural selection to the captive environment. If domesticated animals escape captivity and have an opportunity to interbreed with wild counterparts, then a loss of local adaptation can result leading to reduced fitness of hybrid and introgressed offspring (Rhymer and Simberloff 1996). There are many global examples of domestic–wild hybridization and consequent impacts on wild populations. To provide just a few examples, backcrosses between wild and farmed Atlantic salmon (*Salmo salar* L., 1758) have lower fitness than wild salmon (McGinnity et al. 2003). Crossbreeding between wildcats (*Felis silvestris* Schreber, 1777) and domestic house cats is a major conservation problem in Europe (Oliviera et al. 2008). Finally, native polecats (*Mustela putorius* L., 1758) declined to near extinction in Britain after domestic ferrets began to escape and hybridize with the polecats (Davison et al. 1999).

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The American mink (*Neovison vison* (Schreber, 1777)) represents another example of a species where domestic–wild hybridization appears to present a conservation concern. The mink is a North American member of the Mustelidae that has been harvested as a furbearer for centuries. Because of the quality of its fur and the ease with which it can be raised in captivity, the mink has been domesticated for fur production since the late 1800s. The production of mink on farms expanded greatly beginning in the 1920s to attain an almost global reach (Joergensen 1985).

The global spread of mink farming has brought with it an associated spread of feral American mink, because it is difficult to prevent the escape or intentional release of mink from captivity. Feral mink have colonized many countries around the world including in Europe, Asia, and South America (Medina 1997; Bonesi and Palazon 2007; Zalewski et al. 2011). These escaped domestic mink have led to a variety of biotic impacts on native wildlife, including predation, competition, and spread of potentially deleterious pathogens (Macdonald and Harrington 2003). Numerous taxa, such as the European mink (*Mustela lutreola* (L., 1761)), the water vole (*Arvicola amphibius* (L., 1758)), and various ground-nesting bird species, appear to have been negatively affected by feral mink (Woodroffe et al. 1990; Maran et al. 1998; Ferreras and Macdonald 1999). Extensive control programs have been deployed in many jurisdictions to limit the impacts and spread of invasive feral mink (Bryce et al. 2011).

Nearly all studies about the distribution and biotic effects of feral American mink have taken place in Europe (e.g., Macdonald and Harrington 2003; Bonesi and Palazon 2007; Zalewski et al. 2011). Presumably, because feral mink are more obvious to detect in locations where the American mink is exotic, the occurrence of feral American mink in North America, where the mink is native, has not been assessed until recently. Bowman et al. (2007) first showed that the signature of escaping domestic mink could be detected across Canada in wild mink harvest data. Subsequently, studies in Ontario, Canada, used genetic methods to document the extent of escaped domestic mink (Kidd et al. 2009; Beauclerc et al. 2013). For example, 78% of free-ranging mink sampled in the Niagara Falls area were either domestic or domestic–wild hybrids (Kidd et al. 2009).

The biotic effects of feral mink should differ somewhat in North America compared with other locations, owing to the occurrence of native conspecific mink. Sampling in Ontario has shown that escaped domestic mink hybridize with wild mink (Kidd et al. 2009; Beauclerc et al. 2013), which may lead to outbreeding depression due to a loss of local adaptation (Bowman et al. 2007; Beauclerc et al. 2013). An interface for potential pathogen transfer exists when there are lapses in biosecurity on mink farms. Aleutian mink disease virus (AMDV) appears to spread back and forth between mink farms and wild mink (Nituch et al. 2012) and may be spread by escape of infected animals from farms (Nituch et al. 2011). AMDV has also been detected in other free-ranging carnivores, and appears to be transmissible across species, potentially having community-level effects (Farid 2013; Nituch et al. 2015).

Other than the two recent studies in Ontario (Kidd et al. 2009; Beauclerc et al. 2013), there are no studies evaluating the effects of escaping domestic mink on wild mink or other biota in North America. More broadly, there have only been three studies of the population genetic structure of native American mink anywhere in North America (Belliveau et al. 1999; Stevens et al. 2005; Beauclerc et al. 2013). Therefore, studies of population genetics of mink in other North American jurisdictions are warranted.

The Canadian Maritimes is a region of eastern Canada that includes Nova Scotia (NS), New Brunswick (NB), and Prince Edward Island (PEI) (Fig. 1). The region has both abundant wild mink populations and prevalent mink farming with an excess of 100 mink producers (Statistics Canada, catalogue 23-013-XIE). Evaluating effects of domestic mink in this area is of particular interest, because NS has the largest mink farming industry in Canada. Bowman et al. (2007) showed that mink harvest by trappers in counties of NS could be predicted by mink farm density, suggesting that mink are escaping from farms and being harvested by trappers. There have been no genetic studies in the Maritimes to evaluate the prevalence of escaped domestic mink, or to investigate the occurrence of domestic–wild hybrids. Furthermore, there have been no studies evaluating the population genetic structure of native mink across the Maritimes. Therefore, we were interested in assessing both the occurrence and effects of escaped domestic mink and the population structure of native mink. We hypothesized that domestic mink are escaping from farms, becoming feral, and hybridizing with wild mink. We also explored the population genetic structure of wild mink across the Maritimes region.

Materials and methods

Study area

The Maritimes region of eastern Canada consists of three provinces: PEI (5 685 km^2), NS (55 284 km^2), and NB (72 908 km^2). The provinces all have a large amount of coastline with many natural habitats for wild mink. Consequently, wild mink populations have historically been abundant. Both NB and NS are heavily forested, and all three provinces have large agricultural industries, including mink farms. In 2011, NS had 118 mink farms containing >400 000 domestic mink. In the same year, NB had 13 mink farms with an estimated 16 800 mink and PEI had 12 farms with 39 500 domestic mink (Statistic Canada, Table 003-0015). Mink farming has taken place in the region since the early 1900s.

Sample collection

Historic mink specimens from NB and NS were sampled in various museums to obtain tissue for DNA analysis (Table 1). Historic samples were from the 1980s or older. We were able to successfully genotype 9 NS samples and 11 NB samples.

We collected contemporary, free-ranging mink samples from the Maritimes either by collecting frozen, skinned carcasses from commercial, licensed, local fur harvesters from 2008 to 2012, or by sampling pelts prior to auction. We sampled free-ranging mink from NB in 2010 by sampling pelts being held for sale at North American Fur Auction (NAFA) in Rexdale, Ontario, Canada. We sampled free-ranging mink from PEI at NAFA in 2010 and 2012, and also via a collection of skinned carcasses submitted by licensed trappers to the Canadian Wildlife Health Cooperative in Charlottetown, PEI, Canada, opportunistically collected during the 2011–2012 trapping season. We sampled mink in NS during 2008 and 2009 through a design where we stratified our sample by mink farm density. We collected free-ranging samples from wild fur harvesters in western NS, which had a high mink farm density, and eastern NS, which had no mink farms (Fig. 1; Bowman et al. 2007). We also included 14 free-ranging mink sampled in various NS locations for a previous study by Farid et al. (2010).

We collected known domestic mink from NS via a pelting service, which provided carcasses to us in 2009 following removal of pelts. Pelt colour was difficult to determine accurately for all of these mink because their pelts had been removed prior to our collection; however, we expected that they were mostly black mink because at the time of sampling, black was the dominant colour line in the Maritime region. We also included in the study for comparative purposes domestic mink genotypes from mink farms in Ontario, for a variety of different colour lines including buff, demi, pastel, iris, and mahogany. These genotypes were the same ones used in an earlier study (for Ontario collection details see Beauclerc et al. 2013). It is worth noting that domestic mink tend to be line bred within colour types, such that genotyped domestic mink tend to cluster together by colour (Kidd et al. 2009).

Fig. 1. Study area in eastern Canada where both domestic and free-ranging American mink (*Neovison vison*) were sampled for a study of population genetics. Free-ranging mink were sampled from New Brunswick (n = 13), Nova Scotia (n = 84), and Prince Edward Island (n = 85). Additionally, historic mink samples from the region were sampled in museums (n = 20) and mink were genotyped from farms in Nova Scotia (n = 173). The majority of the free-ranging mink in Nova Scotia were sampled in strata that were either close (open circle) or far (solid circle) from the region with extensive mink farming.

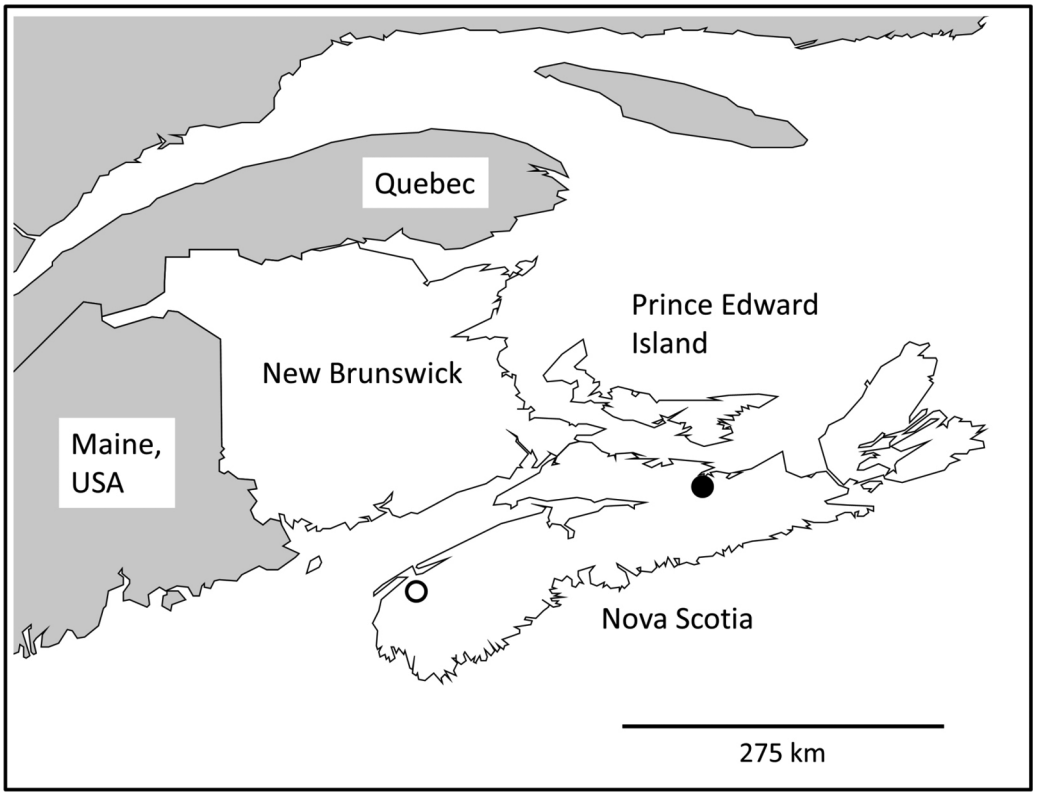


Table 1. Historic American mink (*Neovison vison*) sampled in a study of population genetics of mink in the Canadian Maritimes.

ID	Sex	Province	Site description	Year	Museum
AM ND 2	—	—	—		Acadia University Museum
CMNA 4257	F	NB	Miramichi Rd., 15 mi. (24.14 km) from Bathurst	1921	Canadian Museum of Nature
CMNA 4197	M	NB	Miramichi Rd., 15 mi. (24.14 km) from Bathurst	1921	Canadian Museum of Nature
FMNH 5454	M	NB	Trousers Lake	1894	Field Museum of Natural History
NBM 1340	M	NB	Sunbury Co.; Maugerville	1975	New Brunswick Museum
NBM 2172	M	NB	York Co.; Fredericton	1958	New Brunswick Museum
NBM 2776	F	NB	Saint John Co.; Foster Thurston Drive	1983	New Brunswick Museum
NBM 5456	M	NB	York Co.; Mactaquac	1975	New Brunswick Museum
NBM 5941[a]	M	NB	Charlotte Co.; Campobello Island	1894	New Brunswick Museum
NBM 890	M	NB	Charlotte Co.; Pocologan	1971	New Brunswick Museum
NBM 926	F	NB	York Co.; Ayers Lake	1970	New Brunswick Museum
NBM 4550	M	NB	Newcastle	1965	New Brunswick Museum
CMNA 12754	—	NS	Nipisiquit River near Bathurst	1935	Canadian Museum of Nature
CMNA 32731	M	NS	Cape Breton Co.; Louisburg	1962	Canadian Museum of Nature
NSM 10706	F	NS	—	—	Nova Scotia Museum
NSM 10707	—	NS	Halifax Co.; Spryfield	1963	Nova Scotia Museum
NSM 10708	—	NS	Cumberland Co.; Halfway River	1977	Nova Scotia Museum
NSM 972.312.001	F	NS	Halifax Co.; Oathill Lake, Dartmouth	1919	Nova Scotia Museum
NSM 972.312.014	—	NS	Lunenburg Co.; LaHave River	1921	Nova Scotia Museum
NSM 976.037.000	—	NS	Inverness Co.; Whycocomagh	1976	Nova Scotia Museum

Note: The Canadian Maritimes provinces are New Brunswick (NB) and Nova Scotia (NS). F, female; M, male.
[a]Collection date and sex for this sample are not firm.

Laboratory analysis

All mink were genotyped using the methods described by Beauclerc et al. (2013). Briefly, approximately 10 mg of muscle was digested and whole genomic DNA extracted using the DNeasy Blood and Tissue Kit (Qiagen Inc., Toronto, Ontario, Canada) according to the manufacturer's directions, or an automated magnetic bead procedure using MagneSil PMPs (Promega, Madison, Wisconsin, USA). Purified DNA was quantified with a NanoDrop 8000 or PicoGreen dye (Invitrogen Molecular Probes) with a FLUOStar Galaxy fluorometer (BMG Labtech).

Fifteen microsatellite loci were amplified in eight multiplex polymerase chain reactions (PCR). All reactions were 10 μL and

Table 2. Summary statistics for microsatellite loci used in a study of American mink (*Neovison vison*) in the Canadian Maritimes.

Locus	Range[a]	N_A	H_O	H_E	HWE p
Multiplex 1					
Mvi111	84–110	9	0.663	0.762	>0.0001
Mvi1006	136–168	14	0.652	0.806	>0.0001
Mvi1272	163–181	10	0.707	0.819	>0.0001
Mvi99	316–356	17	0.730	0.830	>0.0001
Mvi4001	223–233 (221)	7	0.580	0.615	0.0267
Mvi114	62–84 (86)	12	0.670	0.768	>0.0001
Mvi1302	207–221 (201)	9	0.475	0.785	>0.0001
Multiplex 2					
Mvi1016	216–236	11	0.756	0.815	>0.0001
Mvi1321	88–108	11	0.657	0.807	>0.0001
Mvi2243	123–155	10	0.608	0.704	>0.0001
Multiplex 3					
Mvi1354	176–198	12	0.590	0.764	>0.0001
Mvi002	176–188	4	0.126	0.131	0.0023
Mvi072	255–269	7	0.517	0.621	>0.0001
Mvi1342	136–174	16	0.684	0.788	>0.0001
Mvi075	111–131	10	0.759	0.831	>0.0001

Note: Allele size ranges are shown, along with number of alleles (N_A), observed heterozygosity (H_O), expected heterozygosity (H_E), and probability of deviations from Hardy–Weinberg equilibrium (HWE p).

[a]Values in parentheses were found only in historic samples originating from museums.

contained 5 ng DNA, 1× PCR buffer, 1.5 mmol/L $MgCl_2$, 0.2 mmol/L each dNTP, 0.2 μg/mL bovine serum albumin (BSA), and 0.5 U (1 U ≈ 16.67 nkat) *Taq* polymerase (BioShop Canada Inc., Burlington, Ontario, Canada). Thermal cycling used an Eppendorf Mastercycler Pro thermal cycler with an initial denaturation of 94 °C for 5 min, followed by 30 cycles of denaturation at 94 °C for 30 s, annealing for 1 min, and extension at 72 °C for 1 min, and a final extension of 60 °C for 45 min. Reactions were pooled into three groups (Table 2) with GeneScan 500 ROX for genotyping on an ABI 3730 automated sequencer. Fragment sizes were scored using GENEMAPPER version 4.0 (Applied Biosystems, Foster City, California, USA). For additional details including primer concentrations and sources, annealing temperatures, and multiplex conditions see Beauclerc et al. (2013).

DNA from historic samples was extracted in a laboratory dedicated to processing ancient DNA at Trent University in Peterborough, Ontario, Canada. Fresh reagents and supplies were used at all stages. Samples were extracted with the Qiagen DNeasy Tissue kit according to the manufacturer's instructions, with blanks incorporated for every 10 samples. PCR was carried out with the same conditions as the contemporary samples, except 50 cycles were used for the denaturing, annealing, and extension steps.

Statistical analysis

Individuals with 10 or more scored loci were used for analyses. The program MICRO-CHECKER version 2.2.3 (van Oosterhout et al. 2004) was used to check for genotyping errors, large allele dropout, and the presence of null alleles. Significant deviations from Hardy–Weinberg equilibrium (HWE) and linkage disequilibrium (LD) were calculated in GENEPOP on the Web version 4.2 (Rousset 2008). For HWE, the estimation of exact p values for deficiency of heterozygotes and F_{IS} (Weir and Cockerham 1984) was conducted using a Markov chain method with 5000 dememorization steps, 1000 batches, and 5000 iterations each. For LD, a log-likelihood ratio statistic was used. Summary statistics (number of alleles, allele frequencies, observed and expected heterozygosities, and pairwise population differentiation (F_{ST})) were estimated using GenAlEx version 6.5 (Peakall and Smouse 2012). Mean allelic richness per locus and private allelic richness were calculated by applying the rarefaction method in HP-Rare version 1.1 (Kalinowski 2005) to account for uneven sample sizes (Kalinowski 2004). Allelic richness was calculated for sampling locations (i.e., predefined groups), historic samples from NS and NB, as well as for clusters identified using K = 5 during the Bayesian assignment analysis described below.

To assess the most probable number of inferred genetic population clusters (K) and assign individuals to their likely population of origin, the program STRUCTURE version 2.3.3 (Pritchard et al. 2000) was run on a high-performance computing cluster using the admixture model with correlated allele frequencies (Falush et al. 2003). Five simulations for each value of K from 1 to 10 were run using a burn-in of 10^6 and Markov chain Monte Carlo of 10×10^6 iterations without prior geographical information. The program STRUCTURE HARVESTER version 0.6.93 (Earl and vonHoldt 2012) was used to summarize STRUCTURE runs and determine the most likely number of clusters based on ΔK (Evanno et al. 2005). Bar plots of individual membership coefficients (q) for K = 2 and K = 5 were generated with DISTRUCT version 1.1 (Rosenberg 2004). An individual was assigned to a single cluster if it had a $q > 0.8$, or jointly to two or more clusters such that the minimum sum of $q_i + q_j + \ldots q_n \geq 0.80$ for admixed individuals, thereby ensuring that at least 80% of an individual's genome was assigned to the inferred cluster(s) (Verardi et al. 2006). This threshold was based on a simulation analysis undertaken by Kidd et al. (2009) in which $q > 0.8$ was shown to be of sufficient power to correctly assign 96% of mink to either domestic, wild, or hybrid groups.

We used a second clustering method to confirm patterns observed in the STRUCTURE analysis. We used adegenet version 2.0.1 (Jombart 2008) to perform a principal component analysis (PCA), and we then inspected the distribution of domestic, wild, and hybrid individuals (as identified using STRUCTURE) on the PCA biplot.

We also conducted an assignment test with STRUCTURE using only the individuals assigned as wild ($q_{wild} \geq 0.80$) in the initial STRUCTURE analysis. We did this to observe whether there was any substructure across the study area among wild mink. As in the first assignment test with the full data set, we used STRUCTURE version 2.3.3 (Pritchard et al. 2000) for the analysis of wild mink.

Finally, using the STRUCTURE results from the full data set for K = 5, we grouped individuals for which $q \geq 0.8$ and removed all hybrid individuals. We treated the historic specimens as a separate group, regardless of their assignment in STRUCTURE. We then constructed a neighbour-joining tree for these six groups using genetic distance (D_A) in POPTREE2 (Takezaki et al. 2010). Significance was assessed using 1000 bootstrap replications.

Results

We sampled and successfully genotyped 173 mink from farms in NS and 133 mink of various colour lines from Ontario, including mahogany, pastel, demi, buff, and iris. We genotyped 13 free-ranging mink from NB, 84 from NS, and 85 from PEI. Additionally, we genotyped 20 historic Maritime mink samples, varying in year of origin from 1894 to 1983 (Table 1). Overall, we evaluated 508 genotyped mink samples in the study.

All 15 loci were polymorphic with 4–17 alleles each (Table 2). In total, 150 tests for HWE were performed, and of these, 50 showed a significant deviation from expected proportions. After Bonferroni correction ($\alpha = 0.05/150 \leq 0.0003$), 12 tests remained significant, which were due to heterozygote deficiencies. Accordingly, F_{IS} values per population revealed a similar pattern with values ranging from 0.018 to 0.17 (Table 3). LD was found in 130 comparisons at the population level. However, only 23 loci pairs deviated significantly from LD after Bonferroni correction. Notably, 16 of these linkage pairs were found in free-ranging mink from NS. There were signs of null alleles in 26 of 150 comparisons, but none of the loci showed null alleles in a majority of sampled populations. Null allele proportions ranged from 4% to 28%.

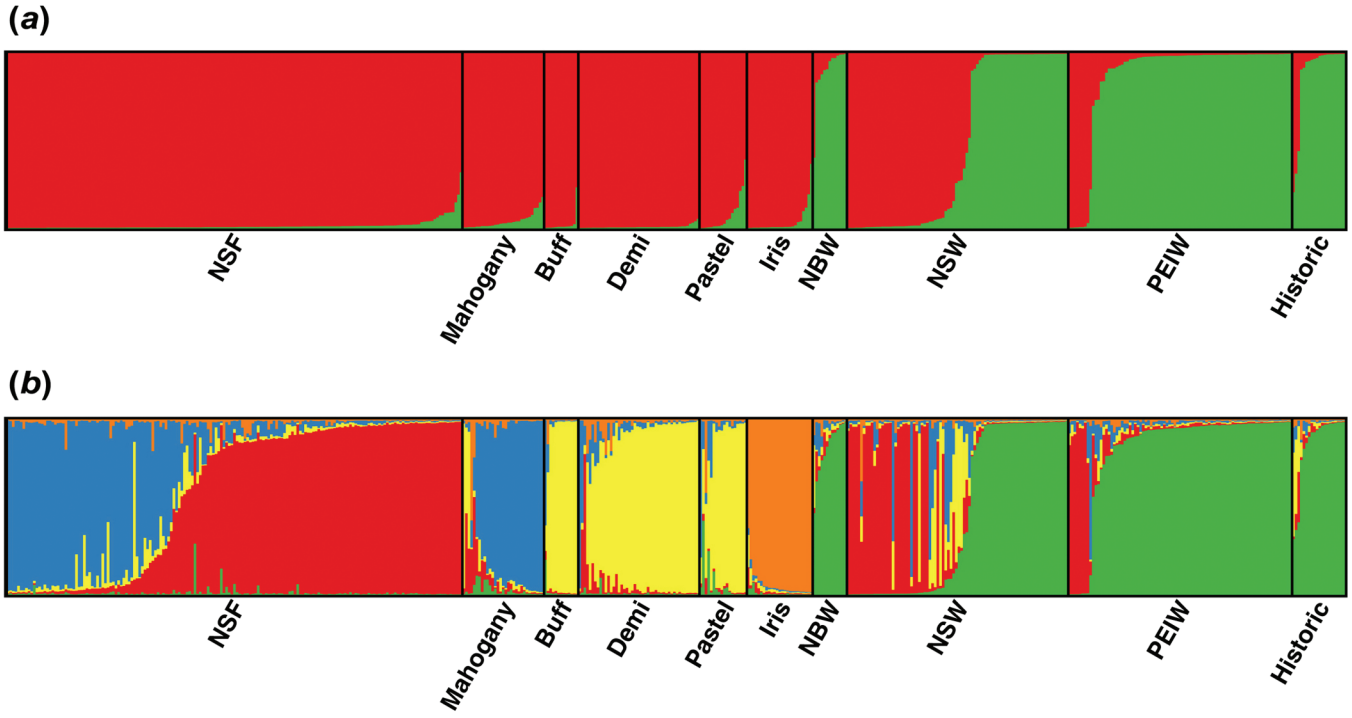
Table 3. Summary statistics for microsatellite loci at the level of sampled populations, used in a study of American mink (*Neovison vison*) in the Canadian Maritimes.

Sampled group[a]	N	N_A	A_R	N_{AP}	A_{RP}	H_O	H_E	SE	HWE p	F_{IS}
Buff	13	4.80	4.64	0.000	0.02	0.569	0.582	0.058	0.0166	0.0648
Demi	46	6.47	4.97	0.067	0.06	0.654	0.658	0.041	0.4498	0.0181
Iris	25	4.87	4.34	0.133	0.09	0.519	0.581	0.060	>0.0001	0.1141
Mahogany	31	6.27	5.09	0.133	0.11	0.624	0.667	0.052	0.0001	0.0872
Pastel	18	4.93	5.7	0.000	0.04	0.593	0.604	0.063	0.0284	0.0468
NSF	173	7.60	4.67	0.467	0.07	0.601	0.636	0.051	>0.0001	0.0546
NBW	13	5.80	5.56	0.067	0.29	0.615	0.685	0.040	0.005	0.1411
NSW	84	8.67	5.79	0.400	0.13	0.614	0.717	0.043	>0.0001	0.1483
PEIW	85	7.47	5.11	0.067	0.13	0.629	0.704	0.036	>0.0001	0.1115
Historic	20	6.87	5.96	0.200	0.31	0.597	0.694	0.044	>0.0001	0.1695

Note: Number of individuals in each group (N) are shown, along with number of alleles (N_A), allelic richness (A_R), number of private alleles (N_{AP}), private allelic richness (A_{RP}), observed heterozygosity (H_O), expected heterozygosity (H_E), standard error (SE) of H_E, and probability of deviations from Hardy–Weinberg equilibrium (HWE p), and inbreeding coefficient (F_{IS}).

[a]Buff, Demi, Iris, Mahogany, and Pastel mink refer to different colour lines sampled from farms in Ontario, Canada. NSF refers to domestic mink from farms in Nova Scotia. NBW refers to free-ranging mink sampled in New Brunswick. NSW refers to free-ranging mink sampled in Nova Scotia. PEIW refers to free-ranging mink sampled in Prince Edward Island. Historic refers to Maritime mink from the 1980s or older and sampled in museums.

Fig. 2. Bar plots for population structure analysis and identification of admixed American mink (*Neovison vison*) individuals using STRUCTURE version 2.3.3 (Pritchard et al. 2000) at $K = 2$ (*a*) and $K = 5$ (*b*). Individuals are grouped by known sampling locations and (or) colour phase (x axis) separated by black vertical lines. Each coloured vertical bar represents one individual and different colours show group membership coefficients (q). $K = 2$ discriminated domestic mink (red) from wild minks (green), whereas $K = 5$ identified wild mink (green) as a separate group and additionally differentiated according to fur colour within domestic farm mink (black mink = red; brown mink = yellow; iris mink = orange; mahogany mink = blue). NBW, New Brunswick free-ranging mink; NSW, Nova Scotia free-ranging mink; PEI, Prince Edward Island free-ranging mink; NSF, Nova Scotia domestic mink.



STRUCTURE analysis

Following the Evanno method (Evanno et al. 2005), maximal ΔK was found at $K = 2$, where domestic mink were differentiated from wild mink. However, additional differentiation may be present at higher K values, so we also studied the assignment results for $K = 5$, which was found in a previous study (Beauclerc et al. 2013). We found four domestic groups (black, brown, iris, and mahogany), along with a fifth group that we perceived to be a wild genotype, consisting of free-ranging mink from all three Maritime provinces (Fig. 2).

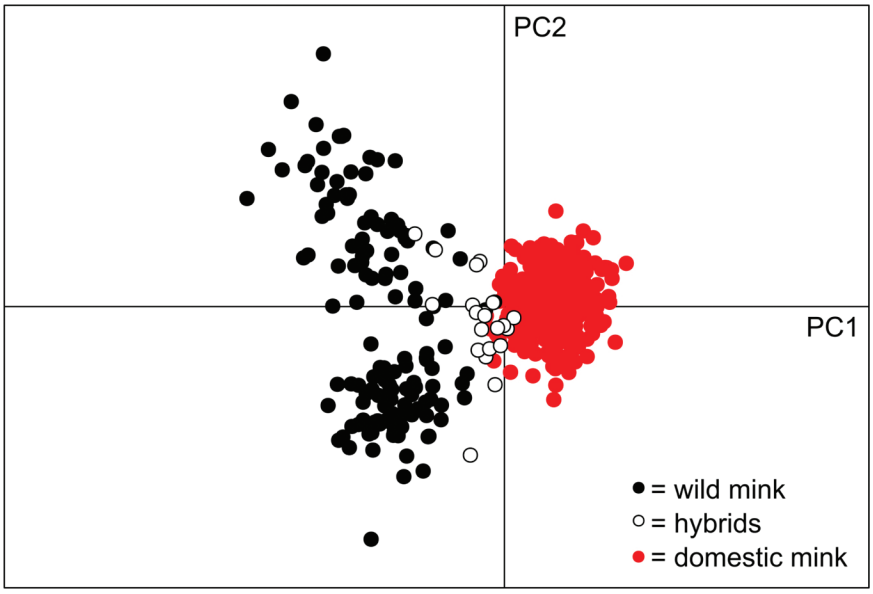
Of all free-ranging Maritime mink ($n = 182$), 26.9% were identified as farm escapees ($n = 49$, $q_{wild} < 0.20$), while 6.0% ($n = 11$) were either domestic–wild hybrids or backcrosses ($q_{wild} = 0.20–0.80$). Importantly, of all farm escapees and hybrids, 41 (83.7%) and 6 (54.5%) were found in NS (PEI: 16.3% and 36%, respectively; NB: 0% and 9.1%, respectively). Overall, 59.5% of free-ranging mink sampled in NS were either domestic escapees, domestic–wild hybrids, or introgressed animals. The mean membership coefficient (q) of free-ranging Maritime mink in the wild group was 0.66 and in the black group was 0.21. Free-ranging mink from NS had a low membership coefficient to the wild group of 0.44. Notably, free-ranging mink from NS had only a slightly lower membership coefficient in the black group (0.37). Thus, free-ranging mink from NS consisted

Table 4. Mean (±SE) membership coefficients of sampled American mink (*Neovison vison*) populations to each group identified in a STRUCTURE analysis when $K = 5$.

	Population				
Sampled group[a]	Black	Brown	Mahogany	Iris	Wild
Nova Scotia Farm ($n = 173$)	0.585±0.031	0.040±0.006	0.345±0.030	0.020±0.002	0.011±0.002
Buff ($n = 13$)	0.012±0.004	0.884±0.066	0.067±0.046	0.016±0.010	0.021±0.015
Demi ($n = 46$)	0.054±0.018	0.828±0.035	0.076±0.024	0.033±0.008	0.009±0.002
Iris ($n = 25$)	0.015±0.005	0.022±0.008	0.013±0.002	0.940±0.016	0.010±0.003
Mahogany ($n = 31$)	0.082±0.023	0.089±0.038	0.758±0.056	0.045±0.019	0.026±0.006
Pastel ($n = 18$)	0.019±0.005	0.784±0.060	0.104±0.045	0.034±0.023	0.058±0.026
NS, NB, and PEI free-ranging ($n = 182$)	0.214±0.027	0.056±0.011	0.059±0.011	0.013±0.002	0.658±0.031
Historic ($n = 20$)	0.036±0.014	0.072±0.032	0.026±0.008	0.016±0.006	0.851±0.048

[a]Buff, Demi, Iris, Mahogany, and Pastel mink refer to different colour lines sampled from farms in Ontario, Canada. Historic refer to Maritime mink from the 1980s or older and sampled in museums.

Fig. 3. Biplot from a principal components analysis (PCA) comparing microsatellites from American mink (*Neovison vison*) with different assignments (domestic, wild, or hybrid) based on a STRUCTURE analysis. Figure appears in colour on the Web.



of a mixture of black farm escapees, introgressed and hybrid mink, and wild mink. Introgression in the NS samples appeared to be greater in the direction of domestic parentals, as indicated by the transition in wild assignment probabilities between low (0.2) and moderate (0.5) values (Fig. 2). There were more individuals with probabilities between 0.2 and 0.5 ($n = 5$) then there were between 0.5 and 0.8 ($n = 1$). At $K = 5$, brown, black, iris, and mahogany individuals had high mean membership coefficients to their respective groups (Table 4). In contrast, individuals from NS mink farms had relatively lower mean membership to the black (0.58) and mahogany (0.34) groups, indicating that our domestic mink samples from NS consisted of both black and mahogany individuals. As with the $K = 2$ analysis, free-ranging Maritime mink did not show any significant substructure among sampling locations (NS, NB, PEI) at $K = 5$. Historic mink samples had a higher membership to the wild group than did contemporary free-ranging mink samples (Table 4).

PCA supported our STRUCTURE assignments for $K = 2$, as there was generally a good separation between domestic and wild mink (as identified using STRUCTURE) in the PCA biplot and hybrid mink tended to be intermediate (Fig. 3). The separation between wild and domestic mink occurred mostly along the first principal component (PC1).

When we considered only mink identified as wild in our analysis of the full data set, we found evidence for some substructure of wild mink. An assignment test of these wild mink demonstrated that $K = 2$, where PEI mink were assigned as a distinct group separated from mainland NS and NB mink (STRUCTURE plot not shown). The mean (±SE) assignment probabilities to their respective groups were 0.977 ± 0.006 for PEI and 0.954 ± 0.009 for the mainland (NS and NB). Some separation between PEI and mainland mink was also apparent from the PCA biplot, appearing as a split between wild mink along PC2 (Fig. 3).

Genetic diversity

The historic samples mostly assigned with the wild group, although three individuals from NB were assigned as domestic–wild hybrids. However, historic samples did have both the highest allelic richness and the highest private allelic richness. Likewise, wild mink showed higher (private) allelic richness when compared with different farm mink varieties (Table 5). Furthermore, wild mink showed highest observed and expected heterozygosity values, and therefore, the highest genetic diversity of all groups compared. Within domestic mink, mahogany animals had the highest genetic diversity (Table 5), indicated by the greatest mean number of alleles, allelic richness, private allelic richness, and expected heterozygosity, which is probably consistent with their hybrid origin. Mahogany mink are a result of mixing brown and black lines (Kidd et al. 2009). This may also explain the fairly high F_{IS} value for the mahogany group, as a Wahlund effect was likely present due to the admixture of two different populations.

Table 5. Summary statistics for genetic groups of American mink (*Neovison vison*) identified in STRUCTURE when $K = 5$.

Population	N	N_A	A_R	N_{AP}	A_{RP}	H_O	H_E	SE	HWE p	F_{IS}
Black	129	7.400	5.48	0.400	0.15	0.604	0.638	0.051	0.0000	0.0540
Brown	60	6.800	5.88	0.133	0.23	0.607	0.660	0.049	0.0001	0.0885
Mahogany	70	8.467	6.97	0.267	0.32	0.587	0.719	0.051	0.0000	0.1908
Iris	24	5.933	5.84	0.000	0.15	0.593	0.652	0.040	0.0000	0.1125
Wild	129	8.667	6.64	0.533	0.56	0.619	0.726	0.037	0.0000	0.1491

Note: Data include historic samples, but all hybrid individuals ($0.2 < q > 0.8$; $N = 96$) were removed. Number of individuals in each group (N) are shown, along with number of alleles (N_A), allelic richness (A_R), number of private alleles (N_{AP}), private allelic richness (A_{RP}), observed heterozygosity (H_O), expected heterozygosity (H_E), standard error (SE) of H_E, and probability of deviations from Hardy–Weinberg equilibrium (HWE p), and inbreeding coefficient (F_{IS}).

Table 6. Pairwise F_{ST} values for all groups of American mink (*Neovison vison*) identified through STRUCTURE analysis.

	Black	Brown	Mahogany	Iris	Wild
Black	—	*0.000*	*0.000*	*0.000*	*0.000*
Brown	0.050	—	*0.000*	*0.000*	*0.000*
Mahogany	0.049	0.043	—	*0.000*	*0.000*
Iris	0.022	0.068	0.064	—	*0.000*
Wild	0.089	0.099	0.056	0.078	—

Note: Hybrid individuals were removed for calculations. F_{ST} values are below the diagonal and probabilities (based on 9999 permutations) are above the diagonal. Values in italic type indicate significance at $p < 0.0001$.

Black and iris were the least differentiated of the $K = 5$ groups according to pairwise estimates of F_{ST} (Table 6). Conversely, wild mink were the most differentiated of all the groups, with a mean F_{ST} compared with the four other groups of 0.081 (Table 6). A neighbour-joining network based on allele frequencies for the $K = 5$ groups, with the historic mink included as a separate, 6th group, demonstrated that historic mink were the most distant from the domestic mink, whereas wild mink were intermediate between the four domestic groups and the historic group (Fig. 4).

Discussion

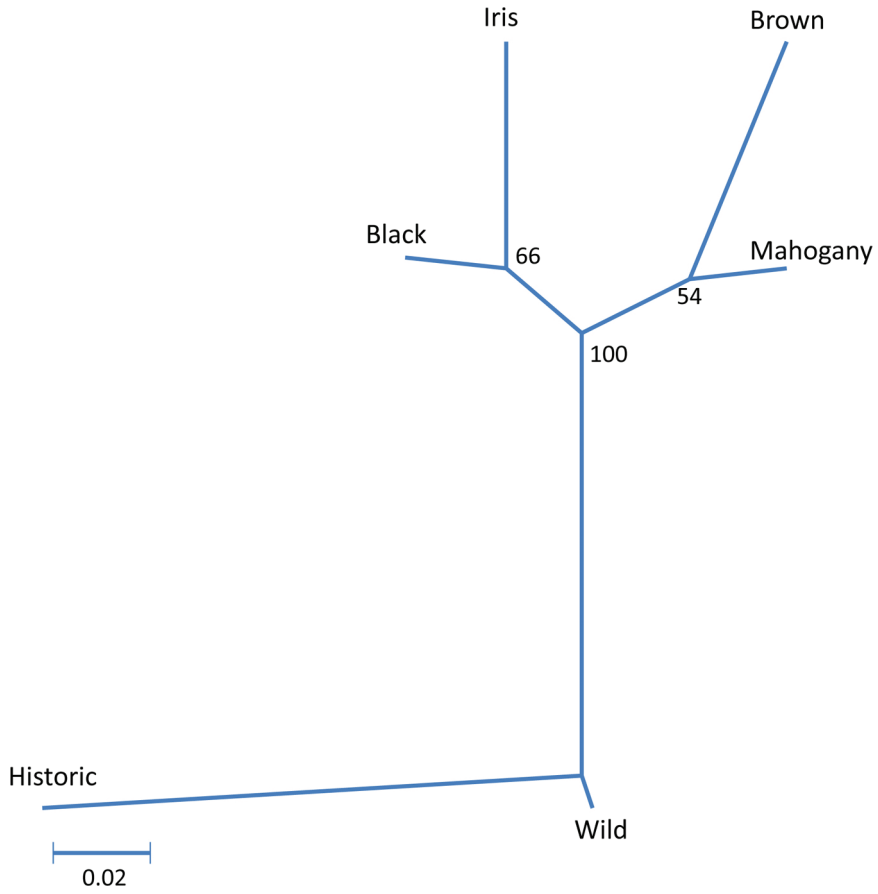
Based on genetic data, our study confirmed that domestic mink have either been escaping, or have been released, from mink farms in the Canadian Maritimes and are hybridizing with wild American mink. We found that 26.9% of 182 free-ranging mink sampled were domestic escapees, and a further 6.0% were domestic–wild hybrids. The impact of mink farming appeared greatest in NS, which contained >80% of the escaped domestic mink that we sampled. This is not surprising insofar as NS had the largest mink farming industry in Canada at the time of our study. Nevertheless, escaped domestic or hybrid mink were detected in all three Maritime provinces. The prevalence of escaped domestic and hybrid mink in the Maritimes is similar to the escapee and hybrid prevalence (13% and 5%, respectively) measured across an Ontario study area (Beauclerc et al. 2013). However, it is notable that in both situations, impacts of domestic mink, such as hybridization, appear to be greater at sites in close proximity to mink farms. In Beauclerc et al.'s (2013) study, southwestern Ontario had the highest mink farm density, and 28% of free-ranging mink sampled in that area were domestic–wild hybrids, while 36% were domestic escapees. NS had the highest prevalence in the Maritimes of domestic escapees (51% of free-ranging mink sampled) and domestic–wild hybrids (8%). The proximate relationship between mink farms and the impacts of domestic mink is consistent with the idea that feral domestic mink have low fitness in a North American context, which may help to limit the spatial extent of their impacts on native biodiversity (Nituch et al. 2011; Beauclerc et al. 2013). However, the idea that escaped domestic mink in North America have low fitness has not been directly tested. It is clear that feral mink are capable of exhibiting high fitness in non-native contexts (Macdonald and Harrington 2003). It is also important to note that various effects of domestic mink such as the spread of pathogens and the diffusion of genes can have widespread impacts (e.g., Nituch et al. 2012, 2015).

Escaped domestic mink can have deleterious effects on native biota through a number of mechanisms. Genetic diversity in wild mink may be reduced by escaped domestic mink because domestic mink show lower genetic diversity than wild mink as the comparison with historic samples indicated. Even if hybrid mink have low fitness, hybridization may have ongoing consequences on wild mink due to the continuous presence of free-ranging domestic mink on the landscape over many generations. Hybridization and introgression of domestic traits into the wild mink population could lead to outbreeding depression and reduced fitness through loss of local adaptation and co-adapted gene complexes (McGinnity et al. 2003). In addition, mink farms may serve as point sources of pathogens such as AMDV and escaping domestic mink may spread AMDV to wild mink and other species such as striped skunks (*Mephitis mephitis* (Schreber, 1776)) and short-tailed weasel (*Mustela erminea* L., 1758) (Nituch et al. 2011, 2012, 2015; Farid 2013). Domestic mink may also have impacts on native biota through predation and competition (Macdonald and Harrington 2003; Bonesi and Palazon 2007). For example, predation by feral American mink appears to have reduced abundance of the water vole (Jefferies et al. 1989), a species which is now of conservation concern in some European jurisdictions. Competition between feral mink and European mink is one hypothesis for the cause of endangerment of the European species (Maran and Henttonen 1995).

Wild mink had the highest private allelic richness, and some of this appeared attributable to historic mink, which had the highest private allelic richness of any sampled group. We observed the presence of two private alleles in our historic samples (Table 1) that did not appear in any other samples. Between our study and the earlier work of Beauclerc et al. (2013), we have now identified five private alleles from 41 individual mink samples obtained from historic collections in museums. Although it is possible that these missing alleles are due to sampling or genetic drift, we must also consider that they represent a loss of genetic diversity due to reduced population size or even due to the effects of introgression with domestic mink. The historic samples had the highest membership coefficient to the wild group of all our samples, including the contemporary free-ranging animals (0.85 versus 0.66). Overall, the wild mink group had high allelic richness and high expected heterozygosity compared with various domestic colour lines (although mahogany mink had the highest allelic richness). Domestic mink generally had lower values for measures of genetic diversity than did wild mink in our study, which was not surprising given that domestic mink are line bred. This contrasts with Belliveau et al.'s (1999) work, which reported that wild mink trapped from one area in NB had a lower expected heterozygosity than various lines of domestic mink, although this may have been attributable to the small geographic area sampled for wild mink (Belliveau et al. 1999).

We observed weak evidence of spatial genetic structure among contemporary wild mink in the Maritimes. All STRUCTURE anal-

Fig. 4. A neighbour-joining network based on allele frequencies for the five genetic groups of American mink (*Neovison vison*) identified in a study of mink population genetics in the Canadian Maritimes. Figure appears in colour on the Web.



yses of the full data set suggested a single group containing all of the presumed wild mink. There was some substructure that emerged when we assessed only mink with a wild assignment probability, suggesting that mink in PEI are somewhat distinct from mink on the mainland. The extensive network of waterways available to mink in the Maritimes suggests that populations of this semiaquatic animal should be well connected throughout the region (e.g., Stevens et al. 2005; Laurence et al. 2013), which is generally what we observed; however, it seems that the Northumberland Strait separating PEI from the mainland may be somewhat of a barrier to mink. We were not able to test for isolation by distance because of the low accuracy of spatial locations associated with some of the samples that we obtained from trappers. Isolation by distance was not observed in wild mink over a relatively similar spatial extent in Ontario (Beauclerc et al. 2013). It may be worthwhile comparing the genotypes of Maritime mink to those from other regions of North America to evaluate whether there is extensive panmixia, as has been observed in some similar species such as the American marten (*Martes americana* (Turton, 1806)) (Kyle and Strobeck 2003).

The prevalence of mink escaping or being released from farms suggests that more attention should be paid to biosecurity, both to prevent mink from escaping and people from accessing mink farms. In all Canadian jurisdictions where this topic has been studied, there is evidence of free-ranging domestic, hybrid, or introgressed mink. It is notable that European studies have also shown domestic mink to escape and hybridize with long-established feral mink (e.g., Zalewski et al. 2011), suggesting that the escape of farmed mink is a widespread process with an extensive footprint. In Canada, biosecurity on farms tends to be a provincial responsibility and measures vary by province. Available measures to reduce risk of escape or release include establishing minimum fencing standards and licensing of mink farmers.

Conservation implications

Our analysis demonstrated that free-ranging domestic mink are present in the Maritimes region of Canada. This is consistent with previous indications that feral domestic mink in NS supplement the wild mink harvest (see Fig. 3 in Bowman et al. 2007). Domestic mink occurred throughout the region, but were most prevalent in close proximity to mink farms. The findings of our study are also consistent with earlier work from Ontario that found prevalent domestic–wild hybridization in close proximity to mink farms. The long-term implications of escaped domestic mink are unknown, although an analysis of harvest data across the country suggested that mink farms were associated with population declines in wild mink (Bowman et al. 2007). A variety of mechanisms are possible that could lead to declines in wild mink, including outbreeding depression due to hybridization and introgression with domestic mink and increased spread of pathogens such as AMDV (Bowman et al. 2007; Kidd et al. 2009). The reduced number of alleles in contemporary versus historic mink is also consistent with the idea that there has been a loss of genetic diversity in wild mink, possibly due to introgression with domestic animals. We suggest that mink farm biosecurity should be an important priority for jurisdictions with mink farms to mitigate potential effects of escaping domestic mink on wild mink.

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