Mink gone wild: hybridization between escaped farm and wild American mink (*Neovison vison)* in a natural context

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Abstract

The release of cultured organisms into natural populations may adversely affect the population through predation, resource competition and disease introduction. Of even greater concern is the potential for hybridization between wild and farmed conspecifics, which may alter the evolutionary integrity of the infused populations. Wild American mink (*Neovison vision*) populations may be threatened not only by the presence of domestic mink originating from farms in Canada, but by breeding with such escapees. Using 10 microsatellite loci I genotyped mink sampled from two farms, two putatively mixed populations in regions surrounding the mink farms and two from wild populations with no history of farming in the region. Genotypes were employed in Bayesian population assignment. I identified four population clusters, including one wild, and three farmed populations. The latter were not clustered by farm but rather by distinct line-bred colour phases. Population clustering further identified farmed and hybrid mink in the wild populations. Over 50% of the mink sampled in the populations in close proximity to mink farms (81% and 43%) were either farm escapees or descendants of escapees. Analysis of body mass and length of mink indicated that the hybrid individuals were intermediate in size to the relatively small wild and the large farmed mink. However, behavioural comparisons showed no differences between farm and wild mink, possibly because of a lack of statistical power due to low number s of true wild mink determined after population assignment. The colour of the wild caught animals was 92.5% concordant with the genetic population assignment. The overwhelming presence of farmed animals and their hybridization with wild mink in natural populations is a great concern for the future sustainability of mink. I suggest that the province should maintain a relationship with mink farms, either through licensing or assessment of biosecurity standards.

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Introduction

The release of cultured organisms is widely considered to be a threat to native biodiversity and the integrity of natural communities (Rhymer and Simberloff, 1996; Garant *et al.*, 2003; McGinnity *et al*., 2003). Natural populations are primarily affected by the introduction of such cultured organisms through predation, resource competition and disease introduction (Manchester and Bullock, 2000). However, when cultured organisms have wild conspecifics, one of the most detrimental impacts is the infusion of domestic genes via interbreeding (Rhymer and Simberloff, 1996; McGinnity *et al*., 2003). Interbreeding may (a) introduce genes favoured under the artificial selection associated with the domestic or cultured context that are maladaptive in the natural environment, (b) disrupt locally adaptated gene complexes, and/or (c) reduce the fitness of wild populations through mechanisms such as juvenile recruitment (Rhymer and Simberloff, 1996; Allendorf *et al*, 2001; McGinnity *et al*., 2003; Hutchings and Fraser, 2008). These issues are not trivial – the impact of introductions and farming on wild environments and their potential contributions to the loss of biological diversity ranked among the top 100 ecological concerns of interest to policy makers in the UK (Sutherland *et al*., 2006). Interbreeding between cultured and wild counterparts has been observed in terrestrial carnivores, ungulates, fowl, anurans and many fish species (Rhymer and Simberloff, 1996; Williams *et al*., 2002). Current research into these hybridization events has raised concerns that the hybridization with cultured conspecifics may alter the evolutionary integrity of the infused populations (Williams *et al*., 2002; Latch *et al*., 2006; Hutchings and Fraser, 2008; Randi, 2008).

Domestic populations have low genetic variation because they are subjected to intense selection and line breeding (Price, 1984; Belliveau *et al*., 1999). Even if intentional selection is avoided the captive environment itself acts as a selective force for many traits through food supplementation (foraging behaviour), mate selection and mating behaviours as well as the reduction or absence of predation risks (Price, 1984; Snyder *et al*., 1996; Belliveau *et al*., 1999). Furthermore, unintentional and drag-along selection is common not only through the environment but by linked traits (Price, 1984; Snyder *et al*., 1996). Intensive management is employed to limit active selection in captive populations designed for reintroduction programs, yet despite this management, released animals may still contribute to genetic homogenization and/or result in outbreeding depression in wild populations by disrupting the genetic diversity generated by divergent evolution (Snyder 1996; Fleming and Einum, 1997; Randi, 2008). However, agricultural breeding programs do not have the same concerns or management foci. In such breeding programs the intent is active selection for high production as well as for behaviour and morphological characteristics desired by humans (Price, 1984; Rauw *et al*., 1998). Therefore it is the desired characteristics and the health of the cultured population that is the management focus, which may result in the amplification of the cultured animals' capacity for disrupting the evolutionary integrity of any wild population that they come into contact with. Active selection and adaptation to captivity promotes the fixation of alleles that may be deleterious in nature and therefore if infused into the natural environment may have impacts on the structure of local communities (Allendorf *et al*, 2001; Lynch and O'Healy, 2001; Randi, 2008). Further, domestic alleles introduced into the wild may

result in genetic transformations that may eventually result in the natural population being incapable of sustaining itself (Lynch and O'Hely, 2001).

Perhaps the most profound and best studied examples of the introduction of cultured organisms into the natural environment are the introductions of cultured fish, especially salmonids (*Salmo* spp.) (Hutchings and Fraser, 2008). Farmed salmon are reared in a predator free and high density environment and are selected for rapid growth; all of these features affect their morphology, behaviour, and life history (Fleming and Einum, 1997; McGinnity *et al.*, 2003). Farmed salmon have been intensively managed, and although they have been intentionally selected for increased growth rate, size and resistance to diseases, other changes have been observed including reduced survival, changes in spawning time, embryo development time, egg size, fecundity, body size, age at maturity as well as changes to behaviour both for aggression and predator avoidance (Hutchings and Fraser, 2008). The introduction of these farmed salmonids to natural populations has resulted not only in resource competition with their wild counterparts but also in 'hybridization' between farmed and wild animals (Fleming and Einum, 1997; McGinnity *et al*., 2003; Hutchings and Fraser, 2008). The list of observed consequences of farm-wild hybridization is extensive and includes reduced survival and fitness of the F1 and F2 generations, accelerated growth rate, decreased predator avoidance behaviours and increased agonistic behaviours of both the farmed and the farm-wild hybrids (McGinnity *et al*., 2003; Wessel *et al*., 2006; Hutchings and Fraser, 2008). A recent study by McGinnity *et al*. (2003) examined the lifetime success of wild, farmed and hybrid Atlantic salmon (*Salmo salar*) over two generations. Juvenile hybrid and introgressed

individuals displaced the wild parr individuals because of their altered life history characteristics despite reduced overall survival. Moreover, distinct outbreeding depression occurred in F2 hybrids. This study further established the link between repeat introductions (escapees) and the risk to the persistence and fitness of native populations, because of continuous opportunity for hybridization and introgression causing cumulative fitness depression (McGinnity *et al.,* 2003)

In addition to the well studied plight of wild salmon, concerns surrounding hybridization between farmed animals and their wild progenitors has been raised for several terrestrial carnivore species including the gray wolf (*Canus lupus*), Scottish wild cat (*Felis silvestris*), European wild cat (*Felis silvestris*), polecat (*Mustela putorius*) and Arctic fox (*Alopex lagopus*) (Norén *et al*., 2005; Randi, 2008).

The American mink (*Neovison vison*) is a semi aquatic mustelid endemic to North America that has been trapped for its fur for centuries (Joergensen, 1985). Owing to high demand for fur, the wild trapping of mink was supplemented by the farming of mink in the late 1800s (Joergensen, 1985; Belliveau *et al.,* 1999; Kruska and Sidorovich, 2003). Farmed mink have been bred intensively (line-breeding) for artificially selected traits including fur colour, size and temperament (Belliveau *et al.,* 1999; Kruska and Sidorovich, 2003). This active selection has led to very large and colourful farm mink that have been habituated to human presence (Belliveau *et al.,* 1999; Malmkvist and Hansen, 2002). Farm mink have been line-bred to include a broad range in colours, from snow white to jet black with the varieties being referred to as colour phases (Shackelford,

1948; Joergensen, 1985). Many of the colour phases are exclusively inbred because the colour is recessive to the standard brown, while others may be blended, such as chocolate brown which is achieved by breeding the black and standard brown phases together (Joergensen, 1985). Artificial selection has not been restricted to physical attributes. Fearfulness and fear induced aggression in mink (a physiological response to situations or stimuli that may be perceived as dangerous) may be beneficial in a natural context but may be detrimental to the animal's welfare in the captive environment and was therefore one of the first traits selected against in the history of mink domestication (Trapezov, 2000; Malmkvist and Hansen, 2002). Malmkvist and Hansen (2002) found that active selection for confident individuals, or those individuals that did not display overt fearfulness or fear-induced aggression resulted in proportionally more confident animals in a farm.

Through accidental escapes and deliberate releases caused primarily by animal rights activists, farmed mink have become established in regions outside their native range; in other words, they have become 'feral' (Joergensen, 1985; Lodé *et al*., 2001; Kruska and Sidorovich, 2003; Reynolds *et al*., 2004; McDonald *et al*., 2007). These feral, invasive mink have had serious impacts on local endemic mustelids through competition as well as a detrimental impact by predation on many birds, voles, amphibians, and invertebrates in Europe, Iceland and South America (Lodé *et al*., 2001; Reynolds *et al*., 2004; Bonesi and Palazon, 2007). Several studies have been conducted on the ecological impacts of feral mink in regions where they have been introduced, such as Europe (Reynolds *et al*., 2004). However, the fur farming industry is also active within the natural range of

American mink. Recently, Bowman *et al*. (2007) statistically examined the relationship between the wild mink harvest and mink farm density in Canada. They found a positive relationship between mink farm density and density of wild trapped mink per province, which have both been in decline in recent years. This study described a disturbing phenomenon whereby up to 34% of wild trapped mink in a province taken to auction in a year were graded as farmed animals. Their results strongly suggested that not only were farmed mink being caught by wild mink trappers but that these feral animals may be contributing to a decline in American mink populations, perhaps via outbreeding depression or disease introduction.

I tested the hypothesis that, as with regions of the world where mink are farmed but not endemic, farm mink escape (or are released) and persist in the wild environment where American mink are endemic (e.g., Bowman *et al*. 2007). Furthermore, I hypothesized that these feral mink would breed with the wild mink and produce farm-wild hybrids (hereafter referred to as hybrids). I collected tissue samples from mink from 6 populations in 4 regions (Figure 1). These populations were composed of (a) two wild mink populations, which I considered to be composed of entirely wild mink based on the lack of nearby fur farms (b) two farm populations and (c) two putatively mixed free ranging populations that were adjacent to the mink farms (Figure 1). I examined population structure and performed Bayesian population assignment using 10 microsatellite loci. I predicted that I would identify not only feral mink living in the natural populations adjacent to farms but also hybrids. I recorded colour, body mass and body length of samples animals for comparison between the different genetically

identified populations, predicting that hybrids would be intermediate to the farmed and wild mink. Subsequently, three behavioural tests were conducted on a subset of mink from a farm and the two putatively mixed populations in an effort to examine proportional differences in behaviour between the wild and farmed mink and any hybrids identified in relation to them.

Methods

Sample collection

Six populations of American mink were sampled in 2005 and 2006; two each of farmed, wild, and 'putatively mixed' (Figure1, Table 1). Putatively mixed populations were free ranging populations in regions containing mink farms that I predicted would be composed of not only wild mink but also farm escapees and potentially hybrids. Wild mink were sampled by obtaining trapped carcasses during winter 2006 from trappers in both the Kirkland Lake (13,698 km^2, hereafter referred to as Wild 1) and Peterborough (15,381 km^2 , hereafter referred to as Wild 2) administrative districts of the Ontario Ministry of Natural Resources (OMNR). Both populations were assumed to be composed only of wild mink due to the absence of mink farms in these districts, either presently, or in the recent past. The two farms sampled were in the regions of Essex (1720 $km^{2,}$ hereafter referred to as Farm 1) and Niagara (1850 km^2, hereafter referred to as Farm 2) in southern Ontario, Canada. Four colour strains were sampled from the farm in Essex and two colour strains from the Niagara farm (Table 1). Furthermore, the free ranging populations in the regions adjacent to the sampled farms (hereafter referred to as

Mixed 1 and Mixed 2), which were considered a priori to be putatively mixed, were sampled by live trapping during 2005 and 2006.

Mink sampled from Mixed 2 were by-catch from the OMNR's Rabies Research and Development Unit (RRDU) live trapping program. Live traps used included Tomahawk live traps (models 108 and 106 in Mixed 2, and models 105 and 106 in Mixed 1) and wooden Edgar live traps were also used in Mixed 1 because of the risks associated with winter trapping (Edgar, 1962). Both trapping programs employed daily overnight trapping where traps were checked every morning and no animal would be left in a trap for longer than 12 hours. Traps were placed based on habitat suitability and access permission. Mink were transported from the field collection site to the field lab in their live traps, which were covered in towels to muffle noise and then placed in a large lidded Rubbermaid container that were secured in the bed of field trucks. The colour of each captured animal was noted by the trapper upon capture. The colour description was used for comparison and proportional agreement with the genetically assigned population and/or colour phase (methods below). Mink from Mixed 1 were put through behavioural tests (methods below) and then immobilized with a 10:1 mixture of ketamine hydrochloride to xylazine hydrochloride at a dosage of 20mg/kg of animal weight. The xylazine was reversed after sampling with Yohimbine at a dosage of 0.1 mg/kg. Animals were then marked with 1-g Monel ear tags. Body mass (+/- 1 g measured with 2 or 5 kg Pesola scale) and body length (+/- 5 mm measured with 500 mm callipers or a 1000 mm ruler when body length exceeded that of the callipers) measurements were obtained from all sampled individuals. Tissue samples collected included blood obtained by cutting the

toe nails just far enough to nick the quick and hair samples collected by plucking hairs with roots attached. Body length measurements were unavailable for the Mixed 2 population because these animals were not sedated. The weights of Mixed 2 were taken with the animal in the live trap and the weight of the trap subtracted later. All animals were then released at their point of capture.

The farm mink were sampled by collecting carcases from the farms when the furs were being harvested and therefore already sorted by colour phase. The carcases were then returned to the lab and processed in the same manner as described for the wild caught mink.

Microsatellite Genotyping

Whole DNA was extracted following the modified Qiagen (Qiagen Mississauga, ON) extraction protocol described by Guglich *et al*. (1994) (Appendix 1). Stock DNA was visualized using gel electrophoresis stained with ethidium bromide in order to determine presence and relative quality of extracted DNA (Appendix 2). DNA yield was quantified using a FLUOstar Optima fluorometer (BMG Labtechnologies) and Hoeschts Dye (Bio-Rad). Extracted DNA concentrations were calculated based on their relationship to the standard curve produced by the fluorescence of the calf thymus DNA. Calculated DNA concentrations were used to create standardized dilutions of 2.5ng/ul (where yield was in excess of standard) for each sample, as a working dilution for PCR amplification.

All samples were amplified using primers for twelve (of which two were dropped from analysis due to incomplete genotyping and the possible presence of null alleles) polymorphic microsatellite loci (Table 2) and pooled in four groups for genotyping. Forward primers were fluorescently labelled with either 6Fam or Hex (Integrated DNA Technologies) (Table 2). Amplifications were performed in 10ul total volumes containing a final concentration of 1X PCR Buffer,1.5mM MgCl2, 0.2mM dNTPs, 0.1-0.3mM forward and reverse primer depending on individual locus (Table 2), 5ng DNA and 0.5units of Taq polymerase.

Amplification was carried out with an initial denaturing period of 4 minutes at 94^{o}C followed by 30 cycles of 94^{o}C for 1 minute, primer specific annealing temperature (Table 2) for 30 seconds and 72^{o}C, followed by a final extension of 4 minutes at 72^{o}C. All samples were amplified using an Eppendorf Mastercycler. All amplifications were conducted alongside positive and negative controls in order to ensure the reliability of the reaction and its reagents. Mvi 1016 and Mvi 1321 were run for 30 seconds at 94^{o}C denaturing for each cycle, and Mvi 114 and Mvi 111 were run for 35 cycles.

All PCR amplified products were precipitated in ethanol to remove excess salts and resuspended in 10ul ddH_2O (Appendix 3). A pooled dilution of 0.5ul was then resuspended to 10ul high HiDi formamide and Rox size standard 350 in a new plate and analyzed with an ABI3730 automated sequencer using GeneMapper 4.0 (Applied Biosystems) at the OMNR genetics lab at Trent University.

Analysis of genotypes

Only individuals that had a minimum of 8 genotyped loci were used in subsequent analyses. I tested for the presence of null alleles at all loci and genotyping errors as suggested by Pemberton *et al*. (1995) using the program Micro-Checker (Oosterhout *et al*., 2003). Significant deviations from Hardy-Weinberg equilibrium (HWE) for both individual populations and globally for each locus were evaluated using an exact probability test in GENEPOP 4.0 (Rousset, 2008). Allele frequencies, observed and expected heterozygosity, F_{IS}, linkage disequilibrium and pair-wise population differentiation (F_{ST}) were analysed using FSTAT 2.9.3.2 updated from Goudet (1995). A three level analysis of molecular variance (AMOVA) was completed using GenAlEx 6 (Peakall and Smouse, 2006) to estimate variance among individuals within populations (V_I), variation among the six populations (Wild 1, Wild 2, Mixed 1, Mixed 2, Farm 1, and Farm 2) within the four geographic regions (where Mixed 1 and Farm 1 occupy the same region and likewise for Mixed 2 and Farm 2;V_P) and variance among the four regions (V_R; Figure 1).

Bayesian clustering using Structure 2.2 with the admixture model was employed to evaluate the number of inferred genetic population clusters (K) and to assign individuals to their likely population of origin, using no prior information (Pritchard *et al*., 2000; Falush *et al*., 2003). The number of inferred populations was assessed using the entire sample set (n=215). Results were generated using five repetitions of 50 X 10^4 iterations following a burn in period of 50 X 10^4 iterations. Burn in length was selected based on the convergence in summary statistics in a series of trial runs. I selected the ideal K

(population clusters) using the posterior probability of the data (Ln $P(D)$) the formula [Ln $P(D)_k$ – Ln $P(D)_{k-1}$] suggested by Garnier et al. (2004). Individuals were assigned probabilistically to populations or jointly to two or more if their genotypes indicated that they were admixed by their membership coefficient (q). Individuals were assigned to clusters with a minimum membership of $q \geq 0.80$ or jointly to two or more clusters when $q_1 + q_2 \geq 0.80$ for admixed individuals, thereby ensuring that at least 80% of an individual's genome was assigned to the inferred cluster(s) (Pierpaoli *et al*., 2003; Verardi *et al*., 2006). These individual population (cluster) assignments were subsequently used to compile groups of purely wild, farm and hybrid individuals for size and behaviour comparisons as well as their relationship to the trappers' description of their colour. Population differentiation (pairwise F_{ST}) was assessed using cluster assignments, including a hybrid cluster composed of individuals of mixed assignment. Hybrids were identified from the mixed (Mixed 1 and Mixed 2) groups, and assigned to their own group (Hybrid 1 and Hybrid 2). Mixed 1 and Mixed 2 clusters were thus composed of only wild mink for a pair-wise F_{ST} analysis of the geographic groups. Degree of population differentiation using pair-wise F_{ST} was described as directed by Wright (1978), where F_{ST} values of 0.05-0.15 indicate moderate genetic differentiation, 0.15-0.25 is considered high and >0.25 is very highly differentiated.

Size comparison

Morphological analysis compared the mass and length of the mink from the 4 populations described by genetic clustering and the hybrid individuals whose genotypes were assigned to more than one cluster. This was done in an effort to compare the mass and

length of wild, farmed, and hybrid animals. The populations were compared for mean differences in body mass and body length with a Kruskal-Wallis non-parametric analysis of variance for genetically assigned populations in Statistica 6.1 (StatSoft Inc.). Non-parametric comparisons were employed because the data violated the assumption of homogeneity of variance and a significant sex by location interaction was observed. This analysis was conducted for the sexes separately because mink are sexually dimorphic (Eagle and Whitman, 1984). The mass of skinned carcasses was corrected for the loss of the hide by adding 17% as suggested by Sherburne and Dimond (1969). Any animals that may have been still juveniles when sampled (females under 400 g and males under 700 g) were removed from the analysis. The only population where this was an issue however was Mixed 2 because the trapping dates for this population (late summer to fall) may have coincided with the dispersal period of juveniles (Eagle and Whitman, 1984).

Behavioural Testing

The individuals sampled from the Mixed 1, Mixed 2 and Farm 2 were tested for behavioural differences using three behavioural tests prior to any other sampling or measurements (Table 1; Appendix 4). Trials were conducted in a testing arena of approximately 4 m^2 that was erected using tarps and existing walls of a shed. The trials were conducted inside a Tomahawk live trap (model 106). Prior to testing, all individuals were left isolated in the testing arena for a 10 minute acclimation period. The behavioural response of the mink to all test stimuli was evaluated based on a categorical scoring system. Categories of behaviour included: explorative, fearful, aggressive and unscorable (mixed or non response) as described by Malmkvist and Hansen (2001) for the stick test.

The three tests employed were: the 'stick test' (Malmkvist and Hansen, 2001) scoring the mink's response to the insertion of ½ inch dowel into cage, the 'mirror test', or response to a mirror image (modified from Svendsen and Armitage, 1973) and the 'approach test', or response to close human proximity, where a person would walk into the arena and place a gloved hand adjacent to the cage (approximately 2 cm from the cage wall, close enough to be sniffed but not bitten; Appendix 4). All responses to test stimuli were observed for 30 seconds and both the mirror and stick tests were employed with the observer outside of the arena to minimize any effect of their presence (Appendix 4). Behavioural tests were conducted in random order except for the approach test, which was always completed last so as to limit the observer effect in the first two tests. Observed behaviours were compared for differences between the genetically determined populations using Chi-squared analysis.

Colour

The colour of the captured animals from both putatively mixed populations was noted by the trapper upon capture. The colour description was used for comparison with the genetically assigned populations. The genetic populations were expected to express specific phenotypes: brown to dark brown coat colours were expected for the wild and standard brown farmed mink, black coat colour for black farmed mink and grey coat colour for iris mink. The expected phenotype was compared to the observed phenotype and percent concordant descriptions were described in an effort to establish whether colour phase is a reliable indicator of genetic population.

Results

Genetic variation and population diversity

Genotyped positive controls showed no sign of genotyping error (<1%). Samples that did not amplify or genotype successfully the first time were re-run to fill the missing genotypes. Analysis of genotyping data in Micro-Checker (Oosterhout *et al*., 2003) identified two loci (Mvi 1010 and Mvi 4052) that may have shown signs of null alleles based on an homozygous excess. Further these two loci had a high proportion of missing genotypes and for these two reasons they were omitted from my analyses. Individual genotypes were determined for 215 mink for the 10 microsatellite loci. All loci were polymorphic with 6-15 alleles per locus (Table 2). The mean number of individuals successfully genotyped per locus was 90.4% and only individuals with 8 of 10 loci successfully genotyped were retained for analysis (Table 2). No loci were in linkage disequilibrium at Bonferroni-corrected $p < 0.05$. A significant deviation from HWE was observed ($p<0.01$), caused by a deficiency in heterozygotes globally. The groups were separated for population HWE analysis and several showed a deficiency of heterozygotes, including both Wild 1, both Mixed 1 and Mixed 2, as well as all of the farm colour phases except the Brown and Black colour phases (Table 3). F_{IS} values per population reflected this deficiency and indicated the presence of low to strong inbreeding with values ranging from 0.003 to 0.175 per population (Table 3).

Hierarchical analysis of molecular variation (AMOVA) demonstrated that the molecular variance was distributed principally within populations (V_I: 92%, Rst 0.089, , d.f. 651, p= 0.01) and significantly, but to a smaller extent between populations within geographic

regions (in this analysis Farm 1 and Mixed 1 are examples of populations that exist in the same geographic region; V_P: 6%, Rsr 0.066, d.f. 6, p= 0.01) and between regions (V_R: 2%, Rrt 0.025, d.f. 3, p= 0.01).

Population Assignment

The second-order rate of change (Evanno *et al*., 2005; Garnier et al. 2004) suggested strong population signals at K=2, K=4 and K=6 (Figure 2; Appendix 5). The two genetic populations clusters separated at K=2 were the wild and farmed genotypes (Figure 2). The four inferred populations identified at K = 4 included one wild group and three farmed colour phases: Black, Brown (including Standard Brown and Pastel individuals) and Iris (Table 4; Figure 2). The signal indicating six population clusters (K=6) identified two wild groupings, a more northern one (Wild 1 and some of Wild 2) and a southern strain including most of the Wild 2 individuals as well as mink in Mixed 1 and Mixed 2 that were not clustered into a farm population (Figure 2). There were 4 farmed colour phases identified by K=6: including the same Black, Brown and Iris, but also Mahogany as its own group. In the K=4 grouping the Mahogany individuals were assigned as Black-Brown hybrids, which is consistent with the breeding of the Mahogany colour phase (Table 4). Where K=6, only 69% of the Mahogany genotypes assigned to the Mahogany cluster. The rest was still divided between black and brown. At all three population levels 2, 4 and 6 the individuals from the mixed populations assigned to both farm and wild groupings, with overall group membership being partitioned between wild and the Black and Brown farmed clusters (Table 4).

I continued analysis principally with K=4 because it was a strong signal (Appendix 5), with greater resolution than K=2 but with greater confidence than K=6. Four populations clearly distinguished between farmed and wild mink as well as among the basic domestic colour types with stronger membership coefficients than K=6.

Mean F_{ST} calculated using the 'F-model' for K=4 populations estimates the drift from the inferred common ancestor, and indicated that the wild population was least diverged while the Farm Iris population was the most diverged from the inferred common ancestor (Table 4; Falush *et al.*, 2003).

I ranked the individual q-values to the wild population cluster for all mink sampled in the putatively mixed populations and ordered them from lowest to highest where an individual with q = 1 would describe a purely wild mink and q = 0 would be a purely exogenous (100% farmed) individual (Susňik *et al*., 2004). The 54 individuals sampled from mixed populations appeared to consist not only of wild and feral individuals but also of hybrids, or individuals with blended genotypes with cumulative q's > 0.80 (Figure 3). Individuals sampled from Mixed 1 included 57% (6 females, 5 males and 1 unknown sex) wild individuals, 14% (1 f, 2 m) hybrids, and 29% (1 f, 5 m) farmed individuals (Figure 4a). Mixed 2 included 19% (1 f, 5 m) wild individuals, 53% (9 f, 8 m) hybrids, and 28% (4 f, 3 m, and 3 unknown sex) farmed individuals (Figure 4b). Admixed (hybrid or introgressed) mink composed 38% of the mink sampled from the putatively mixed groups (Mixed 1 and Mixed 2). While 28% were most likely escapees from local farms and only 34% of the mink sampled from both populations were identified as wild mink. .

Pair-wise F_{ST} analysis was completed for the K=4 groupings with a separate group for the hybrid individuals. All populations were significantly differentiated ($p<0.05$) after 1000 permutations (Table 5). The wild mink population demonstrated moderate to high differentiation from the farmed mink populations, with the greatest difference observed with the Iris phase population. The Iris population displayed the greatest differentiation from all other populations (Table 5). The Brown and Black farmed mink were moderately differentiated. The Hybrid group displayed low to moderate differentiation from all populations except from the Iris population, which only contributed to the genotype of one of the hybrids in each of Mixed 1 and Mixed 2 (Table 5).

Pair-wise F_{ST} analysis with 1000 permutations was also completed using the geographic groupings. Population differentiation was not observed between the wild populations, but it did indicate moderate structuring between them and the putatively mixed populations (Table 6). All of the farm colour groups were moderate to highly differentiated from all wild mink groups, as well as between themselves (Table 6). Although not differentiated from each other, the mixed populations were on average moderately differentiated from almost all other groups with the exception of much stronger differentiation from the Iris farmed mink (Table 6).

Size

The removal of potential juveniles and division of the sexes left only 11 hybrid mink for comparison of mass and only 3 for length (Tables 7-10). The body masses of wild male and female mink were not significantly different from the hybrids, but they were significantly smaller than all farm populations ($p<0.01$) (Table 7, Table 8, Figure 5). The hybrid mink were not significantly different from any other populations (Figure 5). The farm mink populations were not significantly different from each other with the exception of Farm-Brown females, which were significantly smaller than the other two female farmed populations ($p<0.05$; Table 8).

As observed with the mass, both the females and males from the wild population were significantly smaller in body length than the farmed mink populations ($p<0.01$), but not significantly different from the hybrids (Table 9, Table 10, Figure 6). The hybrid mink of both sexes were significantly smaller ($p<0.01$) than all of the farm mink populations (Table 9, Table 10, Figure 6). The length of both male and female farmed populations did not differ significantly (Figure 6).

Behaviour

After genetic clustering, the sample size of 30 farmed mink tested became 39 because of the identification of 9 farm escapees tested in the two putatively mixed populations. Furthermore, of the original 21 individuals in the mixed populations another 10 were identified as hybrids, leaving only 2 behaviourally tested individuals in the wild group

(Table 11). There were no significant differences in the proportion of observed behaviours between groups when compared using χ^2 analysis (Table 11; Table 12).

Colour

The colour of each individual was described by the trapper upon collection. In 74% of the cases, described colours were in agreement with the assigned populations. The wild mink were all described as either brown (80%) or dark brown (20%) and therefore 100% of the descriptions within the expected phenotype of wild mink. The Farm-Black mink were correctly described as 'black' by trappers 7 out of 10 times (70%) and the remaining two were described as 'dark brown'. The Farm-Brown mink were all described as brown or dark brown (100%), while the mink that assigned partly Farm-Brown and partly Farm-Black, or the Mahogany colour phase mink, were all described as black or dark brown (100%). There was one mink that was genetically assigned to the Farm-Iris population that was described by the field technician as 'dark brown'. With the exclusion of this aberrant Iris mink, the overall colour phenotypes described were 92.5% concordant with the genetic population assignment. The hybrids were described as ranging in colour from brown (20%), to dark brown (55%) and black (25%)

Discussion

Our results support the hypothesis that farmed mink are escaping and persisting within wild mink populations. Furthermore, our results provide strong evidence that these feral mink are hybridizing with wild mink. The persistence of farmed mink in the natural environment and the evidence of their interbreeding with wild conspecifics is of serious concern. The consequences of such introductions may include reduced fitness, population viability and survival as well as disruption of local adaptation and life history characteristics such as reproductive and foraging behaviours via the introduction of maladaptive gene complexes (Rhymer and Simberloff, 1996; Allendorf *et al*., 2001; McGinnity *et al.*, 2003; Hutchings and Fraser, 2008). Work on salmonids shows that the risk to the persistence of native populations increases with repeat introductions (McGinnity *et al.*, 2003). In the case of mink, the kind of chronic escapement from ranches suggested by Bowman *et al*. (2007) could have a similarly depressing effect. Introgressive hybridization of wild populations with cultured animals may contribute to genetic homogenization, disrupt population structure and contribute to local extinctions by the disruption of divergent local adaptations (Rhymer and Simberloff, 1996; Allendorf *et al*., 2001; Randi, 2008). Therefore, I consider farm-wild mink hybrids a threat to natural American mink populations. The threat is magnified owing to the potential geographic extent of the problem. Although this study occurred over a small spatial scale, mink ranches occur across much of the native range of mink, meaning that hybridization could be widespread (Joergensen, 1985).

The overall proportion of feral and hybrid mink was more than wild mink caught in both Mixed 1 and Mixed 2. Of the individuals sampled from Mixed 1 only 57% were wild and even more dramatic was that only 19% of the mink sampled in Mixed 2 were wild animals. Thus, over 40% or in the case of Mixed 2 over 80% of the mink sampled in close proximity to mink farms were either farm escapees or descendants of escapees. This is a great concern for the future sustainability of the populations.

Although I observed a significant deficiency of heterozygotes globally, this is to be expected for many of the farmed populations because of line-breeding practices (Joergensen, 1985; Belliveau *et al.,* 1999). However, two of the farm colour phases (Farm 1, Black and Standard Brown) were in Hardy -Weinberg equilibrium (HWE) (Table 3). Belliveau *et al.,* (1999) also found high levels of genetic variability in both brown and black farmed mink. They suggested that this variability results from several factors. First, black farmed mink may be more diverse because they were originally bred from the combination of three subspecies of American mink (Dunstone, 1993). In my study the brown mink exhibited greater variability than the Black. Brown or Standard mink are sometimes crossed with other colour phases to produce new colour phases such as Pastel and Mahogany (Joergensen, 1985). Further, the breeding of these new colour phases often produces a portion of standard brown offspring because this colour is genetically dominant to the other colours and therefore the brown colour phase may be composed of individuals from more than one breeding line (Joergensen, 1985; Belliveau *et al.,* 1999; mink farmer *pers. comm.*). Furthermore, higher vigour and reproductive performance has been observed in the brown mink, which may result in less selective pressure and drift

than other colour phases (Joergensen, 1985; Belliveau *et al.*, 1999). In addition to the colour phase specific impacts on the genetic variability, farmers will purchase animals from other farms for breeding in an effort to mitigate the impact of inbreeding and maintain healthy viable stock without affecting the selected traits such as colour (Joergensen, 1985; Belliveau *et al.*, 1999; anonymous mink farmer *pers. comm.*). It appears therefore that the farms sampled have successfully maintained some genetic variability particularly in the more standard colours, which may be easier to maintain than the more line-bred and divergent colour phases such as Iris.

I suggest that the deficit of heterozygotes in the Mixed populations may result from the observed admixture, or a Wahlund effect. Such an effect would result from the interbreeding of individuals from different subpopulations, such as the feral mink breeding with the wild mink in Mixed 1 and Mixed 2 (Weir and Cockerham, 1984).

Population Differentiation

I observed significant genetic differentiation between the wild, hybrid, and farm populations. Logically, the hybrids were described as moderately differentiated from the populations that had contributed to their blended genomes while they were highly differentiated from the Farm-Iris population. The Farm-Iris population displayed the greatest divergence from all other populations and the most drift from a shared common ancestor. Given their specialized colour and the intensive assortative mating required to maintain it, this is hardly surprising (Joergensen, 1985). However, not only were the Iris mink the most distinct genetically but they did not contribute to the genomes of any of

the 20 hybrids captured. Moreover, out of 15 feral mink identified in the mixed populations only one was Iris. Iris mink are relatively rare in ranches, and therefore I expect that relatively few mink of this colour phase escape. Furthermore, their rarity in the wild may be related to lower in situ survival or reproductive success.

Pair-wise F_{ST} among locations presented a similar trend as the genetic populations resulting from the clustering method, which is reflective of a century of genetic isolation and differing selective pressures. The farm colour phases displayed the same trend in this analysis as in the pair-wise F_{ST} by genetic population. Moderate differentiation was observed between the wild and mixed populations. The Wild populations were moderate to highly differentiated from the farmed populations, with the greatest difference observed with the Iris mink as would be expected given that groups divergence. The Mixed populations were not differentiated from each other and less differentiation from the farm populations than the wild mink, in fact they displayed as much differentiation form the wild populations as from the farm groups that were primarily represented in the populations, which considering the blended nature of these populations is logical.

Size

Body mass and length results displayed almost identical patterns with wild mink being the smallest, farmed mink the largest and hybrids intermediate. The Black and Iris females were significantly heavier than the Brown colour phase as well. The pattern observed where the hybrids were not significantly different from any other group in weight, but shorter than the farmed mink, suggests that the hybrids are intermediate to the

farmed mink and the wild mink and therefore are blending size characteristics when interbreeding. However, the number of hybrid mink was too small, especially for the length data, place too much emphasis on these findings. Furthermore, analysis was inhibited by the interaction effect between population and mass. This difference violated the assumption of homogeneity of variance and of slopes and I suggest is related to differences in body condition. Farmed mink are fed regularly on an optimal diet for growth and rarely leave their cages, as such exhibit very different fat content and length to weight relationships (Joergensen, 1985; Kruska and Sidorovich, 2003; Hammershøj *et al*., 2004).

Behaviour

Wild mink would be expected to display more fearfulness than farmed (Malmkvist and Hansen, 2002). However, a general lack of fearful behaviour in the farmed mink when compared to wild mink is consistent with both the farmed mink's habituation to human presence and breeding techniques employed by farmers. Fearfulness is a predisposition to perceive and react similarly to potentially dangerous situations or stimuli (Malmkvist and Hansen, 2002). This physiological reaction in mink is selected against in the captive environment because mink farmers tend to select confident individuals for their breeding programs (Malmkvist and Hansen, 2002, mink farmer, *pers*. c*om*.). Malmkvist and Hansen (2002) found that active selection over 10 generations for confident individuals resulted in proportionally more confident animals in a farm. However, farmers may not directly test fear or confidence. Instead, mink farmers may be more prone to removing animals with behaviours such as tail-sucking that can be easily identified as a nervous

behaviour that is destructive to the pelt (mink farmer, *pers*. c*om*.).The hybrids observed were neither significantly different from the farmed or the wild mink in terms of their behaviour. However, a greater sample size and potentially more behavioural tests would help to further assess differences resulting from the blending of farm and wild genes in nature. Furthermore, any repercussions of observed differences in behaviour must be examined in the context of the wild environment.

Colour

The evaluation of colour and its concordance with the assigned population's colour indicated that the field descriptions, or colour phenotypes were good indicators of the source population. Certainly, a detailed evaluation of fur quality and colour characteristics by trained personnel can reliably distinguish between wild and farmed mink (Obbard 1987). However, for less trained personnel in a field situation, this brief description would be most useful for the identification of obvious farmed colour phases, such as black, which are dramatically different from the wild type colour. The one mink of farmed origin that was genetically identified as an iris colour phase may be the result of either mis-identification or an error in record keeping. The hybrids were described as a ranging in colour from brown, dark brown, black and grey-brown suggesting that hybrids may not automatically express the wild type brown, but rather some may retain all or some of their farm parental colour characteristics and therefore, may be cryptic hybrids. With the use of more detailed analysis of fur colour and quality, the identification of hybrids using phenotype may be more effective.

Conclusions and Future Directions

Bowman *et al.* (2007) provided evidence of declining mink populations in Canada despite the apparent supplementation of wild populations by farmed mink based on grading of pelts provided to fur auction houses. I have confirmed that wild populations are indeed being supplemented by feral farmed animals, which is consistent with the suggestion of Bowman *et al.* (2007) that instead of increasing numbers, escapees may be directly linked to declines in wild mink populations. There are two avenues by which population declines of wild mink may be induced by the mink escaping from mink farms. First, as I have shown, introgressive hybridization with wild mink can occur. This may lead to the introduction of maladaptive genes into the natural mink population, or the disruption of locally adapted gene complexes (Rhymer and Simberloff, 1996; Allendorf *et al* l., 2001; Randi, 2008). Second, diseases such as Aleutian disease, a highly infectious and often fatal parvovirus found in many mink farms across the country (Bloom *et al*., 1980) may be introduced into natural mink populations via contact with domestic mink.

I believe that the farmers must be required to mitigate their potential impact on adjacent natural ecosystems, because the constant infusion of domestic genes has been linked to reduced fitness and a threat to persistence of natural populations, resulting from a continuous opportunity for hybridization and introgression (McGinnity *et al*., 2003). There are some potentially sufficient regulations in Ontario under the province's Fish and Wildlife Conservation Act such as the requirement by farmers to report escapees and to be held liable for costs associated with their recovery. However, there has been little

enforcement of this policy since the removal of licensing authority over mink farms during the 1990s. Licensing allowed the government to communicate with farmers about such policies and to keep track of active farms. I suggest that the province should maintain a relationship with mink farms, either through licensing or assessment of biosecurity standards.

An explicit policy concerning minimum fencing standards around mink housing facilities should be enforced. I suggest, as in some European jurisdictions, that fencing of smooth boards that continues below ground for at least 10cm and has a top runner that discourages climbing over would act as an effective deterrent to mink leaving farm properties. Furthermore, animal rights groups that release farmed mink should be more aware of the detrimental impacts of their activities. While not all of the domesticated animals released are expected to survive in the wild, the ones that do survive harm natural populations through not only competition, or the introduction of new disease, but also the influx of potentially maladaptive genes (Rhymer and Simberloff, 1996; Allendorf *et al*., 2001; Randi, 2008). I believe that there should be a policy enforcing a minimum standard of biosecurity both preventing human entrance and mink exit.

Eradication and control programs in Estonia, Belarus, Finland and portions of the United Kingdom have shown success in curbing or eliminating their feral mink problem (Bonesi and Palazon, 2007). Hunting programs in Iceland and Lithuania have been less successful. In fact, in Iceland the feral mink population appears to have increased (Bonesi and Palazon, 2007). Unfortunately, whereas these programs may provide some guidance

for dealing with a feral mink problem, the issue is much more complex within the endemic range of the American mink. For example, eradication programs would have to be carefully targeted to avoid wild mink. Finally, feral mink are not just threatening natural populations through resource competition or disease but introgressive hybridization and their genes may be much harder to deal with and have a more lasting effect on the long term viability of endemic mink populations.

Introgressive hybridization has been shown to be a threat to recovery efforts for several species including the rock partridge (Barilani *et al.*, 2007), the Grey wolf (Randi and Lucchini, 2002; Verardi *et al.*, 2006), the Red wolf (Kelly et al., 1999; Adams *et al*., 2007), the wildcat (Randi *et al*., 2001; Pierpaoli *et al*., 2003; Lecis *et al*., 2006), as previously discussed in Atlantic salmon (McGinnity *et al*., 2003; Susnik *et al*, 2003; Hutchings and Fraser, 2008) and has been implicated as a threat to the endangered arctic fox in Sweden and Norway (Noren *et al*., 2005).

The grey wolf in Europe suffered dramatic population declines from human persecution and deforestation, but in the 1980s these meagre populations began to grow and expand in range (Randi, 2007). However, they are growing in number and expanding their range in the presence of an abundance of feral dogs, which could have serious consequences on the recovery of this species through introgressive hybridization. Sporadic hybridization has so far been observed between the grey wolf and domestic dogs, though in a recent study by Verardi *et al.* (2006) found 5% of the wolves sampled in Italy were likely admixed. Hybridization with coyotes is widely considered the greatest threat to the

recovery of the red wolf in North America (Kelly *et al*., 1999), in 2007 Adams *et al*., (2007) found 6 of 23 (26%) known individuals were coyote hybrids, 5 of which were removed from the population.

The central and western European wild cat populations were likewise depleted and fragmented by hunting and trapping as well as deforestation in the 18th and 19th centuries (Randi, 2007). Anthropogenic hybridization has been observed between the wild cat and it's domesticated subspecies in many European countries including in Bulgaria (17% admixed), Belgium (5% admixed), Portugal (14%) (Oliveira *et al*., 2007), Italy (8% admixed), in Hungary (31% admixed (Lecis *et a*l., 2006) and in Scotland (41% admixed; Beaumont *et al*., 2001). These findings have led to the suggestion that a main threat to the genetic integrity of wild cat populations is such hybridization and a request for a sound long-term wildcat conservation strategy directed at control of the feral domestic cats and minimizing opportunities for hybridization (Lecis *et al*., 2006).

The rock partridge is hunted heavily through out Greece and had led to declined in the wild populations, therefore Greece began breeding red-legged partridge hybrids for massive stocking programs. Barilani *et al*., (2007) recently found that up to 20% hybridization and introgression between the stocked partridges and the wild rock partridge population. Based on these findings Barilani *et al*., (2007) suggested an interruption of the stocking programs.

In all above examples the overriding trend is wild populations that have been depleted by anthropogenic activities and the current recovery efforts, their genetic integrity and their future sustainability of the wild populations are being compromised by anthropogenic-induced introgression (Allendorf *et al*., 2001; Randi, 2007). We have found that the wild American mink populations are likewise being genetically polluted by anthropogenic hybridization and introgression and at greater rates in some populations than has been observed in many similar studies. Therefore, I suggest that there is an urgent need for addressing this issue if we are to preserve the genetic integrity and fitness of our native mink populations.

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Table 1: List of samples used in all analyses listed by geographic region and by colour phases for farmed populations, where n represents the number of animals employed in genetic and morphological analysis while n(b) represents those used in behavioural tests and the mean body mass (g) and mean body length (mm) for both sexes with standard deviations in brackets for each group.

Population	**Region**	**n**	**n (b)**	**Mean Weight (g)**		**Mean SVL (mm)**	
				Male	Female	Male	Female
Wild 1	Kirkland Lake	30	~	849.9 (111.1)	474.3 (36.9)	383 (19.1)	335 (14.7)
Wild 2	Peterborough	20	~	936.7 (169.1)	495.3 (75.1)	401 (16.8)	327 (11.7)
Mixed 1	Essex *(All adult)*	21	5	1442.8 (416.6)	585.6 (285.5)	405 (136.1)	336 (34.0)
	(wild adult)	4 - 6		1297.1 (230.1)	492.5 (59.6)	440 (21.6)	328.8 (15.7)
	(hybrid *adult*)	2 - 1		1350 (141.4)	450 (N/A)	420 (77.8)	303 (N/A)
Mixed 2	Niagara *(All adult)*	33	22	1278.6 (646.7)	937.5 (410.4)	~	~
	(wild adult)	1		800 (N/A)	N/A	~	~
	(hybrid *adult*)	4 - 4		1462.5 (838.0)	1075 (567.9)	~	~
Farm 1 *Black*	Essex	29	~	1569.1 (142.9)	758.1 (167.4)	449 (16.1)	379 (24.6)
Farm 1 *Standard Brown*	Essex	28	~	1684.4 (173.9)	708.1 (224.8)	467 (16.1)	394 (15.9)
Farm 1 *Pastel*	Essex	15	~	1852.7 (165.6)	676.5 (n/a)	474 (12.1)	390 (n/a)
Farm 2 *Iris*	Niagara	20	10	1877.5 (283.1)	1087.2 (178.8)	472 (19.1)	403 (16.0)
Farm 2 *Mahogany*	Niagara	20	10	2358.0 (156.7)	1442.4 (241.0)	507 (17.4)	426 (15.3)
Farm 2 *Black*	Niagara	~	10	~		~	

Table 2: Mink primers used in genotyping analysis including range of allele size, number of alleles (A), fluorescent primer label, primer specific annealing temperature (T_A) concentration of primer used in each 10ul reaction, the % of individuals that were successfully genotyped per locus, observed (Ho) and expected heterozygosity (Hs), F_{IS} with bold indicating those loci that exhibited a significant deficiency of heterozygotes determined by the Markov chain method.

Primer	Range	Alleles	Label	T_A (^oC)	Primer [mM]	% typed	Ho	He	F_{IS}	Source
Mvi 2243	123-157	12	6-FAM	63.5	0.25	86	0.588	0.707	**0.128**	Vincent et al., 2003
Mvi 1016	218-236	10	6-FAM	63	0.25	83	0.730	0.782	**0.059**	Farid et al., 2004
Mvi 111	84-106	10	HEX	55	0.20	98	0.656	0.692	0.056	O'Connell et al., 1996
Mvi 1006	136-168	14	6-FAM	59.1	0.25	97	0.617	0.746	**0.168**	Farid et al., 2004
Mvi 099	324-356	15	6-FAM	60	0.15	99	0.767	0.771	0.008	Fleming et al., 1999
Mvi 1302	203-223	10	HEX	61	0.20	99	0.694	0.728	**0.058**	Vincent et al., 2003
Mvi 1321	88-116	12	6-FAM	63	0.15	95	0.679	0.739	**0.069**	Vincent et al., 2003
Mvi 4001	223-233	6	HEX	60	0.10	78	0.521	0.535	0.045	Anistoroaei et al., 2006
Mvi 1014	125-143	11	6-FAM	61	0.20	94	0.608	0.698	**0.145**	Farid et al., 2004
Mvi 114	62-82	9	HEX	61	0.30	75	0.685	0.727	**0.063**	O'Connell et al., 1996

Table 3: Summary of genetic results for each geographic grouping and colour phase including the number sampled from each population, the number of loci typed, the mean number of alleles (Na), Observed heterozygosity (Het) and F_{IS} for each as well as the unbiased estimates of Hardy-Weinberg equilibrium (exact P-values by the Markov chain method (HWE p) for deficiency of heterozygotes).

Population of origin	**N**	**loci typed**	**Na**	**Het**	**F_{IS}**	**HWE p**
Wild 1	30	10	7.0	0.662	0.057	**0.002**
Wild 2	20	10	6.0	0.706	0.003	0.589
Mixed 1	21	10	6.6	0.615	0.170	**<0.01**
Mixed 2	33	10	7.7	0.708	0.091	**<0.01**
Farm 1 – Black	29	10	5.8	0.651	0.045	0.101
Farm 1 – Standard Brown	28	10	6.2	0.703	0.025	0.514
Farm 1 – Pastel	15	10	5.4	0.621	0.110	**0.005**
Farm 2 – Iris	20	10	5.1	0.622	0.037	**0.013**
Farm 2 – Mahogany	20	10	5.9	0.604	0.175	**<0.01**

Table 4: Bayesian clustering analysis for 326 mink samples performed using Structure 2.2 (Pritchard *et al.*, 2000) including the membership coefficients (q) with the assigned cluster in bold as well as the given mean F_{ST} for each cluster that refers to the estimated drift from inferred common ancestor of all populations.

Population of origin	**Cluster I** Wild	**Cluster II** Farm - Brown	**Cluster III** Farm - Black	**Cluster IV** Farm - Iris
Wild 1	0.861	0.03	0.088	0.021
Wild 2	0.919	0.02	0.039	0.022
Mixed 1	0.613	0.147	0.204	0.037
Mixed 2	0.358	0.096	0.481	0.065
Farm 1 – Black	0.043	0.063	0.858	0.035
Farm 1 – Standard Brown	0.068	0.725	0.172	0.036
Farm 1 – Pastel	0.032	0.904	0.044	0.02
Farm 2 – Iris	0.012	0.031	0.029	0.929
Farm 2 – Mahogany	0.126	0.161	0.557	0.156
Mean F_{ST}	**0.060**	**0.131**	**0.111**	**0.240**

Table 5: Pair-wise F_{ST} for the 4 populations assigned using Structure 2.2 (Pritchard *et al.*, 2000) and the wild caught hybrids that assigned to more than one population cluster (the mink for the mahogany colour phase line were excluded as they assigned as Farm-Brown/Black hybrids), of which all were statistically significant ($p<0.05$) after 1000 permutations.

	Hybrid	**Farm - Brown**	**Farm - Black**	**Farm - Iris**
Wild	0.0341	0.0927	0.1134	0.1843
Hybrid		0.0631	0.0512	0.1426
Farm - Brown			0.0597	0.1431
Farm - Black				0.1596

Table 6: Pair-wise F_{ST} values for mink from all localities without population assignment.

	1	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**
1: Wild 1	~	0.045	0.060	0.069	0.126	0.093	0.139	0.209	0.110
2: Wild 2		~	0.045	0.052	0.131	0.115	0.167	0.202	0.116
3: Mixed 1			~	0.040	0.070	0.050	0.090	0.150	0.100
4: Mixed 2				~	0.050	0.060	0.090	0.140	0.050
5: Farm 1 Black					~	0.057	0.103	0.160	0.088
6: Farm 1 Demi Brown						~	0.052	0.138	0.095
7: Farm 1 Pastel							~	0.190	0.109
8: Farm 2 Iris								~	0.152
9: Farm 2 Mahogany									~

Table 7: Z scores for pair-wise comparison of adult male mink body mass (g) per population, including the number (n) of the groups used in the analysis, with significant scores marked in bold.

	Wild	**Hybrid**	**Farm - Brown**	**Farm - Black**
Wild n=52				
Hybrid n=6	1.987			
Farm - Brown n=47	8.390**	1.919		
Farm - Black n=53	8.131**	1.880	0.060	
Farm – Iris n=16	6.932**	2.350	1.013	1.044

* denotes a significance at $p<0.05$ ** denotes a significance at $p<0.01$

Table 8: Z scores for pair-wise comparison of adult female mink body mass (g) per population, including the number (n) of the groups used in the analysis, with significant scores marked in bold.

	Wild	**Hybrid**	**Farm - Brown**	**Farm - Black**
Wild n=29				
Hybrid n=5	2.731			
Farm - Brown n=30	4.125**	0.514		
Farm - Black n=25	7.159**	1.289	3.249*	
Farm – Iris n=13	6.036**	1.315	2.832*	0.178

* denotes a significance at $p<0.05$ ** denotes a significance at $p<0.01$

Table 9: Z scores for pair-wise comparison of adult male mink body length (mm) per population, including the number (n) of the groups used in the analysis, with significant scores marked in bold.

	Wild	**Hybrid**	**Farm - Brown**	**Farm - Black**
Wild n=52				
Hybrid n=2	0.382			
Farm - Brown n=47	8.194**	4.184**		
Farm - Black n=43	7.152**	3.761**	0.828	
Farm – Iris n=16	6.169**	4.028**	0.395	0.988

* denotes a significance at $p<0.05$ ** denotes a significance at $p<0.01$

Table 10: Z scores for pair-wise comparison of adult female mink body length (mm) per population, including the number (n) and mean body mass (x) of the groups used in the analysis, with significant scores marked in bold.

	Wild	**Hybrid**	**Farm - Brown**	**Farm - Black**
Wild n=29				
Hybrid n=1	1.242			
Farm - Brown n=30	5.211**	4.054**		
Farm - Black n=25	6.410**	4.799**	1.449	
Farm – Iris n=13	5.147**	4.408**	1.087	0.092

* denotes a significance at $p<0.05$ ** denotes a significance at $p<0.01$

Table 11: Proportion (%) of observed behaviours of mink from farmed and mixed populations observed in three behaviour trials and grouped based on their genetic clustering into wild, farmed or blended/hybrid genotypes for each of the three behaviour tests.

	Explorative	**Fearful**	**Aggressive**	**Unscorable**
Stick test				
Wild (n=2)	0.0	0.0	0.0	100.0
Hybrid (n=10)	0.0	10.0	30.0	40.0
Farm (n=39)	48.7	0.0	12.8	38.5
Mirror test				
Wild (n=2)	0.0	0.0	0.0	100.0
Hybrid (n=10)	30.0	0.0	0.0	60.0
Farm (n=39)	51.3	2.6	5.1	41.0
Approach test				
Wild (n=2)	0.0	50.0	0.0	50.0
Hybrid (n=10)	0.0	70.0	10.0	20.0
Farm (n=39)	41.0	15.4	5.1	38.5

Table 12: χ^2 comparison of genetic groups for the three behaviour tests with 3 degrees of freedom where the critical value for $p<0.05$ is 7.815, none of the group comparisons displayed significant differences in observed behaviours.

	Stick Test	**Mirror Test**	**Approach Test**
Wild - Hybrid	2.40	1.54	0.18
Wild - Farm	2.97	2.69	1.50
Farm - Hybrid	2.98	5.19	5.19

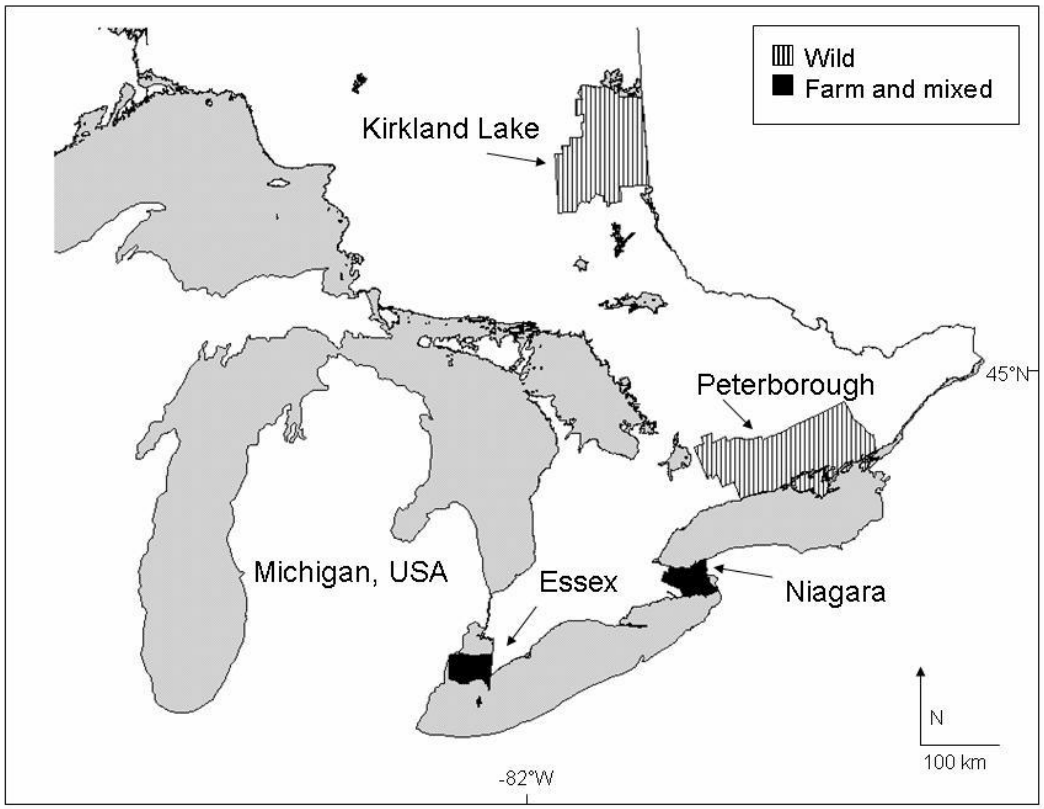


Figure 1: Locations in Ontario, Canada where mink were sampled including the two wild populations (Wild 1 mink from Kirkland Lake and Wild 2 from Peterborough), the overlapping farms and surrounding putatively mixed populations (Mixed 1 and Farm 1 in Essex County; Mixed 2 and Farm 2 in the Municipality of Niagara).

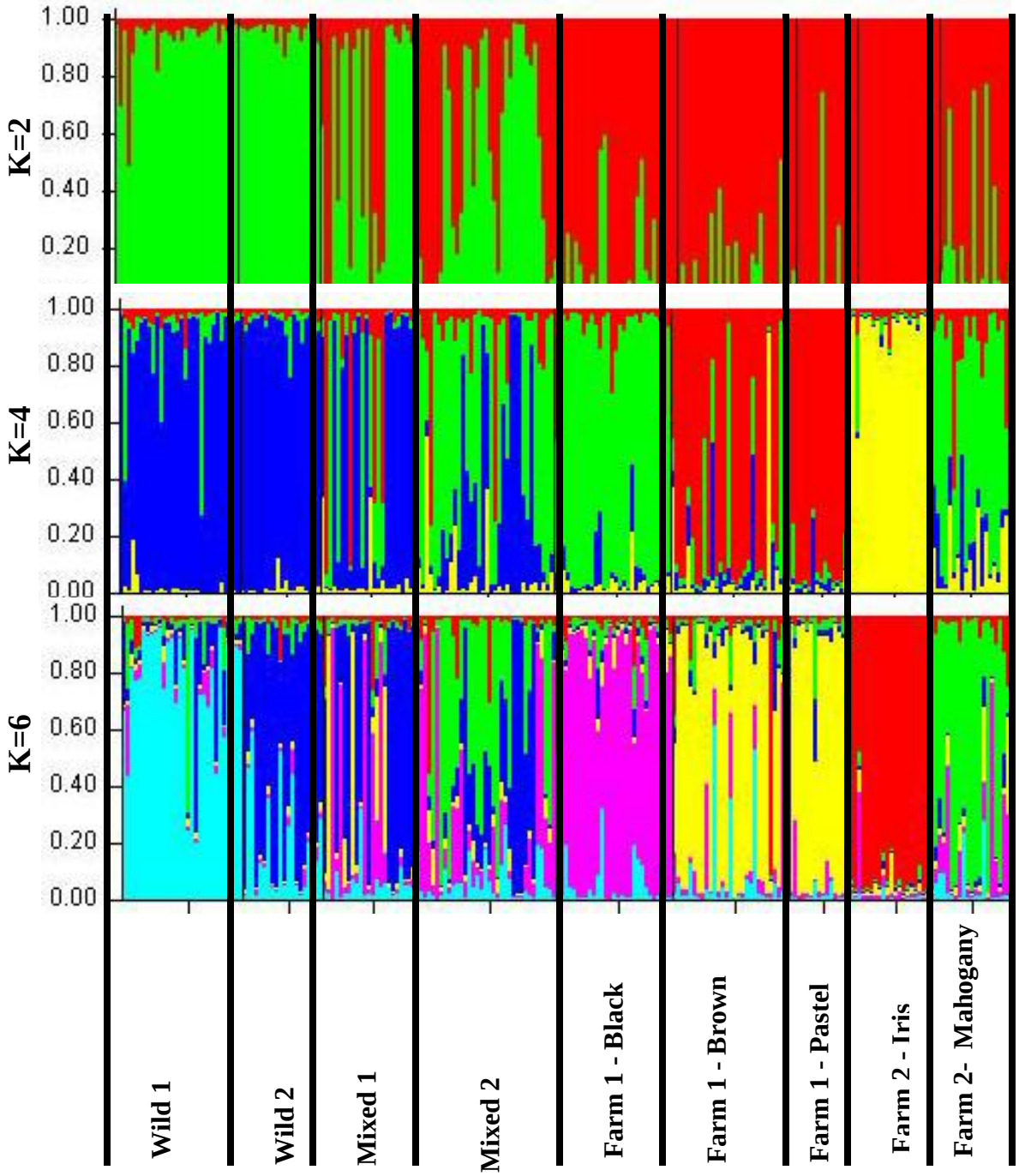


Figure 2: Genetic cluster comparison for admixture analysis performed using Structure 2.2 for K=2, K=4 and K=6 clusters grouped by geographic origin, with each vertical bar representing the membership coefficient (q) for each individual.

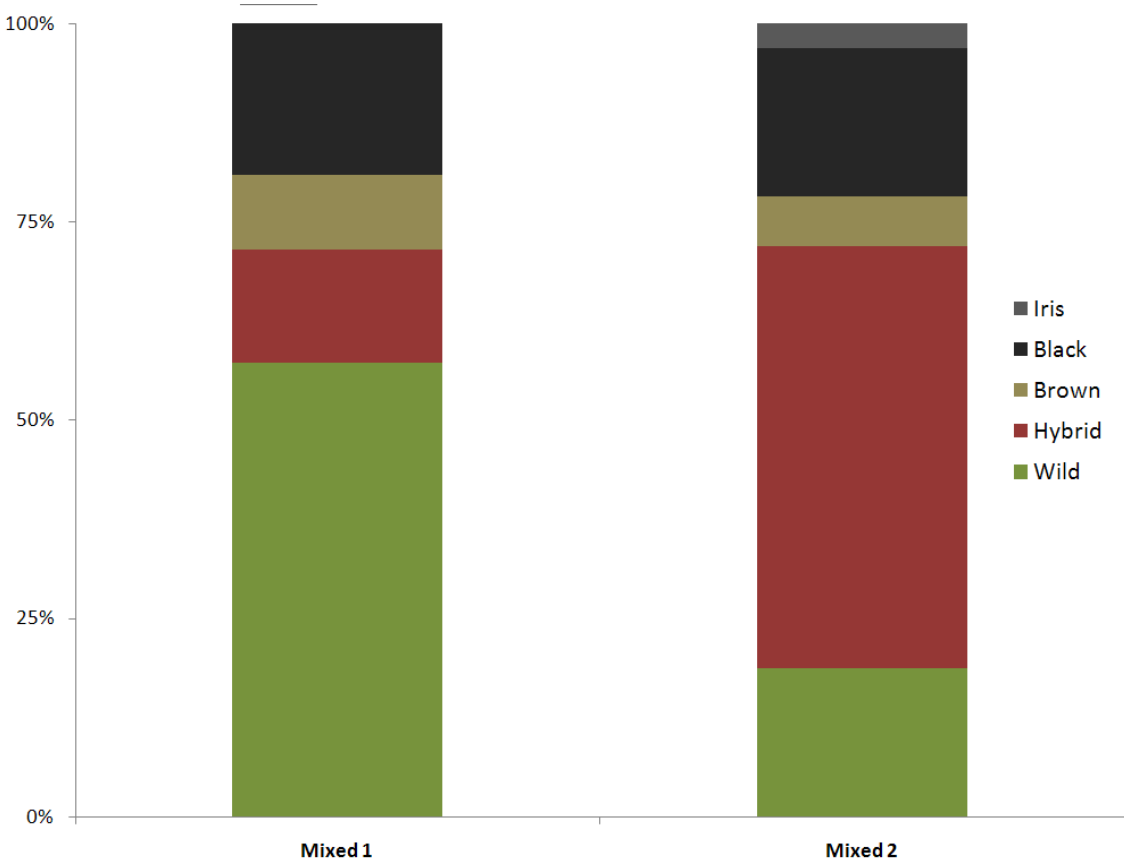


Figure 3: The genetic composition of the Mixed populations based on K=4 population assignment and describing the proportion of mink that were identified as wild, one of the three colour phase populations (Brown, Black and Iris) or as a blend between farm and wild genotypes (hybrids).

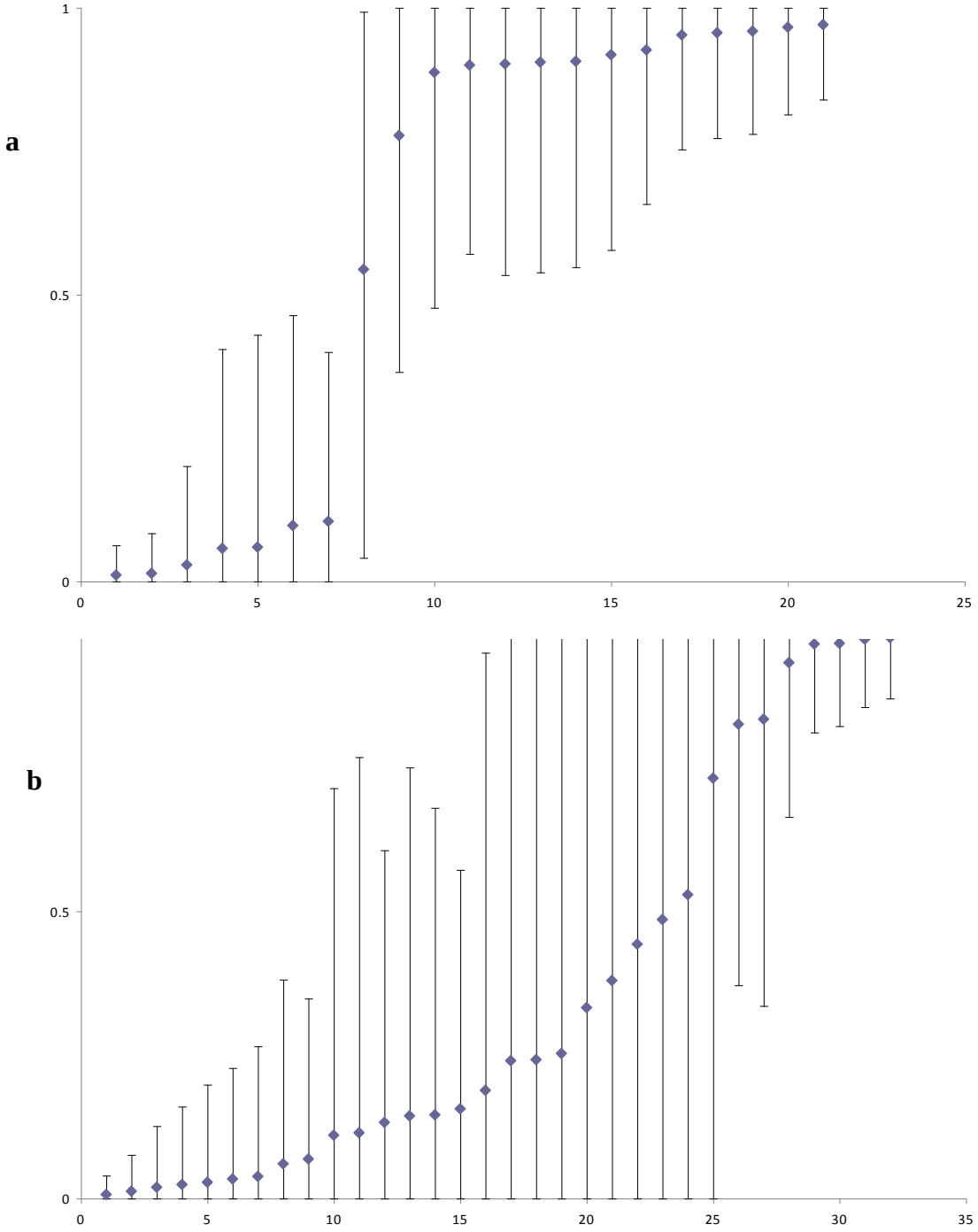


Figure 4: Membership coefficients (q) including their 90% probability limits to the wild cluster averaged from 5 replicate runs of K=4 in Structure 2.2 for all individuals from Mixed 1 (a) population and Mixed 2 (b) population displaying wild and farmed individuals as well as hybrids and introgressed individuals, where a q of $\geq$ 0.80 is considered a 'wild' mink and a contribution of <0.1 was needed to exclude wild mink contribution to genome.

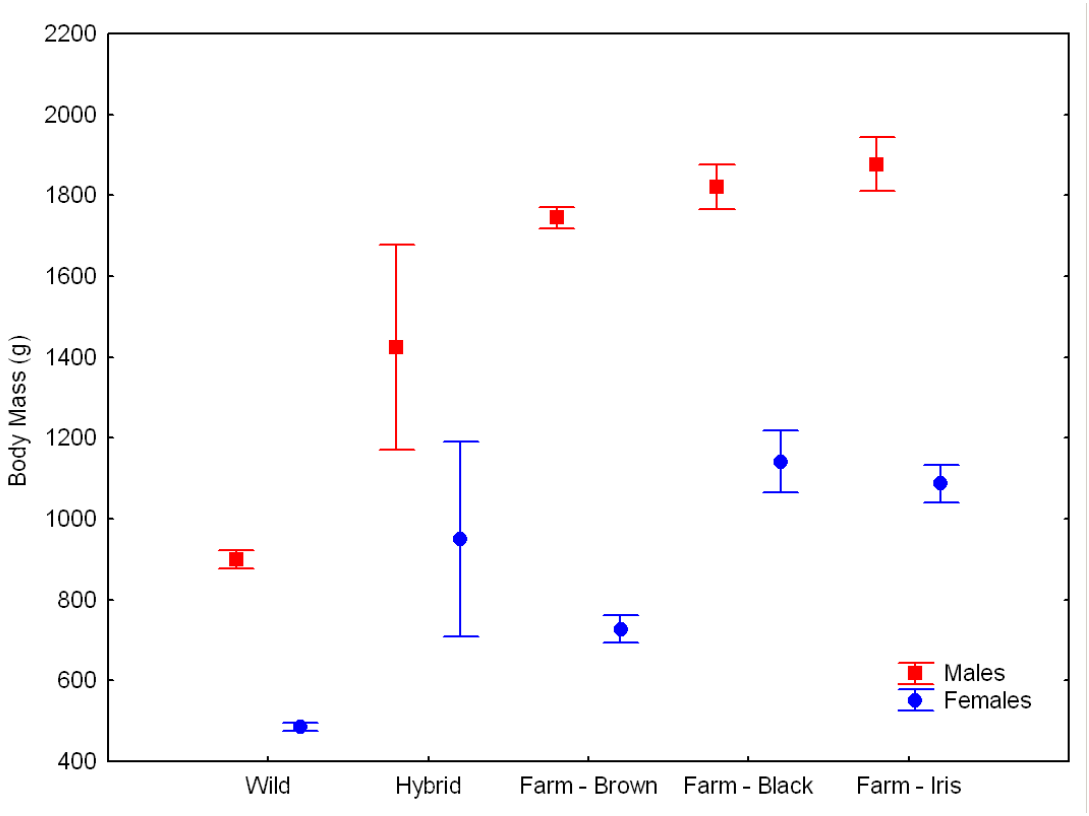


Figure 5: Mean body mass (g) and standard error bars for both males and females per population assigned using Bayesian clustering and including the free living hybrid mink.

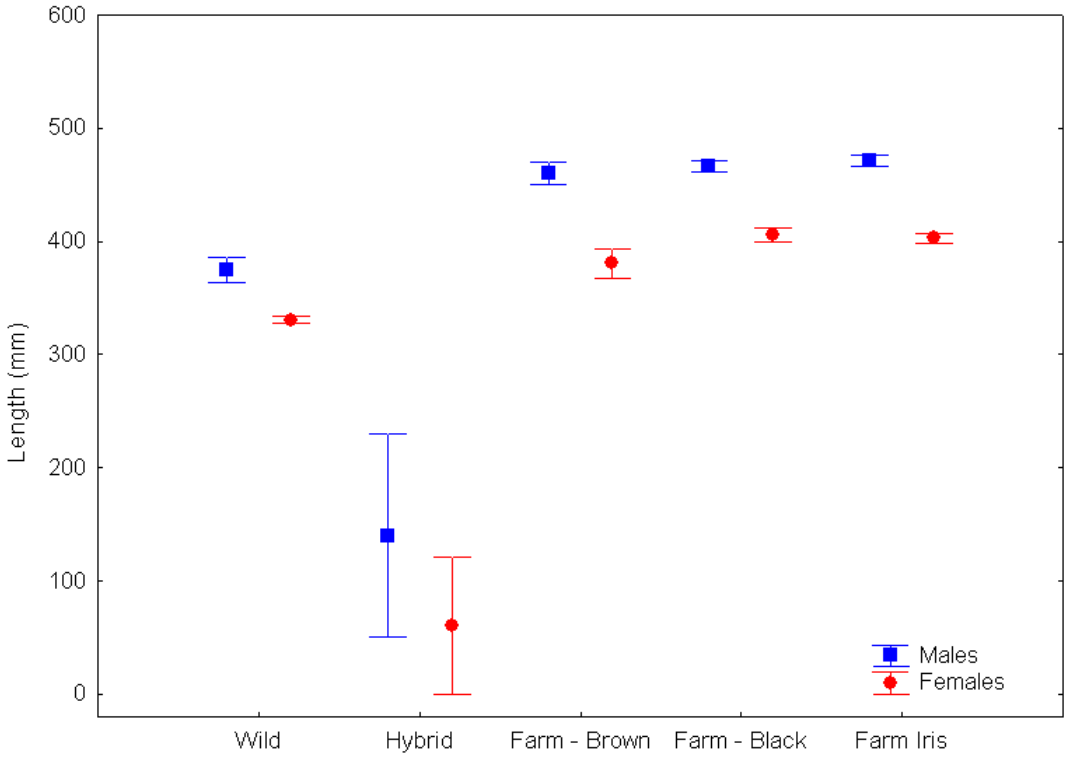


Figure 6: Mean body length (mm) and standard error bars for both males and females per population assigned using Bayesian clustering and including the free living hybrid mink.

Appendix 1

Mink Behavioural Trials Protocol

Set up an 'arena', the arena can be anything that blocks outside visual stimuli (I blocked off an area with the corner of a room and tarp. Animal should be placed in set up arena and left undisturbed for 10 minutes before starting trials.

There are four trials, three of which (stick, mirror and approach) should be completed in random order while the fourth is a measure of time taken to leave the release area (5m radius from trap). The three first tests are scored on a scale of one to four (listed on the form for easy reference) and observations are within a specific time limit. Both the mirror and stick test observations should be made as discretely as possible (quiet, as little movement as possible and as much out of site as possible).

Scoring

1 = explorative (curious, investigative, smelling, observing)
2 = fearful (evasive movement or crouching in corner, screaming)
3 = aggressive (biting, bearing teeth, … aggressive)
4 = undetermined (either unresponsive or combination of above responses)

Stick test

Time: 30 seconds

Insert long doweling into the trap from outside the arena (only needs to be sticking in btw 2-5inches). Start timer and observe behaviour.

Mirror test

Time: 30 seconds

Set up mirror abutting the back end of the trap and cover (with towel or what ever is available) prior to the 10 minute acclimation period. To start test quietly uncover the mirror, start timer and observe.

Approach test

Time: 30 seconds

Approach cage looking directly at the animal, at some point during the test (near mid way point) hold hand up to the cage just out of grasping distance (i.e. don't get your hand so close that the animal could bite you) for a few seconds.

Release test

Return to point of capture, if there is an open trap at the site then release the animal 10-20 meters away to discourage immediate recapture.

Mark out a 5 meter radius from release site with flagging tape. Open the trap. Time departure from the point at which the animal places first paw out of cage and end time when animal crosses the flagged perimeter.

Included are processing forms which have a quick reference table of the scoring system.

Thank you very much!

Anne Kidd

Behavioural Trials Record Sheet

Date _____

Trapper _____

Trap # _____

Individual # _____

Tag # _____

_____

Scoring

1 = explorative

2 = fearful

3 = aggressive

4 = undetermined

Response to Approach: _____

_____

_____

Stick Test (30 sec)

Order: _____

Score: _____

Comments: _____

_____

_____

Mirror Test (30 sec)

Order: _____

Score:_____

Comments: _____

_____

_____

Release Test

Time: _____

Comments: _____

_____

Appendix 2

Extraction Protocol:

Lyse tissue in 500µl 1X Lysis Buffer (4M Urea, 0.2M NaCl, 0.5% n-lauroyl sacrosine, 10mM 1,2-cyclohexanediaminetetraacetic acid CDTA).
Spike with Proteinase K (Qiagen) (25µl for tissue and 10µl for hair)
Vortex
Incubate in a water bath for 2 hours at 65^{o}C
Second spike (as above)
Vortex
Incubate for no less than 12 hours at 37 ^{o}C

Add 200µl AL Buffer and 200µl lysed sample to 1.5 ml centrifuge tubes and incubate in water bath for 10 minutes at 70 ^{o}C.
Add 200µl 95% EtOH to tubes and transfer all to silica columns (Qiagen).
Centrifuge for 1 minute at 8000 rmp.
Change collection tubes.
Add 200µl AW1 to colµmn.
Centrifuge for 1 minute at 8000 rmp.
Add 200µl AW2 to column.
Centrifuge for 1 minute at 8000 rmp.
Centrifuge for 3 minute at 14 000 rmp.
Change collection tubes.
Add 100µl 1X TRIS/EDTA Buffer (BioShop) that has been heated to 70 ^{o}C.
Centrifuge for 1 minute at 8000 rmp.
Transfer collected DNA in TE buffer (stock) from collection tube to labelled 0.5ml centrifuge tubes.
Heat tubes in water bath or on PCR plate with lids open to burn off any remaining EtOH in sample.

Appendix 3

Gel Electrophoresis

Add 0.8g of Agarose (BioShop) to 80ml TBE buffer (0.089M Tris Base, 0.089M Borate, 0.002M EDTA, EMD Chemicals Inc.) into Erlenmyer 500ml flask
Swirl in flask
Heat in microwave for 30 seconds.
Swirl contents.
Heat in microwave again for ~30 seconds or until solution begins to boil. Remove wearing oven mitts.
Place on stir plate and stir for ~10 minutes or until flask is cooled enough to touch with bare hands.
Pour into gel rig (OWL), remove any bubbles with disposable tip and place in well combs.
Let sit till cooled (gel will appear cloudy).
Lift and turn gel plate and replace in the gel rig.
Pour TBE buffer into gel rig until it reaches the 'fill line' (~720 ml
Remove well combs.
Add 5µl Low mass ladder (exACTGene) to the first well of each row.
Add 15µl including 10µl sample with 5µl loading dye into remaining wells (one sample per well!).
Attach gel rig lid with electrodes to power supply with the current running top to bottom from negative to positive (wells at top). Run for 45-50 minutes at 100 volts.
Remove gel and place in water bath with 5ul Ethidium Bromide.
Gently rinse off with a bit of dH_2O.
Take photographic image using a Multi Image Light Cabinet and scanned using FLURO CHEM version 2.0 (Alpha Innotech Corporation).

Appendix 4

ETOH Clean-up for Sequencing and/or Genotyping:

Add 1.0µl Sodium Acetate/EDTA to 10µl product and spin/tap to make sure the Sodium Acetate/EDTA is in the product.
Add 40µl of 95% EtOH.
Centrifuge at top speed:

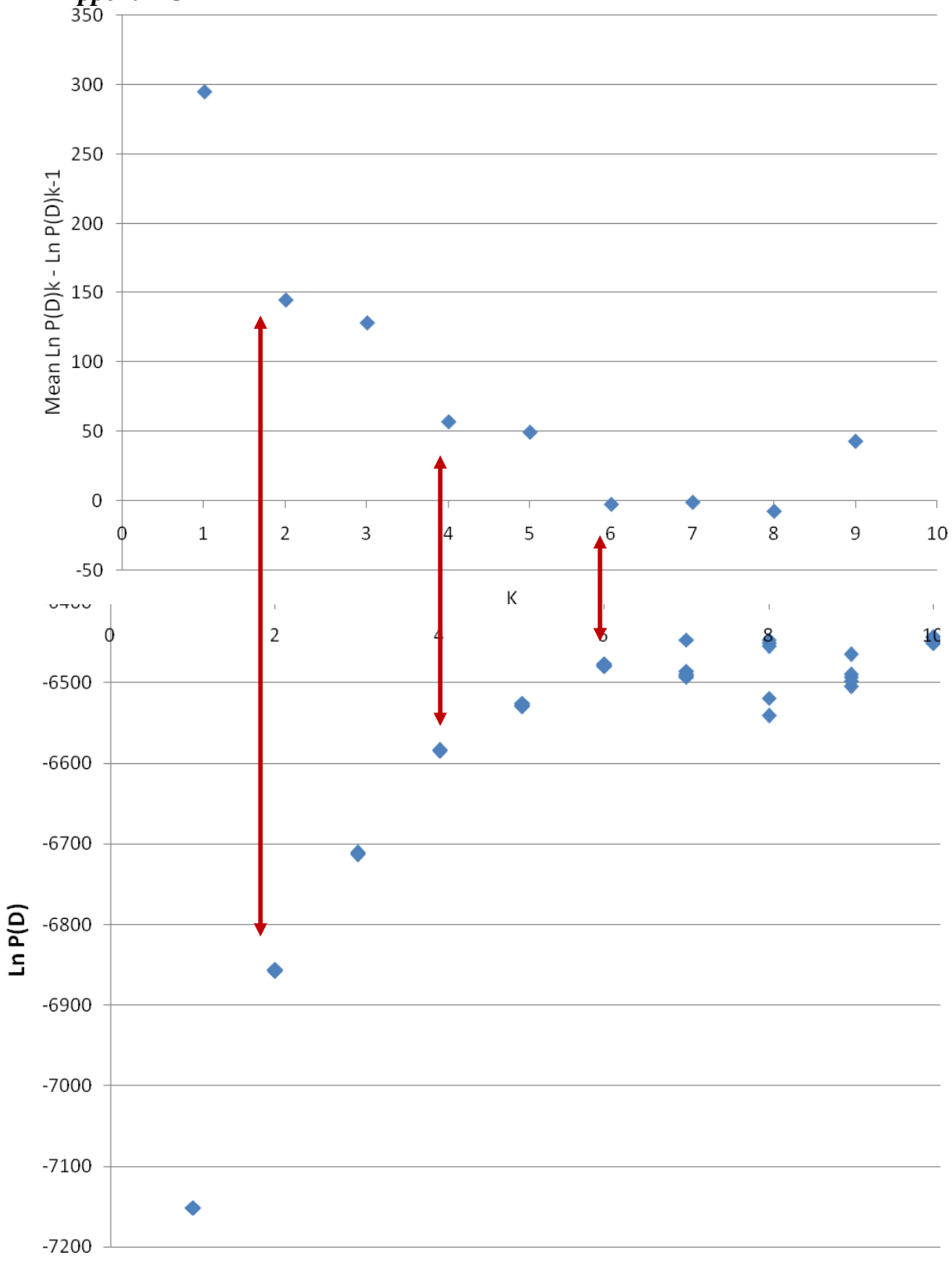
Plates: centrifuge for 45 minutes at 6200 rpm and set temperature to 0^{o}C.
Tubes: centrifuge for 15 minutes at 15 000 rpm.

Pour/flick off supernatant.
Add 150µl of 70% EtOH.
Centrifuge at top speed:

Plates: centrifuge for 20 minutes at 6200 rpm and set temperature to 0^{o}C.
Tubes: centrifuge for 10 minutes at 15 000 rpm.

Pour/flick off supernatant.
Air dry for 30 minutes OR dry in 80^{o}C block for 1 to 2 minutes.
Re-suspend in 10µl ddH2O

Appendix 5



Posterior probability of the data, Ln P(D) of 5 runs with a 500 000 burn in and 500 000 itterations against the range of K 1-10 considered (below), and the mean increase in Ln P(D) between K 1 through 10 (above), both indicating three steps showing a marked decrease in the variation between successive K.