Microsatellite DNA Analysis of Genetic Variation and Population Structure of American Mink from PCB-Contaminated and Non-Contaminated Locales in Eastern North America

Final Report to the Hudson River Foundation
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Final Report for Project Entitled: *An Assessment of Genetic Variation in PCB-Contaminated Mink from the Hudson*
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I have attached three documents that constitute our final report for our American mink project. They are:

1. An overview of the final report
2. A draft of a manuscript entitled: Microsatellite DNA Analysis of Genetic Variation and Population Structure of American Mink from PCB-Contaminated and Non-Contaminated Locales in Eastern North America
3. A PDF of our published paper on CYP1A characterization and expression in mink treated with dietary PCBs entitled: Development and Use of Real-Time Reverse Transcription-Polymerase Chain Reaction Assay to Quantify Cytochrome P4501A1 Expression in American Mink

OVERVIEW

Below I have copied the overall hypothesis and individual work objectives verbatim from our original proposal and have new text addressing our success in achieving these objectives. .

Overall Hypothesis: Genetic diversity and genetic population structure have been altered in PCB-contaminated mink from the Hudson River corridor compared to mink from elsewhere in New York State.

Work objectives:

1. **Levels of total PCBs and congeners have been or will be measured in the livers of field-trapped mink from populations immediately along the Hudson River (within 1 km), other sites in the Hudson River ecozone, and elsewhere in New York State. Subsamples of these tissues will be processed for genetic analysis.**

   Levels of total PCBs and TCDD TEQs were determined by the NYSDEC for livers of mink collected along and more distant from the main stem Hudson River. DNA was isolated from muscles of field trapped mink from around New York State with an emphasis on animals from the Hudson River watershed but also including mink from New Brunswick, five watersheds in Maine, two sites in Rhode Island, and four locales in Ontario.

2. **Allelic frequencies at 8-10 informative microsatellite DNA loci will be determined in > 600 mink samples archived by the NYDEC over the past several years. Levels of genetic diversity and genotype frequencies will be compared between mink populations with high, medium, and low tissue burdens of total PCBs. Genetic diversity will be related to actual Aroclor or congener-specific PCB burdens in these populations.**

Microsatellite genotypes were determined at 9 microsatellite loci in more than 800 mink samples described above from New York State and elsewhere in eastern North American and levels and patterns of allelic diversity compared to tissue burdens of PCBs among individual collections with high and lower levels of PCBs. For the first time, we also determined the population structure of mink from this area in the largest population genetics study of American mink to date

3. **If possible, bioavailability and early bioactivity of PCBs to a subset of the above samples will be confirmed by measuring levels of endothelial CYP1A expression in skin and heart using immocytochemistry.**

Because all of our Hudson River samples were from muscle and because of their less than optimal preservation, we choose not to measure their CYP1A protein expression. But, instead for the first time we partially characterized CYP1A cDNA in American mink, used this sequence information to develop a real-time RT-PCR assay to quantify CYP1A mRNA expression in mink tissues, and then successfully applied the assay to livers of mink kits or juveniles experimentally exposed *in utero* or by diet to graded doses of PCB contaminated carp from Saginaw Bay, Lake Michigan. Results from this study were published in *Arch. Environ. Contam. Toxicol*. (57:608-615, 2009) and showed that CYP1A mRNA expression was dose responsively induced and sensitive to exposure to environmentally relevant levels of PCBs (and perhaps other non-measured contaminants) and that higher level toxicities to mink probably resulted from activation of the aryl hydrocarbon receptor pathway. Induced CYP1A mRNA expression our study was correlated with the development of lesions in the mandible and maxilla of the same treated animals, alterations that are probably incompatible with existence in natural environments. Thus, our results suggest that elevated levels of CYP1A expression are probably predictive of higher level toxic effects in mink.

In this report, we describe in detail our DNA microsatellite study because it has yet to be published and attach our paper on mink CYP1A expression that was published in *Archives of Environmental Contamination and Toxicology*.

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Abstract

American mink *Neovison vison* is common in aquatic ecosystems throughout North America and local populations may be potentially sensitive to toxicities of persistent contaminants such as PCBs because of their aquatic-based diet, position near the top of the food web, and small sizes of their localized demes. Furthermore, ranched mink have been shown sensitive to reproductive toxicities of PCB contaminated fish diets from polluted sites. The upper Hudson River, New York, is highly contaminated with PCBs and previous studies have shown elevated hepatic burdens of total and coplanar PCBs in mink collected in close proximity to the river compared to those from more distant locales. However, few studies have evaluated potential biological effects of PCBs on contaminated mink from the Hudson River. We hypothesized that bioaccumulation of PCBs in Hudson River mink had reduced their levels of genetic diversity or altered their genetic population structure. To address this possibility, we conduced microsatellite DNA analysis at nine previously isolated loci on mink collected in close proximity to the Hudson River and from more distant locales in the Hudson River watershed, elsewhere in New York State, and at other sites in eastern North America including New Brunswick, four locales in Ontario, multiple watersheds in Maine, and two ecozones in Rhode Island. We did not find reduced genetic diversity at the individual or population levels in mink collected in close proximity (< 5 km) to PCB hotspots in the Hudson River nor evidence of altered population structure by PCB exposure. Consistent with their distribution in small localized and isolated demes, we found significant genetic population structure among many mink collections in New York State and elsewhere. Depending on the analytical approach used, genetically distinct populations numbered between 15 when using STRUCTURE to 20 when using Fisher's exact test. Genetically distinct population units were found among major ecozones and minor ecozones in New York State, among different collections on the Hudson River, among spatially separate locales in Ontario, and among most watersheds in Maine. However, despite this localization and potential heightened impact of stressors, genetic diversity and genetic population structure in mink does not seem to be affected by exposure to and their bioaccumulation of high levels of PCBs of Hudson River origin.

Introduction

The Hudson River Estuary has a long legacy of contamination with PCBs and other organic and metal pollutants (Wirgin et al. 2006). Almost 200 miles of the main stem Hudson River was designated by the EPA in 1984 as a U.S. federal Superfund Site, the largest in the nation, because of human and ecological risks from exposure to PCBs. A record of decision by the EPA to remediate the most contaminated upriver sites was issued in 2002, Phase I dredging was conducted in 2009, and Phase II is to begin in spring 2011.

PCBs contamination of the Hudson River resulted from the release from 1947 to 1977 of 0.2 to 1.3 million pounds of PCBs at two General Electric (GE) facilities that manufactured electrical capacitors at Hudson Falls (River mile 197 (RM197) and Ft. Edwards, New York (RM195) (Limburg et al. 1986). Downriver flow and the removal in 1973 of a low dam at Ft. Edwards resulted in a gradient in sediment levels of PCBs from these upriver locales to near New York City where PCB levels spike again because of local wastewater treatment plant and combined sewer overflow sources (Farley and Thomann 1998). Atmospheric deposition may also contribute to sediment accumulations of some PCBs to some regions of the Hudson River (Totten et al. 2004). Tissue burdens of PCBs in resident Hudson River fishes such as brown bullhead, goldfish, pumpkinseed sunfish, and largemouth bass follow a similar, although less dramatic, geographic pattern with levels generally diminishing from upriver to downriver locales (Armstrong and Sloan 1988; Tams/Gradient 1995). Tissue burdens of PCBs in resident and anadromous species have shown a general decline since the cessation of the release of PCBs (Armstrong and Sloan 1988), however, levels in individuals from resident and anadromous species still often exceed FDA guidelines for human consumption and EPA thresholds for ecotoxicological effects.

PCBs are composed of 209 different congeners that differ in the number (1-10) and positions of chlorine (Cl) substitutions on their biphenyl rings. PCBs are highly persistent in the environment because of their lipophilicity and resistance to microbial and metabolic degradation. Depending on the number of their Cl substitutions, the half-

lives of PCBs in biota may range from days to years. It has been estimated that the half lives of Aroclors in human tissues range from 2-6 years (Shirai and Kissel 1996) and perhaps less in fishes (Gooch and Hamdy 1982). PCBs in the U.S. were marketed as Aroclors and the chlorine composition of Aroclor mixtures differed such that A1260 has a higher Cl content than A1242. Because of their lipophilicity and resistance to metabolism, PCBs are highly persistent in impacted ecosystems and their concentrations biomagnify with the highest concentrations seen in taxa at the apex of the food chain.

Toxicities of PCBs are congener-specific. Congeners that are coplanar and that best bind the aryl hydrocarbon receptor (AHR) often exhibit the greatest toxicities. Thus, congener-specific concentrations of PCBs in environmental samples better predict their toxicities than total PCB levels. Those PCB congeners that most resemble 2,3,7,8 tetrachlorodibenzo-*p*-dioxin (TCDD) best bind the AHR, have the greatest toxicities and are assigned the highest toxic equivalency factors (TEFs). Toxicities of coplanar PCBs in environmental samples are often expressed as TCDD toxic equivalency quotients (TEQs) which reflects the TEFs and concentrations of the individual congeners in environmental samples. Toxic effects of PCBs in vertebrate animals include wasting syndrome, tumor promotion, reproductive impairment, teratogenicity, endocrine disruption, and immune system dysfunction.

American mink *Neovison vison* is a common and abundant mammalian species that is broadly distributed in or near aquatic ecosystems throughout much of North America and has been ranched in Europe and South America where escapees have established natural populations (Lecis et al. 2008) and detrimentally interacted with other related and non-related mammalian taxa (Bowman et al. 2007). There is also concern about interactions, including genetic introgression between ranched and natural populations of mink in North America primarily around the Great Lakes (Kidd et al. 2009). Their distribution is in narrow bands along the shores of lakes, ponds, rivers, ditches, and marshes. Mink is carnivorous and its aquatic-based diet is composed of crustaceans, fishes, reptiles, amphibians, waterfowl, and small mammals (Wise et al. 1981). As a result of this diet and its position near the apex of the food web, and because of the environmental

persistence of PCBs, mink can bioaccumulate high levels of PCBs in contaminated ecosystems such as the Hudson River.

Mink live in dens and animals from several adjacent dens comprise single demes. Females are usually restricted to their natal demes for the entire year while males tend to exhibit greater movement during mating season when they may foray among neighboring dens and even beyond their resident demes to seek additional female breeders (Niemimaa 1995). The linear home range of mink has been estimated to be between 1 and 5 km, with population densities between 0.1 and 0.7 animals/square kilometer (Lariviere 1999). Thus, because of their limited movement, mink from individual demes are probably exposed to localized concentrations of PCBs. If population processes are affected by exposure, these effects might be restricted to individual demes because of their reproductive isolation from adjoining population units. Also, the generation time (1 year) and maximum life expectancy (3-4 years) of mink in the wild is brief, therefore accentuating the possibility of rapid evolutionary change from anthropogenic impacts.

Domesticated mink are highly sensitive to PCBs toxicity. Studies in the 1970s demonstrated that ranched mink that were fed fish collected from the Great Lakes suffered reproductive and developmental abnormalities (Aurelich and Ringer 1977; Aurelich et al. 1971). This was manifest as reduced litter size, reduced kit survivorship, and reduced whelping success in exposed animals. Further studies demonstrated that these toxicities were related to PCB contamination and that toxicities were PCB congener-specific (Aurelich et al. 1985) and AHR mediated. It was estimated in controlled laboratory studies that the LC50 for mink chronically exposed for 28 d to TCDD was 4.2 μg TCDD/kg b.w. (Hochstein et al. 1988). Similarly, the lowest observable adverse effect level (LOAEL) in mink exposed in diet and expressed as TCDD TEQ was reported as 189 ng TEQ/kg liver weight (Blankenship et al. 2008). Furthermore, an LOAEL of dietary exposure of mink to PCB 126 contaminated fish diet (the most toxic coplanar congener) on reproduction, kit survivability and growth was 2.4 μg PCB 126/kg feed (Beckett et al. 2008). In summary, it might be expected that mink from the Hudson River would bioaccumulate high levels of PCBs, that mink would be

sensitive to PCBs-induced toxicities, and that these effects would be felt at the population level at impacted sites.

It has been speculated that PCB levels in wild mink collected at contaminated sites across North America, approach and sometimes, exceed those concentrations shown to elicit toxicities under controlled laboratory conditions (Basu et al. 2007). Empirical studies have demonstrated that the kits of ranched mink fed *in utero* and during lactation a diet of PCB contaminated goldfish and carp from the Housatonic River, MA, suffered reduced survivability between three and six weeks of age (LC10 and LC20 were 0.231 and 0.984 μg total PCB/g feed, respectively) (Bursian et al. 2006a). Similarly, ranched mink fed a diet supplemented with PCB contaminated carp (1.1 mg total PCBs/kg feed) from the Saginaw River, MI, experienced maxillary and mandibular squamous epithelial proliferation, a condition that could lead to tooth loss and unknown effects on survival in the wild (Bursian et al. 2006b). However, studies evaluating the toxic effects of PCB exposure on natural populations are still lacking.

Levels of genetic diversity and allelic frequencies in natural populations may be sensitive of their exposure to xenobiotic chemicals. Natural selection may be operative to alter allelic frequencies at selected or linked loci. Reduced genetic variation may be manifest at the individual (level of heterozygosity across loci) or population (number and frequencies of alleles across loci) levels. Genetic diversity is thought to be important to the vitality of individuals and populations as a whole. At the individual level, reduced heterozygosity has been correlated with decreased growth rate and vulnerability to environmental stressors. At the population level, genetic diversity provides the plasticity to exist in heterogeneous or rapidly changing environments and has been correlated with population growth rates (Theodorakis and Wirgin 2002). Suboptimal levels of allelic diversity may be associated with inbreeding depression, particularly in small populations such as in mink, and may impair the fitness of a population by increasing the frequencies of deleterious or lethal genotypes (Frankham et al. 2002). For example, Frankel and Soule (1981) have suggested that a 10% decrease in genetic variation may result in a 10-25% reduction in a population's reproductive output and thereby threaten its ability to

persist. Conversely, mutagenic xenobiotics such as metabolites of PAHs and radiation may increase levels of genetic variation in exposed populations. However few if any studies have demonstrated this effect from PCB exposure (Stapleton 2001). Finally, unlike other damage, genetic alterations cannot be rapidly repaired and are persistent in populations for generations.

Reduced genetic diversity may occur if the abundance of a population is severely cropped and rarer alleles lost. Effects of population "bottlenecks" in the distant and more recent past serve to reduce contemporary genetic variation. This scenario may be particularly relevant for chemically-impacted mink populations in which the reproductive unit is composed of a small number of individuals with limited exchange of alleles with neighboring demes under undisturbed conditions. Also, reduced abundance of a population may result in a "sink" in which immigration of individuals from elsewhere (a source) may result in altered allelic frequencies, increased heterozygosity and Hardy Weinberg disequilibrium. Alternatively, extreme exposure to xenobiotics may result in strong natural selection for tolerant phenotypes and increased frequencies of alleles that are advantageous and the loss of those that are not (Wirgin et al. 2011). Mink may be particularly sensitive to these effects because of the small number of individuals within individual demes and the unlikelihood of gene flow among demes. Thus, contaminants that impair the reproductive potential of populations may influence its level of genetic diversity and allelic frequency patterns.

Microsatellites are one form of genetic variation that has proven informative in population genetics studies. Microsatellites are tandemly repeated short DNA motifs (usually in nuclear DNA), commonly two-to-four bases in length that are hypervariable within vertebrate populations. Polymorphism at microsatellite loci results from variable copy numbers of the repeat unit. Microsatellite loci are scattered throughout the vertebrate genome and are thought usually to be selectively neutral. In microsatellite studies, a panel of non-linked loci (frequently 4-12) are screened and allelic frequencies at each locus and across all loci are determined. Because they are selectively neutral, patterns of genetic variation at microsatellite loci reflect stochastic historical processes

(mutation rate, genetic drift, population size, population age, migration) that have impacted the population and altered its genetic population structure. As a result, microsatellites provide a sensitive tool to evaluate and compare genetic diversity among natural populations.

Microsatellites have been identified in American mink by several groups of investigators and these provide a battery of loci available for population genetic analysis (Belliveau et al. 1999; Fleming et al. 1999; Anistoroaei et al. 2007). These markers were primarily developed to establish a genetic map for identification of monogenic and quantitative trait loci that could be used to improve ranching practices. However, a subset of these loci have been used to evaluate the extent of hybridization between escaped ranched and native American mink in Ontario, Canada (Kidd et al. 2009) and to determine the genetic population structure of American mink in Spain (Lecis et al. 2008) and Poland (Zalewski et al. 2010). To date, only one study has investigated the natural population structure of American mink within their native North American distribution (Stevens at al. 2005) and that focused on a limited number of specimens from Arkansas and Tennessee.

In this study, we used multi-locus microsatellite analysis to evaluate the genetic population structure and genetic diversity in American mink from various collection locales in the northeastern U.S. and eastern Canada, with a focus on animals collected from major and minor ecozones from throughout New York State. We were particularly interested in comparing patterns of genetic diversity in mink collected proximate to the PCB-contaminated upper Hudson River to those in mink collected more distant from the Hudson River in New York State and upriver of Hudson River sites with high levels of PCBs contamination. Additionally, for the first time we provide comparisons of genetic divergence between native populations of mink from within tributaries, between tributaries and among ecozones in eastern North America.

Methods

Unlike samples typically taken from discrete locations for genetic studies, collections for this investigation were fortuitously taken by trappers across broad landscapes. Mink

were collected from the locales listed in Table 1. In New York State these collections were coordinated by the New York State Department of Environmental Conservation (NYSDEC). Mink were also obtained from New Brunswick, Canada; multiple watersheds in Maine; two ecozones in Rhode Island; and four sites in Ontario including western Lake Erie, St. Clair River, Lake St. Clair and the Saint Lawrence River. Mink from New York were collected by professional trappers and maintained by them at -20^{o} C until carcasses were retrieved by NYSDEC personnel. Muscle plugs were taken from all specimens and retained at -80^{o} C in the NYSDEC facility at the Hale Creek Field Station, Gloversville, NY. Muscle plugs were subdivided by NYSDEC personnel and shipped via overnight courier to the NYU School of Medicine where they were maintained at -80^{o} C until further subdivided for microsatellite DNA analysis.

DNA was extracted from each muscle plug using standard phenol/chloroform extractions following tissue incubation in CTAB buffer (Saghai-Maroof, 1984) and digestion with Proteinase K at 65^{o} C for at least 2 hr. Total DNA was precipitated in isopropanol, washed in 70% EtOH, air dried, and resuspended in $_{dd}H_20$. Purity and concentration of each DNA sample was determined with a Nanodrop ND-1000 spectrophotometer (NanoDrop Technologies, Inc., Wilmington, DE). DNA concentrations were adjusted to 50 ng/μl and dilutions were subsequently stored at -20^{o} C in the wells of 96 well plates.

Analyses were conducted on nine previously identified mink microsatellite loci (Table 2). These were mink 2243, 480845, AF474, 1341D3, 1354, 1302, AY24, MV1381, MV1099. PCR reactions were in 12.5 μl total volumes that contained 87.5 ng of template DNA, 0.375 to 0.7 μl of each primer (1 μM) (Invitrogen Life Technologies, Carlsbad, CA) (Table 2), 1.25 μl of 10 x reaction KlenTaq 1 PC2 reaction buffer (50 mM Tris-HCl pH 9.1, 16 mM ammonium sulfate, 3.5 mM $MgCl_2$, and 150 mg/ml BSA), 0.1 μl dNTPs (250 mM stocks) (Pharmacia Biotech, Piscataway, NJ), 0.025 μl of KlenTaq 1 enzyme (0.75 units), (AB Peptides, Inc., St. Louis, MO) and $_{dd}H_20$ to volume.

Amplification parameters were an initial denaturation at 95^{o} C followed by 65 cycles of denaturation at 95^{o} C for 30 sec, annealing at temperatures in Table 2 for 30 sec,

extension at 72^{o} C for 60 sec, followed by a final extension at 72^{o} C for 7 min. One primer at each locus was fluorescently labeled with one of three dyes (D2-D4/PA) (Proligo, Boulder, CO) and the second primer was unlabeled. PCR amplifications were done in 96 well plates in MJ PTC-100^{TM} cyclers (Waltham, MA).

Characterization of microsatellite genotypes was done on a Beckman Coulter (Fullerton, CA) CEQ8000^{TM} capillary-based DNA sequencer. Multi-pooled PCR amplicons from 3-4 loci were diluted 1:3 with Sample Loading Solution (Beckman Coulter) and 0.5 to 2 μl of diluted PCR reactions were loaded into 96 well plates with 0.5 μl of CEQ DNA Size Standard-400 (Beckman Coulter) and run with the FRAG 1 program.

Statistical analysis

Multi-locus microsatellite genotypes were compiled for all individuals, and allele and genotype frequencies were determined for each collection using Genepop version 4.0.10 software (Raymond and Rousset, 1995; Rousset 2008). Micro-checker version 2.2 (Van Oosterhout et al. 2004) was used to test for scoring errors due to stuttering, large allele dropout and null alleles within each of the individual collections. Deviations from Hardy-Weinberg expectations were tested for individual collections and across all samples at individual loci and across all loci using Hardy-Weinberg exact tests (Guo and Thompson 1992); the null hypothesis tested was the random union of gametes. *P*-values were generated using Markov chain default parameters with Genepop. Linkage equilibrium for each pair of loci in each population was evaluated in Genepop. Genetic variation was assessed as the number of alleles and observed heterozygosities per locus in Genepop and as allelic richness using FSTAT version 2.9.3 (Goudet et al. 1995; Goudet 2001).

We used the program Bottleneck (vers. 1.2.02) (Cornuet and Luikhart 1996) to determine if chronic exposure to historically high levels of PCBs resulted in a genetic bottleneck in the effective population size of mink collected in closest proximity (< 5 km) and downstream of sources of Hudson River PCBs. We analyzed for bottlenecks in mink collected more distal (> 5 km) from the main stem Hudson and upstream of the PCBs sources as reference controls. As recommended by the authors, we used the Two-phased

mutation model (TPM) with the Wilcoxon sign-rank test (recommended for < 20 loci and any number of specimens. To test for very recent bottlenecks (within the past 20 generations), we also used the more qualitative graphical mode-shift indicator method to distinguish between bottlenecked and more stable populations. This approach requires only characterization of 5 to 20 polymorphic loci and approximately 30 individuals (Luikart et al. 1998).

Tests of allelic differentiation among collections were conducted using Fisher's exact probability test as implemented in Genepop with default Markov chain parameters to test the null hypothesis of a homogenous distribution of alleles across samples. Population comparisons were initially made between all pairs of samples and when no differences were observed within sampling locations these samples were pooled for subsequent analysis of spatial heterogeneity. All tests were performed for single loci as well as all loci combined. For all tests with simultaneous multiple comparisons (Hardy-Weinberg, linkage disequilibrium, and population differentiation), significance ($P < 0.05$) was adjusted using the standard Bonferroni correction.

Population structure based on relative measures of genetic variation among samples was also characterized using Wright's F_{ST} (Wright 1951) using the estimator θ of Weir and Cockerham (1984) in FSTAT. Significance of F_{ST} values was determined with 300 permutations.

The underlying population structure in these data sets was also analyzed empirically using the software STRUCTURE v.2.3.3 (Pritchard et al. 2000). STRUCTURE infers the number of genetic clusters K within a set of genetic data. The software uses a Monte Carlo Markov chain Bayesian clustering method that maximizes the within-cluster Hardy–Weinberg and linkage equilibrium. It assumes Hardy-Weinberg proportions within populations and independence and linkage equilibrium between loci. These assumptions are necessary to use the observed allele frequencies to compute the probability of origin of a genotype in a population. STRUCTURE provides options to analyze allele frequencies under two models, one assuming the allele frequencies are

independent draws from a distribution. An alternative model assumes that allele frequencies are correlated, so that frequencies in different populations are likely to be similar because of migration or shared ancestry. The default is the uncorrelated allele frequency model and we report these results except where indicated. We also used an additional new option in STRUCTURE which incorporates sampling site data to aid the model in detecting subtle population structure. This model can provide inference of population structure in cases where the signal is too weak for the standard STRUCTURE model. In all instances, we used burnin lengths of 100,000 and run lengths of 100,000 as recommended in the program. Ten replicates were done for each run. The best value of K was determined from values of lnP(D) and ΔK (Evanno et al. 2005).

Analysis of molecular variance (AMOVA) was performed in ARLEQUIN 2.00 to evaluate the different models of population structure suggested by population differentiation tests in Genepop Fisher's exact test, F_{ST}, and STRUCTURE analyses. AMOVA evaluates different models of population structure by quantifying the extent of genetic variation within populations, among populations within a group, and among groups. The optimum model in AMOVA is that which minimizes genetic variation among populations within groups and maximizes genetic variation among groups.

We used the Mantel's test with 10,000 permutations as implemented in Arlequin on a subset of collections (Hudson River watershed locales, Maine watersheds, locales in Ontario to investigate the relationship between genetic distance (F_{ST} *and* $F_{ST}/1\text{-}F_{ST}$) and geographic distance. The relationship between $F_{ST}/1\text{-}F_{ST}$ and log of geographic distance was graphically depicted and the r values for these analyses were calculated in Excel. For each collection, geographic position was determined as the mean latitude and longitude position for all animals collected in the sample. This allowed us to compare this relationship among collections made along a single waterway (Hudson River) to those among watersheds that are not directly connected by waterways. Because mink are thought to migrate much more regularly along watersheds than between watersheds, our hypothesis was that genetic and geographic distance would be significantly inversely

related among Hudson River collections as implied by the Isolation by Distance (IBD) model but show no relationship among collections made elsewhere.

Results

Overview of genetic variation

849 individual mink were screened at nine microsatellite loci in this study. In total, 106 alleles were observed across all individuals in all populations at the nine loci studied. A range of 4 to 16 alleles per locus (Table 3) was detected across the loci with a mean of 11.8 alleles observed per locus across all populations. Mean observed heterozygosity across all loci in all specimens was 0.679 (Table 4).

Linkage disequilibrium can be due to physical proximity of loci, natural selection for single haplotypes, admixture of populations, or random genetic drift. After Bonferroni correction ($p < 0.001$), 3 of 36 possible pairs of loci exhibited linkage disequilibrium across all collections combined. These included 2243 and AF474, 480845 and 1354, and 1341D3 and 1354. However, when these significant associations were investigated within individual collections only one remained consistently significant ($p < 0.05$), 2243 and AF474. Thus, data from all loci were used in population analysis.

Comparisons of genetic diversity in highly PCB exposed and less exposed mink

We compared a variety of measures of genetic diversity in mink collected downstream of PCB sources and near the Hudson River (< 5 km) to those collected more distant (> 5 km) from the Hudson River. In addition, we also compared these measures in mink that were collected < 5 km of the main stem Hudson and that had high tissue burdens of PCBs (> 5.0 μg Aroclor/g lipid) with mink collected from the same region but > 5 km from the main stem Hudson. Measure of genetic diversity included number of alleles sampled, allelic richness, and observed heterozygosity.

All three measures of genetic diversity were similar in mink collected < 5 km and > 5 km from the main stem Hudson River. For example, the mean number of alleles observed were 7.55 and 7.00, respectively but allelic richness (which considers sample sizes) was

almost identical, 6.75 and 6.98, respectively. Observed heterozygosity was also almost identical between the two collections, 0.697 and 0.692, respectively.

All three measures of genetic diversity was also similar when comparing mink collected < 5 km and with high tissue burdens of PCBs to those collected > 5 km from the Hudson River. For example, although the mean number of alleles observed was lower in the contaminated proximal collection compared to those collected more distant from the Hudson, 6.44 alleles and 7.55 alleles, respectively this difference was not seen when comparing allelic richness. The mean allelic richness in contaminated mink collected near the Hudson River was 6.367 compared to 6.102 in mink collected more distant from the Hudson. Similarly, observed heterozygosity was similar between the two groups, 0.697 and 0.684. Thus, none of the indices of genetic diversity revealed decreased variation in mink that probably experienced the greatest exposure to Hudson River borne PCBs

Bottleneck analysis

We used both the observed heterozygosity excess method and the mode-shift model to examine for potential genetic bottlenecks in mink from the most PCB contaminated collections (< 5 km from the Hudson River). Using the Two-phased model, we saw significant evidence of a bottleneck in the distant-from-the Hudson River reference collections (both mid and north Hudson) using the Wilcoxon test (two tail test for heterozygosity excess or deficiency, $p = 0.02, 0.014$), however this was not accompanied by a graphical mode shift which deviated from a normal L-shaped distribution. We saw no evidence of a bottleneck using either approach in the collection of mink (all mink or only those with high PCBs burdens) from adjacent to the Hudson (Wilcoxon two tail test for heterozygosity excess or deficiency, $p = 0.30$ and a normal L-shaped distribution in the mode-shift test). Thus, there was no evidence of a genetic bottleneck in highly exposed mink collected proximal to this contaminated stretch of the Hudson River.

Genetic diversity in Ontario

Because of concerns over possible effects of pollutants on populations in western Lake Erie, we evaluated genetic variation in our four collections in Ontario. We found that mink from western Lake Erie had a smaller number of alleles (51) and reduced allelic richness (5.7) across all nine loci compared to mink from the other three locales. In comparison, our collection from Lake St. Clair exhibited 60 alleles and its allelic richness was 6.67. However, mink from western Lake Erie exhibited only slightly reduced heterozygosity (0.681) compared to those from Lake St. Clair (0.693).

Genetic population structure

Maine

Mink were collected from four separate river drainages in Maine; the Kennebec, Androscoggin, Penobscot, Saint John and from coastal locales. Using Fisher's exact test, six of ten collection pairwise comparisons were significantly distinct with the exceptions of the Kennebec versus the Androscoggin, coastal locales versus Kennebec, Androscoggin versus Penobscot, and Saint John versus Penobscot. Subsequently, because of their proximity, connectivity, and genetic similarity, we pooled collections from the Kennebec and Androscoggin (X^2= 16.57, p = 0.550), for additional comparisons with other systems. In these analyses, all collections proved significantly genetically distinct with the exception of the Saint John and Penobscot in northern Maine (p = 0.152). The mean F_{ST} value across all Maine collections was 0.022 and F_{ST} values for pairwise comparisons ranged between 0.0411 (coastal locales-Saint John) to 0.0019 (Penobscot-Saint John). F_{ST} analysis of four populations revealed that three of six pairwise comparisons were significantly different with the exceptions being the Androscoggen-Kennebec and coastal locales; coastal locales and the Saint John; and the Saint John and Penobscot. In contrast, STRUCTURE indicated only one cluster for the four river drainages and coastal locations in Maine (Table 5). But, the use of AMOVA strongly supported the presence of three genetically distinct groups among these collections in Maine (Table 6). These three included the Kennebec-Androscoggin, coastal locales, and Penobscot-Saint John.

Ontario

Mink were analyzed from four separate locales in Ontario including western Lake Erie, Lake St. Clair, St. Clair River and St. Lawrence River. Using Fisher's exact test, highly significant genetic heterogeneity was observed among these collections with 8 of 9 loci exhibiting statistically significant differences ($p < 0.001$) in allelic frequencies (except for locus 480845 at which $p = 0.037$). All pairwise comparisons of allelic frequencies among collections were highly significant different ($p < 0.001$) with the exception of western Lake Erie versus Lake St. Clair ($p = 0.014$). The mean F_{ST} among these four collections was 0.054 with individual F_{ST} values ranging from 0.0089 (western Lake Erie-Lake St. Clair) to 0.0757 (St. Lawrence-St. Clair River). All F_{ST} values were statistically significant except for the western Lake Erie and Lake St. Clair comparison. All iterations of STRUCTURE indicated four distinct clusters (Table 5) for the Ontario collections (Table 4), a result that was confirmed by use of AMOVA (Table 6).

Major ecozones in New York State

We obtained a sufficient number of specimens to compare the genetic differentiation of collections representative of seven major ecozones in New York State including the Appalachian Plateau, Great Lake Plains, Mohawk Valley, Hudson River Valley, Taconic Highlands, Tug Hill Transition, and the Adirondacks. Pairwise allelic differentiation tests indicated that there was highly significant genetic differentiation among the seven major ecozones at all nine loci investigated. Furthermore, Fisher's exact allelic differentiation tests revealed highly significant differentiation among all 21 possible pairs of collections from these seven major New York ecozones.

The overall mean F_{ST} value among these seven collections was 0.019. F_{ST} values among individual collection pairs ranged between 0.0037 and 0.0526. F_{ST} comparisons among pairs of major ecozones collections were highly significantly different except two; the Hudson River Valley-Taconic Highlands ($p= 0.029$) and Appalachian Plateau-Mohawk Valley ($p= 0.017$). All iterations of STRUCTURE revealed less heterogeneity than shown by the genetic distance comparisons, indicating only two clusters (Table 5) among the New York major ecozones. AMOVA analysis indicated the presence of 2 or 3 genetically groupings among the major ecozones (Table 6). The two-group model

contains the Adirondacks, Hudson Valley, Taconic Highlands and Mohawk Valley as one grouping and the Appalachian Plateau, Great Lakes Plains, and Tug Hill Plateau as the second. The three-group model is similar except that the Mohawk Valley forms a third distinct group.

However, Hardy-Weinberg analysis within many of these seven collections revealed Hardy-Weinberg disequilibrium suggesting that it is possible that these collections from major ecozones including the Adirondacks were comprised of genetically heterogeneous assemblages of specimens. However, with the exception of the Adirondacks major ecozone, collection sizes at minor ecozones within the seven major ecozones were too small to make meaningful statistical comparisons among them.

Minor ecozones within the Adirondack's major ecozone

We next investigated the extent of genetic differentiation among collections from five minor ecozones within the Adirondacks major ecozone previously analyzed. These minor ecozones included Central Adirondacks, Eastern Adirondacks Foothills, Eastern Adirondacks Transition, Western Adirondacks Foothills, and Western Adirondacks Transition. Fisher's exact allelic differentiation tests indicated the presence of highly significant genetic heterogeneity among these five minor ecozones. Four of nine loci exhibited significant heterogeneity among the minor ecozones. Eight of ten possible comparisons of the Adirondack minor ecozone collection pairs yielded significant genetic differentiation. The two pairs that were not significantly genetic differentiated included Eastern Adirondacks Transition-Western Adirondacks Foothills and Western Adirondacks Foothills-Western Adirondacks Transition. The mean F_{ST} value across all minor ecozone comparisons was 0.017. F_{ST} analysis also revealed significant genetic differentiation in six of ten pairwise comparisons. With F_{ST} analysis, in addition to the two groupings above, the eastern Adirondacks foothills-western Adirondacks foothills and eastern Adirondacks transition-western Adirondacks foothills failed to display significant genetic differentiation

In comparison, STRUCTURE indicated only one group under the independent alleles model and two under the correlated alleles and sampling site identifier models (Table 5). Use of AMOVA indicated the presence of three distinct genetic groups (Table 6) including central Adirondacks-eastern Adirondacks foothills, eastern Adirondacks transition-western Adirondacks foothills, and western Adirondacks transition.

Hudson River tributaries

Genetic differentiation was evaluated among the 16 collection sites in the Hudson River watershed. Overall allelic differentiation among these Hudson River locations was highly significant with all nine loci exhibiting significant differentiation. Allelic differentiation was next evaluated using Fisher's exact test across all 120 pairs of collections from Hudson River drainages. In total, 48 of 120 (40%) comparisons of collection pairs were significant (after conservative Bonferroni correction, p was significant at <0.0004).

The mean F_{ST} among these 120 pairs of collections was 0.024 with F_{ST} values at individual loci ranging from 0.006 (MV1381) to 0.048 (AY24). F_{ST} comparisons among pairs of collections revealed that 29 of 120 were statistically significantly different (after Bonferroni correction, p was significant at < 0.000417). In contrast, STRUCTURE analysis indicated homogeneity among collections from the 16 Hudson River tributary locations (Table 5). Use of AMOVA resulted in the identification of three genetically distinct groups of mink from Hudson River drainages (Table 6)

Northern Hudson River

Genetic differentiation was evaluated among five collections from the northern Hudson River. Initial use of the allelic differentiation test revealed highly significant heterogeneity among the collections. After Bonferroni correction, significant allelic differentiation was observed at 2 of 9 loci. The extent of allelic differentiation was then evaluated among all 10 pairs of collections from the northern Hudson using Fisher's exact test. Of these, 4 of 10 comparisons proved statistically significant after Bonferroni correction ($p < 0.005$). The mean F_{ST} value across all 9 loci among these 10 pairs of

collections was 0.034. Individual F_{ST} values among these 10 comparisons ranged from 0.0192 to 0.0657. Two of 10 pairwise F_{ST} comparisons of collections from the northern Hudson River proved significantly different.

Mid- northern Hudson River

Genetic differentiation was also evaluated among 7 collections of mink from the mid-northern Hudson River region. Overall allelic differentiation among these was collections was highly significant ($p< 0.001$) with 5/9 loci exhibiting allelic heterogeneity after Bonferroni correction. Of 21 pairwise comparisons, three were statistically significant ($p < 0.00238$). The mean F_{ST} value among these comparisons across all loci was 0.010. with single pair comparisons ranging between 0.0003 to 0.0253. F_{ST} values for two of the 21 pairwise comparisons proved statistically significant.

Mid-Hudson River and surrounding regions

The collection from the mid Hudson area were divided into those made within 5 km (n=35) and those more distant than 5 km (n=106) from the Hudson River. We hypothesized that those PCB exposed demes from proximal to the river would be genetically distinct from those collected more distant from the river. However, both the Fisher's exact test and F_{ST} tests failed to detect any evidence of significant overall or locus-specific genetic heterogeneity between these two collections of mink (overall $p = 0.326$ for Fisher's exact test). We next investigated if there was significant heterogeneity between mink with high burdens of PCBs (> 5 mg/g lipid) that were collected within 5 km of the Hudson (n=19) and those collected from locales more distant (n=106) from the mid Hudson River. Once again there was no significant heterogeneity between these two collections using either the Fisher's exact test ($p = 0.124$) or F_{ST} tests.

Other comparisons

When STRUCTURE analysis was used to compare Hudson Valley collections with all other collections from New York State, two groupings were indicated (Table 4). This result was confirmed with AMOVA. Both Fisher's exact test and F_{ST} analyses revealed that our collections from New Brunswick, Rhode Island and pooled Maine collections

were genetically distinct. When STRUCTURE analysis was used to compare among these three collections its results suggested the presence of three groupings (Table 4). This result was also confirmed with AMOVA.

Across all regions

We summed the total number of genetically distinct units of mink that we identified across all collections by each of the four analyses that we used; Fisher's exact test, F_{ST}, AMOVA, and STRUCTURE. In total, these analyses identified 20, 18-19 18-19, and 14-15 genetically distinct units of mink, respectively (Table 5).

Relationships between genetic and geographic distances

Interestingly, we found a significant positive relationship between genetic (F_{ST} or F_{ST}/1-F_{ST}) and geographic distance among the 16 Hudson River collections (r = 0.635, p = < 0.000) (Fig. 1) and weakly so for the four locales in Ontario (r = 0.571, p = 0.044) (Fig. 2). In contrast, we failed to find a significant relationship among mink collections from Maine (r = 0.522, p = 0.127) (Fig. 3).

Discussion

Our study provided two major findings. First, mink that reside in close proximity to the Hudson River and that have been previously shown to bioaccumulate high levels of Hudson River-borne PCBs and presumably are most affected by them are not genetically distinguishable from collections made at sites more distant from the river. Additionally, exposure of these animals to high levels of PCBs and their bioaccumulation has not significantly reduced their individual or population levels of overall genetic variation. This suggests that demes in closest proximity to the Hudson did not suffer catastrophic mortality resulting in severe genetic bottlenecks.

Second, mink from throughout eastern North America exhibit high levels of genetic population structure, sometimes even over small geographic distances. This genetic differentiation was observed among collections from geographically distant and even proximal sites, major watersheds, different tributaries of individual watersheds, major

ecozones, and minor ecozones. Many comparisons of mink collections in this study yielded significant genetic differentiation. However, the number of genetically distinct populations detected within specific geographic areas and across all collections varied depending on the means of analyses used except for locales in Ontario for which all analyses identified four genetically distinct populations. Estimates made with STRUCTURE invariably proved more conservative than those made with F_{ST}, Fisher's exact test, or AMOVA. That is because the STRUCTURE approach is based on optimizing the extent of Hardy-Weinberg and linkage equilibrium within clusters whereas the latter approaches evaluate whether alleles are drawn from the same distribution in all populations.

We feel that this discrepancy in results among approaches may be largely due to the sampling regime used in our study (Schwartz and McKelvey 2009). Our collections did not respect specific geographic or temporal borders and instead were mostly fortuitous samples made by part-time trappers for other purposes. Thus, it is likely that our collections frequently sampled animals drawn from more than a single population. That is evidenced by the observation that on occasion our collections were not in Hardy Weinberg equilibrium. Schwartz and McKelvey (2008) showed that when individuals were distributed continuously over a landscape and mated preferentially with individuals from neighboring demes, STRUCTURE sometimes produced unpredictable clustering results. Studies with mink have demonstrated that although they preferentially mate with females from dens within their home demes, it is not unusual for males to venture outside their own deme to mate with females from neighboring demes. Furthermore, Latch et al. (2006) suggest that STRUCTURE does not perform well at assigning clusters when F_{ST} values are less than 0.05. In most cases, F_{ST} comparisons in our study did not achieve that threshold. For example, we observed mean F_{ST} values of 0.022, 0.019, and 0.017 for comparisons among Maine watersheds, major ecozones, and minor ecozones, respectively. Furthermore, it has also been suggested that STRUCTURE does not perform well when too many populations (>10) are included in the analysis (Pearse and Crandall 2004). Additionally, other investigators have found that STRUCTURE on occasion yields spurious results because of small or unequal sample sizes and the relative

amount of differentiation among populations is unequal (Kidd et al. 2011). All of these factors are common among our collections. We are not in a position to resolve the discrepancies in the results from our data achieved by application of our four analytical approaches. It may be best at this time to consider the results of the these approaches to constitute the upper and lower bounds for the geographic structuring of the mink collections included in our study.

We used Mantel's test to determine if genetic distance (F_{ST} and $F_{ST}/1\text{-}F_{ST}$) and geographic distance were significantly correlated among collection sites and found differing results dependent if collections were distributed along a single waterway or not. Collections made along the main stem Hudson River corridor exhibited a highly significant positive correlation between genetic and geographic distances ($p < 0.000$) while those made as-the-crow-flies among watersheds in Maine did not. The relationship among the four locales in Ontario proved marginally significant ($p = 0.044$) reflecting the proximity and potential aquatic connectivity among 3 of the 4 collection locales. Our results largely confirmed that gene flow in mink followed the isolation by distance model longitudinally along waterways such as the Hudson but that non-riparian habitats proved at least to be partial barriers to gene flow.

Previous studies in other taxa and with other contaminants have provided mixed results regarding the effects of pollutants on levels of genetic diversity. It has been suggested that chronic exposure to non-mutagenic contaminants may erode genetic variation by severely cropping population size or by strongly selecting for advantageous homozygote genotypes. For example, Fratini et al. (2008) recently reported a decrease in microsatellite variability in an intertidal crab *Pachygrapsus marmorat*us that bioaccumulated elevated levels of four metals along the Tuscan coast. In contrast, Berckmoes et al. (2005) found no evidence of reduced microsatellite diversity in wood mouse *Apodemus sylvaticus* populations in Belgium exposed to high levels of five metals in soil. Meta analysis across taxa and contaminants by DiBattista (2008) found no significant association between contaminant exposure and levels of allozyme or microsatellite variation.

It is not surprising that reductions in neutral genetic diversity are not frequently found in these studies because bottlenecks that will generate decreased variation must be severe, often in the order of 50 to 1000 individuals. However, because of their documented exposure, small reproductive units, relative isolation, and known sensitivity to reproductive impairment, it is surprising that mink proximal to contaminated Hudson River locales did not exhibit reduced genetic variation. However, our highly exposed collection showed no evidence of a genetic bottleneck nor the influx of migrants from nearby non-contaminated source populations; two possible explanations for their maintenance of robust levels of genetic diversity.

How do levels of genetic variation and population differentiation that we report compare to that previously observed among mink populations in North America? Kidd et al. (2009) used a panel of ten microsatellites to determine the extent of introgression of ranch escapees on proximal natural populations of mink. They observed an almost equal number of alleles per locus (6-15 alleles) and observed heterozygosity (0.654) as us and that nearly two-thirds of the mink in these two populations were either escapees from nearby ranches or their descendents. To date there are only two small-scale reports on microsatellite variation among populations of native American mink in North America. Surprisingly, Belliveau et al. (1999) reported a lower level of genetic diversity at 7 microsatellite loci in wild mink from Nova Scotia with a mean 4.43 alleles/locus and mean expected and observed heterozygosities of 0.498 and 0.370, respectively compared to mink from four nearby ranches. They attributed this finding to the likelihood that their small collection area was populated by a limited number of male mink since each demands considerable territory (Dunstone 1993). Stevens et al. (2005) analyzed microsatellite variation at four loci in a small number of mink (n=76) distributed among 8 localities in Arkansas and Tennessee. They observed a smaller number of alleles (7.25 alleles/locus) and lower expected heterozygosity of (0.643) than we did with no significant departure from Hardy-Weinberg equilibrium. Based on F_{ST} analysis, they failed to observe significant differentiation among 6 riparian localities in Arkansas-only comparisons between the distant site in Tennessee (204 km distant) and those in

Arkansas proved significant. Furthermore, genetic distance was positively correlated with straight-line geographic not riverine distance. They hypothesized that the lack of differentiation was due to a propensity of young males to migrate greater distances than originally thought and across non-riparian habitat.

Larger studies of microsatellite variation have been conducted among introduced populations of American mink in Europe. Lecis et al. (2008) quantified microsatellite diversity at 10 loci among 5 collections of mink in Spain. They observed 6.1 alleles/locus with mean expected and observed heterozygosities of 0.609 and 0.583, respectively. Zalewski et al. (2010) have recently reported on variation at 12 microsatellite loci in American mink collected from 10 sites in Poland and at several nearby ranches. They observed a mean of 5.3 alleles/locus and with expected and observed heterozygosities of 0.638 and 0.599, respectively. They found strong genetic differentiation among collections using both pairwise F_{ST} and STRUCTURE analyses. They identified at least 5 distinct clusters of mink-a surprising result given the recent origin of these populations. They attributed their genetic differentiation to isolating landscape features and the different origins of mink in ranches across Poland.

Summary

We found no evidence that exposure to high levels of bioactive PCBs and their bioaccumulation impacted levels of overall genetic diversity or the population structure of mink collected in close proximity to the Hudson River. Consistent with their limited vagility and non-riparian barriers to gene flow, many collections of mink in eastern North America exhibited strong genetic differentiation.

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Figure Legends

Figure 1

Depiction of results of Mantel's test of significant inverse relationship between genetic distance (F_{ST}/1-F_{ST}) and mean geographic distance among 16 collections of American mink made along the main stem Hudson River, New York.

Figure 2

Depiction of results of Mantel's test of non-significant inverse relationship between genetic distance (F_{ST}/1-F_{ST}) and mean geographic distance among 5 collections of American mink made among watersheds in Maine.

Figure 3

Depiction of results of Mantel's test of significant inverse relationship between genetic distance (F_{ST}/1-F_{ST}) and mean geographic distance among 4 collections of American mink made in Ontario, Canada.

Table 1

Collections of mink analyzed at microsatellite loci in this study

Locale	Sub locale	Number of animals
Canada	Western Lake Erie	20
	St. Clair River	20
	Lake St. Clair	20
	St. Lawrence River	20
	New Brunswick	14
Rhode Island	Inland	39
	Coastal salt ponds	8
Maine	Lower Androscoggin	19
	Kennebec	8
	Coast	9
	Upper Penobscot	29
	Saint John	13
New York	Appalachian Plateau	132
	Great Lake Plain	47
	Mohawk Valley	29
	Hudson Valley	89
	Taconic Highlands	64
	Central Adirondacks, Adirondacks Transition and foothills	182
	Tug Hill Plateau	127
	Champlain Transition	1

Table 2
Characterization of mink microsatellite loci used in this study

Locus name	Genbank identifier	Repeat motif	Primer sequences	Number alleles	Molecular size (bp)
Mvi 2243	AY053518	$(TG)_4TA(TG)_{14}$	CGGACATTTGTTCTAAGAGGT AGATTAACAAGCCATGCTC	14	132-164
Mvi 1354	AF480852	$(CA)_{22}$	CCAACTGGAGCAAGTAAAT CATCTTTGGGAAAGTATGTTT	16	176-216
Mvi 1302	AF480846	$(GT)_{17}$	CATAGGTTCCAGGGATTAGAA ATGCCATTACAGTACGACTCA	11	210-224
Mvi 1381	AF421188	$(AC)_{19}$	CCATCGGAGTTTCTCATCGT CCAGGTGCCCCTTACATT	12	185-198
Mvi 099	AF132106	(CA)16	TGAGGCAAGAGGAGCAAAAG TTTGCATTTCCCTGATGAGG	16	321-351
Mvi 1273	AF480845	$(GGAA)_6$	ATAGCATCCCTAAAATGG TCCCCCTCCAGACCTCTA	16	183-215
Mvi 9700	AF474150	$(CCTT)_{10}$	AGCCTTCCTGGGTATCTA TGAAAAGTTTATTGGTCT	8	252-268
Mvi1341	AF480850	(CA)17	GTGGGAGACTGAGATAGGTCA GGCAACTTGAATGGACTAAGA	16	148-176
Mvi 3402	AY244352	$(ATC)_5(TGC)_9$	CAAAGGAGTATTCACAGG CCGACACCGCCACAGCAG	3	290-296

Table 3

Allelic Frequencies at each Microsatellite Locus Across All Populations

Locus 2243	1	2	3	4	5	6	7	8	9	10	11	12	13				# Alleles Scored
	0.007	0.001	0.009	0.241	0.072	0.001	0.001	0.036	0.159	0.462	0.009	0.001	0.001				1630
Locus 480845	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	
	0.09	0.001	0.155	0.001	0.218	0.001	0.057	0.109	0.002	0.144	0.001	0.076	0.027	0.111	0.008	0.001	1628
Locus AF474	1	2	3	4	5												
	0.243	0.395	0.124	0.151	0.087												1624
Locus 1341D3	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15		
	0.002	0.001	0.010	0.026	0.252	0.173	0.181	0.024	0.100	0.113	0.015	0.063	0.035	0.002	0.003		1622
Locus 1354	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	
	0.001	0.205	0.022	0.024	0.158	0.016	0.014	0.066	0.15	0.085	0.115	0.086	0.048	0.004	0.001	0.004	1594
Locus 1302	1	2	3	4	5	6	7	8	9	10							
	0.040	0.170	0.080	0.159	0.141	0.197	0.162	0.040	0.010	0.002							1638
Locus AY24	1	2	3	4													
	0.023	0.785	0.191	0.001													1634
Locus MV1381	1	2	3	4	5	6	7	8	9	10	11	12					
	0.042	0.001	0.009	0.279	0.039	0.131	0.355	0.079	0.056	0.002	0.001	0.007					1636
Locus MVI099	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15		
	0.001	0.001	0.005	0.001	0.001	0.130	0.411	0.017	0.046	0.263	0.075	0.001	0.028	0.018	0.004		1640

Table 4

Number of alleles, observed (H_o) and expected (H_e) heterozygosity, F_{ST} and allelic differentiation (p values) among all American mink collections at 9 microsatellite loci. Bold text indicates significance at the $p = 0.05$ level.

Locus	# Alleles Sampled	Mean Allelic Richness	H_o	H_e	F_{ST}	p
2243	13	3.71	0.628	0.700	**0.035**	< 0.0001
480845	16	5.35	0.814	0.866	**0.024**	< 0.0001
AF474	5	3.91	0.863	0.739	**0.048**	< 0.0001
1341	15	5.20	0.789	0.845	**0.024**	< 0.0001
1354	16	5.32	0.782	0.875	**0.048**	< 0.0001
1302	10	4.94	0.656	0.852	**0.029**	< 0.0001
AY24	4	1.84	0.347	0.334	**0.015**	< 0.0001
MV1381	12	4.28	0.730	0.767	**0.015**	< 0.0001
MV1099	15	4.13	0.702	0.736	**0.019**	< 0.0001
Mean	11.78	4.29	0.701	0.746	**0.037**	Infinity

Table 5

STRUCTURE analysis of the number of clusters among mink sample collections

Sample collections	**Number of specimens**	**# Collection locations**	**Correlated alleles – best K**	**Independent alleles – best K**
ME, NB, RI	130	3	3	3
Hudson River vs. other NY	600	2	2	2
Major NY Ecozones	608	7	2	2
All Hudson Collections	305	16	1	1
Adirondacks Minor Ecozones	182	5	2	1
Ontario Locales	78	4	4	4
Maine Drainages	78	4	1	1

Table 6

Sample collections	**Number of specimens**	**# Collection locations**	**Fisher's Exact Test**	F_{ST}	**AMOVA**
ME, NB, RI	130	3	3	3	3
Major NY ecozones	608	7	4	3-4	2-3
16 Hudson Locales	305	16	1	3	3
Minor Ecozones Adirondacks	182	5	4	3	3
Ontario Locales	80	4	4	3	4
Maine Drainages	78	5	4	3	3

Figure 1

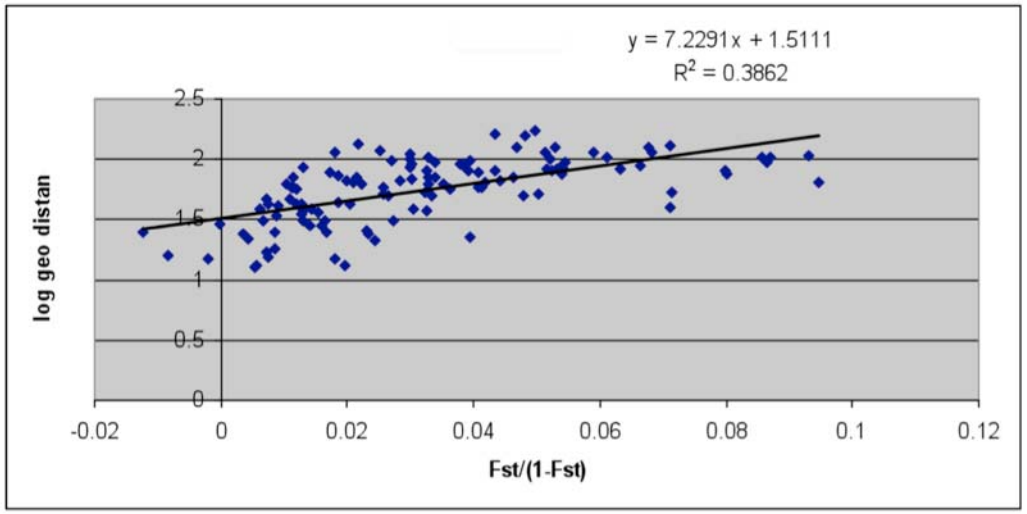

y = 7.2291x + 1.5111
R2 = 0.3862
log geo distan
2.5
2
1.5
1
0.5
0
-0.02
0
0.02
0.04
0.06
0.08
0.1
0.12
Fst/(1-Fst)

Figure 2

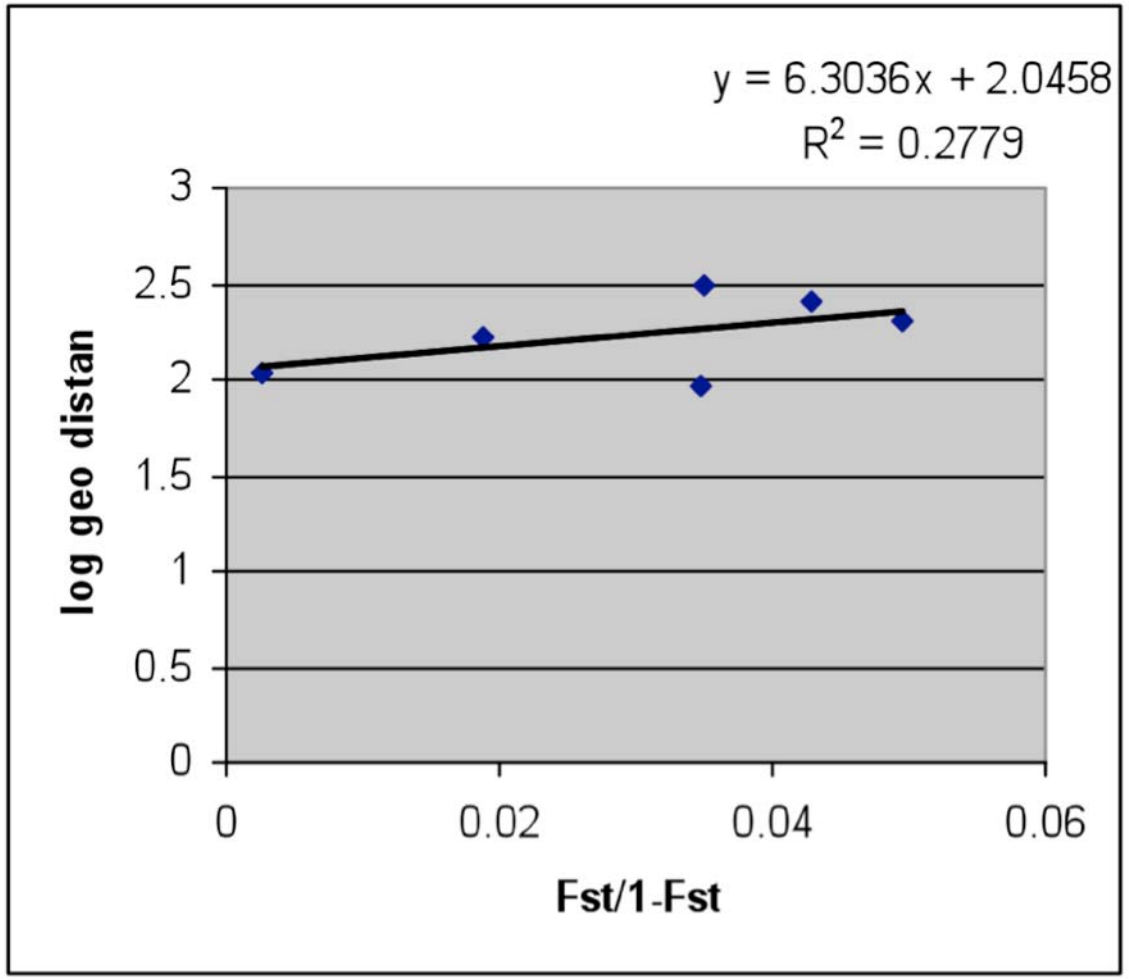
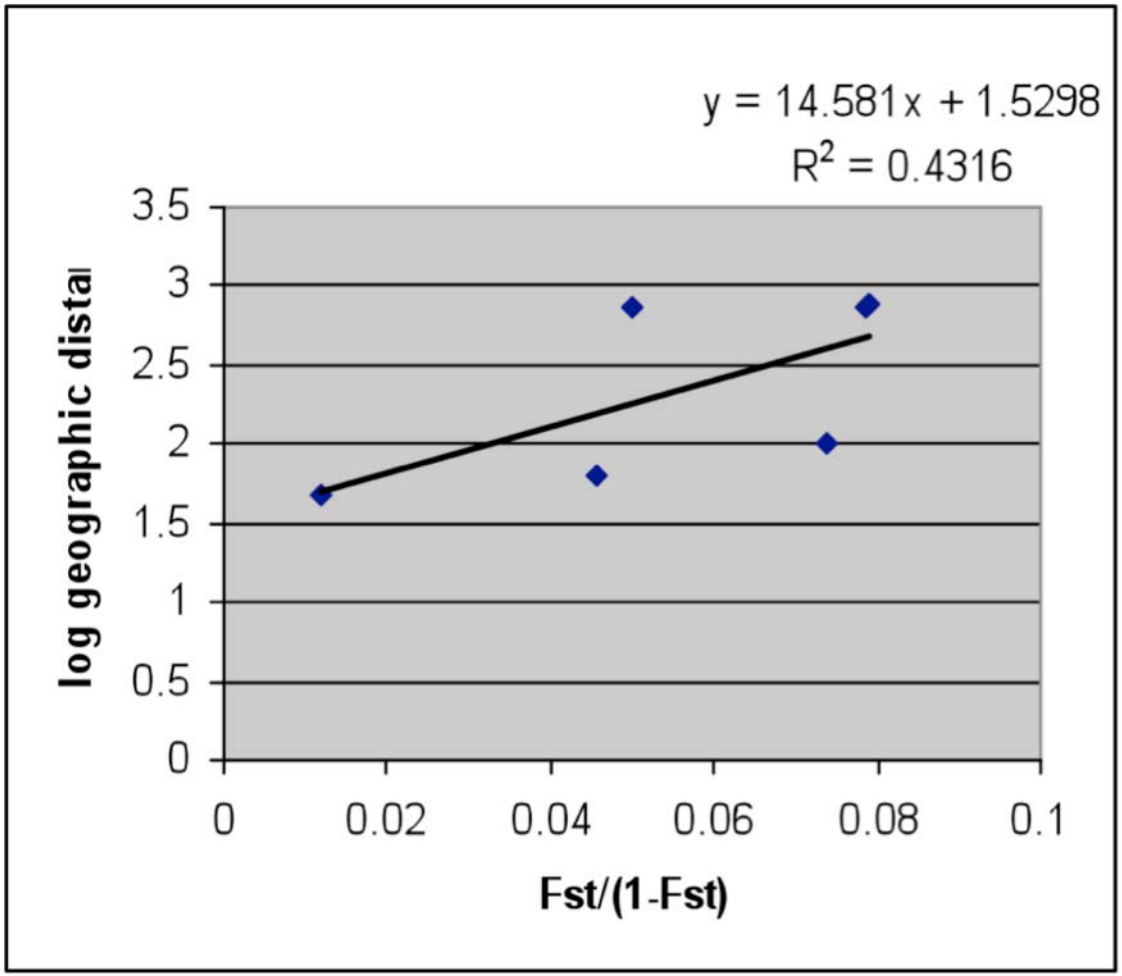

y = 6.3036x + 2.0458
R2 = 0.2779
log geo distan
3
2.5
2
1.5
1
0.5
0
0
0.02
0.04
0.06
Fst/1-Fst

Figure 3


y = 14.581x + 1.5298
R2 = 0.4316
log geographic distal
3.5
3
2.5
2
1.5
1
0.5
0
0
0.02
0.04
0.06
0.08
0.1
Fst/(1-Fst)