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Genetic Variation and Population Structure of American Mink *Neovison vison* from PCB-Contaminated and Non-Contaminated Locales in Eastern North America

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Abstract

American mink *Neovison vison* may be particularly vulnerable to toxicities of persistent contaminants such as PCBs because of their aquatic-based diet, position near the top of the food web, and small deme sizes. Furthermore, ranched mink are sensitive to reproductive toxicities of fish diets from PCB-polluted sites. The upper Hudson River is highly contaminated with PCBs and previous studies have shown elevated hepatic burdens of total and coplanar PCBs in mink collected near the river compared with those from more distant locales in New York and elsewhere. We hypothesized that bioaccumulation of PCBs in Hudson River mink has reduced their levels of genetic diversity or altered their genetic population structure. To address this, we conducted microsatellite DNA analysis on collections made in proximity to 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 drainages in Maine, and two ecoregions in Rhode Island. We did not find reduced genetic diversity at the individual or population levels in mink collected near (< 6 km) to PCB hotspots in the Hudson River nor evidence of altered population structure. Consistent with their distribution in small localized and isolated demes, we did find 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 16 when using STRUCTURE to 19-20 when using Exact G tests,

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Contributions of authors

I. Wirgin conceptualized the study; I. Wirgin and J. Waldman statistically analyzed the genetic data and wrote the manuscript; L. Maceda conducted microsatellite DNA analysis; and D. T. Mayack, as an employee of New York State Department of Environmental Conservation, provided tissue samples and associated ecological data.

Conflict of interest

The authors declare that they have no conflict of interest.

F_{ST}, or AMOVA analyses. Genetically distinct population units were found among major ecoregions and minor ecoregions in New York State, among different hydrologic subunits within the Hudson River watershed, 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 their bioaccumulation of high levels of PCBs of Hudson River origin.

Keywords

Hudson River; PCBs; genetic population structure; genetic diversity; DNA microsatellites

Introduction

Three hundred and seventeen km of the Hudson River was designated a federal Superfund Site by the U.S. EPA in 1984, because of human and ecological risks from PCB exposure (Wirgin et al. 2006). Most of this contamination resulted from release from 1947 to 1977 of 0.2 to 1.3 million pounds of PCBs at two General Electric facilities that manufactured electrical capacitors at Hudson Falls (river km 317 (rk) and Ft. Edwards, New York (rk 314) (Limburg et al. 1986). Downriver transport and removal in 1973 of a low level 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 and combined sewer overflow sources (Farley and Thomann 1998). Tissue burdens of PCBs in resident Hudson River fishes follow a similar, although less pronounced pattern (Armstrong and Sloan 1988; Tams/Gradient 1995).

American mink *Neovison vison* is a broadly distributed opportunistic carnivore usually associated with aquatic habitats (Gerell 1967, Erlinge 1969, Gilbert and Nancekivell 1982). Mink activity is generally confined to narrow bands along wetlands and shorelines of lakes, rivers, and the sea (Gerell 1970; Dunstone and Birks 1983; Arnold and Fritzell 1990). Estimated mink home ranges are between 1 and 6 km (Harris et al. 2008) and vary due to population density, vegetative cover, food availability, trapping activity, gender, age, and season (Mitchell 1961; Yamaguchi et al. 2003). Given adequate prey availability, the linear home ranges of adult males are considerably larger than those of females (Eagle and Whitman 1987). Males may expand their search for females well beyond their normal home ranges during the mating season (Gerell 1970; Arnold and Fritzell 1987; Niemimaa and Pokki 1994) and they actively patrol and maintain non-overlapping territories (Birks and Linn 1982; Yamaguchi, Rushton et al. 2003). Similarly, territories of females do not overlap with those of other females (Mitchell 1961; Dunstone and Birks 1983) and territorial defense by females suggest that except during breeding, they maintain territories that, although they may lie within, are distinct from territories of males (Gerell 1970; Linn and Birks 1980). Restricted mink distributions at a landscape level due to dependence on disjunct aquatic habitats coupled with relatively small, well-defended, non-overlapping home ranges suggest that mink populations are likely made up of small demes.”

Mink show a high degree of intraspecific morphological variation. Much of the phenotypic variation related to size, fur, and skin characteristics is controlled genetically. Furthermore, the adaptation of mink to domestication has demonstrated the genetic plasticity of a range of morphological and biochemical traits (Berg 1993ab; Dunstone 1993; Kruska and Schreiber 1999). The effect of selective pressures in the wild is enhanced by the short generation times and short life expectancies of mink (Eagle and Whitman 1987). Because of these factors, ecosystem-specific selective pressures acting on reproductively isolated assemblages may be expected to foster genetically diverse populations of mink at the landscape level.

Domesticated mink are highly sensitive to PCBs toxicity. Ranched mink that were fed Great Lakes fishes suffered reproductive and developmental abnormalities that were related to PCB levels including reductions in litter size, kit survivorship, and whelping (Aurelich and Ringer 1977; Aurelich et al. 1971). Toxicities were congener-specific and aryl hydrocarbon receptor mediated (Aurelich et al. 1985) with coplanar congeners being the most toxic. In controlled laboratory studies, the LC_{50} for mink chronically exposed for 28 d to TCDD was 4.2 μg TCDD/kg body weight (b.w.) (Hochstein et al. 1998). The lowest observable adverse effect level (LOAEL) of dietary exposure of mink to a coplanar PCB126 contaminated fish diet on reproduction, kit survivability and growth was 2.4 μg PCB 126/kg feed (Beckett 2008). Levels of total PCBs in whole carp *Cyprinus carpio* collected at three sites downstream of the two GE facilities was 36 μg PCBs/g wet wt (Bursian et al. 2013b). Thus, it might be expected that mink from the Hudson River corridor would bioaccumulate high levels of PCBs, be sensitive to PCBs-induced toxicities, and that reproductive effects might be felt at the population level.

PCB levels in wild mink collected at contaminated sites across North America may exceed those shown to elicit toxicities under controlled laboratory conditions (Basu et al. 2007). Ranched mink kits fed *in utero* and during lactation of goldfish *Carassius auratus auratus* and carp from the PCB-contaminated Housatonic River suffered reduced survivability between 3 and 6 weeks of age (LC_{10} and LC_{20} were 0.231 and 0.984 μg total PCB/g feed, respectively) (Bursian et al. 2006a). Similarly, those fed a diet supplemented with PCB-contaminated carp (1.1 mg total PCBs/kg feed) from the Saginaw River, Michigan, experienced maxillary and mandibular squamous epithelial proliferation, a condition that could lead to tooth loss and reduced survival in the wild (Bursian et al. 2006b). Furthermore, ranched mink fed diets containing 2.5 to 20% of Hudson River-collected fish suffered a significant increase in stillborn kits at the two highest doses, significantly decreased survivorship by 31 weeks of age at two lower doses, and jaw lesions at the lowest dose of PCBs used (Bursian et al. 2013ab).

Levels of genetic diversity and allelic frequencies in natural populations may be sensitive to exposure to toxic chemicals. Reduced genetic variation may be manifest at the levels of the individual (reduced heterozygosity) or population (number and frequencies of alleles across loci). At the individual level, reduced heterozygosity has been correlated with decreased growth rate and vulnerability to environmental stressors. At the population level, rich genetic diversity provides the plasticity to persist in changing or heterogenous 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, and may impair fitness by increasing the frequencies of deleterious or lethal genotypes (Frankham et al. 2002). Conversely, mutagenic xenobiotics such as metabolites of PAHs and radiation may increase levels of genetic variation in impacted populations (Wirgin and Theodorakis 2002). However, few studies have demonstrated this effect from exposure to total PCBs (Stapleton et al. 2001).

Reduced genetic diversity may occur if the abundance of a population is severely cropped and rare alleles lost. This may be particularly relevant for 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 may result in altered allelic frequencies, increased heterozygosity and Hardy Weinberg disequilibrium. Exposure to toxicants may also 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).

Microsatellite DNA analysis has proven informative in population genetics studies. Because microsatellite loci are usually selectively neutral, patterns of genetic variation at loci reflect stochastic historical processes (mutation, drift, population size, population age, migration) that have impacted the population and molded its genetic population structure. As a result, microsatellites provide a sensitive tool to evaluate genetic diversity and evolutionary processes among and within natural populations.

In this study, we used microsatellite analysis to evaluate the genetic population structure and diversity in mink from locales in the northeastern U.S. and eastern Canada. We hypothesized that overall levels of genetic diversity would be significantly reduced in mink collected proximal to the Hudson River and immediately downstream of PCB sources compared to collections made more distant from the Hudson or upstream of PCB sources. We also hypothesized that the genetic population structure of mink within the Hudson River corridor would be altered historically by exposure to high levels of PCBs, resulting in differentiation between proximal and distant collections. Finally, we placed genetic results for Hudson River collections in a larger context by evaluating the effects of ecological and historical factors in determining the genetic architecture of mink populations collected from New York and elsewhere in northeastern North America.

Methods

Sample collections

In total, 828 mink were analyzed in this study. Unlike samples typically taken for genetic studies from discrete locations, our collections were largely taken by trappers across broad landscapes. In New York, these were coordinated by the New York State Department of Environmental Conservation (NYSDEC). Mink were also obtained from New Brunswick, Canada; five watersheds in Maine; two regions in Rhode Island; and four sites in Ontario, Canada including western Lake Erie, the St. Clair River, Lake St. Clair and the Saint Lawrence River (Table 1). Mink from New York were retrieved from trappers or as road kills by NYSDEC personnel and stored at −20° C. Subsequently, sections of pectoral muscle

or kidney were taken. These samples and muscle plugs from mink taken elsewhere were retained at −80° until processing.

The 611 mink collected from New York State were assigned to eight major ecoregions based on the designations made in Dickinson (1983) and Will et al. (1982) (Fig 1; Table 2A). Collections of specimens from the Adirondacks major ecoregion (n=182) were also assigned to 5 minor ecoregions (Fig. 1, Table 2B). A subset of these New York specimens (n=262) were also assigned to 16 small hydrologic units in the Hudson River drainage as described in NYSDEC (2003) (Table 2C). Finally, mink from the Upper Hudson River watershed collected proximal to the river (< 6 km) and downstream of PCB sources (n=27) or with high hepatic burdens of total PCBs (> 16-174.6 μg total PCBs/g lipid) (n=15) were compared to mink collected more distant from the river (> 6 km) or upstream of PCB sources (n=98) and with low hepatic burdens of PCBs (< 16 μg total PCBs/g lipid) (n=98) (Fig. 2). Six km was chosen as the distance at which to compare genetic diversity and allelic patterns between potentially highly and less exposed mink because the home range of mink is believed not to exceed that distance. A hepatic concentration of total PCBs > 16 μg/g lipid was chosen to distinguish animals with high and lower exposures because that was a threshold concentration that induced toxicity in mink under controlled laboratory conditions (Bursian et al. 2013ab). Hepatic burdens of total PCBs in Upper Hudson River and other New York State collections of mink that we genetically characterized were previously reported by Mayack and Loukmas (2001) and by the Hudson River Natural Resource Trustees (2013) and are available at http://www.dec.ny.gov/docs/wildlife_pdf/mnk_prg.pdf and http://www.dec.ny.gov/docs/fish_marine_pdf/pcbhudsonecosys.pdf, respectively.

DNAs isolation and microsatellite DNA analysis

DNAs were extracted using phenol/chloroform extractions following tissue incubation in CTAB buffer (Saghai-Maroof, 1984) and digestion with Proteinase K and alcohol precipitations. DNA purities and concentrations were determined with a Nanodrop ND-1000 spectrophotometer (NanoDrop Technologies, Inc., Wilmington, DE) and concentrations were adjusted to 50 ng/μl for standardization of subsequent procedures.

Microsatellite analysis was conducted at nine previously characterized mink microsatellite loci including Mvi2243, Mvi1354, Mvi1302, Mvi1381, Mvi099, Mvi1273, Mvi9700, Mvi1341, and Mvi3402 (Table 3) (Fleming et al. 1999; Wisely et al. 2002; Vincent et al. 2003; Vincent et al. unpublished data). PCR reactions were in 12.5 μl total volumes containing 87.5 ng of template DNA, 0.375 to 0.7 μl of each primer (1 μM stock) (Invitrogen Life Technologies, Carlsbad, CA), 1.25 μl of 10 × KlenTaq 1 PC2 reaction buffer, 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° C followed by 60 cycles of denaturation at 95° C for 30 sec, annealing at temperatures in Table 3 for 30 sec, extension at 72° C for 60 sec, followed by a final extension at 72° 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™ thermal cyclers (Waltham, MA).

Characterization of genotypes was done on a CEQ8000™ capillary-based DNA sequencer (Beckman Coulter, Fullerton, CA). 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 on the sequencer with the FRAG 1 program (Beckman Coulter).

Statistical analysis

Multi-locus genotypes were compiled for all individuals, and allele and genotype frequencies were determined for each collection using GenAlEx 6.5 (Peakall and Smouse (2006). 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 Exact tests in Genepop (Rousset 2008); the null hypothesis tested was the random union of gametes. Linkage equilibrium for each pair of loci in each population was evaluated in Genepop. Genetic diversity was assessed as the number of alleles in collections, expected and observed heterozygosities per locus and across all loci in Genepop, and as allelic richness using FSTAT version 2.9.3 (Goudet et al. 1995; Goudet 2001).

The program ML-Relate (Kalinowski et al. 2006) was used to compare the genetic relatedness between each pair of mink specimens collected < 6 km of the main stem Mid-Northern Hudson River (tributary stream distance) and immediately downstream of the upriver PCB hotspots (n=27) to those collected > 6 km from the main stem Mid-Northern Hudson in the same area (n=98). Of the 98 mink collected > 6 km from the main-stem Hudson, hepatic PCB levels were quantified in 49 specimens. We also compared mink collected < 6 km of the main stem Mid-Northern Hudson with elevated levels of hepatic PCBs (> 9 μg total PCBs/g lipid) (n=15) to those collected > 6 km of the Hudson with baseline (< 9 μg total PCBs/ g lipid) hepatic PCB levels. Our hypothesis was that mink with high PCB burdens collected proximal to the Hudson would be more closely related than those collected more distant from the Hudson. ML-Relate calculates maximum likelihood estimates of relatedness and relationships.

The Bottleneck program (vers. 1.2.02) (Cornuet and Luikhart 1996) was used 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 (< 6 km (tributary stream distance) and downstream of sources of Hudson River PCBs (n=27). We analyzed for bottlenecks in mink collected more distal (> 6 km) from the Hudson and upstream of the PCBs sources as reference controls (n=116). 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 Exact G tests implemented in Genepop with default Markov chain parameters to test the null hypothesis of

a homogenous distribution of alleles across collections. Population comparisons were initially made between all pairs of samples and when no differences were observed within sampling locations these were pooled for subsequent analysis of spatial heterogeneity. For all tests with simultaneous multiple comparisons (Hardy-Weinberg, linkage disequilibrium, and population differentiation), significance ($P < 0.05$) was adjusted using sequential Bonferroni correction.

Population structure was also evaluated based on relative measures of genetic variation among samples as characterized using Wright's F_{ST} (Wright 1951) in FSTAT using the estimator θ of Weir and Cockerham (1984). Significance of F_{ST} values was determined in FSTAT (Goudet 2001) with 300 permutations.

Underlying population structure within the genotypic data was also analyzed using the STRUCTURE v.2.3.3 program (Pritchard et al. 2000). STRUCTURE infers the number of genetic clusters, *K*, within a set of genetic data using a Monte Carlo Markov chain Bayesian method that maximizes the within-cluster Hardy–Weinberg and linkage equilibria. We used the admixture model using sampling locations as a prior with allele frequencies correlated. In all instances, we used burn-in lengths of 10,000 and run lengths of 100,000. Ten replicates were done for each run. The best value of K was determined from values of lnP(D) (Pritchard et al. 2000) and ΔK (Evanno et al. 2005) using STRUCTURE HARVESTER (Earl and vonHoldt. 2012).

Analysis of molecular variance (AMOVA) was performed in Arlequin ver 3.5 (Excoffier and Lischer 2010) to evaluate the different models of population structure suggested by population differentiation tests using the Exact G 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 Mantel's test with 10,000 permutations as implemented in Arlequin on a subset of collections (small hydrologic units within the Northern Hudson River and Mid-Northern Hudson River subdrainages of the Upper Hudson River, Maine watersheds, and sites within 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 the relationship among hydrologic subunits within a single drainage (Upper Hudson River) to the relationship among hydrologic units from disjointed drainages. Because mink are thought to migrate more regularly along water courses within a watershed rather than across watersheds, our hypothesis was that genetic and geographic distance would be significantly inversely related among Upper Hudson River collections as implied by the Isolation By Distance (IBD) model but show no genetic-distance relationship among collections made from independent watersheds.

Results

Overview of genetic variation

In total, 106 alleles were observed among the 828 individuals at the nine microsatellite loci screened in this study. A range of 4 to 16 alleles per locus (Table 4) was detected across all populations with a mean of 11.8 alleles observed per locus. 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, rate of recombination, admixture of populations, or random genetic drift (Slatkin 2008). After Bonferroni correction ($P < 0.001$), 3 of 36 possible loci pairs exhibited significant linkage disequilibrium across all collections combined. These included loci Mvi2243 and Mvi474, Mvi480845 and Mvi1354, and Mvi1341 and Mvi1354. However, when these significant associations were investigated within individual collections only one remained consistently significant ($P < 0.05$), Mvi2243 and Mvi474. We found no evidence of Hardy Weinberg disequilibrium at 8 of the 9 loci screened. At Mvi1302, we found disequilbrium after Bonferroni correction in 6 of the 18 collections that were screened. Thus, data from all loci were used in population analysis.

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

We compared measures of genetic diversity in Mid-Northern Hudson mink collected immediately downstream of PCB sources and proximal to the Hudson River (< 6 km tributary stream distance) (n=27) to those collected more distant (> 6 km) (n=98) from the river. In addition, we compared these measures in collections made < 6 km of the Mid-Northern Hudson River and that had high hepatic PCB levels (16 to 174.6 μg total PCBs/g lipid) (n=15) with collections from the same region but > 6 km from the Mid-Northern 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 < 6 km and > 6 km from the main-stem Hudson River. The mean number of alleles observed was 6.67 and 7.78, respectively, but allelic richness (which considers sample sizes) between the two collections was almost identical, 6.57 and 6.63. Observed heterozygosity was also almost identical between the two collections, 0.699 and 0.698, respectively.

All three measures of genetic diversity were also similar when comparing mink collected < 6 km and with high tissue burdens of PCBs to those collected > 6 km from the Mid-Northern Hudson River. For example, although the mean number of alleles observed was lower in the contaminated proximal collection, (6.22 alleles compared to 7.78 alleles), this difference was not seen when comparing allelic richness. The mean allelic richness in contaminated mink collected near the Mid-Northern Hudson River was 6.14 compared to 5.83 in mink collected more distant from the Hudson. Also, observed heterozygosity was similar between the two groups, 0.699 and 0.705, respectively. Thus, none of the indices of genetic diversity revealed decreased variation in mink that probably experienced the greatest exposure to Hudson River borne PCBs.

Genetic relatedness

To test whether demes of mink residing near the Mid-Northern Hudson River in areas of high PCB contamination suffered declines because of PCB bioaccumulation and toxicity, we compared the genetic relatedness among mink collected within 6 km of the Mid-Northern Hudson River (n=27), within 6 km of the Mid-Northern Hudson River with high hepatic burdens of PCBs (n=15), and mink sampled more distantly (> 6 km) from the Mid-Northern Hudson (n=98). After determining the number of pairs of individuals within the three collections that were full sibs, half sibs, parent-offspring, or unrelated we then compared these results among the three collections (Fig. 3). We found no increase in relatedness of highly contaminated mink collected proximal compared to mink collected more distant from the Hudson and in fact the percentage of unrelated pairs was highest in the proximal collection with high PCB burdens (91.6%) compared to the more distant collection (81.1%)

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 (< 6 km from the Mid-Northern 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-Northern Hudson, comprised of mink upstream of PCB sources and > 6 km from the main-stem Hudson downstream of sources [n=116] and Northern Hudson [n=86] 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) downstream of PCB sources, < 6 km from the main-stem Hudson River (Wilcoxon two tail test for heterozygosity excess or deficiency, p = 0.30 and a normal L-shaped distribution in the mode-shift test).

Genetic distinctiveness of highly exposed and matched control mink

The collection of mink from the Mid-Northern Hudson River area was divided into those made within 6 km (n=27) and those more distant than 6 km (n=98) from the Hudson River. We hypothesized that those PCB exposed demes near the river would be genetically distinct from those collected more distant from the river. However, both the Fisher's exact test and F_{ST} analysis failed to detect any evidence of significant overall or locus-specific genetic heterogeneity between these two collections of mink (overall P = 0.552 for Fisher's exact test). There also was no significant heterogeneity between mink with high hepatic burdens of PCBs that were collected within 6 km of the Hudson (n=15) and those collected from locales more distant (n=98) from the Mid Northern Hudson River using either the Fisher's exact test (P = 0.577) or F_{ST} tests.

Genetic population structure

Maine—Mink were collected from the Maine coast and four major river drainages in the state: the Kennebec, Androscoggin, Penobscot, and Saint John. Using Fisher's exact test, six of ten pairwise comparisons between collections 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, watershed connectivity, and genetic similarity (X^2= 16.57, P = 0.550), collections from the Kennebec and Androscoggin were pooled for additional comparisons with other regions. All of these comparisons proved significantly different with the exception of the Saint John versus Penobscot rivers 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 (Maine coast-Saint John) to 0.0019 (Penobscot-Saint John). F_{ST} analysis of four populations (Androscoggin and Kennebec combined) revealed that three of six pairwise comparisons were significantly different, with the exceptions being the Androscoggin-Kennebec versus Maine coast; coastal locales versus Saint John; and Saint John versus Penobscot. In contrast, lnP(D) and ΔK analyses in STRUCTURE indicated only one combined cluster for the four river drainages and coastal locations in Maine (Table 5). However, AMOVA strongly supported the presence of three genetically distinct groups among these collections (Table 5); the Kennebec-Androscoggin, Maine coast, and Penobscot-Saint John.

Ontario—Mink collections were compared among four sites in Ontario, including western Lake Erie, Lake St. Clair, St. Clair River and St. Lawrence River. Highly significant genetic heterogeneity was observed among these collections with 8 of 9 loci (except for locus 480845 at which P = 0.037) exhibiting statistically significant differences in allelic frequencies (Fisher's exact test; P < 0.001). All pairwise comparisons of allele frequencies among collections were highly significant different (P < 0.001), except for 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. Both lnP(D) and ΔK analyses of the STRUCTURE results indicated four distinct clusters for the Ontario collections, a result that was supported by use of AMOVA (Table 5).

Because of concerns over 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 lower 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).

Major ecoregions in New York State—We compared genetic differentiation of collections representative of seven major ecoregions 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 overall genetic differentiation among these major ecoregions at all nine loci investigated. Furthermore, Fisher's exact test showed highly significant differentiation among all 21 possible pairs of collections from these seven major

New York ecoregions. The overall mean F_{ST} value among these seven ecoregion collections was 0.019. F_{ST} values among these individual collection pairs ranged between 0.0037 and 0.0526. After Bonferroni correction, F_{ST} comparisons among all 21 pairs of major ecoregions zone collections were highly significantly different except for two: the Hudson River Valley versus Taconic Highlands (P = 0.029) and Appalachian Plateau versus Mohawk Valley (P = 0.017). Both STRUCTURE analyses results revealed less heterogeneity than shown by the genetic distance comparisons, indicating only two clusters (Table 5) among the seven New York ecoregions. AMOVA indicated the presence of 2 or 3 genetical groupings among the ecoregions (Table 5). 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 disequilibrium suggesting that these collections from ecoregions including the Adirondacks were comprised of genetically heterogeneous assemblages of specimens

Minor ecoregions within the Adirondacks—With the exception of the Adirondacks major ecoregion, collection sizes at minor ecoregions within the seven major New York ecoregions were too small to make meaningful statistical comparisons. Therefore, we were restricted to investigating the extent of genetic differentiation among collections from five minor ecoregions within the Adirondacks major ecoregion including the Central Adirondacks, Eastern Adirondacks Foothills, Eastern Adirondacks Transition, Western Adirondacks Foothills, and Western Adirondacks Transition. Fisher's exact test revealed highly significant genetic heterogeneity, with four of the nine loci exhibiting significant heterogeneity among the minor ecoregions. With Fisher's exact test, 8 of 10 comparisons of minor ecoregion collection pairs yielded significant genetic differentiation. The two pairs that were not significantly differentiated included Eastern Adirondacks Transition versus Western Adirondacks Foothills and Western Adirondacks Foothills versus Western Adirondacks Transition. The mean F_{ST} value across all minor ecoregion comparisons was 0.017. F_{ST} analysis also revealed significant genetic differentiation in 6 of 10 pairwise comparisons. With F_{ST} analysis, in addition to the two groupings above, the Eastern Adirondacks Foothills versus Western Adirondacks Foothills and Eastern Adirondacks Transition versus Western Adirondacks Foothills failed to display significant genetic differentiation. STRUCTURE indicated two clusters among the Adirondacks minor ecoregions, whereas AMOVA revealed three distinct genetic groups (Table 5) including Central Adirondacks, Eastern Adirondacks Foothills, and Western Adirondacks Transition.

Hudson River watershed hydrologic subunits—Overall allelic differentiation among collections from 16 hydrologic subunits containing main-stem tributaries of the Upper Hudson River was highly significant, with all nine loci exhibiting significant differentiation. In total, using Fisher's exact test, 48 of 120 (40%) comparisons of allelic differentiation between collection pairs were significant (after conservative Bonferroni correction, P was significant at < 0.0004).

The mean F_{ST} among the 120 pairs of collections was 0.024 with F_{ST} values at individual loci ranging from 0.006 (Mvi1381) to 0.048 (Mvi3402). F_{ST} comparisons among pairs of collections revealed that 29 of 120 were statistically significantly different (after Bonferroni correction, P was significant at < 0.0004). Similarly, both lnP(D) and ΔK analyses of STRUCTURE results indicated heterogeneity among collections from the 16 hydrologic units within the Upper Hudson River (Table 5). Both analyses detected four distinct clusters within this data set. Use of AMOVA resulted in the identification of three genetically distinct groups of mink from these Upper Hudson hydrologic subunits (Table 5).

Other comparisons—Both Fisher's exact test and F_{ST} analyses revealed that pairwise comparisons of collections from New Brunswick, Rhode Island and pooled Maine collections were genetically distinct (Table 5). When STRUCTURE was used to compare among these three collections, lnP(D) analysis indicated the presence of three clusters, whereas two clusters were observed with ΔK analyses. Use of AMOVA suggested the presence of three distinct groups among these collections.

Summary of results from four different analyses—We summed the total number of genetically distinct units of mink that we identified across all collections by each of the four analyses that were 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—We found a significant positive relationship between genetic (F_{ST} or $F_{ST}/1\text{-}F_{ST}$) and geographic distance among the 16 Hudson River collections (r = 0.635, P = < 0.001) (Fig. 4a) and weakly so for the four locales in Ontario (r = 0.571, P = 0.044). In contrast, we failed to find a significant relationship among geographically disjointed mink collections from Maine (r = 0.522, P = 0.127) (Fig 4b).

Discussion

Our study provided two major findings. First, mink that reside in close proximity to the Hudson River and that bioaccumulate high levels of Hudson River-borne PCBs were not genetically distinguishable from collections made at control sites more distant from the river. 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 proximity to the Hudson either did not suffer elevated rates of mortality from PCB exposure resulting in genetic bottlenecks or if severe mortality did occur demes proximal to the river served as a sink for immigrants from elsewhere. At this time, we cannot empirically differentiate between the two scenarios.

Second, mink from throughout eastern North America exhibit high levels of genetic population structure. We investigated the patterns and magnitude of genetic structuring among populations throughout eastern North America to provide a framework with which to evaluate the significance of differentiation between highly exposed and less exposed Hudson River demes. We detected significant genetic differentiation among collections from

geographically distant and even proximal sites, individual drainage units, different tributaries of individual watersheds, and major and minor ecoregions. 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 sites near Lake Erie in Ontario for which all analyses identified four genetically distinct population units). 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 equilibria within clusters whereas the latter approaches evaluate whether alleles are drawn from the same distribution in all populations.

Previous studies in other taxa and with other contaminants have provided mixed results regarding the effects of pollutants on levels of genetic diversity in exposed populations compared to reference locales. Chronic exposure to non-mutagenic contaminants may erode genetic variation by severely cropping population size and thereby eliminating rare alleles or by strongly selecting for advantageous homozygote genotypes. For example, Fratini et al. (2008) reported a decrease in microsatellite variability in an intertidal crab *Pachygrapsus marmorat*us that had bioaccumulated elevated levels of four metals along the Italian Tuscan coast. Similarly, populations of brown trout (*Salmo trutta* L.) in rivers in metal mining impacted regions of southwest England demonstrated lower levels of microsatellite diversity than those in nearby cleaner rivers (Paris et al. 2015). Also, metal impacted trout populations had suffered significant declines and were genetically distinct from those in proximal cleaner rivers. Finally, the historical divergence of metal contaminated and clean populations dated to the onset of the Industrial Revolution. 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. Similarly, Stapleton et al. (2001) failed to find an effect of PCB exposure on minisatellite DNA mutation rate in tree swallows from the upper Hudson River compared to control sites. 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 to be detected. However, because of their documented PCB exposure, small reproductive units, relative isolation, and known sensitivity to reproductive impairment from PCBs exposure, it is surprising that mink proximal to PCB-contaminated Hudson River locales failed to exhibit reduced genetic variation or altered population structure. This is particularly intriguing because of results of recent laboratory studies which have demonstrated the sensitivity of ranched mink to impaired reproductive performance, decreased kit growth, development of jaw lesions, and decreased juvenile survivorship from exposure to diets of 2.5 to 20% of Hudson River-collected fishes (Bursian et al. 2013ab). The authors concluded that a maternal diet of 10% or less of Hudson River fishes would result in a 20% increase in kit mortality. Natural populations of mink that we analyzed in our study were dietarily exposed and

bioaccumulated hepatic PCB levels (expressed as TCDD Toxic Equivalency Quotients) that often exceeded those that incurred reproductive failure in these laboratory studies (Bursian et al. 2013ab). Thus, it would be anticipated that the sensitivity of mink to reduced reproductive success and survivorship at these exposure levels would crop census and effective population size in Hudson River populations. For example, the mean maternal hepatic burden of PCBs that induced a 20% incidence of jaw lesions in these laboratory studies was 2.8 μg total PCBs/g liver (Bursian et al. 2013b). This concentration of total hepatic PCBs is exceeded by the hepatic concentrations quantified in mink collected proximal to river in the Mid-Northern Hudson River region between Hudson Falls and Troy, New York, suggesting that toxicity has occurred from PCB exposure in the Hudson River mink population.

However despite this prediction, our highly-exposed collection from the Mid-Northern Hudson River showed no evidence of a genetic bottleneck nor an influx of migrants from nearby non-contaminated source populations; two possible explanations for their maintenance of robust levels of genetic diversity. It is also possible that the sensitivity of natural populations of mink to PCB-induced toxicities is significantly less than that of ranched mink perhaps resulting from physiological acclimation or genetic adaptation as seen in other organisms from the Hudson River and elsewhere (Wirgin and Waldman, 2004; Wirgin et al. 2011). Alternatively, the carrying capacity of habitat to demes proximal to the Hudson River may have been reached and even limited recruitment due to PCB exposure may be sufficient to maintain populations.

As expected, because of the effects of ecological barriers to gene flow, their division into small isolated demes, and short generation times, mink exhibited high levels of population structuring. However, use of a variety of different statistical approaches yielded differeing results in the number of distinct populations detected. We feel that this discrepancy in population structure results among approaches may be in part due to the sampling regime used in our study. Our collections were opportunistic, largely made by part-time trappers for other purposes, and, consequently, did not respect specific geographic or temporal borders. Thus, it is likely that our individual collections sometimes sampled animals drawn from more than a single population. That is supported by our finding that some of our collections from within major or minor ecoregions were not in Hardy Weinberg equilibrium. Schwartz and McKelvey (2009) showed that when individuals were distributed continuously over a landscape and mated preferentially with individuals from neighboring demes, STRUCTURE sometimes produced surprising 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, STRUCTURE does not perform well at assigning clusters when F_{ST} values are less than 0.05 (Latch et al. 2006) and most pairwise 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 ecoregions in New York, and minor ecoregions within the Adirondacks major ecoregion, respectively.

Furthermore, STRUCTURE may not perform well when too many populations (>10) are included in the analysis (Pearse and Crandall 2004). Other investigators have found that

STRUCTURE may yield 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-F_{ST}) and geographic distance were significantly correlated among collection sites and found differing results between collections made along a single waterway or among multiple drainages. Collections made along the main-stem Hudson exhibited a highly significant positive correlation between genetic and geographic distances ($P < 0.001$), while Euclidean distances among watersheds in Maine did not. The relationship among the four sites bordering Lake Erie in Ontario proved marginally significant ($P = 0.044$) reflecting the proximity and potential aquatic connectivity among 3 of the 4 collection sites. Our results largely confirmed that gene flow in mink followed the isolation-by-distance model longitudinally along waterways such as the Hudson River but that non-riparian habitats proved at least to be partial barriers to gene flow.

How do levels of population differentiation and genetic variation that we report compare to those previously observed among mink collections? Using microsatellite analysis, Kidd et al. (2009) determined that nearly two-thirds of mink in two populations in Ontario, Canada, were either escapees from nearby ranches or their descendants. Belliveau et al. (1999) reported a lower level of genetic diversity at seven microsatellite loci in wild mink from Nova Scotia compared to mink from four nearby ranches probably due to their small collection area which was populated by a limited number of male mink and because each demands considerable territory (Dunstone 1993). Stevens et al. (2005) analyzing microsatellite variation among eight localities in Arkansas and Tennessee, USA, did not find significant differentiation among six riparian localities in Arkansas; only comparisons between the distant site in Tennessee and those in Arkansas proved significant. 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. Furthermore, genetic distance was positively correlated with straight-line geographic, not riverine distance.

Larger studies of microsatellite variation have been conducted among introduced populations of American mink in Europe. For example, Zalewski et al. (2010) reported on microsatellite variation in American mink collected from ten sites in Poland and at several nearby ranches. Strong genetic differentiation among collections was found using both pairwise F_{ST} and STRUCTURE analyses. They identified at least five distinct clusters of mink and concluded that this was surprising 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. Thus, levels of microsatellite DNA variation and genetic discontinuity that we found in mink across locales in eastern North American was at the high end of that observed in mink elsewhere. For the first time, we demonstrate that a

variety of natural barriers can serve to reproductively isolate mink demes, sometimes even those that are spatially proximal.

Conclusions

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. Population structuring in mink was strong and usually respected ecological features thought to promote reproductive isolation. Gene flow followed the isolation-by-distance model longitudinally along waterways such as the Hudson but non-riparian habits proved at least to be partial barriers to gene flow.

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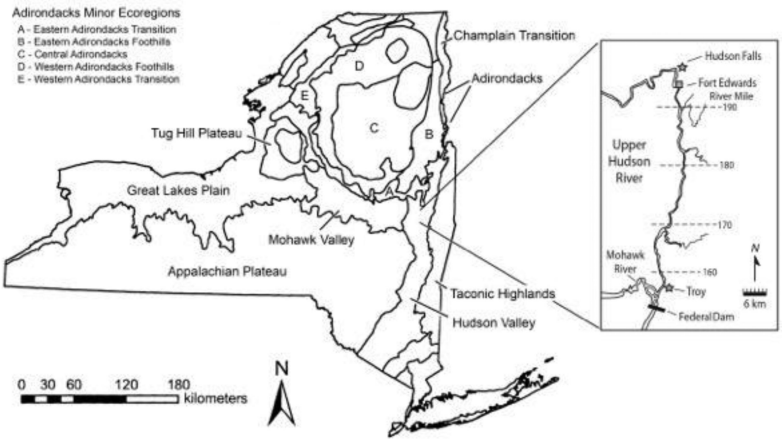

Figure 1.

A map depicting eight major ecoregions in New York State and five minor ecozones within the Adirondacks major ecoregions where mink were collected and genotyped for this study. The inset depicts the Upper Hudson River from Hudson Falls to the Federal Dam in Troy where mink where collected within 6 km stream distance of the Hudson.

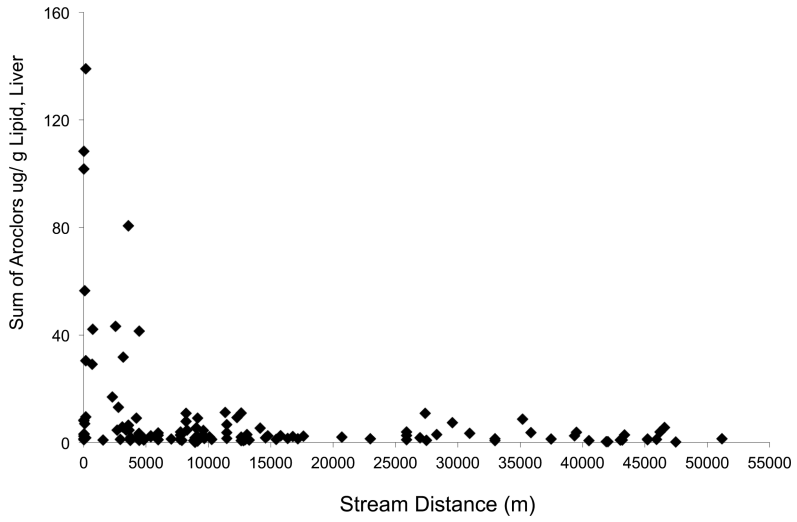

Figure 2.

Levels of sum of hepatic Aroclors (μg/ g lipid liver) in individual mink (both genders) collected proximal and more distant from the Upper Hudson River between Hudson Falls and Troy, New York between 1998 and 2001 and analyzed for microsatellite genotypes in this study. Levels of the sum of Aroclors in each individual are depicted in relation to stream distance (0 to 55 km) that each mink was collected from the main stem Upper Hudson River.

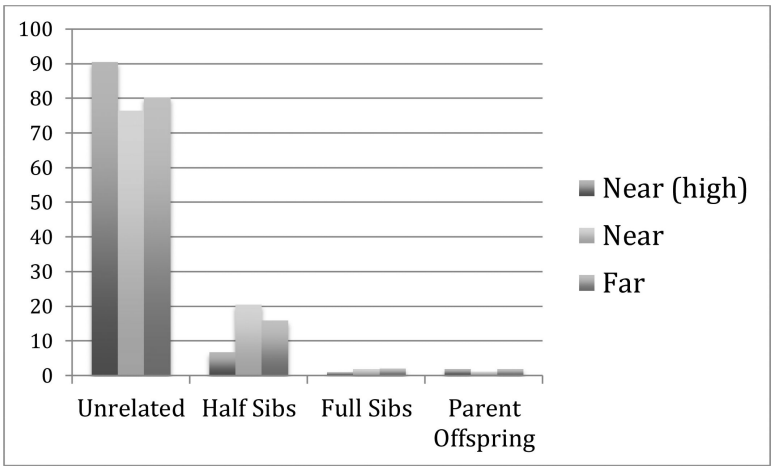

Figure 3.

Relatedness of all American mink collected within 6 km (stream distance) of the mid-Northern Hudson River (N=27), mink collected within 6 km with high PCBs burdens (16 to 174.6 μg total PCBs/g lipid) (n=15), and mink with low PCB burdens collected greater than 6 km from the Hudson River (N=98). Pairwise comparisons of the relatedness of individual specimens were made with the ML-Relate program. All pairwise comparisons were characterized as unrelated, half sibs, full sibs, or parent-offspring.

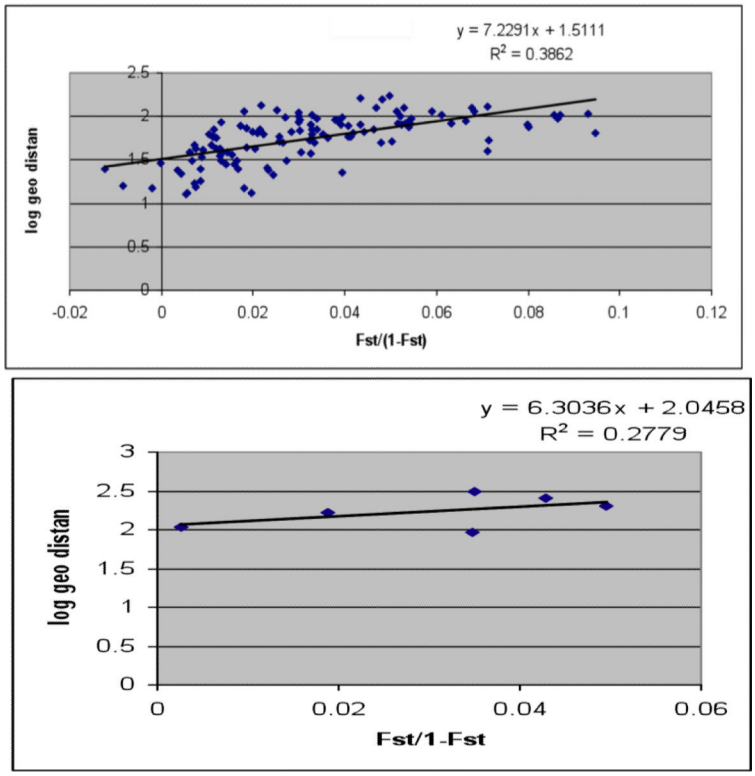

Figure 4.

A. 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 within Hudson River, New York watershed.

B. 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.

Table 1

Collection locales, drainage unit or ecoregion, and number of American mink analyzed at nine microsatellite loci in this study from each drainage unit or ecoregion.

Locale	Drainage unit or ecoregion	Number of animals analyzed
Canada	Western Lake Erie	19
	St. Clair River	20
	Lake St. Clair	19
	St. Lawrence River	22
	New Brunswick	14
Rhode Island	Inland	31
	Coastal salt ponds	8
Maine	Lower Androscoggin	20
	Kennebec	10
	Maine Coast	9
	Upper Penobscot	30
	Saint John	15
New York	Hudson River watershed and elsewhere	611

Table 2

Subdivisions of New York State collections by (A) 8 major New York ecoregions (*n*=611), B) 5 minor ecoregions in the Adirondacks major ecoregion (*n*=182), and (C) 16 hydrologic subunits within the Hudson River watershed (*n*=261). New York State ecoregions are defined in Dickinson (1983) and Will et al. (1982) and Hudson River hydrologic subunits in the New York State Master Habitat Database (NYSDEC 2003).

A) Major New York ecoregions	Number mink Analyzed
Appalachian Plateau	131
Great Lakes Plain	47
Mohawk Valley	29
Hudson Valley	87
Taconic Highlands	64
Adirondacks	182
Tug Hill Plateau	70
Champlain Transition	1
B) Minor ecoregions in the Adirondacks major ecoregion	
Eastern Adirondacks Transition	32
Eastern Adirondacks Foothills	45
Central Adirondacks	65
Western Adirondacks Foothills	27
Western Adirondacks Transition	13
C) Hydrologic subunits in the Hudson River watershed	
Northern Hudson, Indian River	14
Northern Hudson, Cedar River	11
Northern Hudson, Mill Creek-Hudson River	13
Northern Hudson, Patterson Brook-Hudson River	11
Northern Hudson, Stewart Brook-Hudson River	19
Northern Hudson, North River	7
Mid-Northern Hudson, Sacandaga River to Clendon Brook	13
Mid-Northern Hudson, Clendon Brook to Snook Kill	22
Mid-Northern Hudson, Batten Kill and Hudson River-Snook Kill to Battern Kill	15
Mid-Northern Hudson, Hudson River-Snook Kill to Fish Creek	35
Mid-Northern Hudson, Hudson River –Fish Creek and Batten Kill to Hoosic River	13
Mid-Northern Hudson, Hoosic River and Little Hoosic River	40
Mid-Northern Hudson, Hudson River-Hoosic River to Mohawk River and Hudson River-Hoosic River to Troy, NY	6
Scanadaga Reservoir, Scanadaga Reservoir	25
Mid-Hudson, Claverac Creek	8
Mid-Hudson, Lower Kinderhook	9

Table 3

Characterization of nine American mink microsatellite loci used in this study

Locus name	Genbank identifier	Repeat motif	Primer sequences	Annealing temperature	Molecular sizes (bp)
Mvi 2243	AY053518	$(TG)_4TA(TG)_{14}$	CGGACATTTGTTCTAAGAGGT AGATTAACAAGCCATGCTC	59° C	132-164
Mvi 1354	AF480852	$(CA)_{22}$	CCAACTGGAGCAAGTAAAT CATCTTTGGGAAAGTATGTTT	59° C	176-216
Mvi 1302	AF480846	$(GT)_{17}$	CATAGGTTCCAGGGATTAGAA ATGCCATTACAGTACGACTCA	60° C	210-224
Mvi 1381	AF421188	$(AC)_{19}$	CCATCGGAGTTTCTCATCGT CCAGGTGCCCCTTACATT	60° C	185-198
Mvi 099	AF132106	$(CA)_{16}$	TGAGGCAAGAGGAGCAAAAG TTTGCATTTCCCTGATGAGG	60° C	321-351
Mvi 1273	AF480845	$(GGAA)_6$	ATAGCATCCCTAAAATGG TCCCCCTCCAGACCTCTA	56° C	183-215
Mvi 9700	AF474150	$(CCTT)_{10}$	AGCCTTCCTGGGTATCTA TGAAAAGTTTATTGGTCT	56° C	252-268
Mvi1341	AF480850	$(CA)_{17}$	GTGGGAGACTGAGATAGGTCA GGCAACTTGAATGGACTAAGA	59° C	148-176
Mvi 3402	AY244352	$(ATC)_5(TGC)_9$	CAAAGGAGTATTCACAGG CCGACACCGCCACAGCAG	60° C	290-296

Table 4

Locus name, number of alleles, mean allelic richness, mean observed (H_O) and expected (H_e) heterozygosities, mean F_{ST} and allelic differentiation (P values) among all American mink collections at nine microsatellite loci. Bold text indicates Bonferroni corrected significance at the $P = 0.05$ level.

Locus	# Alleles Sampled	Mean Allelic Richness	H_o	H_e	F_{ST}	P
Mvi2243	13	3.71	0.628	0.700	**0.035**	< 0.0001
Mvi1273	16	5.35	0.814	0.866	**0.024**	< 0.0001
Mvi9700	5	3.91	0.863	0.739	**0.048**	< 0.0001
Mvi1341	15	5.20	0.789	0.845	**0.024**	< 0.0001
Mvi1354	16	5.32	0.782	0.875	**0.048**	< 0.0001
Mvi1302	10	4.94	0.656	0.852	**0.029**	< 0.0001
Mvi3402	4	1.84	0.347	0.334	**0.015**	< 0.0001
Mvi1381	12	4.28	0.730	0.767	**0.015**	< 0.0001
Mvi099	15	4.13	0.702	0.736	**0.019**	< 0.0001
Mean	11.8	4.29	0.701	0.746	**0.037**	Infinity

Table 5

A comparison of genetic divergence among collections of American mink from various major ecoregions, minor ecoregions, and drainage units in North America based on DNA microsatellite analysis at nine loci

Sample collections	Number of specimens	# Collection locations	Fisher's Exact Test	F_{ST}	STRUCTURE	AMOVA
ME, NB, RI	137	3	3	3	3	3
Major NY Ecoregions	610	7	4	3-4	2	2-3
16 Hudson River Locales	305	16	1	3	4	3
Minor Ecoregions Adirondacks	182	5	4	3	2	3
Ontario Locales	80	4	4	4	4	4
Maine Drainage Units	84	5	3	3	1	3
Total			19	19-20	16	18-19