# THE GENETIC VARIATION OF PEREGRINE FALCONS (FALCO PEREGRINUS) IN THE CHESAPEAKE BAY

by

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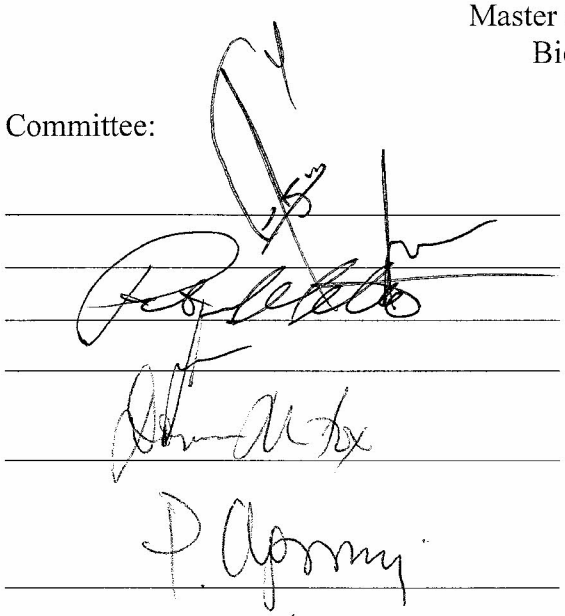

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# The Genetic Variation of Peregrine falcons (Falco peregrinus) in the Chesapeake Bay

A Thesis submitted in partial fulfillment of the requirements for the degree of Master of Science at George Mason University

by

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## DEDICATION

This is dedicated to all of the Peregrine falcons and wildlife that have suffered as a result of mankind's continued progress. It is my hope that my research will inspire other like-minds and pave the way for progress in the field of environmental remediation.

Also to my family and friends, I could not have gotten here without you. Thank you for all of your continued love, support, patience, and ability to ask me critical, thought provoking questions about my research. Thank you to my mother and father who helped me cultivate my scientific mind by always pushing me to be critical and ask questions in every situation.

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## ABSTRACT

THE GENETIC VARIATION OF PEREGRINE FALCONS (FALCO PEREGRINUS) IN THE CHESAPEAKE BAY

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George Mason University, 2014

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For the last century, the local population of Chesapeake Bay Peregrine falcons (*Falco peregrinus*) has been fluctuating in its abundance, making genetic diversity difficult to assess (Johnson et al., 2010). Recently, the well-being of falcon populations in Coastal Virginia has improved, and their population numbers have been stabilizing. The extent of the Peregrine falcon genetic variation in the Chesapeake Bay population of falcons is unknown, yet the possibility of a genetic bottleneck does exist. To assess levels of allelic heterozygosity in this population, genetic analyses were performed on blood and feather samples taken from breeding pairs in the Bay during 2007 and 2013. DNA was extracted from these samples then fingerprinted at microsatellite loci with Peregrine falcon specific labeled primers (Nesje 2000b). Fingerprint results were analyzed using the GenAlEx 6.5 software, then observed alleles ($A_O$), expected heterozygosity ($H_E$), observed heterozygosity ($H_O$), and fixation index ($F_{ST}$) were assessed. At each locus studied, there

were similarities in $H_O$ between contemporary populations of falcons in the Chesapeake Bay and other global reintroduction programs in southern Scandinavia and southern Norway. At locus NVH *fp54,* observed heterozygosity in the Chesapeake Bay population was dramatically lower than that in the European populations. In the Chesapeake Bay population, $F_{ST}$ values were close to zero at each locus, suggesting complete panmixis of this population. The cluster analyses performed with the STRUCTURE 2.3.4 software confirmed that the contemporary populations of Peregrine falcons in the Chesapeake Bay are genotypically one genetic cluster. Comparisons were made between the historic population of Peregrine falcons endemic to the east coast (the Appalachian mountains) and the contemporary population of birds reintroduced to and currently living in the Chesapeake Bay. These comparisons showed no significant difference when levels of observed heterozygosity, expected heterozygosity, and number of alleles were assessed. This suggests that the reintroduced population of peregrines is no more able to cope with anthropogenic or environmental disturbances than their historic counterparts. Known susceptibility of Peregrine falcons to environmental contamination as well as relatively low heterozygosity levels in the contemporary Chesapeake Bay population of Peregrine falcons suggest that continued monitoring and further conservation efforts of this population are warranted.

## CHAPTER ONE: INTRODUCTION

American Peregrine falcon (*Falco peregrinus*) populations comprise one of the largest reintroduction programs in the history of endangered species management (Barclay, 1980 and Cade et al., 1988). Starting at the end of World War 2, peregrines experienced massive declines as a result of insecticides, in particular, DDT/DDE and Dieldrin, which nearly led to the global extinction of several bird species (Barclay, 1980 and Cade et al., 1988). When ingested, these insecticides bioaccumulate weakening the integrity of the structure of avian eggshells causing frequent reproductive failures. This inability to reproduce, in turn, severely limited the amount of genetic diversity in affected populations, resulting in a genetic bottleneck effect (Barclay, 1980 and Cade et al., 1988).

During 1964, surveys of 70% of Peregrine falcon nests east of the Mississippi River demonstrated that they were completely deserted, while by 1965 a decline in peregrine populations in Europe and North America was observed (Barclay, 1980). DDT was banned for agricultural use in 1972 in the U.S.A.. The global population of peregrines experienced a steady recovery since then and efforts have been focused on the recovery of Peregrine falcon populations in North America, in particular the East Coast.

Historically, out of the 19 subspecies found globally, two subspecies of falcons were native to the east coast of North America: *Falco peregrinus anatum*, and *Falco*

*peregrinus pealei (*Barclay, 1980). These same two subspecies were used in the majority of conservation and reintroduction efforts (Johnson et al., 2010). However, the population of peregrines that currently exists in the Chesapeake Bay is a conglomeration of populations from seven different subspecies and several different geographic areas (Barclay, 1980 and Cade et al., 1988).

## 1: Background

Peregrine falcons have recently gained additional popularity from their struggles with pesticides and other organocontaminants. Once a bird associated with royalty, the Peregrine is now seen as a representative of the effects man-made toxins can have on the natural world. Seventeen to nineteen subspecies of Peregrines exist globally with two being endemic to North America (*Falco peregrinus anatum* and *Falco peregrinus pealei*). In 1999, Peregrine falcons were federally delisted from the Endangered Species List due to recovery.

Peregrines are large falcons, often the size of crows, and the most widespread raptor occurring everywhere on the globe except the Arctic, Iceland, and New Zealand. Peregrines prey primarily on other birds, preferentially marine and shore birds, and favor a variety of habitats so long as they are open, have tall cliffs, and are near riparian areas. They prefer to hunt during the daytime. Their method of attack involves diving straight down from the sky at high speeds and knocking their prey unconscious with their legs and/or bodies then retrieving the incapacitated prey from the ground.

As typical for most raptors, the peregrines exhibit sexual dimorphism, with females being larger than males. Female peregrines can be 45-51 cm long, weigh 0.8-1.1 kg, and have a

wingspan of 94.5-102 cm while males can be 40-46 cm long, weigh 0.45-0.70 kg, and have a wingspan of 79-91 cm. They are the fastest animal in the world with hunting dives reaching upwards of 322 kilometers per hour.

Peregrines are monogamous birds that show high site fidelity (philopatry) and are sexually mature between two and three years of age. The timing of breeding can vary depending on location, but in the Chesapeake Bay region eggs are laid in March and April, hatch May through June, and young fledge in June and July. A typical female will lay eggs every two to three days during the breeding season and have an average clutch size of four. Out of each clutch, typically one egg will fail to hatch (Barclay, 1980). Females are responsible for incubating the eggs (Barclay, 1980) and can re-build the nest should it happen to be destroyed early in the breeding season. During this time, both males and females will exhibit aggressive territoriality within a 1.5-3.2 km radius of their nesting site ("Ohio Division of Wildlife Life History Notes Peregrine Falcon Scientific Name: Falco Peregrinus." 2013).

Population density in Peregrine falcons is primarily regulated by the availability of nesting sites and secondarily by territoriality (Cade et al., 1988). However an interesting aspect of peregrine populations is that breeding populations remain relatively constant from breeding season to breeding season. This regular occupancy of virtually all available nesting sites suggests that a mobile population of juvenile, non-breeding birds exists that travels with the breeding population and takes over nest sites as the occupants die or fail to return (Cade et al., 1988 and Barclay, 1980).

Nests are often in a scrape and on large structures such as cliffs, bridges, and buildings. Eggs normally hatch after 32 to 34 days of incubation yielding altricial young that fledge anywhere from 35 to 42 days after hatching. On average, one to three young are fledged and one brood is produced each breeding season ("Ohio Division of Wildlife Life History Notes Peregrine Falcon Scientific Name: Falco Peregrinus." 2013). Unfortunately, 70% of nestlings do not survive their first year after hatching and 25% of those survivors perish each subsequent year (Barclay, 1980). This can mainly be attributed to collisions with man-made objects in cities and predation in nature. Birds that survive are quite long-lived, living 12-18 years in the wild. ("Ohio Division of Wildlife Life History Notes Peregrine Falcon Scientific Name: Falco Peregrinus." 2013).

## 2: Motivation

Peregrine falcon populations first began to decline in the late 1940s as a result of the introduction of dichlorodiphenyltrichloroethane (DDT) to the agricultural community (Barclay, 1980 and Cade et al., 1988). DDT was primarily used as a potent insecticide by troops and civilians overseas during World War II to control diseases such as malaria typhus, as it is highly effective against arthropods. Unfortunately DDT made it into the food chain through prey animals, such as mammals and birds, which caused it to bioaccumulate in the tissues and eggshells of top predators such as Peregrine falcons and Bald eagles (*Haliaeetus leucocephalus*). The bioaccumulation of DDT in Peregrine falcons and other raptors causes reproductive failure (Barclay, 1980, and Cade et al., 1988) through the thinning of eggshells by interfering with shell synthesis in the shell

gland of the bird (Barclay, 1980). With weaker eggs, the weight of the female bird incubating the eggs causes the eggs to crack and the embryos to perish.

As a result of the pervasive usage of DDT in agricultural enterprises, by 1964 the *F. p. anatum* populations were extirpated in the East (Barclay, 1980) and by 1979 *F. p. tundrius* populations had been reduced by 50-60%, while populations of *F. p. pealei* remained largely unaffected (Johnson et al., 2010). In 1969, DDT was banned in Canada and in 1972 it was banned in the United States. The banning of DDT in conjunction with re-introduction efforts of captive-bred individuals in declining or extirpated populations aided in the recovery of peregrine populations (Barclay, 1980 and Cade et al., 1988). Peregrine falcons were delisted from the Federal List of Endangered Species in the United States in 1999 because of recovery. While overall peregrine populations have recovered, the Chesapeake Bay population has continued to fluctuate dramatically in distribution and relative abundance, thereby making their genetic diversity difficult to assess (Johnson et al., 2010).

## 3: State of Research

A number of other peregrine populations were assessed to aid in elucidating potential challenges facing the Chesapeake Bay. Franke et al. (1996) assessed a population of *F. p. tundrius* near Rankin Inlet, Nunavut, Canada to determine long-term trends of persistent organochlorine pollutants (POPs) and their effects on reproductive success. It was found that peregrines in this part of Canada had much higher levels of organochlorine (OC) contamination including DDE, polychlorinated biphenyls (PCBs), and dieldrin, than their counterparts in Greenland and Alaska. The original explanation

for this was that this population spent their winters in South America, where they fed on contaminated prey, as DDT was not banned there yet during this study. Assessments of eggshell thickness and breeding successes for two time periods demonstrated that from 1991-1994, eggshells were on average, 15% thinner than those shells produced before DDT was introduced. Moreover, in 1981-1985, 10% of breeding attempts were unsuccessful as a result of pesticide contamination (Johnstone et al., 1996). Interestingly, the "overwintering contamination" hypothesis was not supported by evidence, as year-round resident Canadian waterfowl specimens also displayed high levels of OC contamination and toxic residues. However, while the resident waterfowl were highly contaminated, the resident mammals were relatively not contaminated. This led to the discovery that habitat and trophic level have a heavier contribution to contaminant levels than to the species' migration range (Johnstone et al., 1996).

Further studies done on the Rough-legged hawk (*Buteo lagopus*), another raptor native to this area whose primary prey sources are mammalian, discovered that levels of OCs and toxin residues in this hawk were significantly lower than those in peregrines from the same area. Through the investigation of tissue and blood samples, scientists were able to decipher that the most contaminated birds in this habitat are seabirds, followed by waterfowl and shorebirds, while passerines exhibit the least amount of contamination. Amongst the seabirds, the Long-tailed duck (*Clangula hyemalis*) was found to be the most contaminated and its contamination levels have increased over decades while the contamination levels of other birds have decreased. Unfortunately, this one species of duck accounted for 25% of the diet in Rankin Island peregrines and 0-5%

of the diet in Alaskan peregrines (Johnstone et al., 2006). As a result, 28% of the eggshells of Rankin Island peregrines exhibited thicknesses below critical levels and PCB levels in the nestlings had continued to increase since the 1980s. Overall, the levels of contamination in the Rankin Inlet peregrine population had not decreased as was expected after OCs were restricted. In other locations, OC levels have decreased in *F. p. anatum* and *F. p. tundrius* subspecies. However, researchers were able to deduce that the contamination in the Rankin Inlet population came mainly from highly contaminated prey from diverse habitats, not from resident species prey, not from differences in the birds' migration route, and not from prey in South America where the birds overwinter (Johnstone et al., 1996).

In a follow-up study done by Franke et al. (2010) on the Rankin Inlet population of peregrines in Canada, the effects of long-term trends of POPs were assessed once again as well as the effects of weather on the reproductive successes of the falcons. In this study as with the previous Rankin Inlet study, the scientists assessed the birds during two time periods: 1982-1989, and 2002-2009. They found evidence of a decrease in the number of offspring, the number of young that reached banding age, and the number of territories occupied during the breeding season. Assessments showed that OC loads in the falcons were decreasing over time; blood plasma DDE levels had decreased from the 1980s to the 1990s, average PCB levels had decreased from the 1980s to the 1990s and then increased again in the 2000s, and dieldrin levels decreased dramatically from the 1980s to the 1990s, then leveled off (Franke et al., 2010). This data caused the researchers to explore another possible explanation for the loss in reproductive

productivity (number of attempted breedings and number of offspring to survive to banding age); a changing climate. The researchers looked at local weather patterns and found that from 1982-2009, the average summer temperature in Rankin Inlet had increased by 1.5°C and total precipitation had declined slightly, but not significantly. However, they did notice a slight trend towards wetter Julys and drier Junes and Augusts. This is critical, as peregrines tend to hatch around July 11$^{th}$ in this geographic range (Franke et al., 2010). This means that birds were hatching during the wettest part of the summer. Researchers set up cameras near the nests and found that with the increase in precipitation, nests were flooding causing nestlings to drown, and even when nestlings did not drown they often perished from the cold, wet conditions. When cameras were not present, scientists performed nest surveys and found significant numbers of dead and/or missing nestlings. They also found evidence of starvation in nestlings as a result of a lack of prey sources presumably killed by inclement weather (Franke et al., 2010). In this population, it is critical to continue to monitor hatching successes and organocontamination as well as work to continue the ban on DDT. Lobbyists have recently been trying to resurrect the usage of DDT to combat malaria and other insect-borne diseases in Mexico and Central America, which are overwintering locations for many peregrines. If DDT were to be resurrected there, the contamination effects would be devastating to Peregrine falcons and other raptors (Franke et al., 2010).

Johnson et al. (2010) assessed the level of genetic variation in migratory populations of peregrines in North America and Greenland and performed genetic analyses to approximate an effective population size as the number of breeding

individuals. These analyses included the measurement of the changes in allelic richness and heterozygosity as well as the degree of differentiation in the population. The migratory population was measured over 7 generations in their migratory range of Texas and over 4 generations in their range in Southern Greenland. Genetic analyses found the falcons to be polymorphic at 8 out of 11 microsatellite loci which supported the hypothesis that this population does exhibit allelic richness, however populations of *F. p. pealei* were found to exhibit less allelic richness than populations of *F. p. anatum* and *F. p. tundrius*. Analyses also found that *F. p. anatum* and *F. p. tundrius* are genetically identical species historically but contemporary populations in Greenland and North America exhibit genetic differentiation while contemporary populations in Alaska and Canada do not (Johnson et al., 2010).

The contemporary genetic variation in North American populations is commonly attributed to the captive reintroductions of peregrines of a different subspecies whose genetic stock came from Europe, South America, or Australia. These reintroductions were done to help the populations to rebound from declines that resulted from agricultural usage of DDT in efforts known as the Eastern Peregrine Reintroduction Plan. Indeed, the allele frequencies and levels of genetic diversity in contemporary samples are the same as those from historic samples, thereby proving that there is no real loss of allelic richness or genetic diversity in contemporary peregrine populations in this region. This could be attributed to the fact that peregrines are relatively long-lived birds and this quality could help to buffer genetic drift and/or a loss of genetic diversity (Johnson et al., 2010).

Overall, a majority of the studies supported the assessment that this population is large enough and stable enough to offset genetic drift and maintain gene flow. As of 2004, 77% of peregrines in the population had lower than detectable DDE concentrations (compared to 1% between 1978-1994). While estimation of exact effective population size for this population was not possible, the lower limits of the number of breeding individuals were placed at is not less than 500, which supports the notion that this population lacks genetic drift (Johnson et al., 2010).

Non- DDT contaminants causing problems in peregrine populations are polybrominated diphenyl ethers (PBDEs) and polybrominated biphenyls (PBBs). PBDEs and PBBs are persistent organic pollutants (POPs) and PBDEs are often found in indoor furniture and electronics while PBBs are used as flame-retardants. PBDEs are found to bioaccumulate in high doses in raptors from Europe, Asia, and North America, particularly in peregrines, yet like OCs, levels are found to be low in mammals (Potter et al., 2009). PBDEs are found globally, but levels are highest in North America (where the demand is highest) and China (where the materials are made) and levels are highest in raptors (Chen & Hale, 2010). PBDE is a compound with many related chemicals, all of which have yet to be banned. For example, deca-BDE and octa-BDE may be the most abundant in abiotic materials such as soil, sediments, sewage, and indoor dust while tetra- and penta-BDE are highest in fish, and therefore Peregrine falcons (Potter et al. 2009). Unfortunately, even when all PBDE-like compounds are banned, they will still escape into the environment for quite some time because products containing these compounds will still be in use (Potter et al., 2009).

In a lab study, American kestrels (*Falco sparverius*) exposed to PBDEs experienced lowered hatching success, an increase in adult size and appetite, modified reproductive behaviors, and delayed egg-laying (Potter et al., 2009). Unfortunately, the Chesapeake Bay population is particularly at risk because these birds do not avoid locations that are heavily populated with humans and in fact often preferentially use city structures as nest sites (Barclay, 1980 and Cade et al., 1988). Potter et al., 2009 found that in Virginia in 2002, only 21 of 36 eggs hatched and only 8 of those birds survived to fledge. They also found that at all sites in the Chesapeake Bay, PBDE levels were substantial for piscivorous birds that are the main prey source for falcons near water. They also found that at some sites in the Chesapeake Bay, the levels of PBDE-like contaminants had doubled in the past 5 years. To prove the point that PBDEs will still escape into the environment decades after they are banned, PBB was banned in 1976, but PBB-like compounds were found in all eggs in the Chesapeake Bay in 2002 (Potter et al., 2009). Surprisingly enough, Potter et al., (2009) found that few PBDE congeners are positively correlated with human density, yet congener levels are strongly correlated with proximity to water, which poses a problem for the Chesapeake Bay peregrines. Currently, deca-BDE is the only congener still in production yet PBDEs continue to flow into the environment from electronics recycling, plastics manufacturing, and wastewater treatment (Chen & Hale, 2010).

Lastly, a genetic problem that could affect Peregrine falcons as a result of their sharp declines in recent history is genetic bottlenecks. In a study done by Brown et al., (2007), genetic analyses were performed to appraise the effect of a DDT-induced genetic

bottleneck on populations of Canadian falcons. Peregrines in the Eastern United States are the products of reintroductions of 7 different subspecies, 5 of which contributed to breeding (Barclay, 1980 and Cade et al., 1988). Genetic analyses of contemporary populations found low levels of microsatellite diversity and very low levels of diversity in mitochondrial DNA. However this observation itself does not support evidence of a genetic bottleneck. Indeed, when the results of this study were compared with data from museum specimens and data from contemporary populations from Greenland (where captive re-introductions did not occur), it was found that peregrines populations naturally lack diversity in both of these locations (Brown et al., 2007).

The lack of evidence of a genetic bottleneck in Canadian populations of *F. p. anatum*, *F. p. tundrius*, and *F. p. pealei* could be the result of their long-lived nature which could aid in buffering genetic degradation. However, it is difficult to identify the actual level of genetic erosion that took place in Eastern North American populations because it is likely that captive reintroduced birds artificially replenished their genetic stocks. Estimates of effective population size could be artificially inflated because of genetic introgression from reintroduced birds, however effective population size in Eastern North American populations should decline as the populations reach their mutation-drift equilibrium. Overall, evidence from Canadian populations of peregrines suggests that a DDT/DDE bottleneck did not decrease the evolutionary capacity of the birds however these populations need to continue to be monitored and counted conservatively because their effective population size is still low (Brown et al., 2007).

## 4: Restoration Efforts

Since the initial decline of Peregrine falcon populations in the late 1940s, several types of conservation efforts have been initiated to help protect the species. After initial declines, captive breeding programs were instituted in order to reintroduce captive-bred peregrines to the wild in the hopes of increasing the population size of extirpated East Coast populations (Barclay, 1980 and Cade et al., 1988). This method of conservation has been very successful in stabilizing and increasing the population sizes of wild peregrines particularly on the East Coast of the United States.

Another conservation method often used in concert with captive breeding is biological monitoring. Many conservation groups including, federal, state, and local governments and thousands of scientists perform biomonitoring globally. Locally, the Chesapeake Bay peregrine population is monitored by the Center for Conservation Biology (an institution founded by William and Mary and Virginia Commonwealth University). Scientists at the Center for Conservation Biology have been monitoring the peregrines since 1982 and publish annual reports summarizing the population health, breeding success, number of breeding pairs, number of offspring, and environmental contaminant levels of the population.

In conjunction with monitoring, conservation biologists have created conservation management plans in order to help states increase and maintain their peregrine populations. Florida in particular has a very succinct and thorough plan for managing their population of migrant peregrines. Their main goals are to maintain and increase protected habitat for peregrines and to manage the mortality risk of migratory falcons in Florida. In order to accomplish this, the state plans to implement regulations and permits

to decrease the amount of illegal takes, use habitat management of undeveloped public lands, lands under conservation easements, and wetlands to attract the falcons, acquire land from the Florida Keys and revert it to its natural state to attract falcons, provide incentives in the form of tax write-offs for people who put their land under permanent conservation easements, initiate monitoring plans using migration counts and 3 year surveys to record occupancy, nest success, and productivity, increase education and outreach to land managers, airports, and the general public, and implement research to determine the origins of the migrating peregrines (Florida Fish and Wildlife Conservation Commission, 2009).

In Britain, the Royal Society for the Protection of Birds has helped to conserve and protect historical ranges of peregrines as well as encourage the birds to use cities as this attracts media attention and increases interest in and appreciation of the species. There is even a local pair of city-nesting peregrines in Richmond, VA that people can come and observe or even watch via webcam from their homes.

Conservation on the federal level falls under the purview of the Migratory Bird Treaty Act, which serves to protect threatened or endangered birds. This act federally protects the peregrines by limiting the taking of peregrines from the wild for use in falconry. Currently in the east, the U.S. Fish and Wildlife Service permits the taking of 36 fall migrant peregrines east of 100 degrees longitude per year (“Migratory Birds Permits” 2013).

One more recent development in the area of Peregrine falcon conservation is the “greening” of utilities. In Iowa, the Raptor Resource Group began a power plant nesting

program in 1990. The goal of this program was to make smokestacks attractive to falcons by making them tall, placing them near water, and having made-to-order smokestacks. This program has proven to be successful as in 1999, 33% of all peregrines hatched in the Midwest were hatched on power plants (Baker, 1999). Nearby in Minnesota in 1998, 41% of fledglings hatched at utilities, 18% hatched on buildings, 20% hatched on cliffs, and 20% hatched on bridges (Baker, 1999). Preliminary studies performed in 1999 sought to assess whether using utilities as nesting and hatch sites negatively affected falcons. While preliminary analyses showed no decrease in bird health (Baker, 1999), there has since been worry that the readiness of the birds to adopt anthropogenic structures could be a result of non-native alleles reducing population fitness and/or that the utilities could be modifying the distribution and spatial structuring patterns of the falcons (Johnson et al., 2010).

### 4.1: Restoration Project

As a result of DDT and lindane being released for public use after 1945, 70% of eyries east of the Mississippi River were surveyed and found to be deserted by 1964 (Barclay, 1980). This realization catalyzed the formation of the Eastern Peregrine Reintroduction Plan; a joint effort by Cornell University and The Peregrine Fund. The Plan was implemented from 1974-1999 and its main goal was to reintroduce Peregrine falcons to eastern North America. In order to accomplish this, the Plan utilized collective efforts from institutional programs, falconers, and private projects to begin a captive breeding program to produce birds that would replace the extirpated eastern North American population (Cade et al., 1988). In 1965, falconers and biologists implemented a

Peregrine falcon breeding program in order to save the species from likely extinction (Cade et al., 1988). By 1975, more than 200 captive Peregrine falcons were being produced by private breeders and research centers per year and that same year the release of captive-bred falcons into the wild began (Cade et al., 1988). First the birds were released using fostering or hacking methods at widely spaced sites up and down the east coast in order to assess which sites were more preferred by the falcons in terms of numbers of returning birds and numbers of successful releases (Barclay, 1980). After assessing these values, continuing reintroductions were done in the Chesapeake Bay, coastal New Jersey, and in inland areas of New England and southeast New York (Barclay, 1980).

Birds that have been reintroduced using the hacking technique typically have as high or higher mortality rates than that of naturally fledged young (Cade et al., 1988). In North America, naturally fledged young have a mortality rate of approximately 70% in their first year and 25% after their first year (Barclay, 1980). In the populations of reintroduced falcons, first year mortality is 50-70%, and mortality after the first year is 20-30%. However out of the 775 peregrines released in the east between 1975-1985, only 10% attempted to breed while 70-80% did not live long enough to attempt to breed (Cade et al., 1988).

Grier (1976) developed a stochastic model in order to assess the impact mortality rates would have on survivorship of the reintroduced eastern population (Table 1). This stochastic model is especially useful for small founder populations and it was used it to predict the outcome and growth of peregrines in eastern North America. Barclay and

Cade, 1988 developed a deterministic model (**Table 2**) to accompany the stochastic model and used the two together to find basic patterns and probabilities in the eastern population. (Cade et al., 1988). Using approximate numbers of falcons released in the east through 1983 and assuming sustained releases of 80 birds per year for 1984 and 1985, they were able to calculate that unless drastic environmental changes occurred or new significant factors emerged, there was a 100% likelihood that Peregrine falcons would exist in eastern North America in the year 2010 even if no more releases were made (Cade et al., 1988).

**Table 1**
**Stochastic simulations of Peregrine falcon reintroductions based on a moderate rate of population growth[a] and different numbers of released birds (Cade et al., 1988, pg. 93)**

| | No. of young released[b] | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | 10 | 20 | 50 | 100 | 500 | 752[c] | 1000 | 1074[d] | 1074[d] |
| No. of simulations | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 |
| No. that failed[c] | 85 | 61 | 11 | 0 | 0 | 0 | 0 | 0 | 0 |
| Probability of success[c] | 0.15 | 0.39 | 0.89 | 1 | 1 | 1 | 1 | 1 | 1 |
| Average population size at end of year 2010[f] | 10.3 | 9.15 | 24.7 | 61.6 | 439 | 676 | 942 | 1014 | 1021 |
| Standard deviation | 14.4 | 9.9 | 21.3 | 33.6 | 96 | 120 | 137 | 148 | 153 |
| Coefficient of variation[g] | 140 | 108 | 86 | 55 | 22 | 18 | 15 | 15 | 15 |
| 95% confidence interval | 0-39 | 0-29 | 0-67 | 0-129 | 247-631 | 436-916 | 688-1216 | 718-1310 | 715-1327 |

[a] Survival and reproduction as in second column of life tables, Table 1.

[b] Male : female sex ratio 50 : 50.

[c] The number actually released in the eastern United States as of 1985.

[d] The number simulated to be released through 1990 (see Table 2 and Figure 1). In the first 100 simulations of 1074 releases (as well as for all lesser releases to the left) all released birds were released at once during 1985 then followed to the year in 2010. In the second 100 simulations of 1074 birds (last column of this table), the releases were spread out from 1975-90, as in Table 2.

[e] As of the year 2010.

[f] Of successful simulations.

[g] S.D. as a precent of average population size.



Source: Cade, T. J., J. H. Enderson, C. G. Thelander, and C. M. White. 1988. Peregrine Falcon populations: their management and recovery. Peregrine Fund, Boise, ID, Table 3.

**Table 2**
**Deterministic simulations of Peregrines in eastern United States, based on actual releases and compared to recent observations (Cade et al., 1988, pg. 90)**

| | No. released ($n$=752) | No. used in simulations[a] | No. under "stationary" conditions[b] | No. under conditions of age 2 breeding[t] | No. sighted[c] | No. known nesting attempts | $R_P$[d] | $R_2$[e] | $R_{KS}$[f] | $R_{KN}$[g] |
|---|---|---|---|---|---|---|---|---|---|---|
| 1975 | 16 | 16 | 6 | 6 | | | | | | |
| 1976 | 37 | 38 | 20 | 20 | 5 | 0 | 3.33 | 3.33 | | |
| 1977 | 46 | 46 | 34 | 36 | 5 | 0 | 1.7 | 1.8 | 1 | |
| 1978 | 53 | 52 | 49 | 55 | 10 | 0 | 1.44 | 1.53 | 2 | |
| 1979 | 52 | 52 | 64 | 74 | 14 | 1 | 1.31 | 1.35 | 1.4 | |
| 1980 | 65 | 66 | 85 | 100 | 14 | 2 | 1.33 | 1.35 | 1 | 2 |
| 1981 | 84 | 84 | 112 | 133 | 24 | 4 | 1.32 | 1.33 | 1.71 | 2 |
| 1982 | 79 | 80 | 135 | 165 | 31 | 5 | 1.21 | 1.24 | 1.29 | 1.25 |
| 1983 | 79 | 80 | 158 | 199 | 42 | 9 | 1.17 | 1.21 | 1.35 | 1.8 |
| 1984 | 124 | 80[h] | 182 | 235 | 65 | 16 | 1.15 | 1.18 | 1.55 | 1.78 |
| 1985 | 117 | 80[h] | 206 | 273 | 94 | 25 | 1.13 | 1.16 | 1.45 | 1.55 |
| 1986 | | 80[h] | 231 | 312 | (146)[i] | (39)[i] | 1.12 | 1.14 | (1.55)[i] | (1.55)[i] |
| 1987 | | 80[h] | 255 | 354 | (226)[i] | (60)[i] | 1.1 | 1.13 | (1.55)[i] | (1.55)[i] |
| 1988 | | 80[h] | 280 | 398 | (350)[i] | (93)[i] | 1.1 | 1.12 | (1.55)[i] | (1.55)[i] |
| 1989 | | 80[h] | 304 | 443 | | | 1.09 | 1.11 | | |
| 1990 | | 80[h] | 329 | 492 | | | 1.08 | 1.11 | | |
| 1991 | | | 323 | 511 | | | 0.98 | 1.04 | | |
| 1992 | | | 322 | 538 | | | 1 | 1.05 | | |
| 1993 | | | 327 | 565 | | | 1.02 | 1.05 | | |
| 1994 | | | 330 | 594 | | | 1.01 | 1.05 | | |
| 1995 | | | 332 | 625 | | | 1.01 | 1.05 | | |

[a] Equal nos. for each sex, 1074 birds total through 1990.
[b] Refer to Table 1; no. in population at end of year.
[c] No. of birds sighted in wild (pairs and singles) during spring and summer.
[d] $R$ from previous year via "stationary" model.
[e] $R$ with first breeding at age 2.
[f] $R$ for known sightings.
[e] $R$ for known nesting attempts.
[h] Conservative value based on a number of considerations; see text.
[I] Predictions nased on continued $R$ of 1.55 for observations.

Source: Cade, T. J., J. H. Enderson, C. G. Thelander, and C. M. White. 1988. Peregrine Falcon populations: their management and recovery. Peregrine Fund, Boise, ID, Table 2.

In an attempt to quantify genetic variation and divergence in reintroduced Peregrine falcons, electrophoretic variability was observed at 10 of 60 gene products in order to match allele frequencies of endemic birds with the most genetically similar captive birds in order to avoid inbreeding. However, as can be seen (**Table 3**), common loci occur in virtually all samples worldwide, thereby making it problematic to differentiate natal origins of falcons using these genetic markers. (Cade et al., 1988).

**Table 3**
**Global allele frequencies of three polymorphic loci (Cade et al., 1988, pg. 90)**

| | NP | | | | LDH1 | | MPI | |
|---|---|---|---|---|---|---|---|---|
| **Race if known or locality**[a] | **0** | **1** | **2** | **3** | **1** | **2** | **1** | **2** |
| Colville River ($n$ = 22) | 0.023 | 0.023 | 0.932 | 0.023 | 0.045 | 0.955 | 0.026 | 0.974 |
| *F.p. pealei* ($n \leq 5$) | | | 0.75 | 0.25 | 0.125 | 0.875 | | 1 |
| Yukon-Tanana Rivers ($n$ = 20.5) | | | 0.854 | 0.146 | | 1 | | Unfinished |
| Alberta ($n \leq 8$) | | | 0.94 | 0.06 | | 1 | | 1 |
| Colorado ($n \leq 11$) | 0.045 | | 0.773 | 0.136 | | 1 | | 1 |
| Quebec ($n \leq 4$) | | | 1 | | | 1 | | 1 |
| Greenland ($n$ = 13) | | 0.208 | 0.792 | | | 1 | | 1 |
| *F.p. peregrinus* ($n \leq 3$) | | 1 | | | | 1 | | 1 |
| *F.p. brookei* ($n \leq 3$) | | 0.125 | 0.375 | 0.5 | | 1 | | 1 |
| *F.p. macropus* ($n$ = 2) | | 0.75 | 0.25 | | | 1 | | 1 |
| *F.p. cassini* ($n$ = 2) | | | 1 | | | 1 | | 1 |
| Assateague Island ($n$ = 24) | | 0.083 | 0.854 | 0.063 | | 1 | | 1 |
| Padre Island: Fall 1982 ($n$ = 51) | | 0.041 | 0.918 | 0.041 | 0.039 | 0.961 | 0.02 | 0.98 |
| Padre Island: Spring 1984 ($n$ = 47) | | 0.011 | 0.936 | 0.053 | 0.021 | 0.979 | | 1 |
| Padre Island: Fall 1984 ($n$ = 39) | | 0.048 | 0.935 | 0.016 | 0.026 | 0.974 | | 1 |

[a] Colville River, Alaska; Yukon River and Tanana River, Alaska; province of Alberta, Canada; Assateague Island, Maryland; South and North Padre Islands, Texas; *F.p. pealei - Aleutian and Queen Charlotte Islands, Alaska and British Columbia*; *F.p. peregrinus - Scotland*; *F.p.brookei - Spain*; *F.p. macropus - Australis*; *F.p. cassini - Argentina and Chile*



Source: Cade, T. J., J. H. Enderson, C. G. Thelander, and C. M. White. 1988. Peregrine Falcon populations: their management and recovery. Peregrine Fund, Boise, ID, Table 2.

One hypothesis made by Barclay (1980) was that the reintroduction of Peregrine falcons to the east coast of North America would give rise to new subspecies as a result of the high levels of philopatry falcons display. In order to quantify subspecies, it is generally considered that there will be readily recognizable traits in 75% of a population that allow them to be distinguished from 75% of individuals from a separate population (Cade et al., 1988). Peregrine falcons typically display a significant amount of geographic variation when their external traits are assessed. This is a product of their highly philopatric tendencies which cause traits to become fixed in a population. This population differentiation consequently gives rise to new subspecies whose external traits tend to follow both Bergmann's and Gloger's Rules (Cade et al., 1988).

Reintroductions were made using peregrine stock from seven different subspecies: *Falco peregrinus cassini*, *Falco peregrinus brookei*, *Falcon peregrinus anatum*, *Falco peregrinus pealei*, *Falco peregrinus peregrinus*, *Falco peregrinus tundrius*, and *Falco peregrinus macropus* (Cade et al., 1988). As the goal of the Eastern Peregrine Reintroduction Plan was to re-establish populations of Peregrine falcons on the east coast of North America, it was decided that the best way to accomplish this would be by introducing several subspecies endemic to different global regions in order to provide enough genetic variation for the reintroduced falcons to have the ability to adapt to challenges they may face in their new range (Barclay, 1980). It was not the goal of the Eastern Peregrine Reintroduction Plan to reintroduce the native subspecies, but rather to

produce “the most promising ecologically-preadapted stock for reintroduction” (Barclay, 1980, pg. 61).

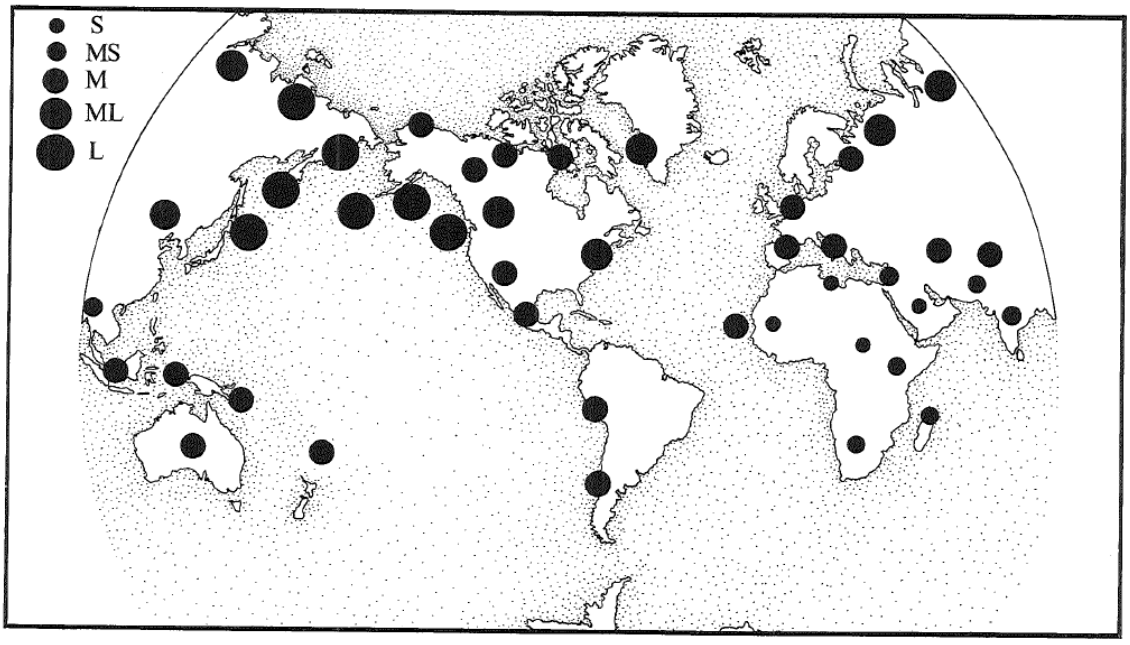


**Figure 1**
**Global size trends of Peregrine falcons from Cade et al., 1988, pg. 119.** Source: Cade, T. J., J. H. Enderson, C. G. Thelander, and C. M. White. 1988. Peregrine Falcon populations: their management and recovery. Peregrine Fund, Boise, ID, Figure 2.

**Table 4**
**Adult peregrine comparative sizes (Cade et al., 1988, pg. 118)**

| Subspecies (largest to smallest) | Body size | Dominant color dorsal/ventral | Breast barring size[a]/density[b]/spots[c] | Breeding latitudes[d] (region) |
|---|---|---|---|---|
| *F.p. pealei* | 100 | black/black | W/M-S/Sp | NL-I (North America) |
| *F.p. calidus* | 98 | blue/white | M-C/M-S/Sp | NL (Eurasia) |
| *F.p. japonensis* | 98 | black/blue-buff | W-M/M/Sp | NL (East Asia) |
| *F.p. anatum* | 97 | blue/rust | M-N/M/Sp | NL (North America) |
| *F.p. furuitii* | 97 | black/black-buff | W/M/Sp | NL-I (Volcano-Bonin Island) |
| *F.p. peregrinus* | 97 | blue/white-buff | M/M/Sp | NL (Eurasia) |
| *F.p. madens* | 96 | blue-brown/buff | M/M | ML-I (Cape Verde Island) |
| *F.p. tundrius* | 96 | blue/white | M-C/M-S/Sp | NL (North America) |
| *F.p. cassini* | 96 | blue/buff(blue/white) | M-N,(C)/D-M,(S) | SL (South America) |
| *F.p. brookei* | 92 | blue/buff | M/M/Sp | ML (Mediterranean) |
| *F.p. babylonicus* | 90 | blue-brown/buff-white | N-C/M-S | ML (Mideast - South Asia) |
| *F.p. nesiotes* | 89 | black/black-rust | M-N/M-D/Sp | SL-I (Southwest Pacific Islands) |
| *F.p. ernesti* | 89 | black/black-blue | M-S/M-D/Sp | ML/SL (Indonesia-Papuan) |
| *F.p. macropus* | 88 | blue/rust | M-N/D-M | SL (Australia) |
| *F.p. submelanogenys* | 88 | blue/rust | M-N/D-M | SL (Australia) |
| *F.p. minor* | 88 | blue/black-white | M/M/Sp | SL (Africa) |
| *F.p. peregrinator* | 88 | black/rust | M-C/M-S | ML (India - South Asia) |
| *F.p. radama* | 86 | blue/black-white | M/M/Sp | SL-I (Madagascar) |
| *F.p. pelegrinoides* | 85 | blue-brown/buff-white | N-C/M-S | ML (North Africa - Arabia) |

[a] Dark breast cross-barring represents size of bar: W = wide; M = medium; N = narrow; C = clear (without conspicuous barring).
[b] Bar density: D = densely barred; M = moderately dense; S = sparse or widely spaced.
[c] Sp = presence of spots (or bars becoming circular) on breast, usually in crop or midline.
[d] NL = northern latitudes; ML = middle latitudes; SL = southern latitudes; I = insular forms. Compare with Figures I and 2.

Source: Cade, T. J., J. H. Enderson, C. G. Thelander, and C. M. White. 1988. Peregrine Falcon populations: their management and recovery. Peregrine Fund, Boise, ID,

Table 1.

Captive-bred peregrines were reintroduced to the east coast up until 1992. By this time, approximately 1,250 peregrines had been reintroduced to east coast with 98 established breeding pairs (Heinrich, 2009). One issue with these reintroductions has to do with the philopatry of Peregrine falcons. As a result of the falcons' tendency to breed only within their natal range, it is likely that reintroduced populations have undergone separate genetic bottlenecks independent of those bottlenecks occurring in other localized populations (Cade et al., 1988). As a result of this, Cade et al., 1988 suggested that forcing gene flow between peregrine populations by exchanging eggs or nestlings among eyries might help to manage the populations by mitigating levels of inbreeding within populations. They postulated that as a result of philopatry, allele frequencies will become fixed and regional subpopulations will conserve their unique traits which will give rise to new subspecies in the future. It was predicted that this new subspecies will be genetically unlike that of the endemic *F. p. anatum* subspecies because the new subspecies will exhibit a novel gene pool that has been molded by genetic bottlenecks, diverse genetic backgrounds, and selective pressures from new environments which will give rise to a new phenotype as well (Barclay, 1980; Cade et al., 1988).

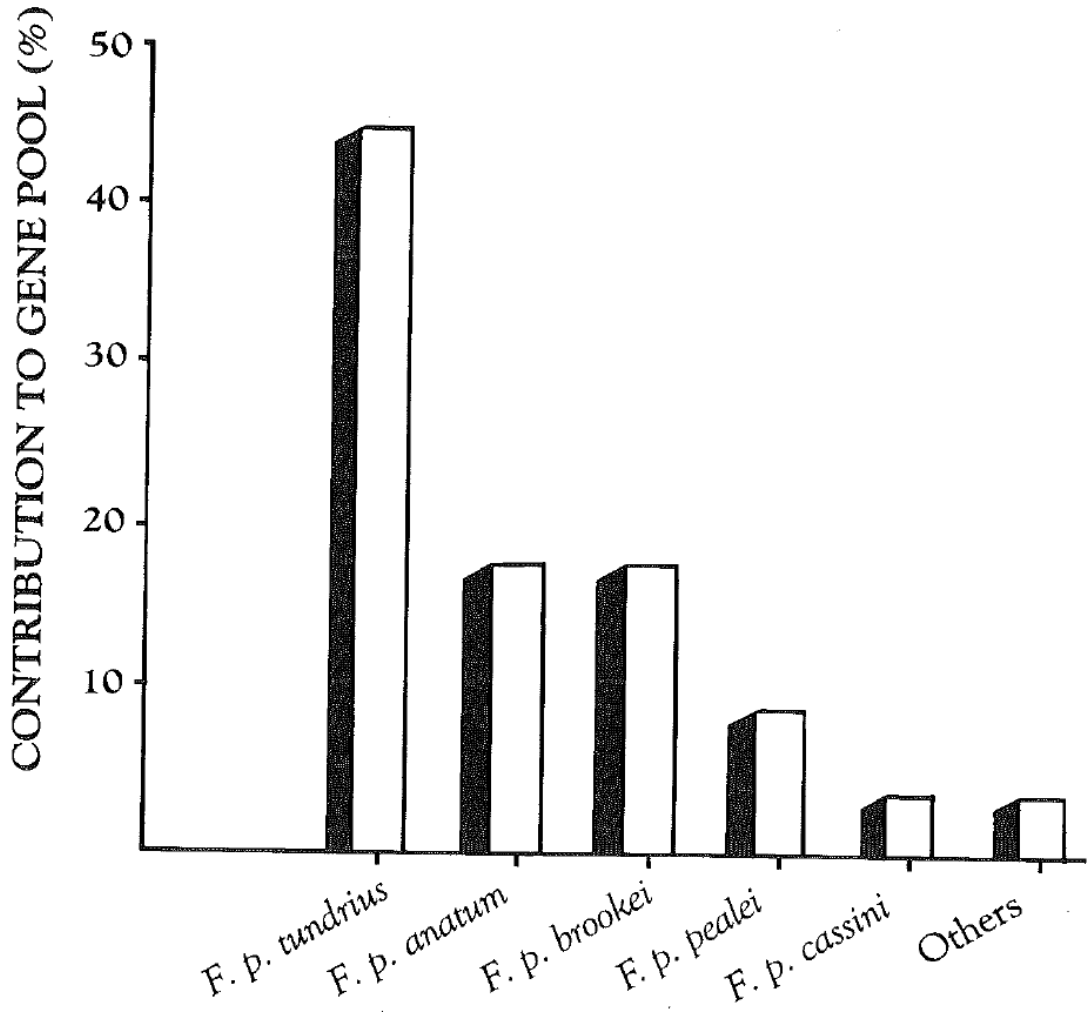


**Figure 2**
**Subspecies contributions to gene pool of captive-bred falcons from Cade et al., 1988, pg. 146.** Cade, T. J., J. H. Enderson, C. G. Thelander, and C. M. White. 1988. Peregrine Falcon populations: their management and recovery. Peregrine Fund, Boise, ID, Figure 1.

## 5: Goals and Outline

### 5.1: Hypotheses

1. The contemporary peregrine population in the Chesapeake Bay will exhibit more diversity when compared to historic stocks.
2. The amount of heterozygosity assessed at pre-determined microsatellite loci from the Chesapeake Bay population should be comparable to the amount of heterozygosity found in reintroduced Peregrine falcon populations in Europe (southern Norway and southern Scandinavia).
3. The contemporary population demonstrates a greater buffering capacity than the historic population to anthropogenic/ecological disturbances.
4. The contemporary population in the Chesapeake Bay in one population.

### 5.2: Outline

The overarching goal of this experiment was to assess overall genetic diversity between historic and contemporary populations of peregrines on the eastern shore of North America. Subsequent aims included assessing the number of genetic clusters that exist within the Chesapeake Bay population, and comparing levels of heterozygosity to birds from other reintroduction programs (southern Norway and southern Scandinavia). The final question was of course, what is the heterozygosity of the contemporary population? Was the Eastern Peregrine Reintroduction Plan successful at providing reintroduced birds with enough genetic diversity to combat novel challenges?

These findings will give insight into the buffering capacity of the contemporary population in response to anthropogenic or environmental disturbances.

## CHAPTER TWO: MATERIALS AND METHODS

In order to assess the levels of genetic diversity in the historic and contemporary populations of peregrines, blood, feather or tissue samples from each population were tested with genetic analyses. Blood and feather samples from the contemporary population are provided by the Center for Conservation Biology in Williamsburg, Virginia. Historic population tissue and feather specimens were provided by Smithsonian National Museum of Natural History, Washington, D.C., the American Museum of Natural History, New York and the Western Foundation of Vertebrate Zoology, town & state.

QIAGEN DNeasy® Blood & Tissue kits (QIAGEN) were used in order to extract nuclear DNA and analyze genetic variation in both populations. Each individual specimen was genotyped at 12 polymorphic microsatellite markers (Fp5, Fp13, Fp31, Fp46-1, Fp54, Fp79-1, Fp79-4, Fp82-2, Fp86-2, Fp89, Fp92-1, Fp107) that were originally developed for Peregrine falcons (Nesje et al., 2000b). Polymerase chain reactions (PCR) were performed in order to amplify the DNA sequences. These PCR products were appropriately diluted and run on a fingerprinting machine for analyses of microsatellites. Microsatellite genotypes were tested within each population and at each of the aforementioned loci. Allelic diversity (mean number of alleles per locus), observed

and expected heterozygosity values, and allelic richness were calculated using the GenAlEx 6.5 computer program.

# 1: Methods

## 1.1: Contemporary Population Sampling

The Center for Conservation Biology has monitored the Chesapeake Bay population of peregrines since 1982 and they have assembled a catalog of blood and feather samples from each breeding season since 2007. For this study, samples were withdrawn from the catalog using William and Mary's facilities. Feather and blood samples were taken from each individual for the years 2007 and 2013. Blood samples were stored on cellulose paper (Advantec Nobuto Blood Filter Strip (Advantec)) and contained at most 0.1 mL of blood.

Most individuals that were sampled had both blood and feather samples available in the catalog but when not available, either blood strip or feather sample was used. All the feather samples in the catalog were taken from the breast of the bird and the blood samples were taken from the feather samples. When the feather sample was taken, a blood droplet remained in the base of the quill. These blood droplets were then squeezed out by flattening the base of the quill and the droplet was then stored on an Advantec Nobuto Blood Filter Strip (Advantec) strip. This study required only the very base of the quill that had been flattened as that is the portion of the feather that was attached to the skin and therefore contained skin cells as well as residual blood cells. Laboratory dissecting scissors and laboratory forceps were used to remove the lower portion of the quill which was then stored in 2 mL collection tubes at room temperature. The 2 mL

collection tubes had been previously radiated with ultraviolet light in order to get rid of any DNA in a Stratagene UV Stratalinker 1800 (Stratagene) for twenty minutes. Scissors and forceps were cleaned with 10% bleach followed by a rinse of de-ionized water between each sample to prevent contamination. Both the Advantec Nobuto Blood Filter Strip (Advantec) and the feather sample from the same individual (when available) were stored in the same collection tube. The collection tubes were then stored at room temperature until recalled for use (**Table 5**).

**Table 5**
**List of Samples Collected**

| Band Number | Location | Sex | Date | Feather | Strip |
|---|---|---|---|---|---|
| 2206-81664 | Benjamin Harrison Bridge | M | 5/31/07 | TRUE | TRUE |
| 1807-65095 | Benjamin Harrison Bridge | F | 7/10/13 | TRUE | TRUE |
| 1807-65078 | Berkley Bridge | F | 5/16/13 | TRUE | FALSE |
| 1807-65079 | Berkley Bridge | F | 5/16/13 | TRUE | FALSE |
| 1807-65080 | Berkley Bridge | F | 5/16/13 | TRUE | FALSE |
| 1807-65081 | Berkley Bridge | F | 5/16/13 | TRUE | FALSE |
| 1126-11929 | Cedar Island | M | 7/9/13 | TRUE | FALSE |
| 1126-11930 | Cedar Island | M | 7/9/13 | TRUE | FALSE |
| PEFA - 0005 | Cedar Island | U (Adult) | 7/9/13 | TRUE | FALSE |
| 2206-81667 | Cobb Island Tower | M | 6/11/07 | TRUE | TRUE |
| 1807-02733 | Cobb Island Tower | F (Adult) | 4/3/13 | TRUE | FALSE |
| 1807-65096 | Cobb Island Tower | F | 6/29/13 | TRUE | TRUE |
| 1807-65097 | Cobb Island Tower | F | 6/29/13 | TRUE | TRUE |
| 1126-11903 | Elkins Marsh Chimney | M | 5/13/13 | TRUE | TRUE |
| 1126-11904 | Elkins Marsh Chimney | M | 5/13/13 | TRUE | TRUE |
| 1807-02748 | Elkins Marsh Shack Tower | F | 5/23/07 | TRUE | TRUE |
| 2206-81655 | Elkins Marsh Shack Tower | M | 5/23/07 | TRUE | TRUE |
| 2206-81656 | Elkins Marsh Shack Tower | M | 5/23/07 | TRUE | TRUE |
| 1126-11919 | Elkins Marsh Shack Tower | M | 6/18/13 | TRUE | TRUE |
| PEFA - 0008 | Elkins Marsh Shack Tower | U (Adult) | 6/18/13 | TRUE | FALSE |
| 1126-11912 | Finney's Island Tower | M | 6/4/13 | TRUE | TRUE |
| 1807-65084 | Finney's Island Tower | F | 6/4/13 | TRUE | TRUE |
| 1807-65085 | Finney's Island Tower | F | 6/4/13 | TRUE | TRUE |
| 1807-65090 | Finney's Island Tower | F | 6/4/13 | FALSE | FALSE |
| 2206-81669 | Godwin Island Box | M | 6/11/07 | TRUE | TRUE |
| 1126-11915 | Godwin Island Box | M | 6/11/13 | TRUE | TRUE |
| 1807-65099 | Godwin Island Box | F | 6/11/13 | TRUE | TRUE |
| 1807-65100 | Godwin Island Box | F | 6/11/13 | TRUE | TRUE |
| PEFA - 0009 | Gull Marsh Tower | U (Adult) | 5/13/13 | TRUE | FALSE |
| PEFA - 0007 | Gull Marsh Tower | U (Adult) | 6/18/13 | TRUE | FALSE |
| 1126-11920 | Gull Marsh Tower | M | 6/29/13 | TRUE | TRUE |
| PEFA - 0006 | Gull Marsh Tower | U (Adult) | 6/29/13 | TRUE | FALSE |
| 1807-02744 | James River Bridge | F | 5/18/07 | TRUE | TRUE |
| 2206-81649 | James River Bridge | M | 5/18/07 | TRUE | TRUE |
| 2206-81650 | James River Bridge | M | 5/18/07 | TRUE | TRUE |
| 2206-81651 | James River Bridge | M | 5/18/07 | TRUE | TRUE |
| 2206-81652 | James River Bridge | M | 5/18/07 | TRUE | TRUE |
| 1807-65076 | James River Bridge | F | 5/16/13 | TRUE | FALSE |
| 1807-65077 | James River Bridge | F | 5/16/13 | TRUE | FALSE |
| 1807-02750 | James River Ghost Ship 2 | F | 5/30/07 | TRUE | TRUE |
| 1807-02751 | James River Ghost Ship 2 | F | 5/30/07 | TRUE | TRUE |
| 1807-02752 | Mills Godwin Bridge | F | 5/30/07 | TRUE | TRUE |
| 2206-81660 | Mills Godwin Bridge | M | 5/30/07 | TRUE | TRUE |
| 1126-11907 | Mills Godwin Bridge | M | 5/16/13 | TRUE | FALSE |
| 1807-65082 | Mills Godwin Bridge | F | 5/16/13 | TRUE | FALSE |
| 1807-65083 | Mills Godwin Bridge | F | 5/16/13 | TRUE | FALSE |
| 2206-81665 | Mockhorn Island Tower | M | 6/7/07 | TRUE | TRUE |
| 1126-11916 | Mockhorn Island Tower | M | 6/11/13 | TRUE | TRUE |

| | | | | | |
|---|---|---|---|---|---|
| 1126-11917 | Mockhorn Island Tower | M | 6/11/13 | TRUE | TRUE |
| 1126-11918 | Mockhorn Island Tower | M | 6/11/13 | TRUE | TRUE |
| 1807-65098 | Mockhorn Island Tower | F | 6/11/13 | TRUE | TRUE |
| 1807-02745 | Norris Bridge | F | 5/18/07 | TRUE | TRUE |
| 2206-81653 | Norris Bridge | M | 5/18/07 | TRUE | TRUE |
| 2206-81654 | Norris Bridge | M | 5/18/07 | TRUE | TRUE |
| 1126-11906 | Norris Bridge | M | 4/26/13 | TRUE | TRUE |
| 2206-81662 | Upsher Bay Tower | M | 5/31/07 | TRUE | TRUE |
| 2206-81663 | Upsher Bay Tower | M | 5/31/07 | TRUE | TRUE |
| 1807-02756 | Wachapreague Shack Tower | F | 5/31/07 | TRUE | TRUE |
| 2206-81661 | Wachapreague Shack Tower | M | 5/31/07 | TRUE | TRUE |
| 1126-11910 | Wachapreague Shack Tower | M | 5/29/13 | TRUE | TRUE |
| 1126-11911 | Wachapreague Shack Tower | M | 5/29/13 | TRUE | TRUE |
| 1807-65091 | Wachapreague Shack Tower | F | 5/29/13 | TRUE | TRUE |
| 1807-65092 | Wachapreague Shack Tower | F | 5/29/13 | TRUE | TRUE |
| 1807-02747 | Watts Island Tower | F | 5/23/07 | TRUE | TRUE |
| 2206-81657 | Watts Island Tower | M | 5/23/07 | TRUE | TRUE |
| 1126-11913 | Watts Island Tower | M | 6/4/13 | TRUE | FALSE |
| 1126-11914 | Watts Island Tower | M | 6/4/13 | TRUE | FALSE |

### 1.2: Contemporary Population – Extraction

To extract DNA from the contemporary samples (those obtained from William and Mary), the QIAGEN DNeasy® Blood & Tissue kits (QIAGEN) were used. DNA was also extracted from eight samples using the FastDNA Spin Kit for Plant and Animal Tissues (MP Biomedicals, LLC). This was done to see if one kit was more effective at extracting DNA than the other. The results were that both kits were equally effective for DNA extraction so extractions were continued using the QIAGEN DNeasy® Blood & Tissue kit (QIAGEN). For both kits, the manufacturer's protocols were followed except the samples for this study were allowed to digest in the Buffer ATL and Proteinase K mixture overnight in a Thermomixer® 5436 (Eppendorf) at 55°C with gentle agitation as was recommended by current literature.

### 1.3: Contemporary Population – Amplification

After extraction, the DNA was visualized on a 1% agarose gels. The PCR was run on 1:5 and 1:10 dilutions of the extracted DNA.

PCR amplifications were performed in a final reaction volume of 20 uL. Each reaction mixture contained Taq Gold 1X Buffer, 2.5 mM of Magnesium++ mix, 200uM dNTPs, 0.1% BSA, 0.5 units of *Taq* polymerase (Taq Gold), and 0.5 uM of each primer (forward and reverse). Wild tundra genomic DNA was used as a positive control for PCR reactions. For preliminary analyses, swan-specific 12s primers were used (forward primer H1753: AAACTGGGATTAGATACCCCACTA reverse primer L2258: CCTTCCGGTACACTTACCTTRTTACG). The loci were amplified by PCR using a T100™ Thermal Cycler (Bio-Rad). The cycling conditions for the PCR were initial

denaturation at 95°C for 11 minutes and 30 seconds, followed by 35 cycles of 30 seconds at 50°C, 2 minutes at 72°C, and 10 minutes at 72°C, and then held at 4°C.

The products from the amplification were visualized on 1% agarose gel using ethidium bromide for 20-30 minutes. After electrophoresis, amplification products were visualized using a Kodak DS EDAS 120 Camera and a Transilluminator 4000 (Stratagene).

An amplification with 12S RNA swan-specific primers confirmed that the contemporary samples were yielding DNA. After that, all 12 unlabeled primer pairs previously developed for the Peregrine falcons (Nesje et al. 2000b) were ordered. The primers were received as dry pellets, so they were spun down and then dissolved in DEPC-treated water. After adding the water, the primers were allowed to sit at room temperature for several minutes and were then spun down again. The primers were diluted to 10 uM concentrations and stored in the fridge at 4°C; the stock solutions were stored in the freezer at -20°C.

Using the R 3.0.2 randomization software, one individual per nest site per year was randomly selected. These samples were used for testing the labeled primers. PCR amplifications were performed in a final reaction volume of 20 uL with each reaction mixture containing Taq Gold 1X Buffer, 2.5 mM of Magnesium++ mix, 200uM dNTPs, 0.1% BSA, 0.5 units of *Taq* polymerase (Taq Gold), and 0.5 uM of each primer (forward and reverse) using a T100™ Thermal Cycler (Bio-Rad). The standard conditions that were used for the 12s primers were not yielding consistent product, therefore PCR conditions were optimized by increasing the amount of Mg++ to 3.125 uM Mg and

increasing the number of PCR cycles from 35 to 40. The microsatellite loci were also typed in a positive control sample of wild tundra swan genomic DNA. The cycling conditions for all twelve unlabeled primers were 95°C for 11 minutes and 30 seconds, followed by 40 cycles of 30 seconds at 54°C, 2 minutes at 72°C, and 10 minutes at 72°C, and then held at 4°C.

The products from the amplification were visualized on 1% agarose using ethidium bromide after running the gel for 20-30 minutes. After electrophoresis, amplification products were photographed using a Kodak DS EDAS 120 Camera and a Transilluminator 4000 (Stratagene). Identifying the product bands was difficult because the amplified sequences were so small that the product bands often ran together with the primer dimer bands. For this reason, the amplification products were also visualized on 1.5% agarose gel for 30-40 minutes in an attempt to separate the primer bands from the product bands. These 1.5% gels did provide better separation of primer dimer bands from product bands. Running 1% agarose gel for 40-60 minutes was also often effective at distinguishing whether the amplification reaction had produced product.

Each primer pair was assessed for suitability to this study based on the quality of respective amplification product, expected level of average heterozygosity, and their average number of alleles. Based on these criteria, 6 of the 12 primer pairs were selected for the profiling of the Chesapeake Bay Peregrine falcon population (**Table 6**).

Labeled primers *Fp13, Fp31, Fp54, Fp82-2, Fp86-2*, and *Fp89* were ordered as dry pellets, spun down and then dissolved in DEPC water. After adding the water, the primers were allowed to sit at room temperature for several minutes then they were spun

down again. The primers were diluted to final concentrations of 10 uM, then stored them in the fridge at 20°C.

Two individuals per nest site were randomly selected (using the R 3.0.2 software) to be used in fingerprinting and final statistical analyses. Two individuals were selected from every nest site with the exception of Gull Marsh Tower as the Center for Conservation Biology had tissue from only one individual cataloged from this location. PCR was run on all final samples with the 6 labeled primers.

**Table 6**
**List of Primers Used**

| Locus | Repeat motif | Primer sequences 5′–3′ | Range in size (bp) | Number of alleles | Expected heterozygosity |
|---|---|---|---|---|---|
| First parent | | | | | |
| NVH fp5 | (GT)11 | F: CCGTTCTGGAGTCAAAAC<br>R: CATGCAGCACTTTATTCAG | 105–107 | 2 | 0.16 |
| NVH fp13 | (CA)12 | F: AGCTTGATTGAGGCTGTG<br>R: CCAAATTCCCTGCTGAAG | 96–108 | 5 | 0.76 |
| NVH fp31 | (CA)17 | F: ATCACCTGCACATAGCTG<br>R: TTTAGCTCCTCTCTCTCAC | 154–160 | 5 | 0.73 |
| NVH fp46–1 | (CA)11 | F: TTAGCCTCGCAGCTTCAG<br>R: GTAATGAAAAGTCTTTGGGG | 123–125 | 2 | 0.37 |
| NVH fp54 | (GT)16 | F: TGATTGCAGGAACTAAGAC<br>R: TACATTCGCCAAAGGACG | 104–110 | 3 | 0.53 |
| NVH fp 79–1 | (CA)11 | F: TTCTCCCTAACACCTTGC<br>R: TCATCATGCTGCTGCTGC | 145–147 | 2 | 0.12 |
| NVH fp79–4 | (CA)16 | F: TGGCTTCTCTTATCAGTAAC<br>R: GGCTGGGTGGAATTAAAG | 151–171 | 11 | 0.89 |
| NVH fp82–2 | (GT)10 | F: CTGCACGAGGAGATGATG<br>R: CCAGATAGCTGTGAAATGG | 132–140 | 3 | 0.28 |
| NVH fp86–2 | (CA)11 | F: GTAAATAAGCCTCCAAAAGG<br>R: CATGCTTCCTGATTACTTC | 143–149 | 3 | 0.52 |
| NVH fp89 | (AT)12 | F: CTCTGCCCTGAATACTTAC<br>R: GAATCTTGTTTGCATTGGAG | 121–139 | 8 | 0.83 |
| NVH fp92–1 | (CA)10 | F: TTACTAGAAGGCTGCTCAG<br>R: CGTATTCCAAACTTTATGGC | 116–128 | 5 | 0.51 |
| NVH fp107 | (GT)11TT(GT)8 | F: ACAGATTTGATTGCCAGG<br>R: TGCCATGTCACATTCATAC | 204–206 | 2 | 0.45 |

**Table 7**
**Final Samples,** *n* = sample size

| Location | *n* |
|---|---|
| Ben Harrison Bridge | 2 |
| Berkley Bridge | 2 |
| Cedar Island | 2 |
| Cobb Island Tower | 2 |
| Elkins Marsh Chimney | 2 |
| Finney's Island Tower | 2 |
| Godwin Island Box | 2 |
| Gull Marsh Tower | 1 |
| James River Bridge | 2 |
| Mills Godwin Bridge | 2 |
| James River Ghost Ship 2 | 2 |
| Mockhorn Island Tower | 2 |
| Norris Bridge | 2 |
| Upsher Bay Tower | 2 |
| Wachapreague Shack Tower | 2 |
| Watts Island Tower | 2 |
| Elkins Marsh Shack Tower | 2 |

PCR amplification was performed in a final reaction volume of 20 uL with each reaction mixture containing 1X Buffer, 3.125 mM of Magnesium mix, 200uM dNTPs, 0.1% BSA, 0.5 units of *Taq* polymerase (Taq Gold), and 0.5 uM of each primer (forward and reverse). The positive control remained the same as a sample of wild tundra genomic DNA and the microsatellite loci were amplified by PCR using a GeneAmp PCR System 9700 (Applied Biosystems®). The cycling conditions for all six labeled primers were 95°C for 11 minutes and 30 seconds, followed by 40 cycles of 30 seconds at 54°C, 2 minutes at 72°C, and 30 minutes at 72°C, and then held at 4°C.

The products from the amplification were visualized on 1% agarose gel using ethidium bromide for 40-60 minutes. After electrophoresis, amplification products were visualized using a Kodak DS EDAS 120 Camera and a Transilluminator 4000 (Stratagene).

Identifying the product bands was once again difficult because the amplified sequences were so small the product bands were often present at the same location as the primer bands. However, it was often possible to assess whether or not product was present based on the brightness and width of the product bands.

### 1.4: Contemporary Population – Fingerprinting

Fingerprint analyses for the final samples of the contemporary population were run on an ABI PRISM 3130*xl* using the Length Heterogeneity PCR (LH-PCR) fingerprinting process. Products of LH-PCR were added to a mix of ILS-600 (internal lane standard from Promega Corp.) and HiDi formamide (Applied Biosystems®) in 1:10 ratio (1 part diluted PCR product and 9 parts ILS mix). The ILS-600 and HiDi

Formamide mix was made in a 1:20 ratio (1 part ILS600 and 19 parts HiDi Formamide). This mixture then was run on the ABI PRISM 3130*xl* capillary electrophoresis instrument for fingerprinting. The heterozygosity of the population was assessed by determining the size and presence of microsatellite peaks. PCR products of each primer were run with duplicates for comparison purposes.

After the fingerprinting analysis, data was analyzed with the Genemapper v4.1 software (Applied Biosystems®) software. Allele calls were taken from the Genemapper output table. These numbers were then made into tables and analyzed with the STRUCTURE 2.3.4 software. The STRUCTURE software performs genetic cluster analyses which were used for assessment of overall heterozygosity and amount of inbreeding in the population. Information from the Genemapper v4.1 output was also analyzed with the GenAlEx 6.5 software. With the GenAlEx 6.5 software, we calculated values for observed alleles ($A_O$), expected heterozygosity ($H_E$), observed heterozygosity (HO), and fixation index ($F_{ST}$), which were also used for assessment of overall heterozygosity and amount of inbreeding in the population.

### 1.5: Historic Population Sampling

Historic samples were provided by the American Museum of Natural History, the Carnegie Museum, the Smithsonian Institution, and the Western Foundation of Vertebrate Zoology. Samples were found using the ORNIS2 search database that catalogs museum specimens for research purposes. For this study, samples were withdrawn from collections by museum employees and sent to Manassas, VA for genetic analyses (**Table 8**). Either feather (from the breast) or toe pad samples were provided for each individual

with toe pad samples being more common because toe-pad sampling is a non-invasive sampling method. Both feather and toe pad samples were stored in 1.5-2uL tubes at room temperature.

This study required only the very base of the quill for feather samples as that is the portion of the feather that was attached to the skin and therefore contains skin cells. A scalpel and laboratory forceps were used to remove the lower portion of the quill, which was used for DNA extraction. Scalpel and forceps were cleaned with 10% bleach followed by a rinse of de-ionized water between each sample to prevent contamination. Samples were stored at room temperature until extraction. After extraction, any remaining tissue was stored at 4°C.

**Table 8**
**Historic samples**

| Identification | Location | Subspecies | Sex | Date |
|---|---|---|---|---|
| CM P50183 | Canada: Quebec | - | Male | 6/31/15 |
| WFVZ 20804 | New York:West Point | *F.p. anatum* | Female | 5/10/10 |
| WFVZ 20803 | Maryland: Assateague I. | *F.p. tundrius* | Female | 5/5/50 |
| SI 352723 | Mayland | - | Female | 3/15/38 |
| SI 272872 | New Jersey | - | Male | 5/1/20 |
| SI 309471 | New York | - | Female | 4/5/25 |
| SI 311757 | New York | - | Male | 4/5/18 |
| SI 309456 | New York | - | Male | 4/2/16 |
| SI 311758 | New York | - | Female: Adult | 4/5/18 |
| SI 309457 | New York | - | Female: Immature | 5/30/16 |
| SI 414339 | Virginia | - | Female | 3/15/22 |
| AMNH 352934 | Pennsylvania:Campbell's Ledge | *F.p. anatum* | Male | 4/2/1903 |
| AMNH 352940 | Virginia:Ship Shoal | *F.p. anatum* | Female | 5/26/1889 |
| AMNH 436357 | New York:Seaford, L.I. | *F.p. anatum* | Female | 5/16/14 |
| AMNH 470359 | Pennsylvania:Pittston | *F.p. anatum* | Male | 3/5/1900 |
| AMNH 816357 | Maryland | - | Female | 5/17/05 |

### 1.6: Historic Population – Extraction

To extract DNA from the historic samples, the QIAGEN DNeasy® Blood & Tissue kit (QIAGEN) was used first. Extractions were performed in a separate laboratory from the contemporary samples and using different equipment in order to avoid cross-contamination. Extractions were done using the manufacturer's protocols except the samples were allowed to digest in the Buffer ATL and Proteinase K mixture overnight in a Thermomixer® 5436 (Eppendorf) at 55°C with gentle agitation as was recommended by current literature.

### 1.7: Historic Population – Amplification

After the DNA had been extracted, PCR was run on 1:5 dilutions of the extracted DNA.

PCR amplification was performed in a final reaction volume of 20 uL. Each reaction mixture contained 1X Buffer, 3.125 mM of Magnesium mix, 200uM dNTPs, 0.1% BSA, 0.5 units of *Taq* polymerase (Taq Gold), and 0.5 uM of each primer (forward and reverse). Wild tundra genomic DNA was used as a positive control for PCR reactions. For preliminary analyses, swan 12s primers were used (forward primer H1753: AAACTGGGATTAGATACCCCACTA reverse primer L2258: CCTTCCGGTACACTTACCTTRTTACG). The loci were amplified by PCR using a T100™ Thermal Cycler (Bio-Rad). The cycling conditions for the PCR were 95°C for 11 minutes and 30 seconds, followed by 35 cycles of 30 seconds at 50°C, 2 minutes at 72°C, and 30 minutes at 72°C, and then held at 4°C.

The products from the amplification were visualized on 1% agarose gel using ethidium bromide for 20-30 minutes. After electrophoresis, amplification products were visualized using a Kodak DS EDAS 120 Camera and a Transilluminator 4000 (Stratagene).

After amplification with 12s swan primers there was no evidence that the historic samples were yielding any DNA. Therefore, several more PCRs were run in an attempt to optimize the conditions in order to visualize any PCR products. In order to optimize the conditions, the GeneAmp® PCR System 9700 (Applied Biosystems®) was used instead of the T100™ Thermal Cycler (Bio-Rad), dilutions with lower amounts of DNA were tried as well as using higher amounts of DNA template per PCR reaction. Samples were extracted again using the QIAGEN DNeasy® Blood & Tissue kit (QIAGEN), DNA was purified using Microcon® Centrifugal Filters for Protein and DNA Concentration, both lower (3.125 uM to 2.5 uM) and higher (3.125 uM to 3.75 uM) Magnesium concentrations were tested. Also, we attempted doubling the amount of Taq Polymerase, increasing the number of cycles during PCR from 35 to 40, and changing the extraction kit to the FastDNA™ SPIN Kit for Soil (MP Biomedicals) or the QIAamp® DNA FFPE Tissue Kit (QIAGEN).

The products from all amplifications were visualized on 1% agarose gel using ethidium bromide for 20-30 minutes. After electrophoresis, amplification products were visualized using a Kodak DS EDAS 120 Camera and a Transilluminator 4000 (Stratagene).

Using the camera and the Transilluminator, PCR products were assessed for presence of DNA bands. DNA bands were present in PCR products that had been optimized by increasing the amount of *Taq* polymerase, increasing the amount of DNA and by increasing the amount of Magnesium mixture reagent. Only historic samples 2, 6, 9, 10, 11, and 16 were successfully amplified using 12S and peregrine specific primers.

Identifying the product bands was once again difficult because the amplified sequences were so small the product bands were often present at the same location as the primer bands.

### 1.8: Historic Population – Fingerprinting

Fingerprint analyses for the final samples of the historic population were run on an ABI PRISM 3130*xl* using the Length Heterogeneity PCR (LH-PCR) fingerprinting process. Products of LH-PCR were added to a mix of ILS-600 (internal lane standard from Promega Corp.) and HiDi formamide (Applied Biosystems®) in 1:10 ratio (1 part diluted PCR product and 9 parts ILS mix). The ILS-600 and HiDi Formamide mix was made in a 1:20 ratio (1 part ILS600 and 19 parts HiDi Formamide). This mixture then was run on the ABI PRISM 3130*xl* capillary electrophoresis instrument for fingerprinting. The heterozygosity of the population was assessed by determining the size and presence of microsatellite peaks. PCR products of each primer were run with duplicates for comparison purposes.

After the fingerprinting analysis, data was analyzed with the Genemapper v4.1 software (Applied Biosystems®) software. Allele calls were taken from the Genemapper output table. These numbers were then made into tables and analyzed with the

STRUCTURE 2.3.4 software. The STRUCTURE software performs genetic cluster analyses which were used for assessment of overall heterozygosity and amount of inbreeding in the population. Information from the Genemapper v4.1 output was also analyzed with the GenAlEx 6.5 software. The GenAlEx 6.5 software calculates values for observed alleles ($A_O$), expected heterozygosity ($H_E$), observed heterozygosity (HO), and fixation index ($F_{ST}$), which were also used for assessment of overall heterozygosity and amount of inbreeding in the population.

# CHAPTER THREE: RESULTS

## 1: A Comparison Between European Populations and the Chesapeake Bay

Using the GenAlEx 6.5 software, values for expected observed alleles ($A_O$), heterozygosity ($H_E$), observed heterozygosity ($H_O$), and fixation index ($F_{ST}$) were calculated for the contemporary falcon population in the Chesapeake Bay. Using data provided by Jacobson 2008 and Nesje 2000b, similarities in $H_O$ at each locus were seen between the contemporary populations in southern Scandinavia and southern Norway and the contemporary population of falcons in the Chesapeake Bay with the exception of locus NVH *fp54* whose observed heterozygosity in the Chesapeake Bay population was dramatically lower than that of the European populations (**Table 9**). The total number of observed alleles for the Chesapeake Bay population was 27, which is the same as the total number of observed alleles in the contemporary European populations (**Table 9**). In the Chesapeake Bay population, $F_{ST}$ values were close to the zero value at each locus, thus, suggesting complete panmixis of this population (**Table 9**).

**Table 9**
**$A_O$, $H_O$, and $H_E$ of southern Scandinavia, southern Norway, and Chesapeake Bay populations**

Historical Population – Southern Scandinavia

| Locus | *n* | $A_O$ | R | $H_O$ ($H_E$) |
|---|---|---|---|---|
| NVH *fp13* | 38 | 6 | 5.87 | 0.87 (0.76) |
| NVH *fp31* | 36 | 5 | 4.33 | 0.78 (0.67) |
| NVH *fp54* | 38 | 3 | 2.45 | 0.29 (0.47) |
| NVH *fp82-2* | 38 | 5 | 3.57 | 0.24 (0.24) |
| NVH *fp86-2* | 38 | 4 | 3.95 | 0.55 (0.62) |
| NVH *fp89* | 38 | 7 | 6.35 | 0.76 (0.79) |

Captive Population – Southern Scandinavia

| Locus | *n* | $A_O$ | R | $H_O$ ($H_E$) |
|---|---|---|---|---|
| NVH *fp13* | 20 | 6 | 5.85 | 0.75 (0.75) |
| NVH *fp31* | 19 | 5 | 4.9 | 0.79 (0.74) |
| NVH *fp54* | 20 | 4 | 3.83 | 0.25 (0.37) |
| NVH *fp82-2* | 20 | 2 | 1.99 | 0.15 (0.14) |
| NVH *fp86-2* | 20 | 3 | 2.99 | 0.55 (0.54) |
| NVH *fp89* | 20 | 8 | 7.55 | 0.8 (0.79) |

Contemporary Population – Southern Scandinavia

| Locus | *n* | $A_O$ | R | $H_O$ ($H_E$) |
|---|---|---|---|---|
| NVH *fp13* | 44 | 5 | 4.41 | 0.55 (0.59) |
| NVH *fp31* | 44 | 5 | 4.75 | 0.61 (0.73) |
| NVH *fp54* | 44 | 4 | 3.16 | 0.39 (0.46) |
| NVH *fp82-2* | 41 | 2 | 2 | 0.15 (0.31) |
| NVH *fp86-2* | 44 | 3 | 2.39 | 0.48 (0.51) |
| NVH *fp89* | 43 | 8 | 7.55 | 0.86 (0.85) |

Contemporary Population – Southern Norway

| Locus | *n* | $A_O$ | R | $H_O$ ($H_E$) |
|---|---|---|---|---|
| NVH *fp13* | – | 5 | – | 0.83 (0.76) |
| NVH *fp31* | – | 5 | – | 0.67 (0.73) |
| NVH *fp54* | – | 3 | – | 0.42 (0.53) |
| NVH *fp82-2* | – | 3 | – | 0.30 (0.28) |
| NVH *fp86-2* | – | 3 | – | 0.52 (0.52) |
| NVH *fp89* | – | 8 | – | 0.83 (0.83) |

Contemporary Population – Chesapeake Bay

| Locus | *n* | $A_O$ | R | $H_O$ ($H_E$) | $F_{ST}$ |
|---|---|---|---|---|---|
| NVH *fp13* | 33 | 6 | – | 0.76 (0.68) | -0.119 |
| NVH *fp31* | 33 | 6 | – | 0.67 (0.81) | 0.178 |
| NVH *fp54* | 33 | 3 | – | 0.09 (0.09) | -0.037 |
| NVH *fp82-2* | 33 | 3 | – | 0.15 (0.24) | 0.379 |
| NVH *fp86-2* | 33 | 3 | – | 0.45 (0.46) | 0.021 |
| NVH *fp89* | 33 | 6 | – | 0.58 (0.74) | 0.224 |

Sources: Johnson, Jeff A., Sandra L. Talbot, George K. Sage, Kurt K. Burnham, Joseph W. Brown, Tom L. Maechtle, William S. Seegar, Michael A. Yates, Bud Anderson, and David P. Mindell. "The Use of Genetics for the Management of a Recovering Population: Temporal Assessment of Migratory Peregrine Falcons in North America." *PLoS One* 5.11 (2010): n. pag. National Center for Biotechnology Information. U.S. National Library of Medicine, 18 Nov. 2010. Web. 22 Apr. 2013, Table 1 and Nesje, M., K. H. Roed, J. T. Lifjeld, P. Lindberg, and O. F. Steen. "Genetic Relationships in the Peregrine Falcon (Falco Peregrinus) Analysed by Microsatellite DNA Markers." *Molecular Ecology* 9.1 (2000b): 53-60. Wiley Online Library. 25 Dec. 2001. Web. 15 Oct. 2013, Table 1.

## 2: A Comparison Between Historic Appalachian and Contemporary Chesapeake Bay Populations

Importantly, despite the fact that several different subspecies from different geographical regions were used in reintroduction efforts, the contemporary population of

Peregrine falcons in the Chesapeake Bay does not possess high levels of heterozygosity (**Figure 3B**). Further investigation comparing historic and contemporary levels of heterozygosity could aid in discovering how or when bottlenecking occurred and heterozygosity decreased. Our attempts to extract DNA from museum specimens of birds collected from the Appalachian mountain population of Peregrine falcons yielded quantifiable DNA from some specimens. The purpose of this study was to compare genetic diversity and overall heterozygosity between the Chesapeake Bay population and that of the historic population endemic to the Appalachian mountains in an attempt to prove that the reintroduced population has more heterozygosity than the historic population.

Using the GenAlEx 6.5 software, values for expected observed alleles ($A_O$), heterozygosity ($H_E$), observed heterozygosity ($H_O$), and fixation index ($F_{ST}$) were calculated for the contemporary falcon population in the Chesapeake Bay and the historic falcon population endemic to the Appalachians. A total of 40 alleles were detected across both populations and loci. Comparisons showed that values for observed heterozygosity ($H_O$), expected heterozygosity ($H_E$), and number of alleles ($N_E$) were not significantly different ($P = 0.68$, $P = 0.82$, and $P = 0.12$, respectively). Using a two-tailed heteroscedastic t-test to determine significant difference between the $H_O$ of the historic and contemporary populations, $P = 0.97$. This shows that there is no significant difference between the observed heterozygosity of the two populations.

## 3: Population Structure in the Chesapeake Bay

The cluster analysis provided using the STRUCTURE 2.3.4 software confirmed the hypothesis that the contemporary populations of Peregrine falcons in the Chesapeake Bay are effectively one population. Furthermore, the variance showing the estimated log-likelihood of each number of inferred genetic clusters suggests very high levels of inbreeding; to the point that the cluster analysis had difficulty in distinguishing between genetic distinctions and inferring clusters (**Figure 3**). Hence, we proved that the Chesapeake Bay population constitutes a single genetic cluster. Moreover, our observations suggest that introduction of captive bred falcons of diverse stock was not successful at increasing the genetic diversity in the contemporary population. However, an influence of recent genetic drift for now cannot be excluded. The depression in heterozygosity may have taken place after 1992, when new introductions of diverse, captive bred stock were stopped.

In the future, further studies comparing the levels of heterozygosity in the Chesapeake Bay should be performed. For these, future studies, our work has substantial value as our study was the first genetic study performed on the Chesapeake Bay population of Peregrine falcons that validated a set of heterozygous genetic markers and defined baseline heterozygosity values on which later studies can rely upon.

### 3.1: Population Structure in the Appalachian Mountains

The cluster analysis provided using the STRUCTURE 2.3.4 software was unable to deduce the number of populations that existed in the population of Peregrine falcons endemic to the Appalachian mountain range prior to their extirpation. This could be the result of a small available sample size ($n$ = 6) or it could be that like the contemporary

population of Peregrine falcons in the Chesapeake Bay, the Appalachian population lacked enough genetic variability for the STRUCTURE 2.3.4 software to definitively infer clusters (**Figure 5).**

When cluster analyses were performed on the contemporary and historic populations together using the STRUCTURE 2.3.4 software, the software was unable to infer the presence of distinct genetic clusters (**Figure 6**). Figure 6 shows an equal probability of there being anywhere from one to ten genetic clusters when the contemporary and genetic populations are compared to each other. The fact that the STRUCTURE software could not infer any distinct genetic clusters when data from the two populations were combined shows that the two populations are too genotypically similar to distinguish from each other.

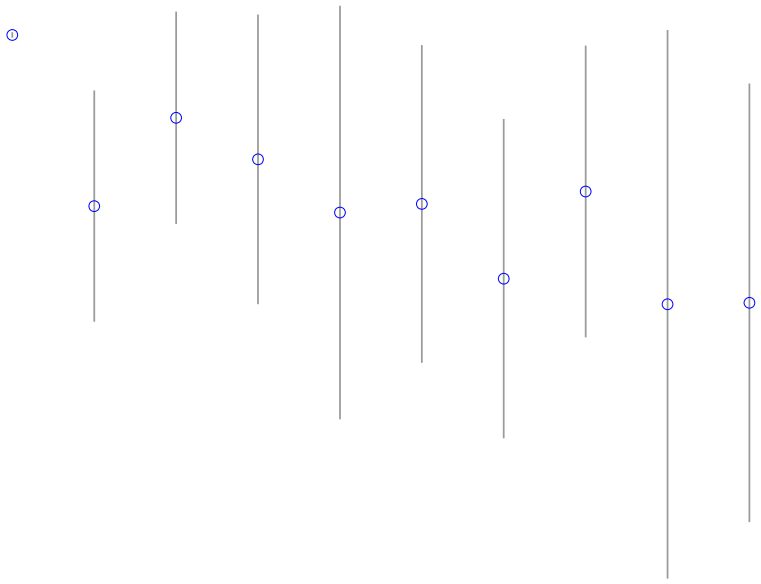

**Figure 3**
**STRUCTURE analysis of Peregrine falcon populations in the Chesapeake Bay. Scatter plot showing the estimated log likelihood of each number of inferred genetic clusters.**

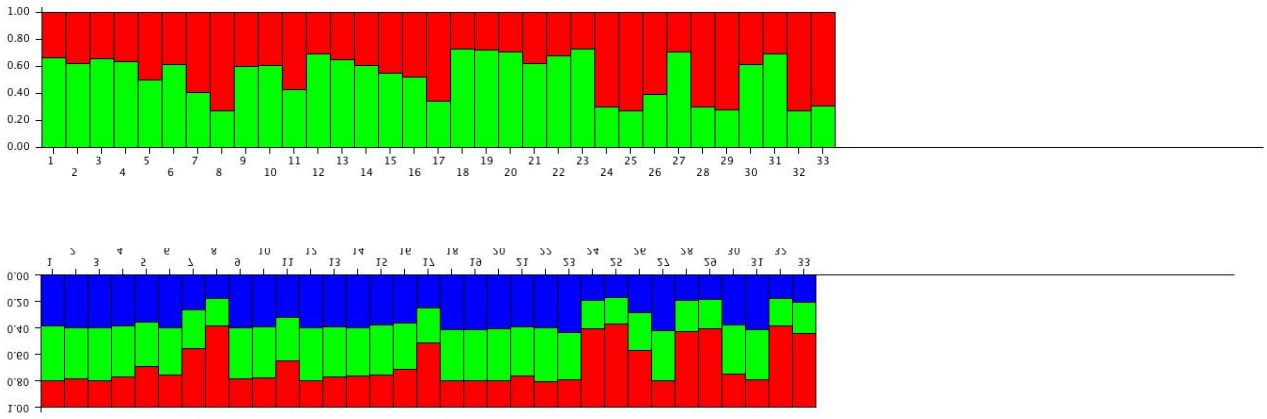


**Figure 4**
**Bayesian assignment of individuals to *K = 2* (top) and *K = 3* (bottom) clusters. Each bar represents the estimated posterior probability of each individual belonging to each inferred cluster. It can be seen that due to similarity of individuals, cluster analyses could not decisively construct clusters.**

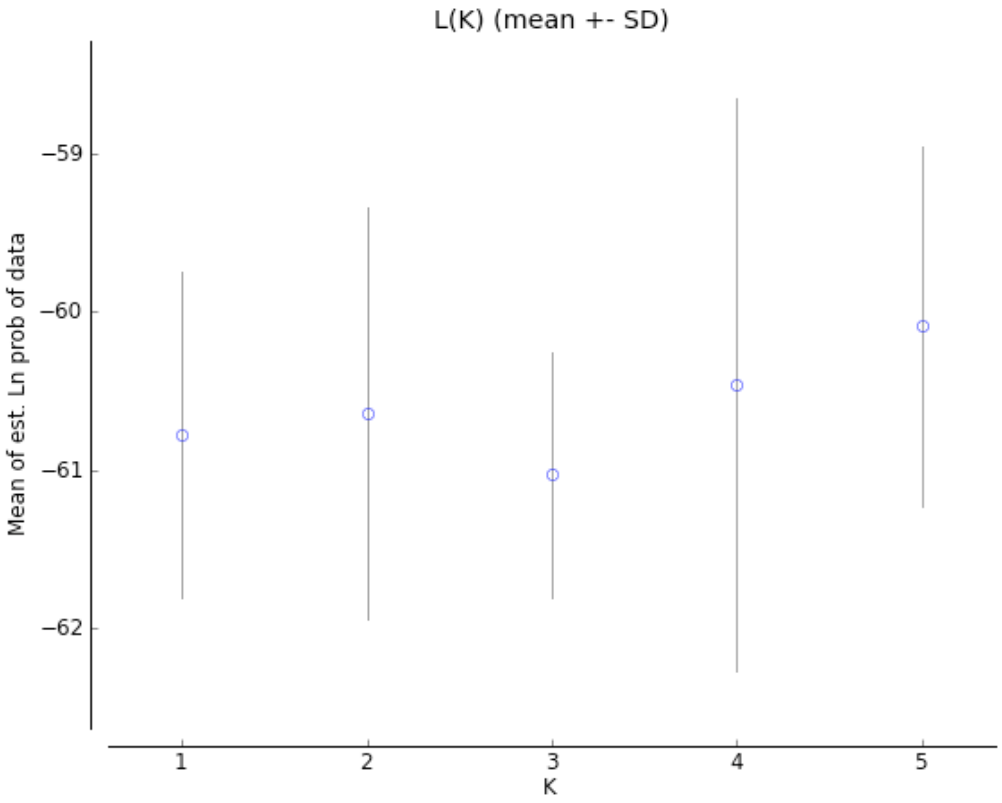


**Figure 5**
**STRUCTURE analysis of Peregrine falcon populations in the Appalachian mountains. Scatter plot showing the estimated log-likelihood of each number of inferred genetic clusters.**

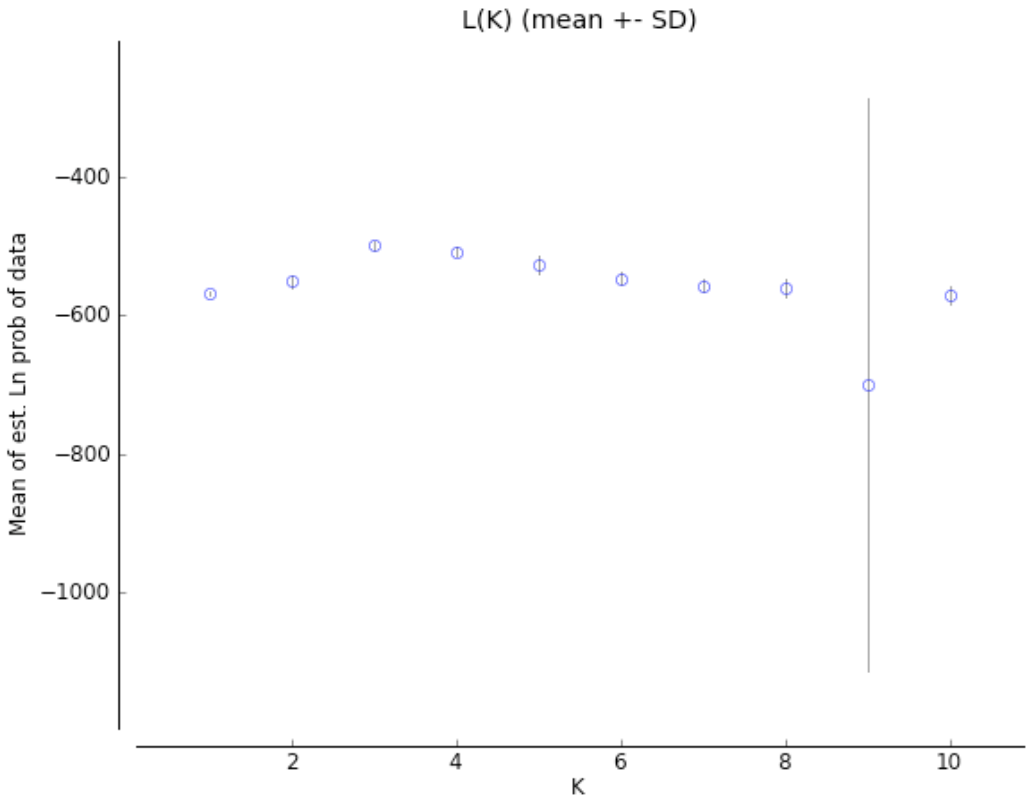


**Figure 6**
**STRUCTURE analysis of both Peregrine falcon populations in the Chesapeake Bay and the Appalachian mountains. Scatter plot showing the estimated log-likelihood of each number of inferred genetic clusters.**

## 4: The Structure of East Coast Subpopulations

Barclay, 1980 and Cade et al., 1988 postulated that when enough time had passed after the reintroduction, the Peregrine falcon populations on the East Coast of the United States would form distinct subspecies. As it has been 40 years since the first reintroductions took place, it is possible that subspecies distinctions between the reintroduced populations in the Chesapeake Bay, coastal New Jersey, and in inland areas of New England and southeast New York may now have formed. As these birds were all introduced from the same genetic stock (Barclay, 1980), future studies that compare heterozygosity between these three populations could be helpful in determining whether they have undergone any genetic drift or bottlenecking independently of each other. Studies comparing the heterozygosity of these populations could also be helpful in assessing whether some populations are more at risk than others.

It is possible that bottlenecking occurred during the reintroduction of Peregrine falcons to the East Coast regions. As can be seen in Figures 7 and 8, out of approximately 1200 birds introduced to the region, only 98 breeding pairs were established. Even if we were to generously assume a mobile population the same size as the breeding population that leaves roughly 800 birds that are unaccounted for.

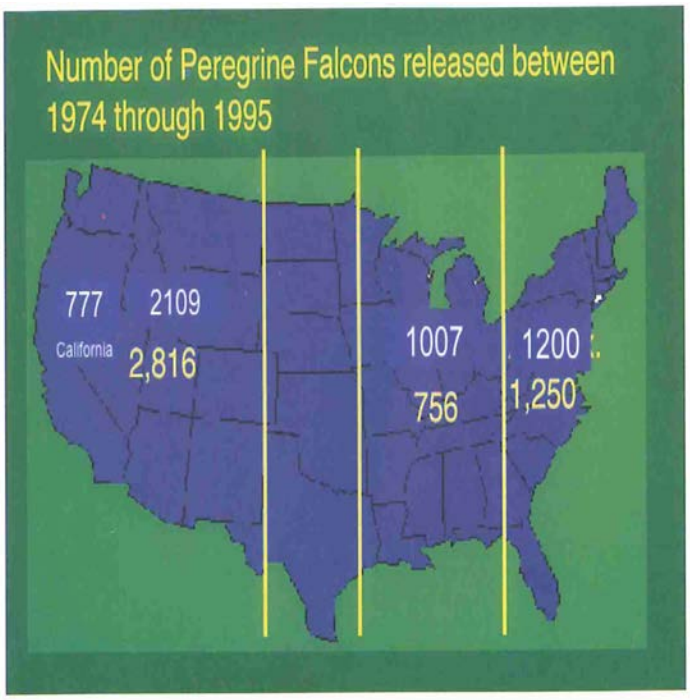


**Figure 7**
**Peregrine falcons released by the Eastern Peregrine Reintroduction Plan from 1974-1999.** Heinrich, William. "Peregrine Falcon Recovery in the Continental United States 1974-1999, with Notes on Related Programs of The Peregrine Fund." Turul/Poznan University of Life Sciences Press (2009): 431-44. Print, Figure 4.

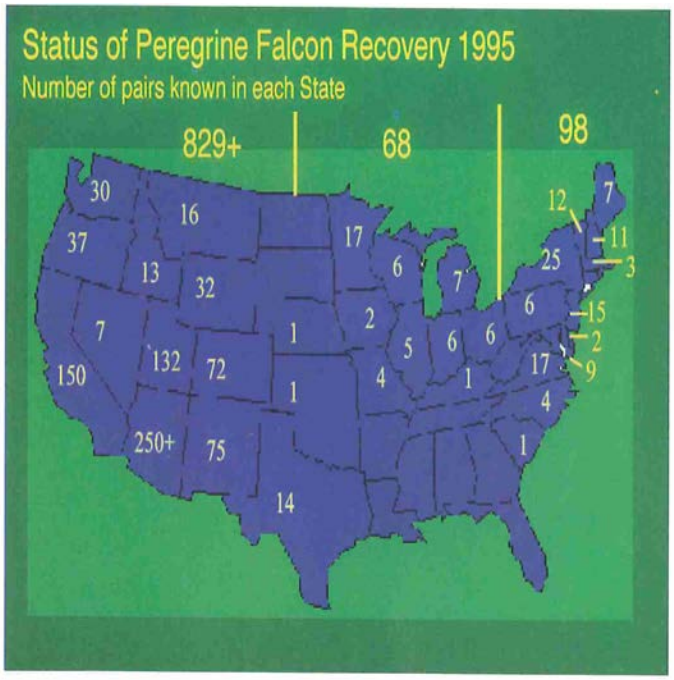


**Figure 8**
**Number of known Peregrine Falcon pairs in each state during 1995.** Heinrich, William. "Peregrine Falcon Recovery in the Continental United States 1974-1999, with Notes on Related Programs of The Peregrine Fund." Turul/Poznan University of Life Sciences Press (2009): 431-44. Print, Figure 5.

## CHAPTER FOUR: DISCUSSION

The effects of DDT were particularly devastating to Peregrine falcons on the East Coast of North America. Bioaccumulation and biomagnification caused reproductive failures which led to complete extirpation east of the Mississippi River by 1964. However, as a result of the banning of DDT and reintroduction efforts by Cornell University and The Peregrine Fund, Peregrine falcon populations have increased in size to the point of permitting delisting of the animal in 1999.

This study serves to document the effects of reintroducing an extirpated population from exogenous source populations. Would indigenous as well as exogenous source populations have been used, it is possible that contemporary genetic diversity would have been higher. Unfortunately, this option was not feasible as the indigenous population was extinct before the reintroduction program had begun.

The Eastern Peregrine Re-introduction Plan was successful at introducing falcons back to their historic ranges and at increasing population sizes (**Figure 9**). However, based on cluster analyses, one must question whether genetic variability need be a factor in the de-listing and subsequent monitoring of an endangered species. Despite evidence of Peregrine falcon populations increasing in many locations across the East Coast, the STRUCTURE cluster analysis suggests that the genetic variation of these birds is very low (**Figure 3** and **Figure 4)**.

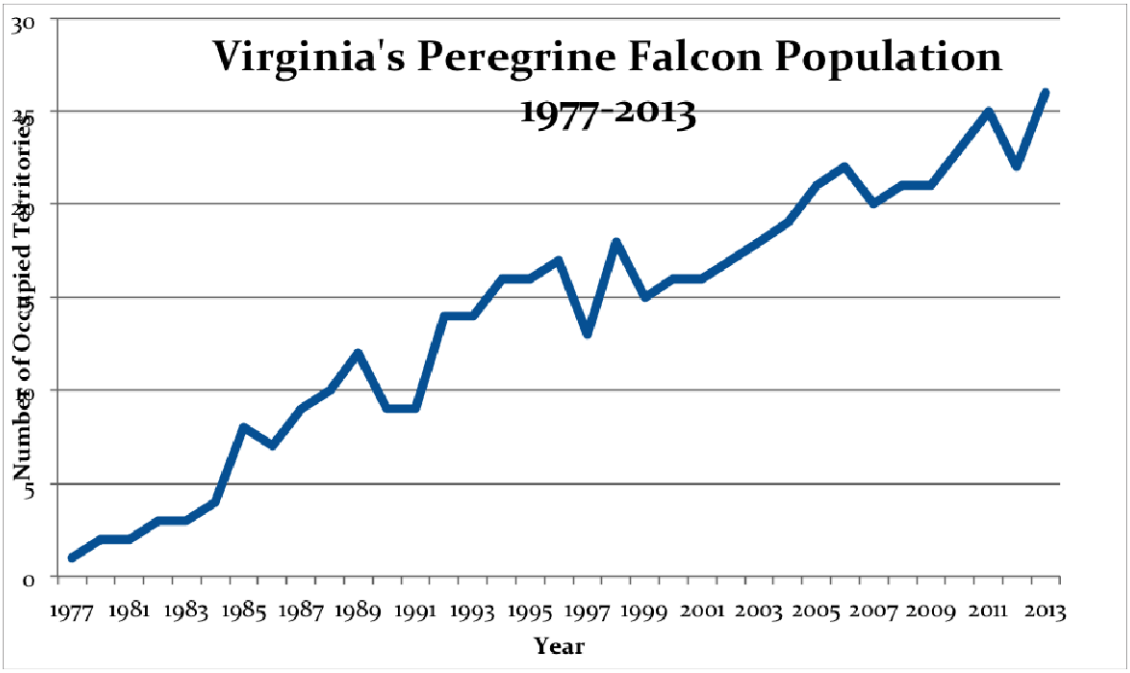


**Figure 9**
**Peregrine falcon population in Virginia from 1977-2013.** Mojica, E.K., B.D. Watts, and S.M. Padgett. 2012. Virginia Peregrine Falcon monitoring and management program: Year 2012 report. Center for Conservation Biology Technical Report Series, CCBTR-12-10. College of William and Mary & Virginia Commonwealth University, Williamsburg, VA. 18 pp. Figure 1.

As there have been no prior genetic studies of Peregrine falcons in the Chesapeake Bay region it is impossible to derive whether genetic variation has been decreasing or remaining the same in the population. However it is unlikely that the level of genetic variation has been increasing along with population size. Results show that levels of observed heterozygosity, expected heterozygosity, and number of alleles do not differ significantly between historic and contemporary populations of falcons. This suggests that the falcons in the Chesapeake Bay today may be just as well prepared to face environmental or anthropogenic pressures as were their predecessors in the Appalachians.

It was unexpected that not all peregrine specific primers were successful at amplifying DNA in the Chesapeake Bay population and that the levels of observed heterozygosity were so similar between the European and Chesapeake Bay populations. These results suggest that philopatry typical of Peregrine falcon populations has led to genomic evolution thereby making primers synthesized for peregrines in the past unhelpful at locating heterozygosity in present populations of peregrines. Should new peregrine specific primers be developed at different loci, differences in observed heterozygosity between peregrine populations may be more significant. The results found in this study suggest high levels of inbreeding and potential population-specific bottleneck events in contemporary populations of Peregrine falcons.

When compared to other raptors that were extirpated as a result of OCs and repopulated using subsequent reintroduction plans, the numbers of peregrines in reintroduced populations is dramatically lower. Henny et al., 2010 investigated North American Osprey (*Pandion haliaetus)* populations during the post-DDT-era and their subsequent increases in numbers as a result of reintroduction programs. Osprey populations nationwide contained ~8,000 breeding pairs in 1981, ~14,200 in 1994, and ~16,000-19,000 in 2001 (Henny et al., 2010). Osprey populations declined as a result of DDT just as peregrine populations did and both species were reintroduced in similar manners (using artificial nest structures in the Chesapeake Bay). No studies have been done documenting the reason for the large difference in population numbers between the two species, ~995 Peregrine falcon pairs nationwide in 1995 compared to ~14,200 Osprey pairs nationwide in 1994, but the discrepancy does merit investigation. It is

possible that Osprey have an easier time finding nest sites as they typically build their own nests whereas peregrines typically utilize nests made by other birds of prey. However it is doubtful that this one strategy alone is responsible Osprey having roughly fourteen times more breeding pairs nationwide than Peregrine falcons.

In Peregrine falcons, low levels of heterozygosity (**Table 9**) are particularly worrisome because of their known history of population demise due to anthropogenic factors and the birds' preference to inhabit areas near humans.

While DDT has been banned, toxic PBDE-like compounds have yet to be banned and could pose a serious threat to wildlife populations lacking genetic diversity. The fact that PBDE-like compounds are present in high levels in areas near water and the tendency of PBDE-like compounds to bioaccumulate makes this chemical eerily similar to DDT. Levels of PBDEs are highest in North America and have been found in high levels in raptors in Europe, Asia, and North America. Furthermore, fish have been found to have very high levels of PBDEs. As peregrines preferentially feed on marine and shore birds, this presents another opportunity for bio-magnification of the organotoxin in the tissues of peregrines. Already in 2002 PBDE levels were substantial for piscivorous birds (Potter, Watts, La Guardia, Harvey, & Hale, 2009). These factors coupled with the falcons' lack of genetic diversity and the fact that PBDEs will persist in the environment even after they have been banned suggest real threats to the Chesapeake Bay peregrine population.

Results from this study indicate that it is crucial that heterozygosity in this population be monitored for us to identify whether it is increasing, decreasing, or

remaining the same so that we may take steps to increase it for the future success of the population.

When directly comparing the Chesapeake Bay population with those in southern Norway and southern Scandinavia using the same microsatellite markers, we can see similarities in heterozygosity (**Table 9**). According to the results of STRUCTURE cluster analysis of Chesapeake Bay population, these similarities warrant further investigation into contemporary populations in Europe. A more extensive investigation into the levels of heterozygosity in contemporary populations of peregrines in North America, Norway, and Scandinavia would allow us to evaluate the global genetic adaptability of Peregrine falcons. Only once we have done this can we evaluate the success of reintroduction programs and make informed decisions about ensuring the survival of Peregrine falcon populations on a global scale. It will also aid in future restoration projects for other endangered species and populations.

# CHAPTER FIVE: CONCLUSIONS

Peregrine falcons have a history of being a charismatic species that is attractive to humans. Past declines have spurred humans to decrease our toxicity on the environment to preserve our species of interest. While DDT and other insecticides have since been banned and the health of our falcons and ecosystems have improved, PBDEs and other new chemicals continue to be produced that will continue to threaten the health of the Chesapeake Bay ecosystem. Results indicating a depression in genetic diversity of these species should serve as a red flag indicating that peregrines continue to be susceptible to chemicals and anthropogenic disturbances as it was a lack in genetic diversity that caused their extirpation in the past.

Peregrine falcons can serve as sentinel species for the health of the Bay as well as charismatic megafauna that can and should be used to encourage humans to preserve and restore the natural state of the Bay. While programs have been successful at bringing peregrines back to the East Coast of North America, our results demonstrate that they are still at risk from environmental contaminants and it is our responsibility to utilize this information to preserve the continued growth of our repopulated peregrines and the ecosystems they rely on for survival.

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# APPENDIX

## Allele Calls – Contemporary Population

| Individual | Population | AT12 | CA11c | CA12 | CA17 | GT10 | GT16 |
|---|---|---|---|---|---|---|---|
| BB1a | 1 | 115 | 140 | 90 | 148 | 134 | 99 |
| BB1b | 1 | 115 | 140 | 98 | 150 | 134 | 99 |
| BB2a | 1 | 115 | 140 | 88 | 148 | 134 | 99 |
| BB2b | 1 | 115 | 140 | 98 | 148 | 134 | 99 |
| BHB1a | 1 | 115 | 136 | 90 | 148 | 134 | 99 |
| BHB1b | 1 | 115 | 142 | 98 | 152 | 134 | 99 |
| BHB2a | 1 | 115 | 136 | 88 | 152 | 134 | 99 |
| BHB2b | 1 | 115 | 142 | 98 | 154 | 134 | 99 |
| CI1a | 1 | 115 | 140 | 98 | 156 | 134 | 99 |
| CI1b | 1 | 131 | 142 | 98 | 156 | 134 | 99 |
| CI2a | 1 | 129 | 140 | 96 | 152 | 132 | 99 |
| CI2b | 1 | 129 | 140 | 98 | 156 | 134 | 99 |
| CIT1a | 1 | 115 | 140 | 98 | 143 | 134 | 99 |
| CIT1b | 1 | 127 | 142 | 98 | 143 | 134 | 99 |
| CIT2a | 1 | 131 | 140 | 88 | 143 | 134 | 99 |
| CIT2b | 1 | 131 | 140 | 98 | 152 | 134 | 99 |
| EMC1a | 1 | 115 | 136 | 98 | 152 | 134 | 99 |
| EMC1b | 1 | 127 | 140 | 98 | 156 | 134 | 99 |
| EMC2a | 1 | 115 | 140 | 98 | 152 | 134 | 99 |
| EMC2b | 1 | 127 | 142 | 98 | 156 | 136 | 99 |
| EMS1a | 1 | 123 | 140 | 88 | 152 | 134 | 99 |
| EMS1b | 1 | 123 | 140 | 88 | 152 | 134 | 99 |
| EMS2a | 1 | 127 | 140 | 90 | 150 | 132 | 99 |
| EMS2b | 1 | 131 | 142 | 96 | 150 | 134 | 99 |
| FIT1a | 1 | 127 | 136 | 90 | 150 | 134 | 99 |
| FIT1b | 1 | 131 | 140 | 100 | 152 | 134 | 99 |
| FIT2a | 1 | 115 | 136 | 98 | 150 | 134 | 99 |
| FIT2b | 1 | 129 | 140 | 98 | 152 | 134 | 99 |
| GIB1a | 1 | 115 | 140 | 88 | 150 | 134 | 99 |
| GIB1b | 1 | 123 | 140 | 90 | 152 | 134 | 99 |
| GIB2a | 1 | 123 | 140 | 88 | 150 | 134 | 99 |
| GIB2b | 1 | 131 | 140 | 96 | 152 | 134 | 99 |
| GMTa | 1 | 131 | 140 | 90 | 152 | 134 | 99 |
| GMTb | 1 | 131 | 140 | 98 | 156 | 134 | 99 |
| JRB1a | 1 | 115 | 140 | 96 | 154 | 132 | 99 |
| JRB1b | 1 | 129 | 140 | 98 | 154 | 132 | 99 |
| JRB2a | 1 | 115 | 140 | 96 | 150 | 132 | 99 |
| JRB2b | 1 | 131 | 140 | 98 | 150 | 132 | 101 |
| JRG1a | 1 | 115 | 140 | 90 | 150 | 134 | 99 |
| JRG1b | 1 | 123 | 140 | 98 | 154 | 134 | 101 |
| JRG2a | 1 | 115 | 140 | 90 | 150 | 134 | 99 |
| JRG2b | 1 | 131 | 140 | 98 | 154 | 134 | -9 |
| MGB1a | 1 | 115 | 140 | 90 | 148 | 134 | 99 |
| MGB1b | 1 | 115 | 140 | 98 | 150 | 136 | 99 |
| MGB2a | 1 | 115 | 140 | 94 | 152 | 134 | 99 |
| MGB2b | 1 | 125 | 142 | 98 | 154 | 134 | 103 |
| MIT1a | 1 | 131 | 140 | -9 | 143 | 134 | 99 |
| MIT1b | 1 | 131 | 140 | 98 | 143 | 136 | 99 |

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| MIT2a | 1 | 131 | 140 | 98 | 143 | 134 | 99 |
| MIT2b | 1 | 131 | 140 | 98 | 143 | 134 | 99 |
| NB1a | 1 | 129 | 140 | 90 | 152 | 134 | 99 |
| NB1b | 1 | 131 | 140 | 98 | 156 | 134 | 99 |
| NB2a | 1 | 115 | 136 | 98 | 154 | 134 | 99 |
| NB2b | 1 | 123 | 140 | 100 | 156 | 134 | 99 |
| UBT1a | 1 | 129 | 140 | 88 | 143 | 134 | 99 |
| UBT1b | 1 | 129 | 142 | 90 | 143 | 134 | 99 |
| UBT2a | 1 | 129 | 140 | 88 | 143 | 134 | 99 |
| UBT2b | 1 | 131 | 142 | 90 | 143 | 134 | 99 |
| WIT1a | 1 | 123 | 142 | 96 | 152 | 134 | 99 |
| WIT1b | 1 | 131 | 142 | 98 | 154 | 134 | 99 |
| WIT2a | 1 | 115 | 140 | 96 | 154 | 134 | 99 |
| WIT2b | 1 | 123 | 142 | 98 | 156 | 134 | 99 |
| WST1a | 1 | 131 | 140 | 88 | 143 | 134 | 99 |
| WST1b | 1 | 131 | 140 | 98 | 152 | 134 | 99 |
| WST2a | 1 | 131 | 136 | 88 | 143 | 134 | 99 |
| WST2b | 1 | 131 | 142 | 98 | 152 | 134 | 99 |

## Allele Calls – Historic Population

| Individual | Population | AT12 | CA11c | CA12 | CA17 | GT10 | GT16 |
|---|---|---|---|---|---|---|---|
| NY_051010a | 1 | 137 | -9 | -9 | -9 | -9 | -9 |
| NY_051010b | 1 | 137 | -9 | -9 | -9 | -9 | -9 |
| NY_040525a | 1 | 114 | 140 | 91 | 146 | 117 | 99 |
| NY_040525b | 1 | 131 | 140 | 101 | 148 | 133 | 103 |
| NY_040518a | 1 | 114 | 140 | 82 | 152 | 133 | 99 |
| NY_040518b | 1 | 137 | 142 | 101 | 152 | 133 | 99 |
| NY_053016a | 1 | 114 | 140 | 82 | 152 | 132 | 99 |
| NY_053016b | 1 | 126 | 140 | 101 | 154 | 133 | 99 |
| VA_031522a | 1 | 114 | 140 | 91 | 152 | 133 | 99 |
| VA_031522b | 1 | 114 | 140 | 91 | 152 | 133 | 99 |
| MD_051705a | 1 | 126 | 140 | 88 | 152 | 132 | 99 |
| MD_051705b | 1 | 137 | 142 | 91 | 152 | 133 | 99 |

## BIOGRAPHY

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