Genetic structure in Peregrine Falcons (*Falco peregrinus*): An assessment using multiple markers

BY

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B.A., Universidad Nacional de Buenos Aires, 2002

THESIS

Submitted as partial fulfillment of the requirements

for the degree of Doctor of Philosophy in Biological Sciences

in the Graduate College of the

University of Illinois at Chicago, 2012

Chicago, Illinois

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To my husband, Dr. Ricardo Eusebi, who showed me how to entertain a thought without accepting it.

ACKNOWLEDGEMENTS

I am indebted to my committee members for their invaluable help and guidance from beginning to end: Mary V. Ashley, John M. Bates, Roberta J. Mason-Gamer, Miquel A. Gonzalez-Meler, Christopher J. Whelan, and Tanya Y. Berger-Wolf. I am also grateful to the numerous people that I had the privilege to meet during the course of graduate studies not only in the University of Illinois at Chicago but also at the Field Museum of Natural History. Both institutions made me feel at home throughout all these years.

I am very grateful to Mary V. Ashley for believing in my potential to do a Ph.D., welcoming me as a graduate student in her lab and guiding me throughout the course of my studies both inside and outside her lab. I am very thankful to John M. Bates whom I met previous to my admission to UIC and saw me evolved from a ‘volunteer’ to the Museum to a full-fledged researcher. His enthusiasm and optimism, present in all our discussions, were very much helpful during the course of my thesis writing. To both my ‘academic parents’ I say it was an honor to work with both of you and I only hope to be like you some day.

My husband, Ricardo, has been the greatest support of my life, not only morally but also intellectually. I thank him for putting up with me all these years. Without him this quest would probably have been less exciting. Not a day passes that I think how lucky I am that I met him even though I do not say it out loud. His passion for science and his untamed curiosity has always been an endless source of inspiration. When I look back at all the events that led to this moment there is no doubt in my mind that I could not have done it alone.

I have a profound gratitude for my family that endured this process from far away but nonetheless I felt they were all here with me. I am thankful to my father who inherited me his

love for books while my mother gave me her tenacity. I could not forget my sisters, Gabi and Caro, and my brother, Pablo, who always made me laugh and were there for me and whom I miss so much.

I could not forget to mention the ‘gang’ at UIC-FMNH who made this a wonderful experience from day one. We spent many years together learning, discussing ideas and sharing our views about life and science; Fusun Ozer, Diane McCarthy, Gabi Nuñez Iturri, Pepe Tello, Paul Velazco, Luca Borghesio, and Jace Robinson.

I am grateful to all the people at UIC who helped me smoothly navigate through all the paperwork processes at different points of my graduate studies. Special thanks go to Beth Brand who was very helpful during the final stages of my thesis writing and submission process.

Numerous institutions and granting agencies supported field work, laboratory work, and museum work. Many people shared their knowledge, spent time with me engaging in meaningful discussions, and showed me how wonderful Peregrines are. They are specially thanked in the acknowledgments section of the following chapters.

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LIST OF ABBREVIATIONS

°C	Centigrade
AFLP	Amplified Length Polymorphisms
AIc	Assignment Index corrected
AMNH	American Museum of Natural History, New York,
AMOVA	Analysis of Molecular Variance
bp	Base Pair
BSA	Bovine Serum Albumin
CI	Confidence interval
CR	Control Region
cyt *b*	Cytochrome *b*
DDT	Dichloro-diphenyl-trichloroethane
DNA	Deoxyribonucleic acid
dNTP	Deoxyribonucleotide Triphosphate
EPC	Extra Pair Copulation
EPF	Extra Pair Fertilization
ESA	Endangered Species Act
ESU	Evolutionarily Significant Unit
FMNH	Field Museum of Natural Museum, Chicago
gDNA	Genomic DNA
HWE	Hardy-Weinberg Equilibrium
IAM	Infinite Alleles Model
IBD	Isolation By Distance

LIST OF ABBREVIATIONS (Continued)

ICZN	International Code of Zoology Nomenclature
IUCN	International Union for Conservation of Nature
KCl	Potassium Chloride
km	Kilometer
LD	Linkage Disequilibrium
LOD	Overall Likelihood Ratio
M	Molarity
MCMC	Monte Carlo Markov Chain
mg	Milligram
MgCl2	Magnesium chloride
MHC	Major Histocompatibility Complex
ml	Milliliter
mM	Millimolar
MNHN	Muséum National d'Histoire Naturelle, Paris
mtDNA	Mitochondrial Deoxyribonucleic Acid
ND2	NADH Dehydrogenase Subunit 2
ND3	NADH Dehydrogenase Subunit 3
ng	Nanogram
PCA	Principal Coordinate/Component Analysis
PCR	Polymerase Chain Reaction
PID	Probability of Identity
RsaI	*Rhodopseudomonas sphaeroides*
SDS	Sodium Dodecyl Sulfate

LIST OF ABBREVIATIONS (Continued)

SMM	Stepwise Mutation Model
SMNH	Swedish Museum of Natural History
SOM	Suspension of Migration
sqrt	Square Root
SSC	Saline-Sodium Citrate
T_a	Annealing Temperature
tan	Tangent
Taq	*Thermus aquaticus*
TPM	Two-Phase Model
Tris-HCl	Hydroxymethyl-aminomethane-Hydrochloride
U	Units
uM	micro-Molar
UMMZ	University of Michigan Museum of Zoology, Ann Arbor, Michigan
USFWS	United States Fish and Wildlife Service
XmnI	*Xanthomonas camprestris pv. manihotis* 7AS1
Δ	Delta
µl	Microliter
µM	Micromolar

SUMMARY

This thesis explores mating system, effects of reintroductions, and worldwide population structure of the Peregrine Falcon (*Falco peregrinus*). The following chapters are based on a set of questions focused on different spatial and geographical scales, making use of nuclear and mitochondrial data to address these questions.

The Peregrine Falcon is a bird of prey with one of the largest natural distributions of any avian species. This species breeds on every continent except Antarctica, and on all major islands except New Zealand and Iceland. Earlier studies of Peregrine phylogeography as well as genetic effects after reintroduction efforts following pesticide use, and familial genetic relationships only focused on the northern hemisphere. My research questions touch all these aspects and employ extensive sampling and advanced statistical techniques to make robust inferences. I begin my thesis with a literature review on avian phylogeography and population genetic studies and recent advances made on statistical phylogeography.

Chapter 1: Subspecies delimitations, population differentiation and phylogeography in birds. Phylogeographic studies uncover patterns where genealogy and geography can be connected, revealing a great deal about the demographic and historical nature of intraspecific evolution. In contrast, population genetics paired with coalescent theory has the power to look back in time to aid in discerning which processes were involved in the evolutionary history of the species of interest.

This chapter focuses on why birds make good subjects for these kinds of studies, why genetic data is used to reveal geographical patterns in widespread species, and the use of genetic data to validate subspecies designations. I present case studies highlighting the use of both mitochondrial DNA, the marker of choice for phylogeographic studies, and nuclear markers,

which are used in population and landscape genetics. I describe my study species, the Peregrine Falcon, a cosmopolitan top-order predator which has faced several ecological and human generated factors that shaped population distribution and stability.

Chapter 2: Breeding and dispersal in urban Peregrines: integrating field and molecular data. The use of the pesticide DDT had negative impacts on Peregrine Falcons populations causing extirpation or sharp reduction in numbers. Following the banning of the pesticide and through captive breeding and hacking programs Peregrines made a successful comeback. I investigate the breeding and dispersal patterns in urban- dwelling reintroduced Midwestern Peregrine Falcons using molecular tools.

Data for eleven microsatellite DNA markers, including eight newly developed for the species, were gathered from a total of 282 chicks and 68 additional birds from nine Midwestern cities representing 20 nest sites, with most extensive sampling from Chicago.

I found high nest site fidelity, long-term mate fidelity, and two instances of extra-pair paternity. Field observations suggest that males preferred to breed close to their natal site, but this behavior left no clear genetic signal. My results are in accordance with previous findings for other raptors that genetic monogamy is the rule and nest site fidelity is frequent.

Chapter 3: Genetic evaluation of a successful recovery: the case of the extirpated Midwestern Peregrine Falcon (*Falco peregrinus anatum*). This chapter starts with an historical review of the extirpated Midwestern Peregrine, the nature of the recovery and how the reintroduced population successfully recovered.

Samples included museum specimens (*n*= 32, collected 1881-1963) representing the Midwestern-Eastern historical population, and contemporary samples (*n*=80, collected 1986-2009) from the recovered urban Midwestern population.

I detected differences in levels of genetic diversity for both periods. Average observed heterozygosity was significantly lower for the historical population while haplotype and nucleotide diversity were significantly higher for the historical population. Bayesian clustering analysis of microsatellite data revealed a genotypic shift between historical and contemporary populations. The cause of this shift is likely the result of the use of non-native stock in the recovery process.

Chapter 4: Inferring genetic discontinuities and testing predictions of ecological modeling in the recovered Peregrine Falcon (*Falco peregrinus anatum*) from the Western United States. Peregrine Falcon populations across the US abruptly declined in the 1950's due to the bioaccumulation of chlorinated hydrocarbon insecticides. Beginning in the 1970s, different populations had very different management histories.

In southern California, intense management began with two local pairs. In northern California, Oregon, and Western Washington remnant populations recovered on their own. In the Midwest, management was conducted using non-native stock. I used genetic markers to assess whether contrasting reintroduction strategies led to differences in genetic composition following recovery. Analyses were carried out on 192 birds, 112 individuals representing recovered Western US populations and 80 individuals from the Midwest, using one mitochondrial DNA gene (control region) and 11 polymorphic microsatellite loci.

I found genetic differentiation among populations, and similar levels of genetic diversity across regions. Microsatellite data separate Western Peregrines into three distinct genetic clusters: (i) Channel Islands and Southern California; ii) Northern California and Oregon; and iii) Washington. These genetic patterns were concordant with previous ecological modeling studies. My results do not support the hypothesis that different management techniques have left an imprint on these recovered populations, but there is genetic structure consistent with the original groups on which management plans were focused.

Chapter 5: Global scale genetic structure and the inference of seasonal migration and gene flow in biogeographical patterns. Worldwide Peregrines exhibit regional differences in behavior, morphology, and demographic history. A mix of migratory and resident breeding populations occurs in the northern hemisphere while exclusively resident breeding populations occur in the southern hemisphere where northern migrants come to winter.

This chapter focuses on understanding how Peregrines evolved such a wide-ranging distribution and how changes in migratory behavior or breeding dispersal have led to the formation of a distinct population structure. Two alternative hypotheses of range expansion and colonization are considered, Isolation by Distance, and Suspension of Migration.

Genetic results based on 11 microsatellite loci show low to medium degree of genetic differentiation among northern hemisphere Peregrines while significant differentiation was observed between northern migrants and southern residents in both Old and New World. Mitochondrial control region data reveals a similar picture with lack of differentiation between northern Peregrines and significant differentiation between northern and southern populations.

SUMMARY (Continued)

Worldwide genetic patterns derived from multiple type of analysis supported the Isolation by Distance hypothesis. Contiguous populations were less differentiated than far distant ones. Even though Suspension of Migration was not supported, mitochondrial DNA migration analysis detected a west to east historical gene flow in northern migrants, and a south to north direction of gene flow for Old World Peregrines.

1. SUBSPECIES DELIMITATIONS, POPULATION DIFFERENTIATION AND PHYLOGEOGRAPHY IN BIRDS

Modern evolutionary genetic studies which are concerned with describing both phylogeographic patterns and evolutionary processes require mitochondrial as well as nuclear data. Phylogeography uncovers patterns where genealogy and geography can be connected, revealing a great deal about the demographic and historical nature of intraspecific evolution. In contrast, population genetics paired with coalescent theory has the power to look back in time to aid in discerning which processes were involved in the evolutionary history of the species of interest. *This chapter is focused on why birds make good subjects for these kinds of studies, why use genetic data to reveal geographical patterns in widespread species, and the use of genetic data to validate subspecies designations. I present case studies highlighting the use of both mitochondrial DNA, the marker of choice for phylogeographic studies, and nuclear markers, which are used in population and landscape genetics. I describe my study species, the Peregrine Falcon, a pandemic top-order predator which has faced several ecological and human generated factors that shaped population distribution and stability. My research objectives include revealing genetic aspects associated with breeding behavior in a new reintroduced urban population, historical patterns in an extirpated population and direct comparisons with a recovered population, and the elucidation of evolutionary relationships among Peregrines distributed across the New and Old World.*

1.1. Introduction

One of the basic attributes of any species is its geographical range, yet we often have little understanding how each species achieved and maintains its current distribution. The study of the historical processes that explain the contemporary geographic distributions of individuals is the focus of a discipline known as *phylogeography*. This is accomplished by considering the geographic distribution of individuals together with patterns associated with a gene genealogy. Since its inception in the mid-1980s (Avise et al. 1987), thousands of studies have been carried out and new analytical tools continue to be developed. In the related fields of population and landscape genetics, allele frequencies and distributions of highly variable nuclear markers are also used to infer historical population and demographic processes. My dissertation carries on the phylogeographic tradition. In particular, the aim of my dissertation research is to give insight into phylogeographic patterns and population genetic processes of one globally distributed species that can be found on all continents except Antarctica; the Peregrine Falcon (*Falco peregrinus* Tunstall 1771).

Birds offer particular advantages for phylogeographical research. Their evolutionary relationships are relatively well understood; recent advances in molecular techniques have made possible to work with museum skins, and genetic data have been coupled with long-term observational field studies (Newton 2003). Not only have the major relationships of birds been reappraised at the levels of orders and families (Hackett et al. 2008), but many groups have been studied at the specific and intraspecific level (Milá et al. 2007; Wenink et al. 1996). Although still based on incomplete sampling, this has allowed researchers to make broad comparative studies to assess major patterns of avian evolution (Mittelbach et al. 2007; Ricklefs 2007). Compared with most other organisms, birds are highly mobile, and some are able to fly long

distances over sea, deserts and other inhospitable terrain. In this sense, birds are considered good colonizers that may exhibit adaptive radiations (Grant & Grant 2006).

The study of genetic patterns requires the use of molecular markers. Early phylogeographic studies used mitochondrial DNA (mtDNA) to produce haplotype trees, which rooted with a sister taxon reveals whether closely related haplotypes occur locally or are widespread (Zink & Barrowclough 2008). Nowadays, there have been an increasing number of studies that also include estimates of population parameters that are central in evolutionary processes. Even if the incorporation of nuclear markers such as amplified length polymorphisms (AFLP) and microsatellites might compromise the "phylo" component in phylogeographic studies they possess the potential of obtaining multiple gene estimates of evolutionary patterns and processes (Zink & Barrowclough 2008). My dissertation work uses both mtDNA, the traditional marker used for avian phylogeography, as well as highly variable microsatellite markers, a more recent molecular tool for assessing nuclear variation in order to infer population structure and patterns of gene flow.

1.2. Molecules and Genealogical History

Animal mtDNA has proven to be a valuable molecule for understanding the evolutionary relationships among individuals, populations, and species (Irwin et al. 1991; Zink & Barrowclough 2008). Mitochondrial DNA possesses a great number of variable characters with known genetic basis. Gene rearrangements rarely occur; it has maternal inheritance and a fast rate of evolution (Lowe et al. 2004). It has rarely shown recombination (Berlin & Ellegren 2001), and thousands of copies are present in somatic cells (Avise 2009). Concerns have been raised by different authors about the exclusive use of mtDNA to infer phylogeographic patterns (Ballard &

Whitlock 2004; Zhang & Hewitt 2003; Zhang & Hewitt 1996). These concerns primarily relate to the fact that the mitochondrial genome evolves as a linkage unit and no matter how many genes are sequenced only a single tree is recovered as a result of analyses. This may lead to a possible misrepresentation or a biased portrayal of the overall lineage history for species that have sex-biased dispersal (Zink & Barrowclough 2008), as well as it traces only one marker's genealogy. Another concern is that lineage sorting could obscure the interpretation of a single tree when multiple speciation events occurred closely spaced in time. Although all these concerns can potentially be the source of what patterns emerge, others have found that this is an infrequent phenomenon (McKay & Zink 2010).

When studies include closely related species or subspecies –as is the case for this study- the resultant mtDNA haplotype tree often is geographically unstructured, with haplotypes from different areas scattered on a shallow tree (Avise 2009). To obtain further resolution or to study recent events, many researchers incorporate nuclear markers based on the observed frequencies of phylogenetically unordered alleles, such as microsatellites. Advocates of mtDNA-only studies argue that the use of frequency markers might provide less direct information about population histories. However, when such data are accumulated across multiple unlinked loci, these can in turn be used in spatial genetic analyses at the intraspecific level (Avise 2009; Zink 2008; Zink & Barrowclough 2008).

Nuclear markers such as microsatellites are presumed to be neutral and provide an independently inherited set of molecular characters for revealing intraspecific genetic structure and assessing population history (Ashley & Dow 1994). Microsatellites are useful to investigate evolutionary questions at a variety of levels. Questions at the individual-level include determining gender (Longmire et al. 1993; Nesje & Roed 2000). For birds, microsatellites

located in the W chromosome serve as an unequivocal marker for females (McDonald & Potts 1997) since females are the heterogametic sex. Microsatellites are also useful at familial levels, including inferring parentage and relatedness (Blouin 2003; DeWoody 2005). At higher scales, these markers are variable enough to distinguish population-level processes such as gene flow, and admixture (Haig 1998), and they also have been used in comparisons among closely related species (Wandeler et al. 2007) because microsatellite primers will often work across closely related species (McDonald and Potts 1994). On the other hand, they are subject to homoplasy due their high mutation rate, and do not provide clear genealogies (Hewitt 2004b).

1.3. Factors Influencing Phylogeographic Patterns

Phylogeographic studies are concerned with the evolutionary history within species. More of a descriptive field, phylogeography focuses on discerning patterns of phylogroups – groups that share a common ancestry. These patterns are often correlated with climatic factors (glacial, dry-wet cycles, etc.), geographic (rivers, mountains, habitat discontinuities), and less often behavioral (changes in dispersal or migration routes). For studies focused on population processes, additional analyses using the same data seek to determine whether genetic discontinuities (or lack of them) are attributed to dispersal, gene flow, bottlenecks, or hybridization. Below, I will expand on these ideas and explain in more detail how the combination of these two fields, phylogeography and population genetics, have helped disentangle the recent evolutionary history of avian species.

A number of studies, particularly in Europe and North America, have invoked the influence of glacial cycles as one of the main causes for explaining present species distribution and inferring past distributional patterns (Stewart et al. 2010). This is not unexpected since

climatic change causes habitat alterations (such as fragmentation) that in turn affect population distributions. However, for changes leading to speciation, surviving patches of habitat had to be large enough to sustain the population for a sufficient period of evolutionary time to permit divergence, and far enough apart to prevent gene flow (Newton 2003). If the isolated populations later expand, in response to spreading habitat, they may come into contact again (secondary contact), with results ranging from complete interbreeding if they are not sufficiently different from one another to formation of co-occurring non-interbreeding units that may be considered species. All these stages: isolation, spread, and subsequent reconnection are represented in current distribution patterns of many Eurasian and North American bird species (Hewitt 2004b). In the north temperate zone, it is believed that climatic refuges have provided places where populations survived difficult periods (such as an ice age) and aided by this isolation there has been subsequent reconnection of such populations as climate improved, with speciation resulting in some, but not all cases. Since the avian fossil record is highly fragmentary, the range changes of most modern bird species can only be inferred from their present distributions, and the inferred location of past refuges (Newton 2003).

In the south temperate zone, only species from southern South America and New Zealand are in geographic positions comparable with glaciated areas on the northern hemisphere (Glasser et al. 2008). Pleistocene glaciations in South America were mostly restricted to the Southern Andes, and the shallow shelf of the Patagonian Atlantic coast was partly exposed when sea level dropped during glacial maxima. It has been suggested that the Patagonian steppe communities occurred several degrees of latitude farther north during Pleistocene cold cycles and shifted southward when the climate warmed up again (Jakob et al. 2009). The number of avian phylogeographical studies conducted in the Southern hemispheres is negligible compared to

those from the North hemisphere (Newton 2003), and lower latitude regions conformed by tropics and savannah (Africa, South America, South East Asia and North Australia) are rich in species diversity but little is known of their phylogeography or their paleobiology (Hewitt 2004a).

There is some empirical evidence that the geographical distributions of species have expanded and contracted in a cyclical manner according to the Quaternary climatic changes (Stewart et al. 2010). The concept of 'refuge areas' was first proposed to explain the immense variety of the Amazonia forest bird fauna which underwent dry-wet cycles during the Pleistocene and post-Pleistocene (Haffer 1969). Some genetic evidence suggests that young species are the result of not just one vicariant event, but of a succession of such events in the general areas, as described by glacial-non-glacial cycles. Nowadays, the refugia hypothesis has been modified from molecular evidence mostly from the dating of divergence events (Newton 2003) between species, which often appear to have occurred much earlier than the glacial or dry periods and for some species date back to the Pliocene, where we have little data on climatic cycles.

1.3.1. Colonization Patterns, Secondary Contact, and Response to Barriers

Many current bird distributions are in concordance with areas that remained favorable throughout the glaciations, as well as more northern areas that could have been occupied only since the last glacial retreat. A number of bird studies are in agreement with this reporting higher values of mtDNA sequence divergence in the refugia than in the recent colonized areas (Newton 2003). Merilä et al's (1997) genetic study on European Greenfinches, *Carduelis chloris*, found a deep split between northern and southern populations in Europe and low genetic diversity in northern populations. One possible explanation for this is that northern populations may have

descended from an Iberian stock that expanded north rapidly (Merilä et al. 1997). Their data, which shows a progressive decline of nuclear and mtDNA variability for these populations in more northern samples, supports a post-Pleistocene process. In contrast, other species from deglaciated parts of North America have shown low levels of phylogeographic differentiation, despite high levels of phenotypic differentiation, such as Red-winged Blackbirds, *Agelaius phoenicus* (Ball et al. 1988), Song Sparrows, *Melospiza melodia* (Zink & Dittmann 1993), and Sandhill Cranes, *Grus canadensis* (Jones et al. 2005). This could suggest that individuals from limited areas (microrefugia; Rull 2009) colonized glaciated areas and underwent morphological adaptations to local environmental conditions (Newton 2003). However, not all widespread bird species occupying recent deglaciated areas show low genetic differentiation. For example, the Yellow Warbler, *Dendroica petechia* shows clear eastern and western mtDNA haplotypes (Milot et al. 2000), Fox Sparrows, *Passerella iliaca* have four phylogeographic groups that correspond to groups defined by plumage characters (Zink 1994), and the Dunlin, *Calidris alpina* has up to nine subspecies recognized based on plumage and genetic data across a circumpolar distribution with low levels of gene flow making this species highly structured geographically (Wenink et al. 1996; Wenink et al. 1993).

The joining of formerly separated populations is frequently evident when the taxa involved have diverged in color patterns or calls. They often form a distinct contact zone where the two forms hybridize. For example, two well differentiated subspecies of Yellow-rumped Warblers, *D. c. auduboni* (western form) and *D. c. coronata* (eastern form) meet along a ~1000 km line that runs from Alberta to southeastern Alaska (Price 2008), and 0.2% mtDNA divergence has been reported (Milá et al. 2007). However, there are also species with no plumage variation that still show evidence of contact zones based on genetic data. In a study of

the Common Raven, *Corvus corax*, Omland et al (2000) reported a deep genetic break between birds from the Western US and those from the rest of the northern hemisphere based on cytochrome *b* sequences divergence (>4%) and microsatellites (F_{ST}= 0.067). Although ravens may have formerly consisted of two allopatric groups, they come into contact over a wide area in the western US, where they intermix (Omland et al. 2000).

A growing number of avian molecular evolution studies provide evidence for barriers as promoters of population differentiation. Such barriers may be areas of completely inhospitable or unsuitable habitat. Divergence on opposite sides of a barrier produces first geographically distinct populations, secondly subspecies and later allospecies (Price 2008). Examples of barriers for bird species are mountain ranges, deserts, rivers, and sea channels. Deserts can operate as barriers for non-deserts species, while wetter habitats can act as barriers for desert species. Bird species distributed throughout the desert in the Southwestern United States show different patterns of genetic differentiation (Newton 2003). The Canyon Towhee, *Pipilo fuscus* and Curve-billed Thrasher, *Toxostoma curvirostre* show substantial mtDNA differentiation between the Sonoran and Chihuahuan deserts, whereas several other bird species are not differentiated (Zink 1997).

In conclusion, mtDNA and nuclear DNA studies reveal past population splits that are not always apparent in the morphology of populations, and with sufficient sampling, they can elucidate the degree to which these populations are mixing. It thus becomes possible to calculate what proportion of species in an area have undergone past population splits, how divergent the different forms (or subspecies) are, and approximately estimate when their divergence began. The combination of genetic and geographical data helps to highlight the locations of past refuges and dispersal routes more precisely and in greater detail than can morphological data alone.

1.4. Genetic Evidence from Current Distributions

As previously stated, one of the focal points in current phylogeography studies is centered on whether existing sister taxa arose from a common ancestor mainly during the last glacial cycle (~ 100,000 years ago) or over multiple cycles extending through the Pleistocene or even earlier. A widely used approach is to estimate divergence time by measuring the 'genetic distance' between different populations and then working back on the assumption of a constant rate of nucleotide sequence divergence. This has been termed a "molecular clock" (Zuckerkandl & Pauling 1962). The mitochondrial gene cytochrome *b*, has been widely used in bird species that separated up to 4-5 million years ago**.** Faster evolving mtDNA control region sequences or nuclear microsatellites are useful for dating separations that happened in the past 10,000 years, since the end of the last glaciation (Newton 2008).

I reviewed avian case studies involving mitochondrial and nuclear DNA to assess the congruence of these markers in detecting phylogeographic patterns as well as tracing back origin of populations and routes of colonization (Table I). I compared studies that used only mtDNA sequences and/or microsatellites since these are currently the markers most commonly used for bird studies. One of the advantages of supplementing mtDNA phylogeographic studies with microsatellite markers is to obtain multiple gene estimates of evolutionary patterns and processes (Zink & Barrowclough 2008). Table I summarizes the general outcomes of comparisons of mtDNA and nuclear data. Overall, there was good correspondence between markers. However, in some cases there were slight differences. For example, there was partial agreement between microsatellites and the mtDNA gene tree for the Fox Sparrow, *P. iliaca* (Zink 2008). Since microsatellites recovered no more than three groups –instead of four mitochondrial based parapatric groups (Zink & Weckstein 2003), the discrepancy was explained by insufficient time

for nuclear allele sorting or hybridization at group boundaries. Some studies reported conflicting patterns in identifying population structure (Johnson et al. 2007), assessing levels of genetic diversity (Martinez-Cruz et al. 2007), and uncovering patterns of postglacial colonization (Brito 2007). Studies that showed agreement between the markers were the ones that pointed to isolation and limited gene flow as factors causing population structure including Tawny Owl, *Strix aluco* (Brito 2007), and Burrowing Owl, *Athene cunicularia* (Desmond et al. 2001, Korfanta et al. 2005). More congruent cases involved geographically structured species such as the Common Raven (Olmand et al. 2000), or included species with low levels of genetic diversity like the Gyrfalcon, *Falco rusticolus* (Johnson et al. 2007). Finally, congruence between markers was observed in species lacking monophyly as a whole like the Saker Falcon, *F. cherrug* (Nittinger et al. 2007).

TABLE I. EXAMPLES OF AVIAN PHYLOGEOGRAPHIC STUDIES EXHIBITING CONTRASTING OR CONCORDANT PATTERNS BETWEEN NUCLEAR AND MITOCHONDRIAL DATA

Species	*Location*	*N° populations/individuals*	*Markers*	*Mitochondrial data*	*Nuclear Data*	*Congruence?*	*Explanation*	*References*
Burrowing Owl (*Athene cunicularia floridana, A. c. hypugaea*)	Western and Southern North America (FL)	15 pop./201 individuals	297 bp cytochrome *b*, 7 microsatellite loci	0.7% seq. divergence between western forms and Florida pop.	Substantial genetic differentiation between western pop. and FL pop.	Yes	*Limited gene flow among populations. * Isolation	(Desmond et al. 2001; Korfanta et al. 2005)
Common Raven (*Corvus corax*)	North America, Northern Europe	72 individuals	314 bp Control Region, 307 bp cytochrome *b*, 3 microsatellite loci	> 4% seq. divergence between 2 clades; California and Holarctic	Significant differentiation (F_{ST}) for both clades	Yes	*Geographically structured species with wide contact zone	(Omland et al. 2000)
Dunlin (*Calidris alpina alpina, C. a. arctica, C. a. centralis and C. a. schinzii*)	Western Palearctic and East Greenland	19 pop./328 individuals	505 bp control region, 7 microsatellite loci	Svalbard pop. linked to East Greenland and around the Baltic Sea	Svalbard pop. only linked to East Greenland	Yes, but slight difference	* Slow rate mutation of mtDNA compared to microsatellites	(Marthinsen et al. 2008)
Fox Sparrow (*Passerella iliaca iliaca, P. i. unalaschcensis, P. i. schistacea, P. i. megarhynca*)	US, Canada	46 pop./333 individuals	308 bp Control Region, 1,030 bp ND2, 348 bp ND3, 433 bp, cytochrome *b*; 5 microsatellite loci	Reciprocal monophyly in mt DNA gene tree for four groups	Significant pop. structure but support for only three groups	Yes, but one clade not supported	*Insufficient time for nuclear gene lineage sorting or introgression. *Hybridization in contact zone	(Zink 2008; Zink & Weckstein, 2003)

TABLE II, CONTINUATION

Species	*Location*	*N° populations/individuals*	*Markers*	*Mitochondrial data*	*Nuclear Data*	*Congruence?*	*Explanation*	*References*
Saker Falcon (*Falco cherrug)*	Africa, Europe, Asia	12 pop./244 individuals	412 bp Control Region, 6 microsatellite loci	Two shallow non-monophyletic clades	Populations not clearly differentiated	Yes	*Low number of nuclear markers used. *Incomplete sorting of mtDNA lineages. *Insufficient time	(Nittinger et al. 2007)
Spanish Imperial Eagle (*Aquila adalberti*)	Spain	2 pop./113 individuals	345 bp Control Region, 10 microsatellite loci	Levels of genetic diversity reduced by recent decline	Constant levels of genetic diversity at the nuclear genome	No	* Mitochondrial effective population size four times lower than nuclear genome	(Martinez-Cruz et al. 2007)
Tawny Owl (*Strix aluco aluco; S. a. sylvatica*)	Western Europe, North Africa	14 pop./187 individuals	1,425 bp Control Region, 7 microsatellite loci	1- Three major refugia and Balkan origin supported. 2-Low genetic diversity (northern pop.)	1- Idem. 2- High genetic diversity (northern pop.)	Yes (population structure). No (postglacial colonization)	*Isolation (population structure). *Recent bottleneck recovery (postglacial colonization).	(Brito 2007)

1.5. Empirical Avian Phylogeographic Patterns

Species that inhabit large areas often exhibit clinal variation across their ranges, possibly due to temperature variation and other climatic factors. This is a common pattern described for a number of avian species and subspecies. There are many well documented morphological clines for birds based on body size, coloration, and presence-absence of color morphs (Soltis et al. 2006; Zink 1996). One explanation for the existence of clines is local adaptation, which is inhibited by gene flow from neighboring populations, resulting in a gradual trend of certain characters across its distribution (Winker 2010). In continental species the absence of barriers makes intergradations with one another more likely as seen in Alaska Song Sparrows (Pruett & Winker 2010) but can also be equally common in widespread species that are capable of using a broad niche like the Slate-throated Redstart, *Myioborus miniatus* (Pérez-Emán et al. 2010). Fine-scale genetic studies usually detect clines and also disjunctions in populations through interconnections and gradual changes in genetic patterns (Manel et al. 2003), and by how genetic diversity (i.e. neutral and adaptive component) is affected by spatial and environmental variables (Segelbacher et al. 2010). Finally, one factor often ignored is a species' dispersal behavior which is directly linked to the degree of clinal and subspecific differentiation (Belliure et al. 2000). For example, migratory species or populations typically show less geographic variation in color and size than non-migratory groups (Newton 2003). In some cases, as shown in the next section, genetic evidence defining clines or genetic entities often correspond to previously morphological described subspecies. Subspecies may be seen as useful entities that contributed to a better understanding of the geographic variation of taxa subjected to microevolutionary processes and selection pressures that vary across the geographic landscape (Mayr 1980).

1.6. Validating Morphological Subspecies with Genetic Data

The concept of subspecies has undergone many changes, from describing "varieties" in the Linnaean period, to describing well-defined entities at a lower taxonomic level than species at the end of the 19th century, to a prevalent unit with the emergence of the biological species concept (Mayr 1980). In this sense, subspecies have functioned as units of classification, as subjects to test evolutionary theories, and more recently as targets for conservation plans (Zink 2004). Subspecies are defined as a taxonomic category in the official International Code of Zoology Nomenclature (ICZN; Ride 1999), being the lowest rank that is consistent with the biological species concept (Mayr 1963).

Subspecies are usually divided by a steep character gradient or cline over a small geographic space (Endler 1977), that may be correlated with environmental factors, past or present. Traditionally, subspecies of birds have been defined by morphological traits or color variations (Haig et al. 2006) and the concept has been widely applied by ornithologists. Nevertheless, recent avian subspecies debates have arisen motivated from studies of genetic population structure that differed from morphology-based subspecies delineations (Zink 2004). This has led to an increasing number of studies where genetics patterns have been compared to morphological traits and/or geographical variation. Avian studies have covered numerous species and thus far a few main patterns have emerged. Five main patterns, the first four modified from Newton (2003), are presented with associated case examples:

1- No geographical pattern in either mtDNA, microsatellites or in morphology –attributed to recent expansion from a limited area too small to allow for geographical structuring (Gyrfalcon; Johnson et al. 2007), insufficient time for differentiation to occur (Saker

Falcon; Nittinger et al. 2007), panmixia (Snowy Owl; Marthinsen et al. 2009), or high gene flow across a wide area (Swainson Hawk; Hull et al. 2008).

2- Geographical structuring in mtDNA and microsatellites matching the geographical variation in morphology as reflected in clines and/or subspecies –attributed to long residence in the area (Dunlin; Marthinsen et al. 2008) or residence in a number of cores areas (past refugia) within it (Tawny Owl; Brito 2007).

3- Geographical structuring detectable in mtDNA and microsatellites but no morphological differentiation –attributed to past population fragmentation but no morphological differentiation followed by subsequent expansion and fusion (Common Raven; Omland et al. 2000), or asymmetric gene flow (Spanish and Eastern Imperial Eagles; Martinez-Cruz & Godoy 2007).

4- No detected geographical structuring in mtDNA but apparent clinal or subspecific variation in morphology and microsatellites –attributed to recent expansion from a single area followed by rapid morphological adaptation to local conditions (Red-winged Blackbird; Ball et al. 1988; Loggerhead Shrike; Eggert et al. 2004; Red-winged Blackbird; Williams et al. 2004) which may subsequently be affected by hybridization (Loggerhead Shrike; Patten & Campbell 2000).

5- Detected geographical structure in mtDNA and concordant subspecific morphological separation but lack of differentiation in microsatellites –attributed to insufficient time for nuclear allele sorting or hybridization at group or cluster boundaries (Fox Sparrow; Zink 2008).

Dissecting patterns observed in nature depends heavily on the interpretations researchers make about the data. The authors of each of the studies mentioned above pointed out at least two

causes that might explain their results. While genetic data can be tested against models, these models also rely strongly on assumptions. In any case, the combination of datasets provides a more accurate depiction of the notion of 'subspecific boundaries' species biogeography than is discernible from morphology alone.

1.7. Study Species

The Peregrine Falcon is a crow-sized bird of prey with one of the largest natural distributions of any avian species. This species breeds on every continent except Antarctica, and on all major islands except New Zealand and Iceland (Hickey & Anderson 1969; White & Boyce 1988). Geographic variation in external morphology (i.e. size and color) has led to the description of 19 subspecies (Stresseman & Amadon 1979). A mix of migratory and resident populations occurs in the northern hemisphere while exclusively resident populations live in the southern hemisphere. This study will concentrate on samples that have previously been categorized into seven subspecies. The northern migrants include European Peregrine (*F. p. peregrinus* Tunstall 1771), American Tundra Peregrine (*F. p. tundrius* White 1968), and Siberian Tundra Peregrine (*F. p. calidus* Latham 1790). The northern residents include American Peregrine (*F. p. anatum* Bonaparte 1838) and Mediterranean Peregrine (*F. p. brookei* Sharpe, 1873). Finally, the southern residents contain South American Peregrine (*F. p. cassini* Sharpe, 1873) and African Peregrine (*F. p. minor* Bonaparte 1850) that inhabit the New and Old World, in that order. Genetic data was collected from current populations except for the historical appraisal on Midwestern Peregrines populations (Chapter 3), and phylogeographical assessment (Chapter 5) from Peregrines around the world.

To date, several research papers have investigated Peregrine phylogeography in the northern hemisphere (Brown et al. 2007; Johnson et al. 2010; Nesje et al. 2000a), genetic effects after reintroduction efforts (Brown et al. 2007; Jacobsen et al. 2008; Johnson et al. 2010), and genetic relationships (Nesje et al. 2000b). These studies focused on restricted geographic regions as well as presumably distinct subspecies. Two of these studies incorporated data on historical almost extirpated populations and investigated how the supplement of indigenous (Brown et al. 2007) and exogenous (Jacobsen et al. 2008) captive breeding stock may have altered the present genetic composition of Peregrine populations. The study of Brown et al. (2007) covered the entire geographical range of *F. p. pealei* and *F. p. tundrius,* it did not include the complete distribution of *F. p. anatum*. Nesje et al. (2000a) and Jacobsen et al. (2008) studies dealt only with the northern European range of *F. p. peregrinus*. These past studies only used Nesje's microsatellites markers (2000b). For this study, I use only three of Nesje's markers along with eight new microsatellite loci I developed specifically for Peregrine Falcons.

1.8. Research Objectives

I use genetic markers to gain insight into the individual behavior and evolutionary processes that distinguish and shape Peregrine populations. In Chapter 2, I determine familial relationships, and infer breeding behavior in a newly established urban population in Chicago, IL. In Chapter 3, I investigate whether changes in genetic variation and genetic structure in an extirpated and reintroduced urban population in Midwestern North America occurred. Chapter 4 includes an analysis of fine scale population genetic patterns of Western North American populations. Finally, in Chapter 5, I test coalescent models against genetic data from current geographical patterns in both the Old and New World Peregrine populations to ascertain whether

similar processes have occurred in both hemispheres. Globally, the assessment of the evolutionary relationships among migratory and resident populations of Peregrines is used to test the role of long distance dispersal and migration in achieving their current distribution. Widespread species such as Peregrines fall at one end of the spectrum of possible distributions and may harbor cryptic genetic structure (Omland et al. 2000). In order to decipher the phylogeographic history of a species it is essential to take into consideration its natural history and how it expanded and contracted in its particular geographic distribution (Hewitt 2004b). I take advantage of advances in coalescent theory which now allows model-based parameter estimation as well as hypothesis testing that has increased the statistical rigor of phylogeography (Hickerson et al. 2010).

In summary, this study undertakes specific and broad questions using three different tiers of molecular markers (i.e. individual genotypes, population allele frequencies, and gene genealogies). As such, it is an ambitious project but guided by population genetics and phylogenetics theory, and the long-standing discipline of biological sciences, I am confident my work sheds light on not yet well-known evolutionary and biological aspects of this charismatic and intriguing species.

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2. BREEDING AND DISPERSAL IN URBAN PEREGRINES: INTEGRATING FIELD AND MOLECULAR DATA

Peregrine Falcons were extirpated by the pesticide DDT in the 1960s but have rebounded following the banning of the pesticide and successful captive breeding and hacking programs. This reestablishment has been accompanied by the creation of essentially new populations of Midwestern Peregrines that inhabit urban centers. I investigate the breeding and dispersal patterns in urban- dwelling Midwestern Peregrine Falcons using molecular tools. Data for eleven microsatellite DNA markers, including eight newly developed for the species, were gathered from a total of 282 chicks and 68 additional birds from nine Midwestern cities representing 20 nest sites, with most extensive sampling from Chicago. To document mating patterns, parentage was inferred by likelihood techniques when both parents were sampled and by parental genotype reconstruction when only one parent was sampled. In cases where neither parent was sampled, a sibship reconstruction approach was used. I found high nest site fidelity and long-term mate fidelity in urban Peregrines. Two instances of extra-pair paternity are discussed in light of long-term monitoring at the nest sites. Field observations suggest that males preferred to breed close to their natal site, but no conclusive evidence for this was observed in the genetic analysis. My results are in accordance with previous findings for other raptors that genetic monogamy is the rule and nest site fidelity is frequent.

2.1. Introduction

The application of molecular techniques to study breeding behavior has expanded our knowledge about many aspects of avian reproductive strategies that have been difficult to assess

from field observations alone. For example, we can ask whether social and genetic mating systems match and whether long-term mate and/or site fidelity is common. Identification of the entire set of active breeders can be determined through parentage analysis and sibship reconstruction, which in turn allows fitness of individuals and pairs to be measured. Molecular approaches can be used also to investigate patterns of dispersal, including the prevalence of sex-biased dispersal (Mossman & Waser 1999; Goudet et al. 2002). In any study a combination of field observations and molecular data can provide more robust inferences. Banding reports, field observations, and nesting chronology, analyzed together with data from molecular markers, provides a more comprehensive assessment of the behavior of the target species than either type of data taken alone.

I use such an integrative approach to evaluate reproductive and dispersal behavior of recently established urban populations of a formally endangered species, the Peregrine Falcon (*Falco peregrinus*) in the Midwestern US. Using both genetic and observational data, I identify unknown and/or unsampled breeders through analysis of their progeny. These in turn allows me measure nest site and mate fidelity, and assess dispersal in an urban environment. Several distinctive features characterize this urban Midwestern population: 1) it is monitored closely, with a region-wide network of observers that has made it possible to obtain detailed field data on individuals; 2) population density has been growing steadily since the reintroduction, leading to intraspecific interactions over territories, including fights between neighboring territory holders and the occurrence of floaters of unknown origin (Tordoff & Redig 1997); and 3) it is not uncommon for urban Peregrines to have 4-5 young per nest (Redig et al. 2007; Redig et al. 2008), whereas typical clutch size for the species in natural habitat is closer to three (Burnham et al. 2003). Thus, increased population density and aggressive territorial interactions may facilitate

swapping among breeders and increase the likelihood of local extra-pair fertilizations (EPFs) (Tordoff & Redig 1999b). In addition, a large portion of North American Peregrines breeds at high latitudes regions in Canada and Alaska and migrates annually to South America to winter. As a result, each year migrant birds interact with Midwestern birds as they pass through this region northward (Fuller et al. 1998). This would suggest another source of EPFs in these mid-latitude urban systems. There are observations of territorial birds copulating with northbound migrant Peregrines (Tordoff & Redig 1997), which suggests that EPFs may be facilitated and thus more common in these urban systems. Using microsatellite data, I characterize patterns of dispersal, reproduction, and mating that have emerged as a result of a major shift in the ecology and habitat of a re-established species.

2.1.1. Demise and Recovery of Midwestern Peregrine Populations

The original *Falco peregrinus anatum* populations in the Eastern-Midwestern US were extirpated (Berger et al. 1969). Their demise was due to the effects of chlorinated hydrocarbon insecticide contamination (DDT; 1,1,1-thrichloro-2,2-bis[p-chlorophenyl]-ethane) during the 1950s and 60s that caused the thinning of eggshells, preventing reproduction (Ratcliffe 1967; Hickey & Anderson 1968). The current urban Peregrine populations of the Midwest result from a captive breeding reintroduction program launched in the 1980s under the coordination of the Raptor Center in Minnesota. Over 1,000 progeny of captive pairs were released from 1982 through 1998 (Tordoff et al. 2003). The Midwestern Peregrine reintroduction program included twelve states and two Canadian provinces (Redig et al. 2007). My study is centered on Chicago, Illinois, one of the urban areas that have shown a sustained growth rate. From 1986-1990, the Chicago Peregrine Program (http://fieldmuseum.org/explore/illinois-peregrines) released a total

of 46 Peregrines from four different hack sites. Starting with a single breeding pair at the Wacker site in 1988, as of 2009 there were 14 breeding pairs and eight non-breeding territories across the Chicago region (Redig et al. 2009). Banding started with the breeders in 1985 and blood sampling from chicks started in 1990 (Redig & Tordoff 1990). Most nestlings in Chicago nests have been banded and sampled for DNA analysis, providing an unprecedented opportunity for studying these birds.

2.1.2. Parentage and Sibship

Parentage analysis using genetic markers has become a fundamental tool for research in behavioral ecology (Jones et al. 2010). Two key factors responsible for the boost of this relatively young discipline are the discovery of microsatellites about 20 years ago (Tautz 1989) and the refinement of statistical techniques for the analysis of these data in parentage studies (Jones et al. 2010). Sibship reconstruction, a more recent approach, is commonly used when parental data are incomplete or lacking (Wang 2004; Berger-Wolf et al. 2007; Ashley et al. 2009).

To investigate questions about breeding patterns, such as whether the same parents come to breed to the same nest site during consecutive years and, whether EPFs occur, I used a combination of molecular and analytical approaches, including parentage assignment and parentage and sibship reconstruction. These approaches are particularly valuable when applied to long-term studies with extensive sampling across generations which provide data about familial relationships that cannot be inferred otherwise.

The study of mating systems starts with the observation of breeding behavior. In general, birds of prey are large and perform conspicuous copulation displays (Ratcliffe 1980; Sodhi

1991). A great majority copulate at very high rates (up to several hundred times a clutch) over extended periods of time (Mougeot 2004). Peregrines are no exception. Tordoff and Redig (1997) reported that territorial male Peregrines seem willing to accept any female as a mate. Through a combination of observational data, parentage, sibship reconstruction of offspring based on microsatellites, I determine mate and nest site fidelity, and the rate of switching among pairs, even in cases where breeders are unbanded and unidentified for Midwestern urban Peregrines. For monogamous birds, it is also possible to look for evidence of single or multiple mating during consecutive years using parental reconstruction techniques as a way of identifying the most productive breeders, and long-term reproductive output.

2.1.3. Dispersal Patterns

Dispersal is important as it is a primary determinant of abundance, range limits, and genetic composition of animal populations (Greenwood 1980; Newton 2003, 2008). Two types of dispersal need to be recognized; *natal dispersal*, measured by the linear distances between natal and first breeding sites and *breeding dispersal*, measured by the linear distances between breeding sites in successive years (Newton 2008). Tests for sex-biased dispersal using bi-parentally inherited genetic markers such as microsatellites have not been done in many avian species (Goudet et al. 2002). The most common pattern in birds is for young females to disperse further between natal sites and breeding sites than males (Newton 1979; Greenwood 1980). This tendency holds true for owls and raptors, but also for many for many passerines, waders and colonial seabirds (Newton 2003).

Previous work on natal dispersal, based on movements of banded Peregrines has shown that natal dispersal differs between sexes. For the urban Midwestern populations, Tordoff and

Redig (1997) calculated a mean natal dispersal distance of 320 km for females compared to 176 km for males. The direction of dispersal appeared random, although many pairs settled close to the Great Lakes, indicating a tendency to remain near large bodies of water, possibly because these areas concentrate migrating avian prey. With regard to breeding dispersal, raptors tend to return to nest in the same areas in consecutive years (Steenhof & Peterson 2009).

I use field data to estimate sex bias in natal dispersal for breeders banded as chicks and compare these estimates to measurements based on a genetic assignment method (Favre et al. 1997; Mossman & Waser 1999). This study provides a rare opportunity to follow the expansion of a recently established urban population of Peregrines. I present the first data on the genetic patterns associated with breeding behavior and dispersal on a species that continues to adapt to a growing urban landscape, prompted by past reintroduction strategies but probably maintained by intrinsic aspects of the biology of the species.

2.2. Materials and Methods

2.2.1. Sampling

Since reintroduction programs started in the 1980s, most Peregrines born in the Midwestern region have been banded as chicks and blood samples were taken. Peregrines banded in the Midwest wear a bicolor project band, black over green (b/g) or black over red (b/r) on the left leg **(**Figure 1**)**. In addition, wild-produced birds have a purple anodized United States Fish and Wildlife Service (USFWS) band on the right leg; 'hacked' birds (born in captivity, later released in the wild) have a gold anodized USFWS band also on the right leg. The project bands have proven to be readable in the field given the right circumstances (i.e. many hours of

observations or multiple visits to the nests) and appropriate optics. However, as the population expands, there are a growing number of unbanded birds. There are many reasons for this; some nests go undiscovered or are unreachable, and other nests are located outside urban areas where they are monitored less frequently. In my study, parentage analysis was conducted at nests where breeders were known (banded) and when blood samples were available from one or both. When only one parent was sampled the genotype of the other parent could be reconstructed from the genotypes of the offspring. Blood samples are taken during banding when nestling Peregrines are

Figure 1. Types of bands used for Midwestern Peregrine Falcons. US Fish and Wildlife Service shown on right side and Midwestern band (b/r) on left side.

21 to 35 days old. Nestlings also can be sexed at this time (by weight and measurements), and are different size bands used for male and female chicks. Blood samples are deposited at the Bell Museum, and/or Raptor Center in Minnesota. Identity of banded birds, productivity, and locations of nests can be retrieved from an online database maintained by the Midwest Peregrine Society (http://www.midwestperegrine.org/). No adults were sampled at the nest. Blood samples, in these cases, were obtained only if the adult was sampled and banded as chick. When samples

could not be obtained from banded adults, I incorporated this information into the analysis and considered the adult as "known." Finally, adults that had no bands (or could not be identified) were tagged as "unknowns." For these unknowns, it is possible to look for evidence of parentage during consecutive years using parental reconstruction techniques as a way of identifying the most productive breeders, and long-term reproductive output.

The location of nests (latitude and longitude) was accessed from the Peregrine database. From this, I determined natal dispersal distance, as defined by the straight-line distance from hack or natal site to the first breeding site. For determining preferred breeding orientation relative to the natal site I converted the latitude and longitude rectangular values to polar coordinates using the following expression: $R = sqrt\ [x^2 + y^2]$ and $tan\ t = y / x$**,** where x and y are the longitude and latitude rectangular values, respectively, R is the radius, and t is the polar angle.

2.2.2. Microsatellite Screening and DNA Extraction

I used an enrichment protocol (Glenn & Schable 2005) to isolate microsatellites from two Midwestern Peregrine Falcons. Genomic DNA (gDNA) was digested with two different restriction enzymes (RsaI and XmnI), and linkers (SuperSNX24) were used to ligate the ends of gDNA fragments. Biotinylated probes were hybridized to gDNA. Magnetic beads (Invitrogen) were added and the mixture was washed twice with 2xSSC, 0.1% SDS and four times with 1xSSC, 0.1% SDS at 55°C. A magnetic particle-collecting unit was used to capture the magnetic beads between washes, which are attached to the biotin-gDNA complex. The enriched fragments were removed from the biotinylated probe by denaturation at 95°C and precipitation with a mix of 95% ethanol and 3 M sodium acetate. Enriched fragments were amplified using PCR, and

cloned using the TOPO-TA Cloning® kit following the manufacturer's protocol (Invitrogen). Bacterial colonies containing a vector with gDNA were used as template for subsequent PCR in a 25 µl reaction containing 1xPCR buffer, 1.5 mM $MgCl_2$, 0.12 mM of each dNTP, 10xBSA, 0.25 µM of the M13 primers (Forward: 5'GTTTAAGGCCTAGCTAGCAGAATC3'; Reverse: 5' CAGAATGGCTATGGTATTGGGAAAC3 '), and 1 U *Taq* DNA polymerase. Thermal cycling was as follows: 95°C for 20 s, 50°C for 7 min followed by 35 cycles of 95°C for 20 s, 50°C for 20 s, and 72°C for 90 s. DNA sequencing from cleaned PCR products was performed using the BigDye® Terminator v3.1 kit (Applied Biosystems), and sequencing reactions were run on an ABI3730 analyzer. Primers flanking core microsatellite repeats were developed using Primer3; http://frodo.wi.mit.edu/primer3/.

Genomic DNA was extracted from blood samples using the Qiagen DNeasy Tissue Kit® following manufacturer's protocol. Multilocus genotypes for 11 loci were obtained for 350 individuals. Eight of the loci were developed as described above, and three were previously developed by Nesje et al., (2000a) (Table I**).** PCR reactions in a total volume of 10 µl consisted of 1xPCR Buffer (10 mM Tris-HCl, 1.5 mM $MgCl_2$, 50 mM KCl, pH 8.3), 0.6 µM of each primer, 200 µM each dNTP, 0.6 U *Taq* and approximately of 10ng/µl gDNA. Thermal cycling was a follows: 94°C for 4 min, 35 cycles at 94°C for 30 s, T_a° for 20 s (Table II) and 72°C for 15 s**.** For fragment analyses 1 µl of PCR products were mixed with the GeneScan™ 500 LIZ™ Size Standard (Applied Biosystems) with formamide, and run on an ABI3730 DNA Analyzer. Genotyping was carried out using the STRand Analysis Software v.2.4 (Toonen & Hughes 2001).

2.2.3. Statistical Analyses

Deviations from Hardy-Weinberg equilibrium, and the presence of null alleles were tested in CERVUS 3.0 (Marshall et al. 1998), and linkage equilibrium was tested in GENEPOP on the web (Raymond & Rousset 1995; Rousset 2008). To obtain population allele frequencies, analyses of population summary statistics were carried out with all 350 unique individuals for 11 microsatellite loci (Table III).

Field and genetic data were incorporated in the analysis of sibships, parentage and to detect events of EPFs, using a mixture of methods (Figure 2). Maternity, when the mother was sampled, was confirmed by exclusion using CERVUS v.3.0 (Kalinowski et al. 2007). When samples were not available, maternity was inferred through parentage and/or sibship reconstruction with GERUD2.0 (Jones 2005), and COLONY v.2.0 (Wang & Santure 2009). Paternity was assigned through likelihood in CERVUS. This method considers the proportion of candidate parents sampled, allows for genotyping errors (null alleles, mistypes), and calculates statistical confidence based on the difference in LOD scores of candidate parents, Delta Δ, (i.e. the natural log of the likelihood ratio). Tending males from a given nest and adjacent nests from the same year were considered as candidate fathers. For nests where no tending male was genotyped, adult sampled males from adjacent nests and neighboring nests in the same city were considered as candidates and tested.

TABLE I. REPEAT MOTIFS, PRIMER SEQUENCES, SIZES, AND SOURCE FOR 11 PEREGRINE FALCONS MICROSATELLITES MARKERS.

		Primers (5' → 3')				
Locus	Repeat motif	Forward (label)	Reverse	T_a°	Size range	Source
CAB *fp*18-2	$[AGTTAAGTT]_{28}$	ACAGGATACATGCTTTGTAGCTC (FAM)	GCCACCAGGACCAAATTTCT	54	158 – 482	present study
CAB *fp*24	$[TATTC]_{17}[TGTCC]_6$	AACCCCCATGATGAACAAGA (NED)	CATTGCAAACATCTCCATAGTCA	54	211 – 286	present study
CAB *fp*77	$[TGGA]_6GA[TGGA]GA[TGGA]_8$	TCTTCCATCTGGGCTTCATT (NED)	ATCCTCCTGCCAAAGCAACT	51	241 – 281	present study
CAB *fp*85	$[TATC]_{10}$	TGCCAGTCAGGTCACAATTT (VIC)	CCCCACGGAAATTAATAGACTT	55	238 – 258	present study
CAB *fp*117	$[CATA]_7$ CATG $[CATA]_4$	TGTGCCTACCCAAAAGCAAT (PET)	ACATCCAAAAGTGGCACCTC	56	246 – 270	present study
CAB *fp*120	$[TATC]_{15}$	TGGTAGGCATTTGGATGTGA (NED)	CGCAGGTTTCTTGTGCTGTA	55	261 – 277	present study
CAB *fp*157	$[TCTA]_{14}$	GAGGGGAAAAATTGTGGGATA (FAM)	TTGGAAAGCATATTGCATCG	55	200 – 236	present study
CAB *fp*181	$[TCTTA]_{11}$	CCTGAAAACCTGTCATGTCCT (PET)	TCAGGCCCCTTTGAGATTAAGA	57	178 – 368	present study
NVH *fp*13	$[CA]_{12}$	AGCTTGATTGAGGCTGTG (VIC)	CCAAATTCCCTGCTGAAG	61	115 – 131	Nesje et al 2000b
NVH *fp*31	$[CA]_{17}$	ATCACCTGCACATAGCTG (NED)	TTTAGCTCCTCTCTCTCAC	51	165 – 177	Nesje et al 2000b
NVH *fp*89	$[AT]_{12}$	CTCTGCCCTGAATACTTAC (FAM)	GAATCTTGTTTGCATTGGAG	58	142 – 162	Nesje et al 2000b

Males (or mother-father pairs) were allocated parentage if they were assigned at 95% or higher confidence. Males were allocated parentage if they were assigned at 80%, and if there were zero or one parent-offspring mismatches. For those cases where only maternity or paternity was solved and the remaining parentage was unresolved, GERUD was used to reconstruct the unknown parental genotypes. Finally, in cases where neither parent was sampled, a sibship reconstruction approach was used to calculate the most likely number of parent's contribution to each offspring array by inferring sibling relationships among offspring, using the program COLONY (Wang & Santure 2009). Simulations with CERVUS were done to estimate the resolving power of microsatellites and to estimate critical values of Δ. Parallel simulations were done for paternity inference with and without maternal genotypes. Simulation parameters values were empirically calculated from my data set (in parentheses) and included: 1- number of candidate males estimated from field data (*n*=25), 2- proportion of candidate males sampled (0.641); 3- proportion of loci typed, averaged across all loci and individuals (0.991), and 4- error rate, which accounts for the fraction of loci typed incorrectly averaged across all loci and individuals (0.022). The final phase of the simulation finds critical values of Δ for relaxed (80%) and strict (95%) confidence levels based on 10,000 tests. I conducted the CERVUS analysis for a subset of chicks born between 1993 and 2009. There were few mismatches across multiple loci so maternity was confirmed for all cases when the presumed maternal genotype was available. The few mismatches were interpreted as genotyping errors. My estimate for the overall rate of typing error based on CERVUS is 2% which is similar to most studies.

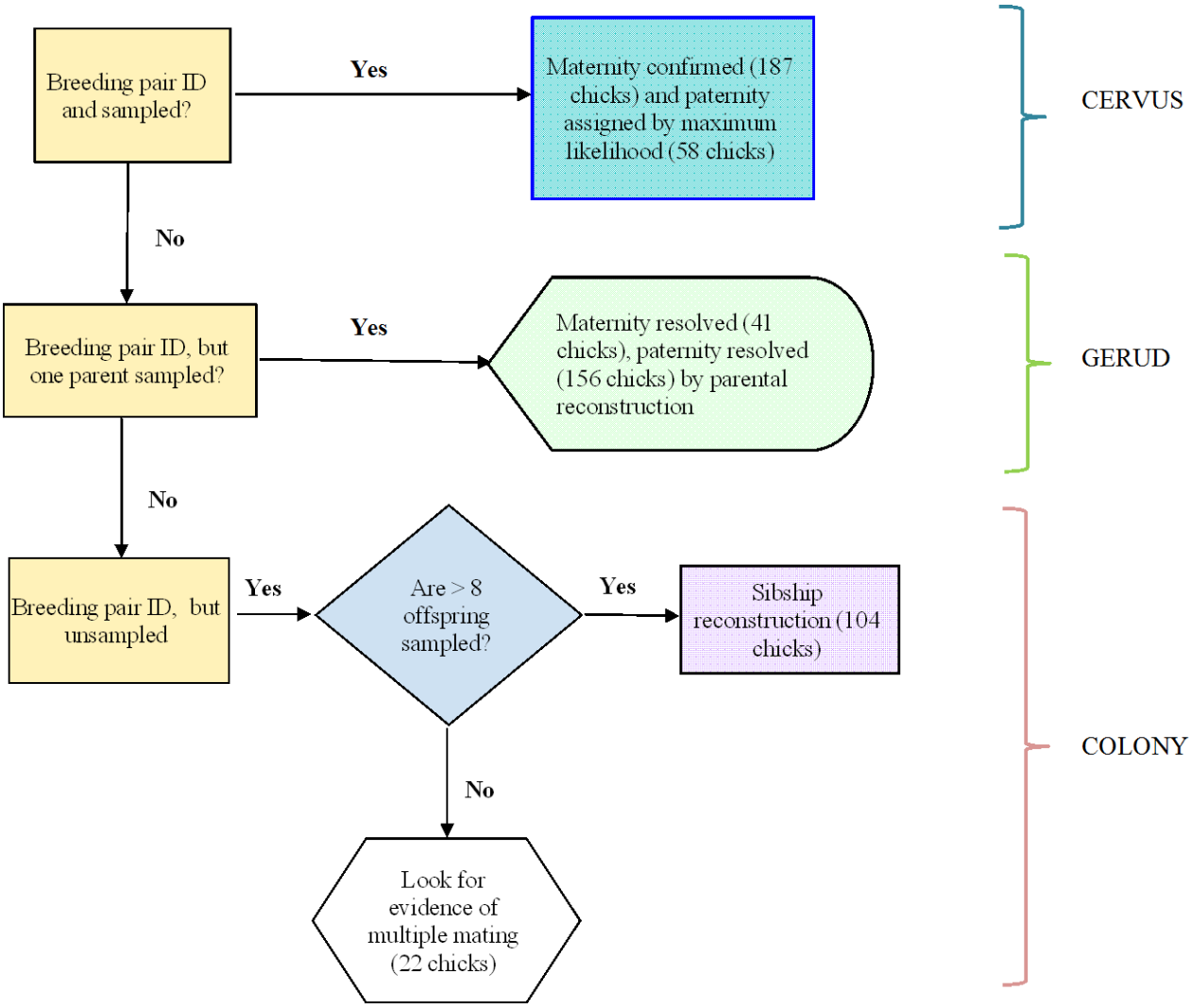

Figure 2. Flow chart illustrating the path of analyses and software used in this study. ID means that adult was banded as a chick.

I also ran simulations for the second method (GERUD) which uses an exhaustive algorithm to reconstruct the minimum set of parents that can explain the progeny array (Jones 2005). Generally, the best results are obtained using the 2-4 most powerful loci, which have the highest exclusion probabilities (Jones 2001). To assess the power of this algorithm, I used GERUDsim2.0 to simulate progeny arrays based on the population allele frequencies. The simulation reports how frequently GERUD recovers the correct number of parents as well the correct paternal and maternal genotypes under the assumed pattern of parentage. GERUD was used in cases when one of the parent's genotype was unknown since its algorithm can reconstruct it from progeny arrays composed of only full or half sibs. The parameters used for these simulations are based on values of the Midwestern Peregrine dataset (Table II).

TABLE II. SEARCH PARAMETERS FOR GERUD SIMULATIONS, WITH KNOWN MOTHERS AND FOUR MOST POLYMORPHIC LOCI (CAB *PF*24, CAB *PF*77, CAB *PF*157, NVH *PF*13). EIGHT RUNS WERE PERFORMED WITH 1,000 ITERATIONS EACH.

	Number of offspring			
	assayed	assigned by sire		
Run	total	1 sire	2 sires	3 sires
1	3	3	2, 1	1, 1, 1
2	5	5	3, 2	2, 2, 1
3	10	10	7, 3	6, 2, 2
4	15	15	10, 5	10, 3, 2
5	20	20	15, 5	13, 4, 3
6	25	25	15, 10	15, 5, 5
7	30	30	20, 10	15, 10, 5
8	35	35	20, 15	15, 10, 10

To test for sex-biased dispersal I used two methods. The first method is based on a traditional method based on a global descriptor of population structure, F_{IS} (Weir & Cockerham 1984), an unbiased statistic that describes how well the genotypes frequencies, within a population, fit with Hardy-Weinberg expectation (Hartl & Clark 1997). F_{IS} is also the most commonly employed statistic for assessment of genetic variation within populations; it was calculated using FSTAT (Goudet 2001). In the case of Peregrines, if only females disperse, as suggested by previous field studies (Tordoff & Redig 1997, 1999a), the females sampled from a patch will be a mixture of two groups: residents and immigrants. Due to the Walhund effect, the sample should show a heterozygote deficit and an $F_{IS} > 0$. Thus, females should display a higher F_{IS} than males. The second method is a new approach that relies on individual genotypes. It is an assignment test that detects an asymmetric natal dispersal between sexes (Favre et al. 1997; Mossman & Waser 1999), implemented in the program GENALEX v6.1 (Peakall & Smouse 2006). For each individual, a log likelihood assignment value is calculated, which corresponds to the expected frequency of its genotype across all loci in the population from which the individual was collected (Paetkau et al. 1995), in this case, the breeding population. Next, these values are corrected by subtracting assignment population means after log-transformation. As a result, Assignment Index corrected values (*AIc*) will average zero for each population considered, while individuals with negative values are potentially migrants or of recent immigrant ancestry (Prugnolle & de Meeus 2002). Under sex-biased dispersal we expect the dispersing sex to have a lower *AIc* on average than the non-dispersing sex. Members of the dispersing sex may include both residents (with common genotypes) and immigrants (with rare genotypes) while the philopatric sex will only include residents, so the variance of *AIc* for the philopatric sex should be greatest (Goudet et al. 2002). The mean and the variance of *AIc* for males and females were

calculated separately and the frequency distribution of *AIc* for both sexes was plotted. A genetic signal of sex biased dispersal would result in gender-differences in the mean *AIc* (*mAIc*) and the variance *AIc* (*vAIc*). To test for homogeneity of means and variances, non-parametric tests were calculated using SYSTAT13 (Systat Software Inc., Chicago, IL).

2.3. Results

My study included a total of 350 individuals, both chicks and adults, collected from Midwestern urban nests (Table III). From this sample, 282 were offspring from 20 nests sampled over consecutive years and 68 were from breeders and chicks from single-year nests. Most of the offspring data came from Chicago (IL), Minneapolis/Saint Paul/Monticello (MN), Cleveland (OH), Detroit (MI), Jefferson/Sheboygan/Milwaukee (WI), while the remaining birds came from Omaha (NE), Cedar Rapids (IA), and Indianapolis (IN).

Birds were on average genotyped at 99% of loci. The mean number of alleles per locus was 8.640 and the mean gene diversity was 0.733. The 11 microsatellite loci in the data set had a low overall probability of identity among siblings ($PID_{sib}= 5.299 \times 10^{-5}$). This means that only 0.005% of full siblings share the same genotype by chance. An identity analysis using CERVUS, whose objective is to find individuals with the same exact genotype returned no matches. All loci

TABLE III. COMPOSITION OF ANALYZED BROODS IN IL, OH, MN, WI FOR SAMPLES TAKEN FROM 1997 TO 2009. VALUES IN PARENTHESES INDICATE THE NUMBER OF GENOTYPED INDIVIDUALS IN EACH CLASS. UNKNOWN CHICKS WERE THOSE OBSERVED BUT UNSAMPLED AND SEX WAS UNDETERMINED.

	Adults		Chicks			
Nests	Female	Male	Female	Male	Unknown	Total
IL						
Broadway	2 (2)	3? (1)	23 (21)	16 (14)	4 (0)	
Evanston	2 (1)	3? (1)	13 (13)	7 (7)	1 (0)	
Hyde Park	1 (0)	1 (1)	8 (8)	5 (5)		
Pilsen	1 (0)	1 (0)	8 (8)	6 (6)		
Prison	3? (0)	3? (0)	10 (7)	11 (4)		
River	2 (1)	2 (0)	4 (4)	4 (3)	2 (0)	
Saint Michael	1 (1)	1 (1)	2 (2)	2 (1)	3 (0)	
UIC	1 (1)	2? (1)	12 (11)	12 (9)	2 (0)	
Uptown	2? (2)	2 (1)	8 (6)	16 (15)	3 (0)	
Wacker	4 (4)	4? (1)	24 (16)	26 (24)	2 (0)	
Waukegan	1 (1)	2? (0)	19 (16)	15 (14)	3 (0)	
South Loop	1 (0)	1 (0)	2 (2)	1 (1)		
Total	22 (13)	27 (7)	133 (114)	119 (103)	20 (0)	321 (237)
MI						
Whittier	1 (1)	1 (0)	0	1 (1)		3 (2)
MN						
Colonnade	3 (1)	1? (0)	14 (11)	23 (20)		
NSP High Bridge	1 (1)	1 (0)	2 (2)	2 (2)		
NSP Monticello	1 (1)	1 (0)	4 (4)	0		
Riverside	1 (1)	1 (0)	1 (1)	1 (1)		
Total	6 (4)	4 (0)	21 (18)	26 (23)		57 (45)
OH						
Terminal Tower	2 (1)	1 (1)	3 (3)	5 (5)		11 (10)
WI						
Cargill Malt	1 (0)	1 (0)	4 (4)	1 (1)		
Froedtert Malt	1 (1)	1 (0)	0	1 (1)		
Landmark on the Lake	1 (0)	1 (0)	3 (3)	0		
WPL Edgewater	1 (1)	1 (0)	4 (4)	2 (2)		
Total	4 (2)	4 (0)	11 (11)	4 (4)		23 (17)

resulted polymorphic with 5-13 alleles per locus (*k*), and high observed heterozygosity (H_O =0.516-0.871) (Table IV). All loci have expected heterozygosity (H_E) values higher than 0.5,which is useful for large-scale parentage analysis (Kalinowski et al., 2007). No linkage disequilibrium was detected for the 11 loci. There were three loci that were not in Hardy-Weinberg equilibrium after applying Bonferroni correction for multiple comparisons (P<0.05) (Rice 1989). Locus CAB *fp*181 exhibited heterozygote deficit and CERVUS indicated the presence of null alleles. The remaining two loci in question were CAB *fp*24, CAB *fp*157, which presented homozygote deficits. Homozygote genotypes in the progeny array showed consistently in 4 out of 20 nests assessed. Once these genotypes were removed no departure from H-W was detected. A deviation from Hardy-Weinberg equilibrium at a single locus may occur because of natural selection acting on a nearby gene. In this case, the deviation detected for only two loci would not be problematic for parentage analysis (see CERVUS documentation).

TABLE IV. SUMMARY STATISTICS OF 11 MICROSATELLITE LOCI FROM 350 TYPED PEREGRINE FALCONS FROM URBAN MIDWESTERN POPULATIONS, NUMBER OF ALLELES (K), OBSERVED HETEROZYGOSITY (H_O), EXPECTED HETEROZYGOSITY (H_E), HARDY-WEINBERG (HW).

Locus	*k*	H_O	H_E	HW
CAB *fp*18-2	11	0.516	0.522	NS
CAB *fp*24	12	0.871	0.832	*
CAB *fp*77	10	0.763	0.783	NS
CAB *fp*85	5	0.575	0.578	NS
CAB *fp*117	9	0.746	0.779	NS
CAB *fp*120	5	0.594	0.651	NS
CAB *fp*157	9	0.804	0.769	***
CAB *fp*181	13	0.699	0.82	**
NVH *fp*13	8	0.836	0.806	NS
NVH *fp*31	6	0.764	0.741	NS
NVH *fp*89	7	0.777	0.777	NS

2.3.1. Simulations

The combined non-exclusion probability (parent-pair) was 6.2×10^{-7}, which means that the probability of not excluding an unrelated parent pair of parentage of a given offspring for this particular set of loci was 0.000062%. Simulations done with CERVUS permitted the calculation of expected number of paternities with in turn was used to compare with observed values (Table V). Paternity for 212 offspring was resolved with 95% confidence for 69 chicks (36%) with sampled mothers, while only one chick was secured at the 80% confidence level. For 21 paternity tests where the mother was unsampled 13 chicks (62%) were secured at the 95% confidence, and the remaining 8 chicks (38%) had an 80% paternity confidence. Given that the majority of the paternities were assigned at 95% confidence level, I consider that the difference between observed and expected assigned paternities (Table V) was due to the true father not being included in the pool of candidate males rather than not having sufficient genetic data (0.99% of individuals were genotyped), or typing additional loci (critical values of Δ equal zero suggest that the power of markers was high).

TABLE V. RESULTS FOR THE PATERNITY INFERENCE USING THE SOFTWARE CERVUS FOR 212 PEREGRINES CHICKS BORN BETWEEN 1999 AND 2009.

	Mother sampled *n*= 191		Mother unsampled *n*= 21	
Number of paternities	80%	95%	80%	95%
Observed	70 (37%)	69 (36%)	20 (95%)	13 (62%)
Expected	128 (67%)	127 (66%)	16 (75%)	12 (55%)

GERUDsim2.0 simulation results depict how parental reconstruction and determination of the number of sires depend on the sample size, given population alleles frequencies (Figure 3). For polymorphic loci with an average of 8 or more allele per locus, GERUD was able to reconstruct both the number of parents and their genotypes. The probability of success when GERUDsim2.0 is used to reconstruct the number of sires decreases with the number of sires but increases with the number of offspring genotyped (Figure 3 a). Paternity (or maternity) of broods was mostly attributed to one sire and the broods analyzed per nest site were composed of 5-30 chicks. Under this scenario, the probability of inferring the correct number of sires given by the algorithm was 90% or higher. The probabilities of correctly reconstructing the genotypes of the sires were lower with increasing number of true sires but higher when more chicks were sampled (Figure 3 b). At a few nest sites, males disappeared (or died) and were replaced by another male. In these situations broods were used to reconstruct the male's genotypes. The proportion of correct reconstructed parental genotypes in these circumstances is 70 % or higher. These simulations support the robustness of the results given by GERUD on the reconstructed parental genotypes.

2.3.2. Parentage and sibship assignments

I analyzed 282 offspring from a total of 149 broods. Broods were defined as one or more chicks found in a specific nest, in the same year. Broods ranged from one to five chicks. Parentage with CERVUS was assessed for a subset of 212 offspring born in 20 nest sites (Table VI). For 191 of these chicks belonging to 66 broods, the genotype of the female tending the nest was known, and maternity for all of them was assigned to the female tending the nest. Mother-

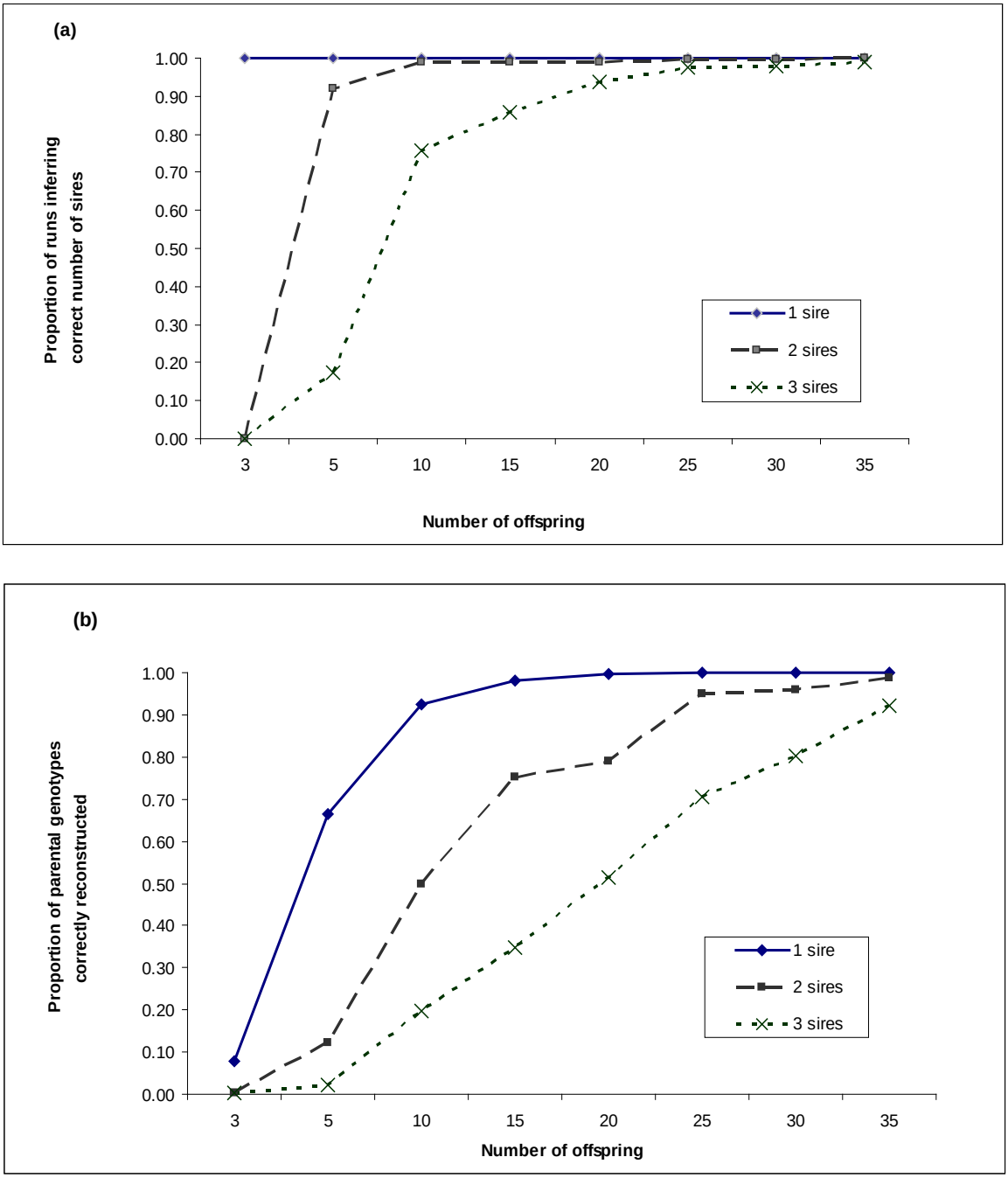

Figure 3. The performance of GERUD 2.0 given the allele frequencies observed. Simulations were carried out with the assumption that the genotype of the mother is known. They show the effect of sample size on the ability of gerud to infer the correct number of sires (a) or to reconstruct the parental genotypes (b). Each point is based on 1000 simulation runs. The panels use the 4 most polymorphic loci with the highest exclusion probabilities. Results are based on loci CAB *fp*24 (k=12, H_E=0.87), CAB *fp*77 (k=10, H_E =0.76), CAB *fp*157 (k =9, H_E =0.80), and NVH *fp*13 (k =8, H_E =0.84).

offspring mismatches occurred for only five chicks at different single locus. These chicks were located in nests in Chicago (1), Cleveland (1), Monticello (2) and St Paul (1). Maternity assignments were confirmed by mother-father-offspring trios assigned at 95% confidence with CERVUS. Maternity was unresolved for the remaining 21 chicks, in Chicago (17), and Cleveland (4), because the adult female was unsampled.

For paternity assignments using CERVUS, 68 of the 191 chicks with resolved maternity were assigned to males genotyped in this study. Seven additional fathers were identified for the 66 broods with resolved maternity (Table VI). Sixty-one paternities were assigned at 95% confidence, while seven paternities were assigned at 80% confidence. To further infer familial relationships that were not resolved with CERVUS, parentage reconstruction with GERUD was used on a subset of 172 chicks. In total, 34 chicks were assigned both maternity and paternity; 126 chicks were assigned only paternity and 12 chicks were assigned only maternity (seven by this GERUD and five using CERVUS). The five remaining chicks from four broods did not have their father's genotypes reconstructed since the size of the broods was equal or less than two, and could not be determined accurately.

Finally, the sibship reconstruction using COLONY identified nine full sib groups from 11 nest sites. These results were in agreement with field data (Table VI). For one nest in Chicago (Prison), the sibship reconstruction yielded three full-sib groups. In this nest, only the last two sampled broods (2007 & 2008) had their parents banded and identified. Four broods from four nests located in Chicago, Detroit, Minneapolis, and Milwaukee, could not have the paternity known even though maternity was resolved because of the small number of chicks in those broods (one or two).

TABLE VI. PARENTAGE ASSIGNMENTS FOR URBAN PEREGRINE CHICKS IN THE MIDWEST

		Maternities resolved				Paternities resolved					
						Categorical allocation					
Nest	No. of chicks[a]	Exclusion	Parental reconstruction	Sibship reconstruction	No. of mothers[b]	80%	95%	Parental reconstruction	Sibship reconstruction	No. of fathers[b]	No. of EPP
IL											
Broadway	35	12+23★	–	–	2	2	16	12+5★	–	3	–
Evanston	20	2	18	–	2	–	5	3+8	4	3	–
Hyde Park	13	–	–	13	1	–	–	–	13	2	–
Pilsen	14	–	–	14	1	–	–	–	14	1	–
Prison	11	–	–	3+8★	2	–	–	–	5+6★	2	–
River	7	1	–	6	2	–	–	–	6+(1)✦	2	–
Saint Michael	3	3	–	–	1	2	1	–	–	1	–
UIC	20	20	–	–	1	–	12	3+5★	–	3	–
Uptown	21	21	–	–	1[c]	3	13	5	–	2	–
Wacker	40	15+11+12 +2★	–	–	4	–	6	26+2+6★	–	4	2
Waukegan	30	30	–	–	1	–	–	18+12★	–	2	–
South Loop	3	–	–	3	1	–	–	–	3	1	
MI											
Whittier	1	1	–	–	1	–	–	–	(1)✦		–

[a] This accounts for chicks genotyped only

[b] Number of mothers (or fathers) determined after analysis

★Each number represents size of full-sib group, and each sib-group is separated by the symbol +

✦Offspring size not enough to resolve paternity

[c] Number of mothers reported (2) differed from results after genetic analysis

[d] Number of mothers reported (4) differed from results after genetic analysis

TABLE VI, CONTINUATION

		Maternities resolved				Paternities resolved					
						Categorical allocation					
Nest	No. of chicks[a]	Exclusion	Parental reconstruction	Sibship reconstruction	No. of mothers[b]	80%	95%	Parental reconstruction	Sibship reconstruction	No. of fathers[b]	No. of EPP
MN											
Colonnade	31	12	12+7★	–	3[d]	–	–	31	–	1	–
NSP High Bridge	4	4	–	–	1	–	–	4	–	1	–
NSP Monticello	4	4	–	–	1	–	–	4	–	1	–
Riverside	2	2	–	–	1	–	–	–	(2)✦		–
OH											
Terminal Tower	8	4	4	–	2	–	8	–	–	1	–
WI											
Cargill Malt	5	5	–	–	1	–	–	5	–	1	–
Froedtert Malt	1	1	–	–	1	–	–	–	(1)✦		
Landmark	3	–	–	3	1	–	–	–	3	1	–
WPL Edgewater	6	6	–	–	1	–	–	4+2★	–	2	–
TOTAL	0	191	41	50	32	7	61	155	59	34	2

[a] This accounts for chicks genotyped only

[b] Number of mothers (or fathers) determined after analysis

★Each number represents size of full-sib group, and each sib-group is separated by the symbol +

✦Offspring size not enough to resolve paternity

[c] Number of mothers reported (2) differed from results after genetic analysis

[d] Number of mothers reported (4) differed from results after genetic analysis

2.3.3. Fidelity to Mate and Territories

To calculate the rate of EPFs, I examined nests where there were at least three years of consecutive offspring data and maternal and/or paternal genetic information. This dataset was comprised of 35 broods from four nesting sites in Chicago with a total of 126 chicks. Parental reconstruction indicated that two nestlings out of 40 at the Wacker site in Chicago were not fathered by the male tending the nest. At all other nest sites in all years, genetic analysis indicated that the tending male was the father. At the Wacker site, one male tended the nest site from 1993-2004. From 1993-1997, he paired with one female, but she disappeared in 1998. That year he paired with a new female. Parental reconstruction for that year indicated that both chicks in the nest were not his. Thus the rate of EPF for Chicago nests was two of 126 (1.58%) young and one of 35 (2.85%) broods.

Comparisons of nest fidelity to mate fidelity were done with the most prolific breeders (Table VII). A total of 170 nesting attempts were made by this group but only seven (4.1%) involved changes to a new nesting site. One of these site-changes involved a male moving to a new territory located in another state. Five changes were to nearby nest sites that occurred after previous breeding attempts had been unsuccessful at one site or when a mate disappeared. The last change involved a ‘divorce’ and both male and female paired with new birds. For the same 170 nesting attempts, a total of 15 changes of mates occurred (8.8%). Nine involved changing after a mate had presumably died, three involved disappearance of mates following territorial fights; and three involved mate changes in association with changing nest sites. In one case, a pair together for ten years changed nest sites after their third breeding year to a nearby site that they used for the next seven years.

TABLE VII. NUMBER OF MATES AND NESTS SITES USED BY URBAN PEREGRINE BREEDERS WITH THE HIGHEST LIFETIME REPRODUCTION IN THE MIDWEST CONFIRMED BY GENOTYPIC DATA.

Lifetime Production	Years nesting	Nests sites used	Mates
Males			
31	11	1	3
26	12	1	3
19	8	2	2
18	5	1	1
17	6	1	1
16	7	1	1
14	8	1	2
13	10	2	1
12	7	2	2
12	3	1	1
12	3	1	1
Females			
34	8	1	2
28	11	2	3
26	11	1	1
23	6	1	1
18	5	1	3
16	4	1	1
14	7	1	1
14	5	1	1
13	16	3	2
13	5	2	3
12	7	1	1
12	5	1	1

2.3.4. Detection of Sex Biased Dispersal

For the genetic detection of sex-biased dispersal, I considered 32 females and 33 males that were confirmed breeders by parentage analyses. These birds were from across the Midwestern region. F_{IS} estimates for males and females were not statistically different (males= 0.066, females= -0.010, P = 0.924). Similarly, male Peregrine Falcons did not have significantly higher *AIc* values (0.048 ± 0.255 (SE)) than females (-0.049 ± 0.246 (SE)), (Mann-Whitney *U*-test: U = 466, P= 0.680). The difference between male and female *AIc* variance also was not significant (F ratio test, F = 0.905, P = 0.787). For both sexes, the frequency *AIc* plot shows a similar pattern with both negative and positive values 9 (Figure 4).

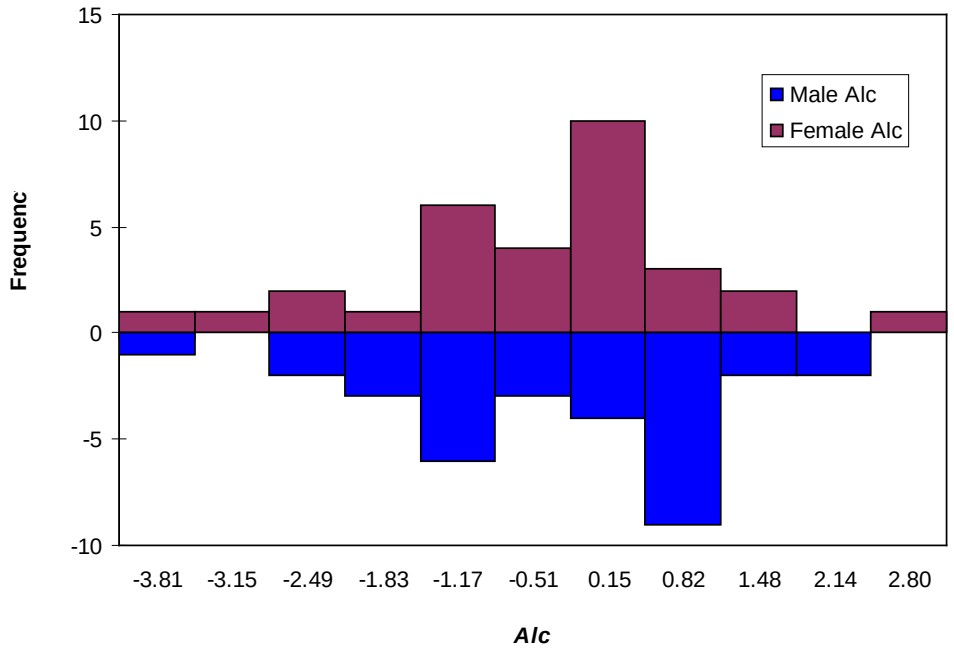

Figure 4. Frequency distribution of corrected assignment indices (*AIc*) for male and female breeders. The genetic signal of sex-biased dispersal is indicated when there is a difference in the frequency distribution of *AIc* values between sexes.

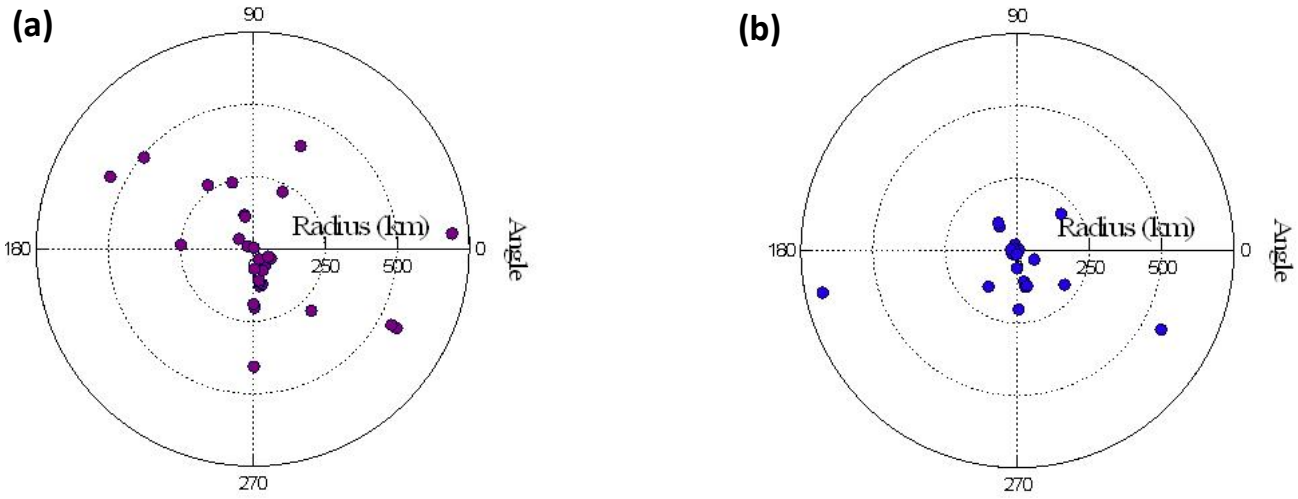

Figure 5. Natal dispersal (km) of Peregrine breeders, females (a) and males (b) banded as chicks and identified in a later breeding season, shown in relation to their natal site (center).

For the appraisal of sex-biased natal dispersal using field data, only 25 males and 29 adult females could be included because both field (banding) and genetic data were available for these individuals. Natal dispersal showed large individual variation (Figure 5) with individuals of both sexes moving short distances less than 100 km (33%) or even settling back at the hack or natal site (4%), while others moved >100 km (63%). Females Peregrines moved on average almost twice as far as males, 226 km vs. 124 km ($\chi^2 = 5.649$, df = 1, $P < 0.05$).

When breeding sites are plotted in relation to the natal site (Figure 5), the number of established nests is concentrated around the natal nest site, and decline progressively with increasing distance. A polar histogram (Figure 6) reflecting the orientation patterns of individuals from natal to breeding site shows that although individuals dispersed in all directions, there was a distinct tendency for both males and females to disperse in a southeast direction. No sampled females dispersed in a southwest direction.

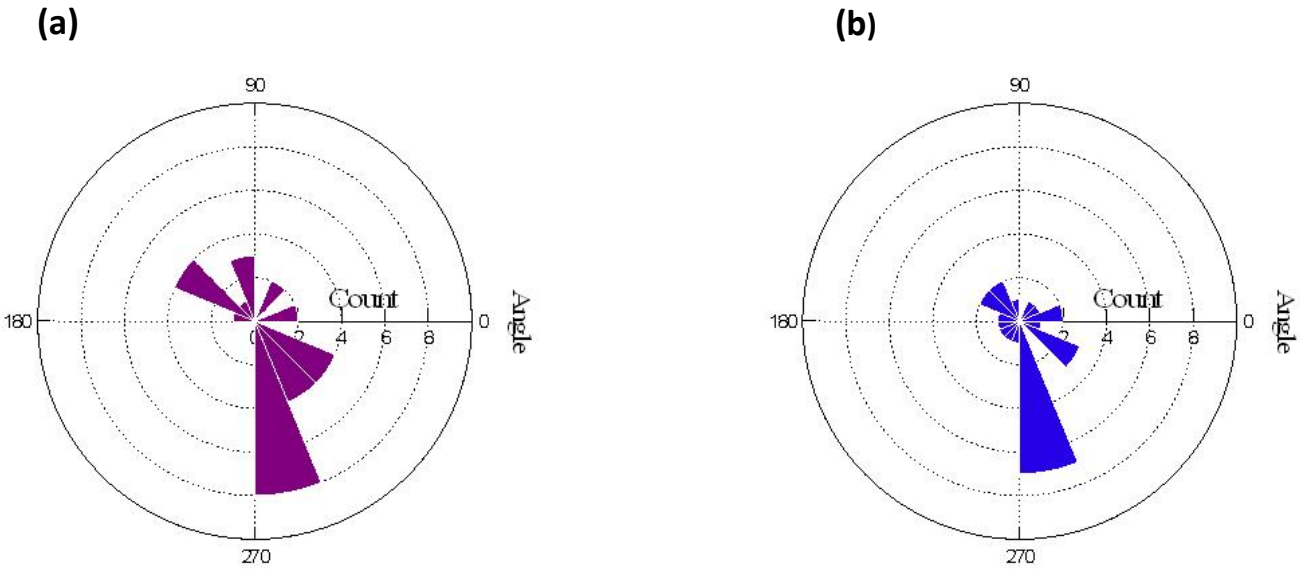

Figure 6. Polar histogram depicting the preferred direction (angle) chosen by Midwestern females (a) and males (b) breeders plotted in relation to the natal site (center).

2.3.5. Origin of Unknown Breeders

From the pool of 109 unrelated Peregrines, there were 12 unbanded or unidentified male breeders. Eight of those males were in Chicago, IL, three males in Sheybogan/Jefferson, WI, and one male in Monticello, MN. I used a frequency-based population assignment test (Paetkau et al. 1995) to establish their origin. Birds whose origin were known but were born in different cities from the same state were clustered as coming from that state to increase sample size for that group. For each assessed individual a log likelihood of assignment to urban centers was calculated based on allele frequencies. Then, each individual was assigned to the urban center with the highest likelihood ratio. The overall rate of individual assignments was calculated based on the known place of birth for banded birds. The rates were not high, and varied greatly by urban centers. The urban centers with the highest correct assignments were Minneapolis/St. Paul,

MN (66%), followed by Chicago, IL (59%), and Milwaukee, WI (56%). None of the seven birds born in several cities in IN could be assigned to any group. The eight breeders from Chicago, IL were assigned as follows, four were assigned to MN, one to IA, one to WI, one to OH, and the last one to IN, respectively. Two out of three males from WI were assigned to the same group while the third bird was assigned to IL. Finally, the male breeder in MN had his origin assigned to WI.

2.4. Discussion

A central theme of this chapter has been to highlight the importance of combining genetic and field data for monitoring and studying a species that until not long ago was in the process of recovery. Peregrine Falcons in the Midwest and other regions in North America have suffered dramatic changes in population abundance over the past half-century (Johnson et al. 2010). Monitoring programs such as the one implemented in the Midwestern region following reintroduction provides decades of data on individuals and populations. There is a limit on the number of questions that can be answered with only monitoring data; adding genetic data allowed me to study the behavior of this species more thoroughly. Understanding basic life history traits of a newly reintroduced species will be useful for future reintroduction programs.

The increased density of urban nest sites in places like Chicago, IL, as well as the observations that the average number of fledglings of Peregrines is among the highest documented in North America (Wakamiya & Roy 2009), and that passing migrant birds were copulating with established breeders, provided the stage to inquiry about the levels of EPFs. This subject is intertwined with questions regarding like nest and mate fidelity, and these questions

could not be answered given that not all breeders were banded. Based on genetic information obtained from offspring in consecutive years, I was able to determine whether the same male (or different males) where tending the nest in question. Dispersal distance and orientation of breeders also was determined, giving insight into dispersal habits of urban Peregrines.

2.4.1. Genetic Mating System

EPCs have been observed for many raptorial birds. For the genus *Falco*, Mougeot (2004) reported that EPCs occurred in five out of seven (71%) studied species and cuckoldry risk was found to covary positively with density. The incidence of EPFs rates, however, was always low (1-5% of young or broods) (Mougeot 2004). EPF values for the Common Kestrel, *Falco tinnunculus* were 1.9% of young; 2.7% of broods (Korpimaki et al. 1996), and for the Lesser Kestrel *Falco naumanni* 7.25% of young; 9.7% of broods (Alcaide et al. 2005), while no EPFs were found for the Eleonora's Falcon, *Falco eleonorae* (Swatschek et al. 1993), the Merlin, *Falco columbarius* (Warkentin et al. 1994), or the American Kestrel, *Falco sparverius* (Villarroel et al. 1998). For Peregrine Falcons, EPFs were expected because there have been observations of males Peregrines in urban environments engaging in copulation displays with migrating females, with anecdotal evidence of floaters as the breeding population increased density (Tordoff & Redig 1997). Sporadic episodes of bigamy have been reported for Peregrines nesting in cliffs in England (Ratcliffe 1980). Another factor that could increase the chance of detecting EPFs events come from the study of captive breeding raptors. Sperm stored in the female oviduct can remain viable there for up to twelve days and can fertilize two or three eggs in a row (Fox 1995). I found only one example of EPF and even that event had special

circumstances associated with it (see below). Peregrines have not been reported to have EPFs, thus making this study the first to provide evidence and quantify its occurrence.

My results suggest several things about urban Peregrine mating systems. Despite some observations to the contrary, migratory birds appear not to contribute to the gene pool. For local birds, neither floaters nor neighboring males are involved in EPFs. The only case of EPFs I found was a male that lost his mate and quickly remated. Paternal effort is critical for the success of the brood, and females may refrain from seeking extra-pair copulations, because males reduce their breeding effort when their paternity is in doubt. Also, high paternal investment may leave males with little time available to engage in extra-pair copulations, which they would have to pursue actively, intruding in already claimed territories and risking injuries or death (Saladin et al. 2007). This is also in agreement with the hypothesis that long-lived birds where males invest substantial effort in rearing their broods should have low level of EPFs (Greenwood 1980).

2.4.2. Nest Site Fidelity and Spatial Pattern of Dispersal

Midwestern Peregrines show strong site fidelity to nesting sites as do Peregrines from other parts of the world. Peregrines do move around before settling to breed for the first time but once a territory is established they seldom move to another territory. These results have never been obtained genetically, and they are in agreement with earlier observational data (Tordoff and Redig 1997).

For the majority of bird species, studies have reported individuals moving greater distances between natal sites and breeding sites than between breeding sites (Paradis et al. 1998; Newton 2008). Female Peregrine Falcons like other raptor species, moved further between natal

and breeding sites than males. This supports Greenwood's (1980) resource competition hypothesis which proposes that females disperse to acquire mates and males are more philopatric to increase their chances of obtaining a territory (Johnson & Gaines 1990). An alternative explanation could be related to the possible avoidance of inbreeding since it has been speculated that Peregrines cannot recognize close relatives when it comes to forming a pair (Tordoff & Redig 1999a). With respect to breeding dispersal, the majority of nest switching in urban Peregrines occurred in the vicinity of the previous breeding site (i.e., in the same city). The geographic range encompassing nests sites has expanded since the reintroduction. Evidence of expansion is reflected in the growing number of nest sites (4 in 1986; 244 in 2008), and nesting pairs (2 in 1986, 190 in 2008).

2.4.3. Microsatellites as Tools for Identification

Overall, combining field and genetic data give a more detailed picture of the breeding behavior in urban Peregrine Falcons. For my dataset, 31 of 33 (94%) of the female breeders were identified via field observations and later confirmed by genetic analyses. Three conflicts involving identity could only be resolved genetically. They involved correct identification of bands that were similar in one case, and solving the identity of two unbanded females in the other. Only 16 of 34 (48%) of the male breeders were identified in the field, while an additional 15 (43%) males were identified after genetic analysis, leaving only three (9 %) males that could not be assigned identity by either method. This shows that combining datasets enhances our understanding of the relationships in this population. Genetic methods also were used to infer the origin of unknown breeders via population assignment techniques. Even though the percentage of correct assignments varied for each of the assayed sub-populations, the problem was not with

the technique as much as the lack of data for some of the urban centers (i.e. Cedar Rapids, Indianapolis). Adding genetic data could improve the origin assignment of unknown breeders in some urban centers where the density of Peregrines is still low.

2.5. Acknowledgments

This study was conducted with the support of the following grants: *Dean Amadon Grant*, Raptor Research Foundation, *2006 Bleitz Award*, The American Ornithologists' Union, and *Endowment Fund 17th Annual Grant*, The International Osprey Foundation. Microsatellite development and optimization by ICC and the subsequent laboratory work was carried out in the Ashley Laboratory at the University of Illinois at Chicago, and the Pritzker Laboratory for Molecular Systematics and Evolution operated with support from the Priztker Foundation. I thank Kevin A. Feldheim for helping in the development of microsatellites, and helpful discussions regarding breeding biology. I am grateful to the curators, collections managers, and their institutions that kindly provided materials for this study: David Willard and Mary Hennen, Field Museum of Natural History, Robert M. Zink and Mike Westberg, Bell Museum of Natural History. Special thanks go to the Chicago Peregrine Program members including Mary Hennen (Director), Matt Gies, Stephanie Ware, and all the monitors I have the pleasure work with during all these years.

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3. GENETIC EVALUATION OF A SUCCESSFUL RECOVERY: THE CASE OF THE EXTIRPATED MIDWESTERN PEREGRINE FALCON (*FALCO PEREGRINUS ANATUM*)

Peregrine Falcons (Falco peregrinus anatum) *were extirpated east of the Mississippi in the 1950's-60 due to the use of pesticides. In the Midwestern US, a successful reintroduction project was launched in 1980, using captive birds of five different subspecies. I examined the genetic composition of the historical population using ten microsatellite markers and one mitochondrial gene. I start with an historical review of the extirpated Midwestern Peregrine, the nature of the recovery and how the reintroduced population came to be. My sampling scheme included museum specimens (n= 32, collected 1881-1963) representing the Midwestern-Eastern historical population, and contemporary samples (n=80, collected 1986-2009) representing the recovered urban Midwestern population. I detected differences in levels of genetic diversity for both periods. Average observed heterozygosity was significantly lower for the historical population while haplotype and nucleotide diversity were significantly higher for the historical population. Bayesian clustering analysis of microsatellite data revealed a genotypic shift between historical and contemporary populations. The cause of this shift is likely the result of the use of non-native stock in the recovery process.*

3.1 Introduction

The Peregrine Falcon (*Falco peregrinus* Tunstall 1771) is a crow-sized bird of prey with one of the largest natural distributions of any avian species. In North America, the effect of chlorinated hydrocarbon (primarily DDT) contamination caused the thinning of eggshells,

impairing reproduction. As a result, Peregrine populations crashed during the late 1950's throughout the continental United States, and were extirpated in states east of the Mississippi by the 1960's. These populations comprised much of the range of one subspecies, *F. p. anatum* Bonaparte 1838 (Cade *et al.*. 1988). Use of DDT was banned in the United States in 1972 and efforts to reintroduce Peregrines began in 1975 with the launching of different programs across North America (Barclay 1988). Today, there are increasing populations of Peregrine Falcons in many urban areas east of the Mississippi. The objective of this study is to evaluate the genetic consequences of the reintroduction efforts and to compare the genetic composition of the current population to the historical population in the Midwestern region.

Historical records of Peregrine Falcons in the Midwestern US are limited. A review of specimen records, reports and ornithological literature suggests that the historical range included North Dakota, South Dakota, Nebraska, Wisconsin, Illinois, Iowa, Indiana, Michigan, Ohio, and Kentucky (Figure 1). Records indicate that Peregrines were sparsely distributed in both the Midwestern and Eastern US prior to the DDT era. For both regions, the south to north progression of their disappearance was correlated (Berger & Mueller 1969) (Figure 1). Peregrines were historically dependent on rocky cliffs and cut-banks although there is some evidence that trees were used as nest sites.

Nesting events from the Dakotas are documented in egg collections (ND, 6 eggs, 1899, 4 eggs 1901, Field Museum of Natural History). Other states that largely lack cliff nest sites such as Ohio, Illinois, and Indiana have report of breeders prior to 1900 (Hickey 1942), but most early records in Ohio occurred during migration season (Sept-Oct & Jan-Mar) (Bezdek 1942) and from 1874 to 1940 (Oberholser 1896; Preble 1945). There are a few records of Peregrines

nesting on trees in Mt Carmel, IL (Ridgway & Forbes 1889) and sightings of residents during the summer (Cory 1909). These nests belonged to a remnant tree-nesting population that

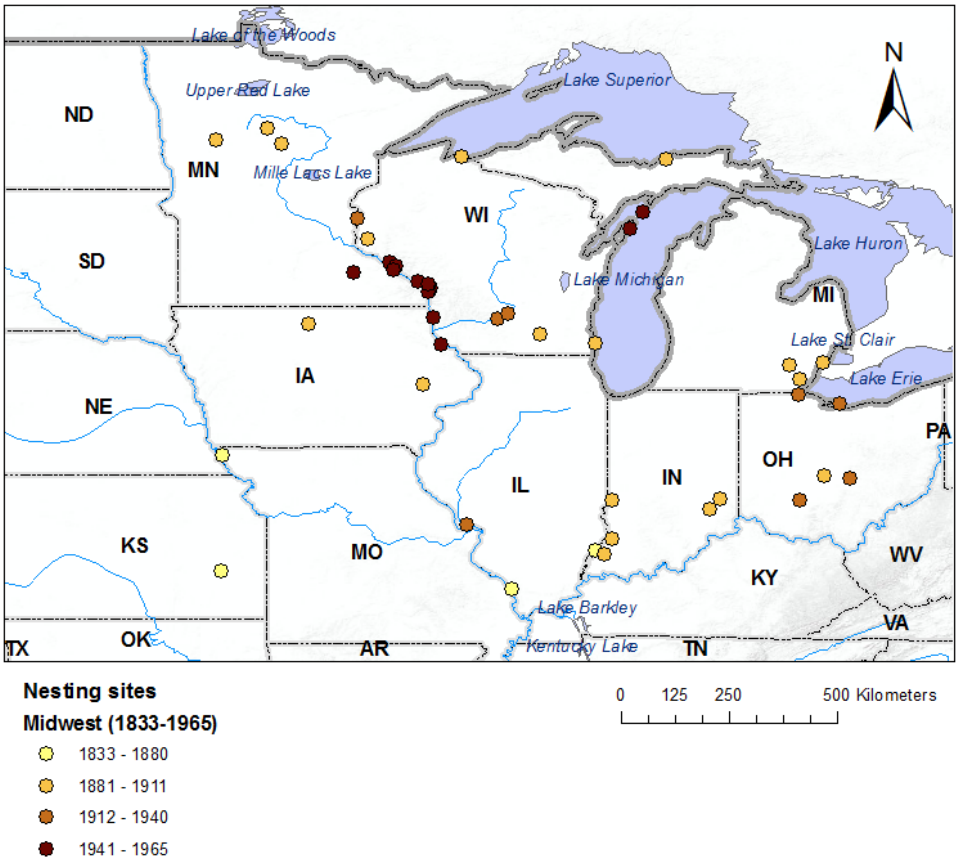

Figure 1. Breeding locations formerly occupied by nesting Peregrines in the Midwestern US based on historical reports (for more details see text). Peregrine nest sites disappeared in a south-north fashion. The progressive disappearance is highlighted with different colors.

extended into Indiana and disappeared with the felling of the great trees (sycamores, cottonwood) (Hickey & Anderson 1969). Records of nests from Indiana also come from the 1900's or earlier, and were from the Wabash Valley (1906) and Boone County (1896) (Sweeney 1907-1908). In Iowa, Peregrines were not common and usually seen at intervals, breeding in Cedar River (1895-1898) (Keyes 1906) and probably in Hancock County (1911) (Bailey 1918).

The nests reported in Michigan were along the south shore of Lake Superior (1906) (Barrows 1912). Other tree nesting Peregrines were reported in the great forest-lake region in Minnesota (Hatch 1892). In the southeastern part of the state six breeding pairs were reported in 1932 (Berger & Mueller 1969). There also were nests along the Niagara Escarpment in upper Michigan and in Door County in northeastern Wisconsin, but these sites were abandoned by 1958 (Berger & Mueller 1969). Nesting in the central part of Wisconsin was reported much earlier near Lake Wisconsin (Stoddard 1921), and the Wisconsin River (Stoddard 1917).

In response to the catastrophic decline of Peregrines in the 1950s, and the specific correlation between eggshell thickness and DDT content in Peregrine eggs (Hickey & Anderson 1968), North American Peregrines were placed on the federal Endangered Species List in the 1970's (Kiff 1988). The original *anatum* populations that occurred in Eastern-Midwest North America were large birds that were extirpated by about 1965 (Berger *et al.*. 1969). As a result, no wild *anatum* were available for reintroduction programs (Tordoff & Redig 2001). Founders used for the reintroduction in the Midwestern US included birds from five subspecies: *F. p. anatum* (western U.S., Canada and interior Alaska), *F. p. tundrius* (Arctic), *F. p. pealei* Ridgway 1873 (northwest Pacific Coast and Aleutians), *F. p. brookei* Sharpe, 1873 (Spain, southern Europe), and *F. p. peregrinus* Tunstall, 1771 (Scotland, northern Europe) (Tordoff & Redig 2001). Fifty-nine individuals were bred in captivity and 1,182 of their progeny were hacked at nest sites across the Midwest from 1982 through 1998, establishing populations in largely urban settings that continue to increase today (Tordoff & Redig 2003).

The subspecific designations of the founders were based on traditional taxonomic assessments of geographic variation in size, plumage coloration, and behavior, which have been historically considered to represent regional adaptations to diverse environments and to have

some genetic basis (Tordoff & Redig 2001). A presumed consequence of re-establishment of Midwestern Peregrine populations is the infusion of genetic variants from the founders that is different from the populations that once inhabited the region. Under this scenario, levels of overall genetic diversity may not have changed, and may even be higher given the different breeders, but the genetic constitution of the population might also have been altered significantly. To determine the genetic contributions made by the different subspecies, Tordoff and Redig (2001) classified 73 active breeders (1987-1998) based on their ancestry. These were classified as *anatum* (54%); *pealei* (22%); *peregrinus (*9%); *brookei* (10%); and *tundrius* (5%). Moreover, 44 of these breeders had mixed ancestry, while 25 birds were pure *anatum*, three were pure *pealei*, and one was pure *brookei* (Tordoff & Redig 2001).

While there have been similar studies of pre-and post- recovery in Peregrines from Canadian and European birds, this is the first assessment of birds from the United States. Populations of reintroduced Peregrines have been examined in Norway (Nesje et al. 2000a; Jacobsen et al. 2008) and Canada (Brown et al. 2007). I evaluate levels of genetic diversity and composition in native and reintroduced Peregrine Falcons in the Midwestern United States based on mtDNA sequence data, and genotypic data from ten polymorphic nuclear microsatellite loci. Based on historical records, I grouped samples collected prior to the crash (1965) as the native population. By comparing genetic data on this historical population with data on the current (urban) population, I address two major issues: First, what was the level of genetic diversity in the historical Midwestern *anatum* Peregrines compared to the modern urban Peregrines and second, what were the genetic consequences of the use of non-native stock in the recovery program?

3.2 Materials and Methods

3.2.1 Sampling Scheme

I analyzed 32 historic Peregrine museum samples collected from 1881 to 1963 and 80 Peregrines sampled from the reintroduced Midwestern population (Figure 2). Due to the scarcity of historical samples, I requested additional toepads from museum collections that given their geographic locations might correspond to the Eastern historical distribution of Peregrines (Appendix A). For the urban population I collected genetic data from confirmed breeders (*n*= 45) and chicks (*n*= 35) (Appendix B).

3.2.2 DNA Extraction

Genomic DNA was extracted from tissue samples using the Qiagen DNeasy Tissue Kit® following manufacturer's protocol. For historical samples from toe pads protocols for DNA extractions and handling of material was modified to prevent contamination. These precautions included but were not limited to: 1) small batches of individuals (<12) were handled during DNA extractions and amplifications at any given time; 2) genetic material was processed in an isolated, designated space with an autonomous ventilation system and UV-irradiation hood that had not been previously exposed to avian DNA; 3) ancient DNA was handled separately from fresh tissue; 4) all plastic material was autoclaved and UV-irradiated prior to use, and only filtered tips were used to avoid cross-contamination; 5) negative controls during DNA extractions and amplification were included with each batch. Modifications of the DNA extraction protocol for toe pads and skin samples consisted of digestion in 180 µl ATL buffer,

and addition of 20-80 µl proteinase K solution for 1-4 days at 56ºC. Final DNA elution was performed with 70 µl of AE buffer preheated to 70ºC (Mundy et al. 1997).

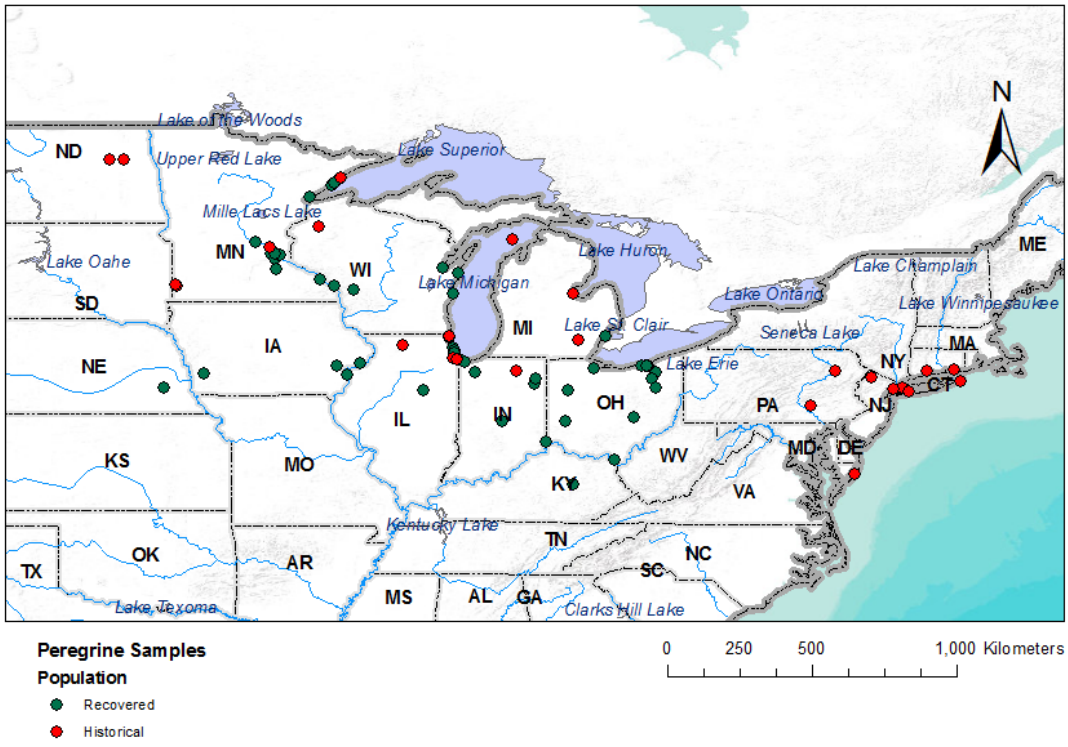

Figure 2. Sampling locations for historical (*n* = 32) Midwestern-Eastern and contemporary (*n* = 80) Midwestern Peregrine Falcons. Circles may represent several individuals.

3.2.3 Genotyping and Sequencing

All samples were genotyped with 10 microsatellite loci primer pairs. Seven microsatellites markers were developed and optimized from an enriched genomic library following Glenn and Schable's protocol (2005). In addition, three published dinucleotide microsatellites for this species developed by Nesje et al. (2000b) were used. Microsatellite screening and polymerase chain reaction (PCR) amplification conditions were the same as

described in Chapter 2. Multilocus genotypes were obtained for a minimum of six loci for all samples. Approximately 25% of individuals within each temporal sample (historical and recovered populations) were re-amplified for each locus to confirm allelic scoring. To check for allelic dropout, amplifications were repeated under less stringent conditions (lower annealing temperature and higher $MgCl_2$ and/or BSA concentration). For fragment analyses, 1.0 µl of PCR products were mixed with 0.25 µl of GeneScan™ 500 LIZ™ Size Standard (Applied Biosystems) and 8.75 µl of formamide and run on an ABI3730 DNA Analyzer. Genotyping was carried out using the STRand Analysis Software v.2.4 (Toonen and Hughes, 2001).

A 405 base pair region of the mtDNA control region (CR) was amplified by PCR using existing primers and published PCR conditions (Brown et al. 2007). Internal primers used for sequencing historical samples were developed using Primer3; http://frodo.wi.mit.edu/primer3/ (L15429 5'-TTTCCAGGATACGGAAGTGC-3', H16793 5'-TTAGGTCCTATGGCCCGTTA-3'). Primer names correspond to the 3' nucleotide position on the published Peregrine Falcon genome (Genbank Accesion No: AF090338) (Mindell et al. 1999). PCR amplifications were performed in 25 µl volume including 0.25 µl of *Taq* polymerase (Roche©), 1x PCR buffer, 50 µM each dNTP, 0.4 µM each primer, 1.5 mM $MgCl_2$ and 25 µg of BSA (bovine serum albumin). After an initial denaturing step at 94ºC for 5 min, 35 cycles of denaturation at 94ºC for 30 s, primer annealing at 58ºC for 1 min, and primer extension for 2 min were performed on PTC-100 Programmable Thermal Controller (MJ Research Inc., Waltham, MA,USA). PCR negative controls were also run to detect contamination. Amplification products were electrophoresed in 1% NuSieve GTG (Cambrix Bio Science) agarose gels containing ethidium bromide. DNA sequencing of cleaned PCR products in both directions was performed using the BigDye® Terminator v3.1 kit (Applied Biosystems), and sequences were run on an ABI3730 Analyzer.

3.2.4 Data analyses

Mitochondrial DNA (mtDNA) sequences were aligned using CLUSTAL W (Thompson et al. 1994) and the positions of polymorphisms refer to the published mitochondrial genome of *F. peregrinus* (Mindell et al. 1999) (Table I). Indices of mtDNA diversity, including nucleotide diversity (π), haplotype diversity (h), and Tajima's D (Tajima 1989) were calculated across temporal samples using DnaSP 5.10 (Librado & Rozas 2009). In addition, I determined the total number of mitochondrial haplotypes as well as the number of unique and shared haplotypes among different temporal population samples.

Partition of variance within and between populations based on mtDNA sequences and microsatellite genotypes was computed in ARLEQUIN v.3.5 (Excoffier et al. 2005) using an analysis of molecular variance (AMOVA) (Excoffier et al. 1992). For mtDNA, pairwise differentiation between sites was calculated using both F_{ST} (calculated using haplotypes frequencies) and Φ_{ST} (using both frequency and the level of sequence variation) (Excoffier et al. 1992). These statistics were compared across time periods to assess whether significant genetic changes have occurred within populations across time (i.e. test for temporal differences in haplotype/genotype frequencies). Significance of variance components was tested with 1000 random Monte Carlo simulations.

Microsatellite genotypes were tested for Hardy-Weinberg equilibrium (HWE) departures and linkage equilibrium within each sampling period and each locus using FSTAT (Goudet 1995). Bonferroni corrections were applied to correct for multiple comparisons (Rice 1989). Allele frequencies, number of alleles, observed heterozygosity (H_O), expected heterozygosity ($H_{E)}$ were also calculated using the computer program FSTAT. Since a larger sample will contain more alleles than a smaller sample, we estimated two other parameters that correct for sample size.

Measures of allelic richness, an estimate of allelic diversity that compensates for unequal sample size, as well as private allelic richness, number of alleles which were unique to a particular time period, were calculated using rarefaction as implemented in HP-RARE (Kalinowski 2005). Statistics for each time period were compared using a Wilcoxon signed-rank test.

Population differentiation between time periods was measured using R_{ST} (Slatkin 1995) using ARLEQUIN v.3.5 (Excoffier et al. 2005). R_{ST} is an analogue of F_{ST} that conforms to the stepwise model of mutation for microsatellite loci. Significance levels were determined using permutation tests in which individuals were permuted between populations 1,000 times. I also incorporated a multivariate technique, Principal Coordinate Analysis to find whether genetic patterns could be detected between the two sampled periods. I used GENALEX v.6.1 (Peakall & Smouse 2006).

The scale of population subdivision between sample locations and temporal sampling periods was examined using a Bayesian clustering method in the program STRUCTURE (Pritchard et al. 2000) . This iterative method takes the information from multilocus genotypes and Markov Chain Monte Carlo (MCMC) sampling, and statistically assigns individuals, based on Hardy-Weinberg expectations, to a user-defined number of anonymous genetic clusters (K). I carried out 10 independent runs per K (K=1,…, 5) using a burn-in period of 10,000 and collected data for 100,000 iterations. These simulations were performed using two different settings. First, I used the default settings, which consisted of an ancestry model allowing admixture and correlated allele frequencies, without including prior information on sampling origin while letting the degree of admixture alpha, (α) be inferred from the data. Second, I used an ancestry model of no admixture and independent allele frequencies while the remaining settings were left unchanged. These user-advanced settings were used because this model is more powerful than

the default model in detecting subtle subdivision (see STRUCTURE documentation). The most likely number of clusters (*K*) for the two settings was assessed measuring the rate of change of the log probability of data following consecutive *K* values (ΔK) (Evanno et al. 2005). Finally, I ran STRUCTURE at the most likely value of *K* for a burn-in period of 100,000 and collected data for 1,000,000 iterations.

Assessment of bottleneck signatures was done using BOTTLENECK 1.2 (Piry et al. 1999). Populations that have experienced a recent ($0.2N_e - 4N_e$ generations) bottleneck exhibit elevated levels of heterozygosity (H_E) (Nei 1987) compared to levels expected under mutation-drift equilibrium (H_{Eq}) (Cornuet & Luikart 1996). Thus, for each population sample the expected equilibrium heterozygosity (H_{Eq}) is calculated from the observed number of alleles (*k*), given the sample size (*n*) under the assumption of mutation-drift equilibrium simulating a coalescent process. I used a two-phase model (TPM) assuming 5% of multiple-step mutations and a variance of 12, as suggested by Piry et al (1999).

3.3 Results

3.3.1 Mitochondrial DNA

Mitochondrial control region sequences were obtained for 31 historical and 31 contemporary individuals. Across the 405 bp of mtDNA CR sequence, there were six polymorphic sites (Table I) and seven haplotypes. The haplotype reported by Mindell et al (1999) appeared in only one historical individual (haplotype CC). Three of the seven haplotypes (CA, CD, and CE) were previously reported by Brown et al. (2007) in three Peregrine subspecies, *anatum, tundrius and pealei.* However, for position 15267, the single base pair is an A (while Brown' study reported G at this position). I consider this a difference in interpretation.

There were three novel haplotypes that occurred in two historical birds (haplotype CJ) and two contemporary birds (haplotypes CO, CR). In sum, there were four haplotypes found only in historical samples and two found only in contemporary samples while the most common haplotype (CA) was found at very high frequency in both time periods (Table I).

Values of genetic diversity for mtDNA were significantly higher for historical birds when compared to contemporary birds. Haplotype diversity (*h*) was 0.351 (±0.107) and nucleotide diversity (π) was 0.00093 (± 0.00031) for the historical population while *h* was 0.127 (±0.080) and π was 0.00032 (± 0.0002) for the contemporary population. For both periods, Tajima's *D* values were non-significant ($D_{historical}$= -1.583, $D_{contemporary}$ = -1.505, P > 0.05), so the null hypothesis of neutrality could not be rejected.

TABLE I. MITOCHONDRIAL CONTROL REGION POLYMORPHIC SITES AND FREQUENCY DISTRIBUTION AMONG TEMPORAL SAMPLES. NUMBERS FOR VARIABLE SITES CORRESPOND TO SITES WITHIN THE PUBLISHED MITOCHONDRIAL GENOME OF *F. PEREGRINUS* (GENBANK ACCESION NO. AF090338).

	Variable site							
Haplotype	15267	15331	15332	15356	15462	15623	Historical	Contemporary
CA	A	T	T	A	G	C	0.806	0.935
CC	G	.	.	.	.	.	0.032	
CD	.	C	.	.	.	.	0.065	
CE	.	.	C	.	.	.	0.030	
CJ	.	.	.	G	.	.	0.065	
CO	.	.	.	.	A	.		0.032
CR	.	.	.	.	.	G		0.032

Haplotype CC corresponds to the Peregrine haplotype previously reported by Mindell et al (1999). Haplotypes CA, CD and CE correspond to haplotypes A, B and C of Brown et al (2007), respectively.

An AMOVA was conducted to assess temporal differences. This analysis indicated that 2.80% of the total variance was explained by variation between historical and contemporary populations while 97.20% of the total variance was apportioned within populations. The pairwise Φ_{ST} was 0.028 and found to be non-significant (P= 0.105). The same value was obtained for pairwise F_{ST} and was not significant (P= 0.117).

3.3.2 Microsatellites

A total of 20 tests for deviations from HWE was performed (400 permutations) for both time periods. The global multilocus Hardy-Weinberg exact test for heterozygote deficiency was significant (P< 0.001). Single locus Hardy-Weinberg tests by population yielded ten significant cases of heterozygote deficiency, but none remained significant after standard Bonferroni corrections (Table II). We found no evidence of linkage disequilibrium between loci in both sampling periods.

A total of 102 microsatellites alleles were detected across 10 loci for both time periods. The total number of alleles was slightly higher for the historical population (85) compared to the contemporary population (79) but was not significantly different. The mean number of alleles (A) (Table II) also was not significantly different between the historical (A = 8.50 ± 1.96) and the contemporary population (A = 7.9 ± 2.60; Z = 0.58, P = 0.56). Allelic richness (AR) did not differ significantly between the historical (AR = 8.32 ± 1.90) and the contemporary populations (AR = 6.82 ± 2.12; Z = 1.67, P = 0.10). Forty alleles were only observed in a single time period (23 alleles in historical samples, and 17 alleles in contemporary samples). All but one of the alleles observed in a single time period had a frequency below 0.10 (Appendix C). Average number of alleles found only in one time period (PA) did not differ significantly for historical

(PA= 2.30 ± 1.57), and recovered populations (PA= 1.70 ± 2.21, Z = 0.70, P = 0.48). However, allelic richness for alleles found in one time period (PAR) was significantly higher for the historical population (PAR = 2.81 ± 1.48) than the contemporary population (PAR = 1.32 ± 1.82; Z = 2.01, $P < 0.05$). Expected heterozygosity (H_E) did not differ significantly between the historical and the contemporary samples (Z = -0.892, P = 0.37) while observed heterozygosity (H_O) was significantly higher in the contemporary population (Z = 2.80, P < 0.01). Allelic frequencies ranges were smaller for the historical population (0.016-0.074) than for the recovered population (0.006-0.114) (Appendix C). Alleles present at both time periods account for 61% of the total sampled alleles, and their frequencies did not differ significantly (Z = -0.785, P= 0.432). This indicates that the recovered population shared many alleles with the historical population.

For the Bayesian cluster analysis using STRUCTURE, the modal value of the distribution of the true K identified a strong peak at ΔK=2 (Evanno et al. 2005), for both set of conditions imposed (default and user-defined). Samples were assigned to either cluster only if they had a high (>0.85) assignment proportion. For the default conditions, the overall proportion of membership for cluster one (green, Figure 3 a) was 75.4% (Eastern), and 63.4% (Midwestern) for historical samples. Similarly, 64.8% of the recovered birds were assigned to cluster two (red, Figure 3 a). The user-defined conditions also resulted in two clusters. The historical samples (Eastern-Midwestern) were mostly admixed, and proportion of membership to cluster one were lower compared to the default analysis, 56.1% (Eastern) and 46.6% (Midwestern). However, the contemporary birds were assigned unequivocally with a 94.5% frequency to cluster two (red, Figure 3 b).

TABLE II. GENETIC DIVERSITY IN HISTORICAL MIDWESTERN-EASTERN (1881-1963) AND RECOVERED MIDWESTERN PEREGRINES (1986-2009).

	Historical population (n = 32)							Recovered population (n = 80)						
Locus	n	A	AR	PA	PAR	H_O	H_E	n	A	AR	PA	PAR	H_O	H_E
CAB *fp* 18-2	31	9	8.10	5	4.92	0.26	0.35	80	10	8.14	6	4.97	0.56	0.52
CAB *fp* 24	31	8	7.86	2	1.99	0.28	0.83	80	11	9.62	5	3.75	0.80	0.85
CAB *fp* 85	32	6	5.98	1	1.65	0.28	0.76	80	5	4.32	0	0.00	0.61	0.60
CAB *fp 1*17	27	9	9.00	1	1.88	0.30	0.84	80	9	7.68	1	0.56	0.74	0.76
CAB *fp* 120	29	7	6.93	3	3.36	0.31	0.71	79	4	3.57	0	0.00	0.51	0.66
CAB *fp* 157	32	7	6.97	0	0.82	0.41	0.78	78	8	6.99	1	0.83	0.56	0.78
CAB *fp* 181	29	13	12.78	4	5.52	0.41	0.86	79	12	9.99	3	2.73	0.73	0.84
NVH *fp* 13	32	10	9.78	3	3.14	0.50	0.84	80	7	6.67	0	0.02	0.78	0.82
NVH *fp* 31	30	8	7.80	3	2.84	0.57	0.70	77	6	5.30	1	0.35	0.74	0.73
NVH *fp* 89	31	8	7.97	1	2.07	0.48	0.82	79	7	5.90	0	0.01	0.76	0.77
Mean	30.40	8.50	8.32	2.30	2.82	0.38	0.75	79.20	7.90	6.82	1.70	1.32	0.68	0.73
SD	1.65	1.96	1.90	1.57	1.48	0.11	0.15	1.03	2.60	2.12	2.21	1.82	0.11	0.11

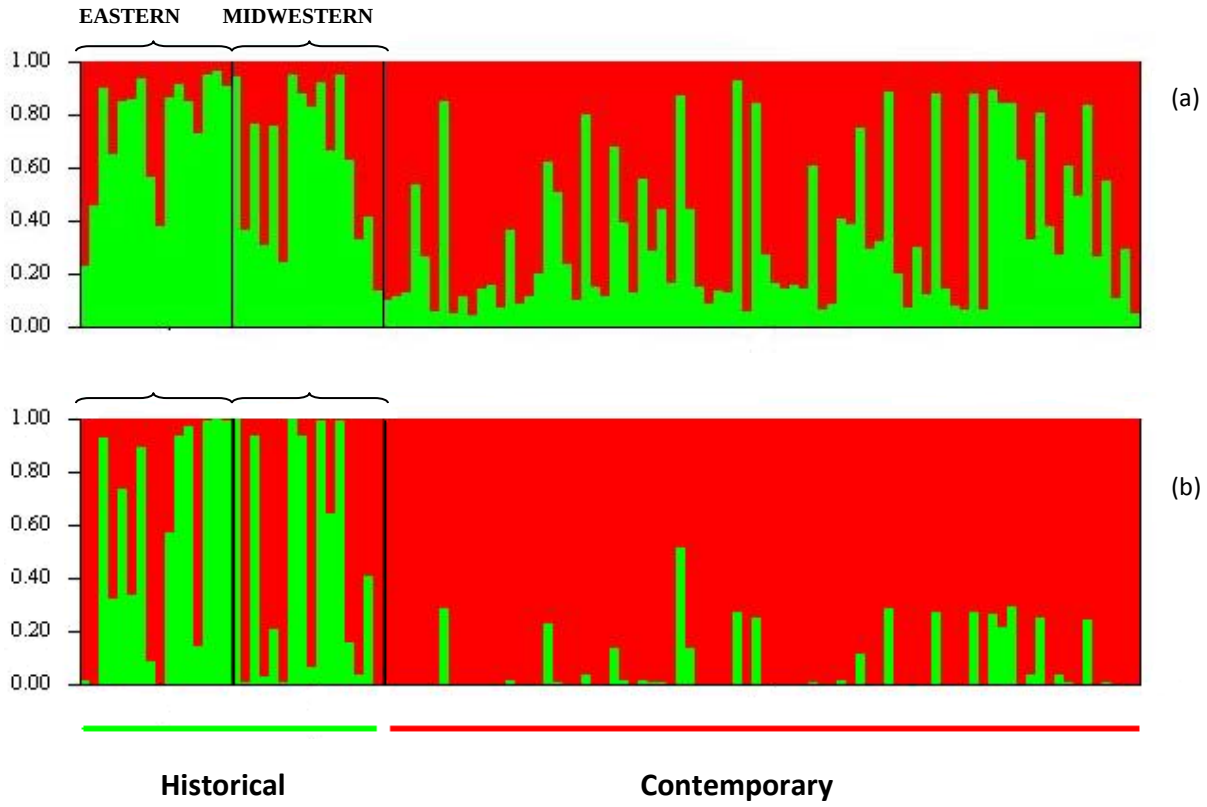

Figure 3. Assignment of temporal sampling of Peregrines using structure with *K*=2. In x-axis each bar represents a single Peregrine sample. Y-axis depicts the individual proportion of membership to each cluster. Results are shown with default (a), and user-defined settings (b).

Principal Coordinate Analysis (PCA) conducted on historical and contemporary birds also shows a shift in genetic composition following reintroduction. Axes 1 and 2 combined explain 42.9 % of the variation for the multilocus microsatellite markers. Contemporary samples are shifted to the right along axis 1 relative to historical samples, and historical samples from the Eastern US are shifted upward along axis 2 (Figure 4), suggesting genetic differentiation across time periods. Sixty two percent of the historical samples are on the left upper quadrant of the graphs and they do not overlap with the contemporary samples. This pattern resembles a cline

rather than a cluster grouped around a central point. Most of the contemporary birds group together while the clustering for the historical birds is more spread out along axis 1 and 2.

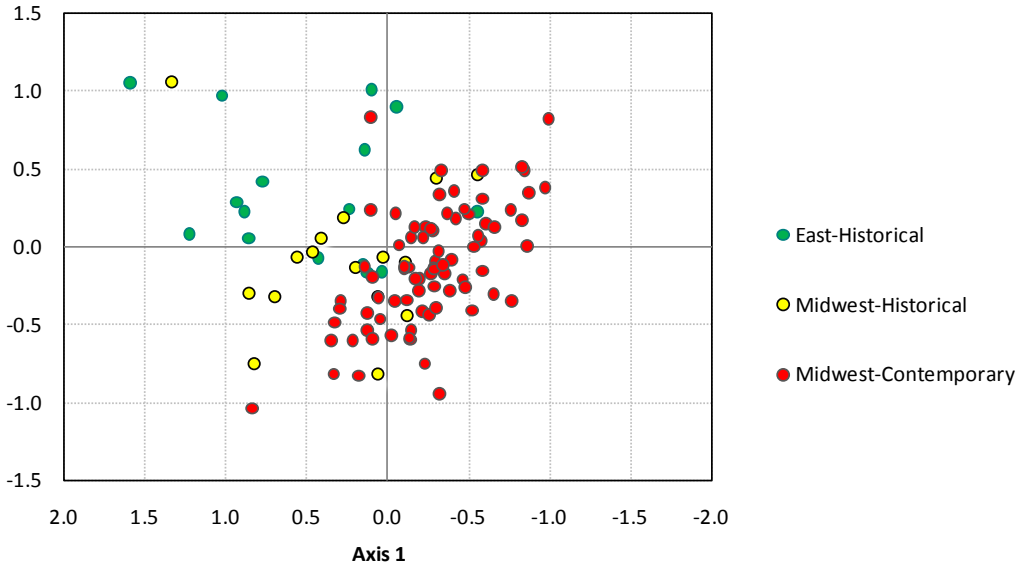

Figure 4. Principal coordinate analysis (PCA) based on genetic distances using microsatellite data. Individuals were coded by time period and plotted on the first two coordinates. Green and yellow circle symbols correspond to 32 historical samples (1881-1963) and red circle symbols represent 80 recovered samples (1986-2009).

The AMOVA showed that variance across time periods explained 3.96% of the total variance with 96.04% of the variance within each time period. The associated F_{ST} was 0.040 and significant (P< 0.05) while R_{ST} was 0.070 and also significant (P<0.05).

None of the three tests implemented in BOTTLENECK detected a signature of elevated heterozygosity indicative of a recent population decline for either temporal sample, historical or contemporary. However, a significant heterozygosity deficit was found for the sign test (P = 0.015) and the Wilcoxon test (P= 0.009) for the historical population. The qualitative graphical

method did not reveal a mode-shift distortion of the allele frequencies distribution that would be expected in a bottlenecked population (Figure 5). In a recovery bottlenecked population fewer alleles would be found in the low frequency class than in one or more intermediate frequency classes (Luikart et al. 1998).

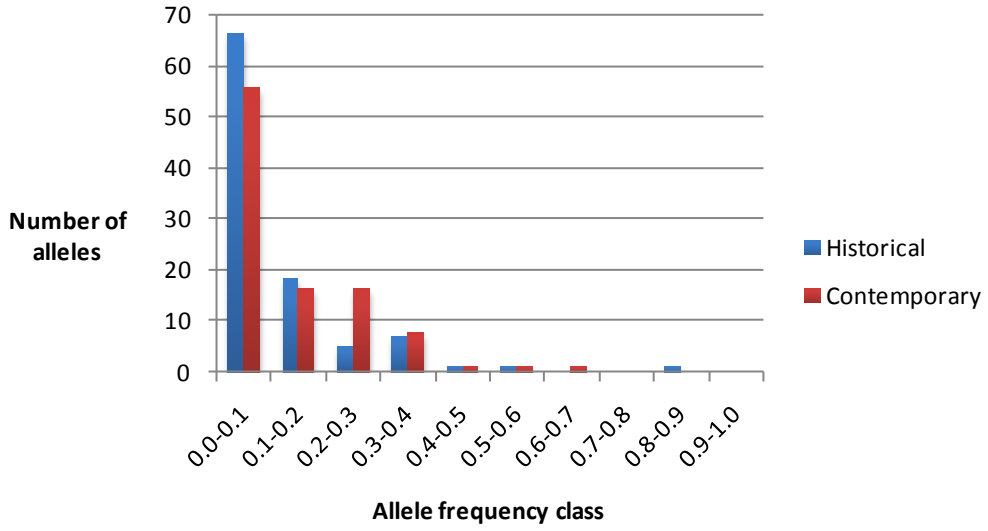

Figure 5. Distribution of allele frequencies from historical (n= 32) and contemporary (n= 80) non-bottlenecked populations.

3.4 Discussion

Widespread use of organochlorine pesticides led to the extirpation of Peregrine populations east of the Great Plains by the 1960s. Protection under the Endangered Species Act (ESA, 1973), the banning of DDT use under the authority of the U.S. Federal Insecticide,

Fungicide, and Rodenticide Act (Doremus & Pagel 2001), and recovery efforts have restored Midwestern Peregrines to most of their historic range. Here, I have used genetic data to examine changes in genetic variation and genetic composition in Midwestern Peregrine populations by comparing living birds to ancestral birds from museum skins that were up to 100 years old. My results based on multiple methodological approaches indicate that the recovered population did not suffer loss of genetic variation as measured by heterozygosity levels or allelic diversity, and loss of genetic diversity could not be detected by measuring bottleneck signals. However, there were some changes in genotypic composition based on multivariate and clustering analyses.

3.4.1 Genetic Diversity and Bottleneck Signatures

Both historical and contemporary samples showed high levels of nuclear diversity. Levels of observed heterozygosity were higher for recovered vs. historical populations. This would not be expected following a severe population crash in a top predator with a long generation time, however, this is likely due to the way these birds were managed, i.e. a constant influx of captive birds into the recovery effort, and the adaptability that Peregrines have exhibited. Seven out of 10 microsatellite loci used were newly developed markers and were chosen because they were highly polymorphic. The three remaining markers were developed by Nesje et al. (2000b) and used in other Peregrine studies. This could explain the higher number of alleles found in my study (102 alleles, n=112). Nesje et al. (2000a) using 12 dinucleotide markers found 76 alleles in a global sampling of Peregrines (n= 146). Similarly, Brown *et al.* (2007) reported a total of 69 alleles (n=279) for Canadian Peregrines, and the same number of alleles was found by Jacobsen *et al.* (2008) for Scandinavian Peregrines (n= 102); all these studies used eleven Nesje's (2000b) microsatellite loci.

Birds of different origins were used for reintroduction in order to maximize genetic variation and to insure that parents were unrelated (Tordoff & Redig 2001). As a result, reintroduced Midwestern Peregrines are potentially a mixture that includes five subspecies from outside their historical native ranges. The assortment occurred not only during the captive breeding phase when it was done intentionally to boost genetic variation but at a later stage due to the fact that wild birds can "freely" choose their mates. Despite the possibility of introducing new alleles from other populations into the recovery, when I calculated the proportion of unique alleles for each sampling period corrected for sample size I found that a higher number of alleles was present in the historical birds. Thus, these alleles were not present in my assessment of the reintroduced population so in a sense they could be considered "lost." This suggests that the reintroduction program did not lead to additional new alleles.

Low levels of mtDNA diversity seem to be a generalized phenomenon not only in Peregrines (Wink et al. 2000; Brown et al. 2007) but in other raptors species as well (Roques & Negro 2005; Johnson et al. 2007; Martinez-Cruz et al. 2007). Values of mtDNA diversity in my analysis are slightly lower than those reported in other raptor studies investigating demographic crashes, such as Canadian Peregrines (Brown et al. 2007), the Red Kite, *Milvus milvus* (Roques & Negro 2005), and the Spanish Imperial Eagle, *Aquila adalberti* (Martinez-Cruz et al. 2007). In my study, comparison with historical samples suggests that the lower levels of mtDNA diversity for contemporary samples probably resulted from a founder event. Since the effective population size of the mitochondrial genome is ¼ that of the nuclear genome, mtDNA mutations are much more susceptible to genetic drift and bottlenecks than nuclear loci (Ashley & Wills, 1987; Allendorf & Luikart 2007).

Haplotype frequencies also varied between time periods. The frequency of the widespread haplotype CA was 80.6% (historical) and 93.5% (contemporary). Haplotype CC, reported previously (Mindell et al. 1999) only appeared in 3.2% of my historical samples. The haplotype CD occurred in only 6.5% of my historical samples, whereas Brown et al (2007) found it in 31.1% of historical *tundrius* samples, and 15.8% of historical *anatum*. Haplotype CE occurred in 3% of the historical samples, while for Canadian Peregrines it was recorded in 2.2% of historical *tundrius* birds and 9% of historical *anatum* birds (Brown et al. 2007). The three remaining haplotypes (CJ, historical; CO and CR, contemporary) are novel haplotypes.

Data on the genetic effects of bottlenecked Peregrine populations has been presented for populations recovered using native stock in Canada (Brown et al. 2007), and for populations in Scandinavia, where a combination of native and non-native stock were used (Jacobsen et al. 2008). The Midwestern birds were re-established using stock from a much more diverse set of founders. Using temporal sampling, I address changes in genetic structure and diversity resulting from the process of reintroduction. Comparisons of mtDNA and microsatellites from these time periods offer evidence that there has been a shift in the genetic composition from the historical to the current population. I found no evidence of a population bottleneck based on microsatellite data for the reintroduced population, indicating a prompt recovery. This may be due to the release of large number of young in the Midwest and may explain the higher values of genetic diversity. However, the bottleneck analysis pointed out an overall deficiency of heterozygotes for the historical population. Interpreting the causes of an observed excess of homozygotes can be difficult. Whenever a deficiency appears in several markers like in this case, the most general claimed cause is population subdivision (i.e. Wahlund effect). However, inbreeding within a single deme (in my case the historical population) will produce a similar genotypic effect. My

historical sampling covered a period of about 50 years, in that sense one can think of samples coming from different “temporal” subpopulations, and the changes in allele frequencies from these might be one explanation for the observed heterozygote deficit. Also, historical samples came from a population that was decreasing; when this happens the probability of mating with a related individual increases and that may be worth considering as an explanation for the observed excess of homozygotes.

3.4.2 Patterns of mtDNA and Microsatellites

My genetic analyses show that the historical population had a certain degree of genetic admixture when compared to the modern population. One possible explanation for this is that my sampling included a relatively small sample of birds (32) and some alleles could have gone undetected. An alternative would be that the sampling included birds from the far north. To be sure of this one would need associated samples from those populations. Previous genetic studies have found differences in allele frequencies, but not fixed differences, for Canadian Peregrines (*anatum, tundrius, and pealei*). Similar results were found for mtDNA (Brown et al. 2007).

Brown et al. (2007) using Bayesian clustering analysis (STRUCTURE) found two clusters, one composed by most *pealei* birds and the other comprised of *anatum-tundrius* samples with different degrees of admixture. Similarly, the analysis for my historical samples identified two clusters, where the historical birds showed a certain degree of admixture, while the reintroduced birds showed high membership to one cluster. The historical birds were presumably *anatum* in origin and there is evidence that although I included Eastern Peregrines these birds were not differentiated genetically. This was corroborated by F_{ST}, PCA, and STRUCTURE analyses.

Microsatellite data provided support that the historical and contemporary populations have shifted their genetic composition. This conclusion is based on the PCA, the Bayesian clustering analysis and AMOVA. The pairwise F_{ST} showed slight but significant differentiation between the historical and contemporary population. Low polymorphism of mtDNA may have influenced the ability to detect temporal differences in these data.

3.4.3 Genetic Consequences of Recovery

Are current Midwestern Peregrines altered genetically by the historical population crash? I found no evidence of a bottleneck in the reintroduced population. Comparison of mtDNA pre- and post- DDT Peregrines shows that recovered Peregrines have low haplotypic and nucleotide diversity when compared to historical Peregrines. This could be because mtDNA has a lower population effective population size and I am seeing the results of a founder effect. My results demonstrate that Peregrines inhabiting the Midwestern US prior to extirpation were somewhat genetically distinct when compared to the recovered population and were genetically indistinguishable from the Eastern Peregrines.

Is there evidence for genetic contribution resultant from the reintroduction program? To address this question one should survey founders that were involved in the reintroduction and see whether these alleles/haplotypes are still present in the current population. The most likely place where European alleles/haplotypes in North American populations will show up is in the Midwestern-Eastern birds since the reintroduction used a limited number of birds (10%) of European ancestry (*peregrinus, brookei*).

One of the strategic goals during the reintroduction phase was for Peregrines to recolonize natural cliffs sites in Illinois and much of the lower Midwestern US. However, the

reintroduced populations remain mostly urban. High levels of heterozygosity and absence of bottleneck signals are indication that Peregrines are doing well. Analyses of population viability shows that Midwestern urban Peregrine Falcon populations are not likely to go extinct, and there is ample evidence that numbers are actually increasing (Redig et al. 2009; Wakamiya & Roy 2009).

In sum, future genetic research should be focused on comparing levels of genetic diversity to other reintroduced US Peregrine populations. I provide a starting point in Chapter 4. Midwestern Peregrines are the product of mixing several subspecies, while the reintroduction efforts for Western Peregrines, relied on the native stock that was available. This information can be used not only to provide better estimates of the contribution of foreign genetic stock but also a baseline data to incorporate in future management decisions. Genetic changes may occur over short periods of time during reintroduction efforts while populations have not reached demographic stability. Genetic monitoring by sampling periodically, facilitated by the fact that these populations are still being monitored closely, can help to understand what these changes mean for recovering populations.

3.5 Acknowledgments

This study was conducted with the support of the Raptor Research Foundation, The American Ornithologists' Union and The International Osprey Foundation. I am grateful to the curators, collections managers, and their institutions that kindly provided materials for this study: David Willard and Mary Hennen, Field Museum of Natural History, Steven Sullivan, Chicago Academy of Sciences, Paul Sweet, American Museum of Natural History, Kristof Zyskowski, Peabody Museum of Natural History, William L. Fink, University of Michigan Museum of

Zoology, Robert M. Zink and Mike Westberg, Bell Museum of Natural History. Additional thanks to Jonathan K. Pritchard for helpful comments and advice. Microsatellite and the subsequent laboratory work was carried out in the Pritzker Laboratory for Molecular Systematics and Evolution operated with support from the Priztker Foundation. I thank Kevin A. Feldheim for technical advice, and helpful discussions.

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4. INFERRING GENETIC DISCONTINUITIES AND TESTING PREDICTIONS OF ECOLOGICAL MODELING IN THE RECOVERED PEREGRINE FALCON (*FALCO PEREGRINUS ANATUM*) FROM THE WESTERN UNITED STATES

Peregrine Falcon populations across the US abruptly declined in the 1950's due to the bioaccumulation of chlorinated hydrocarbon insecticides. Beginning in the 1970s, different populations had very different management histories. Management techniques included captive breeding, combined hacking, fostering and cross-fostering. In southern California, intense management began with two local pairs. In northern California, Oregon, and Western Washington remnant populations recovered on their own. In the Midwest, management was conducted using non-native stock. I used genetic markers to assess whether contrasting reintroduction strategies led to differences in genetic composition following recovery. Analyses were carried out on 192 birds, 112 individuals representing recovered Western US populations and 80 individuals from the Midwest, using one mitochondrial DNA gene (control region) and 11 polymorphic microsatellite loci. To detect genetic discontinuities, I used individuals as the operational unit focusing only on genetic information, and I found genetic differentiation among populations, and similar levels of genetic diversity across regions. Microsatellite data separate Western Peregrine Falcons into three distinct genetic clusters supported by multivariate and Bayesian clustering analysis: (i) Channel Islands and Southern California; ii) Northern California and Oregon; and iii) Washington. These genetic patterns were concordant with previous ecological modeling studies. My results do not support the hypothesis that different management techniques have left an imprint on these recovered populations, but there is genetic structure consistent with the original groups on which management plans were focused.

4.1 Introduction

Defining the limits of populations is not an easy task and remains an active field of research. When ecological or geographical barriers are not clear the task can be quite daunting. If the members of the species under study are highly mobile or good dispersers, then the logical *a priori* hypothesis is that no population structure will exist. The development of statistical methods that use genetic information to measure the cohesiveness of individuals inhabiting a certain area now allows the inference of population limits. Why is this important? Accurate assessment of population boundaries becomes especially important if the species in question is currently endangered or threatened, and also if it is being actively managed, as we seek to assess the impacts of previous management practices (or lack of them) on current population distributions.

The case of the Peregrine Falcon is one of the most remarkable successes of conservation. Through extensive management, including the cessation of use of DDT in the United States, this avian predator was delisted as an endangered species in 1999 (USFWS 1999, Doremus & Pagel 2001), although this did not occur without controversy (Doremus & Pagel 2003). The effects of chlorinated hydrocarbon contamination starting in the 1950s and ending in the 60s caused the thinning of eggshells of many large carnivorous birds, impairing their reproduction (Ratcliffe 1967; Hickey & Anderson 1968). Peregrine populations crashed or diminished throughout the continental United States (Kiff 1988). Use of DDT was banned in the United States in 1972 and reintroduction efforts began in 1975 (Barclay 1988; Redig & Tordoff 1988). In the Western US, the decline mirrored what happened east of the Great Plains but did not lead to complete extirpation (Walton & Thelander 1988; Wooton & Bell 1992). There was a remnant population of *anatum* in the interior of northern California and adjacent Oregon that

received minor augmentation, but largely recovered on its own. In southern California, intense nest site manipulation including cross-fostering, fostering, and hacking used to promote resurgence and recovery began with several wild pairs (D. A. Bell, *pers. comm.*). In the 1990s, birds in mid- and south coastal California still showed extremely low reproduction rates attributed to elevated levels of DDT, whereas some northern interior California birds showed lower levels of contamination (Wooton & Bell 1992), potentially creating sink populations in southern California and source populations in northern California (Kauffman et al. 2004). Other western states such as Oregon and Washington have historically had low-densities of Peregrines pairs, but have also seen increased in numbers since the 70s.

In an attempt to determine viability in Californian Peregrine populations, Wootton and Bell (1992) applied ecological modeling based on a series of class matrix models. These incorporated published parameters values and accurately estimated the size of the southern subpopulation while slightly underestimating the size of northern subpopulation. Their primary finding was one of population subdivision in a source-sink substructure, linked by dispersal from northern populations to southern populations. Kauffman et al. (2004) also incorporated long-term monitoring data to examine patterns, mechanisms and consequences of spatial structure and dispersal in the California subpopulations. They made a further distinction of habitats and recognized an “interior” habitat, relatively underdeveloped, a “coastal” habitat more developed, and characterized by the majority of breeding sites on cliffs and lastly, an “urban” habitat (Los Angeles, San Francisco, and San Diego) where Peregrines nest on man-made structures (Kauffman et al. 2004). Work conducted by the Santa Cruz Predatory Bird Research Group has shown substantial geographical variation in the fecundity of breeding Peregrines in California, and the degree and persistence of the metabolite DDT (Kauffman et al. 2003). Peregrines fledged

in urban environments in California had a higher chance of survival their first year than did birds along the rural coastline and inland areas of southern and central coastal California (0.65 ± 0.15 vs., 0.28 ± 0.05, respectively (Kauffman et al. 2003).

The number of nests based on historical data of western Peregrines before 1970 varies greatly by state. California has the highest number, with approximately 130 reported nests while the historical status in Oregon remains vague. Historical records of Peregrines within the Columbia River Basin suggest at least 60 nest sites or sightings suggesting the presence of nest sites (Pagel 1995). Historical data on Peregrines inhabiting Washington before 1970 are restricted to 14 sites, although historical counts may have greatly underestimated population size (Hayes & Buchanan 2002). Based on these estimates the Recovery Plan for the western Peregrines aimed at reestablishing target numbers of 120 pairs in California, 30 pairs in Oregon and 30 pairs in Washington (Walton & Thelander 1988). By 2000, the number of eyries (nests) known to have been occupied by pairs of Peregrine Falcons in California was estimated to exceed 175 (Walton 2003), in Oregon were 65 reported nests while in Washington the number was 57 (Burnham 2003), thus exceeding target numbers in every western region.

4.1.1 Detecting Genetic Boundaries Using Neutral Multilocus Data

Defining the limits of populations is essential to study evolutionary processes such as local adaptation or gene flow and plan for the preservation and management of species (or subspecies) of concern. The concept of a ‘population’ is central to the fields of ecology, evolutionary biology, and conservation biology, and numerous definitions can be found in the literature (Waples & Gagiotti 2006). For practical as well as biological reasons, ‘populations’ are natural focal units for conservation and management (Palsboll et al. 2007), and identification of

population boundaries should influence management practices (Schwartz et al. 2007). The ecological paradigm emphasizes demographic cohesion as valid criterion to define a population whereas the evolutionary paradigm focuses on reproductive cohesion (Waples & Gagiotti 2006). Multilocus genetic data have been used with success in many raptor species to provide information on the relationship between geographical features and microevolutionary processes (Alcaide et al. 2008; Ewing et al. 2008; Funk et al. 2008). The field of landscape genetics identifies cryptic boundaries that could exist due to breaks in gene flow across demes without any apparent reason, or secondary contact among previously isolated populations (Manel et al. 2003). Connectivity between demes by dispersal is important because for it can lead to range expansion, and it promotes gene exchange between individuals from different localities, reducing inbreeding (Newton, 2003). In general, the degree of genetic differentiation between two populations of a species correlates with the distance that separates them (Peterson 1992; Nesje et al. 2000) because individuals are most likely to disperse to nearby sites i.e., Isolation by Distance, IBD, (Wright 1943, 1946). The IBD model has been used to infer the extent of genetic connectivity within and between populations (Lowe et al. 2004).

To study patterns of genetic differentiation in Peregrines, I started with the ecology-based designation proposed by Wooton and Bell (1992), and placed Californian samples into three groups: the South Coast population (SOC), the middle interior-coast population (MIC), and the Channel Islands (CHI) population. I also assigned samples collected in Oregon (ORE) and Washington (WAS) to separate populations, and included the Midwestern population (MIW) as a sixth group. Thus, I analyze the samples from the perspective of considering pre-defined populations as units for analysis but also I incorporate methods that are individual-based that might reveal alternative or cryptic population structure. These two complementary approaches

allow for the detection of genetic boundaries and/or measure the degree of connectivity among populations as well as individuals. Since management strategies were different in each western state, and different Peregrine stocks were used in the Western and Midwestern regions, I also assess the genetic consequences of reintroduction regarding genetic diversity, evidence for bottlenecks, and compare those results with other genetic studies of Peregrines.

4.2 Materials and Methods

4.2.1 Sampling Scheme

Blood samples representing 112 different nest sites in the Western US were collected from breeders and chicks in nests by licensed raptor biologists in California, Oregon and Washington from 1991 to 2007. Specifically, we obtained samples from the following locations: Channel Islands (CHI, Sta. Barbara Island/Anacapa Island/Sta. Cruz Island/Sta. Rosa Island/San Miguel Island), South Coastal (SOC, Los Angeles/San Diego), Middle Interior-Coast- (MIC, San Francisco/Santa Barbara/Monterey/Santa Cruz/Siskiyou/Humboldt), Oregon (ORE, Columbia River/Cascades/Umpqua), Washington (WAS, San Juan Islands/Seattle/Spokane) (Appendix D). For the Midwestern population (MIW) 80 samples from breeders and chicks were collected from 1986 to 2009 as detailed in Chapter 3 (Appendix B).

4.2.2 Genotyping and Sequencing

For the mtDNA analysis, a total of 93 individuals along the sampled range from Western US and 31 individuals from the Midwestern region were sequenced. A 405 base pair region of the mitochondrial DNA (mtDNA) named control region (CR) was amplified by PCR using

primers and PCR conditions as described in (Brown et al. 2007). Mitochondrial DNA sequences were aligned using CLUSTAL W (Thompson et al. 1994) and position of polymorphisms refer to the published mitochondrial genome of *F. peregrinus* (Mindell et al. 1999).

Eight new microsatellites markers were developed from an enriched genomic library (Glenn & Schable 2005). Primers flanking core microsatellites repeats (> six repeats) were inspected by eye and developed via Primer 3 software (Rozen & Skaletsky 2000) and tested using AmplifX v1.11 (Chapter 2, Table I). In addition, three published dinucleotide microsatellites for Peregrines developed by Nesje *et al*. (2000) were used. For labeling purposes, fluorescent labeled M13 primers are incorporated during PCR (Schuelke 2000). Then, labeled PCR products are pooled together with and internal size standard (GENESCAN™ 500 LIZ™ 50-500 bp ladder) and run on an ABI 3730 DNA Analyzer. Genotyping was carried out using the STRand Analysis Software v.2.4 (Toonen & Hughes 2001).

4.2.3 Data Analyses

Basic statistics describing mtDNA diversity and structure, including nucleotide (π) and haplotype diversity (*h*), and Tajima's *D* (Tajima 1989) were computed using DNASP 5.10 (Librado & Rozas 2009). Pairwise F_{ST} (Weir & Cockerham 1984) between populations were computed, using an analysis of molecular variance as implemented in ARLEQUIN v.3.5 (Excoffier *et al.*, 2005).

Microsatellite data was tested for linkage equilibrium and departure from Hardy-Weinberg equilibrium (HWE) within each population at each loci using GENEPOP (Raymond & Rousset 1995). Bonferroni corrections were applied to correct for multiple simultaneous comparisons (Rice 1989). Mean heterozygosity values were calculated using GenAlEx v.6.4

(Peakall & Smouse 2006). Measures of allelic richness (*AR*), and private allelic richness (*PAR*) were calculated using HP-RARE (Kalinowski 2005) to control for differences in sample size.

For detecting and quantifying genetic structure I used several approaches. Beginning with multivariate methods, which do not have biological assumptions, I used Principal Coordinates Analysis (PCA) with the software GenAlEx v. 6.4 (Peakall & Smouse 2006). This method was first applied to human genetic data (Cavalli-Sforza & Feldman 2003) and it has become a standard tool in population genetics (Novembre & Stephens 2008). Typically, PCA has been applied to data at the population level, but more recently, it has become common to use it at the individual level (Patterson et al. 2006). At the population level it offers an independent measure of relationships among demes. At the individual level it explores within population variation and helps in identifying outliers. It also identifies genetic clusters and their cohesion which can be easily seen graphically with individuals color-coded by population. This can later be compared to other individual-clustering techniques. The software GenAlEx v. 6.4 was also used to perform a Mantel test (Mantel 1967) with 1,000 permutations to examine the correlation of between genetic matrices of linear genetic distance and geographic distance of breeding sampling location of Western Peregrines.

The calculation of N_em (migrants per generation) from the expression $F_{ST} = 1/ (4N_em+1)$ (Wright, 1969) is a popular method for estimating gene flow from genetic data. However, there are certain assumptions of the underlying island model that are rarely met, i.e., no selection, no mutation, infinite number of populations of equal size having an equal probability of exchanging migrants, and populations in migration-drift equilibrium. Because of this, a newly developed group of methods (*assignment tests*) are being widely used. These methods assign samples to their most likely population of origin by comparing their genotypes to the genetic profiles of

various populations based on the allele frequencies in those populations (Paetkau et al. 1995). This differs from the assessment of gene flow (N_em) in that it identifies individuals that have dispersed from their natal population, as opposed to comparing overall genetic similarities between populations. Assignments tests have been improved by the application of Bayesian methods (Pritchard et al. 2000; Wilson & Rannala 2003) that are based on subjective statements of probability. Bayesian statistics often provide multiple probabilities meaning that numerous scenarios can be compared simultaneously (Freeland 2005).

I used three methods that provide an estimate of the number of populations while estimating gene flow. The first method, implemented in the software STRUCTURE v.2.3 (Pritchard et al. 2000), uses an estimation procedure to run a user-defined number of genetic clusters or populations *K*, and then compares the estimated log probability of data under each trial number, $\hat{K}$. The true number of clusters (*K*) is the value with the highest posterior probability. Since occasionally the estimated log probability of data provided by STRUCTURE might overestimate the most likely value of *K*, I also calculated an *ad hoc* statistic, ΔK, based on the rate of change in the log probability of data between successive $\hat{K}$ values (Evanno et al. 2005). Calculations in STRUCTURE were conducted with a burn-in period of 10^6, followed by 10^6 MCMC iterations. Following Evanno's method (2005), each simulation corresponding to a particular value of $\hat{K}$ $\hat{K}$ = 1-10 was performed 20 times using the default settings of STRUCTURE. These include an ancestry model incorporating admixture, lambda fixed to 1, independent calculation of alphas for each population, and a correlated model of allele frequencies without *a priori* information of the origin population. Later, the 20 runs corresponding to the inferred value of *K* were averaged using the software CLUMP v.1.1.2 (Jakobsson & Rosenberg 2007) applying the greedy algorithm

and the G' pairwise matrix similarity. Finally, the resultant clusters were plotted using DISTRUCT (Rosenberg 2004).

The second method used to investigate spatial genetic structure includes individual georeferenced multilocus genotypes, without prior knowledge on population geographical boundaries. Here, the group of sampled individuals is modeled as a spatial mixture of panmictic populations while the spatial connectivity of populations is modeled through the colored Voronoi tessellation (Guillot et al. 2005a). Like the first method, this approach estimates the number of populations in the studied area, assigns individuals to the population of origin, and detects migrants between populations. Unlike the first method, it quantifies the amount of spatial dependence in the dataset while considering uncertainty on the location on sampled individuals. This approach was implemented in the R computer package GENELAND (Guillot et al. 2005b). During preliminary analysis I followed authors' recommendations (Guillot et al 2005b) to perform inferences in two steps: a first run where *K* is treated as unknown and is left variable (*K*=1-10), followed by a second run where *K* is fixed at the value estimated in the first run to estimate individual cluster memberships. These preliminary runs allows checking for MCMC convergence issues and consistency on the parameters inferred. Once I determined *K* fixed at the modal value I ran the MCMC again 10 times with 10^6 iterations, a maximum number of nuclei in the Poisson-Voronoi tessellation fixed to 300, and an uncertainty associated with the spatial coordinates of 5 km. I used the Dirichlet model of allelic frequencies (D-model) as it has been shown to perform better than the F-model (see Guillot et al. 2005b). These 10 runs were post-processed (with a burn-in of 10^5 iterations) in order to obtain more accurate posterior probabilities of population membership for each individual.

The third method I used estimates recent immigration rates, *m* (i.e. proportion of migrants), among the populations analyzed using microsatellite data. The program BAYESASS v.1.3 (Wilson & Rannala, 2003), also a Bayesian method, does not require the populations to be in either migration-drift or HWE, which is an assumption that in our populations likely does not hold. The MCMC was run for a total of 3×10^6 iterations, with a burn-in of 10^6 to allow the chain to reach stationarity. Data was collected every 2000 iterations to infer posterior probability distributions of migration rates.

To date, there have been no assessments of the genetic impacts on the decline of Western US Peregrine populations. Nuclear loci are expected to be less sensitive to bottleneck effects than mitochondrial genomes because they have an effective population size (N_e) that is four times as large. Loss of genetic diversity depends strongly on the duration of a bottleneck. Population genetics theory predicts that the majority of diversity can be retained in populations suffering a severe decline if the duration of the bottleneck is brief (Nei et al. 1975). Moreover, the time since a bottleneck took place will influence whether genetic signatures will be detected (Cornuet & Luikart, 1996). To test for bottleneck signatures I used BOTTLENECK 1.2 (Piry et al. 1999). I assumed a two-phase model (TPM) with 5% multiple-step mutations and a variance of 12, as suggested by Piry et al (1999). Populations that experienced a recent bottleneck ($0.2N_e$ – $4N_e$ generations) exhibit temporal elevated levels of heterozygosity (H_E) (Nei 1987) compared to levels expected under mutation-drift equilibrium (H_{Eq}) (Cornuet & Luikart 1996). Thus, for each population H_{Eq} is calculated from the observed number of alleles (*k*), given the sample size (*n*) under the assumption of mutation-drift equilibrium simulating a coalescent process.

4.3 Results

4.3.1 Genetic Diversity

For the 405 base pairs of mtDNA control region sequence, there were four polymorphic sites and five haplotypes for Western US Peregrine samples, and three haplotypes for the Midwestern US Peregrines (Table I). One haplotype, CA, was previously reported by Brown et al. (2007). Haplotype CC corresponds to the haplotype from the mitochondrial Peregrine genome reported by Mindell et al. (1999). The remaining five haplotypes, CB, CI, CJ, CO and CR have not been reported previously. The first three haplotypes CB, CI, and CJ, were found in the Western sampling locations, and the last two, CO and CR, in the Midwest population. Number of haplotypes per location sampled ranged from one in Washington (WAS) to a maximum of four in California (MIC) (Table I). Similarly, Peregrine haplotype diversity (h) ranged from 0 in WAS, where only the common haplotype CA was found, to $h = 0.489$ in the MIC population, and nucleotide diversity (π) was consistently low across all Western Peregrine sampling locations (0.000-0.001) (Table II). Observed polymorphisms within each population were consistent with neutral expectations: Tajima's D values ranges were -0.399 to -1.506, $P > 0.10$.

No instances of linkage disequilibrium were found in pairwise comparisons of the 11 microsatellite loci**.** After adjusting for multiple comparisons ($n = 66$), tests for departure from Hardy-Weinberg equilibrium indicated that eight loci showed heterozygote deficiency in four out of six populations (Table III). None of those loci showed deficiencies in Oregon (ORE) and Midwest (MIW) sampling locations. Only one locus (CAB *fp* 157) showed deficiency in California (MIC), and another (CAB *fp* 181) showed deficiency in California (SOC). One possible explanation is that these two loci contain null alleles. Two other populations showed

deficits in various loci, California (CHI) (CAB *fp* 24, 117, 120 and NVH *fp* 31), and WAS (CAB *fp* 18-2, 85, 117). In these cases, I consider the possibility of inbreeding ($F_{IS} > 0$) but also this outcome could be attributed to the small sample size of these two populations ($n_{CHI} = 25$, $n_{WAS} = 21$).

A total of 123 alleles were observed among all geographic locations for Western and Midwestern Peregrines across 11 loci (Appendix E). The total number per location ranged from a minimum of 69 alleles in the Channel Islands (CHI) to a maximum of 88 alleles in MIW. Wilcoxon sign-rank tests did not show significant differences for allelic richness or private allelic richness among the sampled locations for individual loci (Table III). The lowest average value of allelic richness was for CHI (5.49 ± 1.68) and highest for SOC population (6.32 ± 1.71). Interestingly, the average value of private allelic richness of ORE (0.09 ± 0.15) was significantly

TABLE I. HAPLOTYPES OBSERVED AMONG WESTERN AND MIDWESTERN PEREGRINE FALCONS. SEE FIG. 1 FOR GEOGRAPHIC DISTRIBUTION BY POPULATION FOR WESTERN PEREGRINES. NUMBERS INDICATE THE POSITIONS OF VARIABLE NUCLEOTIDES WITHIN THE 405 BP SEQUENCE OF CR ACCORDING TO THE MITOCHONDRIAL GENOME OF THE PEREGRINE. DOTS UNDER NUCLEOTIDE POSITIONS DENOTE THE SAME NUCLEOTIDE SPECIFIED AS HAPLOTYPE CA.

	Variable site						West	Midwest
Haplotype	15265	15267	15355	15356	15462	15623	Peregrines	Peregrines
CA	C	A	G	A	G	C	0.819	0.968
CB	G	.	.	.	.	.	0.012	−
CC	.	G	.	.	.	.	0.133	−
CI	.	.	C	.	.	.	0.012	−
CJ	.	.	.	G	.	.	0.024	−
CO	.	.	.	.	A	.	−	0.032
CR	.	.	.	.	.	G	−	0.032

TABLE II. MITOCHONDRIAL GENETIC DIVERSITY FOR CR (405 BP) IN WESTERN PEREGRINE FALCONS SAMPLING LOCATIONS, *N* REFERS TO NUMBER OF SAMPLED INDIVIDUALS. STANDARD ERROR VALUES ARE GIVEN IN PARENTHESES.

Sampling location	*N*	No. of haplotypes	Haplotype diversity (*h*)	Nucleotide diversity (π)	Tajima's $D^{\dagger}$
SOC	15	2	0.248 (0.012)	0.000 (0.000)	-0.399
CHI	26	3	0.394 (0.011)	0.001 (0.000)	-0.432
MIC	20	4	0.489 (0.013)	0.001 (0.000)	-0.975
ORE	18	2	0.209 (0.014)	0.000 (0.000)	-0.528
WAS	14	1	0.000 (0.000)	0.000 (0.000)	n.a.
MIW	31	3	0.127 (0.080)	0.000 (0.000)	-1.506

†All values P > 0.10

higher than WAS (0.96 ± 0.90) (t-test, Z = -4.96, $P < 0.001$). The number of private alleles varied greatly by location. Six alleles were unique to SOC, two alleles were unique to MIC, and a single allele was unique to both CHI and ORE. The highest number of unique alleles were 11 and 12 from WAS and MIW populations, respectively (Appendix E). Average observed heterozygosity (H_O) varied for populations. Values of H_O were similar for SOC (0.58 ± 0.05) and CHI (0.56 ± 0.08). Estimates of H_O were higher for MIC (0.64 ± 0.05), WAS (0.66 ± 0.05), and MIW (0.70 ± 0.04) (Table III). Peregrines from ORE had the highest H_O estimate (0.77 ± 0.06). However, none of the values were significantly different when making pairwise comparisons.

4.3.2 Population Structure

Most of the Peregrine populations defined by geographical designations appear distinct, according to significant pairwise differentiation (F_{ST}) for microsatellites (Table IV). Only two pairwise comparisons, those between Californian SOC-CHI and Californian-Oregonian MIC-

ORE populations, were not significant. Sampling locations that are further apart have higher values of F_{ST}. Within the Western US, the northernmost population in Washington, WAS, exhibited significant differentiation from all other regions. Likewise, pairwise comparisons to the Midwestern population were significant, and corresponded with the highest F_{ST} values: MIW-CHI (0.111), and MIW-ORE (0.085). Also, significant population structure was detected among neighboring populations MIC-SOC and Channel Island, CHI. In contrast, F_{ST} based on mtDNA data resulted significant in only three instances. The highest F_{ST} value corresponded to adjacent populations MIC-WAS (0.146), followed by populations separated by the Great Plains, MIC-MIW (0.131) and CHI-MIW (0.071) (Table IV).

Little structure in the mtDNA sequence data was observed among Western and Midwestern Peregrine samples. The parsimony network of haplotype relationships was starlike, with the haplotype CA, common to all populations, in the center of the network (results not shown). Specifically, haplotypes CB and CI were only found in California, MIC. Similarly, one haplotype, CJ was found in Channel Islands, CHI. Haplotype CC was found in all of the Western populations excluding Washington, WAS (Figure 1). Haplotypes CO and CR were only found in Midwestern Peregrines.

TABLE III. ANALYSIS OF MICROSATELLITE GENETIC DIVERSITY IN RECOVERED PEREGRINE POPULATIONS.

	California									Oregon			Washington			Midwest		
	SOC			CHI			MIC			ORE			WAS			MIW		
Locus	AR	PAR	H_O/H_E	AR	PAR	H_O/H_E	AR	PAR	H_O/H_E	AR	PAR	H_O/H_E	AR	PAR	H_O/H_E	AR	PAR	H_O/H_E
CAB *fp* 18-2	5.53	1.39	0.47/0.53	4.31	0.43	0.29/0.42	3.66	0.05	0.35/0.34	4.27	0.12	0.46/0.47	4.87	2.36	**0.52/0.60**	5.59	2.46	0.56/0.51
CAB *fp* 24	7.00	0.19	0.64/0.79	6.98	0.63	**0.45/0.76**	6.51	0.09	0.59/0.80	6.42	0.11	0.96/0.74	6.96	1.72	0.76/0.78	7.62	0.73	0.80/0.84
CAB *fp* 77	8.89	1.17	0.75/0.83	8.63	0.07	0.94/0.83	9.04	0.00	1.00/0.88	8.89	0.00	0.83/0.88	6.97	0.00	0.72/0.79	7.54	0.00	0.87/0.80
CAB *fp* 85	4.95	0.18	0.38/0.42	4.22	0.01	0.46/0.57	4.38	0.01	0.61/0.67	3.38	0.00	0.75/0.59	5.87	1.56	**0.33/0.51**	3.89	0.00	0.61/0.60
CAB *fp* 117	6.94	0.72	0.56/0.77	6.27	0.07	**0.46/0.78**	6.36	0.05	0.67/0.78	6.10	0.00	0.83/0.79	6.22	0.62	**0.60/0.72**	5.86	0.27	0.73/0.75
CAB *fp* 120	2.96	0.00	0.43/0.55	2.73	0.00	**0.13/0.52**	2.50	0.00	0.41/0.52	2.71	0.00	0.38/0.48	3.00	0.00	0.58/0.62	3.26	0.26	0.51/0.65
CAB *fp* 157	5.75	1.36	0.67/0.60	3.99	0.03	0.79/0.68	4.82	0.00	**0.61/0.70**	5.61	0.48	0.88/0.77	6.87	1.50	0.67/0.80	5.84	0.32	0.56/0.77
CAB *fp* 181	8.84	2.53	**0.50/0.85**	6.28	0.08	0.48/0.80	7.02	0.00	0.70/0.82	7.23	0.01	0.88/0.84	9.29	2.12	0.71/0.88	7.74	1.07	0.73/0.83
NVH *fp* 13	5.37	0.23	0.94/0.77	5.61	0.21	0.77/0.77	5.20	0.09	0.78/0.77	4.96	0.00	0.92/0.78	5.71	0.42	0.80/0.79	5.97	0.43	0.78/0.81
NVH *fp* 31	6.59	0.41	0.40/0.75	4.73	0.00	**0.46/0.70**	5.66	0.87	0.61/0.75	5.66	0.01	0.88/0.79	5.96	0.14	0.88/0.82	4.70	0.02	0.74/0.73
NVH *fp* 89	6.67	0.11	0.62/0.79	6.69	0.10	0.91/0.76	7.77	0.96	0.74/0.82	5.34	0.25	0.67/0.71	7.08	0.12	0.71/0.78	5.19	0.02	0.76/0.76
Mean	6.32	0.75	0.58/0.70	5.49	0.15	0.56/0.69	5.72	0.19	0.64/0.71	5.51	0.09	0.77/0.72	6.25	0.96	0.66/0.74	5.74	0.51	0.70/0.73
SD	1.71	0.78	0.05/0.04	1.68	0.20	0.08/0.04	1.88	0.36	0.05/0.05	1.73	0.15	0.06/0.04	1.56	0.90	0.05/0.03	1.48	0.73	0.04/0.03

AR= allelic richness, PAR= private allelic richness, H_O= observed heterozygosity, H_E= expected heterozygosity. Values in bold indicate significant heterozygote deficiency following Bonferroni corrections (n= 66 comparisons, critical P= 0.0009)

TABLE IV. PAIRWISE POPULATION FST VALUES FOR MICROSATELLITE (BELOW THE DIAGONAL) AND MTDNA (ABOVE THE DIAGONAL) ANALYSIS. SIGNIFICANT VALUES ARE INDICATED WITH BOLD AND ASTERISKS

Sampling	SOC	CHI	MIC	ORE	WAS	MIW
SOC	–	-0.033	-0.010	-0.063	0.065	0.024
CHI	0.020	–	-0.022	-0.016	0.088	**0.071***
MIC	**0.043****	**0.043****	–	0.015	**0.146***	**0.131***
ORE	**0.085****	**0.091****	0.004	–	0.039	0.009
WAS	**0.069****	**0.084****	**0.072****	**0.071****	–	-0.017
MIW	**0.085****	**0.111****	**0.086****	**0.108****	**0.066****	–

*$P < 0.05$, **$P < 0.01$, *** $P < 0.001$

TABLE V. ESTIMATES OF RECENT MIGRATION RATES AMONG SAMPLING LOCATIONS IN WESTERN AND MIDWESTERN PEREGRINE FALCONS BASED ON THE BAYESASS ANALYSIS. MEANS OF THE POSTERIOR DISTRIBUTION OF M ARE DISPLAYED ALONG WITH THEIR RESPECTIVE 95% CONFIDENCE INTERVALS IN PARENTHESES. VALUES ON THE DIAGONAL IN BOLD REPRESENT THE PROPORTION OF INDIVIDUALS DERIVED FROM THE SOURCE POPULATION EACH GENERATION. MIGRATION RATES ≥ 0.10 ARE IN ITALICS**.**

	Migration from			
Migration into	SOC/CHI	MIC/ORE	WAS	MIW
SOC/CHI	**0.734 (0.693-0.789)**	*0.238 (0.179-0.289)*	0.012 (0.000-0.042)	0.015 (0.000-0.048)
MIC/ORE	0.003 (0.000-0.017)	**0.990 (0.964-0.999)**	0.003 (0.000-0.019)	0.004 0.000-0.023)
WAS	0.021 (0.000-0.078)	*0.121 (0.038-0.219)*	**0.732 (0.673-0.834)**	*0.124 (0.041-0.226)*
MIW	0.004 (0.000-0.017)	0.003 (0.000-0.018)	0.003 (0.000-0.012)	**0.990 (0.966-0.999)**

Principal Coordinates Analyses were conducted using the covariance matrix in two ways: in one matrix the rows were indexed by individuals (Figure 2) while in another matrix the rows were indexed by populations (Figure 3). For the first PCA, individuals were color-coded by population only for comparative purposes. The majority of individuals (85.5%) form one cluster along axis 2. The remaining individuals (14.5%) are dispersed along axis 1, and more than half of them are from the Channel Island, CHI population. Combined, axis 1 and 2, explained 52. 3% of the genetic diversity (PC1: 35.91%, PC2: 16.35%), and no clear population subdivision was evident (Figure 2). Population-based PCA, however, does show some patterns. In some cases, populations that are geographically adjacent to one another such as the Californian, SOC and CHI as well as MIC and the Oregonian, ORE, are closer on the graph. In this analysis, the northern Washington population, WAS, clustered with the Midwestern population, MIW. Population scores were plotted on PC1 and PC2 which explained 77.96 % of the total genetic diversity (PC1: 42.28%, PC2: 35.68%) (Figure 3). Significant isolation by distance was found only for birds from California (MIC, r_{yx}= 0.275, Mantel Test P= 0.000). Isolation by distance was not supported for the birds in Southern California (SOC, r_{yx}= 0.111, Mantel Test P= 0.141), and Channel Islands Peregrines (CHI, r_{yx}= 0.072, Mantel Test P= 0.070). Similarly, northern Western Peregrines did not show and IBD pattern in Oregon (ORE, r_{yx}= 0.109, Mantel Test P= 0.166) and Washington (WAS, r_{yx}= 0.179, Mantel Test P= 0.113).

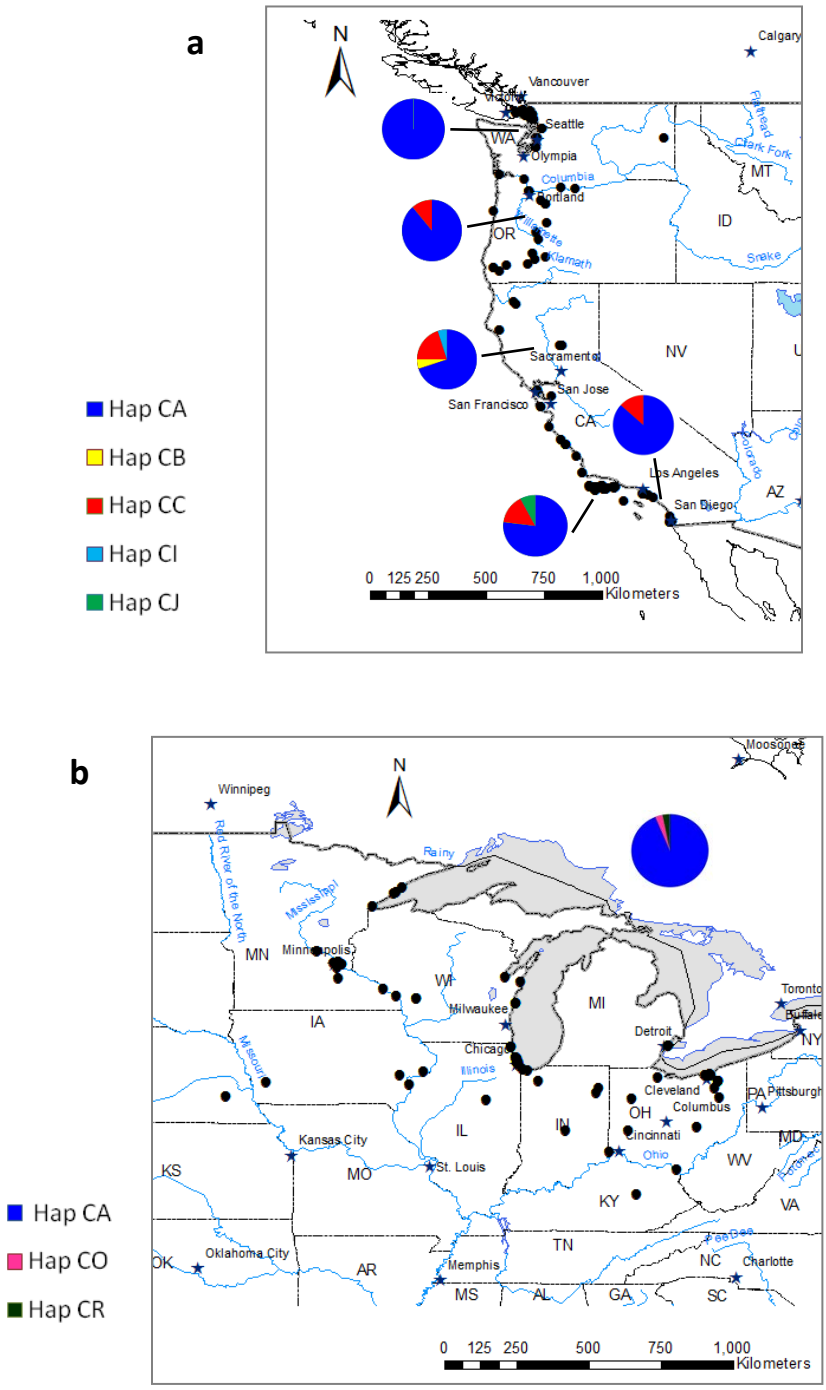


Figure 1. Geographical distribution of mitochondrial DNA Control region haplotypes from Western (a) and Midwestern (b) Peregrines. Black round points represent locations of individuals sampled.

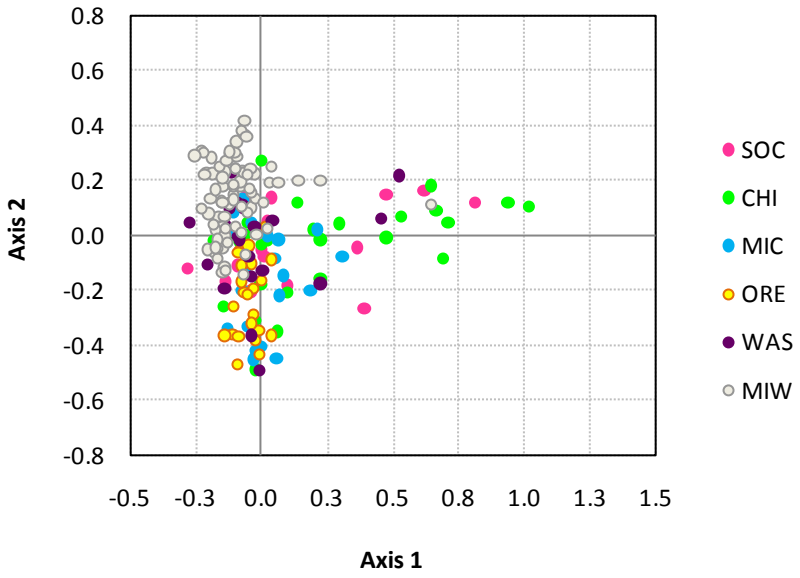


Figure 2. Individual-based PCA for Western and Midwestern Peregrines. Birds from California (SOC, CHI and MIC), Oregon (ORE), Washington (WAS) and Midwest (MIW) are color coded according to their breeding/natal origin.

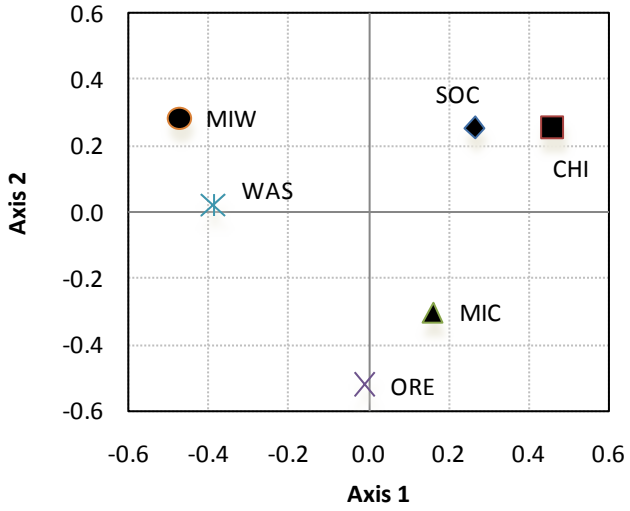


Figure 3. Population-based PCA for Western and Midwestern Peregrine populations.

Results from Bayesian analyses using the software STRUCTURE (Figure 4) showed increasing posterior probabilities values with increasing *K* values, peaking at *K*= 5; (Figure 5 a). Thus, the value of the choice criterion suggested by Pritchard et al (2000) agreed with the calculated *ad hoc* statistic, Δ*K* (Evanno et al. 2005) (Figure 5 b). However, the value of *K* that captured most of the structure of my data and seemed biologically sound corresponded to *K*=3. This is clearly seen in the population membership values assigned by cluster (Figure 4, Table VI). Only Peregrines from Washington (WAS) were consistently assigned to a cluster with relatively high assignment proportions (69-82%), when compared to the remaining populations. These assignment values decreased at higher values of *K* (Table VI).). For *K* = 3, the southernmost population (SOC) and Washington (WAS) were

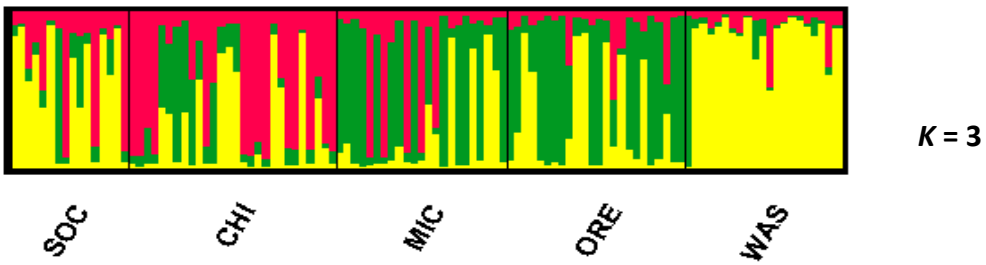


Figure 4. Assignment of Peregrine Falcons to clusters based on genotypic data. Samples are from Californian (SOC, CHI, MIC), Oregon (ORE), and Washington (WAS) *K* = 3 illustrates three inferred clusters (yellow, red and green). Bars represent proportion of membership for a single individual sample.

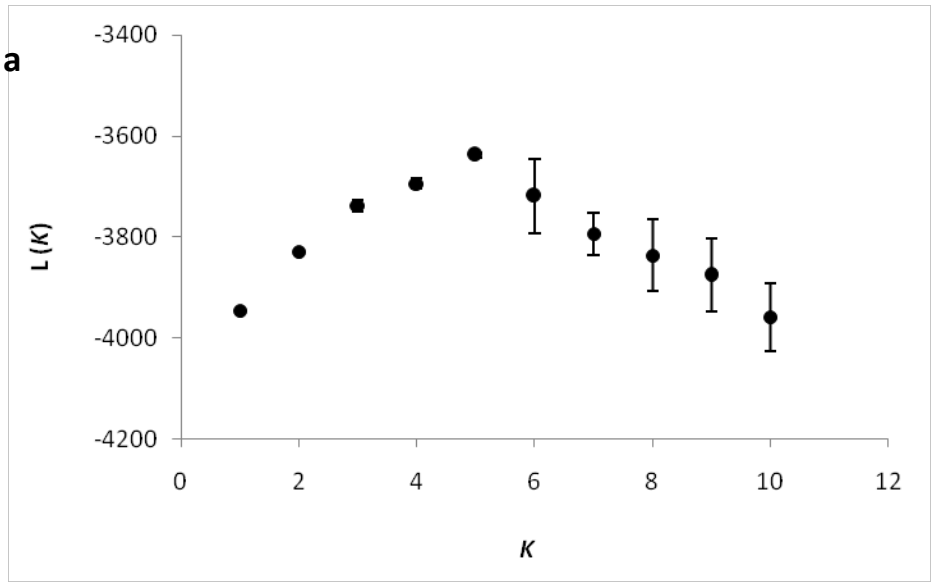
assigned to the same cluster (cluster 3, Table VI). The MIC/ORE populations were assigned to a single group (cluster 2), and Peregrines from the Channel Islands (CHI) were grouped alone (cluster 1). The assignments for all these three populations were low (54-60%). For $K = 5$, which corresponded with the maximal value of L(K), and ΔK, the assignment proportions decreased for each cluster but did not changed how populations were assigned to them. The only exception was the birds from southern California (SOC) that were in a different cluster than WAS. The posterior distribution in GENELAND gave a modal value at $K = 3$, and are somewhat concordant with STRUCTURE ($K = 3$) (Figure 6. Maps of the posterior probability for each cluster for the Western Peregrine data (GENELAND). The three plots represent the assignment of pixels to each cluster, from left to right: SOC-CHI cluster, MIC-ORE cluster, and WAS cluster. The highest membership values are in light yellow and the isoclines illustrate the spatial changes in assignment values. The Western coastline is depicted in blue, and black arrows indicate each one of the clusters.). This spatial method gives evidence for the presence of three Western clusters; the same number previously detected using non-spatial statistical approaches (F_{ST}). Pairwise F_{ST} values between the five populations were not significant for the boundary among the SOC-CHI and the MIC-ORE populations.

TABLE VI**.** PROPORTION OF MEMBERSHIP FOR EACH POPULATION TO EACH CLUSTER WITH INCREASING NUMBER OF *K*, USING THE PROGRAM STRUCTURE. BOXES INDICATE CLUSTERED POPULATIONS.

	Proportion of membership (*K*=3)		
Population	Cluster 1	Cluster 2	Cluster 3
SOC	0.298	0.154	0.548
CHI	0.536	0.209	0.255
MIC	0.245	0.555	0.200
ORE	0.108	0.603	0.289
WAS	0.087	0.091	0.823

	Proportion of membership (*K*=4)			
	Cluster 1	Cluster 2	Cluster 3	Cluster 4
SOC	0.570	0.222	0.108	0.100
CHI	0.263	0.517	0.149	0.071
MIC	0.155	0.221	0.500	0.124
ORE	0.113	0.076	0.551	0.261
WAS	0.196	0.033	0.071	0.701

	Proportion of membership (*K*=5)				
	Cluster 1	Cluster 2	Cluster 3	Cluster 4	Cluster 5
SOC	0.361	0.205	0.094	0.077	0.263
CHI	0.172	0.494	0.132	0.047	0.155
MIC	0.051	0.213	0.475	0.113	0.147
ORE	0.031	0.062	0.534	0.244	0.128
WAS	0.014	0.024	0.064	0.687	0.212



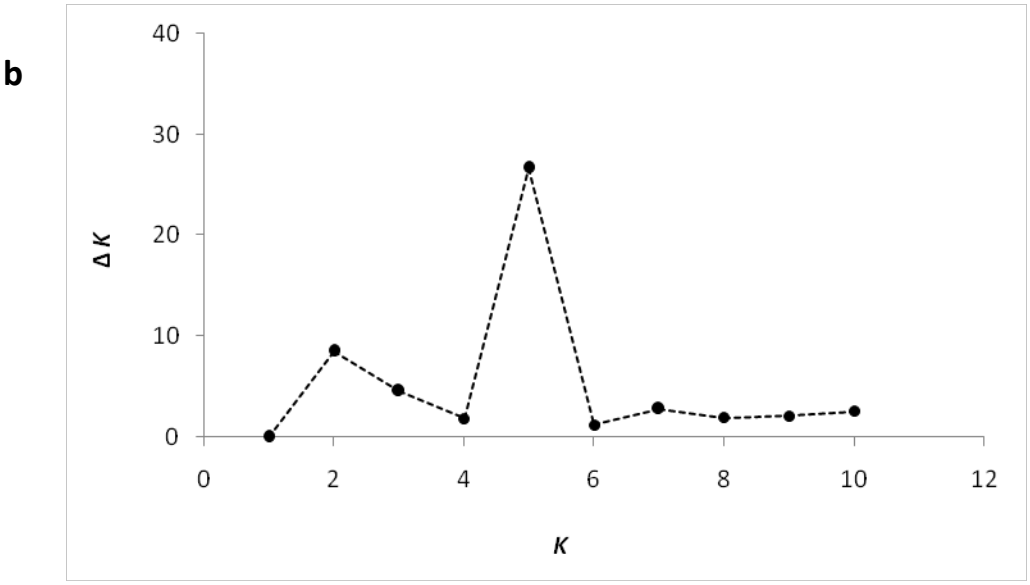


Figure 5. Bayesian inference of the number of clusters (*K*) for Western Peregrines. *K* was estimated in two ways, using the (a) posterior probability of the data given each *K*, based on 20 replicates, and (b) the distribution of Δ*K*.

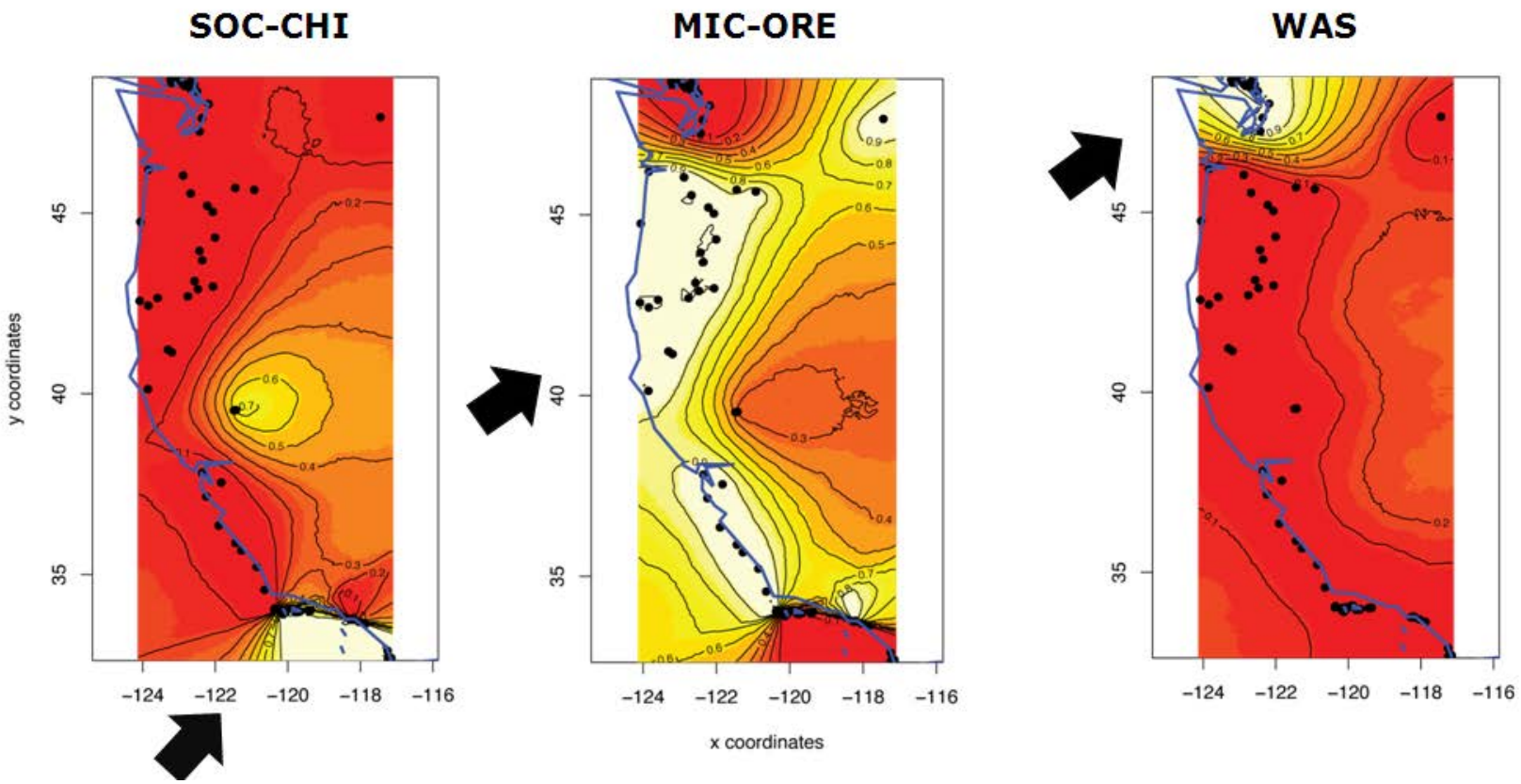


Figure 6. Maps of the posterior probability for each cluster for the Western Peregrine data (GENELAND). The three plots represent the assignment of pixels to each cluster, from left to right: SOC-CHI cluster, MIC-ORE cluster, and WAS cluster. The highest membership values are in light yellow and the isoclines illustrate the spatial changes in assignment values. The Western coastline is depicted in blue, and black arrows indicate each one of the clusters.

Recent migration rates were estimated between all Peregrines (Western and Midwestern) using the software BAYESASS. When simulating the effect of having no information in the data from which to estimate migration rates, we obtained a 95% confidence interval (CI) of 0.675-0.992 for the proportion individuals derived from the source populations each generation (non-migrant rates) and a CI of 0.000-0.218 for migration rates. Confidence intervals obtained from the data were considerably smaller than those obtained from the null hypothesis (Table V), suggesting that the data are informative for assessment of migration rates between populations. Sampling locations were pooled based on F_{ST} values and Bayesian clustering analyses. In general, each one of the sampling locations has a high proportion of non-migrants. The highest proportion of non-migrants is located in MIC/ORE (m = 0.99) which also is supported as a source for the remaining Western populations. The other location with a high proportion of non-migrants is the Midwestern population, which is not supported as a source for most Western populations (Table V). The SOC/CHI and the WAS populations had a lower proportion of individuals derived from their own population (m = 0.734 & m = 0.732, respectively). Within the Western US populations, we do observe two cases with rather high proportion of immigrants between sampling locations. This movement seems to be asymmetric with birds from MIC/ORE, moving into both southwestern SOC/CHI (m = 0.238), with basically no movement in the opposite direction (m= 0.003) (Table V). Lower immigration rates were observed from MIC/ORE into WAS (m = 0.121). Immigration in the opposite direction between these location was low (m = 0.003). The migration rates between Midwestern and Western Peregrines were low as is expected because these populations are separated by the Great Plains, and in not contact whatsoever. However, immigration from MIW to WAS (m = 0.124) was considerably higher than in the opposite direction (m = 0.003) and the lower bound for CI in this case not different

from zero. This result is rather unexpected because these populations are apart 2,800 km and there are no reports of Midwestern birds moving to Washington and vice versa. However, founders from the Midwestern populations included birds from this area and that could explain the "inflated" rate of migration from MIW to WAS. An alternative explanation may be related to homoplasy and the fact that these populations have many common alleles.

None of the three tests implemented in BOTTLENECK detected a signature of elevated heterozygosity indicative of a recent population decline for any of the surveyed locations, and the qualitative graphical method did not reveal a mode-shift distortion of the allele frequencies distribution that would be expected in a bottlenecked population.

4.4 Discussion

Peregrine Falcons have had a remarkable recovery from extirpation in the Midwestern US and greatly reduced numbers in the Western US. Management practices, although different in these regions, have not resulted in disparate levels of genetic diversity. I studied two different types of genetic markers, mtDNA sequences and microsatellites, and obtained contrasting results.

The mtDNA control region sequence data that I gathered largely showed minimal structure. Genetic diversity was low in all populations. A single haplotype, CA, was present in high frequency in both the Western and Midwestern regions, while the remaining six haplotypes were found in one to a few individuals in only one region (Western: CB, CC, CI, CJ, Midwestern: CO, CR). Because the reintroduced Midwestern birds may stem from a mix of subspecies with a higher proportion of *pealei* and Western *anatum* (Tordoff & Redig 2001) the common haplotype was expected to be at high frequency, but there is no evidence that significant

novel haplotype diversity was introduced as a result of the recovery program. The WAS population, which has always been designated *anatum* based on plumage, had only the common haplotype, CA, which has been reported previously for *pealei*, a Pacific marine subspecies that inhabits northwestern Washington state and the Aleutian Islands, in both current and historical populations (Brown et al. 2007). Other bird species from the same region that are devoid of multiple mitochondrial haplotypes are the Western Sage-grouse, *Centrocercus urophsianus phaios* (Benedict et al. 2003), and the Northern Saw-whet owl, *Aegolius acadicus* (Topp & Winker 2008). Minimal population structure was recovered from the mtDNA F_{ST} analyses. Only two Western populations (CHI & MIC) were differentiated with respect to the Midwestern population. This lack of mtDNA population structure is concordant with control region datasets for avian species with similar distributions. These include the Yellow-rumped Warbler, *Dendroica coronata* complex, *D. c. audoboni* (Western form), *D. c. coronata* (Eastern form) (Milá et al. 2007), Screech-Owls, *Megascops kennicottii* (Western form), *M. asio* (Eastern form) (Proudfoot et al. 2007), and *Passerina* Buntings, *P. amoena* (Western form), *P. cyanea* (Eastern form) (Carling et al. 2010).

Contrary to what would be predicted given the reduction in numbers these populations experienced, levels of genetic diversity (H_O, AR, PAR) for microsatellites in both regions are similar but slightly higher when compared to other recovered populations of Peregrines from Canada, and Europe, specifically, Sweden and Norway (Nesje et al. 2000; Brown et al. 2007; Jacobsen et al. 2008). However, the comparability of population variability data for these studies might be problematic because different loci were used.

The microsatellite data revealed evidence for population structure that was not apparent in the mtDNA data. Significant population structure exists between Western and Midwestern

Peregrines. While the Midwestern US revealed no signal of substructuring, across the Western US, three major clusters were detected (SOC-CHI, MIC-ORE and WAS) based on georeferenced Bayesian clustering analysis (GENELAND) and F_{ST} values. This pattern is somewhat consistent with the results obtained from STRUCTURE analysis where the only distinct birds were the Washington Peregrines (WAS). The estimation of *K* for this dataset was not straightforward. Pritchard's procedure for estimating *K* is suitable for datasets with a small number of discrete populations (see STRUCTURE documentation). The fact that both Pritchards' method and the *ad hoc* measure of Evanno's inferred that *K*=5 may be indicative of the nature of my dataset. It has been suggested that isolation by distance data might lead to overestimation of *K*. Because the Middle Coast Peregrines from California (MIC) showed significant IBD, this might have influenced the calculation of *K* resulting in a overestimation.

It is believed that the recovery in Southern California and the Channel Islands was also influenced by the influx of birds from Baja California (D. A. Bell, *pers. comm.*), a population I did not sample. This could explain the assignment of some birds from SOC and CHI to cluster 5 (blue) evidenced by the STRUCTURE analysis (Figure 4). Based on the same results, individual assignments proportions also helped to investigate whether Peregrines were of mixed ancestry or migrants. Across all five clusters, 25.9% of the individuals were deemed admixed while 14.2 % was considered migrants. Migrant birds were (in most cases) from a nearby population. The populations that registered the highest number of migrants were SOC-CHI (*n*=10) while MIC-ORE had the lowest (*n*=2). The pattern of genetic structure and movement of individuals are consistent with previous ecological predictions for Western Peregrines identifying source-sink populations (Wooton & Bell 1992, Kauffman et al. 2004).

Microsatellite data supported significant genetic structure at a regional level (Western-Midwestern) and at a local level (within Western populations). I found that regional-level F_{ST} values indicated low, but significant population differentiation among the all the Western sampled locations and Midwestern US population based on microsatellite data (F_{ST} = 0.066-0.111). Brown et al. (2007) also reported low, but significant F_{ST} values for recent samples of *tundrius-anatum* and *pealei* from Canada (F_{ST} = 0.013-0.081). Other raptors species such as Swainson's Hawks (*Buteo swainsoni*) have been reported to have low, but significant F_{ST} values as well (F_{ST} = 0.009-0.030) between populations located in coastal California and the Northern Plains (Hull et al. 2008a). Similar significant population differentiation (F_{ST} = 0.031) has been reported for Red-tailed Hawks populations (*Buteo jamaicensis*) located in the Western and Eastern US (divided by the Rocky Mountains) (Hull et al. 2008b).

Within the western U.S., the presence of geographic structure in Peregrines may be related to one or a combination of the following factors: 1) ecological factors acting within the established range to prevent a population from increasing and spreading to adjacent areas, 2) ecological barriers (i.e. adverse climate or habitat) acting at the limits of the existing range to prevent spread, or 3) physical barriers, such as oceans or mountain ranges that impede dispersal and isolate populations. The first two factors may fluctuate over time scales of hundreds to thousands of years (the timing over which structure in Peregrines likely evolved) while the last factor is largely fixed over these periods. The dispersal capability of Peregrines suggests that physical barriers are unlikely to influence regional distribution while ecological factors such as availability of suitable nest sites, abundance of prey, changes in climate and sex-biased dispersal could shape the boundaries of populations. In Midwestern Peregrines, it is documented that females disperse on average two or up to three times farther than males (Tordoff & Redig 1997).

The limits of genetic discontinuities between two of three Western populations (WAS and SOC/CHI) (Table IV) are roughly similar to these average values of dispersal calculated for Midwestern females Peregrines (≈ 300 km). My dataset suggests two genetic discontinuities based on microsatellite data. The first boundary, in central California, divides the CHI population from the MIC population, north of the Channel Islands, the second boundary occurs along the southern limit of Washington. In contrast, the mtDNA data detected significant population differentiation (based on F_{ST} values) between MIC and WAS sampled locations. This suggests that Western Peregrines inhabiting Washington and Oregon are different from the ones in California. This is a biogeographic pattern found in other bird species, including Spotted owls (*Strix occidentalis caurina,* and *S. o. occidentalis*) whose mtDNA data suggests a break in northern California in a region of low density (Haig et al. 2004) and Fox sparrows which present two different contact zones among three subspecies in the same region (Zink 1994, 2008).

Western Peregrines are considered to be permanent residents (they do not annually migrate south to wintering grounds). This behavior maybe the strongest argument for ecological differences at a regional scale explaining two genetic discontinuities (zones of limited gene flow). Results of gene flow (migration) analysis on the microsatellites data suggest asymmetrical movement across these zones, with birds from inland regions (ORE) moving to the coastal regions (MIC) without much movement in the opposite direction. While the microsatellite data suggest significant differentiation, the level of dispersal likely prevents additional divergence between SOC-CHI and MIC-ORE.

Population size contractions brought on by the use of pesticides occurred across all North America. In the Western US, Peregrine populations were greatly reduced, while extirpation occurred in the Midwest US. Management practices used in stimulating the recovery in these two

regions were different. Most importantly, the source of birds used for captive breeding was different. In the West, recovery came from remnant local birds while for the Midwestern region non-native genetic stock was used. My results do not find obvious genetic signals that suggest the different recovery programs led to novel genetic structure, but at the same time, microsatellite data do distinguish between Midwestern and western populations, a pattern not previously documented. Levels of genetic diversity were similar but genotypic variation was different enough to be able to detect differences in population structure. In addition, no genetic effects of recent bottlenecks were detected in any population based on microsatellite data. Future work, including samples of historical birds from both regions, may shed additional light on these results.

In this chapter I focused mainly on Western Peregrine Falcons. The genetic structure found in these populations is significant and generally matches regional genetic structure for other avian species that have been sampled across this part of the continent. It also matches previously hypothesized population designations based on ecological attributes. The lack of evidence supporting bottlenecks in these recovered populations, as well as other Peregrine populations, may be the result of a fairly fast recovery. Future work should include the assessment of historical populations and additional unsampled Western regions, i.e. populations from Baja California which are suspected to have played a fundamental role in the recovery of Southern California Peregrines.

In contrast to some recent reports on other birds (Zink & Barrowclough 2008), my results demonstrate the utility and sensitivity of microsatellite data for studies on population structure in a case where mtDNA sequence data exhibits little or no genetic structure in a widespread and highly mobile bird.

4.5 Acknowledgments

This study was conducted with the support of the Raptor Research Foundation, The American Ornithologists' Union and The International Osprey Foundation. Microsatellite development by ICC and the subsequent laboratory work was carried out in the Pritzker Laboratory for Molecular Systematics and Evolution operated with support from the Priztker Foundation. I thank Kevin A. Feldheim for technical advice, and helpful discussions. Special thanks go to the Oregon Department of Fish and Wildlife, the California Department of Fish and Game for cooperation and permission to draw blood, and to the US Forest Service for their support of portions of field work in the Pacific Northwest.

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5. GLOBAL SCALE GENETIC STRUCTURE AND THE INFLUENCE OF SEASONAL MIGRATION AND GENE FLOW ON BIOGEOGRAPHICAL PATTERNS

Peregrine Falcons have a worldwide distribution but exhibit regional differences in behavior, morphology, and demographic history. A mix of migratory and resident breeding populations occurs in the northern hemisphere while exclusively resident breeding populations occur in the southern hemisphere where migrants from northern populations come to winter. This chapter focuses on understanding how Peregrines evolved such a wide-ranging distribution and how changes in migratory behavior or breeding dispersal have led to the formation of a distinct population structure. Two alternative hypotheses of range expansion and colonization are considered, Isolation by Distance, and Suspension of Migration. Genetic results based on eleven microsatellite loci show low to medium degree of genetic differentiation among reintroduced Peregrine Falcons and European-Asian migrants while significant differentiation was observed between northern migrants and southern residents in both Old and New World. However, the mitochondrial DNA control region data reveals lack of differentiation between the Old and New World Peregrines while differentiation between northern and southern populations were significant. Worldwide genetic patterns derived from multiple type of analysis supported the Isolation by Distance hypothesis. Populations that overlapped in distribution were less differentiated than populations without overlap. Even though Suspension of Migration was not supported, migration analysis based on mitochondrial DNA detected directionality of historical gene flow in northern migrants from west to east, and in Old World Peregrines from south to north.

5.1. Introduction

Many bird species are capable of long distance dispersal and presumably range expansion, however, such species have distributional ranges that vary from locally restricted to cosmopolitan. Cosmopolitan distributions must involve a history of substantial range expansion. In migratory bird species that are capable of long-distance dispersal to different hemispheres, we would like to know how their current distributional range came about, and what limits their distributions. Do drastic changes in distribution, say from the northern hemisphere to the southern hemisphere, result from migratory behavior? If wintering migrants occasionally stay and breed, and thus effectively colonize sites thousands of kilometers away, the genetic structure of the species will be much different than expected through range expansion. There is a possibility that populations or subspecies at the extremes of a widespread species range may be evolutionarily related, and genetically more similar to each than geographically adjacent populations or subspecies. Thus, we can gain insight on important processes of colonization and range expansion by examining the phylogeographic patterns and genetic differentiation within these geographically widespread species.

One cosmopolitan species, the Peregrine Falcon (*Falco peregrinus* Tunstall 1771), is ideally suited to studying the relationship between genetic structure, migratory behavior, dispersal, and range expansion. It possesses one of the largest natural distributions of any avian species. Peregrines breed on every continent except Antarctica, and on all major islands except New Zealand and Iceland (Hickey & Anderson 1969; White & Boyce 1988). Regional differences in behavior, morphology, and demographic history have led to the description of 19 subspecies. In this chapter, I mainly focus on seven described subspecies, three northern migrants, American tundra Peregrine (*F. p. tundrius* White 1968), European Peregrine (*F. p.*

peregrinus Tunstall 1771), and Siberian Tundra Peregrine (*F. p. calidus* Latham 1790); two northern residents/partial migrants, which move shorter distances than migrants, American Peregrine (*F. p. anatum* Boanaparte 1838) and Mediterranean Peregrine (*F. p. brookei* Sharpe 1873); and two southern residents, South American Peregrine (*F. p. cassini* Sharpe 1873) and African Peregrine (*F. p. minor* Bonaparte 1850), which inhabit the New and Old World, respectively (Figure 1).

5.1.1. Distribution of Peregrine Falcon Populations

While evolutionary relationships have not been studied among northern and southern Peregrine Falcons, some ecological and demographic differences are noteworthy (White et al. 2011). First, *minor* (Africa) and *cassini* (South America) have a wide north-south distribution. Pairs are usually resident and territorial through the year, and both subspecies are darker and smaller when compared to others. These shared features could reflect shared ancestry or ecological convergence. Second, notable features of *calidus* (Northern Europe, Asia), which are mirrored in *tundrius* (Canada, Greenland), are long distance migration to wintering grounds in the southern hemisphere, as well as morphological characters (long, narrow wings, bill shape and size) (Johansson et al. 1998). Third, populations of *peregrinus* (Western Europe) and *anatum* (Canada, USA) are both considered residents and partial-migrants. Both populations were dramatically reduced by the effects of pesticides (DDT) from the 1950s into the early

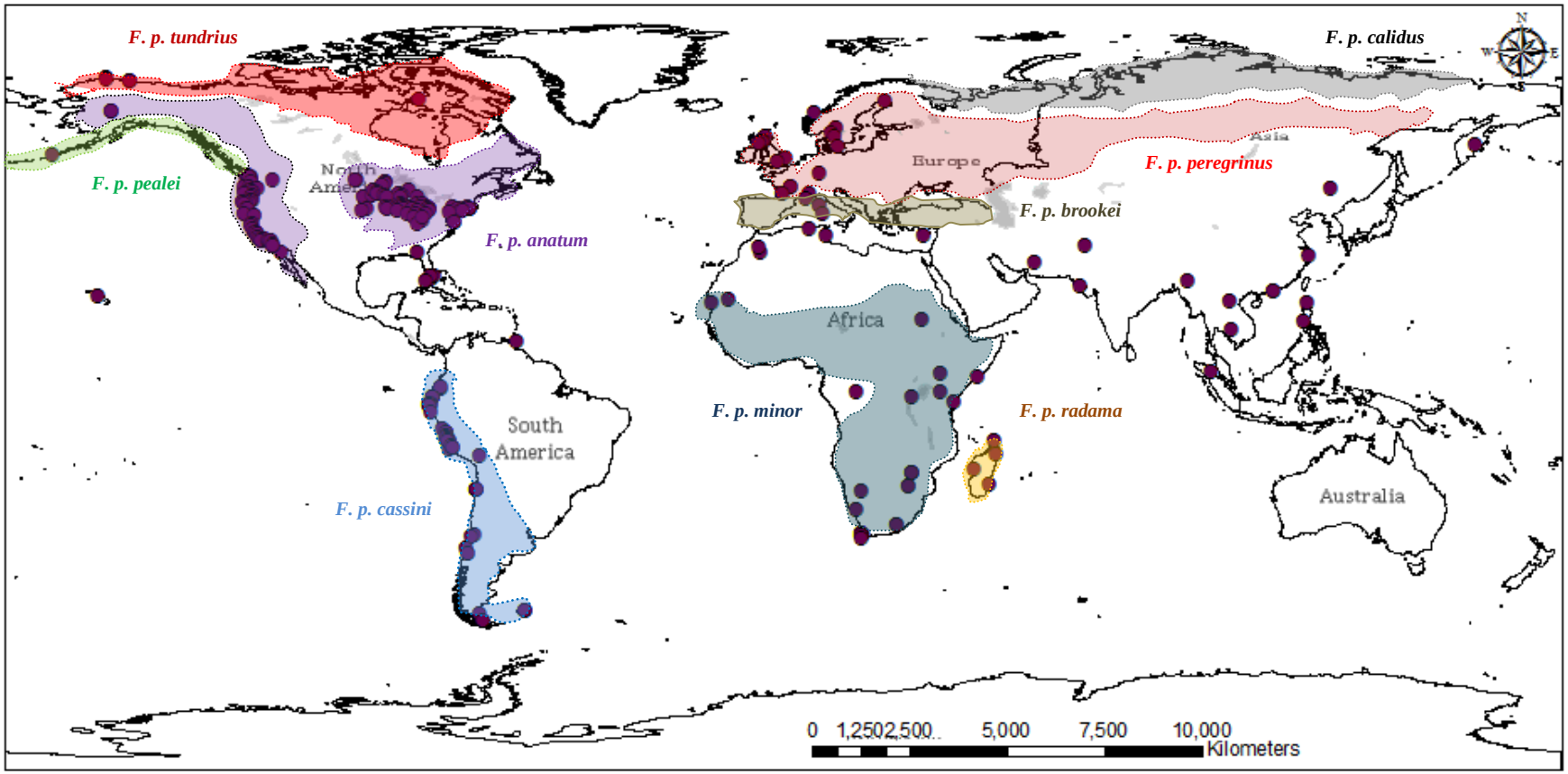

Figure 1. Distribution of Peregrine Falcon subspecies represented in this study (based on White and Boyce 1988). Dots represent sampling sites.

1970s, more than other subspecies. Finally, *brookei* (southern Europe) and *anatum* (Western, Eastern US) are residents/partial migrants and some populations are found at coastal locations.

The widespread use of pesticides (DDT) from the 1950s into the early 1970s had a well-documented negative impact on northern Peregrine populations (Ratcliffe 1967) while the actual effects suffered by southern Peregrines were understudied. In North America the *anatum* populations in Eastern-Midwestern US were extirpated (Berger et al. 1969), while in the Western US populations declined dramatically. Mirroring what happened in North America, the waning in Peregrine numbers in Europe became obvious in the late 1950s (Newton 1988). Declines for *peregrinus* were least marked in resident populations living in mountain areas away from agricultural land and most marked in populations occurring in farmland (Germany) or wintering in farmland and estuaries (Fennoscandia). As in North America, the recovery in these populations followed reductions in organochlorine use (Newton, 1988). Studies of southern hemisphere Peregrines seemed to suggest that pesticides effects were less detrimental than in the northern hemisphere but this remains an understudied issue (Mc Nutt et al. 1988). Ecological studies have revealed that the Cape Peninsula, South Africa, supports a remarkable population of Peregrine Falcons (*minor*). The density on the Peninsula exceeds any other recorded on mainland Africa (Jenkins & van Zyl 2005). Peregrines in South America (*cassini*) (Ecuador, Peru, central and southern Chile and Argentina) have suitable nesting cliffs, and abundance of potential prey (Schoonmaker et al. 1985; Mc Nutt et al. 1988) but have been reported to be in low numbers until recently (Beingolea Masaveu 2003). Studies assessing pesticides levels have reported low values of DDT in eggs corresponding with high reproductive values in Argentina (Ellis 1985) and Ecuador (Jenny et al. 1983).

5.1.2. Migratory Pathways and Connectivity

Migration is defined as a regular seasonal movement of birds between breeding and non-breeding areas (Newton 2008) while dispersal is a movement from where an individual was born or has bred to a new location where it breeds (South et al. 2002). In a biogeographical context, dispersal can lead to range expansion and to colonization of new areas, promoting gene exchange and reducing inbreeding (Newton 2003). In general, the degree of genetic differentiation between two populations increases with the distance that separates them (Peterson 1992; Nesje et al. 2000) because individuals are most likely to disperse to nearby sites i.e., Isolation by Distance (IBD) (Wright 1943, 1946). However, migratory birds, such as some northern Peregrines, that migrate thousands of kilometers every year can potentially establish and maintain genetic connectivity over extremely long distances (Avise 1994), reducing large scale genetic structure and differentiation (Johnson & Gaines 1990). In this case, IBD will not be evident because the most distant populations may be genetically more similar, if they arose through loss of migratory habits. Each year, millions of Holarctic migratory birds go to winter in the southern hemisphere portions of the New and Old World. Occasionally some wintering birds suspend migration and fail to return in spring, a phenomenon that has been termed Suspension of Migration (SOM) (Leck 1980). However, it is largely unknown how frequently SOM has led to range expansion and establishment of new populations. Range expansion through SOM would be an evolutionary recent phenomenon, occurring after migration patterns of Holarctic birds were established (Leck 1980).

In the New World, extensive banding and recovery records for migrant Peregrines *tundrius* and some *anatum* provide insight into the movements during fall and spring migrations from various geographic regions, as well as their wintering areas in the southern hemisphere

(Yates et al. 1988). The major migratory routes studied in North America occur along the Gulf of Mexico coast in Texas, the Atlantic coast, the Great Lakes region, the Canadian prairies of Alberta, and along the Pacific coast (Anderson et al. 1988). In the Old World, Eurasian subspecies *peregrinus* and *calidus* migrate to Central/South Africa and South Asia using two routes, the Western European-West African Flyway and the Eurasian-East African Flyway (Bildstein 2006). These migrants may spend 6-7 months in Southern breeding latitudes (Bierregaard et al. 1994). It has long been hypothesized that southern non-migratory populations evolved from lingering northern migrants individuals. The Mediterranean subspecies *brookei* which is a partial migrant has been suggested to be the oldest among Peregrine subspecies (White & Boyce 1988), and may have colonized northern Africa which is the northern range of *minor*.

Using two types of genetic data, I examine the evolutionary relationships among migratory and resident populations and use these relationships to test the role of dispersal and migration in achieving current distributions. I test these patterns and ascertain whether similar processes have occurred in both Old and New World Peregrines. I investigate whether changes in migratory behavior or dispersal have led to the establishment of southern range populations.

5.1.3. Research Hypotheses

I test the following hypotheses, which consider two plausible (though not necessarily mutually exclusive) mechanisms of range expansion:

H_1: Southern populations are established when migrants do not return to breeding grounds, but stay to breed in “wintering grounds” (SOM). This hypothesis predicts that migrant populations from the far north and southern resident populations are genetically more similar in both the Old

World and New World. This assumes that migration and the establishment of founder populations distant from the ancestral range are only likely to happen when migration reaches regions where resources allow year round survival (Salewski & Bruderer 2007). A correlate of H_1 is that differentiation between the eastern and western hemispheres occurred first with northern residents leading to partial or full migrants, which in turn led to the evolution of the southern hemisphere residents. The southern birds will constitute a genetic 'subset' of the former and possibly be less diverse (due to founder effects and SOM).

H_2: Range expansion occurs through gradual dispersal into new areas (IBD). This hypothesis predicts greater similarity among nearby northern populations (migrant and resident) while distant southern resident populations will be divergent. This presupposes that birds move to favorable adjacent areas and range expansion occurs gradually through dispersal (Bell 2000). A correlate of H_2 is that differentiation evolved between northern and southern resident populations in the eastern and western hemispheres with migration evolving recently in northern populations (IBD).

These hypotheses are tested through assessment of genetic structure in migratory and resident populations from the Old and New Worlds using two types of molecular markers, mitochondrial DNA sequences and DNA microsatellite genotypes.

5.2. Materials and Methods

5.2.1. Sampling

The sampled birds represent much of the breeding range of Peregrine populations (Figure 1). A subset of the sampling considered in this chapter was discussed in Chapters 3 (Appendix

A) and 4 (Appendix D). The remaining samples were obtained from the following museums and institutions (see Appendix F, Appendix G): Muséum National D'Histoire Naturelle, Paris (*n* = 17), Swedish Museum of Natural History, Stockholm (*n* = 35), Chicago Academy of Sciences (*n* = 8), Field Museum of Natural History, Chicago (*n* = 26), Peabody Museum of Natural History (*n* = 6), American Museum of Natural History New York (*n* = 15), University of Michigan Museum of Zoology (*n* = 4), Universidad Alas Peruanas, Lima (*n* = 25), Stellenbosch University (*n* = 37), and Falcon Research Group (*n* = 110).

5.2.2. Genotyping and Sequencing

Isolation of genomic DNA was done from tissue (muscle, blood) samples using the Qiagen DNeasy Tissue Kit® following manufacturer's protocol. Toepads samples were extracted with some minor modifications to the DNA extraction procedure as detailed in Mundy et al (1997). Measures for prevention of cross contamination for the historical samples were the same as those detailed previously (see Chapter 3).

A fragment of 405 bp from the mitochondrial control region was sequenced using primers published in Brown et al (2007). Sequence data was gathered from a total of 335 birds from the New and Old World. PCR reactions conditions, reagents used and internal primers for historical samples were the same as detailed in Chapter 3. Mitochondrial DNA sequences were aligned using CLUSTAL W (Thompson et al. 1994) and positions of polymorphisms refer to the published mitochondrial genome of *Falco peregrinus* (Mindell et al. 1999).

Samples from around the world consisted of a total of 490 birds genotyped at eight microsatellite loci (CAB *fp* 18-2, CAB *fp* 24, CAB *fp* 77, CAB *fp* 85, CAB *fp* 117, CAB *fp* 120, CAB *fp* 157, CAB *fp* 181) (see chapter 2 for primer information) that I designed from a

Peregrine Falcon genomic library following Glenn and Schable's protocol (2005), and three additional loci (NVH *fp* 13, NVH *fp* 31, NVH *fp* 89) developed by Nesje et al (2000). Microsatellite PCR conditions, concentration of reagents, and multiplex scoring followed procedures previously used for blood and toepads samples (Chapter 2). Genotypes were scored using the STRand Analysis Software v.2.4 (Toonen & Hughes 2001).

5.2.3. Statistical Analyses

Indices of mtDNA diversity, including nucleotide diversity (π) (Tajima 1983), and haplotype diversity (h) (Nei 1987), and tests assessing deviation from neutral expectations were calculated using DnaSP 5.10 (Librado & Rozas 2009) and ARLEQUIN v.3.5.1 (Excoffier & Lischer 2010). I calculated Fu's F_S test (Fu 1997), which identifies deviations from neutrality in situations characterized by an excess of rare alleles and young mutations in non-recombining sequences (Milá et al. 2007), and Tajima's D statistic (Tajima 1989).

For microsatellite data, allele frequencies, observed/expected heterozygosity (H_O/H_E), testing for genotypic departures (Hardy-Weinberg equilibrium, HWE), and linkage equilibrium were done for each locus using the computer programs FSTAT (Goudet 2001) and ARLEQUIN. Sequential Bonferroni corrections were applied to correct for multiple comparisons (Rice 1989). An additional index that account for different samples sizes, allelic richness, was calculated using HP-RARE (Kalinowski 2005). Computed statistics for each population were compared via Wilcoxon signed-rank tests with the software SYSTAT 13 (Systat Software Inc., Chicago, IL).

The genetic structure of populations was initially investigated by an analysis of variance framework (Weir & Cockerham 1984) for both mtDNA sequences and nuclear microsatellites. I used the analysis of molecular variance (AMOVA) (Excoffier et al. 1992) implemented in

ARLEQUIN. By defining groups of populations, I tested a hierarchical structure where the total genetic variance is partitioned into covariance components corresponding to intra/inter-individual differences, and/or intra/inter-population differences. Significance of all these tests was assessed with 10,000 permutations. For mtDNA, pairwise differentiation between populations was calculated using Φ_{ST} which is based on haplotype frequencies and levels of sequence variation and F_{ST} which takes into account only haplotype frequencies. Pairwise differentiation for microsatellites was assessed with F_{ST} and R_{ST}. R_{ST} is based on the mean square differences in the number of repeats between alleles at each locus. Calculation of Φ_{ST} and R_{ST} (in addition to F_{ST}) may be useful when mutations have contributed significantly to allelic differences among populations (Holsinger & Weir 2009). To visualize the genetic relationships among populations, a multivariate method, Principal Component Analysis (PCA), was done based on microsatellite data with PCAGEN v1.2 (http://www2.unil.ch/popgen/softwares/pcagen.htm) with 10,000 randomization tests, and compared with results provided using other clustering techniques.

To detect genetic structure, I used the software STRUCTURE v.2.3 (Pritchard et al. 2000) which relies on Bayesian statistics and uses individual multilocus genetic data to cluster individuals into K groups. It is assumed that within populations, the microsatellite loci are at Hardy-Weinberg equilibrium and linkage equilibrium. To estimate K, I ran a series of trial values of the number of populations from 1 to 20, and then compared the estimated log probability of data, Ln[Pr(X|K)], under each K. Each run used 10^6 iterations and a burn-in period of $5x10^6$, using the admixture model and correlated allele frequencies. To check for convergence of the Markov chain Monte Carlo simulations (MCMC), I performed 20 replicated for each value of K and then checked for the consistency of results. The number of clusters (K) was determined

considering two criteria: 1- the value of *K* with the highest posterior probability given by STRUCTURE and 2- the ad hoc statistic ΔK proposed by Evanno et. al (2005).

I estimated pairwise gene flow from mtCR sequence data as the asymmetric female migration rate among populations, M, defined as $M = m/\mu$ where *m* is the immigration rate per generation and μ is the neutral mutation rate per site per generation. Parameters were calculated using a Monte Carlo Markov chain (MCMC), maximum likelihood procedure on the program LAMARC v.2.1.6 (Kuhner 2006). LAMARC estimates directional gene flow and works better with populations that have shown evidence of population structure (such as clusters obtained from STRUCTURE, see LAMARC guidelines). Consequently, population samples were combined and individuals tested were randomly chosen. I ran three replicates for each run, with a burn-in of 1,000 steps, followed by one initial chain of 10,000 steps; sampling 500 trees every 20 steps, and one final chain of 200,000 sampling 10,000 trees per 20 steps. Three runs were performed and the median values were used for the final estimates.

Estimation of migration rates, *m* (i.e. proportion of migrants) over the last several generations between Peregrine populations was done from microsatellite data using the Bayesian procedure implemented with MCMC in BAYESASS v.1.3 (Wilson & Rannala 2003). This approach does not require populations to be in either Hardy-Weinberg equilibrium or migration-drift equilibrium. To examine the strength of the information in the Peregrine microsatellite dataset, 95% confidence intervals (CI) were determined for migration rates and compared to a situation where all proposed changes throughout the Markov chain are accepted (thus simulating the scenario where any information from the data is insufficient to affect the posterior distribution of migration rates). The MCMC chains were run 5 times for a total of $9x10^6$ iterations, with a burn-in of $2x10^6$ allowing the chain to achieve stationarity. Samples were

collected every 2,000 iterations to infer posterior probability distributions of parameters of interest.

Investigation of past demographic history for Peregrines was done using Fu's F_S test of neutrality (Fu 1997) to detect past sudden changes in effective population size (N_e). Significantly negative values of F_S indicate an excess of recent mutations and reject population stasis regarding changes in population size (Fu 1997). I used ARLEQUIN to generate values of F_S for each population using mtCR data. To complement this method, I calculated the statistic R_2 which has been shown to be a more powerful test than F_S for small sample sizes (Ramos-Onsins & Rozas 2002). R_2 was calculated in DnaSP v.5 and significance was estimated based on coalescent simulations. Ramos-Onsins and Rozas (2002) suggested that these statistics have greater power under a diverse set of conditions, especially when population sample sizes are large (≈ 50, F_S) or when sample sizes are small (≈ 10, R_2). The significance of both statistics was assessed by examining the null distribution of 10,000 coalescent simulations. Significant large negative values of Fu's F_S and positive R_2 values maybe evidence of population expansion.

I also investigated the historical demography of populations through the comparison of the observed distribution of nucleotide differences between pairs of haplotypes within demes (mismatch distributions). These distributions are usually multimodal (ragged) in samples obtained from populations at demographic equilibrium for a relatively long period of time (Rogers & Harpending 1992), and unimodal (Poisson-shaped) in populations experimenting a demographic exponential growth (Barrowclough et al. 2004) or having passed a range expansion with high migration levels between nearest demes (Excoffier 2004). I generated mismatch distributions for the different populations using ARLEQUIN v. 3.5 to test for demographic expansion in all suitable populations. The model also generates an estimate of the time (τ)

elapsed between the effective population size before the expansion (N_0), and the present effective population size (N_1). The validity of the estimated expansion model was tested by obtaining the sum of the square deviations (SSD) between the observed and the expected mismatch as a test statistic (Schneider & Excoffier 1999). A significant SSD value was interpreted as a deviation from the estimated demographic model, which is here population expansion when $\tau > 0$ and $\theta_1 > \theta_0$. The time since expansion was estimated by applying a substitution rate of 0.032 per site per million years, following the results reported for *F. peregrinus* (Nabholz et al. 2009). These substitution rates are third position codon changes for cytochrome *b* (cyt*b*). Previous work on mtDNA in species of the hierofalco complex (*F. biarmicus, F. cherrug, F. jugger and F. rusticolus)* has shown that the substitution rates for CR seem to be similar to those obtained for *cytb* (Nittinger et al. 2005). Peregrines first breed at two years of age (Tordoff & Redig 1997), and consequently, previous population genetic studies have applied a generation time (*T*) of three years (Brown et al. 2007) or four years (Johnson et al. 2010). More accurately, the generation time of a population can be defined as the average age of mothers of newborn individuals in a population with a stable age distribution, hence, $T = \alpha + s/ (\lambda - s)$, where α is the age in years at first breeding, λ is the annual geometric growth rate of the population, and *s* is the annual probability of survival (Lande et al. 2002). Assuming the population is in demographic equilibrium ($\lambda = 1$) and using an estimated value of $s = 0.77$ for Western Peregrines (Wooton & Bell 1992), the resulting estimate for generation time is $T = 5.35$ years.

5.3. Results

5.3.1. Genetic Diversity

A total of 335 individual sequences were obtained from ten populations (Table I). Within the 405 bp of the mtDNA CR, 16 nucleotide positions were polymorphic, 12 were transitions and 4 were transversions, generating 18 haplotypes (Table I). The number of haplotypes per population ranged from one in Washington (WAS) to a maximum of six in Europe-Asia (CAL, PER/BRO) (Table II, Figure 2). Haplotype CA was present in all populations (Table I). Haplotype CD was shared by five populations, haplotype CE and CC by four populations, and haplotype CJ was present in three populations. Two haplotypes, CB and CF, were found in two different populations. The remaining 10 haplotypes, CI, CO, CR, CK, CL, CN, CQ, CH, CM and CP occurred in single populations (Table I). Peregrine haplotype diversity (*h*) ranged from 0.105 in South Africa (MIN) to 0.713 in Europe (PER/BRO). Nucleotide diversity (π) was consistently low across all populations (0.000-0.0002), and polymorphism within each population was consistent with neutral expectations for all populations based on Tajima's *D* (Table II).

TABLE I. MITOCHONDRIAL DNA HAPLOTYPES AND FREQUENCIES OBSERVED AMONG PEREGRINE GEOGRAPHIC SAMPLES. VERTICAL NUMBERS INDICATE THE POSITIONS OF VARIABLE NUCLEOTIDES WITHIN THE PUBLISHED MITOCHONDRIAL GENOME OF FALCO PEREGRINUS (MINDELL ET. AL 1999). DOTS UNDER NUCLEOTIDE POSITIONS INDICATE AN IDENTICAL NUCLEOTIDE AS GIVEN IN HAPLOTYPE CA.

Haplotypes	Variable site																			North America						S. America	Asia, Europe		S. Africa
	15265	15267	15531	15532	15338	15354	15355	15356	15383	15390	15403	15410	15462	15511	15536	15623													
																	MIG *n*= 42	WAS *n* = 14	MIC/ ORE *n* = 38	SOC/ CHI *n* = 41	ANA *n* = 32	MIW *n* = 31	CAS *n* = 27	CAL *n* = 22	PER/B RO *n* = 47	MIN *n* = 37
CA	C	A	T	T	A	C	G	A	A	A	A	T	G	G	A	C	24	14	30	33	25	29	25	14	18	35
CB	A	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	-	-	1	-	-	-	-	-	1	-
CC	.	G	.	.	.	.	.	.	.	.	.	.	.	.	.	.	-	-	6	6	1	-	-	-	-	2
CD	.	.	C	.	.	.	.	.	.	.	.	.	.	.	.	.	13	-	-	-	4	-	1	3	17	-
CE	.	.	.	C	.	.	.	.	.	.	.	.	.	.	.	.	3	-	-	-	1	-	-	1	6	-
CF	.	.	.	.	.	.	.	.	.	G	.	.	.	.	.	.	-	-	-	-	-	-	1	-	1	-
CG	.	.	.	.	G	.	.	.	.	.	.	.	.	.	.	.	1	-	-	-	-	-	-	-	-	-
CH*	.	.	.	.	.	T	.	.	.	.	.	.	.	.	.	.	-	-	-	-	-	-	-	-	-	-
CI	.	.	.	.	.	.	C	.	.	.	.	.	.	.	.	.	-	-	1	-	-	-	-	-	-	-
CJ	.	.	.	.	.	.	.	G	.	.	.	.	.	.	.	.	1	-	-	2	1	-	-	-	-	-
CK	.	.	.	.	.	.	.	.	G	.	.	.	.	.	.	.	-	-	-	-	-	-	-	1	-	-
CL	.	.	.	C	.	.	.	.	.	G	.	.	.	.	.	.	-	-	-	-	-	-	-	1	-	-
CM*	.	.	.	.	.	.	.	.	.	.	G	.	.	.	.	.	-	-	-	-	-	-	-	-	-	-
CN	.	.	.	.	.	.	.	.	.	.	.	C	.	.	.	.	-	-	-	-	-	-	-	2	-	-
CO	.	.	.	.	.	.	.	.	.	.	.	.	A	.	.	.	-	-	-	-	-	1	-	-	-	-
CP*	.	.	.	.	.	.	.	.	.	.	.	.	.	C	.	.	-	-	-	-	-	-	-	-	-	-
CQ	.	.	.	.	.	.	.	.	.	.	.	.	.	.	G	.	-	-	-	-	-	-	-	-	4	-
CR	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	G	-	-	-	-	-	1	-	-	-	-

* These haplotypes appeared in Peregrines from Madagascar (*F. p. radama*), they are shown but were not used in the mtDNA analyses

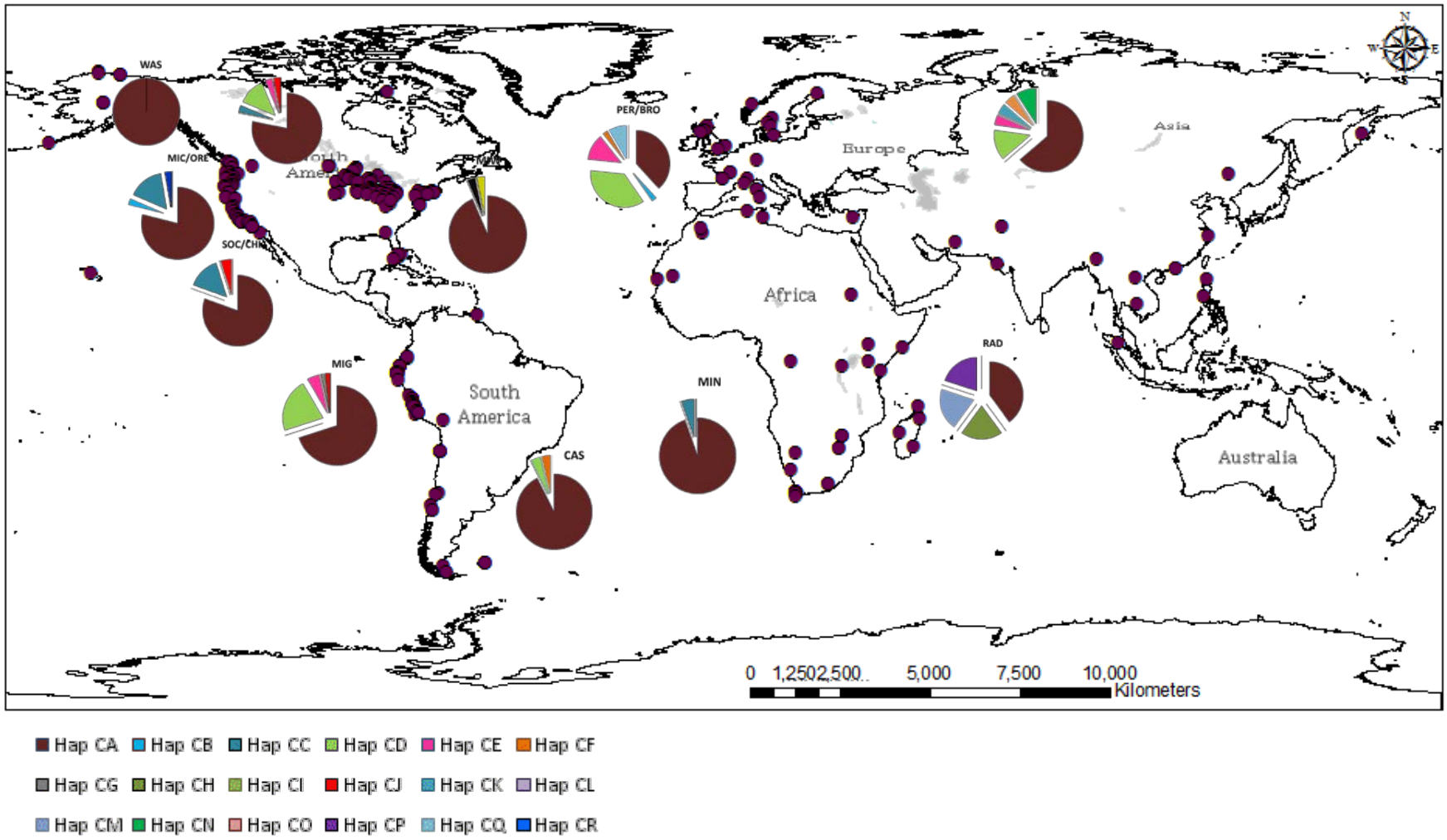

Figure 2. Geographic distribution of haplotypes and sampling scheme.

All eleven microsatellite loci were polymorphic in the ten sampled populations (see Appendix H). A total of 207 microsatellite alleles were observed among all samples, ranging from a maximum average of 12.55 ± 1.50 (± s.e.) alleles per population in North America (MIG) to a minimum average of 6.73 ± 0.79 alleles in South Africa (MIN) (Table III). Overall, 47 private alleles were found in 11 loci; 31 alleles were unique to North America (MIG, WAS, MIC/ORE, SOC/CHI, ANA, MIW), six to South America (CAS), seven to Europe-Asia (CAL, PER/BRO), and three to South Africa (MIN) (Appendix H). Allelic richness was lowest in South Africa, 4.20 ± 0.56 (MIN), and highest in Europe-Asia 6.09 ± 0.60 (CAL) but not significant. All the remaining locations had similar and non-significant different values ranging from 4.92 ± 0.45 to 5.65 ± 0.37 (Table III). Similarly, private allele richness was similar for all populations considered (Table III). None of the variation parameters analyzed (number of alleles, H_O, H_E, allelic richness, and private allelic richness) were significantly different in any population based on Wilcoxon-signed rank tests. After adjusting for multiple comparisons (n = 121), significant departures from Hardy-Weinberg equilibrium in the form of heterozygote deficiencies were observed in five loci across eight sampled populations. Loci in two populations (MIW, CAB *fp* 24 & NVH *fp*13 and CAS, CAB *fp* 157, CAB *fp* 181) exhibited significant linkage disequilibrium after correcting for multiple comparisons (n = 605). Given that these loci did not exhibit linkage disequilibrium across all populations, I conclude that the loci can be treated as independent from each other.

TABLE II. MITOCHONDRIAL GENETIC DIVERSITY FOR PEREGRINE FALCON POPULATIONS AND NEUTRALITY TEST STATISTICS; STANDARD ERROR VALUES ARE GIVEN IN PARENTHESES, BOLD NUMBERS INDICATE SIGNIFICANT RESULTS ($P < 0.05$).

Regions	North America					South America	Asia, Europe		South Africa
Populations	MIG	MIC/ORE	SOC/CHI	ANA	MIW	CAS	CAL	PER/BRO	MIN
No. of haplotypes	5	4	3	5	3	3	6	6	2
Haplotype diversity (*h)*	0.585 (0.058)	0.360 (0.089)	0.337 (0.085)	0.383 (0.103)	0.127 (0.080)	0.145 (0.090)	0.570 (0.114)	0.713 (0.038)	0.105 (0.066)
Nucleotide diversity (π)	0.002 (0.001)	0.001 (0.001)	0.001 (0.001)	0.001 (0.001)	0.000 (0.000)	0.000 (0.000)	0.002 (0.002)	0.002 (0.000)	0.000 (0.000)
Tajima's *D*	-0.661	-1.038	-0.467	-1.474	-1.506	-1.512	-1.293	0.409	-0.820
Fu's F_S	-1.360	-1.668	-0.434	**-2.982**	**-2.397**	**-2.223**	**-2.910**	-1.280	-0.620
R_2	0.089	0.087	0.088	0.078	0.123	0.131	**0.083**	0.095	0.053

TABLE III. MICROSATELLITE DIVERSITY FOR PEREGRINE FALCON POPULATIONS. STANDARD ERRORS ARE GIVEN IN PARENTHESES.

Region	Population	*n*	Average alleles/locus	Allelic richness	Private Allelic Richness	H_O	H_E
North America	MIG	140	12.55 (1.50)	5.59 (0.32)	0.55 (0.19)	0.688 (0.035)	0.775 (0.021)
	WAS	20	7.27 (0.60)	5.22 (0.37)	0.40 (0.14)	0.671 (0.049)	0.749 (0.034)
	MIC/ORE	48	7.27 (0.65)	4.92 (0.43)	0.12 (0.05)	0.701 (0.050)	0.727 (0.044)
	SOC/CHI	42	7.82 (0.80)	5.17 (0.46)	0.34 (0.12)	0.569 (0.063)	0.716 (0.046)
	ANA	36	9.36 (0.77)	5.65 (0.37)	0.49 (0.12)	0.428 (0.031)	0.756 (0.046)
	MIW	80	8.09 (0.77)	4.99 (0.34)	0.22 (0.12)	0.697 (0.035)	0.738 (0.032)
South America	CAS	28	7.91 (0.78)	4.90 (0.34)	0.70 (0.27)	0.421 (0.065)	0.671 (0.048)
Asia, Europe	CAL	24	8.46 (0.81)	6.09 (0.48)	0.50 (0.09)	0.496 (0.041)	0.799 (0.039)
	PER/BRO	30	9.36 (1.11)	5.62 (0.58)	0.41 (0.12)	0.567 (0.050)	0.744 (0.039)
South Africa	MIN	42	6.73 (0.79)	4.20 (0.56)	0.36 (0.37)	0.441 (0.059)	0.602 (0.045)

5.3.2. Population Structure and Gene Flow

Results from a three-level hierarchical AMOVA based on mtDNA sequences recovered no significant differentiation between Old and New World Peregrines (F_{CT} = 0.007, P > 0.05). A similar result was obtained when populations were separated in four regions (Southern-Northern hemispheres, Old-New World, Table IV). However, differentiation among populations within the Northern hemisphere was significant (8.9%, Table IV) as was the level of divergence apportioned within populations, which accounted for the majority of the variation (88.5 %, Table IV). The four-level hierarchical AMOVA based on microsatellites revealed substantial subdivision among Peregrine populations at all levels (Table V). The grouping of individuals into four regions explained a higher percentage of the genetic variance (8.2 %, Table V) than grouped populations within the Northern hemisphere (4.4 %, Table V). However, a large fraction of the variation was found within populations (87.2 %, Table V). Overall, I found that both markers were able to detect population structure with the exception of the mitochondrial data at the hemispheric level.

For CR data, pairwise Φ_{ST} and F_{ST} values between North American migrants (MIG) and residents (MIC/ORE, SOC/CHI, and MIW), and South African residents (MIN) were significant after sequential Bonferroni corrections (Rice 1983) (Table VI). European migrants (PER/BRO) also exhibited significant Φ_{ST} and/or F_{ST} values when compared to North American residents (ANA, WAS, MIC/ORE, SOC/CHI, and MIW), South American residents (CAS), and South African residents (MIN) but were not differentiated from the North American migrants (MIG). Pairwise Φ_{ST} and F_{ST} were significant between Asian migrants (CAL), and South African

TABLE IV. AMOVA ANALYSIS RESULTS FOR FOUR REGIONS (NORTH VS. SOUTH IN THE EASTERN AND WESTERN HEMISPHERE) IN PEREGRINE FALCONS USING MITOCHONDRIAL CR SEQUENCE DATA.

Source of variation	d.f.	Sum of squares	Variance components	Percentage of variation	Fixation indices	*P*-value
Among hemispheres	3	4.143	0.007	2.57	$F_{CT} = 0.027$	0.30792
Among populations within hemispheres	6	5.480	0.022	8.85	$F_{ST} = 0.115$	< 0.00001
Within populations	321	69.737	0.217	88.49	$F_{SC} = 0.091$	< 0.00001
Total	330	79.360	0.246			

TABLE V. GLOBAL AMOVA RESULTS AS A WEIGHTED AVERAGE OVER 11 MICROSATELLITE LOCI. RESULTS SHOW SIGNIFICANT STRUCTURE AT ALL FOUR HIERARCHICAL LEVELS. HEMISPHERES REFER TO OLD WORLD (NORTH-SOUTH) AND NEW WORLD (NORTH-SOUTH).

Source of variation	Sum of squares	Variance components	Percentage of variation	Fixation indices	*P*-value
Among hemispheres	201.847	0.381	8.21	$F_{CT} = 0.082$	< 0.00001
Among populations within hemispheres	145.975	0.211	4.56	$F_{ST} = 0.128$	< 0.00001
Within populations	3691.864	4.051	87.22	$F_{SC} = 0.050$	< 0.00001
Total	4039.686	4.644			

residents (MIN). A different outcome was observed when pairwise F_{ST} and R_{ST} were calculated from microsatellite data. The majority of comparisons were significant for one or both statistics (Table VII). No significant F_{ST} and R_{ST} values were observed between North American western residents (MIC/ORE, SOC/CHI) and historical residents (ANA). Similarly, European migrants (PER/BRO) were undistinguishable from Asian migrants (CAL). More pairwise comparisons were significant for F_{ST} (40) than R_{ST} (26).

Principal component analysis using microsatellite data showed separation between Old and New World Peregrines, and between Northern and Southern hemispheres (Figure 3).

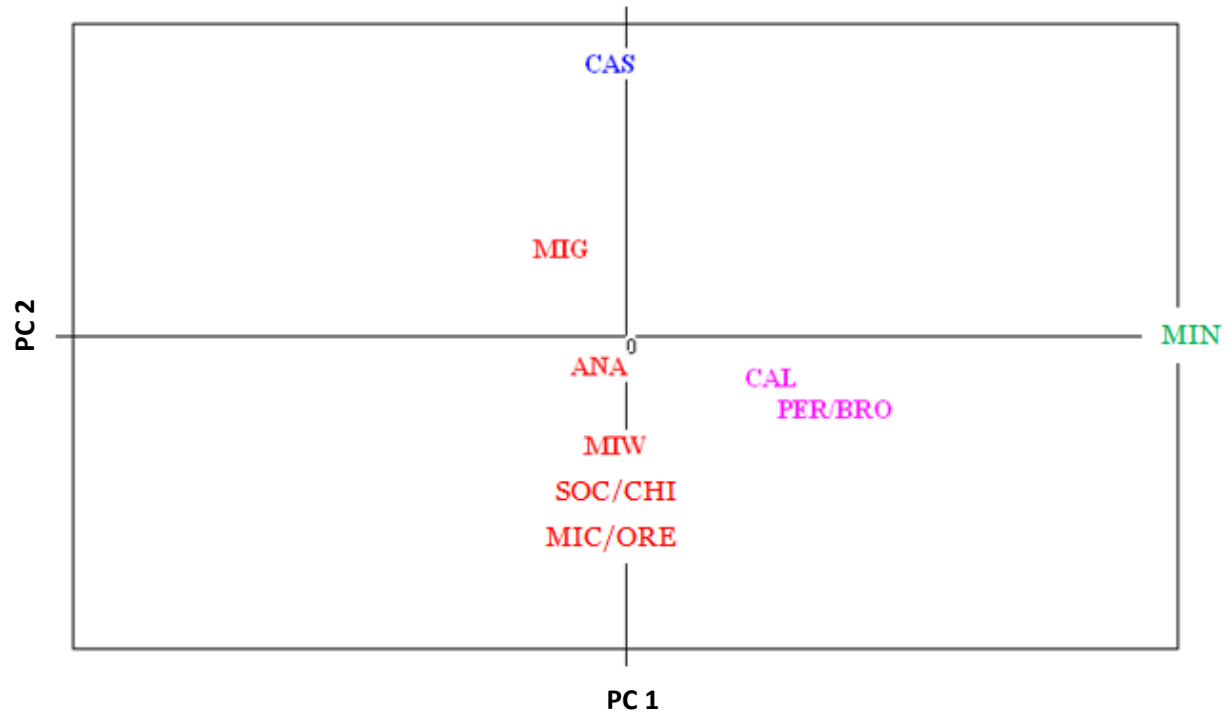

Figure 3. Genetic relationships among Peregrines using PCA of microsatellite data. Populations are colored based on continental distribution (red; North America, purple; Europe-Asia, blue; South America, green; South Africa)

TABLE VI. PAIRWISE Φ_{ST} (WHITE) AND F_{ST} (GREY) VALUES BASED ON MTDNA CONTROL REGION DATA BETWEEN EACH POPULATION. NEGATIVE VALUES ARE EQUIVALENT TO ZERO INDICATING NO DIFFERENTIATION BETWEEN SAMPLES. THICK BORDER POINTS TO BOTH VALUES (Φ_{ST}, F_{ST}) BEING SIGNIFICANT AFTER SEQUENTIAL BONFERRONI CORRECTIONS WHILE DOTTED BORDERS INDICATE ONLY ONE VALUE BEING SIGNIFICANT.

	North America						South America	Asia- Europe		South Africa
	MIG	WAS	MIC/ORE	SOC/CHI	ANA	MIW	CAS	CAL	PER/BRO	MIN
MIG	✳									
WAS	0.133 0.215	✳								
MIC/ORE	**0.178** **0.137**	0.043 0.077	✳							
SOC/CHI	**0.178** **0.144**	0.040 0.069	-0.015 -0.020	✳						
ANA	0.038 0.051	0.012 0.067	0.057 0.018	0.049 0.015	✳					
MIW	**0.172** **0.215**	-0.030 -0.017	0.072 0.064	0.068 0.055	0.042 0.052	✳				
CAS	**0.127** **0.180**	-0.027 -0.012	0.066 0.053	0.063 0.046	0.007 0.025	0.000 -0.017	✳			
CAL	0.027 0.007	0.023 0.144	0.082 0.055	0.084 0.062	-0.006 0.000	0.062 0.136	0.025 0.106	✳		
PER/BRO	-0.004 0.016	0.141 **0.303**	**0.192** **0.223**	**0.197** **0.236**	0.072 **0.149**	**0.184** **0.312**	**0.144** **0.280**	0.044 0.067	✳	
MIN	**0.189** **0.239**	-0.012 -0.012	0.023 0.049	0.020 0.040	0.042 0.063	0.013 -0.007	0.014 -0.007	**0.077** **0.164**	**0.200** **0.337**	✳

TABLE VII. PAIRWISE F_{ST} AND R_{ST} VALUES BASED ON 11 MICROSATELLITE LOCI. NEGATIVE VALUES (FOR R_{ST}) ARE EQUIVALENT TO ZERO. SIGNIFICANT VALUES ARE INDICATED IN BOLD. VALUES MARKED WITH A THICK LINE INDICATES THAT BOTH F_{ST} AND R_{ST} ARE SIGNIFICANT WHILE DOTTED BORDERS INDICATE ONLY F_{ST} (OR R_{ST}) IS SIGNIFICANT.

	North America						South America	Asia- Europe		South Africa
	MIG	WAS	MIC/ORE	SOC/CHI	ANA	MIW	CAS	CAL	PER/BRO	MIN
MIG	✳									
WAS	**0.055** **0.003**	✳								
MIC/ORE	**0.058** **0.186**	**0.053** 0.132	✳							
SOC/CHI	**0.056** **0.127**	**0.047** 0.062	0.013 0.004	✳						
ANA	**0.022** **0.300**	**0.032** **0.310**	0.003 0.023	0.019 0.078	✳					
MIW	**0.046** **0.146**	**0.038** **0.098**	**0.057** 0.005	**0.033** 0.002	0.002 **0.065**	✳				
CAS	**0.099** -0.006	**0.177** 0.013	**0.143** **0.232**	**0.139** 0.163	**0.112** **0.430**	**0.129** **0.183**	✳			
CAL	**0.063** **0.196**	**0.059** 0.142	**0.064** -0.012	**0.060** -0.005	**0.037** 0.023	**0.034** 0.006	**0.145** **0.258**	✳		
PER/BRO	**0.050** **0.346**	**0.049** **0.383**	**0.037** 0.074	**0.037** 0.148	**0.025** 0.030	**0.018** **0.112**	**0.106** **0.488**	0.016 0.094	✳	
MIN	**0.188** **0.427**	**0.229** **0.442**	**0.211** **0.306**	**0.231** **0.272**	**0.182** **0.362**	**0.181** **0.334**	**0.257** **0.549**	**0.113** **0.312**	**0.112** **0.470**	✳

Two clusters were observed among the sample locations, with North America (WAS, MIC/ORE, SOC/CHI, MIW) forming a cluster and Asia-Europe (CAL, PER/BRO) forming a second cluster. Southern populations (CAS, MIN) were well separated from these clusters. Population scores were plotted on two principal axes (PC1 and PC2), which cumulative explained 59.73% of the total genetic diversity (PC1: 38.26%, $P = 0.002$, PC2: 21.47%, $P = 0.002$).

For STRUCTURE analysis, posterior probability values plateau at $K= 6$ to $K= 10$. Posterior probability values from multiple runs at $K= 5$ (SD= 2.64) and $K= 6$ (SD= 6.97) were more consistent across runs compared to $K= 10$ (SD= 81.95) (Figure 4 a). However, five population clusters ($K= 5$) were identified using the ΔK method (Evanno et al 2005) (Figure 4 b). North American Peregrines migrants (MIG) and Midwestern (MIW) had comparable assignment proportions (80% and 89%, respectively, Table VIII) in separate clusters, with migrants showing mixed ancestry (Figure 5). Population assignment proportions varied for Western Peregrines from Washington (WAS, 45%), Oregon-Northern California (MIC/ORE, 88%), and Channel Islands-Southern California (SOC/CHI, 69%) (Table VIII). Peregrines resident in South America (CAS) and South Africa (MIN) were assigned with high proportions (91% and 96%, respectively) to separate clusters (Figure 5). Low assignment proportions were given to European (PER/BRO, 48%) and Asian (CAL, 41%) migrants in two different clusters (Table VIII).

Gene flow immigration rates from microsatellites on BAYESASS in northern migrants (MIG & CAL/PER/BRO) and southern residents (CAS & MIN) revealed that the proportion of individuals in each generation that is not migrating is high ($m > 0.93$) providing evidence that even though northern migrants spend most of the year in southern latitudes they return to breed to northern latitudes (Table IX). Low levels of gene flow movement appeared asymmetric in the

New World where northern Peregrines had a higher mean proportion of migrants per generation (m= 0.07) than the south resident birds which showed no evidence of movement (m= 0.009). Immigration rates in Old World were low and symmetric with migrants and residents exchanging a low proportion of individuals (m= 0.010 & 0.035, respectively).

TABLE VIII. PROPORTION OF MEMBERSHIP FOR EACH POPULATION TO EACH CLUSTER (K= 5) BASED ON THE SOFTWARE STRUCTURE. BOXES SHOWED THE HIGHEST ASSIGNMENT VALUE TO A PARTICULAR CLUSTER.

		Proportion of membership (K= 5)				
Region	Population	Cluster 1	Cluster 2	Cluster 3	Cluster 4	Cluster 5
North America	MIG	**0.803**	0.012	0.065	0.061	0.060
	WAS	0.043	0.012	0.362	**0.453**	0.129
	MIC/ORE	0.019	0.008	0.076	**0.882**	0.014
	SOC/CHI	0.061	0.012	0.166	**0.692**	0.069
South America	ANA	0.134	0.033	**0.356**	0.264	0.214
	MIW	0.022	0.010	**0.887**	0.063	0.017
	CAS	0.045	0.005	0.018	0.014	**0.918**
Asia, Europe	CAL	0.051	0.145	**0.410**	0.163	0.231
	PER/BRO	0.065	0.168	0.135	0.152	**0.480**
South Africa	MIN	0.009	**0.956**	0.007	0.013	0.014

Historical female migration rates as measured in LAMARC (M= m/μ) were asymmetrical and moderate in one direction and low in the opposite direction. Median values of M were 659 (Credibility Interval: 0.02-1086) from Europe-Asia to South Africa, and 160 (0.03-694) in the reverse direction. Likewise, M values were 639 (0.02-1000) from North America to South

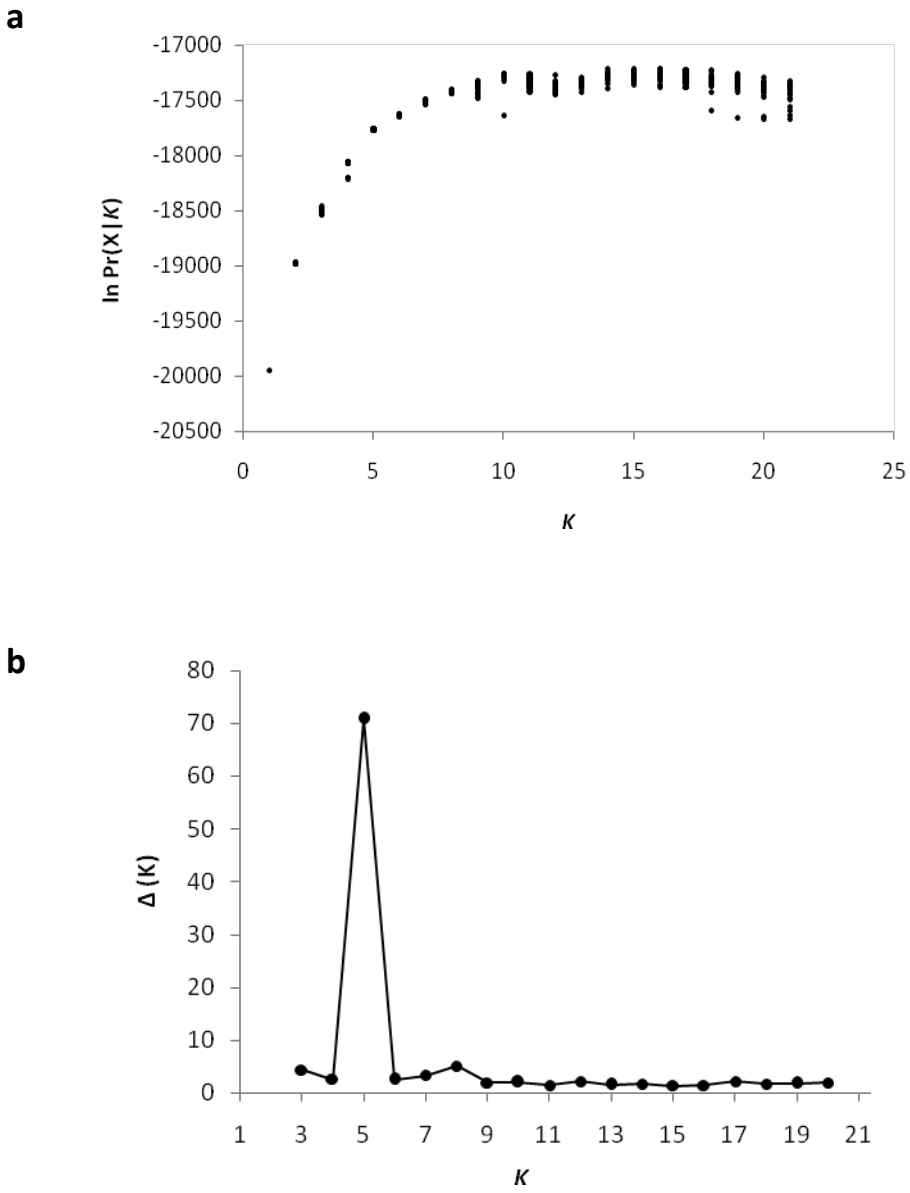

Figure 4. Bayesian inference of the number of clusters (*K*) of Peregrine Falcons. *K* was estimated using the (a) the posterior probability of the data (ln Pr(X|*K*) given each *K* (20 replicated), and (b) the distribution of Δ*K* following the Evanno's method.

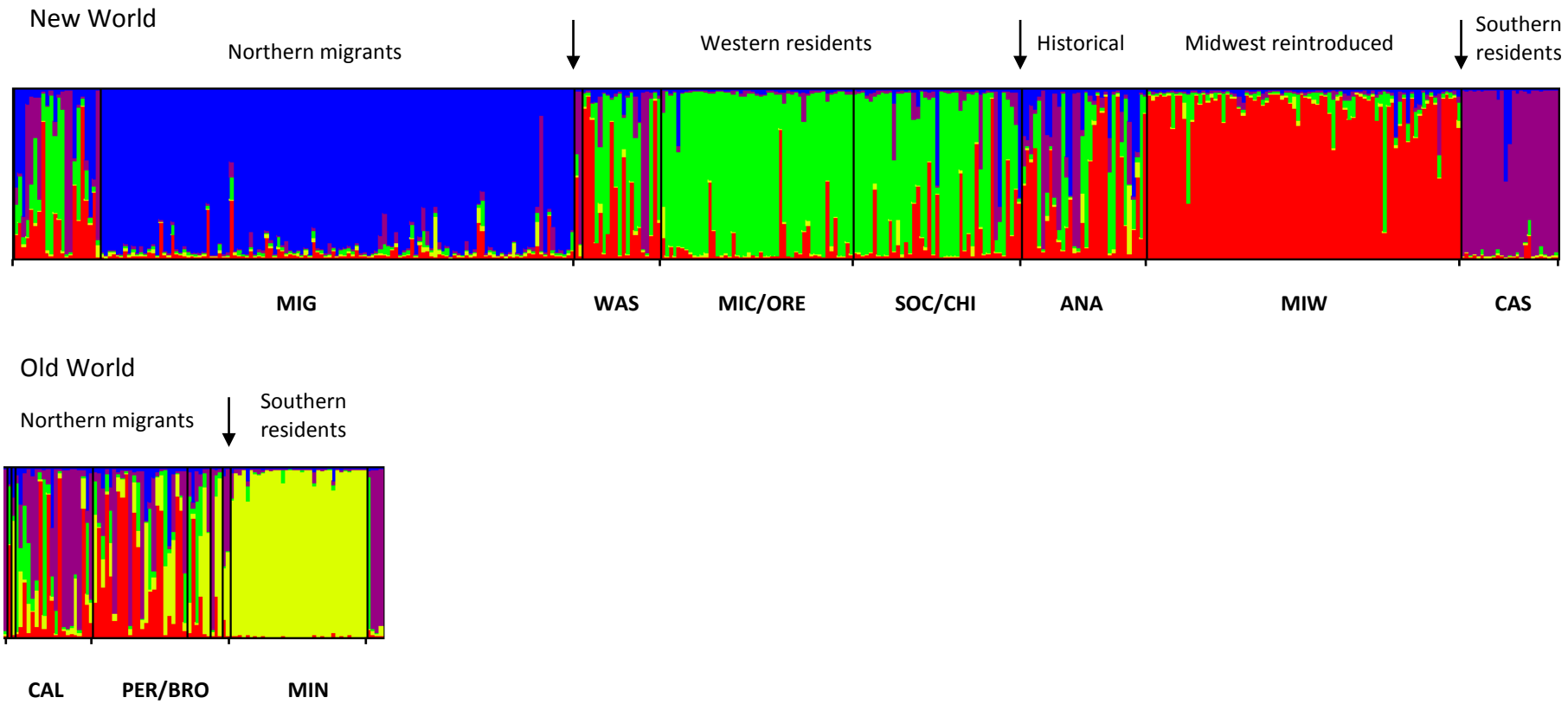

Figure 5. Assignment of Peregrine Falcons by population and *K*= 5 based on STRUCTURE. Clusters are limited with arrows and colors correspond to one of five clusters indicated in Table VIII (blue-cluster 1, yellow-cluster 2, green-cluster 4, red-cluster 3, purple-cluster 5). Each bar represents a single individual.

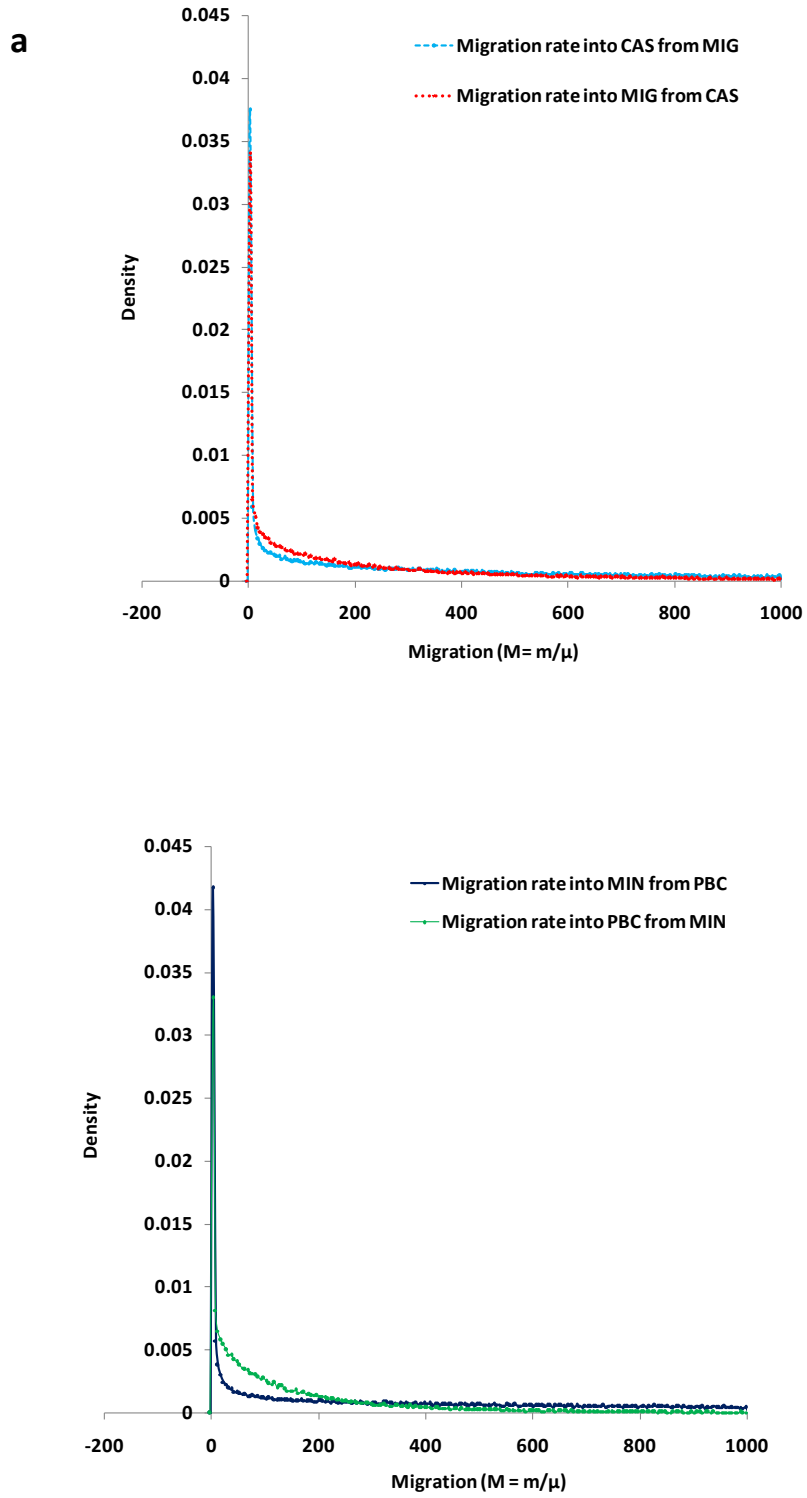

Figure 6. Posterior density distribution from LAMARC for, a) migration between populations, and b) amount of population genetic diversity

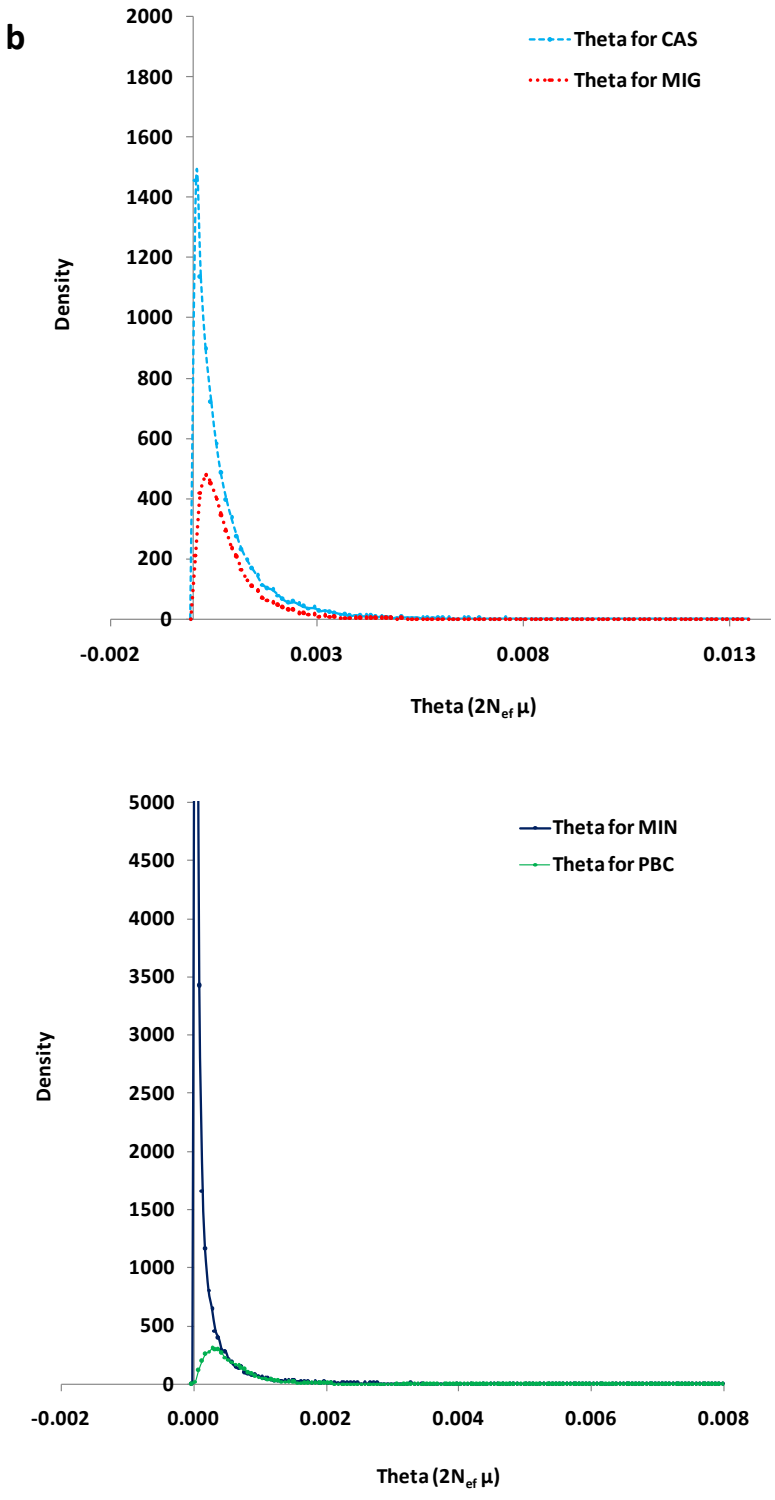

Figure 6, Continuation.

TABLE IX. RECENT MIGRATION RATES AMONG NORTHERN MIGRANTS AND SOUTHERN RESIDENT PEREGRINES. MEANS OF THE POSTERIOR DISTRIBUTION OF *M* ARE DISPLAYED ALONG WITH THEIR RESPECTIVE 95% CONFIDENCE INTERVALS IN PARENTHESES. VALUES ON THE DIAGONAL IN BOLD REPRESENT THE PROPORTION OF INDIVIDUALS DERIVED FROM THE SOURCE POPULATION EACH GENERATION.

		Migration from	
	Migration into	MIG	CAS
North America	MIG	**0.990** **(0.975, 0.998)**	0.009 (0.001, 0.025)
South America	CAS	0.071 (0.02, 0.13)	**0.93** **(0.860, 0.979)**
		Migration from	
	Migration into	CAL/PER/BRO	MIN
Asia-Europe	CAL/PER/BRO	**0.965** **(0.905, 0.997)**	0.035 (0.003, 0.095)
SOUTH AFRICA	MIN	0.010 (0.000, 0.037)	**0.990** **(0.963, 0.999)**

TABLE X. MOST PROBABLE ESTIMATES OF HISTORICAL FEMALE MIGRATION RATES PER GENERATION ($2N_{EF}M$) BASED ON BAYESIAN ANALYSIS IN LAMARC BETWEEN NORTHERN MIGRANT AND SOUTHERN RESIDENT PEREGRINES.

		Migration from	
	Migration into	MIG	CAS
North America	MIG	-	0.401 (0.045, 1.519)
South America	CAS	0.433 (0.015, 2.140)	-
		Migration from	
	Migration into	CAL/PER/BRO	MIN
Asia-Europe	CAL/PER/BRO	-	0.331 (0.070, 1.258)
South Africa	MIN	0.013 (0.007, 0.783)	-

America, and 272 (0.03-870) in the opposite direction (Figure 6 a). However, southern residents populations had a smaller θ (Figure 6 b), which affects these estimates. The population migration rate per generation for mtDNA ($2N_{ef}m$) was asymmetrical for the Old World Peregrines, and symmetrical for the birds of the New World (Table X). Even though CIs partially overlapped for birds in the Old World the migration rate from South Africa into Europe-Asia was higher than in the reverse direction.

5.3.3. Demographic History

Results from different analyses provided evidence of significant past fluctuations in the sizes of populations. The pattern of CR genetic diversity for all groups (excluding WAS) was characterized by the presence of one high-frequency haplotype accompanied by several other closely related haplotypes in lower frequencies (Figure 2). This high frequency haplotype was

present in all populations evidence of the high dispersal capability of this species. Further evidence for a population expansion was shown by Fu's F_S, negative values for all populations, with significant values in North America (ANA, MIW), South America (CAS), and Europe-Asia (CAL) (Table II). In contrast, only a single European population (CAL) had a statistically significant R_2 (Table II) which is also taken as evidence of population expansion. Finally, mismatch distributions corresponding to a demographic expansion (Figure 7) in an unsubdivided population (Rogers & Harpending 1992) showed a good fit to the expansion model curve and appeared as Poisson-shaped wave, as expected for populations that have undergone a sudden expansion in effective population size. Only one North American population (MIG) had a significant *SSD* and did not show a clear pattern of sudden population expansion (Figure 7, top row), indicating a poor fit to the stepwise growth model. The estimates of time since demographic expansion were calculated from τ values resulting from ARLEQUIN (Table XI).

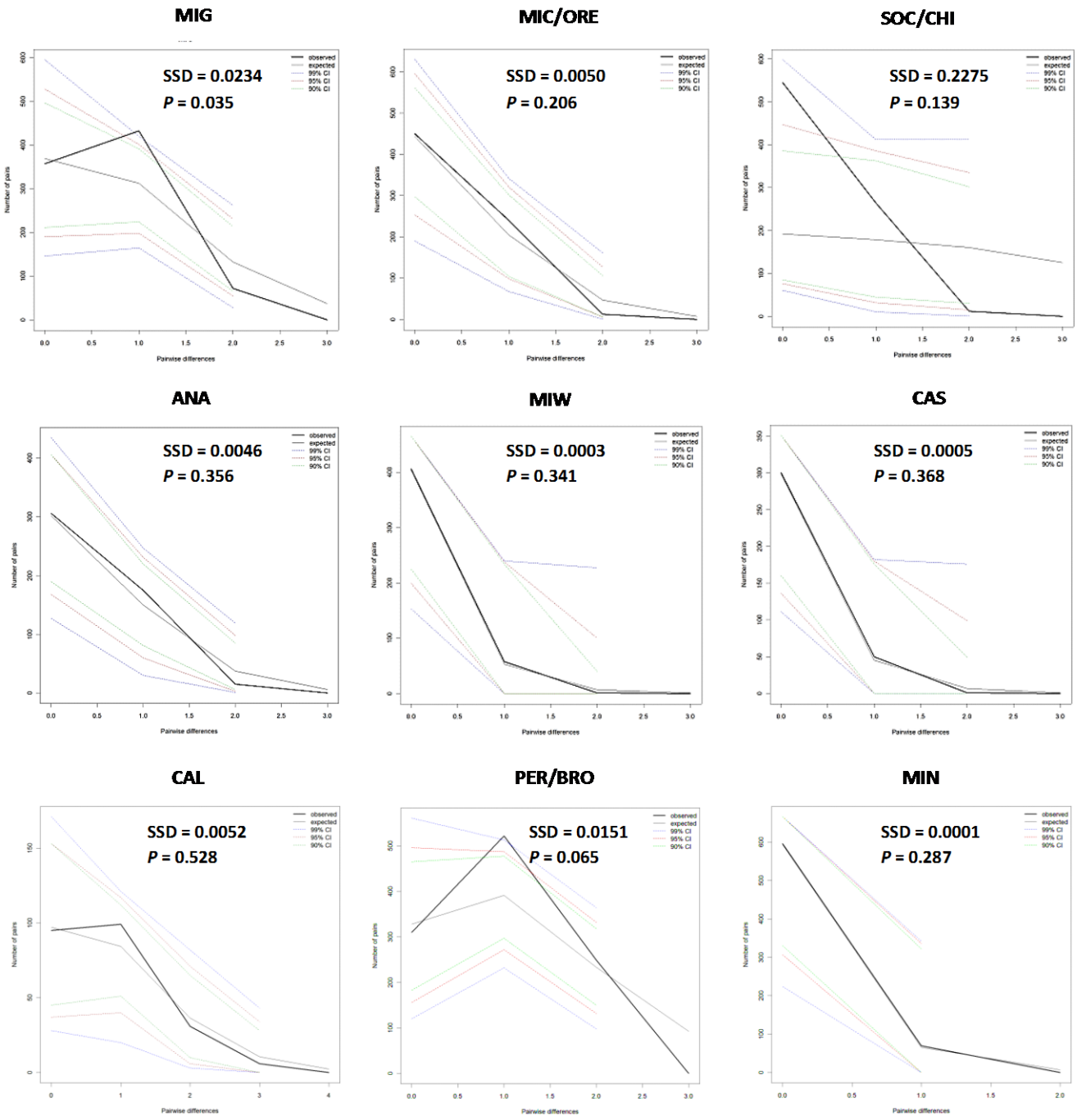

Figure 7. Mismatch distributions from North America Peregrines (MIG, MIC/ORE, SOC/CHI, ANA, MIW), South America (CAS), Asia-Europe (CAL, PER/BRO), and South African (MIN) based on CR data. Thick black lines correspond to observed frequencies of pairwise nucleotide differences while thin black lines represent the expected frequencies under a sudden expansion model. Colored broken lines represent upper and lower bounds for confidence intervals around the observed distribution.

TABLE XI. AGE OF DEMOGRAPHIC EXPANSION (τ) AND TIME (t, IN YEARS) FROM A POPULATION SIZE OF N_0 TO N_1.

	North America			South America	Asia, Europe		South Africa
	MIC/ORE	SOC/CHI	ANA	CAS	CAL	PER/BRO	MIN
Tau (τ)	0.5	3	0.5	3	0.9	1.2	3
CI	(0.20-0.84)	(0-5.20)	(0.15-0.94)	(0.52-3)	(0.38-3)	(0.66-1.79)	(0.29-3)
t ($N_0 \rightarrow N_1$)	19,300.1	115,800.9	19,300.1	115,800.9	34,740.3	46,320.3	115,800.9
CI lower bound	8,066.7	0.0	5,880.4	20,204.9	14,475.1	25,482.4	11,384.0
CI upper bound	32,418.1	200,691.4	36,263.0	115,800.9	59,935.8	68,983.0	115800.9

Since $\tau = 2\mu t$, and t= number of generations since population expansion, to obtain the number of years where N_0 suddenly changed to N_1, I multiplied t by the Peregrine generation time (T= 5.35). Thus, the estimated time to the expansion based on parameter τ ranged between 35,000 to 46,000 YBP for the Asian-European migrants (CAL, PER/BRO), 19,000 YBP for some North American residents (ANA, MIC/ORE), and ~115,000 YBP for the remaining North American residents (SOC/CHI) and southern hemispheres resident (CAL, MIN).

5.4. Discussion

Falco peregrinus is a bird of prey with an extremely widespread geographic range, portions of which were under continental ice sheets during the last glacial maximum. In order to understand the process and history of range expansion for the Peregrine Falcon, I studied genetic diversity and population structure throughout much of the species range. I assayed 490 individuals sampled from four continents at 11 microsatellite loci, and a 405 bp fragment of the

mitochondrial CR from 335 individuals. Microsatellite and mitochondrial data identified shallow but significant population structure of spatially separated demes in the northern and southern parts of the Peregrine's distribution providing information about how migration and expansion have shaped the evolutionary history for this species. These results were evaluated with regard to my two hypotheses, Suspension of Migration which will be evidenced if migrant populations from the far north and southern resident populations are genetically more similar in both the Old World and the New World, and Isolation by Distance which relies upon the detection of genetic differentiation that is correlated with geographical distance. I compare and contrast patterns of genetic diversity in this species with other birds of prey.

5.4.1. Genetic Diversity and Population Structure

Levels of mitochondrial diversity were in general low but typical of raptors. Nucleotide and haplotype diversity will be lost more rapidly than nuclear diversity following either permanent or temporary reductions in population size and fragmentation. Various raptor studies have reported this phenomenon, including the White-bellied Sea-Eagle *Halieetus leucogaster* (Shephard et al. 2005) in Australia, and the Spanish Imperial Eagle *Aquila adalberti* (Martinez-Cruz et al. 2004). Contractions in population size may have occurred not only in recently recovered populations but other populations as well which could explain the generalized observed low diversity levels. This has been cited as the most plausible explanation for low mtDNA diversity in the holarctic Gyrfalcon, *F. rusticolus* (Johnson et al. 2007; Nittinger et al. 2007) and the Snowy Owl *Bubo scandiacus* (Marthinsen et al. 2009). Low genetic diversity could also be the result of historically small population sizes rather than a recent decline such as in the Madagascar Fish Eagle, *Halieetus vociferoides* (Johnson et al. 2009) and may be the

explanation for the low diversity in the southern Peregrines (CAS, MIN). Demographic crashes have also been associated with low mtDNA genetic diversity for the Red Kite, *Milvus milvus* (Roques & Negro 2005) in a similar fashion to what happened to western Peregrine residents (MIC/ORE, SOC/CHI) and Midwestern Peregrines (MIW). Another factor that could explain the lower diversity in raptors is the lower mitochondrial mutation rate observed in non-passerines compared to passerines (Nabholz et al. 2009).

However, some Old and New World raptors have higher reported values of nucleotide diversity, like the Old World Saker Falcon *F. cherrug*, the Lanner Falcon *F. biarmicus*, and the Laggar Falcon, *F. jugger* (Nittinger et al. 2007). New World raptors with higher levels of nucleotide diversity included the Harpy Eagle *Harpia harpyja* (Lerner et al. 2009). The levels of mtDNA diversity in these species were similar to migrant Peregrine populations (MIG, CAL, PER/BRO).

Patterns of genetic diversity recovered the same signal independently of the marker used. Levels of mitochondrial and nuclear genetic diversity were, in general, similar for all populations. However, lower values of gene diversity were registered for southern populations (CAS, MIN), and some recovered populations (MIC/ORE, MIW). Gene diversity was slightly higher for migrants (MIG, CAL, PER/BRO) and some western residents (WAS, SOC/CHI).

Hierarchical analysis showed substantial divergence between Old World and New World Peregrines with the largest fraction of variation within populations (≈87% nuclear, ≈88% mitochondrial data). The level of genetic differentiation recovered by this method was somewhat variant, but with similar patterns agreeing with geographical barriers and distance to a large degree, and to a lesser degree with traditional subspecies distinctions. Further nuclear DNA substructuring was found in the Northern hemisphere where multiple populations were analyzed.

Levels of genetic differentiation found between distant populations were high even though Peregrines are a highly mobile species. All the diverse analytical methods based on microsatellites were more informative in revealing population structure compared to analysis of mtDNA. Population pairwise F_{ST} and R_{ST} showed significant genetic structure among Peregrines at the extremes of the species' distribution (north-south) on both sides of the Atlantic Ocean (east-west). Southern residents, *minor* (MIN) and *cassini* (CAS) were the most genetically differentiated populations from the northern migrants (MIG, CAL, PER/BRO). The continental division of populations (e.g. North American, and South American) was significant based on nuclear AMOVA and the PCA. In the STRUCTURE analysis, the clusters with the highest proportions of membership were the southern residents, *cassini* and *minor*, followed by the North American migrants, indicating that these groups do not intermix. All these results gathered by different methods do not support my SOM hypothesis (H_1). Even though it is known that migrants make an annual trip of 5,000-10,000 km to wintering grounds where they spend 6-7 months (Bierregaard et al. 1994), my results document that little or no genetic exchange occurs among southern residents and northern migrants, in either Old or New World Peregrines.

The patterns of genetic differentiation observed revealed that Peregrines are most likely to disperse to nearby sites. This was the pattern detected by STRUCTURE where geographically isolated populations had higher proportion of membership in clusters (>85% in most cases) and clusters representing continuous distributed populations had lower assignments and admixture. Some admixed Peregrines were *anatum* (ANA), northern *calidus* (CAL), *peregrinus/brookei* (PER/BRO), and some North American migrants (MIG). Many of these specimens were sampled prior to the DDT decline (Figure 5). However, for historical specimens that were included in clusters from isolated populations such as *casssini* and *minor*, admixture was not observed,

suggesting this was not an artifact of analyzing study skins. Migrants sampled in South America were not admixed (Figure 5, blue cluster) in contrast to migrants sampled in the US. These may be pointing out the different geographical origin of the migrants. Geographically distant populations are clearly diagnosable by genetic analysis based on nuclear DNA.

In contrast, my results do find support for my second hypothesis. Populations with overlapping geographical ranges follow an IBD pattern (H_2). No or low differentiation was observed for Peregrines that have continuous distributions, like Western Peregrines, Old World migrants, and some New World migrants. Isolation by distance was observed for Western Peregrines and discussed in Chapter 4. Northern migrants, in particular, have a somewhat continuous distribution in the Old World which is mirrored in the New World. The overall distribution of *peregrinus* extends over 30,000,500 km^2 (north of 35N) intergrading with *calidus* in Eastern Europe. *Peregrinus* also overlaps with *brookei* in Southern Europe while *brookei* overlaps with *pelegrinoides* and *babylonicus* in northern and Eastern Africa. In the New World this situation is somewhat similar; *anatum* and *tundrius* in the northern part of the range (>50N) are indistinguishable and only *pealei* remains well differentiated when analyzed with nuclear markers -although devoid of mtDNA variation.

Effective dispersal was detected in several instances by inferred genetic ancestry not matching the geographic collection site of individual birds. Two Midwestern birds (MIW) showed western ancestry. This is unlikely due to gene flow as there is no evidence from banding data that Pacific Northwest Peregrines disperse to the Midwestern states or vice versa. The genotypes of these individuals probably arose from the captive-bred Western *anatum* birds used in the Midwest reintroduction (Tordoff & Redig 2001). Some *anatum* or *tundrius* Peregrines sampled in South America were placed in the *cassini* cluster (Figure 5, purple cluster) which

could be indicating a misidentification or that these birds could have been effectively exhibited SOM. Peregrines also assigned to the *cassini* cluster but from outside its breeding range included *calidus* samples from Northern Africa and *radama* from Madagascar, which all can be found right of the *minor* cluster in Figure 5. The disparate origin of these samples is possibly illustrating homoplasies reflecting the nature of microsatellites. Finally, three *pelegrinoides* and two *babylonicus* had different levels of *minor* ancestry. Previous studies had presented evidence that *pelegrinoides* and *minor* are morphometrically very close (Kemp & Crowe 1991), with overlapping distributions (Vaurie 1961), particularly in Kenya where they are often mistaken from one another (Thomsett 1988) suggesting that they may represent a single population.

5.4.2. Demographic History and Migration

Demographic expansions were detected for some Peregrine populations by different methods based on mtDNA. The low overall polymorphism and the haplotypes star-like pattern with the resulting mismatch distributions indicated that some populations suffered a rapid size expansion in different time periods. Population growth may have been very recent, as expected given that many parts of the species range was under ice until c. 10,000 years ago. From the mismatch distributions, the sudden expansion for southern resident Peregrines (MIN, CAS) and some south western birds (SOC/CHI) took place the earliest (~115,000 YBP) while the Asian-European migrants occurred later (CAL & PER/BRO, ~35,000-45,000 YBP), as well as Western and historical *anatum* (MIC/ORE & ANA, 19,000 BP). Southern hemisphere populations were largely ice-free in the areas now corresponding to their current distribution. Only the southernmost parts of South America were close enough to the pole to allow extensive glaciations and there is no clear evidence of glaciation in South Africa (Ehlers & Gibbard 2007)

which could explain an earlier demographic expansion inferred by mismatch analyses. Northern population distributions, on the other hand, were under the influence of glaciations since extensive ice sheets covered major parts of North America and Europe, northeastern Canada, Greenland, and Iceland (Ehlers & Gibbard 2007). Canada was largely ice-covered, with the exception of the Yukon Territory (Duk-Rodkin 1999). Only Alaska and Eastern Siberia were major areas that seem to have remained ice-free which may have facilitated the passage of Peregrines from the Old World to the New World (Brigham-Grette et al. 2003).

Evidence that Peregrines reached their current distribution rather rapidly is also recorded in the fossil record. Previous molecular studies based on *cytb* sequences suggests that *F. peregrinus* had a recent origin and separated from an ancestral stock around 2 MYBP (Seibold et al. 1993; Helbig et al. 1994) because it does not exhibit much haplotype variation (Wink & Sauer-Gürth 2004). There are no *F. peregrinus* fossils prior to the Pleistocene (White et al. 2002). The first dated Peregrines remains are from the Early Pleistocene, ~1 MYBP in Victoria, Spain (Sanchez Marco 2004). The majority of fossils are mid- to late Pleistocene, and widespread at known Pleistocene-Holocene prehistoric sites: e.g., Australia, New Caledonia, throughout Europe, Mediterranean region (Tchernov 1968; Sanchez Marco 2004), Caucasus Region (Tyrberg 1998), and the Americas (Brodkorb 1964). Considering the fossil evidence, Peregrines already reached their current distribution when the demographic expansions occurred, based on my mismatch analyses, and remained in separate glacial refugia during glaciations.

Analysis with LAMARC showed that historical female gene flow between northern migrants and southern resident demes in the New World was minimal ($2N_{ef}m$, <1) but symmetrical, meaning that the forces shaping genetic variation are a combination of mutation-drift rather than migration (or long distance dispersal). This provides further evidence against

SOM (H_1). Furthermore, migrants from Asia-Europe had much lower immigration rate than South Africa residents, indicating a south→north direction of gene flow, in the opposite direction as my proposed correlate of H_1.

The lack of mtDNA population structure observed across North America/Northern Asia-Europe was evident from my analysis of historical gene flow, which also supported IBD (H_2). Immigration estimates involving northern migrants were high and slightly asymmetrical. Old World migration rate was 1.80 (CI: 0.33, 7.39) while New World migration rate was 1.28 (0.15, 4.98), implying that gene flow has been west → east. However, the CI upper bounds were close or higher than five, indicating enough gene flow for these two populations to be considered one. Morphological studies have also found striking similarities between New World *tundrius* and Old World *calidus* Peregrines (White & Boyce 1988; Johansson et al. 1998). These similarities may have been due gene flow connecting these populations through the Bering Strait, during Pleistocene, and perhaps the recent past (White 1968).

Inferences of contemporary gene flow from BAYEASS based on microsatellites also showed that long distance dispersal events are rare and the distribution of genetic diversity is apportioned based on breeding grounds and reinforced by philopatry to natal regions (Johansson et al. 1998). Even if Peregrines exhibit displays indicative of breeding (i.e. cooperative hunting, courtship feeding, aerial displays) in stopover sites, such as Cuba (Wardman & Aspinall 1999), or wintering sites like Argentina (Notarnicola & Seipke 2004), the phenomenon of SOM seems rare and points to these populations being long-term isolated. This contrasts with the observations of Peregrines breeding in new places showing the extreme adaptability of these birds to different conditions. These reports include an established pair of migrant Peregrines breeding in Cuba (Regalado & Cables 2000), and southern resident Peregrines extending their

territories into new areas. South American *cassini* have begun breeding in Lima, Peru (Beingolea Masaveu 2003), Australian *macropus* are using atypical nest sites in Victoria (White et al. 1988). While in the Old World, there has been recent colonization of urban areas by European *peregrinus-brookei* (Gainzarain et al. 2002).

My study presents one of the most comprehensive analyses of nuclear and mtDNA structure and diversity of Peregrine Falcons in terms of sample size and geographic coverage. I found for the most part, no support for the SOM hypothesis and my results suggest that major genetic divergence was correlated with geographical distances and barriers, giving support for the alternative hypothesis, IBD. Moreover, minor differentiation was found for populations with overlapping distributions also supporting IBD. The mechanism maintaining southern genetic differentiation in residents and migrants might be related to the high fidelity that northern migrant Peregrines exhibit to breeding areas. Differences detected among populations depended on the marker used, and as with other avian studies microsatellite markers were more informative than mtDNA. This suggests that Zink and Barrowclough's 2008 review may have been overly negative about the utility of microsatellites. Microsatellites were largely consistent in diagnosing migrants sampled in wintering grounds or during migration. In many cases, I confirmed suspected subspecies designation based on morphology or in cases where morphological features were ambiguous. Nuclear data recovered the distinctiveness of subspecies designations of Peregrines in the southern hemisphere (*cassini*, *minor*), northern migrant (*tundrius, calidus*), partially migrant (*anatum*, *brookei*), and other sedentary Peregrines (western *anatum*). I also found support for divergence between continental US Peregrines recovered from population crashes from the Western and Midwest regions.

5.5. Acknowledgments

This study was conducted with the support of the Raptor Research Foundation, The American Ornithologists' Union and The International Osprey Foundation. Laboratory work was carried out in the Pritzker Laboratory for Molecular Systematics and Evolution. I thank Kevin A. Feldheim, who saw how this project evolved, from beginning to end. Special thanks go to all the Peregrine researchers who I have had the pleasure to meet, work, and engage in spirited discussions through the years, Doug Bell, Joel Pagel, Clayton M. White, Bud Anderson, Jeff Johnson, Joseph Brown, Wayne Nelson, David Ellis, Miguel Saggese, Juan Carlos Galvez Aramburu, Oscar Beingolea, and Andrew Jenkins. I am most grateful to the curators, collections managers, and their institutions that kindly provided materials for this study: David Willard and Mary Hennen, Field Museum of Natural History, Steven Sullivan, Chicago Academy of Sciences, Paul Sweet, American Museum of Natural History, Kristof Zyskowski, Peabody Museum of Natural History, William L. Fink, University of Michigan Museum of Zoology, Robert M. Zink and Mike Westberg, Bell Museum of Natural History, Eric Pasquet and Marie Portas, Muséum National d'Histoire Naturelle, Ulf Johansson and Göran Frisk, Swedish Museum of Natural History, Bettine van Vuuren and Andrew Jenkins, Stellenbosch University.

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APPENDIX A. Voucher data for historical museum specimens of Midwestern-Eastern Peregrine falcons deposited at the following museums: AMNH (American Museum of Natural History, NY), FMNH (Field Museum of Natural History, IL), CAS (Chicago Academy of Sciences, IL), UMMZ (University of Michigan Museum of Zoology, MI), BMNH (Bell Museum of Natural History), YPM (Yale Peabody Museum, CT)

Voucher No.	Date	Locality
AMNH298200	1932	Thomasville, GA
AMNH298238	1933	Manhasset, NY
AMNH352934	1903	Campbell's Ledge, PA
AMNH436357	1914	Seaford, L.I., NY
AMNH470359	1900	Pittston, PA
AMNH750432	1926	Kittating Mts, Sussex, NJ
AMNH750440	1921	Fisher's Isd., Suffolk, NY
AMNH750442	1920	Fisher's Isd., Suffolk, NY
AMNH816372	NA	Assateague Island, MD
AMNH816373	NA	Assateague Island, MD
AMNH818117	1942	New York City, NY
BMNH7567	1930	Illgen City, Lake, MN
BMNH7582	1930	Pipestone, MN
BMNH7936	1933	Pipestone, MN
BMNH8669	1937	Carlos Avery Refuge, Anoka, MN
CAS15050	1935	Lake Calumet, Cook, IL
CAS1843	NA	Cook, IL
CAS3765	1881	Cook, IL
CAS4075	1881	Cook, IL
CAS9205	1942	Mineral Springs, IN
FMNH130774	1902	Adler, Nerlson, ND
FMNH130775	1905	Stump Lake, Nelson, ND
FMNH246546	1963	Byron, Ogle, IL
FMNH254897	1940	Dead River, Lake, IL
FMNH364819	1887	Spooner, Washburn, WI
UMMZ104646	1939	South Fox Islan, Leelenau, MI
UMMZ98999	1940	Ann Arbor, Washtenaw, MI
YPM23177	1925	New Haven, CT
YPM773	1931	New London, CT
YPM774	1900	Bay, MI
YPM775	1929	Fisher's Isd., Suffolk, NY

APPENDIX A, CONTINUATION

Voucher No.	Date	Locality
YPM78185	1929	Fisher's Isd., Suffolk, NY

APPENDIX B. Sampling data from Midwestern Peregrine urban populations. Identification refers to United States Fish and Wildlife Service banding data. Most of the birds are sampled and banded as chicks. Year & locality data for breeders correspond to first year of breeding and first successful nesting event (NA= no banding data available).

USFWS #	Year	Locality	Status
R0027351	1986	Hacked bird	Breeder
R0027388	1986	Hacked bird	Breeder
877-42484	1989	Chicago, Cook, IL	Breeder
2206-13814	1990	Glenn Ellyn, Dupage, IL	Chick
987-20710	1992	Chicago, Cook, IL	Breeder
816-21947	1992	Saint Paul, Ramsey, MN	Breeder
2206-18504	1993	Akron, Summit, OH	Breeder
1807-29475	1993	Detroit, Wayne, MI	Breeder
1807-34872	1994	Chicago, Cook, IL	Breeder
2206-35861	1996	Cleveland, Cuyahoga, OH	Breeder
1807-53956	1996	Chicago, Cook, IL	Breeder
NA	1996	Monticello, Wright, MN	Breeder
1807-53883	1997	Bong Bridge, Duluth, MN	Chick
1807-61928	1997	Minneapolis, Hennepin, MN	Chick
1807-61906	1998	Detroit, Wayne, MI	Breeder
1807-53861	1998	Sheboygan, Sheboygan, WI	Breeder
1807-49789	1998	Chicago, Cook, IL	Breeder
1807-77625	1999	Waukegan, Lake, IL	Breeder
NA	1999	Sheboygan, Sheboygan, WI	Breeder
1807-44144	1999	Cleveland, Cuyahoga, OH	Breeder
NA	1999	Chicago, Cook, IL	Breeder
1807-49799	1999	Chicago, Cook, IL	Breeder
1807-61902	1999	Cleveland, Cuyahoga, OH	Breeder
1807-61913	1999	Saint Paul, Ramsey, MN	Breeder
1807-61932	1999	Minneapolis, Hennepin, MN	Breeder

USFWS #	Year	Locality	Status
1807-91943	2000	Omaha, Douglas, NE	Chick
2206-35768	2000	Minneapolis, Hennepin, MN	Breeder
1807-34844	2001	Cleveland, Cuyahoga, OH	Breeder
2206-62723	2001	Eagan, Dakota, MN	Breeder
NA	2001	Chicago, Cook, IL	Breeder
2206-62861	2002	Cincinnati, Hamilton, OH	Chick
1807-91916	2002	Kokomo, Howard, IN	Chick
2206-47671	2002	Cedar Rapids, Linn, IA	Chick
2206-62847	2002	Cleves, Hamilton, OH	Chick
1807-35941	2002	Lakewood, Cuyahoga, OH	Chick
2206-62793	2002	Corundum Point, Lake, MN	Chick
2206-62854	2002	Ironton, Lawrence, OH	Chick
1807-35945	2002	Silver Bay, Lake, MN	Chick
1807-35942	2002	Cleveland, Cuyahoga, OH	Chick
2206-62749	2002	Queen's Bluff, Winona, MN	Chick
1807-34720	2002	Fort Wayne, Allen, IN	Chick
1807-35921	2002	Cleveland, Cuyahoga, OH	Chick
2206-62860	2002	Akron, Summit, OH	Chick
2206-62797	2002	Kewaunee, Kewaunee, WI	Chick
2206-62715	2002	Indianapolis, Marion, IN	Chick
2206-69819	2002	Minneapolis, Hennepin, MN	Chick
1807-62101	2002	Castle Danger, Lake, MN	Chick
NA	2002	Waukegan, Lake, IL	Breeder
2206-47672	2002	MEC Louisa, Louisa, IA	Chick
1807-69727	2002	Green Bay, Brown, WI	Chick
1807-91915	2002	Gary, Lake, IN	Chick
1807-91912	2002	Chesterton, Porter, IN	Chick
1807-35950	2002	Minneapolis, Hennepin, MN	Chick
1807-91965	2002	Quad Cities, Scott, IA	Chick
2206-62885	2002	Faith Bluff, Winona, MN	Chick
2206-62820	2002	Daniel Boone NF, Powell, KY	Chick
987-40220	2003	Jefferson, Jefferson, WI	Breeder
1807-77797	2004	Whiting, Lake, IN	Breeder
2206-35789	2004	Chicago, Cook, IL	Breeder

USFWS #	Year	Locality	Status
1807-62131	2005	Old Town, Cook, IL	Breeder
1807-62141	2005	Evanston, Cook, IL	Breeder
2206-49427	2005	Old Town, Cook, IL	Breeder
2206-28989	2005	Hyde Park, Cook, IL	Breeder
NA	2006	Jefferson, Jefferson, WI	Breeder
2206-72281	2006	Cleveland, Cuyahoga, OH	Chick
1687-02008	2006	Dayton, Montgomery, OH	Chick
1687-02081	2006	Lima, Allen, OH	Chick
7/6 b/b*	2006	Chicago, Cook, IL	Breeder
1687-02091	2006	Canton, Stark, OH	Chick
987-40299	2006	Old Town, Cook, IL	Breeder
2206-72234	2006	Toledo, Lucas, OH	Chick
NA	2006	Sheboygan, Sheboygan, WI	Breeder
2206-62828	2006	Lawndale, Cook, IL	Breeder
1687-01829	2006	Lincoln, Lancaster, NE	Chick
NA	2006	Evanston, Cook, IL	Breeder
NA	2007	Waukegan, Lake, IL	Breeder
NA	2007	Chicago, Cook, IL	Breeder
2206-49420	2007	Evanston, Cook, IL	Breeder
NA	2008	Chicago, Cook, IL	Breeder
NA	2009	Chicago, Cook, IL	Breeder

APPENDIX C. Microsatellite allele frequencies for each locus and historical and recovered Midwestern-Eastern Peregrine populations.

Locus	Allele	Historical	Recovered
	n	31	80
CAB *fp* 18-2	158	0.806	0.688
	194	0.081	0.056
	203	0.016	0.025
	230	0.016	0
	248	0.016	0
	266	0.016	0
	293	0.016	0
	302	0	0.019
	320	0.016	0
	338	0	0.044
	365	0	0.038
	374	0.016	0.013
	410	0	0.081
	473	0	0.031
	482	0	0.006
	n	31	80
CAB *fp* 24	206	0.032	0
	211	0	0.006
	216	0.016	0
	221	0.161	0.131
	226	0.323	0.188
	231	0.065	0.038
	236	0.177	0.231
	241	0	0.013
	251	0.145	0.206
	256	0	0.038
	261	0.081	0.044
	271	0	0.044
	286	0	0.063
	n	32	80
CAB *fp* 85	234	0.031	0
	238	0.203	0.15
	246	0.313	0.206
	250	0.328	0.575

Locus	Allele	Historical	Recovered
	254	0.078	0.006
	258	0.047	0.063
	n	27	80
CAB *fp 1*17	246	0.315	0.231
	248	0.074	0
	254	0	0.013
	258	0.167	0.056
	260	0.185	0.375
	262	0.074	0.019
	264	0.037	0.044
	266	0.074	0.213
	268	0.056	0.038
	270	0.019	0.013
	n	29	79
CAB *fp* 120	249	0.017	0
	253	0.034	0
	261	0.052	0.013
	265	0.31	0.418
	269	0.448	0.209
	273	0.069	0.361
	277	0.069	0
	n	32	78
CAB *fp* 157	200	0.031	0.006
	204	0	0.026
	208	0.094	0.026
	216	0.156	0.135
	220	0.156	0.282
	224	0.063	0.109
	228	0.094	0.083
	232	0.406	0.333
	n	29	79
CAB *fp* 181	111	0	0.025
	183	0	0.114
	193	0.034	0
	203	0.017	0.006
	208	0.276	0.278

Locus	Allele	Historical	Recovered
	213	0.017	0.095
	218	0.052	0.082
	223	0.207	0.101
	228	0.155	0.013
	238	0	0.025
	243	0.034	0.032
	248	0.086	0.215
	253	0.034	0
	263	0.017	0
	323	0.034	0
	368	0.034	0.013
	n	32	80
NVH *fp* 13	109	0.031	0
	115	0.047	0
	117	0.078	0.05
	119	0.188	0.156
	121	0.016	0
	123	0.078	0.225
	125	0.203	0.106
	127	0.031	0.206
	129	0.281	0.238
	131	0.047	0.019
	n	30	77
NVH *fp* 31	149	0.017	0
	157	0.017	0
	165	0	0.006
	169	0.183	0.091
	171	0.05	0.045
	173	0.5	0.37
	175	0.15	0.299
	177	0.05	0.188
	179	0.033	0
	n	31	79
NVH *fp* 89	136	0.032	0
	142	0.339	0.146
	152	0.097	0.297
	154	0.032	0.013
	156	0.21	0.139

Locus	Allele	Historical	Recovered
	158	0.145	0.076
	160	0.097	0.323
	162	0.048	0.006

APPENDIX D. Sampling data from Western Peregrine populations. Identification refers to United States Fish and Wildlife Service banding data. Birds are sampled and banded as chicks. (N/A= no data available)

USFWS #	Year	Locality	Status
1807-54407	1996	San Diego, CA	Breeder
816-64176	1996	San Diego, CA	Breeder
2206-13273	1997	Los Angeles, CA	N/A
1807-03302	1997	Los Angeles, CA	Breeder
1807-54451	1997	Los Angeles, CA	Breeder
1807-22758	1997	Orange, CA	Breeder
1807-54446	1998	Los Angeles, CA	Breeder
987-77420	1998	Los Angeles, CA	Breeder
1807-28172	1998	Los Angeles, CA	Breeder
2206-13289	1998	Los Angeles, CA	Breeder
2206-13290	1998	San Diego, CA	Breeder
2206-48002	1998	Los Angeles, CA	Breeder
816-64176	1996	San Diego, CA	Breeder
1687-22102	1997	San Diego, CA	N/A
1126-02005	NA	Orange, CA	N/A
987-77386	1995	Los Angeles, CA	Breeder
1807-28285	1995	Santa Barbara, CA	Breeder
1807-28286	1995	Santa Barbara, CA	Breeder
1807-28199	1995	Santa Barbara, CA	Breeder
987-93944	1995	Santa Barbara, CA	Breeder
816-64353	1995	Santa Barbara, CA	Breeder
987-77015	1995	Santa Barbara, CA	Breeder
987-77396	1995	Santa Barbara, CA	Breeder
1807-70284	2002	Ventura, CA	Breeder
2206-48180	2002	Ventura, CA	Breeder

USFWS #	Year	Locality	Status
1807-70285	2002	Ventura, CA	Breeder
2206-48139	2002	Ventura, CA	N/A
2206-48181	2002	Ventura, CA	Breeder
1687-22105	2007	Santa Barbara, CA	Chick
1697-22104	2007	Santa Barbara, CA	Chick
1807-96326	2007	Santa Barbara, CA	Chick
1807-96327	2007	Santa Barbara, CA	Chick
1126-02008	2007	Santa Barbara, CA	Chick
1687-22106	2007	Santa Barbara, CA	Chick
1687-22108	2007	Santa Barbara, CA	Chick
1126-02009	2007	Ventura, CA	Chick
1807-28200	2007	Santa Barbara, CA	Breeder
2206-70064	2007	Santa Barbara, CA	Chick
1687-22109	2007	Santa Barbara, CA	Chick
1687-22110	2007	Santa Barbara, CA	Chick
1687-22112	2007	Santa Barbara, CA	Chick
1807-96222	2007	Santa Barbara, CA	Breeder
1687-22114	2007	Santa Barbara, CA	Chick
1126-02014	2007	Santa Barbara, CA	Chick
1807-70255	1997	Humboldt, CA	Chick
2206-13188	1993	San Francisco, CA	Chick
1807-28127	1993	San Francisco, CA	Chick
1807-28253	1994	San Luis Obispo, CA	N/A
1807-28284	1994	Santa Barbara, CA	Breeder
1807-03385	1994	Santa Barbara, CA	Breeder
1807-03323	1994	San Luis Obispo, CA	N/A
987-77240	1995	San Francisco, CA	Breeder
2206-13073	1995	San Luis Obispo, CA	N/A

USFWS #	Year	Locality	Status
987-77347	1995	Monterey, CA	Breeder
987-77436	1995	Monterey, CA	N/A
987-77343	1995	Santa Cruz, CA	Breeder
987-77047	1995	Santa Cruz, CA	Breeder
1807-13241	1995	Alameda, CA	Breeder
1807-54405	1995	San Luis Obispo, CA	Breeder
2206-13246	1996	San Luis Obispo, CA	Breeder
1807-28253	1998	San Luis Obispo, CA	Breeder
1807-54439	1998	Monterey, CA	Breeder
2206-48012	1998	Butte, CA	Breeder
N/A	1994	Humboldt, CA	Chick
1807-70126	1995	Lake Oroville, CA	N/A
N/A	1991	Siskiyou, CA	Chick
N/A	1991	Siskiyou, CA	Chick
1807-70256	1997	Curry, OR	Chick
2206-48037	1996	Jackson, OR	Chick
N/A	N/A	Linn, OR	Chick
1807-70237	1997	Clatsop, OR	Chick
1807-70219	1995	Multnomah, OR	Chick
1807-70171	1995	Douglas, OR	Chick
1807-70184	1996	Lane, OR	Chick
1807-70178	1996	Clackamas, OR	Chick
1807-70182	1996	Columbia, OR	Chick
1807-70230	1996	Wasco, OR	Chick
N/A	1997	Lincoln, OR	Chick
1807-54494	1991	Josephine, OR	Chick
N/A	1994	Jackson, OR	Chick
N/A	1998	NA, OR	Chick

USFWS #	Year	Locality	Status
N/A	1994	Multnomah, OR	Chick
N/A	1994	Hood, OR	Chick
1807-54468	1991	Josephine, OR	Chick
2206-48018	1994	Humboldt, CA	Chick
2206-13202	1994	Multnomah, OR	Chick
N/A	1993	Curry, OR	Chick
N/A	1991	Douglas, OR	Chick
N/A	1994	Klamath, OR	Chick
N/A	1994	Jackson, OR	Chick
N/A	1991	Clackamas, OR	Chick
2206-48088	1997	Spokane, WA	Chick
N/A	2008	San Juan Isd, WA	Chick
987-95199	2003	San Juan Isd, WA	Chick
987-95194	2003	San Juan Isd, WA	Chick
987-95191	2003	San Juan Isd, WA	Chick
1387-77162	2003	San Juan Isd, WA	Chick
N/A	2002	San Juan Isd, WA	Chick
N/A	2002	San Juan Isd, WA	Chick
N/A	2001	Tacoma, WA	Chick
N/A	2002	San Juan Isd, WA	Chick
N/A	2002	Everett, WA	Chick
N/A	2002	San Juan Isd, WA	Chick
N/A	2002	San Juan Isd, WA	Chick
1387-77161	2003	Seattle, WA	Chick
N/A	2002	San Juan Isd, WA	Chick
N/A	2002	San Juan Isd, WA	Chick
1387-77151	2003	Everett, WA	Chick
1687-02765	N/A	San Juan Isd, WA	Chick

USFWS #	Year	Locality	Status
1177-41042	N/A	San Juan Isd, WA	Chick
N/A	N/A	San Juan Isd, WA	Chick

APPENDIX E. Allele frequencies and sample size by populations. Western US populations are SOC, CHI, MIC, ORE, and WAS. The Midwestern population is designated MIW.

Locus	Allele/N	SOC	CHI	MIC	ORE	WAS	MIW
CAB *fp* 18-2	N	15	28	23	24	21	80
	158	0.667	0.750	0.804	0.708	0.548	0.688
	167	-	-	-	-	**0.024**	-
	194	0.067	0.054	0.109	0.083	-	0.056
	203	-	-	-	-	-	0.025
	230	-	**0.018**	-	-	-	-
	275	-	-	-	-	**0.048**	-
	302						0.019
	338						0.044
	365						0.038
	374						0.013
	383	-	-	-	-	**0.024**	-
	410	0.033	0.107	0.022	0.104	0.310	0.081
	419	0.067	0.018	0.022	-	-	-
	428	**0.100**	-	-	-	-	-
	437	-	-	-	-	**0.024**	-
	455	0.067	0.054	-	0.021	-	-
	473	-	-	0.043	0.083	0.024	0.031
	482	-	-	-	-	-	**0.006**

Locus	Allele/N	SOC	CHI	MIC	ORE	WAS	MIW
CAB *fp* 24	N	11	22	22	24	21	80
	211	-	-	-	-	-	**0.006**
	221	0.136	0.068	0.182	0.104	0.071	0.131
	226	0.364	0.227	0.295	0.438	0.119	0.188
	231	0.045	0.045	0.045	0.021	0.119	0.038
	236	0.045	0.409	0.205	0.188	0.357	0.231
	241	0.136	0.091	0.182	0.125	-	0.013
	246	-	0.045	-	0.021	-	-
	251	-	-	0.023	0.042	0.238	0.206
	256	-	-	-	-	0.024	0.038
	261	0.091	0.045	-	-	0.024	0.044
	266	-	0.068	0.045	-	-	-
	271	-	-	-	-	**0.024**	0.044
	281	-	-	-	-	**0.024**	
	286	0.182	-	0.023	0.063	-	0.063
CAB *fp* 77	N	12	18	20	24	18	78
	241	0.125	0.056	0.075	0.042	0.028	0.038
	249	-	0.028	0.125	0.146	0.056	0.064
	253	0.083	-	0.025	0.125	0.167	0.077
	257	0.083	0.056	0.175	0.083	0.083	0.026
	261	0.083	0.028	0.050	0.021	-	0.154
	265	0.083	0.056	0.100	0.104	0.083	0.090
	269	0.083	0.139	0.075	0.083	-	0.032
	273	0.000	0.139	0.175	0.146	0.028	0.128
	277	0.333	0.139	0.075	0.104	0.222	0.365
	281	-	0.306	0.125	0.146	0.333	0.026
	289	**0.083**	-	-	-	-	-

Locus	Allele/N	SOC	CHI	MIC	ORE	WAS	MIW
	293	0.042	0.056	-	-	-	-
CAB *fp* 85	N	16	26	23	24	21	80
	238	0.094	0.173	0.109	0.083	0.024	0.15
	242	-	-	-	-	**0.024**	-
	246	0.031	0.135	0.087	-	0.024	0.206
	250	0.750	0.615	0.457	0.479	0.690	0.575
	254	0.063	0.019	0.022	0.021	0.048	0.006
	258	0.031	0.058	0.326	0.417	0.095	0.063
	262	0.031	-	-	-	0.048	-
	278	-	-	-	-	**0.048**	-
CAB *fp* 117	N	16	28	21	24	20	80
	238	-	-	-	-	0.025	-
	246	0.313	0.143	0.310	0.313	0.375	0.231
	254	0.063	0.036	-	0.021	0.100	0.013
	256	**0.031**	-	-	-	-	-
	258	-	0.196	0.048	0.042	-	0.056
	260	0.094	0.054	0.071	0.146	0.350	0.375
	262	0.031	0.071	0.071	0.104	0.025	0.019
	264	0.125	0.357	0.262	0.250	0.050	0.044
	266	0.313	0.143	0.190	0.125	0.025	0.213
	268	0.031	-	0.048	-	0.050	0.038
	270	-	-	-	-	-	**0.013**

Locus	Allele/N	SOC	CHI	MIC	ORE	WAS	MIW
CAB *fp*120	N	14	23	22	24	19	79
	261	-	-	-	-	-	**0.013**
	265	0.536	0.587	0.477	0.313	0.263	0.418
	269	0.393	0.370	0.500	0.646	0.500	0.209
	273	0.071	0.043	0.023	0.042	0.237	0.361
CAB *fp* 157	N	12	19	23	24	21	78
	200	-	-	-	-	**0.048**	0.006
	204	-	-	-	**0.021**	-	0.026
	208	-	-	0.022	0.042	0.143	0.026
	212	**0.042**	-	-	-	-	-
	216	0.208	0.158	0.348	0.313	0.238	0.135
	220	0.583	0.474	0.391	0.250	0.262	0.282
	224	0.042	-	-	-	0.024	0.109
	228	-	0.237	0.065	0.021	0.024	0.083
	232	0.083	0.132	0.152	0.229	0.214	0.333
	236	0.042	-	0.022	0.125	0.024	-
	244	-	-	-	-	**0.024**	-
CAB *fp* 181	N	16	21	23	24	21	79
	183	**0.031**	-	-	-	-	0.114
	203	-	-	0.130	0.250	0.048	0.006
	208	-	0.167	0.109	0.042	0.024	0.278
	213	0.188	-	-	-	0.095	0.095
	218	0.031	-	0.065	0.146	0.143	0.082
	223	0.094	0.167	0.217	0.125	0.119	0.101
	228	-	-	-	-	**0.048**	0.013
	238	0.125	0.310	0.283	0.146	0.143	0.025
	243	0.125	0.119	0.022	0.063	-	0.032

Locus	Allele/N	SOC	CHI	MIC	ORE	WAS	MIW
	248	0.250	0.167	0.130	0.188	0.190	0.215
	253	-	0.024	0.043	0.042	0.119	-
	263	0.063	0.048	-	-	-	-
	268	-	-	-	-	**0.048**	-
	273	**0.031**	-	-	-	-	-
	278	**0.063**	-	-	-	-	-
	358	-	-	-	-	**0.024**	-
	368	-	-	-	-	-	**0.038**
NVH *fp* 13	N	16	26	23	24	20	80
	115	-	-	0.022	-	0.050	-
	117	0.031	0.038	-	-	-	0.050
	119	0.250	0.135	0.239	0.292	0.125	0.156
	123	0.031	0.135	0.043	0.188	0.225	0.225
	125	0.281	0.365	0.152	0.208	0.300	0.106
	127	0.250	0.212	0.239	0.104	0.075	0.206
	129	0.156	0.115	0.304	0.208	0.225	0.238
	131						**0.019**
NVH *fp* 31	N	15	26	23	24	17	77
	159	0.133	0.115	-	-	-	-
	165	0.033	-	0.022	-	-	0.006
	169	0.067	0.058	0.109	0.146	0.147	0.091
	171	0.167	-	-	0.104	0.176	0.045
	173	0.433	0.442	0.370	0.292	0.206	0.370
	175	0.067	0.096	0.174	0.167	0.206	0.299
	177	0.100	0.288	0.261	0.250	0.176	0.188
	179	-	-	0.022	0.042	0.088	-
	183	-	-	**0.043**	-	-	-

Locus	Allele/N	SOC	CHI	MIC	ORE	WAS	MIW
NVH *fp* 89	N	13	22	23	24	21	79
	142	0.308	0.386	0.261	0.396	0.190	0.146
	144	-	-	0.087	-	0.048	-
	150	0.038	0.045	0.043	-	0.048	-
	152	0.269	0.250	0.239	0.313	0.119	0.297
	154	0.077	0.045	0.022	0.042	0.119	0.013
	156	0.038	0.023	-	-	0.048	0.139
	158	0.115	0.045	0.087	0.021	0.048	0.076
	160	0.154	0.114	0.174	0.167	0.381	0.323
	162	-	0.091	0.043	0.042	-	0.006
	166	-	-	0.022	0.021	-	-
	168	-	-	**0.022**	-	-	-

APPENDIX F. . Voucher data for specimens of worlwide Peregrine falcons deposited at the following museums: AMNH (American Museum of Natural History, NY), FMNH (Field Museum of Natural History, IL), CAS (Chicago Academy of Sciences, IL), UMMZ (University of Michigan Museum of Zoology, MI), YPM (Yale Peabody Museum, CT), MNHN (Muséum National d'Histoire Naturelle), and SMHN (Swedish Museum of Natural History).

Voucher No.	Year	Locality
FMNH 157020	1929	Tillamook, OR
FMNH 157022	1924	Laguna Valley, Baja CA, Mexico
FMNH 191394	1949	Kiambu, Nairobi, Kenya
FMNH 157012	1926	Umnak, Aleutian Isd., AK
FMNH 98031	1937	Forres, Morayshire, Scotland
FMNH 300990	1923	Combie, Perthshire, Scotland
FMNH 25873	1907	Lake Forest, IL
FMNH 157014	1933	Collinsons Pt., AK
FMNH 157015	1929	Romanoff Mts, AK
FMNH 157016	1935	Meade River, AK
FMNH 160300	1929	Romanoff Mts, AK
FMNH 19897	1903	Calayan, Philippine Isd.
FMNH 102303	1939	Kharirohar, Gujarat, India
FMNH 103877	1950	Neckar Gemund, Germany
FMNH 157005	1934	Sidney, Vancouver Isd., Canada
FMNH 229035	1940	Darzin, Kerman, Iran
FMNH 316599	1946	Lautaro, Cautin, Chile
FMNH 316600	1947	Lautaro, Cautin, Chile
FMNH 369372	1958	Loiyangalani, Kenya
FMNH 364660	1993	Boca Grande, Monroe, FL
FMNH 387631	1997	Long Key, Monroe, FL
FMNH 390916	1991	Wisconsin
FMNH 395701	1998	New Ulm, Brown, MN
FMNH 396838	N/A	Dade, FL

Voucher No.	Year	Locality
FMNH 428719	2000	Miami, Dade, FL
FMNH 364819	1987	Spooner, Washburn, WI
CAS 6294	1933	Colville River, AK
CAS 7862	1934	Alaska
CAS 6295	1933	Colville River, AK
CAS 6297	1933	Colville River, AK
CAS 5841	1932	Alaska
AMNH 260263	1927	White Nile, Sudan
AMNH 296622	1930	Lukolela, Middle Congo R.
AMNH 410954	1931	Ambarartobe, Madagascar
AMNH 442648	1933	Barrow River, Melville Penn., AK
AMNH 442651	1933	Lyon Inlet, Melville Penn., AK
AMNH 459071	1947	Lubao, Philippines
AMNH 470372	N/A	Falkland Islands
AMNH 537149	1927	Argyllshire, Scotland
AMNH 537165	1922	Swanton Abbott, Norfolk, England
AMNH 537167	1913	Wingrave, England
AMNH 537232	1926	Sfax and Kerkennah, Tunisia
AMNH 537267	1919	Pantai Cermin, Deli, Sumatra
AMNH 537280	1928	Mt. Elini, Ilbono, Sardinia
AMNH 537288	1910	Campo di loro, Corsica
AMNH 537384	1917	Andranamanitra, Madagascar
AMNH 748512	1939	Cordillera Raneagua, Chile
AMNH 748513	N/A	Cordillera Bio-Bio, Chile
AMNH PRS 2824	2002	United Kingdom
YPM 26674	1951	Kinloch Rannoch, Perth, Scotland
YPM 79786	1965	Kilifi, Kenya
YPM 82411	1960	Santa Cruz, Argentina

Voucher No.	Year	Locality
YPM 22439	1937	Precordillera Colchagua, Chile
YPM 16109	1940	Ban Ban, Laos
YPM 12017	1950	Moirang, Manipur, India
UMMZ 58978	1927	Kristiansund, Norway
UMMZ 60809	1926	Argyll, Scotland
UMMZ 78136	1934	Khinjar Lake, Sind, Pakistan
UMMZ 122126	1927	Harbin, Kirin, China
UMMZ 135900	1954	Falkland Islands
UMMZ 135901	1954	Falkland Islands
SMNH 552559	1946	Limassol, Cypera
SMNH 570189	1914	Constantine, Algeria
SMNH 570187	1911	Afgoi, Somaliland
SMNH 570188	1909	Ulmerhost, Germany
SMNH 552556	1921	Sake, Congo
SMNH 552551	1929	Vohipeno, Madagascar
SMNH 552566	1913	Quito, Chaupicruz, Ecuador
SMNH 552564	1915	Pomasqui, Ecuador
SMNH 552579	1940	Tierra del Fuego, Argentina
SMNH 552581	1921	Punta Delgada, Chile
SMNH 552550	1920	Macao, W. Shansi, China
SMNH 552560	1920	Achonten Bay, Kamtchatka
SMNH 552578	1932	Volcano Isd., Japan
SMNH 956080	1994	Västergötland, Lerum, Sweden
SMNH 986642	1998	Värmland, Arvika, Sweden
SMNH 20056086	2005	Skåne, Malmö, Bunkeflostrand, Sweden
SMNH 20066868	2005	Bohuslän, Munkedal, Hällevadsholm, Sweden
SMNH 20016101	2001	Småland, Kalmar, Tjärhovet, Sweden
MNHN 1903-162	1973	N/A

Voucher No.	Year	Locality
MNHN 1980-248	1931	Montagne de la Sainte-Beaume, France
MNHN 1947-334	1938	Siem Reap, Cambodia
MNHN 1936-471	1933	Ronan tchean
MNHN 19565-418	1961	Banc d' Arguin, Mauritanie
MNHN 1936-470	1933	Kg-rcheon, Macao, China
MNHN 1987-481	1987	Grollean, France
MNHN 1948-605	1933	Maroc
MNHN 1980-781	1980	Mauritania, Afrique du Nord
MNHN 1932-785	1930	Maroantsetra, Madagascar
MNHN 1931-814	1931	Maroc
MNHN 1965-1469	1930	France
MNHN EP-20-29	N/A	Corse, France
MNHN EP-21-09	1993	France
MNHN EP-21-10	1990	Hautes-Alpes, France
MNHN EP-22-18	N/A	France

APPENDIX G. . Sampling data from New World migrant and South African Peregrine Falcons. N/A refers to no data available

ID Number	Year	Locality
P-CAP1	1988	Peru
P-04	1988	Peru
P-23A	1988	Peru
P-24A	1988	Peru
P-28	1988	Peru
P-29	1988	Peru
P-30	1988	Peru
P-31	1988	Peru
P-32	1988	Peru
P-33	1988	km 182.5 south of Lima, Hawai, Peru
P-34	1988	km 18 south of Lima, Villa, Peru
P-35	1988	km 18 south of Lima, Villa, Peru
P-36	1988	Km 66.5 s, Chilca, Peru
P-37	1988	Km 63 s, Chilca, Peru
P-38	1988	Km 63 s, Chilca, Peru
P-39	1988	km 66.5 s, Chilca, Peru
P-40	1988	km 36 s, Lurin desert, Peru
P-41	1988	km 61 s, Playa Iñave, Chilca, Peru
P-42	1988	km 61 s, Papa Leon XIII, Chilca, Peru
P-43	1988	km 65 s, Chilca, Peru
P-44	1988	km 65 s, Chilca, Peru
P-45	1988	km 65 s, Chilca, Peru
P-46	1988	km 66 s, Playa Chilca, Peru
P-47	1988	km 61.5 s, Chilca, Peru
P-48	1988	Km 65 s, Chilca, Peru
P-49	1988	km 63.1 s, Chilca, Peru

ID Number	Year	Locality
P-50	1988	km 70 s, Puerto Viejo, Peru
P-52	1989	Km 40 north Lima, Ancon, Peru
P-53	1989	Km 60 s, Papa Leon XIII, Chilca, Peru
P-54	1989	Km 60 s, Papa Leon XIII, Chilca, Peru
P-55	1989	km 68.5 n Pasamayo, Huaral, Peru
P-56	1989	km 61.5 s, Chilca, Peru
P-57	1989	km 61.5 s, Chilca, Peru
P-58	1989	km 126 n, Playa Rio Seco, Peru
P-59	1989	km 96 s, Asia, Peru
P-60	1989	Paracas, close to Museum, Ica, Peru
P-61	1989	Paracas, Sequion, Ica, Peru
P-62	1989	Paracas, close to Museum, Ica, Peru
P-65	1989	km 37 s, Lurin desert, Peru
P-66	1989	km 37 s, Lurin desert, Peru
P-67	1989	Km 60 s, Papa Leon XIII, Chilca, Peru
p-70	1989	km 120 n, Playa Rio Seco, Peru
P-71	1989	km 120 n, Playa Rio Seco, Peru
P-73	1989	km 450 n, norte del rio Santa, Peru
P-74	1989	Playa Los Organos, Piura, Peru
P-75	1989	Playa Mancora, Piura, Peru
P-76	1989	Playa Los Organos, Piura, Peru
P-78	1989	Playa Punta Sal, Tumbes, Peru
P-79	1989	Quebrada Fernandez, Piura, Peru
P-80	1989	Playa Cabo Blanco, Piura, Peru
P-84	1989	Playa El Ñuro, Piura, Peru
P-85	1989	Playa Mancora, Piura, Peru
P-86	1989	Playa Punta Sal, Tumbes, Peru
P-87	1989	Playa Mancora, Piura, Peru

ID Number	Year	Locality
P-88	1989	Estuario de Virrila, Piura, Peru
P-89	1989	Playa Chicama, Trujillo, Peru
P-90	1989	Km 60 s, Papa LeonXIII, Chilca, Peru
P-91	1989	Km 60 s, Papa LeonXIII, Chilca, Peru
P-92	1989	Km 60 s, Papa LeonXIII, Chilca, Peru
P-94	1990	Paracas, Ica, Peru
P-95	1990	Paracas, Playa La Palmilla, Ica, Peru
P-96	1990	km 189 s, Playa Hawai, Ica, Peru
P-97	1990	Lima city, Peru
P-104	1990	Playa Mancora, Piura, Peru
P-106	1990	Playa Mancora, Piura, Peru
P-119	1990	km 1178, Quebrada Seca,Tumbes, Peru
P-134	1991	Lima city, Peru
JCA-02	2007	Ica, Peru
JCA-03	2007	Ica, Peru
JCA-04	2007	Huaral, Peru
JCA-05	2007	Pachacamac, Peru
JCA-06	2007	Pachacamac, Peru
JCA-07	2008	Chilca, Peru
JCA-08	2008	Pisco, Peru
JCA-09	2008	Chilca, Peru
JCA-10	2008	Mamacona, Peru
JCA-11	2008	Chilca, Peru
JCA-12	2008	Ancon, Peru
JCA-13	2008	Huaral, Peru
JCA-14	2008	Huaral, Peru
JCA-15	2008	La Molina, Peru
JCA-16	2008	Ica, Peru

ID Number	Year	Locality
JCA-17	2008	Huachipa, Peru
JCA-18	2008	La Molina, Peru
JCA-19	2008	Pachacamac, Peru
JCA-21	2008	Pachacamac, Peru
JCA-22	2008	Chilca, Peru
JCA-23	2008	Lurin, Peru
JCA-24	2008	Lurin, Peru
JCA-25	2008	Ssn Bartolo, Peru
JCA-26	2008	Playa Arica, Km 38 Panamericana Sur, Peru
C01	N/A	Chile
C02	N/A	Chile
C03	N/A	Chile
C04	N/A	Chile
C05	N/A	Chile
C06	N/A	Chile
C07	N/A	Chile
C08	N/A	Chile
E-02	N/A	El Moro, Guayas, Ecuador
E-03	1990	El Moro, Guayas, Ecuador
E-04	N/A	El Moro, Guayas, Ecuador
E-05	N/A	El Moro, Guayas, Ecuador
E-06	1989	El Moro, Guayas, Ecuador
E-0690	1990	El Moro, Guayas, Ecuador
E-07	N/A	El Moro, Guayas, Ecuador
E-08	1989	El Moro, Guayas, Ecuador
E-0890	1990	El Moro, Guayas, Ecuador
E-09	N/A	El Moro, Guayas, Ecuador
E-1189	1989	El Moro, Guayas, Ecuador

ID Number	Year	Locality
E-1190	1990	El Moro, Guayas, Ecuador
E-1290	1990	El Moro, Guayas, Ecuador
E-13	N/A	El Moro, Guayas, Ecuador
E-14	N/A	El Moro, Guayas, Ecuador
E-15	N/A	El Moro, Guayas, Ecuador
E-1690	1990	El Moro, Guayas, Ecuador
E-17	N/A	El Moro, Guayas, Ecuador
E-18	N/A	El Moro, Guayas, Ecuador
E-19	N/A	El Moro, Guayas, Ecuador
E-20	N/A	El Moro, Guayas, Ecuador
E-21	N/A	El Moro, Guayas, Ecuador
E-22	N/A	El Moro, Guayas, Ecuador
E-23	N/A	El Moro, Guayas, Ecuador
E-24	N/A	El Moro, Guayas, Ecuador
E-25	N/A	El Moro, Guayas, Ecuador
E-26	N/A	El Moro, Guayas, Ecuador
E-27	N/A	El Moro, Guayas, Ecuador
E-28	N/A	El Moro, Guayas, Ecuador
E-29	N/A	El Moro, Guayas, Ecuador
E-30	N/A	El Moro, Guayas, Ecuador
E-31	N/A	El Moro, Guayas, Ecuador
E-32	N/A	El Moro, Guayas, Ecuador
E-33	N/A	El Moro, Guayas, Ecuador
E-901	N/A	El Moro, Guayas, Ecuador
PF-01	2002	CapeTown, WC, South Africa
PF-02	2002	Tygerberg, WC, South Africa
PF-04	2002	Wolfgat, WC, South Africa
PF-07	2002	Cape point, WC, South Africa

ID Number	Year	Locality
PF-09	2002	Dorstberg, WC, South Africa
PF-11	2002	Ciolli, WC, South Africa
PF-13	2002	Constantiaberg, WC, South Africa
PF-15	2002	Lakeside, WC, South Africa
PF-16	2002	Chapmanspeak, WC, South Africa
PF-19	2002	Athlone, WC, South Africa
PF-20	2002	Eerste Rivier, WC, South Africa
PF-22	2002	Fish Hoek, WC, South Africa
PF-24	2002	Redhill, WC, South Africa
PF-25	2002	Maitland, WC, South Africa
PF-27	2002	Orange River, South Africa
PF-28	2002	Zimbabwe
PF-29	2002	Zimbabwe
PF-30	2002	Zimbabwe
PF-31	2002	Zimbabwe
PF-36	2002	Cape Peninsula, WC, South Africa
PF-37	2002	Cape Peninsula, WC, South Africa
PF-38	2002	Cape Peninsula, WC, South Africa
PF-40	2002	Cape Peninsula, WC, South Africa
PF-41	2002	Cape Peninsula, WC, South Africa
PF-42	2002	Soutpansberg, Gauteng, South Africa
PF-43	2002	Soutpansberg, Gauteng, South Africa
PF-45	2002	Alexandria, PE
PF-47	2002	Alexandria, PE
PF-48	2002	United Kingdom
PF-66	2003	Silvermine Ridge, WC, South Africa
PF-68	2003	Scarborough, WC, South Africa
PF-70	2003	Blackburn, WC, South Africa

ID Number	Year	Locality
PF-75	2004	Hillcrest, WC, South Africa
PF-78	2004	Muizenberg, WC, South Africa
PF-84	2004	Epping, WC,South Africa

APPENDIX H. Microsatellite allele frequencies for each locus and population of worldwide Peregrine Falcons. Private alleles are indicated in bold.

		North America						South America	Asia- Europe		South Africa
	Allele	MIG	WAS	MIC/ORE	SOC/CHI	ANA	MIW	CAS	CAL	PER/BRO	MIN
	n	139	20	48	41	35	80	27	24	29	42
CAB *fp* 18-2	149	-	-	-	-	-	-	**0.019**	-	-	-
	158	0.436	0.525	0.760	0.720	0.814	0.688	0.389	0.729	0.759	0.702
	167	0.007	0.025	-	-	0.029	-	-	0.063	-	-
	176	-	-	-	-	-	-	-	0.021	-	0.012
	185	0.004	-	-	-	-	-	-	0.167	0.052	0.250
	194	0.018	-	0.094	0.061	0.043	0.056	0.019	-	-	-
	203	0.004	-	-	-	0.014	0.025	-	-	-	0.012
	212	-	-	-	-	-	-	-	0.021	-	-
	230	-	-	-	0.012	0.014	-	-	-	0.034	-
	248	-	-	-	-	0.014	-	-	-	-	-
	257	-	-	-	-	-	-	-	-	**0.017**	-
	266	-	-	-	-	**0.014**	-	-	-	-	-
	275	0.004	0.050	-	-	-	-	-	-	-	-
	284	0.007	-	-	-	-	-	0.019	-	-	-
	293	0.011	-	-	-	0.014	-	-	-	-	-
	302	0.011	-	-	-	-	0.019	0.037	-	-	-
	311	0.018	-	-	-	-	-	-	-	-	-
	320	0.007	-	-	-	0.014	-	-	-	-	-
	329	0.014	-	-	-	-	-	0.037	-	0.017	-
	338	0.011	-	-	-	-	0.044	-	-	-	-
	347	0.021	-	-	-	-	-	0.093	-	-	-
	356	-	-	-	-	-	-	**0.037**	-	-	-

		North America						South America	Asia- Europe		South Africa
	Allele	MIG	WAS	MIC/ORE	SOC/CHI	ANA	MIW	CAS	CAL	PER/BRO	MIN
	365	0.007	-	-	-	-	0.038	0.019	-	-	-
	374	0.004	-	-	-	0.014	0.013	-	-	-	-
	383	0.011	0.025	-	-	-	-	0.037	-	-	-
	392	0.018	-	-	-	-	-	-	-	-	-
	410	0.357	0.325	0.063	0.085	0.014	0.081	0.185	-	0.121	0.024
	419	0.007	-	0.010	0.024	-	-	-	-	-	-
	428	-	-	-	**0.037**	-	-	-	-	-	-
	437	-	**0.025**	-	-	-	-	-	-	-	-
	446	0.004	-	-	-	-	-	0.019	-	-	-
	455	0.007	-	0.010	0.061	-	-	0.074	-	-	-
	464	0.004	-	-	-	-	-	0.019	-	-	-
	473	-	0.025	0.063	-	-	0.031	-	-	-	-
	482	0.007	-	-	-	-	0.006	-	-	-	-
	491	**0.004**	-	-	-	-	-	-	-	-	-
	n	138	20	47	33	34	80	28	24	29	41
CAB *fp* 24	191	-	-	-	-	-	-	**0.018**	-	-	-
	206	-	-	-	-	0.029	-	-	-	-	0.122
	211	-	-	-	-	-	0.006	-	0.021	0.034	-
	216	0.068	-	-	-	0.029	-	0.054	0.104	-	-
	221	0.097	0.075	0.138	0.091	0.132	0.131	0.018	0.104	0.276	0.707
	226	0.414	0.075	0.383	0.273	0.338	0.188	0.554	0.250	0.207	0.110
	231	0.043	0.125	0.032	0.045	0.088	0.038	0.196	0.042	0.052	0.037
	236	0.094	0.375	0.191	0.288	0.147	0.231	0.125	0.250	0.017	-
	241	0.004	-	0.149	0.106	0.015	0.013	-	0.063	0.086	-
	246	0.011	-	0.011	0.030	-	-	-	0.042	0.017	-
	251	0.115	0.250	0.032	-	0.162	0.206	-	0.021	0.086	-

		North America						South America	Asia- Europe		South Africa
	Allele	MIG	WAS	MIC/ORE	SOC/CHI	ANA	MIW	CAS	CAL	PER/BRO	MIN
	256	0.022	0.025	-	-	0.015	0.038	-	0.063	0.103	-
	261	0.047	0.025	-	0.061	0.029	0.044	0.018	-	0.034	0.012
	266	0.018	-	0.021	0.045	-	-	-	0.042	-	0.012
	271	0.018	0.025	-	-	-	0.044	-	-	0.017	-
	276	0.007	-	-	-	-	-	-	-	0.017	-
	281	-	**0.025**	-	-	-	-	-	-	-	-
	286	0.043	-	0.043	0.061	0.015	0.063	0.018	-	0.034	-
	316	-	-	-	-	-	-	-	-	**0.017**	-
	n	84	20	45	30	7	78	19	7	15	31
CAB *fp* 77	241	-	0.029	0.056	0.083	-	0.038	-	-	-	-
	245	**0.012**	-	-	-	-	-	-	-	-	-
	249	0.159	0.059	0.133	0.017	0.357	0.064	-	-	-	-
	253	0.135	0.176	0.078	0.033	0.143	0.077	0.079	-	0.033	-
	257	0.053	0.088	0.122	0.067	-	0.026	0.026	0.071	0.033	-
	261	0.147	-	0.033	0.050	-	0.154	-	0.214	0.067	-
	265	0.018	0.059	0.111	0.067	-	0.090	-	0.286	0.367	0.355
	269	0.176	-	0.078	0.117	0.143	0.032	0.632	0.071	0.067	0.290
	273	0.094	-	0.167	0.083	0.286	0.128	0.053	0.071	0.167	0.097
	277	0.088	0.235	0.089	0.217	0.071	0.365	0.079	0.143	0.133	0.016
	281	0.076	0.353	0.133	0.183	-	0.026	-	-	0.067	0.242
	285	0.018	-	-	-	-	-	0.132	-	0.033	-
	289	0.006	-	-	0.033	-	-	-	0.143	0.033	-
	293	0.006	-	-	0.050	-	-	-	-	-	-
	297	**0.012**	-	-	-	-	-	-	-	-	-

		North America						South America	Asia- Europe		South Africa
	Allele	MIG	WAS	MIC/ORE	SOC/CHI	ANA	MIW	CAS	CAL	PER/BRO	MIN
	n	138	20	48	40	35	80	28	22	30	39
CAB *fp* 85	230	-	-	-	-	-	-	**0.089**	-	-	-
	234	0.004	-	-	-	0.029	-	0.018	-	-	-
	238	0.144	0.025	0.094	0.150	0.214	0.150	0.518	0.227	0.150	0.051
	242	0.011	0.025	-	-	-	-	-	0.023	0.050	0.077
	246	0.230	0.025	0.042	0.050	0.271	0.206	0.018	0.409	0.467	0.769
	250	0.504	0.700	0.469	0.700	0.400	0.575	0.268	0.250	0.333	0.038
	254	0.022	0.050	0.021	0.038	0.043	0.006	0.054	0.023	-	-
	258	0.086	0.075	0.375	0.050	0.043	0.063	0.036	0.068	-	0.064
	262	-	0.050	-	0.013	-	-	-	-	-	-
	278	-	**0.050**	-	-	-	-	-	-	-	-
CAB *fp* 117	*n*	135	19	46	42	31	80	27	23	28	39
	238	0.004	0.026	-	-	-	-	-	-	0.018	-
	242	-	-	-	-	-	-	-	**0.022**	-	-
	246	0.261	0.395	0.304	0.214	0.323	0.231	0.167	0.217	0.268	0.141
	248	-	-	-	-	0.065	-	-	0.022	0.036	-
	250	-	-	-	-	-	-	0.019	0.043	0.054	0.026
	252	0.007	-	-	-	-	-	-	0.043	-	-
	254	0.085	0.105	0.011	0.048	0.016	0.013	-	0.022	0.018	-
	256	0.015	-	-	0.012	0.032	-	0.500	0.065	0.107	0.115
	258	0.033	-	0.043	0.131	0.081	0.056	0.019	0.087	-	0.051
	260	0.235	0.368	0.109	0.071	0.161	0.375	0.111	0.217	0.357	0.590
	262	0.066	0.026	0.087	0.036	0.065	0.019	-	0.065	0.071	-
	264	0.029	-	0.272	0.262	0.081	0.044	0.019	0.109	0.018	0.077
	266	0.254	0.026	0.152	0.214	0.113	0.213	0.093	-	-	-
	268	0.004	0.053	0.022	0.012	0.048	0.038	0.019	0.087	0.036	-

		North America						South America	Asia- Europe		South Africa
	Allele	MIG	WAS	MIC/ORE	SOC/CHI	ANA	MIW	CAS	CAL	PER/BRO	MIN
	270	0.007	-	-	-	0.016	0.013	0.056	-	0.018	-
	n	135	18	47	37	32	79	27	22	30	39
CAB *fp* 120	217	-	-	-	-	-	-	0.037	-	-	0.013
	221	-	-	-	-	-	-	**0.037**	-	-	-
	229	-	-	-	-	-	-	-	-	**0.017**	-
	233	0.011	-	-	-	-	-	-	0.045	0.083	0.513
	245	0.004	-	-	-	-	-	-	0.045	-	0.026
	249	**0.004**	-	-	-	-	-	-	-	-	-
	253	-	-	-	-	0.031	-	-	-	0.033	-
	257	-	-	-	-	0.016	-	-	-	0.033	0.026
	261	-	-	-	-	0.047	0.013	-	-	0.033	0.051
	265	0.335	0.278	0.383	0.568	0.266	0.418	0.333	0.295	0.383	0.090
	269	0.412	0.472	0.585	0.378	0.484	0.209	0.241	0.250	0.217	0.064
	273	0.147	0.250	0.032	0.054	0.078	0.361	0.130	0.205	0.167	0.141
	277	0.048	-	-	-	0.063	-	0.111	0.068	-	0.026
	281	0.037	-	-	-	0.016	-	0.111	0.045	0.033	-
	285	0.004	-	-	-	-	-	-	0.045	-	0.051
	n	139	20	48	31	36	78	28	24	30	41
CAB *fp* 157	200	-	0.050	-	-	0.028	0.006	-	0.083	-	-
	204	0.039	-	0.010	-	-	0.026	-	0.021	-	-
	208	0.064	0.150	0.031	-	0.097	0.026	-	0.083	0.033	-
	212	-	-	-	0.016	0.028	-	-	0.063	0.017	-
	216	0.204	0.250	0.323	0.177	0.167	0.135	0.054	0.063	0.217	0.037
	220	0.093	0.250	0.323	0.516	0.153	0.282	0.071	0.125	0.233	0.012
	224	0.100	0.025	-	0.016	0.056	0.109	0.375	0.208	0.100	0.049

		North America						South America	Asia- Europe		South Africa
	Allele	MIG	WAS	MIC/ORE	SOC/CHI	ANA	MIW	CAS	CAL	PER/BRO	MIN
	228	0.082	0.025	0.042	0.145	0.139	0.083	0.232	0.063	0.217	0.549
	232	0.396	0.225	0.188	0.113	0.333	0.333	0.268	0.292	0.133	0.146
	236	0.014	-	0.083	0.016	-	-	-	-	0.033	0.159
	240	0.007	-	-	-	-	-	-	-	-	0.024
	244	-	0.025	-	-	-	-	-	-	0.017	0.024
	n	138	20	48	37	33	79	28	23	29	36
CAB *fp* 181	111	-	-	-	-	-	**0.025**	-	-	-	-
	183	-	-	-	0.014	-	0.114	-	0.022	0.103	-
	188	-	-	-	-	-	-	-	-	-	**0.028**
	193	-	-	-	-	0.030	-	-	0.065	-	-
	198	-	-	-	-	-	-	0.125	0.043	0.017	-
	203	-	0.025	0.198	-	0.015	0.006	0.143	-	0.017	-
	204	**0.032**	-	-	-	-	-	-	-	-	-
	208	0.108	0.025	0.073	0.095	0.227	0.278	0.054	0.109	0.069	-
	213	-	0.100	-	0.081	0.030	0.095	0.054	0.196	0.241	0.097
	216	0.176	-	-	-	-	-	0.036	-	-	-
	218	0.014	0.150	0.104	0.014	0.091	0.082	-	0.065	0.034	-
	220	0.079	-	-	-	-	-	0.018	-	-	-
	223	0.029	0.100	0.177	0.135	0.197	0.101	0.000	0.130	0.155	0.097
	224	0.083	-	-	-	-	-	0.107	-	-	-
	228	0.076	0.050	-	-	0.152	0.013	0.196	0.043	0.017	0.056
	232	0.345	-	-	-	-	-	0.250	-	-	-
	233	-	-	-	-	-	-	-	-	**0.017**	-
	236	**0.014**	-	-	-	-	-	-	-	-	-
	238	0.007	0.150	0.208	0.230	0.030	0.025	-	0.130	0.121	0.056
	240	**0.007**	-	-	-	-	-	-	-	-	-

		North America						South America	Asia- Europe		South Africa
	Allele	MIG	WAS	MIC/ORE	SOC/CHI	ANA	MIW	CAS	CAL	PER/BRO	MIN
	243	0.011	-	0.042	0.122	0.030	0.032	-	0.065	0.017	-
	248	0.011	0.200	0.156	0.203	0.091	0.215	0.018	0.043	0.069	-
	253	-	0.125	0.042	0.014	0.030	-	-	0.043	-	-
	263	-	-	-	0.054	0.015	-	-	-	-	-
	268	-	**0.050**	-	-	-	-	-	-	-	-
	273	-	-	-	0.014	-	-	-	0.043	0.034	0.028
	278	-	-	-	**0.027**	-	-	-	-	-	-
	288	**0.007**	-	-	-	-	-	-	-	-	-
	323	-	-	-	-	**0.030**	-	-	-	-	-
	333	-	-	-	-	-	-	-	-	**0.034**	-
	338	-	-	-	-	-	-	-	-	-	0.014
	343	-	-	-	-	-	-	-	-	0.017	0.181
	348	-	-	-	-	-	-	-	-	-	0.181
	353	-	-	-	-	-	-	-	-	0.017	0.069
	358	-	0.025	-	-	-	-	-	-	0.017	0.069
	363	-	-	-	-	-	-	-	-	-	**0.111**
	368	-	-	-	-	0.030	0.013	-	-	-	-
	398	-	-	-	-	-	-	-	-	-	**0.014**
	n	139	19	48	42	36	80	28	24	30	42
NVH *fp* 13	105	**0.004**	-	-	-	-	-	-	-	-	-
	109	-	-	-	-	0.028	-	-	-	0.017	-
	111	-	-	-	-	-	-	-	0.042	-	0.012
	113	**0.014**	-	-	-	-	-	-	-	-	-
	115	0.061	0.053	0.010	-	0.069	-	0.054	-	0.017	-
	117	0.064	-	-	0.036	0.056	0.050	0.036	0.104	0.050	-
	119	0.171	0.132	0.260	0.179	0.208	0.156	0.018	0.021	0.033	0.012

		North America						South America	Asia- Europe		South Africa
	Allele	MIG	WAS	MIC/ORE	SOC/CHI	ANA	MIW	CAS	CAL	PER/BRO	MIN
	121	-	-	-	-	0.014	-	-	0.042	-	0.024
	123	0.075	0.211	0.125	0.095	0.083	0.225	0.018	0.188	0.367	0.190
	125	0.196	0.316	0.177	0.333	0.181	0.106	-	0.313	0.333	0.393
	127	0.107	0.053	0.177	0.226	0.069	0.206	0.750	0.188	0.067	0.369
	129	0.279	0.237	0.250	0.131	0.250	0.238	0.107	0.104	0.100	-
	131	0.011	-	-	-	0.042	0.019	-	-	-	-
	135	0.018	-	-	-	-	-	0.018	-	-	-
	137	-	-	-	-	-	-	-	-	**0.017**	-
	Allele	MIG	WAS	MIC/ORE	SOC/CHI	ANA	MIW	CAS	CAL	PER/BRO	MIN
	n	130	16	48	39	34	77	24	24	30	39
NVH *fp* 31	149	-	-	-	-	**0.015**	-	-	-	-	-
	155	-	-	-	-	-	-	**0.021**	-	-	-
	157	-	-	-	-	**0.015**	-	-	-	-	-
	159	-	-	-	**0.077**	-	-	-	-	-	-
	165	-	-	0.010	0.013	-	0.006	-	-	-	-
	167	-	-	-	-	**0.015**	-	-	-	-	-
	169	0.107	0.156	0.125	0.064	0.147	0.091	0.021	0.354	0.150	0.667
	171	0.069	0.188	0.052	0.064	0.044	0.045	-	0.125	0.133	0.218
	173	0.401	0.219	0.323	0.462	0.471	0.370	0.792	0.313	0.317	0.013
	175	0.237	0.219	0.167	0.090	0.191	0.299	0.083	0.125	0.367	0.090
	177	0.027	0.125	0.271	0.231	0.029	0.188	-	0.083	0.017	0.013
	179	0.145	0.094	0.031	-	0.044	-	0.083	-	0.017	-
	181	**0.011**	-	-	-	-	-	-	-	-	-
	183	-	-	**0.021**	-	-	-	-	-	-	-
	185	-	-	-	-	**0.029**	-	-	-	-	-
	187	**0.004**	-	-	-	-	-	-	-	-	-

		North America						South America	Asia- Europe		South Africa
	Allele	MIG	WAS	MIC/ORE	SOC/CHI	ANA	MIW	CAS	CAL	PER/BRO	MIN
	n	135	20	48	35	35	79	28	24	29	40
NVH *fp* 89	136	-	-	-	-	**0.029**	-	-	-	-	-
	140	**0.004**	-	-	-	-	-	-	-	-	-
	142	0.191	0.175	0.333	0.357	0.314	0.146	0.018	0.188	0.103	-
	144	-	0.050	0.042	-	-	-	-	-	0.017	0.025
	148	**0.004**	-	-	-	-	-	-	-	-	-
	150	0.007	0.050	0.021	0.043	0.014	-	-	-	-	0.075
	152	0.232	0.100	0.281	0.257	0.100	0.297	0.321	0.333	0.310	0.688
	154	0.004	0.125	0.031	0.057	0.071	0.013	0.036	0.125	0.276	0.150
	156	0.217	0.050	-	0.029	0.186	0.139	0.036	0.125	0.155	-
	158	0.132	0.050	0.052	0.071	0.157	0.076	0.161	0.042	0.069	0.063
	160	0.085	0.400	0.167	0.129	0.086	0.323	0.071	0.063	0.052	-
	162	0.033	-	0.042	0.057	0.043	0.006	0.304	0.083	0.017	-
	164	0.004	-	-	-	-	-	0.018	0.021	-	-
	166	0.029	-	0.021	-	-	-	0.036	-	-	-
	168	0.029	-	0.010	-	-	-	-	0.021	-	-
	170	**0.029**	-	-	-	-	-	-	-	-	-

VITA

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Education

Ph.D. Biological Sciences, Ecology and Evolution, University of Illinois at Chicago (UIC), Chicago, IL, 2012.
B.A. Biology Education, Universidad Nacional de Buenos Aires (UBA), Buenos Aires, Argentina, 2002.

Professional Experience

2007-2010	Research Assistant. University of Illinois at Chicago: Computational Methods for Kinship Reconstruction.
2004-2006	Teaching Assistant. University of Illinois at Chicago: Biology of Cells and Organisms (1 semester), Biology of Populations and Communities (1 semester), Life Evolving (1 semester), Mendelian and Molecular Genetics (1 semester), and The Biological World (2 semesters).
2003-2004	Research Assistant. Field Museum of Natural History: Study of phylogenetic relationships in three genera of Neotropical birds (*Tolmomyias*, *Onychorhynchus* and *Hylopezus*).

Grants and Awards

10/10 Graduate College Travel Award, University of Illinois at Chicago
02/09 Graduate College Travel Award, University of Illinois at Chicago
11/08 Graduate Student Council, Travel Award, University of Illinois at Chicago
10/08 Biological Sciences Department Travel Award, University of Illinois at Chicago
09/08 William C. Anderson Student Presentation Award 1st place, Annual Conference, Raptor Research Foundation
11/07 Women in Science and Engineering Travel Award
11/07 Graduate College Travel Award, University of Illinois at Chicago
10/07 Biological Sciences Department Travel Award, University of Illinois at Chicago
05/07 Student Presentation Award, 1st Place, 98th Annual Meeting Illinois State Academy of Sciences
04/07 The International Osprey Foundation Endowment Grant
10/06 Women in Science and Engineering Travel Award
10/06 Graduate College Travel Award, University of Illinois at Chicago
10/06 Biological Sciences Department Travel Award, University of Illinois at Chicago

04/06 American Ornithologists' Union, Donald L. Bleitz Fund
11/05 International Student Service Award, University of Illinois at Chicago
05/05 Graduate Student Award for Excellence in Teaching, University of Illinois at Chicago
04/05 Dean Amadon Grant, Raptor Research Foundation
04/05 Biological Sciences Department Travel Award, University of Illinois at Chicago

Research Publications

Caballero, I. C., and Ashley, M. V. 2011. Genetic analysis of the endemic island loggerhead shrike, *Lanius ludovicianus anthonyi*. *Conservation Genetics*, 1-9.

Chaovalitwongse, W. A., Chou, C. A., Berger-Wolf, T. Y., DasGupta, B., Sheikh, S., Ashley, M. V., and Caballero, I. C. 2010. New Optimization Model and Algorithm for Sibling Reconstruction from Genetic Markers. *INFORMS Journal on Computing, 22*(2), 180-194.

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Presentations and Workshops

Caballero, I. C., Ashley, M. V, Bates, J. M., Bell, D., Pagel, J. E., and Anderson, C. M. 2010. "Genetic patterns of recovered Western and Midwestern Peregrine Falcons (*Falco peregrinus*)." *Raptor Research Foundation, Annual Conference*. Ft. Collins, Colorado. September 2010. *(Oral presentation)*

ConGen Data Analysis Course, Flathead Lake BioStation, University of Montana. 31 Aug-4 Sept, 2009.

Caballero, I. C., Ashley, M. V, Bates, J. M., and M. Hennen. "Genetic analysis reveals possible extra-pair paternity and sex-biased dispersal in an urban avian predator (*Falco peregrinus*)." *Raptor Research Foundation, Annual Conference*. Missoula, Montana. September 2008. *(Oral presentation)*.

Caballero, I. C., Ashley, M. V and J. M. Bates. "Sibling relationships analyzed using a new set of molecular DNA markers in Reintroduced Peregrine Falcons *(Falco peregrinus)*." *Raptor Research Foundation and Hawk Migration Association of North America, Joint Conference*. Lehigh Valley, Pennsylvania. September 2007. *(Poster presentation)*.

Introduction to Raptor Field Techniques, Linwood Spring Research Station, Steven Points, WI. Jun 20-22, 2007.

Caballero, I. C., Ashley, M. V and J. M. Bates. "Nest site fidelity and genetic relationships in Midwest Urban Peregrine Falcons analyzed by microsatellite DNA markers." *Illinois State Academy of Science, 99th Annual Meeting*. Springfield, Illinois. April 2007. *(Oral presentation)*.

Caballero, I. C., Ashley, M. V and J. M. Bates. "Return of the Peregrine: What microsatellites can tell us about population genetics dynamics in Reintroduced Peregrine Falcons" *Fourth North American Ornithological Conference*. Veracruz, Mexico. October 2006. *(Poster presentation)*.

Caballero, I. C., Bates, J. M., and M. V. Ashley. "Genetic analysis of reestablished urban Peregrine Falcons (*Falco peregrinus*) in the Midwestern United States." *Center for Biodiversity and Conservation's 11th Annual Spring Symposium*. American Museum of Natural History, New York. April 2005. *(Poster presentation)*.

Tello, J. G., Caballero, I. C., Bowie, R. C. K., Schulenberg. T. S. and J. M. Bates. "Preliminary assessment of phylogenetic relationships in *Tolmomyias* flycatchers." *American Ornithologists' Union. 121st Stated Meeting*. Urbana-Champaign, Illinois. August 2003. *(Poster presentation)*.