GENETIC AND MORPHOLOGICAL VARIATION IN NORTHERN SAW-WHET OWL POPULATIONS IN EASTERN NORTH AMERICA

A Thesis

by

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ABSTRACT

GENETIC AND MORPHOLOGICAL VARIATION IN

NORTHERN SAW-WHET OWL POPULATIONS IN EASTERN NORTH AMERICA

(December 1996)

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Allozyme and morphological data were collected to elucidate the relationships among eastern North American populations of northern saw-whet owl (*Aegolius acadicus acadicus*). In the east, northern saw-whet owls are distributed in an archipelago-like manner. There is a large, main-range population inhabiting boreal forests of the northern US-southern Canada and two smaller, potentially disjunct populations further south: one on the Allegheny Plateau (West Virginia and Maryland) and another in the southern Appalachian mountains of North Carolina, Tennessee, and Virginia. These two populations may be glacial relicts, isolated by the retreat of spruce (*Picea* spp.) and fir (*Abies* spp.) northward following the Wisconsin glacial maximum (glacial relict hypothesis). Alternatively, given the placement of the Allegheny Plateau and southern Appalachian populations on the periphery of their breeding range, these populations may be marginal populations, characterized by low genetic and phenotypic variation, and consequently, of low conservation priority (central-marginal hypothesis). Since northern saw-whet owls are highly vagile and may exhibit low breeding philopatry, the three

eastern populations might form a large, randomly mating population in eastern North America (current ecology hypothesis).

Little support, either genetic or morphological, exists for the central-marginal hypothesis. The data provide better support for the two other hypotheses. Low genetic distances, low population genetic differentiation, and high estimated rates of gene flow all support the current ecology hypothesis. However, significant morphological differences among populations, marked morphological differentiation of the southern Appalachian population from other eastern populations as defined by Amadon's (1949) seventy-five percent rule for subspecific delimitation, and patterns of decreasing genetic variability with increasing latitude all support the glacial relict hypothesis.

Existing literature on southeastern US flora and fauna show that a diverse array of taxa, ranging from amphibians to flowering plants, exhibit similar trends of decreased genetic variation with increasing latitude. This provides independent support for the glacial relict hypothesis. The patterns exhibited by these taxa might be explained by founder events associated with post-glacial dispersal out of a southeastern refugium and suggests that southeastern populations may harbor significant levels of variation.

Based on genetic and morphological data, the southern Appalachian population of northern saw-whet owls do not appear to be marginal populations meriting low conservation priority. Rather, this population may be a genetic "reservoir"; the incorporation of plans for the continued presence of southern Appalachian saw-whet owls into regional management strategies is strongly urged.

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Finally, I would like to thank my committee members for their time and assistance. Matthew Rowe was invaluable in the field; I will remember Fudgie-Os, boiled peanuts, stuffed bears, "lost" car keys, and intractable car alarms with delight. Gary Walker kindly let me use his laboratory for the allozyme analyses and helped me troubleshoot my systems. As a result, potato starch took on a new significance. Kelly "Dr. Perky" Steele provided advice, books, and much needed help with the DNA analyses in progress. Without these three people, I would not have made it this far.

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DEDICATION

> I have no patience with scientists who take a board of wood,
> look for its thinnest part, and drill a great number of holes
> where the drilling is easy.
>
> —Albert Einstein

This thesis is dedicated to three people who persisted and succeeded in drilling holes through thick boards, and when required, thick skulls: Dr. Mary U. Connell, Ms. Margo Hearne and Ms. Bev Day.

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INTRODUCTION

Biogeography, as defined by Brown and Gibson (1983), is the study of both the historical and present-day distributions of organisms. This definition incorporates three scales—space, time, and taxonomic level—occupying central roles in the study of biogeography. The spatial scale ranges from local distributions to global ones; biogeographic questions including a temporal component may range from short-term (ecological) to long-term (evolutionary or historical), or encompass the entire spectrum. A third scale, taxonomic level, ranges from sub-population to higher taxonomic levels (Myers and Giller, 1988).

The second scale, time, is of critical importance when attempting to identify processes affecting the distribution of a given group. One consequence of adopting an evolutionary or an ecological time frame is that each perspective invokes different processes to explain species distributions. Despite the observation that evolutionary and ecological perspectives are not mutually exclusive, these two scales and their associated processes have proven so divisive that two sub-disciplines, each adopting a different temporal perspective, arose within the larger discipline of biogeography (Brown and Gibson, 1983; Futuyma, 1986): historical biogeography and ecological biogeography. Historical biogeography explains past and/or present distributions as influenced by past events such as continental drift or glaciation (Futuyma, 1986;

Myers and Giller, 1988). The time scales typically adopted by historical biogeographers range from thousands to millions of years.

In contrast, ecological biogeography explains present-day distributions through shorter-term processes such as competition and dispersal. Note that dispersal, as used by historical biogeographers, differs slightly from that utilized by ecological biogeographers. Historical biogeographers define dispersal in terms of range changes. Ecological biogeographers define dispersal in terms of movement among populations or into a novel environment as a part of the life-history of the organism (Myers and Giller, 1988). Time scales addressed in this sub-discipline range from months to decades.

In the past thirty years biogeography experienced a revitalization, partly as a consequence of increased geological knowledge (*e.g.*, continental drift), advances in biogeographic theory (*e.g.*, MacArthur and Wilson's theory of island biogeography (1967)), and development of molecular techniques for assaying genetic variation in organisms. Beginning with allozymes (Lewontin and Hubby, 1966), the use of molecular genetic techniques to elucidate relationships among groups has proved invaluable to biogeographic studies and led to new and often unexpected insights regarding the biogeographic history of many organisms (Avise, 1992, 1994). For example, one of the best historical biogeographic studies of the influence of past events on genetic structure originates from the southeastern US (see summary by Avise, 1996). Multiple taxa in the southeastern US exhibit a phylogeographic break

(*i.e.,* a distinct geography-related discontinuity or shift in genotypes of organisms) separating eastern and western populations. This break occurs near the middle of the Florida panhandle and extends north, paralleling the Georgia-Alabama border. Taxa displaying phylogeographic breaks in the same area include the Carolina chickadee (*Parus carolinensis*; Gill *et al.,* 1993), the pocket gopher (*Geomys pinetus*; Avise *et al.*, 1979), the pond slider (*Trachemys scripta*; Avise *et al.,* 1992), the eastern woodrat (*Neotoma floridus*; Hayes and Harrison, 1992), and the white-tailed deer (*Odocoileus virginanus*; Ellsworth *et al.,* 1994). Ellsworth *et al.* (1994) speculate that these patterns were the result of dispersal along a "Gulf Coast corridor" from a Neotropic Pleistocene refugium following the height of the last glaciation.

This productive integration of biogeographic theory with population genetics can make valuable contributions to another area of biology dependent upon the effective synthesis of older biological disciplines: conservation biology. Predictions derived from historical biogeography can lay the groundwork for hypotheses regarding the location of populations that are evolutionarily significant units or reveal phylogenetic branches in a species not expressed at the phenotypic level. Evolutionarily significant units are characterized by isolation from other conspecific populations for an historically long time such that these populations harbor significant amounts of genetic variation of the species' genetic diversity (Avise, 1996).

One retrospective case illustrates this point. The dusky seaside sparrow (*Ammodramus maritimus nigrescens*) inhabited areas along the Atlantic coast of

Florida. This subspecies went extinct in 1987. By 1980, stochastic events eliminated females from the population, leaving only males. Based on available phenetic data, the species recovery program attempted captive breeding of remaining males to females from a Gulf coast subspecies, Scott's seaside sparrow (*A. m. peninsulae*). Avise and Nelson (1989) surveyed the seaside sparrow complex using mitochondrial DNA (mtDNA) and found two clades: one consisting of subspecies from Atlantic coastal areas and the other composed of subspecies from the Gulf coast. Although the recovery plan bred morphologically similar subspecies, the dusky seaside sparrow was more closely related to other subspecies on the Atlantic coast than to the the Gulf coast subspecies used in the breeding program. Ironically, the Atlantic-Gulf coast split detected by Avise and Nelson (1989) had already been suggested by Funderburg and Quay in 1983 (cited in Avise, 1996) for the seaside sparrow complex based on historical biogeographic and distributional data (Avise, 1996). If data documenting the Atlantic-Gulf coast split had been available, Avise and Nelson (1989) speculate that the dusky seaside sparrow recovery program would have differed from the original plan in the following ways: (1) the dusky seaside sparrow population may not have merited protection under the Endangered Species Act; (2) hybrids between the dusky seaside sparrow and an Atlantic subspecies would have been introduced into the wild, rather than dusky seaside sparrow-Scott's seaside sparrow hybrids; and (3) preservation of the Atlantic and Gulf coast clades would have been given priority. However, this example illustrates how hypotheses based on the historical

biogeography of an area and supported by molecular data on representative taxa might contribute significantly to developing more effective species management plans (Avise, 1992, 1994).

Spruce-fir forests in eastern North America

The approaches described above could be applied to organisms associated with spruce (*Picea* spp.)-fir (*Abies* spp.) forests of eastern North America. These forests present fascinating problems in ecological biogeography, historical biogeography (Hubbard, 1971), and conservation. The current distribution of spruce-fir forests in the eastern US resembles an archipelago of forest islands (MacArthur and Wilson, 1967; White, 1984b). There is a large, main range population of spruce-fir forest occupying a wide band across the northern US and southern Canada. There are also two disjunct populations of this forest type in the eastern US, one on the Allegheny Plateau of eastern West Virginia, western Maryland, and northwestern Virginia, found above elevations of 975 m (White *et al.*, 1993) and the other in the southern Appalachian mountains of western North Carolina, eastern Tennessee and southwestern Virginia above elevations of 1350 m (White *et al.*, 1993). In the southern Appalachians, this forest type is restricted to higher elevations and, consequently, is further fragmented into ten smaller areas of spruce-fir (Dull *et al.*, 1988). Given this archipelago-like distribution, island biogeographic questions can and have been addressed in this system (*e.g.*, Rabenold, 1984; White *et al.*, 1984).

From an historical biogeographic perspective, eastern North America affords ample opportunities to examine the effects of glaciation on fauna and flora. The ranges of eastern forests changed dramatically throughout the Quaternary Period, when at least sixteen glaciations (Davis, 1983) caused repeated cycles of gradual southward range shift and compression of forest communities during glacial advances and rapid northward shifts during periods of warming. Davis (1983) estimates that some tree species migrated north at rates ranging from 100 m/yr (chestnut, *Castanea dentata*) up to 400 m/yr (red pine, *Pinus resinota*). Spruce and fir were not exceptions, migrating 250 m/yr and 200 m/yr, respectively (Davis, 1983).

The present-day distribution of spruce and fir forests in eastern North America differs dramatically from its distribution over the past 18,000 years. The following descriptions of spruce and fir distributions 18,000 years before present (ybp), 10,000 ybp, and 200 ybp are summarized from Delcourt and Delcourt (1981, 1984).

At the Wisconsin glacial maximum, 18,000 ybp, eastern spruce-fir forests and their associated fauna—in addition to other forest communities—were confined to refugia south of the Laurentide Ice Sheet (Delcourt and Delcourt, 1984; Pielou, 1991; Rogers *et al.,* 1991; Hubbard, 1971; Figure 1). Spruce-fir-jack pine (*Pinus banksiana*) forests extended from the southern perimeter of the tundra belt that bordered the Laurentide Ice Sheet, west to the Ohio River valley and Mississippi River valley and south to Georgia, Mississippi, and Alabama (Figure 1). As a result of climactic warming, many forest types began to disperse out of this refugium and migrated

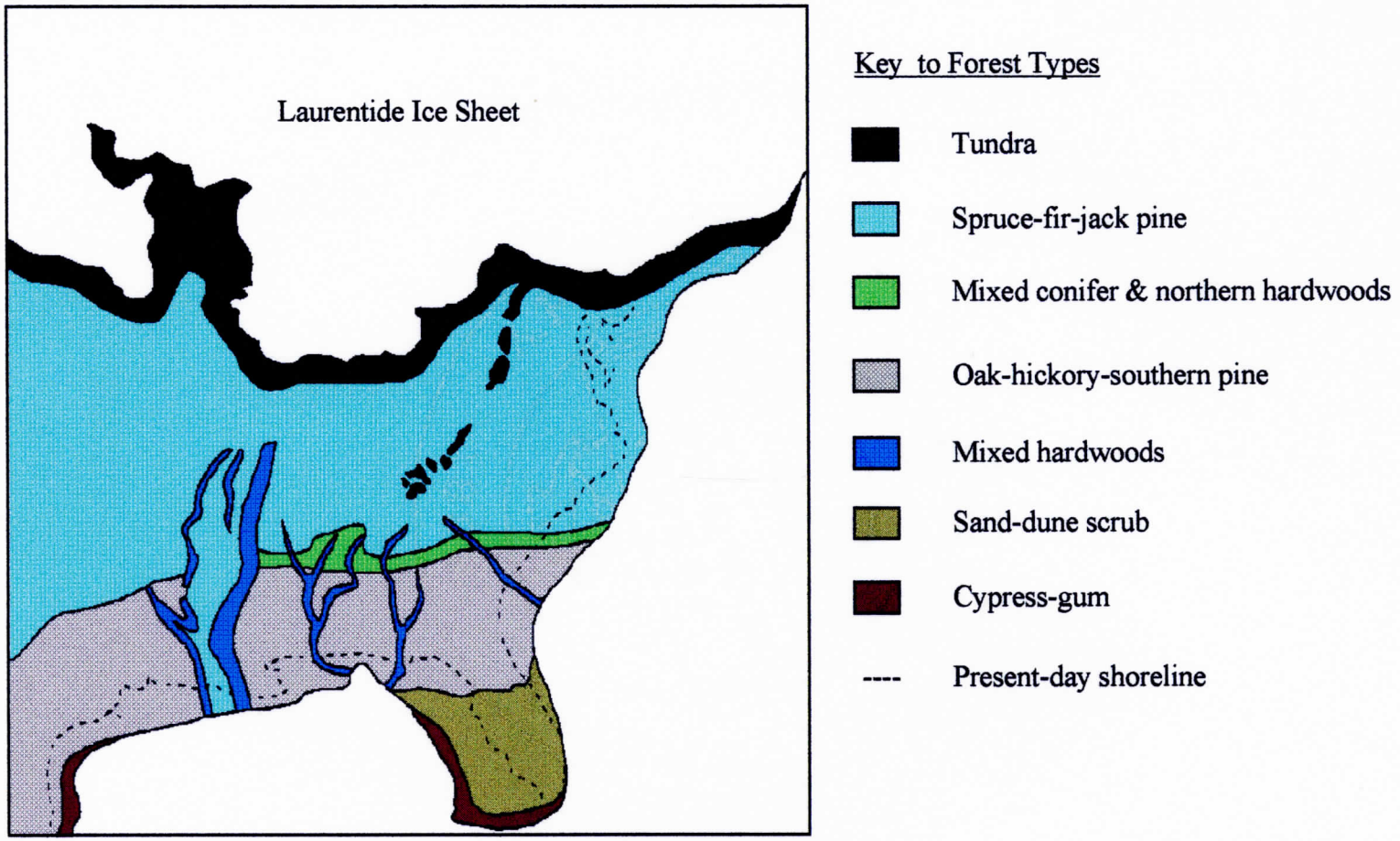

Figure 1. Distribution of forest types in eastern North America at the Wisconsin glacial maximum, 18,000 years before present (modified from Delcourt and Delcourt, 1981)

northward. By 10,000 ybp, spruce and fir were restricted to northern latitudes and to elevations greater than 1500 m in the central and southern Appalachian mountains (Figure 2). These high-elevation southeastern spruce-fir forests persisted on Appalachian mountain peaks through the rest of the Holocene. At 200 ybp, northern boreal forests had moved even further to the north (Figure 3). Although not shown in Figure 3, a small population of spruce and fir also occurs on the Allegheny Plateau (Beltz *et al.*, 1992; White *et al.*, 1993).

In summary, in the last 18,000 years, the distribution of spruce and fir shifted dramatically in response to climactic change (Davis, 1983; Delcourt and Delcourt, 1981, 1984). Initially confined to a southeastern refugia at the peak of glaciation (Rogers, *et al.*, 1991), this forest type migrated rapidly to the north with climate warming. With these dendrological range shifts, the distributions of the flora and fauna associated with particular forest types probably underwent similar changes in distribution (Hubbard, 1971). These historical alterations in range may still affect present-day distributions and population relationships among species associated with eastern spruce-fir forests.

Eastern North American populations of the northern saw-whet owl

Since spruce-fir forests in the southern Appalachians may have been geographically disjunct for more than 10,000 years (Delcourt and Delcourt, 1981, 1984), other organisms strongly associated with these forests may be distinct from

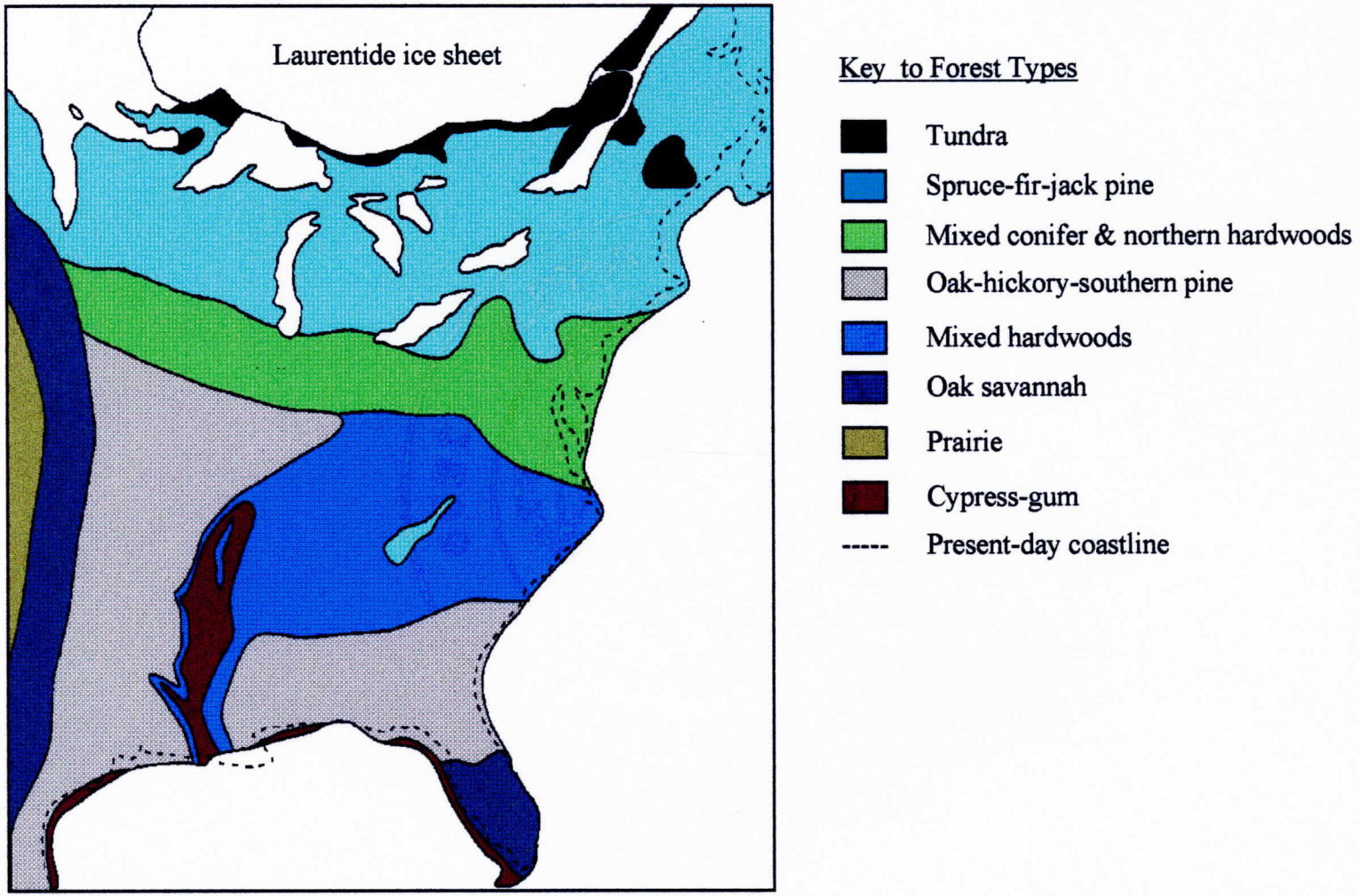

Figure 2. The distribution of forest types at 10,000 years b.p. At this point in time, spruce-fir forest already began to take on its present-day archipelago-like distribution. Spruce-fir in the southern Appalachians were already disjunct from the main range population. Modified from Delcourt and Delcourt (1981).

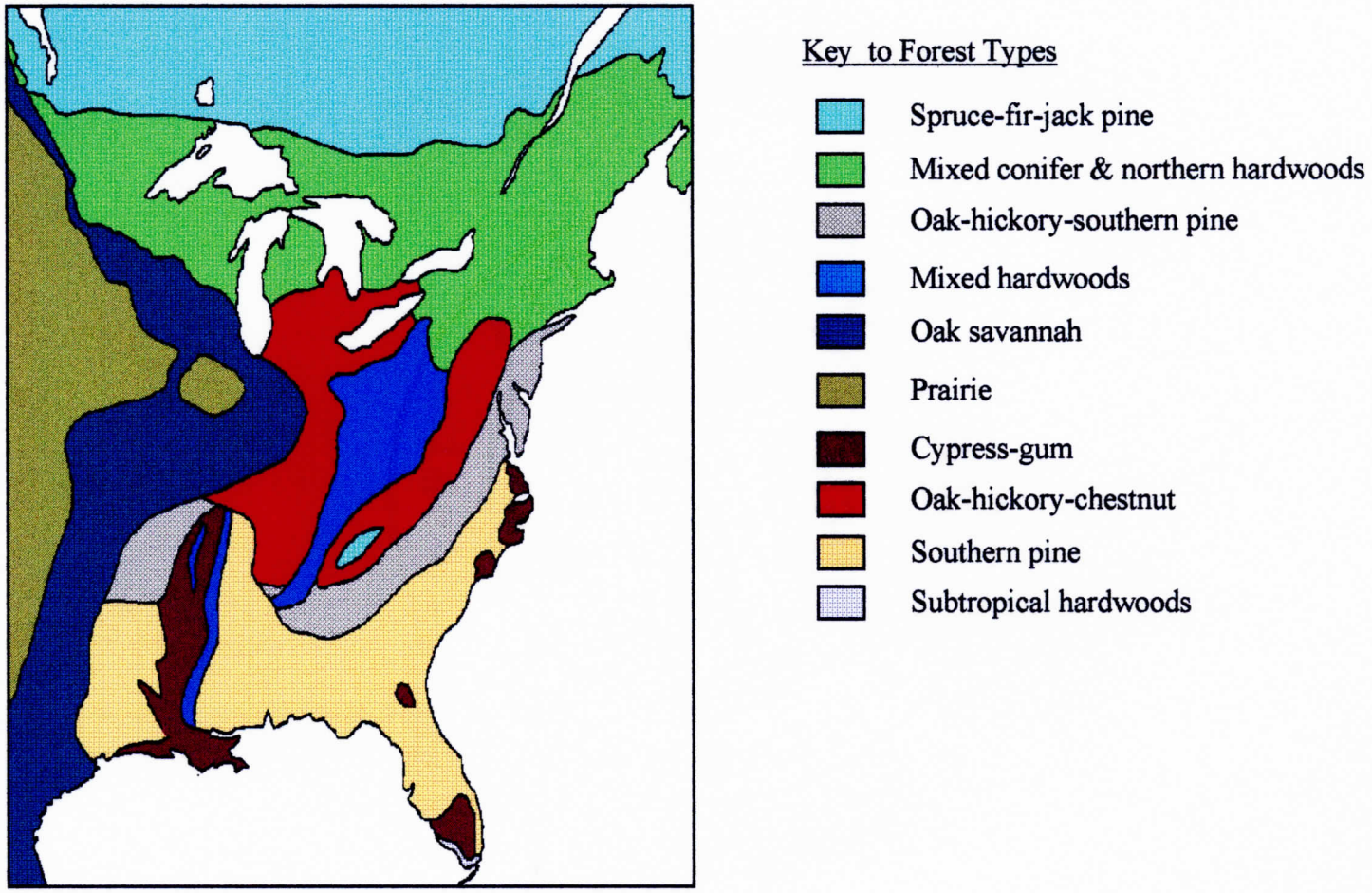

Figure 3. Distribution of forest types in eastern North America at 200 ypb. Modified from Delcourt and Delcourt (1981).

present-day northern conspecifics (Hubbard, 1971; White *et al.*, 1984). Southern Appalachian red spruce (*Picea rubens*) and Fraser fir (*Abies fraseri*) forests already support up to seven endemic subspecies of birds (Rabenold, 1984; American Ornithologists' Union (AOU), 1957) and two cryptic species of red crossbill (*Loxia curvirostra*) (Groth, 1988). Two endemic species of salamander, the imitator salamander (*Desmognathus imitator*) and the pygmy salamander (*Desmognathus wrighti*) (Mathews and Echternacht, 1984) also inhabit these forests. Additionally, numerous plant endemics occur on southern Appalachian peaks, including *Solidago glomerata*, *Houstonia serpyllifolia*, and *Aster chlorolepis*. Of plant species native to the southern Appalachian spruce-fir ecosystem, twenty-six percent are endemics (Ramseur, 1960; White *et al.*, 1993).

Based on glacial history and current high levels of endemism across diverse taxa, the southern Appalachian spruce-fir ecosystem may harbor other, unsuspected endemic taxa. One such organism may be the northern saw-whet owl (*Aegolius acadicus*).

In North America, the species *Aegolius acadicus* is divided into two subspecies: the northern saw-whet owl (*Aegolius acadicus acadicus*) and the Queen Charlotte saw-whet owl (*A. a. brooksi*) (AOU, 1957). The northern saw-whet owl was described by Gmelin in 1788. In 1901, subspecific status for the Queen Charlotte saw-whet owl was proposed by Wilfred Osgood (Fleming, 1916). Ridgway (1914) disputed this proposal, asserting that examination of more specimens from the Queen

Charlotte Islands would show that mainland color morphs (*i.e.*, northern saw-whet owls) also occurred on the Queen Charlotte Islands. He refused to recognize the Queen Charlotte subspecies and listed only one subspecies, *A. a. acadicus* in his *Birds of North America*. However, the AOU, the organization responsible for evaluating the taxonomic status of North American avifauna, considers the Queen Charlotte saw-whet owl as a subspecies distinct from *A. a. acadicus* (AOU, 1957). The classification used by the AOU is adopted herein.

The Queen Charlotte saw-whet owl is distinguishable from the northern saw-whet owl by breast feather coloration and color of the feathers comprising the facial disk. This owl has beige to light brown breast feathers streaked with dark brown (Fleming, 1916; pers. obs.). In contrast, the northern saw-whet owl has a breast with dark brown streaks, but the remainder of breast feathers are off-white. The facial disk of the Queen Charlotte saw-whet owl contains less white than the northern saw-whet owl and a larger number of light brown feathers.

The Queen Charlotte saw-whet owl is endemic to a small archipelago of islands off the coast of British Columbia (Figure 4). This archipelago, known as the Queen Charlotte Islands, is composed of a chain of approximately 150 islands, constituting a total areal extent of approximately 9940 km^2 (Horwood and Parkin, 1989). This subspecies of saw-whet owl may have had a different biogeographic history from its mainland counterpart. Warner *et al.* (1982) used plant macrofossils to demonstrate that the Queen Charlotte Islands were ice-free from at least 16,000 ybp to

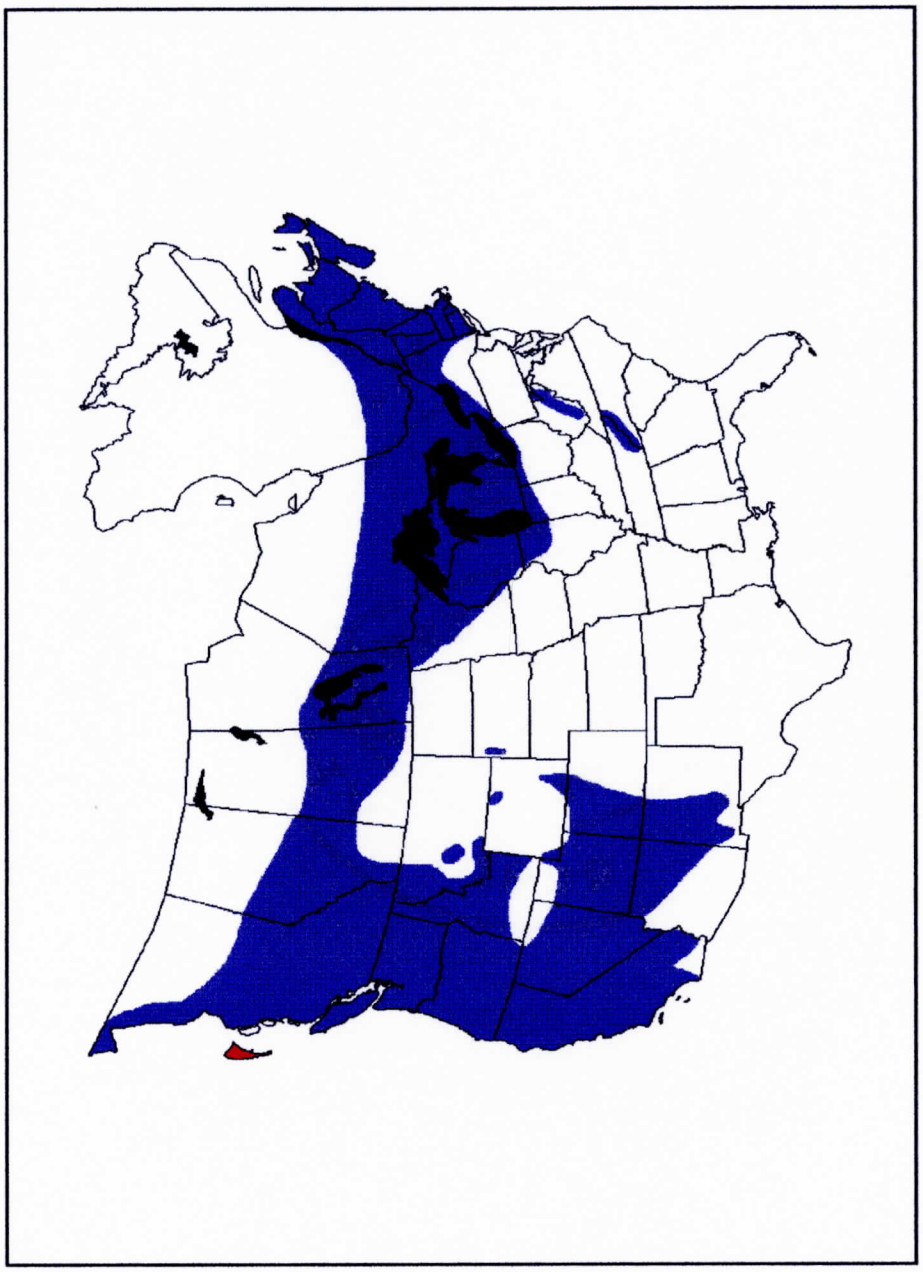

Figure 4. Breeding distribution of northern saw-whet owls (in blue) and Queen Charlotte saw-whet owls (in red) across North America. Figure after Johnsgard (1988).

the present. Based on the plant species and plant species diversity found by Warner *et al.* (1982), Pielou (1991) speculates that the Queen Charlotte Islands may have been ice-free for a considerably longer time. Given that the Queen Charlotte Islands were ice-free and could serve as a refugium, the Queen Charlotte saw-whet owl may have survived on these islands, rather than retreating to another refugium.

The main range population of the northern saw-whet owl extends across southern Canada and the northern United States, ranging from the southernmost tip of Alaska to southern California and northern Pennsylvania (Figure 4). Several potentially disjunct breeding populations of northern saw-whet owl are found in areas of North America (Johnsgard, 1988): the southern Appalachians, the Allegheny Plateau, western South Dakota, northern Wyoming and southwestern Montana (Figure 4). Another population of northern saw-whet owls inhabits the high-elevation montane forests of Mexico (Johnsgard, 1988).

The eastern North American distribution of northern saw-whet owls resembles that of eastern spruce-fir forests: the largest, contiguous population occurs in boreal forests of southern Canada and the northern US (Cannings, 1993; Johnsgard, 1988; Figure 4). Two smaller populations, one on the Allegheny Plateau (eastern West Virginia, northwestern Virginia, and western Maryland) and another in the southern Appalachians (western North Carolina, eastern Tennessee, and southwestern Virginia) are potentially disjunct from the larger northern population. The relationships among these three eastern populations are not clear.

The northern saw-whet owl population in the northern US-southern Canada is known to be highly migratory (Cannings, 1993). Tom Erdman (pers. comm.) banded a northern saw-whet owl during Fall migration of 1995. This owl was caught by another bander on the Outer Banks of North Carolina three weeks later. Additionally, northern saw-whet owls may not exhibit high levels of breeding site philopatry (Cannings, 1993). In light of these observations, it is possible that the high vagility of saw-whet owls result in high migration rates among the three eastern populations, creating a single, large, panmictic population.

Inspection of the breeding distribution of northern saw-whet owls (Figure 4) in eastern North America reveals another potential hypothesis explicating the relationships among these populations. Both southern Appalachian and Allegheny Plateau populations are on the edge of the northern saw-whet owl breeding range in the east. Thus, both populations would be considered "marginal" populations. In contrast, the northern US-southern Canada population would be considered a "central" population. In 1963, Ernst Mayr summarized sets of traits that should typically characterize central and marginal populations (cited in Mayr, 1970). According to Mayr, central populations should exhibit higher population densities, consist of a contiguous population, and have higher levels of phenotypic and genotypic diversity relative to marginal populations. Conversely, marginal populations should have lower population densities, and lower phenotypic and genotypic variation.

Another hypothesis might also explain relationships among the three eastern populations of northern saw-whet owl. Due to their strong association with spruce-fir forests, northern saw-whet owls were probably restricted to southeastern refugia at the Wisconsin glacial maximum. If, following retreat of the Laurentide Ice Sheet, colonizers of newly available northern habitat failed to represent all of the variation present in southeastern refugia, a series of founder events, running from south to north, may have occurred. This would generate a pattern of decreasing population variation with increasing latitude. Preliminary morphological data indicate that male owls from the southern Appalachian population are smaller than males from the larger, northern population (Milling *et al.*, 1993). Thus, the southern Appalachian population may be distinct, morphologically and/or genetically, from northern populations.

Conservation biology of northern saw-whet owls

In addition to addressing questions of biogeographic interest, there is a conservation-related impetus for this study. On a continental scale, the main range population of northern saw-whet owls may be experiencing a decline due to loss of nesting habitat (Cannings, 1993)

As with the northern saw-whet owl in its main range, habitat loss probably poses the most serious threat to the Queen Charlotte Island endemic, the Queen Charlotte saw-whet owl (Mullins and Tedder, 1994). Extensive logging operations are being conducted on Graham and Moresby Islands, the two largest islands in the Queen

Charlotte archipelago. These operations remove old-growth Sitka spruce (*Picea sitchensis*), western redcedar (*Thuja plicata*), western hemlock (*Tsuga heterophylla*) forests on the Queen Charlotte Islands. The Queen Charlotte saw-whet owl is considered dependent upon characteristics associated with these old-growth forests (Mullins and Tedder, 1994).

The southern Appalachian population of northern saw-whet owls may be one of the most threatened populations of these owls in North America. Between the 1880s and 1930s, logging reduced southern Appalachian spruce-fir forest from 50 to as little as 10 percent of its original areal extent (White, 1984b; Korstian, 1937). It is currently considered the second-most endangered habitat in the United States behind the Florida Everglades (Pyle, 1984; White *et al.,* 1993; Noss and Peters, 1995). Prior to legislative protection, logging removed about 25% of the spruce-fir forest in the Great Smoky Mountains National Park (Pyle, 1984). In 1929, logging began on Roan Mountain. Eight years later, all spruce and fir trees over 15 cm in diameter at breast height (dbh) were gone (Warden, 1989). Logging operations in the southern Appalachians ceased by the 1940s, but other threats quickly replaced them.

The balsam woolly adelgid (*Adelges piceae*), an insect native to Europe, was introduced into Maine at the turn of the century (White, 1984b). The first occurrence of the balsam woolly adelgid was documented by Speers (1958) on Mt. Mitchell, North Carolina in 1957. Nine years later, in 1966, approximately 200,000 fir trees were dead due to adelgid infestation (Amman, 1966) in the Mt. Mitchell, NC, area.

The insect has spread and now parasitizes all fir stands in the southern Appalachians (Eagar, 1984). Estimates of standing dead fir trees greater than 12 cm dbh in the southern Appalachians range from 44% on Roan Mountain to 91% in the Great Smoky Mountains (Dull *et al.*, 1988).

The adelgid attacks Fraser fir in the southern Appalachians (Eagar, 1984). It parasitizes the tree by penetrating bark with its stylet and feeds on fluids within the cortical parenchyma cells (Eagar, 1984). Substances secreted during feeding damage cells around the insertion site (Eagar, 1984) and induces changes in xylem, phloem, and vascular cambium of the host, resulting in the pre-mature formation of heartwood (Purtich, 1973). In turn, this disrupts the flow of nutrients, salts, and water from roots to the crown, eventually killing the tree (Purtich, 1973). Estimates of the interval between infestation and tree death range from three to nine years (Amman and Speers, 1965) and two to seven years (Eagar, 1984).

The demise of Fraser fir may result in population declines in fauna and flora dependent upon the microclimate provided by fir, initiating a cascade of linked extinctions (Wilson, 1992). For example, the spruce-fir moss spider (*Microhexura montivaga*), an arachnid endemic to southern Appalachian spruce-fir forests, requires damp areas to survive and inhabits the mosses found under older stands of spruce and fir. With a decrease in canopy shading, moss mats dehydrate, rendering the area uninhabitable for the spider (Harp, 1992). Busing *et al.* (1988) documented the near elimination of Fraser fir from an area on Mt. Collins in the Great Smoky Mountains

National Park due to adelgid infestation. This loss occurred immediately prior to higher levels of wind-related mortality in red spruce on the same site (Busing and Pauley, 1994). The authors speculate that increased gap sizes left by death of Fraser fir increased spruce mortality due to wind exposure.

Thus, the northern saw-whet owl in the southern Appalachians experienced a recent and dramatic decrease in the amount of available habitat due to logging. Moreover, the quality of the remaining spruce-fir is being degraded by the direct and indirect effects of an introduced insect, the balsam woolly adelgid. Human activities may also be impacting this forest type.

Elucidating relationships among eastern North American populations will provide conservation biologists and resource managers with data needed to construct effective management plans for this small owl. If the southern Appalachian population truly is a marginal population as defined by Mayr (1963, cited in Mayr, 1970), or if the three eastern populations form a single, large panmictic population, then the southern Appalachian population might be assigned low conservation priority. If, however, the southern Appalachian population represents the ancestral population to all other northern populations (and consequently a genetic "reservoir") or if this population is truly distinct from all other populations, then management plans should incorporate methods of preserving this threatened population.

Study objectives

A small population of northern saw-whet owls in the southern Appalachian mountains of western North Carolina, eastern Tennessee, and southern Virginia inhabit the second-most endangered ecosystem in the US (White *et al.*, 1993; Noss and Reed, 1995). Since so little is understood about the basic population biology of the species in eastern North America, confident predictions regarding the evolutionary and conservation status of this population are not possible. Three hypothetical scenarios are possible:

(1) **Current ecology hypothesis**: Due to high vagility and low breeding philopatry, eastern North American populations of northern saw-whet owls may form a large, panmictic population. Levels of genetic and morphological variation are expected to be homogenous across all populations.

(2) **Central-marginal hypothesis**: the Allegheny Plateau and southern Appalachian populations of northern saw-whet owls are marginal populations. Levels of genetic and morphological variation in these populations should be lower than that observed in the northern US-southern Canada population due to random drift and/or selection.

(3) **Glacial relict hypothesis**: founder events associated with dispersal out of a southeastern refugium still affect the present-day distribution of morphological and/or genetic variation among eastern North American populations of northern saw-whet owl. Levels of variation should be higher in southern Appalachian populations relative to higher-latitude populations.

This study used genetic data, as revealed by allozyme electrophoresis, and morphological data to determine if patterns in variation or differentiation existed among these eastern populations. If a pattern existed, the data were examined to see which of the above hypotheses best explained the pattern. Table 1 summarizes predicted patterns of genetic and morphological variation for each of the three hypotheses.

Table 1. Distribution of genetic and morphological variation among the three eastern populations as predicted by each of the three hypotheses tested in this study. See text for a description of hypotheses.

	Level of genetic and/or morphological variation		
Hypothesis	Southern Appalachians	Allegheny Plateau	Main range
Current ecology	Equal across populations	Equal across populations	Equal across populations
Central-marginal	Low	Low	High
Glacial relict	High	Moderate	Low

MATERIALS AND METHODS

Sample collection

This study sampled two subspecies of saw-whet owl from five sites and representing four populations across North America (Figure 5). These included: the southern Appalachians; Green Bay, Wisconsin; the Allegheny Plateau; Okanagan Valley, British Columbia (BC); and the Queen Charlotte Islands, BC. While all owls caught on mainland North America were *A. a. acadicus*, owls caught on the Queen Charlotte Islands were all members of the *brooksi* subspecies. Differences in breast feather and facial disk coloration distinguish the two subspecies (Fleming, 1916; Cannings, 1993).

The majority of owls were caught at night using 30 mm x 30 mm mesh mist nets. Birds were located by listening for spontaneous calling, or by playing a taped territorial call of the male saw-whet to elicit responses. Once located, mist nets were erected across flyways near the area and a speaker broadcasting the territorial call was placed at the net's midpoint. In addition to mist-netting, owls from seven nests were sampled (n = 2 from Okanagan Valley, BC; n = 2 from the Allegheny Plateau; and n = 3 in the southern Appalachians). Fledglings were caught in nestboxes; adults were netted in mist-nets situated immediately outside the nestbox entrance. Captured owls were weighed and banded with an United States Fish and Wildlife Service (USFWS)

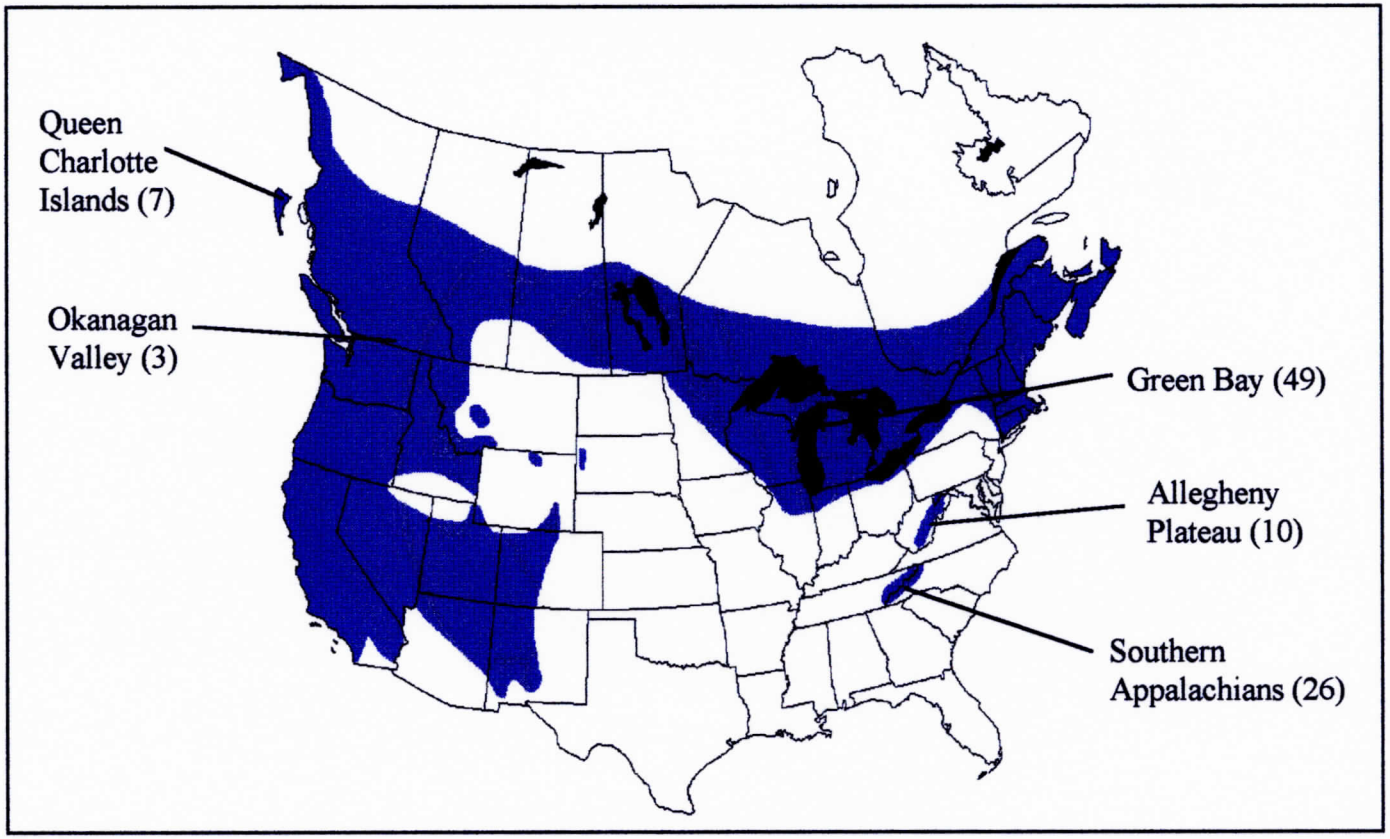

Figure 5. Breeding distribution of saw-whet owls across North America (in blue) locations of study populations, and sample sizes for allozyme electrophoresis (in parentheses). Breeding distribution is after Johnsgard (1988).

aluminum leg band for identification. The following paired morphological measures were taken from owls exhibiting fully grown flight feathers: right and left natural wing chord, right and left flattened wing chord, right and left natural secondary length, right and left alula length, and right and left third toe length. Natural wing chords were taken by measuring the distance between the wing's wrist and the tip of the longest primary, allowing for the natural curve in the bird's wing (see Pyle *et al.,* 1987). Flattened wing chords were taken in a similar manner. However, the arc formed by the feathers was gently pressed against the ruler surface to obtain the straight line distance between the wrist and the tip of the longest primary (Pyle *et al.*, 1987). All measures, excepting natural and flattened wing chords and tail length, were taken using Tajima calipers accurate to the nearest 0.1 mm. Right flattened wing chord, left flattened wing chord, and tail length were taken using a ruler accurate to the nearest 1.0 mm. Culmen length (the anterior tip of the nares to the tip of the bill), bill depth (the distance from the top of the bill, through the anterior tip of the nares to the bottom of the bill) and bill width (the distance between the two lateral sides of the bill running through the anterior portion of the nares) were also recorded using calipers (Figure 6).

In addition to morphological measures, a small blood sample was drawn via the tarsal and, occasionally, the brachial vein for genetic analysis. Other workers (*e.g.*, Bigelow *et al.*, 1977; Colwell *et al.* 1988; Dufty, 1988; Hoysak and Weatherhead, 1991; Stangel, 1986; Stagel and Lennartz 1988) report no adverse effects in other

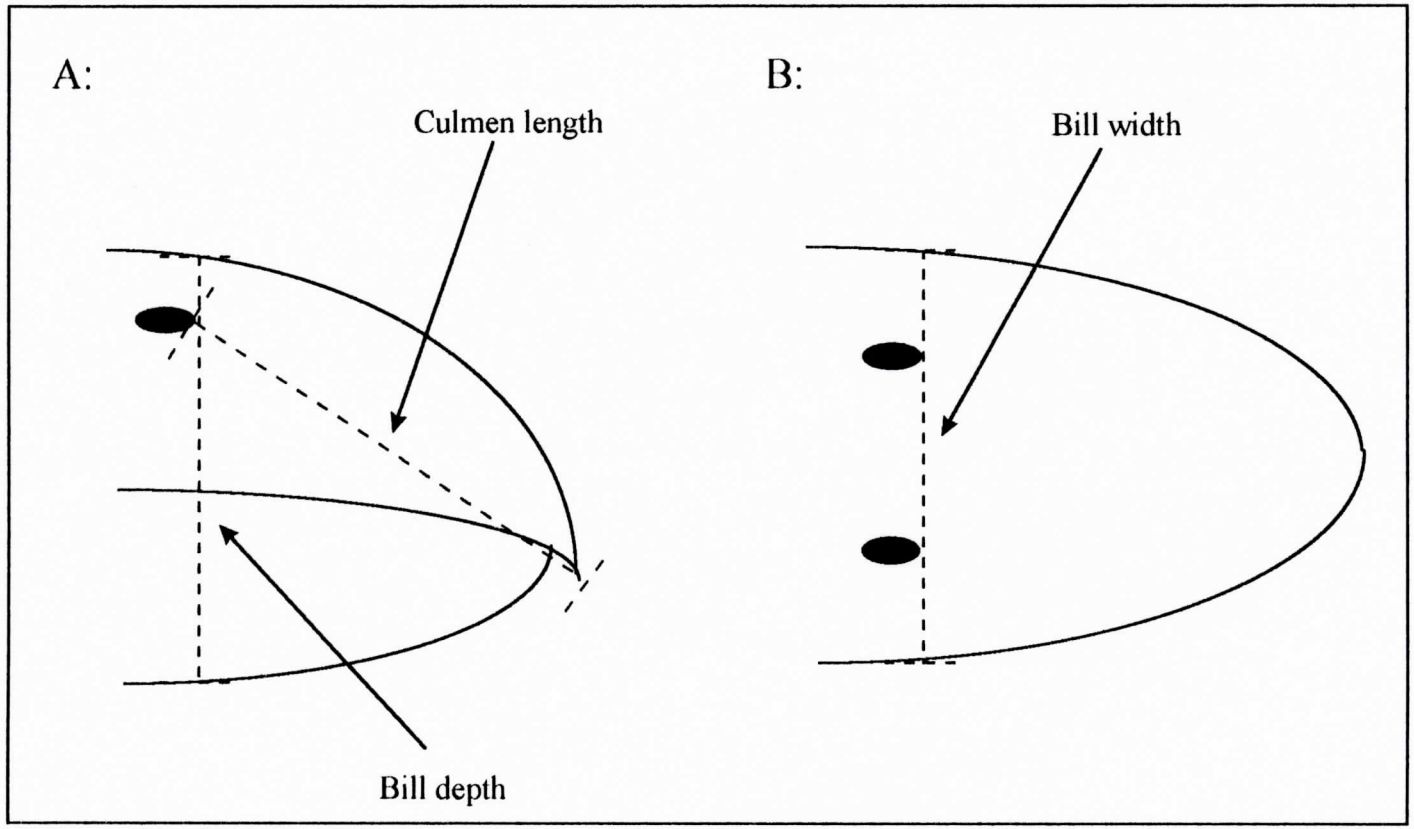

Figure 6. Pictoral representation of bill measures recorded in this study (see Materials and Methods for descriptions of bill measures). A: Lateral view of bill. B: Dorsal view of bill.

avian species (*e.g.*, red-cockaded woodpeckers (*Picoides borealis*) and red-winged blackbirds (*Agelaius phoneiceus*)) where blood was drawn using this technique. Before drawing blood, the maximum safe volume that could be taken was calculated using Evans' (1987) formula.

Blood samples were drawn using a 0.5 cc syringe tipped with a 29 gauge needle. Samples were then divided into two aliquots: one for storage in a DNA lysis buffer for future study using microsatellite DNA and the other for allozyme analysis. Aliquots intended for allozyme analysis were treated with an anti-coagulant (ammonium heparin) by injecting the blood sample into heparinized 250 µl Natelson tubes. The blood was then transferred to a sterile 1.8 ml Nalgene cryovial and frozen in liquid nitrogen within twenty minutes of collection. The samples were transported to the Department of Biology, Appalachian State University, and stored in -80°C freezers until used for genetic analyses.

Allozyme sample preparation

The following procedure was adopted to minimize enzyme degradation due to repeated freeze-thaw cycles. Before being used for allozyme electrophoresis, the samples were thawed in an ice bath. Twelve microliter aliquots of whole blood were pre-soaked onto ~4 mm by ~9 mm dits cut from Whatman #5 filter paper. The soaked dits were wrapped in heavy duty aluminum foil (two dits per foil packet), labeled with the appropriate field identification number or USFWS band number, and replaced in

the -80° C freezer. When needed for allozyme analysis, the foil packet was removed from the freezer, thawed on pre-chilled spot plates, and loaded onto starch gels. This insured that all samples experienced only two freeze-thaw cycles.

Although previous workers centrifuged whole blood samples and ran the serum and red blood cell lysate separately (*e.g.*, Barrowclough and Gutiérrez, 1990; Evans 1987), using whole blood resulted in no discernible loss of band resolution in the present (pers. obs.) and other avian allozyme studies (*e.g.*, Browne, *et al.* 1993).

Allozyme electrophoresis

Allozyme analysis was conducted on horizontal 12% Connaught Laboratories starch gels. Four gel buffers, Poulik (Selander, *et al.*, 1971), tris-citrate 6.3/6.7 (Selander, *et al.*, 1971), morpholine-citrate 6.1 (Clayton and Tretiak, 1972) and tris-hydrochloride (Selander, *et al.*, 1971), were used to screen for 11 presumptive allozyme loci (Enzyme Commission numbers in parentheses): MDH-1 and MDH-2 (1.1.1.37), PEP-B (3.4.11.-), 6PGDH-1 (1.1.1.44), PGI-1 (5.3.1.9), PGM-1 (2.7.5.1), fluorescent EST-1 (3.1.1.-), IDH-1 (1.1.1.42), LDH-1 (1.1.1.27), AAT-1 (2.6.1.1), LAP-1 (3.4.11.1). Electrophoretic conditions and staining procedures followed those described in Werth (1985), Murphy *et al.*, (1990), Selander *et al.*, (1971), and Wendel and Weeden (1989). Gel buffer and staining protocols are in Appendix A.

Allozyme data analysis

Allozyme analysis was conducted on seven individuals from the *brooksi* subspecies and eighty-nine individuals from the *acadicus* subspecies. Within *A. a. acadicus*, twenty-seven were from the southern Appalachians, ten from the Allegheny Plateau, forty-nine from Green Bay, and three from Okanagan Valley, BC (Figure 5).

Data sets were incomplete for some *A. a. acadicus* individuals due to insufficient volumes of sample. Those individuals, however, were still incorporated into the final allozyme dataset for analysis.

Data from adults and nestlings captured at the same nestbox represented non-independent data. To insure the independence of the allozyme data, only data from the parents were used. If only one parent was sampled, the genotype of the absent parent was extrapolated from offspring and mate data. Genotypes of these absentee parents were included in analyses only if the offspring and mate data indicated that a single parental genotype was possible. This was possible for only two nests; both from the southern Appalachian population.

Electrophoretic data were analyzed using BIOSYS-1, version 1.7 (Swofford and Selander, 1981). Four estimates of genetic variation were calculated using the STEP VARIAB function for each population: (1) allele frequencies; (2) percent polymorphism (%P); (3) average heterozygosity (H); and (4) mean number of alleles per locus. The first estimate, allele frequency, was calculated by summing the number

of a given allele at a locus, and dividing by the total number of alleles sampled at that locus. Percent polymorphism was calculated using the following equation:

$$\%P = \frac{\text{number of polymorphic loci}}{\text{number of loci studied}} \times 100\%$$

A locus was considered polymorphic if the frequency of the most common allele did not exceed a given maximum. This study used two criteria, a 0.95 frequency cut-off and a 0.99 frequency cut-off. This permitted comparisons with other avian genetic studies as no single criterion is consistently favored in the literature (Evans, 1987).

The third measure of genetic variation, average heterozygosity (H) was calculated in three ways. The first, $H_{obs\ (observed)}$, is based on the number of heterozygotes observed divided by the total number of individuals surveyed at that locus. The second, $H_{E\ (expected)}$, is derived from Hardy-Weinberg predictions based on observed allele frequencies at a locus and averaged across loci. The third and last—$H_{unb\ (unbiased)}$—incorporates a correction for error due to small sample sizes (Nei, 1978; Levene, 1949).

Genetic structure among populations was determined using Wright's F-statistics (Wright, 1951, 1965, 1978) and genetic distances (Nei 1972, 1978; Rogers, 1972). F-statistics were calculated using STEP FSTAT using the following formula:

$$1-F_{IT} = (1-F_{IS}) \times (1-F_{ST})$$

F_{IS} and F_{IT} are the ratios of the average heterozygosity of the individual relative to the (1) average heterozygosity of the subpopulation and (2) the average heterozygosity of the total population, respectively. These values are described by the following equations:

$$F_{IS} = \frac{H_S - H_I}{H_S} \qquad F_{IT} = \frac{H_T - H_I}{H_T}$$

where H_I is the observed heterozygosity in an individual in a subpopulation, H_S is the expected heterozygosity of an individual in the subpopulation, and H_T is the expected heterozygosity of an individual in the total population (Nei, 1977; Hartl, 1988). Thus, F_{ST} estimates the probability that two alleles drawn at random from a subpopulation have descended from the same ancestral gene (Crow and Kimura, 1970; Barrowclough, 1983). An F_{ST} value of 1 indicates complete genetic identity, whereas a value of 0 indicates complete differentiation.

Departure from Hardy-Weinberg equilibrium within a population was determined by using a chi-square test (Sokal and Rohlf, 1987). If a locus possessed more than two alleles, genotypes were pooled into three classes, one containing all heterozygotes of the most common allele with any other allele, the second containing

homozygotes for the most common allele, and the last containing all other genotypes. Pooling circumvents sampling error problems that arise when some alleles occur at low frequencies (Swofford and Selander, 1981). A statistically significant departure from Hardy-Weinberg equilibrium indicates that one or more of the assumptions (*e.g.*, selection or gene flow) of the model were violated and/or sampling bias due to small sample size (Evans, 1987).

Rogers' (1972) genetic distance (D_R) and similarity (S_R) and Nei's (1978) unbiased genetic distance (D_N) and genetic identity (I_N) were calculated using the STEP SIMDIS function. Dendrograms were constructed using Sneath and Sokal's (1973) unweighted pair group method using arithmetic averages (UPGMA). D_N (Nei, 1978) corrects for small sample size and is the measure most commonly reported in avian population genetic literature (Evans, 1987).

Gene flow acts against genetic differentiation among populations. Two methods were used to estimate levels of gene flow: Slatkin's rare allele method (1985) and Wright's (1951) method based on F_{ST} estimates. Slatkin (1985) derived an equation describing the relationship between the average frequency of private alleles occurring within a deme and *Nm*, the average number of individuals migrating between demes per generation:

$$\ln [p(i)] \cong -0.505[\ln (Nm)] - 2.440$$

N is the number of individuals per deme, m is the migration rate among demes and p(i) is the average frequency of private alleles occurring in a sample. Estimates of Nm were corrected by dividing the average sample size per population by 25 and dividing Nm by the dividend (Slatkin, 1985). Wright (1951) derived another equation for estimating gene flow and this was used as well:

$$F_{ST} \cong 1 / (1 + 4Nm)$$

Morphological analysis

Fifteen morphological measures were recorded; the majority of owls were measured by a single person to reduce inter-observer error (Nisbet, *et al.*, 1970). Prior to analysis, three decisions were made regarding inclusion or exclusion of data. First, owls were not analyzed by sex as saw-whet owls cannot be sexed in the field (D. Brinker, pers. comm.). Second, bird weight fluctuates over the course of a year (Cannings, 1993) and was therefore deemed too variable to include in the morphological analyses. This reduced the dataset to fourteen. Third, Okanagan Valley, BC, was dropped from the morphological analyses due to small sample size (n = 3).

Both one-way analysis of variance (ANOVA) and discriminant function analysis were used to analyze the morphological data using the statistical programs

BMDP, version 7.0 (BMDP Manual, 1983), and SPSS^{x}, version 3.0 (SPSS Manual, 1986). Sample sizes are reported in Appendix B. One-way ANOVAs were conducted on each of the fourteen measures to test for statistically significant differences among populations. Sequential Bonferroni tests were used with ANOVAs to adjust for the possibility of a Type I error due to multiple tests of the same null hypothesis (Rice, 1989).

In addition to the univariate analysis described above, the coefficient of variation for each morphological measure was calculated for eastern North American populations to determine if patterns of morphological variation existed among these populations. The Queen Charlotte Island subspecies was not included in this analysis as it may have had a different biogeographic history from the eastern populations (Pielou, 1991). The Okanagan Valley sample was dropped due to small sample size.

Direct discriminant analysis yields four results of particular interest: canonical variables, loading matrices of the correlations between morphological measures and canonical variables, group centroids, and a table of the frequency of correct classification of individuals to their populations based on equations derived from the morphological data. Before conducting the discriminant analysis, the set of morphological measures needed to be reduced to six (one less than the sample size of the smallest sample, the Queen Charlotte saw-whet subspecies, n = 7) to avoid overfitting the data (Tabachnick and Fidell, 1983). Characters were eliminated based on evaluation of the independence of measures, statistical correlation between pairs of

measures, and the results of a step-wise discriminant function analysis. Ten of the traits were right and left measures of the same anatomical feature (*e.g.,* right alula length and left alula length) and were taken as part of a study of fluctuating asymmetry. Because the same set of genes governs the development of right and left sides of a bilaterally symmetric organism (Waddington, 1942), paired measures were not considered independent. The high correlation between right and left measures of the same morphological feature supports this (Table 2). The right side of a paired measure was retained for analysis, under the assumption that measures of the right side would be more accurate than measures of the left, since all observers were right-handed. Right flattened wing chord and right natural wing chord were also considered non-independent, as they represent two different methods of measuring the same feature (Pyle, *et al.,* 1987). These two measures were, in fact, highly correlated (Table 2). The accuracy of measures of right natural wing chord depended upon the observer's ability to maintain the natural curve of the wing; thus, this measure appeared more sensitive to intra- and inter-observer error than right flattened wing chord, and thus, right natural wing chord was dropped. Based on these criteria, the following six characters were eliminated from further analyses: right natural wing chord, left natural wing chord, left flattened wing chord, left secondary length, left alula length, and left toe length.

A step-wise discriminant analysis was used to reduce the set of predictor variables from the eight remaining variables down to six (Tabachnick and Fidell,

Table 2. Correlation matrix for the fourteen morphological measures taken on captured birds. Correlation coefficients greater than 0.80 (bold print) were assumed to indicate interdependence and one of the variables was eliminated. Abbreviations: RNWC=right natural wing chord; LNWC=left natural wing chord length; RFWC=right flattened wing chord; LFWC=left flattened wing chord; RSL=right secondary length; LSL=left secondary length; RAL=right alula length; LAL=left alula length; RT=right toe; LT=left toe; C=culmen; BW=bill width; BD=bill depth; TL=tail length.

	RNWC	LNWC	RFWC	LFWC	RSL	LSL	RAL	LAL	RT	LT	C	BW	BD	TL
RNWC	1.0000													
LNWC	**0.91235**	1.0000												
RFWC	**0.87396**	**0.83422**	1.0000											
LFWC	0.78718	**0.86795**	**0.86510**	1.0000										
RSL	-0.07906	-0.02606	-0.11739	-0.08107	1.0000									
LSL	0.68435	0.63483	0.60802	0.53640	-0.08849	1.0000								
RAL	0.37187	0.40316	0.38457	0.39357	-0.05889	0.11547	1.0000							
LAL	0.34831	0.35871	0.30933	0.30020	-0.06618	0.13405	0.78956	1.0000						
RT	0.14040	0.10661	0.14159	0.12618	-0.26085	0.17820	0.08157	0.14209	1.0000					
LT	-0.00406	-0.00722	-0.07311	-0.06140	-0.04092	0.24078	0.01040	0.02632	0.16490	1.0000				
C	0.53343	0.53102	0.50629	0.48822	-0.12786	0.31151	0.33328	0.24171	0.18146	0.14896	1.0000			
BW	0.17815	0.20303	0.22151	0.20737	-0.22214	0.01143	0.26715	0.28000	-0.05482	0.00168	0.29659	1.0000		
BD	0.31030	0.35907	0.38345	0.38292	-0.05374	0.29614	0.05366	-0.06104	0.08976	0.02117	0.53392	0.24874	1.0000	
TL	0.59559	0.64362	0.56720	0.55728	-0.03313	0.40784	0.36370	0.27140	0.10982	-0.10369	0.37234	0.06098	0.31319	1.0000

1983). Step-wise analysis, in sequential order, identifies the predictor variable (*i.e.*, morphological measure) that best explains the variation remaining among grouping variables (*i.e.*, population designation) after entry of higher-order variables into the analysis. Step-wise analysis identified bill depth and right alula length as the two least important measures for discriminating among populations; thus, both of the measures were eliminated from further analyses. The remaining set of morphological variables consisted of right flattened wing chord, right secondary length, right toe length, culmen length, bill width, and tail length. This final set was used in the direct discriminant analysis.

RESULTS

Genetic variation within populations

Under the least stringent criterion (see Materials and Methods), a locus was considered polymorphic if the frequency of the most common allele in a population did not exceed 0.99. Using this cut-off, four of the eleven loci (36.4%) were polymorphic across the study populations: PEP-B, 6PGDH-1, Fl-EST-1, and PGM-1 (Table 3). All other loci, LDH-1, MDH-1, MDH-2, AAT-1, GPI-1, IDH-1, and LAP-1, were fixed for the same allele across all populations. Polymorphism levels within each population varied. Levels in the southern Appalachian and Green Bay populations were the highest at 27.27%, followed by the Allegheny Plateau population with a polymorphism level of 9.09%. All loci in the Queen Charlotte population and Okanagan Valley, BC population were monomorphic (Table 3). Under the more restrictive 0.95 criterion, polymorphism levels for each population fell (Table 3); no population, excepting the Allegheny Plateau population, had polymorphic loci.

The average number of alleles per locus per population ranged from 1.00 in the Queen Charlotte and Okanagan Valley populations to 1.36 in the southern Appalachian population (Table 3). Allegheny Plateau (mean = 1.09 alleles per locus) was intermediate between the southern Appalachian and Green Bay (mean = 1.27 alleles per locus) populations.

Table 3. Measures of genetic variation in four populations of northern saw-whet owl and one population of Queen Charlotte saw-whet owl. Standard errors are in parentheses.

Population	Mean # of alleles per locus	%P †	%P ‡	H_E	H_{obs}	H_{unb}
Southern Appalachians	1.36 (0.20)	27.27	0.00	0.013 (0.007)	0.014 (0.008)	0.014 (0.008)
Allegheny Plateau	1.09 (0.09)	9.09	9.09	0.009 (0.009)	0.009 (0.009)	0.009 (0.009)
Green Bay	1.27 (0.14)	27.27	0.00	0.007 (0.004)	0.008 (0.004)	0.008 (0.004)
Okanagan Valley	1.00 (0.00)	0.00	0.00	0.000 (0.000)	0.000 (0.000)	0.000 (0.000)
Queen Charlotte Islands	1.00 (0.00)	0.00	0.00	0.000 (0.000)	0.000 (0.000)	0.000 (0.000)

† Locus considered polymorphic under the 0.99 criterion
‡ Locus considered polymorphic under the 0.95 criterion

The distribution of alleles at polymorphic loci also varied among populations. The sets of alleles in a given population, except for Fl-EST-1, were subsets of the total set of alleles found in the southern Appalachians (Table 4). Two populations possessed unique (private) alleles: Green Bay at Fl-EST-1 and the southern Appalachians at PGM-1. PEP-B varied across all three of the eastern North American populations, but no allele at this locus was characteristic of a particular population. The southern Appalachian population held all three alleles at the locus. The Green Bay population held only two alleles at PEP-B, "1" and "2". The Allegheny Plateau population also held two alleles at the locus, "2" and "3". 6PGDH-1 varied in the southern Appalachians and Green Bay locations. No unique alleles were detected at that locus.

Conformance to Hardy-Weinberg equilibrium was tested using a X^2 test with a correction for small sample size. In the southern Appalachian population, no statistically significant departures from equilibrium were detected (6PGDH-1: X^2 = 0.000, d.f. = 1, p = 1.000; PGM-1: X^2 = 0.000, d.f. = 1, p = 1.000). PEP-B was also tested with pooling (see Materials and Methods section for a description of classes), since all three alleles occurred at this locus in this population. PEP-B genotypic frequencies did not deviate from expected (X^2 = 0.020, d.f. = 1, p = 0.889).

No statistically significant departures from equilibrium were detected at the PEP-B locus in the Allegheny Plateau population (X^2 = 0.000, d.f. = 1; p = 1.000). In the Green Bay population, allele frequencies at the three polymorphic loci did not depart from Hardy-Weinberg equilibrium (6PGDH-1: X^2 = 0.011, d.f. = 1, p = 0.917;

Table 4. The distribution of alleles among the populations studied for all polymorphic loci.

Population	Locus			
	PEP-B	6PGDH-1	PGM-1	Fl-EST-1
Green Bay	1, 3	1, 2	1	1, 2
Allegheny Plateau	2, 3	2	1	1
Southern Appalachians	1, 2, 3	1, 2	1, 2	1
Okanagan Valley	2	2	1	1
Queen Charlotte Islands	2	2	1	1

PEP-B: $X^2 = 0.000$, d.f. = 1, p = 1.000; Fl-EST-1: $X^2 = 0.000$; d.f. = 1, p = 1.000). All loci were monomorphic in the Queen Charlotte Island and Okanagan Valley populations; thus, no tests were necessary.

Mean heterozygosity for each population was calculated using the methods described in the Materials and Methods section: unbiased heterozygosity (H_{unb}), expected heterozygosity (H_E) and observed heterozygosity (H_{obs}). Correcting for small sample sizes did not affect estimates of H_{obs} (Table 3). Heterozygosity levels in the southern Appalachian and Green Bay populations exceeded the predicted levels by a slight amount. When following a north-south transect, heterozygosity estimates in each population increased, regardless of the method used (Table 3).

Genetic variation among populations

The average F_{ST} across all polymorphic loci was 0.018 (Table 5), indicating that approximately 2% of the observed genetic variation was due to among-population differences. Mean F_{IS} across polymorphic loci was -0.012. Negative values indicate heterozygote excess.

Genetic distance between populations

Two measures of genetic similarity and distance were calculated from the electrophoretic data set: Rogers' similarity (S_R) and distance (D_R) (Rogers, 1972), and Nei's identity (I_N) and distance (D_N) (Nei, 1972; Nei, 1978). Genetic distances

Table 5. Summary of Wright's (1978) F-statistics at all polymorphic loci, including mean F-statistics over all polymorphic loci.

Locus	F_{IS}	F_{IT}	F_{ST}
6PGDH-1	-0.021	-0.008	0.012
PEP-B	-0.039	-0.016	0.022
PGM-1	-0.020	-0.004	0.015
Fl-EST-1	-0.010	-0.002	0.008
Mean	-0.030	-0.012	0.018

calculated using Nei's (1978) method were less than 0.001 for all comparisons. D_R ranged from 0.000 to 0.007 (Table 6). Two comparisons were of particular interest: Green Bay versus Queen Charlotte Island and Green Bay versus the southern Appalachians. The former comparison provided an empirical estimate of the genetic distance among subspecies within this species. Between the Green Bay and southern Appalachian populations, D_R was 0.005. In contrast, the estimate of D_Rs between subspecies (*i.e.*, Green Bay vs. the Queen Charlotte Island population) was lower at 0.004. The largest D_R among the pairwise comparisons, 0.007, was between the Green Bay population and the Allegheny Plateau population. In general, D_Rs between the Green Bay and a southeastern population (*i.e.*, Allegheny Plateau or southern Appalachian) were greater than D_R observed when comparing northwestern populations (*i.e.*, the Okanagan Valley, BC or Queen Charlotte populations).

A phenogram using Rogers' (1972) genetic distance was generated using UPGMA (Figure 7). The saw-whet owl populations were grouped as follows: ((((Queen Charlotte Island + Okanagan Valley) + Green Bay) + Allegheny Plateau) + southern Appalachians).

Estimates of gene flow

Two methods of estimating gene flow were used: Slatkin's (1985) rare allele method, and an equation using Wright's F_{ST}. Two loci, PGM-1 in the Green Bay population and Fl-EST-1 in the southern Appalachian population, held private alleles

Table 6. Rogers' genetic distances, D_R, (below diagonal) and Rogers' genetic similarities, S_R,(above diagonal) for pairwise comparisons among populations.

Population	1	2	3	4	5
1. Southern Appalachians	-------	0.994	0.995	0.994	0.994
2. Allegheny Plateau	0.006	-------	0.993	0.995	0.995
3. Green Bay	0.005	0.007	-------	0.996	0.996
4. Okanagan Valley	0.006	0.005	0.004	-------	1.000
5. Queen Charlotte	0.006	0.005	0.004	0.000	-------

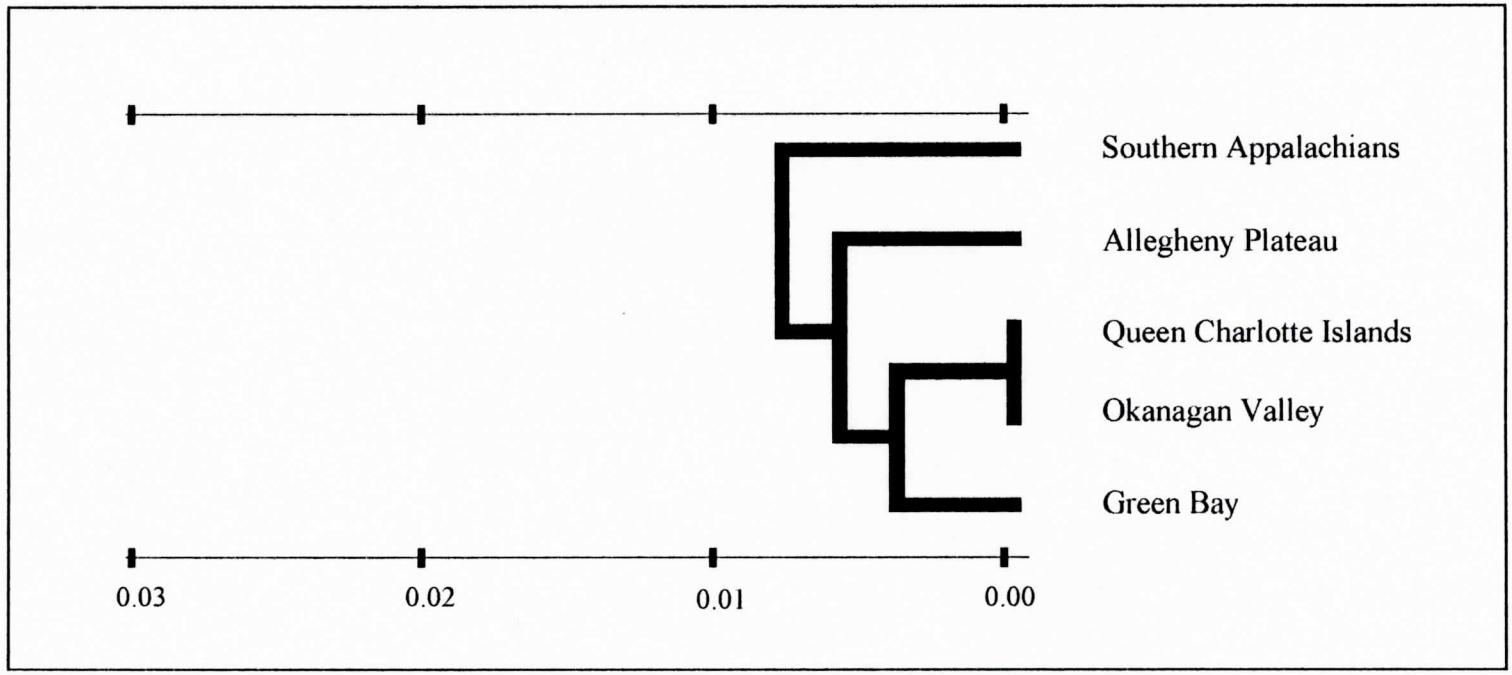

Figure 7. UPGMA dendrogram using Rogers' (1972) genetic distances.

and were used in the calculations. Slatkin's (1985) method using PGM-1 yielded an estimated *Nm* of 22.4 migrants per generation; Fl-EST-1 yielded an *Nm* equal to 79.7 migrants per generation. *Nm* estimates based on Wright's F_{ST} were lower at 13.6 migrants per generation. An *Nm* value greater than 1 indicates high levels of gene flow among populations (Avise, 1994).

Morphological variation among populations

Descriptive statistics were calculated for all morphological measures. Means, standard errors and ranges for these measures are presented in Appendix B. Several trends emerged from this analysis. Means for the Queen Charlotte Island subspecies were generally the lowest. In eight of the fourteen measures, the Queen Charlotte Island sample had the lowest mean. Only four measures, right alula length, left alula length, bill width, and tail length were the smallest in the southern Appalachian population. Right and left toe lengths were smallest in the Allegheny Plateau population. The largest mean values were observed primarily in the Green Bay and Allegheny Plateau populations. In the Green Bay population, the measures with the largest mean values among all populations were left natural wing chord, left flattened wing chord, right and left alula lengths, bill width and bill depth. The Allegheny Plateau population had the largest mean value of the four populations for the following measures: right natural wing chord, right flattened wing chord, right and left secondary lengths and culmen length. Right and left toe lengths were longest in the southern

Appalachian population and tail length was greatest in the Queen Charlotte Island subspecies.

Of the fourteen morphological measures, eight exhibited a pattern of greater morphological variation in marginal populations (*i.e.*, the southern Appalachian and Allegheny Plateau populations) relative to the central population (*i.e.*, Green Bay). These are summarized in Table 7. Coefficients of variation in five other characters were highest in the Allegheny Plateau population, followed by the Green Bay population, and finally, the southern Appalachian population. The last measure, right alula length, showed a different order: variation was highest in the southern Appalachian population, followed by the Green Bay population, and finally, by the Allegheny Plateau population.

Among the eastern populations, six of the fourteen morphological measures increased in magnitude with increasing latitude (Table 8). Right flattened wing chord, left alula, and right alula could be considered multiple measures of the same anatomical feature: the wing. Similarly, bill width and bill length are different measures of the beak. If the same broad perspective was adopted when examining all fourteen morphological measures, the set collapses down into four features: wing, bill, tail, and toe. Thus, in three of the four features—wing, beak, and tail—there is an increase in size with increasing latitude in the eastern populations (*i.e.*, Green Bay, Allegheny Plateau, and southern Appalachian populations).

Table 7. Morphological characters and populations ranked in order of increasing morphological variation. Queen Charlotte saw-whet owls were excluded from this analysis due to the different biogeographic history of this taxon (see Introduction).

Morphological character	Population ranking[†]
Right natural wing chord	AP > GB > SA
Left natural wing chord	AP > GB > SA
Right flattened wing chord	AP > GB > SA
Left flattened wing chord	AP > GB > SA
Right secondary length	SA > AP > GB
Left secondary length	SA > AP > GB
Right alula length	SA > GB> AP
Left alula length	AP > SA > GB
Right toe length	SA > AP > GB
Left toe length	SA > AP > GB
Culmen length	AP > GB > SA
Bill width	AP > SA > GB
Bill depth	SA > AP > GB
Tail length	SA > AP > GB

[†] Key to abbreviations: AP: Allegheny Plateau; GB: Green Bay; SA: southern Appalachians

Table 8. Means of morphological measures taken from the three eastern North American populations of northern saw-whet owl (standard errors in parentheses). Populations are listed in order of decreasing latitude. Sample sizes are listed in Appendix B.

Population	Right flattened wing chord	Left alula	Right alula	Bill width	Bill depth	Tail length
Green Bay	142.6 (± 0.64)	52.9 (± 0.35)	53.0 (± 0.34)	7.2 (± 0.09)	9.3 (± 0.06)	69.4 (± 0.40)
Allegheny Plateau	142.7 (± 2.26)	50.7 (± 0.68)	51.8 (± 0.84)	6.7 (± 0.28)	9.0 (± 0.29)	68.1 (± 1.40)
Southern Appalachians	137.8 (± 0.80)	50.0 (± 0.61)	50.3 (± 0.66)	6.3 (± 0.13)	9.0 (± 0.10)	66.1 (± 0.70)

Univariate analysis of the morphological data revealed significant differences among populations. One-way ANOVAs conducted on each of the fourteen morphological measures revealed that populations differed significantly in twelve of the fourteen morphological measures. The remaining two characters, right natural wing chord and left natural wing chord, approached statistical significance. When α levels were adjusted using the sequential Bonferroni method (Rice, 1989), nine of the fourteen measures showed statistically significant differences among populations (Table 9).

Direct discriminant analysis was conducted on the following set of measures: right flattened wing length, right secondary length, right toe length, bill width, culmen length, and tail length. The procedures used to reduce the original fourteen morphological measures to six are described in the Materials and Methods section.

Direct discriminant function analysis of the six morphological variables yielded three canonical variables; only the first two explained a significant amount of variation, although the third canonical variable approached significance (Table 10). Thus, only the first and second canonical variables will be discussed below. Canonical variable one accounted for 65.1% of the variation among populations. The second canonical variable explained 26.3% of the among-group variance (Table 10).

Plots of the group centroids of the canonical variables showed that the first canonical variable separated the Queen Charlotte and southern Appalachian populations from the Green Bay and Allegheny Plateau populations, although very

Table 9. Results of one-way analysis of variance conducted on each of the fourteen morphological measures using population as the independent variable. Note that with the exception of RNWC and LNWC, all measures show statistically significant differences among populations when using an α level of 0.05. Measures that were significant when one-way ANOVAs were performed with a sequential Bonferroni adjustment (Rice, 1989) are indicated by an ampersand.

Morphological measure[+]	Degrees of freedom	F ratio	p-value
RNWC	3, 86	2.6093	0.0567
LNWC	3, 89	2.6302	0.0550
RFWC	3, 86	8.2442	0.0001 &
LFWC	3, 88	8.9820	< 0.0001 &
RSL	3, 87	5.2350	0.0023 &
LSL	3, 86	7.6851	0.0001 &
RAL	3, 89	5.0332	0.0029 &
LAL	3, 89	5.9542	0.0010 &
RTL	3, 83	2.9661	0.0367
LTL	3, 83	3.0536	0.0329
CULMEN	3, 88	4.2514	0.0075 &
BWIDTH	3, 89	10.6531	< 0.0001 &
BDEPTH	3, 88	3.4610	0.0197
TAIL	3, 89	5.6714	0.0013 &

[+] Key to abbreviations: RNWC=right natural wing chord; LNWC=left natural wing chord length; RFWC=right flattened wing chord; LFWC=left flattened wing chord; RSL=right secondary length; LSL=left secondary length; RAL=right alula length; LAL=left alula length; RT=right toe; LT=left toe; CULMEN=culmen length; BW=bill width; BD=bill depth; TL=tail length.

Table 10. Summary of canonical variables derived from a direct discriminant function analysis conducted using "population" as the grouping variable and six morphological measures (see Materials and Methods for list and selection criteria) as predictor variables.

Function	Percent of variance	Cumulative percent	Chi-squared value	Degrees of freedom	p-value
1	65.12	65.12	80.742	18	< 0.0001
2	26.34	91.46	31.978	10	0.0004
3	8.54	100.00	8.443	4	0.0766

slight separation between the Green Bay and Allegheny Plateau populations is observed (Figure 8). Table 11 shows the matrix of pooled within-group correlations between canonical variables and morphological variables. Bill width and tail length load most heavily on the first canonical variable; these two morphological variables best distinguish among the populations, especially the Queen Charlotte and the southern Appalachian populations.

Right flattened wing chord, bill width, right secondary length, and culmen length load most heavily on the second canonical variable (Table 11). Again, plots of the group centroids show that the southern Appalachian and Queen Charlotte Island populations are distinct from the Green Bay and Allegheny Plateau populations (Figure 8). Note, however, the very slight separation between the Green Bay and Allegheny Plateau population.

The frequency with which an individual owl was assigned to its proper population is listed in Table 12. The Queen Charlotte saw-whet owl was never misclassified as a northern saw-whet owl. In contrast, the Allegheny Plateau sample was correctly classified only 57.1% of the time. Higher rates of correct classification were found for the Green Bay and southern Appalachian populations, at 71.2% and 76.5%, respectively.

Biases in misclassification emerged for the Green Bay and Allegheny Plateau populations (Table 12). Misclassified Allegheny Plateau saw-whet owls were invariably classified as Green Bay birds. Misclassified Green Bay saw-whet owls were

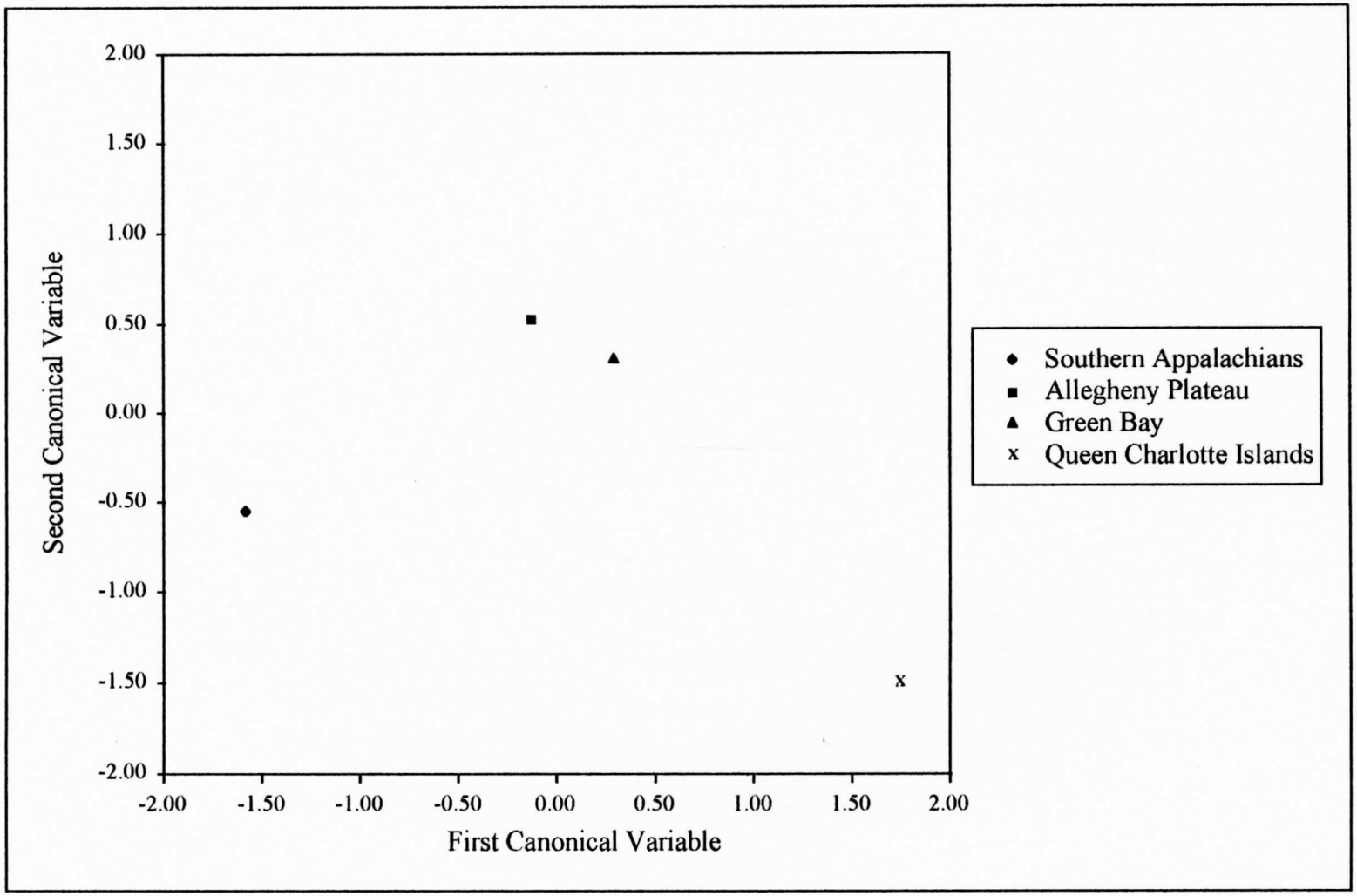

Figure 8. Plots of group centroids for the first and second discriminant functions derived from morphological data using a direct discriminant function analysis.

Table 11. Loading matrix for the three canonical variables derived from an analysis using population as a grouping variable and six morphological variables as predictor variables (see Materials and Methods for a list of morphological variables and selection criteria).

Predictor variable	Canonical variable 1	Canonical variable 2	Canonical variable 3
Tail length	0.40849	0.14783	0.19858
Culmen length	-0.07365	0.61136	0.07416
Bill width	0.45059	0.51732	0.51866
Right toe length	-0.30708	-0.11046	0.69493
Right secondary length	-0.34889	0.59807	0.25606
Right flattened wing chord	0.14664	0.79143	0.07416

Table 12. Percent correct classification tables for direct discriminant analysis using population as the grouping variable. Sample sizes are included in parentheses.

Actual population	Percent correct	Number of cases classified into each population (percent predicted group membership in parentheses)			
		Southern Appalachians	Allegheny Plateau	Green Bay	*A. a. brooksi*
Southern Appalachians (n = 17)	76.5	13 (76.5)	2 (11.8)	1 (5.9)	1 (5.9)
Allegheny Plateau (n = 7)	57.1	0 (0.0)	4 (57.1)	3 (42.9)	0 (0.0)
Green Bay (n = 52)	71.2	3 (5.8)	9 (17.3)	37 (71.3)	3 (5.8)
A. a. brooksi (n = 7)	100.0	0 (0.0)	0 (0.0)	0 (0.0)	7 (100.0)
Mean across populations	73.5				

three times more likely to be classified as Allegheny Plateau birds than as southern Appalachian or Queen Charlotte birds. No obvious biases in misclassification emerged for the southern Appalachian population. Of the four misclassified individuals, one was considered a Green Bay owl, another a Queen Charlotte saw-whet owl, and two were classified as Allegheny Plateau owls.

DISCUSSION

This study quantified genetic and morphological variation within and among northern saw-whet owl populations and attempted to elucidate the relationships among eastern North American populations of this owl. There are five parts to this Discussion. The first section focuses on genetic variation and differentiation among populations of northern saw-whet owls and compares observed levels of variation and differentiation with other avian species. Additionally, this first part evaluates the genetic data in light of the three hypotheses described in the Introduction: current ecology, central-marginal, and glacial relict. The second portion addresses patterns of morphological variation among saw-whet owl populations and discusses the patterns emerging from the data in a variety of contexts, while the third part presents studies of southeastern US taxa displaying patterns similar to those in the present study. The fourth section summarizes the results and conclusions regarding the relationships among eastern populations of northern saw-whet owls. The final portion presents arguments for implementing management plans for conservation of the southern Appalachian population of northern saw-whet owls.

Genetic variation and differentiation

Relative to other estimates of genetic variation reported in the avian literature, the estimates derived for northern saw-whet owls are low. In 1987, P.G.H. Evans published a thorough summary of population-level genetic variation in birds. He reported mean levels of allozyme polymorphism and average expected heterozygosity by avian family and across all species surveyed. The mean percent polymorphism observed across 103 avian species was 24.0% (0.99 frequency criterion). Only the southern Appalachian and Green Bay populations of saw-whet owls show polymorphism levels comparable to Evans' figures: 27.27% in both populations (Table 3). All other populations are considerably lower. As a species, northern saw-whet owls exhibit low levels of polymorphism (%P = 9.09%) relative to other avian species.

Another measure of genetic variation, average heterozygosity, is considered a better measure of genetic variation than polymorphism estimates as it does not rely on an arbitrarily defined criterion and is more precise (see Evans (1987) for a more detailed development of the argument). Evans (1987) reports an average H_{obs} of 0.044 (n = 86 species), ranging from 0.000 to 0.128. This estimate by Evans does not include any studies of strigiform species. In contrast to Evans' (1987) values, H_{obs} across all saw-whet populations was lower: 0.009. Even when comparing the saw-whet population exhibiting the highest H_{obs} (southern Appalachian, H_{obs} = 0.014) to values reported by Evans (1987) for non-strigiform species, H_{obs} is still low.

To compare levels of genetic variation in northern saw-whet owls with more closely related taxa, the avian literature was surveyed for all allozyme studies on strigiform species. Only two other allozyme studies of genetic variation in owls have been conducted: Barrowclough and Gutiérrez's (1990) study of spotted owls (*Strix occidentalis*) and Randi *et al.*'s (1991) survey of eight European owl species. In the spotted owl (*Strix occidentalis*), H_{obs} was 0.022 and percent polymorphism was 4.3% (Barrowclough and Gutiérrez, 1990). Randi *et al.* (1991) did not report percent polymorphism, H_{obs}, or H_E for any of their study species. Thus, for species where more than fifteen individuals were sampled, I calculated H_E and percent polymorphism from the allele frequencies reported by Randi *et al.* (1991). Four species met the $n > 15$ criterion: tawny owls, long-eared owls, barn owls, and little owls. The mean percent polymorphism level in saw-whet owls is comparable to that observed in other strigiform species (Table 13). However, H_E for saw-whet owls is low relative to the other four owl species.

Overall, northern saw-whet owls in this study exhibited reduced levels of genetic variation relative to other avian species, fitting the pattern of low genetic variation reported in other strigiforms (Table 13). At the family level, mean estimates of genetic variability within nine other avian families exceed estimates for the Strigidae (Table 14). This low estimate of genetic variation in owls relative to other avian species might be related to the position of owls as top-level carnivores (Barrowclough and Gutiérrez, 1990). Smaller population sizes may be associated with

Table 13. Levels of polymorphism (0.99 frequency criterion) in six Strigiform species. Data are from the present study, Barrowclough and Gutiérrez (1990) and Randi *et al.* (1991)

Species/Study	% polymorphism	H_E
Spotted owl (*Strix occidentalis*)	4.3%	Not available
Tawny owl (*Strix aluco*)	7.1%	0.031
Little owl (*Athene noctua*)	14.3%	0.029
Long-eared owl (*Asio otus*)	21.4%	0.033
Barn owl (*Tyto alba*)	7.1%	0.018
Saw-whet owl (*Aegolius acadicus*)	9.1%	0.009
Mean of strigiform species[†]	10.8%	0.028

[†] Means of percent polymorphism and H_E exclude values from the saw-whet owl

Table 14. Levels of polymorphism and mean expected heterozygosity found in allozyme studies of avian species in ten families of birds. With the exception of the Strigidae, mean percent polymorphism and mean expected heterozygosity for families are from Evans (1987).

Family	% polymorphism	H_E	References ‡
Procellaridae	32.9%	0.142	9
Anatidae	54.0%	0.236	4, 7, 13, 19
Phasianidae	22.9%	0.034	6, 15, 16, 21, 23
Picidae	13.3%	0.035	31
Tyrannidae	21.7%	0.068	25
Muscicapidae	21.1%	0.047	1, 3, 18
Emberizidae	22.1%	0.040	2, 5, 11, 13, 17, 18, 22, 24, 26
Parulidae	26.2%	0.080	3, 8, 10,
Sturnidae	14.1%	0.032	14
Strigidae†	11.8%	0.026	12, 20, present study

† Means include data from saw-whet owls

‡ 1—Avise *et al.* (1980a); 2—Avise *et al.* (1980b); 3—Avise *et al.* (1980c); 4—Bacon (1979); 5—Baker (1975); 6—Baker and Manwell (1975); 7—Barrett and Vyse (1982); 8—Barrowclough and Corbin (1978); 9—Barrowclough *et al.* (1981); 10—Barrowclough (1980); 11—Barrowclough (1983); 12—Barrowclough and Gutierrez (1990); 13—Corbin (1983); 14—Corbin *et al.*(1974); 15—Gutierrez *et al.* (1983); 16—Gyllensten *et al.* (1979); 17—Handford and Nottebohm (1976); 18—Johnson and Brown (1980); 19—Milne and Robinson (1965); 20—Randi *et al.* (1991); 21—Redfield *et al.* (1972); 22—Sibley and Corbin (1970); 23—Vohs and Carr (1969); 24—Yang and Patton (1981); 25—Zink and Johnson (1984); 26—Zink (1982)

higher trophic levels, and if heterozygosity covaries with population size, then lower levels of genetic heterozygosity might be expected for predatory birds (Barrowclough and Gutiérrez, 1990).

As with comparisons of genetic variation in northern saw-whet owls to other avian species, levels of genetic differentiation among northern saw-whet owl populations are low relative to other values reported in the avian literature. Mean F_{ST} among saw-whet populations was 0.018 across polymorphic loci (Table 5). Evans' (1987) estimate exceeds the saw-whet mean by approximately 2.5 times—mean F_{ST} across 23 avian species was 0.048, ranging from 0.004 to 0.065.

Genetic distances among eastern US northern saw-whet populations and between the two subspecies were also low. All estimates of D_N among saw-whet populations and between the two subspecies were under 0.001. Barrowclough (1980) found an average D_N among avian populations of approximately 0.0024 (n = 117 comparisons). For subspecies and species, mean D_N was 0.0048 (n = 86 comparisons) and 0.0440 (n = 71 comparisons), respectively. Genetic distances among other avian subspecies exceed all D_N values obtained in this study.

Examination of D_R values provides a slightly different perspective. Based on the empirical estimate of D_R between saw-whet owl subspecies, the southern Appalachian and Allegheny Plateau populations are at least as genetically distinct from the main range (*i.e.*, Green Bay) population as main range *acadicus* are from the *brooksi* subspecies (Table 6).

Estimated rates of gene flow among populations are high. These estimates ranged from 13.6 migrants per generation (Wright's method) to 51.1 migrants per generation (Slatkin's method). Regardless of the method used to estimate gene flow, all estimates exceed one, indicating high gene flow among populations (Avise, 1994). One migrant into a population per generation offsets genetic differentiation induced by random genetic drift (Nei, 1987).

The high vagility of birds may result in moderate to large effective population sizes, moderate levels of gene flow, or both (Barrowclough, 1983). This, in turn, might explain the low levels of population differentiation detected in birds as a class (Barrowclough, 1983). With respect to eastern populations of northern saw-whet owls, their high vagility (T. Erdman, pers. comm.) and potentially low breeding philopatry (Cannings, 1993) may result in high migration rates among all populations and thus might explain the low levels of population genetic differentiation.

Taken together, low genetic distances, low F_{ST} values, and high estimated rates of gene flow among populations support the possibility that eastern populations of northern saw-whet owls form a larger, panmictic population (current ecology hypothesis), despite spatial separation of breeding populations (Johnsgard, 1988; Figure 4).

Patterns of the distribution of genetic variation, however, support the glacial relict hypothesis. If, during glacial retreat, owls colonized newly available habitat following a stepping-stone model (Futuyma, 1986), this dispersal pattern might

generate a gradient of genetic diversity that decreased from south to north. That is, if a genetic subset of owls inhabiting southeastern refugia founded a daughter population on the Allegheny Plateau and a subset of the Allegheny population produced another population ancestral to the present-day main range population, lower levels of genetic variation in daughter populations relative to the southern Appalachian population would be expected.

Some of the patterns of genetic variation follow these predictions. For example, the southern Appalachian population holds sixteen of the seventeen alleles detected across all loci in this study (Table 4). All other populations hold subsets of these alleles, with the exception of one allele in the Green Bay population. That population holds an extra, unique allele at the Fl-EST-1 locus. Additionally, genetic variability declines with increasing latitude (Table 3): the southern Appalachian population exhibits the highest levels of genetic heterozygosity, followed by the Allegheny Plateau population, and finally, by the Green Bay population. The central-marginal hypothesis is not supported by these data. It predicts that southern Appalachian and Allegheny Plateau populations would hold lower levels of genetic variation relative to the main range population (Table 1).

Weak support for the central-marginal hypothesis is provided by an UPGMA dendrogram based on D_R. This dendrogram groups the Queen Charlotte Island subspecies with the Green Bay population, followed by the Allegheny Plateau population, and ultimately, the southern Appalachian population (Figure 7). The

effects of genetic drift and/or selection could result in divergence of marginal populations from central populations (Brussard, 1984; Lesica and Allendorf, 1995), and, with respect to this study, low similarity among eastern populations might be expected. However, despite their morphological distinctiveness and potential isolation (Figure 4), the *brooksi* subspecies groups with the main range population before either the southern Appalachian or Allegheny Plateau populations. The glacial relict hypothesis predicts similar results: the southern Appalachian population should be ancestral to higher-latitude populations and that population, followed by the Allegheny Plateau population, should be the least similar to any population included in this study. Under this hypothesis, the following grouping pattern is predicted and was observed: ((Green Bay + Allegheny Plateau) + southern Appalachians). The current ecology hypothesis predicts that if eastern populations form a large, panmictic population encompassing eastern North America, these populations would be grouped together prior to being grouped with any of the other study populations. This pattern was not observed.

In conclusion, genetic data support both the current ecology hypothesis (*e.g.*, high levels of gene flow, low genetic distances, and low differentiation among populations) and glacial relict hypothesis (*e.g.*, allele distribution among populations, increased genetic variability with decreasing latitude, and an UPGMA dendrogram based on D_R). However, only weak support was found for the central-marginal hypothesis. The most explicit prediction made by the central-marginal hypothesis

(Table 1), that lower levels of genetic variation should be observed in the southern Appalachian and Allegheny Plateau populations, was unsupported.

Patterns of morphological variation

The morphological aspect of this study allowed the evaluation of patterns of morphological variation in multiple contexts. First, to test hypotheses regarding relationships among eastern populations (Table 1), the distribution of morphological variation was examined . Second, one-way ANOVAs and a multivariate regression model generated by direct discriminant function analysis determined if significant morphological differences existed among eastern populations and ascertained relative magnitudes of those differences. Based on the multivariate model, populations were examined for adherence to Amadon's (1949) seventy-five percent rule for subspecific delimitation. Finally, morphological data were inspected for patterns reported in other studies of avian populations in mainland and island situations and for consistency with two ecomorphological rules: Bergmann's rule and Allen's rule.

Coefficients of variation were calculated for the eastern populations to examine patterns predicted by central-marginal theory (Table 6; Appendix B). The Queen Charlotte Island population was excluded as that population may have had a different biogeographic history (Pielou, 1991; Warner *et al.*, 1982) and different evolutionary forces may act upon island populations (*e.g.*, Grant, 1965). In nine of the fourteen measures, morphological variation was greater in both of the marginal populations,

southern Appalachian and Allegheny Plateau, than in the central (Green Bay) population. The main range population never exhibited the highest level of morphological variation for any of the measures (Table 6). These results are incompatible with the predictions of the central-marginal hypothesis: levels of morphological variation were not higher, but lower, in central populations relative to marginal populations. Additionally, these results are contrary to the predictions of the current ecology hypothesis (Table 1), which predicts uniform levels of morphological variation across all three eastern populations. Although not completely consistent with the predictions of the glacial relict hypothesis, as a cline of decreasing morphological variation with increasing latitude was not consistently observed, the data may best fit the expectations of this hypothesis.

One-way ANOVAs revealed statistically significant morphological differences among populations in nine of the fourteen characters examined in this study (Table 8). These results were not predicted by the current ecology hypothesis, but may be consistent with the predictions of the other two hypotheses. Under the current ecology hypothesis, which predicts that the three eastern populations form a larger panmictic population, no significant differences among populations should be observed. Results do, however, support the central-marginal and glacial relict hypotheses. Since either a relictual or marginal population might be isolated from the main range population and thus subject to the effects of selection and/or random drift (Brussard, 1984; Lesica and

Allendorf, 1995), morphological divergence of the southern Appalachian and Allegheny Plateau from the main range population is predicted.

To assay the magnitude of morphological differences among populations, direct discriminant function analysis was used. This analysis yields a multivariate regression model that can then be used to classify cases (*i.e.*, individual birds) into populations (Tabachnick and Fidell, 1983). This classification model is useful for the following reason. In 1949, Dean Amadon proposed a seventy-five percent rule for delineating subspecies. The rule states that if a population can be distinguished from all other over-lapping populations seventy-five percent of the time, that population may be distinct enough to be considered a subspecies. Ornithologists still accept and use Amadon's (1949) rule. For example, Orthemeyer *et al.*, (1995) utilized this rule as a first step in delimiting subspecies in greater white-fronted geese (*Anser albifrons*). Two populations, the Queen Charlotte saw-whet owl population and the southern Appalachian population, exceeded Amadon's (1949) rule, with correct classification rates of 100.0% and 76.5%, respectively (Table 12). In view of these results, Queen Charlotte saw-whet owls appear to be distinct from the mainland subspecies with regard to coloration (Fleming, 1916) and external morphology. Additionally, southern Appalachian owls appear morphologically distinct from the two other eastern populations.

Plots of the group centroids for the two statistically significant canonical variates (Figure 8) illustrate that while the southern Appalachian and Queen Charlotte

Island populations are well-separated from other populations, the Green Bay and Allegheny Plateau populations cannot be easily discriminated from each other. Biases in misclassifications also show substantial morphological overlap between the Green Bay and Allegheny Plateau populations (Table 12).

Taken together, the frequency of correct classification and distinctiveness of the southern Appalachian population from other eastern populations support the glacial relict hypothesis. These results only weakly support central-marginal theory. Random drift and/or selection in marginal populations can sometimes lead to divergence from the central population (Brussard, 1984; Lesica and Allendorf, 1995), and generate morphological patterns similar to that observed in eastern populations of northern saw-whet owls. However, these data do not support the current ecology hypothesis, as it predicts that eastern populations should be morphologically uniform.

It is noteworthy that although misclassifications of Green Bay birds are biased toward classification as Allegheny owls and *vice versa*, suggesting high morphological similarity between the populations, D_R between those populations is the greatest (D_R = 0.007) observed in this study. Barrowclough and Johnson (1988) point out that some phenotypically well-differentiated avian species exhibit lower levels of genetic differentiation relative to other avian species not characterized by high levels of phenetic differentiation. For example, Zink and Dittman (1993a) found no concordance between mtDNA differentiation and differences in morphology and/or plumage characters in the song sparrow (*Melospiza melodia*), a species displaying

considerable variation in plumage and morphology. The same was observed for chipping sparrows (*Spizella passerina*, Zink and Dittman, 1993b), despite a sampling design encompassing three subspecies. Zink (1994) did detect four groups of mtDNA haplotypes corresponding to plumage coloration in fox sparrows (*Passerella iliaca*). The disparity between genetic distance and morphology when comparing the Allegheny Plateau and Green Bay populations of northern saw-whet owls might indicate different evolutionary rates between genes controlling plumage characters and loci surveyed.

Although the morphological patterns emerging from the eastern populations appear consistent with at least one of the three hypotheses tested (Table 1), results derived from this study are inconsistent with general patterns observed by other ornithologists performing island versus mainland comparisons. Mainland populations of birds usually exhibit smaller morphological traits, most notably in bill size (Case, 1978), than island populations (*e.g.,* Grant (1965), Freeman-Gallant, (1996)). However, of the fourteen morphological measures recorded, the Queen Charlotte Island saw-whet owl was smallest (n = 8) or second smallest (n = 3) in eleven characters when compared to mainland populations (Appendix B).

The reduced size of Queen Charlotte Island owls relative to mainland conspecifics does, however, fit patterns observed in certain avian species (Thibault *et al.*, 1995) and some non-avian taxa including lagomorphs, artiodactyls, bats, and lizards (see Foster (1964) and Case (1978) for a complete listing). Evolution of

decreased body size in insular situations may be attributed to one of the following (Case, 1978): (1) prey size preferences of predators on a species or population or (2) abiotic and biotic constraints on body size. If predators selectively take larger prey, small size would be selected over larger in the prey species. Predators of Queen Charlotte saw-whet owls might include great horned owls (*Bubo virginianus*), but whether these animals selectively prey upon Queen Charlotte saw-whet owls is unknown. Size in Queen Charlotte saw-whet owls may be constrained by other factors such as adaptations to navigating through heavily forested areas, but data are not available to permit the evaluation of this hypothesis.

In addition to comparisons of island and mainland populations, latitudinal gradients in size were also examined. Among populations of northern saw-whet owls in eastern North America, a cline of increasing character size with latitude was noted (Table 8). If one assumes that appendage length directly correlates with body size (James, 1970), this trend might be explained by Mayr's reformulation (1963, cited in Mayr, 1970) of Bergmann's (1847) ecomorphological rule: races of species inhabiting colder areas are larger than conspecific races occupying warmer areas. A size increase allows animals to conserve heat more efficiently, since larger size decreases the surface area to volume ratio.

Allen's ecomorphological rule predicts shorter appendages in animals occupying colder areas relative to animals in warmer areas for reasons also related to heat conservation. With shorter appendages, there is a decrease in the surface area

available to radiate heat to the environment. Clines of increasing appendage length with increasing latitude in northern saw-whet owl populations (Table 8) are the opposite of that predicted by Allen's rule.

These interpretations must be taken with caution. As illustrated below, morphological differences do not always accurately reflect underlying genetic differences. It should also be noted that twelve of the fourteen morphological measures, excluding right and left toe lengths, consist of structures that do not radiate heat (*e.g.*, feathers). Thus, many of these characters might not be expected to conform to the predictions of Bergmann's or Allen's ecomorphological rules.

In addition, variation in character morphology can be affected by environment. Frances James (1983) elegantly demonstrated that environmental factors can influence characters typically used by ornithologists (*e.g.*, tarsal length and culmen length) to quantify phenetic differences among populations. She reciprocally translocated red-winged blackbird (*Ageliaus phonecius*) nestlings between southern Florida and northern Florida sites and also from Colorado to Minnesota, while maintaining controls at each site. Significant differences between transplant and control groups were detected, indicating a non-genetic component to morphological characters.

Thus, the morphological differences detected among saw-whet populations in this study cannot be assumed to only reflect underlying genetic differences. Differentiating between environmentally-induced and genetically-induced

morphological variation among eastern saw-whet populations would require translocation experiments beyond the scope of this study.

Historical biogeographic patterns in other southeastern US taxa

Both historical events (*e.g.*, a vicariant event due to glaciation) and ecological processes (*e.g.*, immigration) influence levels of variation and mold the distribution of that variation among natural populations. Northern saw-whet owls in the southern Appalachians may be glacial relicts, isolated by the northward retreat of spruce-fir (Hubbard, 1971) following the Wisconsin glacial maximum. If historical events (such as population bottlenecks associated with dispersal out of a southeastern refugium) still affect the distribution of genetic and/or morphological variation, one would predict the existence of a gradient of decreasing variation oriented from south to north. Alternatively, southern Appalachian saw-whet owls may form a marginal population, and thus harbor low levels of variation relative to the central population. Or lastly, these owls may be members of a larger panmictic population, in which case variation should be homogenous across eastern populations.

Genetic and morphological data yielded support for the glacial relict and current ecology hypotheses, while the central-marginal hypothesis was only weakly supported. Discriminating between the glacial relict hypothesis and current ecology hypothesis is difficult based solely on the data from this study. However, if events associated with the retreat of the Laurentide Ice Sheet (*e.g.*, dispersal out of a

southeastern refugia) affected eastern populations of northern saw-whet owls, one might predict that other taxa with comparable biogeographic histories would—and indeed, do—exhibit patterns similar to those observed in this study. Thus, further support for the glacial relict hypothesis stems from population genetic studies of eastern North American temperate flora and fauna. As with eastern populations of northern saw-whet owls, the taxa described below all display patterns of decreased genetic variation with increasing latitude.

Highton and Webster (1976) used allozyme electrophoresis to examine two subspecies of red-backed salamander: *Plethodon cinereus cinereus* and *P. c. serratus* (Figure 9). Within *P. c. cinereus*, samples were grouped into two categories based on glacial history of the area (Figure 9): unglaciated populations (*i.e.*, samples from populations occupying areas that had never been glaciated, even during the Wisconsin glacial maximum), and glaciated populations (*i.e.*, populations inhabiting areas that were glaciated during the Wisconsin glacial maximum). The authors detected a greater diversity of alleles in unglaciated populations of *P. c. cinereus* relative to glaciated populations. Of the 55 alleles detected in all *P. cinereus* populations, only two alleles were unique to glaciated populations of *P. c. cinereus*. Twenty-three alleles, in contrast, were unique to unglaciated *P. c. cinereus* samples. Mean H_{obs} across unglaciated populations was higher than that of glaciated populations, 0.052 and 0.036, respectively. Based on these results, Highton and Webster (1976) conclude

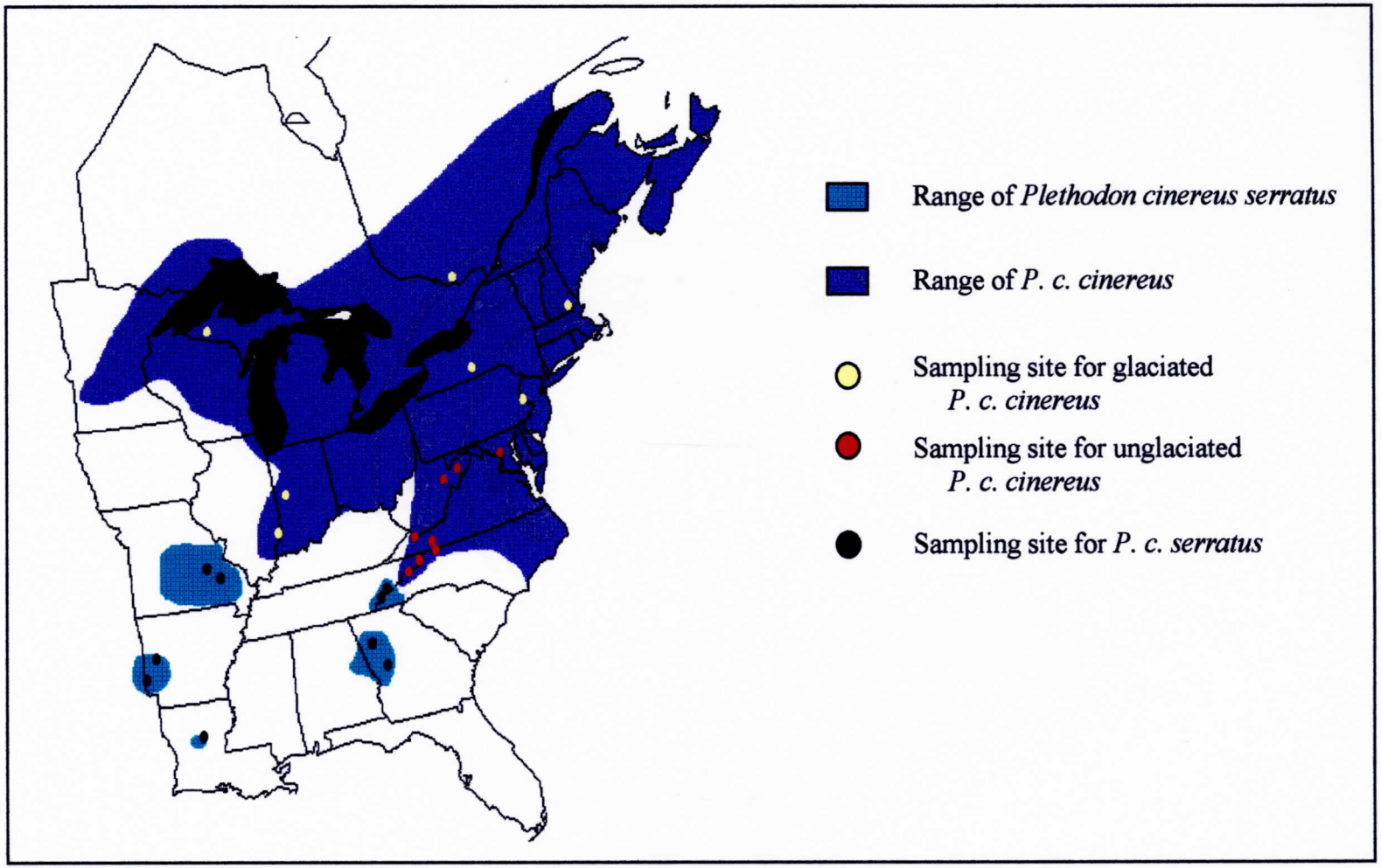

Figure 9. Distribution of *Plethodon cinereus serratus* and *P. c. cinereus*. Dots indicate sampling localities and whether the population was considered "glaciated" or "unglaciated" by Highton and Webster (1976). Map from Highton and Webster (1976).

that a genetic subset of individuals from populations occupying southeastern refugia colonized previously glaciated areas following the retreat of the Laurentide Ice Sheet.

Godt *et al.* (1995) demonstrated that southern Appalachian populations of a threatened wetland species, the swamp pink (*Helonias bullata*), held higher levels of variation, as estimated by mean number of alleles per locus and expected mean heterozygosity, than populations from New Jersey or Virginia. This suggests that dispersal out of a southeastern refugium may have been accompanied by founder events which influenced the distribution of genetic variation among swamp pink populations. It further implies that southern Appalachian populations may be glacial relicts and ancestral to higher-latitude populations.

The same pattern of higher levels of genetic diversity in southern disjunct populations relative to northern populations emerges in northern white cedar (*Thuja occidentalis*) (Walker, 1987). Walker sampled twenty-six sites across eastern North America. Five sites were from south-central Ohio (Ohio disjunct range), nine sites were sampled across the eastern half of the northern US-southern Canada (main range), and twelve sites were distributed across western North Carolina, eastern Tennessee, southeastern Kentucky, central Virginia, and central Maryland (southern disjunct range). Mean heterozygosity among undisturbed populations (*i.e.*, populations with no known history of human disturbance) of northern white cedar are highest in the southern disjunct range, 0.155, followed by the Ohio disjunct range (H = 0.120) and, finally, the main range population (H = 0.114).

Lewis and Crawford (1995) sampled species within a flowering plant genus thought to have occupied a refugium in Florida during the height of the Wisconsin glaciation. Eleven species from this genus, *Polygonella*, were sampled across the eastern United States, encompassing a range from Lake Wales, Florida to Michigan (Lewis and Crawford, 1995). These species included both wide-spread northern species (*Polygonella americana*) and endemics found only in the Lake Wales region. The northern species of *Polygonella* had lower heterozygosity levels relative to congeneric southeastern species occupying small, restricted ranges. These patterns may result from high levels of self-fertilization or rapid migration out of a southeastern refugium following the Wisconsin glacial maximum. Migration across a wide geographic area may result in the loss of alleles and thus, a reduction in genetic diversity.

Arbogast (1996) sequenced 315 base pairs of the cytochrome b mitochondrial gene in northern flying squirrels (*Glaucomys sabrinus*). From these data, he identified two distinct clades in North America. One clade extends from Alaska, across Canada, and down along the eastern US. Disjunct populations in the Alleghenies and southern Appalachians are included in this eastern clade. The second clade encompasses portions of British Columbia, Washington, and Oregon. Phylogenies for populations comprising the eastern clade placed southern populations as basal to more northerly populations, perhaps indicating dispersal out of a southeastern refugium.

At the species level, higher-latitude species of mammals exhibit reduced genetic heterozygosity relative to southern mammalian species (Sage and Wolff, 1986). Rapid expansion over a wide geographic range following the height of the Wisconsin, coupled with insufficient time to accumulate genetic differences and/or variation could explain these patterns (Gill *et al.*, 1993; Zink and Dittman, 1993b). However, Sage and Wolff (1986) adopt a wider time frame, suggesting that cycles of Pleistocene range expansions and constrictions that tracked the glacial advances and retreats resulted in reduced genetic variability in northern mammals.

Distinguishing between the current ecology and glacial relict hypotheses based solely on genetic and morphological data from eastern populations of northern saw-whet owls is difficult. However, as shown above, independent support for the glacial relict hypothesis comes from other genetic studies of southeastern US groups. These studies demonstrated that a taxonomically diverse array of southeastern flora and fauna with similar biogeographic histories exhibit concordant patterns of decreasing genetic variation with increasing latitude.

Summary of results and conclusions

Taken together, patterns of allele distribution, average observed heterozygosity, morphological variation, and the morphological and genetic distinctiveness of the southern Appalachian saw-whet population best support the glacial relict hypothesis. As outlined above, other diverse taxa in eastern North

America exhibit similar trends of increasing genetic variation with decreasing latitude, lending stronger support to the glacial relict hypothesis.

Other genetic data, however, support the current ecology hypothesis. Low levels of genetic differentiation, low genetic distances, and high gene flow rates were observed in the present study; all three are predicted if the three eastern populations are connected by high migration rates. Barrowclough (1983) suggests that low levels of population differentiation and low genetic distances are best explained by high levels of gene flow among demes and moderate to large effective population sizes. However, Robert Zink and other workers propose that rapid, post-glacial colonization coupled with insufficient time to accumulate genetic variation *in situ* might also explain low genetic distances and limited population differentiation in birds (*e.g.*, Zink and Dittman, 1993a; Gill *et al.*, 1993). Post-glacial colonization of higher-latitude areas by northern saw-whet owls in eastern North America may have been similarly rapid. If correct, then insufficient time since population isolation might explain the low levels of genetic differentiation (Table 5) observed among northern saw-whet owl populations sampled.

The morphological and genetic data do not support the predictions of central-marginal theory (*e.g.*, higher levels of genetic variation in marginal populations relative to central populations as estimated by H_{obs}, distribution of alleles among eastern populations, higher levels of morphological variation in the Allegheny Plateau population when compared to the northern US-southern Canada population).

Although geographically peripheral at present, patterns of morphological and genetic variation indicate that the southern Appalachian and Allegheny Plateau populations may better fit characteristics associated with central populations than does the northern US-southern Canada population. For example, the southern Appalachian population exhibits the highest level of genetic variation of any eastern population (Table 3) and the Allegheny Plateau population holds the highest levels of morphological variation of any eastern population (Table 7). These results would, however, support central-marginal theory, however, if viewed from the following historical biogeographic perspective. At the Wisconsin glacial maximum, the central population of northern saw-whet owls shifted into a southeastern refugium. With climatic amelioration, populations of spruce and fir forests migrated out of refugia, attaining their present-day distributions approximately 4,000 ybp (Davis, 1983). Higher-latitude populations of northern saw-whet owls, then, may be daughter populations of the southern Appalachian population, while the current population of southern Appalachian owls may represent remnants of the population that inhabited the southeastern refugium. Dispersal, range expansion of higher-latitude populations, and range contractions in the southeastern US over the intervening 18,000 years may obscure that fact.

Conservation recommendations

In the past, some biologists regarded populations on the edges of their range as low conservation priorities based on predictions from central-marginal theory. This

theory predicts greater genetic and phenotypic variation and higher population densities in central populations relative to marginal populations (Mayr, 1963, cited in Mayr, 1970). However, distinctions between marginal populations (in the sense of Mayr, 1963) and geographically peripheral populations are now being made (*e.g.*, Godt *et al.*, 1995; Hamrick and Godt, 1996). Geographically peripheral populations are populations located on the edge of a species' range and spatially disjunct from central populations (Lesica and Allendorf, 1995). This type of population may be of significant conservation value for a number of reasons. Some geographically peripheral populations are genetically and morphologically distinct from central populations and, therefore, of significant value when attempting to preserve the evolutionary potential of a species. Other geographically peripheral populations may hold more genetic variation relative to central populations due to increased heterozygote advantage in sub-optimal habitats (Lesica and Allendorf, 1992, cited in Lesica and Allendorf, 1995). A number of diverse taxa reach the southernmost limit of their ranges in the southeastern US. The post-glacial history of this area, in addition to genetic studies of extant populations, indicates that these populations may not be marginal populations, but instead, geographically peripheral populations.

The last glaciation, the Wisconsin, confined many plants and animals to a southeastern refugium. Founder events associated with dispersal out of this refugium, taking place over a short period of time and large geographic distance, may have depleted genetic and/or morphological variability in present-day northern populations.

In contrast, since southeastern populations have existed for longer periods of time relative to northern populations and have not experienced periodic bottlenecks due to founder events, these populations may have accumulated higher levels of genetic and morphological variability. Consequently, these southeastern populations may be a "reservoir" of genetic variation for a species (Walker, 1987). Northern saw-whet owls in the southern Appalachians may be one such reservoir.

In light of results from genetic and morphological analyses, management strategies for the southern Appalachian spruce-fir forests and the spruce-fir-deciduous hardwood ecotone must incorporate plans to insure the continued existence of the southern Appalachian population of northern saw-whet owls. There are several reasons for this recommendation. First, the southern Appalachian population of these owls holds the highest levels of genetic variation in a species that might be characterized by low genetic variation (Table 3; Table 13). With the exception of a single, unique allele in the northern US-southern Canada population, this population holds all of the genetic variation present in the other study populations. Thus, the southern Appalachian population may be a genetic reservoir. By ensuring its survival, managers may also be preserving a substantial portion of the evolutionary potential of this species (Beardmore, 1983). Second, contrary to the predictions of central-marginal theory, the present-day southern Appalachian population is not a marginal population in the sense of Mayr (1963, cited in Mayr, 1970). The distributions of genetic and morphological variation among eastern populations (Table 3; Table 7)

suggest that the southern Appalachian population is a geographically peripheral, rather than ecologically marginal, population. Third, the habitat favored by southern Appalachian saw-whet owls is highly endangered. Noss and Peters (1995) consider the southern Appalachian spruce-fir ecosystem as the second-most endangered ecosystem in the entire United States, ahead of other high-profile conservation areas such as the Pacific Northwest and Hawaiian Islands.

In summary, a potentially unique population of northern saw-whet owls meriting high conservation priority inhabits the southern Appalachian mountains. Habitat modification in the southern Appalachians due to the effects of the balsam woolly adelgid and anthropogenic influences (Dull *et al.*, 1988) is extensive. The progenitors of these southern Appalachian owls persisted through dramatic environmental changes during the last glaciation, surviving conditions that formed glaciers nearly a mile thick in some northern areas. It would be unfortunate, having endured such conditions in the past, if this relictual population declined due to human-induced environmental changes and simple neglect in the present.

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APPENDIX A

Gel Buffer, Electrode Buffer and Protein Stain Protocols

Gel and Electrode Buffer Protocols

Morpholine-Citrate 6.1
0.04M citric acid monohydrate 8.4 g/l
Adjust pH with ~10-12 ml of N-(3-aminopropyl)-morpholine to 6.1, then bring to final volume with dH_2O

Electrode buffer Undiluted stock solution

Gel buffer: 1:19 dilution of stock solution

Run the gel at 50 mA for 5 hours

Discontinuous Tris-Citrate (Poulik)
Electrode buffer:
0.07 M sodium hydroxide 2.40 g/l
0.30 M boric acid 18.55 g/l
pH solution to 8.2. Bring to final volume with dH_2O

Gel buffer:
0.076 M Tris-HCl 1.05 g/l
0.005 M citric acid monohydrate 9.2 g/l
Bring to final volume of 1 liter with dH_2O

Run gel at 250V until the borate line reaches the anodal sponge (~5 hours).

Tris-Citrate 6.3/6.7
Electrode buffer:
0.223 M tris 27.00 g/l
0.086 M citric acid monohydrate 18.07 g/l
pH solution to 6.3. Bring to final volume of 1 liter with dH_2O

Gel buffer:
0.008 M tris 0.97 g/l
0.003 M citric acid monohydrate 0.63 g/l
pH solution to 6.7. Bring to final volume of 400 ml with dH_2O

Run gel at 50 mA for five hours.

Tris-hydrochloric acid

Electrode buffer:

0.30 boric acid	18.55 g/l
0.06 M sodium hydroxide	2.4 g/l

Adjust pH to 8.2. Bring to final volume with dH_2O

Gel buffer:

0.01 M tris-hydrochloric acid	1.21 g/l

Adjust pH to 8.5. Bring to final volume with dH_2O

Run gel at 250V for ~5 hours

Protein Stain Protocols

All stains are from Werth (1985) unless otherwise noted. Coenzymes (*e.g.* NAD, NADP, MTT, PMS) used to visualize the stains were added just prior to staining. All gels were incubated in the dark at 37° C unless otherwise noted. Enzyme abbreviations and Enzyme Commission numbers are in parentheses.

Aspartate aminotransferase (AAT; 2.6.1.1)

0.2 M Tris-HCl, pH 8.0	25 ml
AAT substrate*	2.5 ml
Pyridoxal 5'-phosphate	1 mg
Fast Blue BB salt	75 mg

*Consists of 4% L-aspartic acid, 2% *alpha*-ketoglutaric acid at a pH of 10.0.

Cytosol aminopeptidase (CAP; 3.4.11.1)*

0.2 M Tris maleate buffer, pH 5.2	25 ml
2.5% L-leucyl-ß-naphthylamide HCl	0.5 ml

Add to gel slice, incubate in the dark for 15-30 minutes at 37° C. Next, add: 50 mg Fast Black K salt dissolved in ~3 ml of dH_2O

*Werth (1985) lists this as leucine aminopeptidase (LAP)

Esterase (fl-EST; 3.1.1.-): methylumbelliferyl method*

Solution 1

0.50 M sodium acetate, pH 5.0	15 ml
Agar	200 mg

Solution 2

0.50 M sodium acetate, pH 5.0	15 ml
4-methylumbelliferyl acetate	10 mg
dissolved in ~3 ml of 100% acetone	

Heat solution 1 with swirling until it reaches a boil. Cool the solution in an ice bath until the temperature reaches 60°C. Add solution 2 to solution 1, mix and pour on gel slice. Bands can be seen under ultraviolet light in ~2-3 minutes.

*From Wendel and Weeden, 1989

Glucose-6-phosphate isomerase (GPI; 5.3.1.9)

0.2 M Tris-HCl, pH 8.0	25 ml
1 M $MgCl_2 \cdot 6H_2O$	1 ml
Fructose-6-phosphate	25 mg
G6PDH	10 units
1% NAD	1.0 ml
1% MTT	0.5 ml
1% PMS	0.1 ml

Isocitrate dehydrogenase (IDH; 1.1.1.14)

0.2 M Tris-HCl, pH 8.0	25 ml
Isocitrate	60 mg
1 M $MgCl_2 \cdot 6H_2O$	1 ml
1% NADP	1.0 ml
1% MTT	0.5 ml
1% PMS	0.1 ml

L-Lactate dehydrogenase (LDH; 1.1.1.27)*

0.2 M Tris-HCl, pH 8.0	25 ml
DL-lithium lactate	0.25 g
1% NAD	1.0 ml
1% MTT	0.5 ml
1% PMS	0.1 ml

*Modified from Werth (1985).

Malate dehydrogenase (MDH; 1.1.1.?)

0.2 M Tris-HCl, pH 8.0	25 ml
2 M DL malate, pH 8.0	5 ml
1% NAD	1.0 ml
1% MTT	0.5 ml
1% PMS	0.1 ml

Peptidase-B (PEP-B; 3.4.-.-)*

0.2 M Tris-HCl, pH 8.0	25 ml
L-leucylglycylglycine	30 mg
Crotalus atrox venom	8 mg
Peroxidase	20 mg
o-dianisidine dihydrochloride	5 mg

Add peroxidase and *C. atrox* venom just before staining gel slice.
*Modified from Murphy *et al* (1991)

Phosphoglucomutase (PGM; 5.4.2.2)*

0.2 M Tris-HCl, pH 8.0	25 ml
alpha-D-glucose-1-phosphate	50 mg
1 M $MgCl_2 \cdot 6H_2O$	0.5 ml
G6PDH	10.0 units
1% NAD	1.0 ml
1% MTT	0.5 ml
1% PMS	0.1 ml

*Modified from Werth (1985)

Phosphogluconate dehydrogenase (6PGDH; 1.1.1.44)

0.2 M Tris-HCl, pH 8.0	25 ml
1 M $MgCl_2 \cdot 6H_2O$	1 ml
6-phosphogluconate, barium salt	20 mg
1% NADP	0.1 ml
1% MTT	0.5 ml
1% PMS	0.1 ml

APPENDIX B

Descriptive statistics by morphological measure and population

Right natural wing chord[†]

Population	N	Mean (mm)	Standard deviation (mm)	Coefficient of variation	Range (mm)
Southern Appalachians	24	135.7	3.8389	0.0283	129-142
Allegheny Plateau	7	138.9	5.9562	0.0429	132-148
Green Bay	52	138.0	4.3565	0.0316	127-146
Queen Charlotte Islands	7	134.6	4.8255	0.0359	128-140

[†] Descriptive statistics for the Okanagan Valley population are not reported for this and all subsequent measures due to small sample size.

Left natural wing chord

Population	N	Mean (mm)	Standard deviation (mm)	Coefficient of variation	Range (mm)
Southern Appalachians	25	135.3	3.7585	0.0278	130-143
Allegheny Plateau	7	137.9	6.6940	0.0486	130-146
Green Bay	54	137.9	4.9313	0.0357	127-147
Queen Charlotte Islands	7	134.3	4.4240	0.0329	128-140

Right flattened wing chord

Population	N	Mean (mm)	Standard deviation (mm)	Coefficient of variation	Range (mm)
Southern Appalachians	24	137.8	3.9340	0.0286	131-146
Allegheny Plateau	7	142.7	5.9921	0.0420	136-151
Green Bay	52	142.6	4.5977	0.0322	132-152
Queen Charlotte Islands	7	136.9	5.4598	0.0399	131-147

Left flattened wing chord

Population	N	Mean (mm)	Standard deviation (mm)	Coefficient of variation	Range (mm)
Southern Appalachians	26	137.3	4.7518	0.0346	131-151
Allegheny Plateau	7	141.0	5.7446	0.0407	132-148
Green Bay	54	143.2	5.2200	0.0365	132-154
Queen Charlotte Islands	7	137.0	4.0825	0.0298	132-143

Right secondary length

Population	N	Mean (mm)	Standard deviation (mm)	Coefficient of variation	Range (mm)
Southern Appalachians	23	97.6	5.0986	0.0522	90.8-110.0
Allegheny Plateau	7	97.7	3.5194	0.0360	92.0-102.9
Green Bay	54	97.6	3.0140	0.0308	85.3-103.6
Queen Charlotte Islands	7	92.1	2.3307	0.0253	87.8-94.9

Left secondary length

Population	N	Mean (mm)	Standard deviation (mm)	Coefficient of variation	Range (mm)
Southern Appalachians	26	98.1	4.7228	0.0480	90.6-108.0
Allegheny Plateau	7	98.0	3.8839	0.0396	91.9-103.9
Green Bay	54	97.8	2.9447	0.0301	90.9-104.5
Queen Charlotte Islands	7	91.3	2.8235	0.0309	87.5-94.3

Right alula length

Population	N	Mean (mm)	Standard deviation (mm)	Coefficient of variation	Range (mm)
Southern Appalachians	26	50.3	3.1522	0.0624	42.8-55.8
Allegheny Plateau	7	51.8	2.2150	0.0428	47.7-54.1
Green Bay	53	53.0	2.5352	0.0479	46.8-58.4
Queen Charlotte Islands	7	51.4	2.1983	0.0428	48.3-55.0

Left alula length

Population	N	Mean (mm)	Standard deviation (mm)	Coefficient of variation	Range (mm)
Southern Appalachians	26	50.0	2.8631	0.0570	41.6-55.8
Allegheny Plateau	7	50.7	4.4403	0.0876	41.0-53.4
Green Bay	54	52.9	2.5371	0.0479	47.3-59.4
Queen Charlotte Islands	7	53.0	2.6457	0.0499	50.3-57.9

Right toe length

Population	N	Mean (mm)	Standard deviation (mm)	Coefficient of variation	Range (mm)
Southern Appalachians	17	10.5	1.7013	0.1552	8.8-14.2
Allegheny Plateau	7	9.8	0.7521	0.0770	9.1-11.0
Green Bay	54	10.5	0.7134	0.0681	8.2-11.7
Queen Charlotte Islands	7	10.0	0.3132	0.0313	9.6-10.3

Left toe length

Population	N	Mean (mm)	Standard deviation (mm)	Coefficient of variation	Range (mm)
Southern Appalachians	17	10.6	1.6416	0.1500	8.7-14.2
Allegheny Plateau	7	9.8	0.9013	0.0922	8.4-11.2
Green Bay	54	10.5	0.7500	0.0712	8.1-12.3
Queen Charlotte Islands	7	10.0	0.4375	0.0438	9.4-10.7

Bill width

Population	N	Mean (mm)	Standard deviation (mm)	Coefficient of variation	Range (mm)
Southern Appalachians	26	6.3	0.6680	0.1065	5.1-7.6
Allegheny Plateau	7	6.7	0.7313	0.1094	5.3-7.4
Green Bay	54	7.2	0.6398	0.0895	5.6-8.6
Queen Charlotte Islands	7	6.9	0.5563	0.0811	6.0-7.6

Culmen length

Population	N	Mean (mm)	Standard deviation (mm)	Coefficient of variation	Range (mm)
Southern Appalachians	25	10.0	0.4720	0.0471	9.3-11.1
Allegheny Plateau	7	10.4	0.9502	0.0917	8.8-11.4
Green Bay	54	10.3	0.5232	0.0508	8.7-11.3
Queen Charlotte Islands	7	9.6	0.6532	0.0680	8.7-10.2

Bill depth

Population	N	Mean (mm)	Standard deviation (mm)	Coefficient of variation	Range (mm)
Southern Appalachians	24	9.0	0.8302	0.0935	8.1-10.0
Allegheny Plateau	7	9.0	0.7603	0.0846	8.2-10.0
Green Bay	54	9.3	0.4334	0.0468	8.4-10.5
Queen Charlotte Islands	7	8.7	0.5127	0.0586	8.2-9.7

Tail length

Population	N	Mean (mm)	Standard deviation (mm)	Coefficient of variation	Range (mm)
Southern Appalachians	26	66.1	3.7027	0.0559	58-75
Allegheny Plateau	7	68.1	3.6710	0.0539	64-74
Green Bay	54	69.4	3.2602	0.0469	62-75
Queen Charlotte Islands	7	70.4	3.2071	0.0455	66-76

VITA

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GENETIC AND MORPHOLOGICAL VARIATION IN NORTHERN SAW-WHET OWL POPULATIONS IN EASTERN NORTH AMERICA

A Thesis

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