PHYLOGEOGRAPHY AND POPULATION GENETICS OF NORTHERN FLYING

SQUIRRELS (*GLAUCOMYS SABRINUS*) IN SOUTHEAST ALASKA

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SQUIRRELS (*GLAUCOMYS SABRINUS*) IN SOUTHEAST ALASKA

A

THESIS

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ABSTRACT

The Prince of Wales flying squirrel (*Glaucomys sabrinus griseifrons*), a forest associated species, is endemic to several islands in the Alexander Archipelago of Southeast Alaska. Mitochondrial and nuclear markers were examined to assess the genetic uniqueness of this subspecies and its geographic extent and to investigate gene flow among island and mainland populations of flying squirrels. Data from both sets of markers are congruent, and agree with the subspecific designation. The data also indicate that the Prince of Wales subspecies is isolated from other populations in Southeast Alaska, but that there may be gene flow among islands on which it occurs. This island lineage is likely the result of a founder event after the retreat of the Pleistocene ice sheets. The fact that this subspecies is isolated and divergent from mainland populations has potential implications for the design and planning of timber harvests on these islands.

TABLE OF CONTENTS

LIST OF FIGURES

LIST OF TABLES

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INTRODUCTION

Before the explosion of molecular methods in recent years, investigations into microevolutionary processes such as gene flow and population differentiation in animals were somewhat difficult in the field, requiring painstaking care in documenting individual movements, mating, and character states of multiple quantitative traits. DNA sequencing, allozyme and restriction fragment length polymorphism scoring, and nuclear microsatellite typing have all contributed to making these types of studies easier (Avise, 1994). DNA markers may be particularly valuable in identifying isolated stocks or populations; the recognition that certain populations may be unique may lead to management for the maintenance of distinct genetic lineages (Waples, 1991; Moritz, 1994).

My study uses two of these techniques to investigate population divergence and gene flow among flying squirrels (*Glaucomys sabrinus*) on the islands of the Alexander Archipelago in Southeast Alaska. The Prince of Wales flying squirrel (*G. s. griseifrons*) is endemic to several islands, and was originally described on the basis of the pelage coloration of two specimens (Howell, 1934). The new ten-year forest plan mandates the US Forest Service to manage and conserve endemics in the Tongass National Forest, which includes much of the archipelago (USDA, 1997). Until recently there has been little investigation of the distribution, differentiation, or taxonomic status of mammalian endemics. In Chapter 1, I expand a preliminary study (Demboski et al., 1998) of the distribution and extent of molecular divergence of this subspecies using mitochondrial DNA sequences. Chapter 2 explores the history of colonization and gene flow among the islands of the archipelago using microsatellite markers. Conservation and management of this island endemic is addressed in light of the information gathered from this molecular data.

I conducted all laboratory and analytical work described in these chapters, with technical support from the Institute of Arctic Biology Core DNA Sequencing

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Chapter 1

Reduced genetic variation in insular northern flying squirrels (*Glaucomys sabrinus*) along the north Pacific coast[1]

ABSTRACT

Nearshore oceanic archipelagos are valuable laboratories that could provide insight into evolutionary processes such as founder effects and incipient speciation. The Alexander Archipelago of Southeast Alaska is an example of such a complex, yet few biological investigations have been conducted here. For the past fifty years, the region has experienced intense anthropogenic disturbances (particularly timber harvesting), causing habitat fragmentation and potential disruption of biotic communities. As part of a series of studies of mammals endemic to Southeast Alaska, we examined mitochondrial DNA sequences from 118 flying squirrels to investigate genetic diversity across Southeast Alaska. Mitochondrial sequence divergence is in agreement with the subspecific designation of the Prince of Wales flying squirrel (*Glaucomys sabrinus griseifrons*). This island lineage may be the result of an early Holocene founder event, because 52 of 53 animals on Prince of Wales Island and ten islands to the west had identical cytochrome b sequences, and all individuals examined for control region (n=21) were also identical. In contrast, substantial polymorphism and little genetic structure were found in populations across the rest of Southeast and Interior Alaska. Because flying squirrels in the Pacific Northwest are associated with old-growth forest, forest-use plans should aim to conserve this unique lineage of island squirrels.

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[1]Bidlack, A. L. & Cook, J. A. (submitted). Reduced genetic variation in insular northern flying squirrels (*Glaucomys sabrinus*) along the North Pacific Coast. *Animal Conservation.*

INTRODUCTION

Archipelagos play a prominent role in conservation because they often harbor endemic species, which are especially susceptible to extinction (Diamond, 1989), and they may offer refuge for species that are threatened or extinct on the mainland due to predation, competition or habitat loss. Considerable attention has been paid to loss of diversity on remote oceanic archipelagos, like the Hawaiian and Galapagos islands, because of their unique flora and fauna. Less research has focused on nearshore oceanic archipelagos, though similar evolutionary forces shape these ecosystems. Often these islands harbor endemic taxa and unique combinations of plants and animals not found on the mainland (e.g., Cook & MacDonald, in press); they also provide opportunities to explore metapopulation dynamics, such as colonization and extinction (e.g., Lomolino, 1994; Giles & Goudet, 1997; Conroy, Demboski & Cook, 1999). In particular, founder events can impact the genetic variation found in island populations and lead to divergent evolutionary lineages. With the exception of Ranta et al. (1999), conservation on these nearshore archipelagos has received little attention in the literature.

High numbers of nominal endemic species and subspecies occur along the North Pacific Coast of North America. Twenty four taxa of mammals are considered endemic to Southeast Alaska, with 12 others largely confined to the region (MacDonald & Cook, 1996). Southeast Alaska has a dynamic history of glaciations, oceanic transgressions, isostatic rebound and ecosystem change (Ager, 1983; Mobley, 1988; Mann & Hamilton, 1995; Barrie & Conway, 1999), which has contributed to the biogeographic patterns we see today. Many endemics are spread over the Alexander Archipelago, consisting of over 2,000 named islands (Figure 1). This complex landscape has been further fragmented by extensive timber harvests and road building during the past 50 years (Durbin, 1999). Rudimentary inventory work has led to uncertainty over the distribution and abundance of endemic taxa. Recent work is shedding light on the phylogeography of mammals in the region and highlighting the need to incorporate information on endemics and evolutionary processes on islands into management plans (Cook et al., in press).

Northern flying squirrels (*Glaucomys sabrinus*) are highly associated with old-growth forest in the Pacific Northwest (Carey, 1995; Carey, 1996; but see Rosenberg, 1992). They consume and disseminate spores of ectomycorrhizal fungi that are symbiotic with many temperate forest plant species, and they are important prey items of many avian and mammalian predators (Maser, Maser & Trappe, 1985; Maser et al., 1986; Carey et al., 1999). The Prince of Wales flying squirrel (*G. s. griseifrons*) has been the focus of conservation concern (Demboski, Cook & Kirkland, 1998a) because Prince of Wales Island in the Alexander Archipelago of Southeast Alaska has been heavily deforested and extensive future timber harvests are planned (Table 1).

Demboski, Jacobsen & Cook (1998b) provided a preliminary assessment of geographic variation in northern flying squirrels in Southeast Alaska using sequences of the mitochondrial cytochrome b gene. They characterized animals representing three subspecies from Alaska (*G.s. griseifrons*, *G.s. zapheus*, *G.s. yukonensis*), and found that animals from Prince of Wales Island, and eight smaller islands to the west of Prince of Wales (the POW complex), shared two diagnostic base pair mutations. Because the control region of the mitochondrial genome evolves at a higher rate than cytochrome b, it usually provides greater resolution of recent evolutionary and population-level events (e.g., Thomas et al., 1990; Encalada et al., 1996; Pope, Sharp & Moritz, 1996; Good et al., 1997). We examined control region sequences from flying squirrels from the Pacific Northwest and expanded Demboski et al.'s (1998b) data set by adding complete cytochrome b sequences for animals from an additional four islands and three mainland areas. We investigate colonization history, founder effects and genetic diversity in flying squirrels along the north Pacific coast, and discuss the impact of current land uses on the endemic Prince of Wales flying squirrel.

MATERIALS AND METHODS

Heart tissue housed in the Alaska Frozen Tissue Collection at the University of Alaska Museum from 118 specimens (representing 15 islands and eight mainland

populations) was used for DNA extraction. Voucher specimens associated with these tissue samples are also housed in the University of Alaska Museum. Specimens, including many provided by marten trappers, were sampled from 22 locations (sample sizes in parentheses): Prince of Wales I. (21), Tuxekan I. (2), Suemez I. (6), Orr I. (2), Heceta I. (4), El Capitan I. (6), Barrier Islands (12), Kosciusko I. (3), Dall I. (1), Revillagigedo I. (4), Wrangell I. (1), Etolin I. (1), Mitkof I. (9), Chilkat Peninsula (2), Skagway (1), Haines (1), Juneau (3), Rudyerd Bay (1), Cleveland Peninsula (20), Yukon Territory (1), Interior Alaska (15), and Washington state (2). Extraction followed a protocol modified from Miller, Dykes & Polesky (1988). Partial (792 base pairs) and complete (1140 base pairs) cytochrome b gene sequences of 86 animals (n=49 partial; n=37 complete) were amplified using primers MVZ 04/05, 16/37, and 14/23 (Smith & Patton, 1993). 350 base pairs from the 5' end of the mitochondrial control region were obtained from 43 of these 86 individuals using primers TDKD (5' – CCT GAA GTA GGA ACC AGA TG; Kocher et al., 1993) and CTRL-L (5' – CAC YWT YAA CWC CCA AAG CT). Control region sequences were obtained from an additional 34 animals which were not sequenced for cytochrome b. Sequences were amplified by polymerase chain reaction on a Perkin-Elmer 2400 thermocycler using standard protocols (Lessa & Cook, 1998). Both forward and reverse strands were sequenced on an ABI 373 Automated Sequencer and translated and aligned with Sequence Navigator Version 1.0.1 (ABI). All amplifications included negative controls. Control region and cytochrome b sequences have been deposited in GenBank.

RESULTS

Cytochrome b and control region sequences show a division between the POW complex and other populations, amplifying and clarifying the preliminary conclusions of Demboski et al. (1998b). All cytochrome b sequences (but one) from individuals from the POW complex (n=53) are identical. The single sequence that differs is from southern POW; it is identical except that it has one additional base pair transition. All 53

individuals are characterized by two synonymous base pair changes (one transition, one transversion). Cytochrome b sequences from animals from nearshore islands (Wrangell, Etolin, Mitkof and Revillagigedo) and mainland sites (Juneau, Skagway, Haines, Chilkat Peninsula, Cleveland Peninsula and Rudyerd Bay) differ from those of the POW complex by at least these two base pair changes.

Excluding the divergent Washington sequences, there are 24 variable sites in the control region (of 350 base pairs). These comprise a total of 22 haplotypes. All control region sequences from the POW complex (n=21) are identical and comprise a distinct haplotype. Similarly, all five individuals from Mitkof Island share a single haplotype, although this haplotype also occurs on the Cleveland Peninsula. In contrast to the genetically monotypic POW complex samples, two other populations from the Cleveland Peninsula and Interior Alaska with similar sample sizes (n > 14) include seven haplotypes each (Table 2). Even populations for which we were able to sequence only a few animals, such as Juneau, Revillagigedo Island, and Chilkat Peninsula, were variable in control region sequences. Only a single individual was examined for Etolin and Wrangell islands, Rudyerd Bay, Haines, and Skagway.

DISCUSSION

Mitochondrial sequences identify a reciprocally monophyletic clade on the POW complex of islands. This finding demonstrates a genetic uniqueness consistent with the original description of *G. s. griseifrons*, which was based on a morphological assessment of two specimens (Howell, 1934). Given the near genetic homogeneity of the POW clade and low but consistent level of divergence from mainland populations, our analysis indicates that the POW populations have not experienced recent genetic exchange with the mainland populations. Colonization of islands often results in loss of genetic diversity, with subsequent differentiation between the original and founder populations induced by genetic drift or differential selective regimes (Mayr, 1942).

Glaciers covered much of Southeast Alaska repeatedly during the Pleistocene, with subsequent retreat and revegetation. Continental shelf and nunatak refugia along the North Pacific Coast have been hypothesized on the basis of pollen records and plant macrofossils (Warner, Mathewes & Clague, 1982; Peteet, 1991), vertebrate fossils (Heaton, Talbot & Shields, 1996), disjunct populations of extant taxa (Worley & Jaques, 1973; Ogilvie, 1989; Heusser, 1989), and phylogeographic breaks in vertebrate and plant taxa (Wheeler & Guries, 1982; Zink & Dittman, 1993; O'Reilly et al., 1993; Byun, Koop & Reimchen, 1997; Soltis et al., 1997). Despite the large amount of paleobotanical, archaeological, geological, molecular, and morphological data that have been collected, no concrete evidence exists for the presence of unglaciated areas with persistent populations of vertebrates during maximum glaciation. If substantial periglacial refugia existed, it seems doubtful that forest-associated species (e.g., flying squirrels) would have persisted in Southeast Alaska during the last glacial maximum. Pollen records and plant macrofossils indicate only alpine tundra-like vegetation shortly after maximum glaciation in Haida Gwaii (Queen Charlotte Islands) and areas in the Alexander Archipelago (Heusser, 1989; Mann & Hamilton, 1995).

Given this evidence, it seems unlikely that the POW haplotype is due to isolation in a northern coastal refugium that persisted since the last interstadial period (ca. 35,000 yr. BP). Furthermore, divergence between the POW haplotype and the mainland is shallow (~ 0.03%). In contrast, there is some evidence that the POW complex and Haida Gwaii supported refugial populations of ermine (*Mustela erminea*). Cytochrome b sequences indicate a 1.2-3.0% split for ermine populations from the POW complex (including Haida Gwaii) and populations from elsewhere in Beringia and southern North America (Fleming & Cook, submitted). We suspect that the distinctive haplotype that is shared among flying squirrel across the POW complex may be due to a founder event early in the Holocene, after the retreat of the glacial ice. Alternatively, the POW haplotype may have arisen elsewhere along the coast and recolonized following deglaciation. Sampling design for both flying squirrels and ermine may have failed to

detect the POW haplotype elsewhere, and particular effort should focus farther south along the west coast of British Columbia.

Arbogast (1999) reported a major break (4.3-7.2%) between eastern (Maine to Alaska) and western (California to Washington) clades of *G. sabrinus* based on partial cytochrome b sequences. He suggested that these were separated in refugia south of the ice sheets during the last glaciation. He found < 2% sequence divergence within the eastern clade, and suggested a rapid westward expansion across the continent following the retreat of the Laurentide and Cordilleran ice sheets. Our control region sequences indicate little geographic structuring along the mainland, suggesting a rapid expansion into Southeast Alaska and/or high levels of gene flow throughout the region. Sequences from the POW complex of islands show a much different pattern, with near monomorphy across eleven islands.

The extremely low level of variation across populations on the eleven POW complex islands (a single autapomorphic change in one individual) suggests the possibility of a severe bottleneck in the ancestral population (Nei, Maruyama & Chakraborty, 1975). The mutations diagnostic for the POW complex would have accumulated concurrently or prior to the bottleneck, resulting in the distinct island haplotype. Alternatively, lineage sorting of the POW and mainland haplotypes may have occurred, although this seems unlikely given the relatively brief period (< 13,500 years) since the retreat of the glacial ice and the assumed large size of mainland populations. Our sampling of 39 individuals from the mainland and nearshore islands did not reveal the POW haplotype. A third possibility is a selective sweep of the mitochondrial genome in the POW complex populations. Examining nuclear loci, such as microsatellites, may help to assess this possibility because a selective sweep would not be expected to affect these independent markers.

Conservation Implications

The Tongass National Forest covers 6.8 million ha in Southeast Alaska and the current ten-year forest plan mandates the management and conservation of endemic taxa within the Tongass (United States Department of Agriculture, 1997). Flying squirrels have been managed as a single phylogenetic unit; however, mitochondrial DNA sequences identify a distinct island clade of flying squirrels within the Tongass. An evolutionarily significant unit (ESU; Ryder, 1986) is defined as a population that has been historically (and reproductively) isolated from other such populations and, hence, a lineage with distinct evolutionary potential (Waples, 1991; Moritz, 1994). The Prince of Wales flying squirrel fits this definition of an ESU, and therefore careful management is needed. Flying squirrels are associated with old-growth forest (Carey, 1995; Carey, 1996), yet habitat favored by this species has been heavily harvested and fragmented. Up to 46% of the old-growth on Forest Service land has been cut on some islands (Table 1; USDA, 2000), and much of the private land throughout Southeast Alaska (220,000 ha) has been logged (Durbin, 1999). An additional 200 million board feet of timber from old-growth forests is currently scheduled to be harvested from four islands within the range of *G. s. griseifrons* between the years 2000 and 2010 (Table 1). While there are substantial areas of old-growth forest in Southeast Alaska that are largely protected from timber harvests (e.g., Admiralty Island), most of these areas are not within the range of *G. s. griseifrons*.

This study is one of several concurrent studies of endemic mammals in the Alexander Archipelago. Molecular work is revealing a more dynamic picture of faunal colonization and differentiation in Southeast Alaska than previously thought, with both paleoendemics and neoendemics possibly present (Cook et al., in press). These biogeographic investigations provide insight into incipient speciation, gene flow, and founder effects, and reveal patterns relevant to conservation. The pronounced signal of a founder event on the POW complex highlights the value of documenting intraspecific diversity across nearshore archipelagos, particularly when large-scale anthropogenic

disturbance may impact insular endemic populations. We suggest that *G. s. griseifrons*, like some other Alexander Archipelago taxa, may be on a distinct evolutionary path. Current land use plans in the region do not adequately address the management or conservation of genetic diversity in this north Pacific coast endemic.

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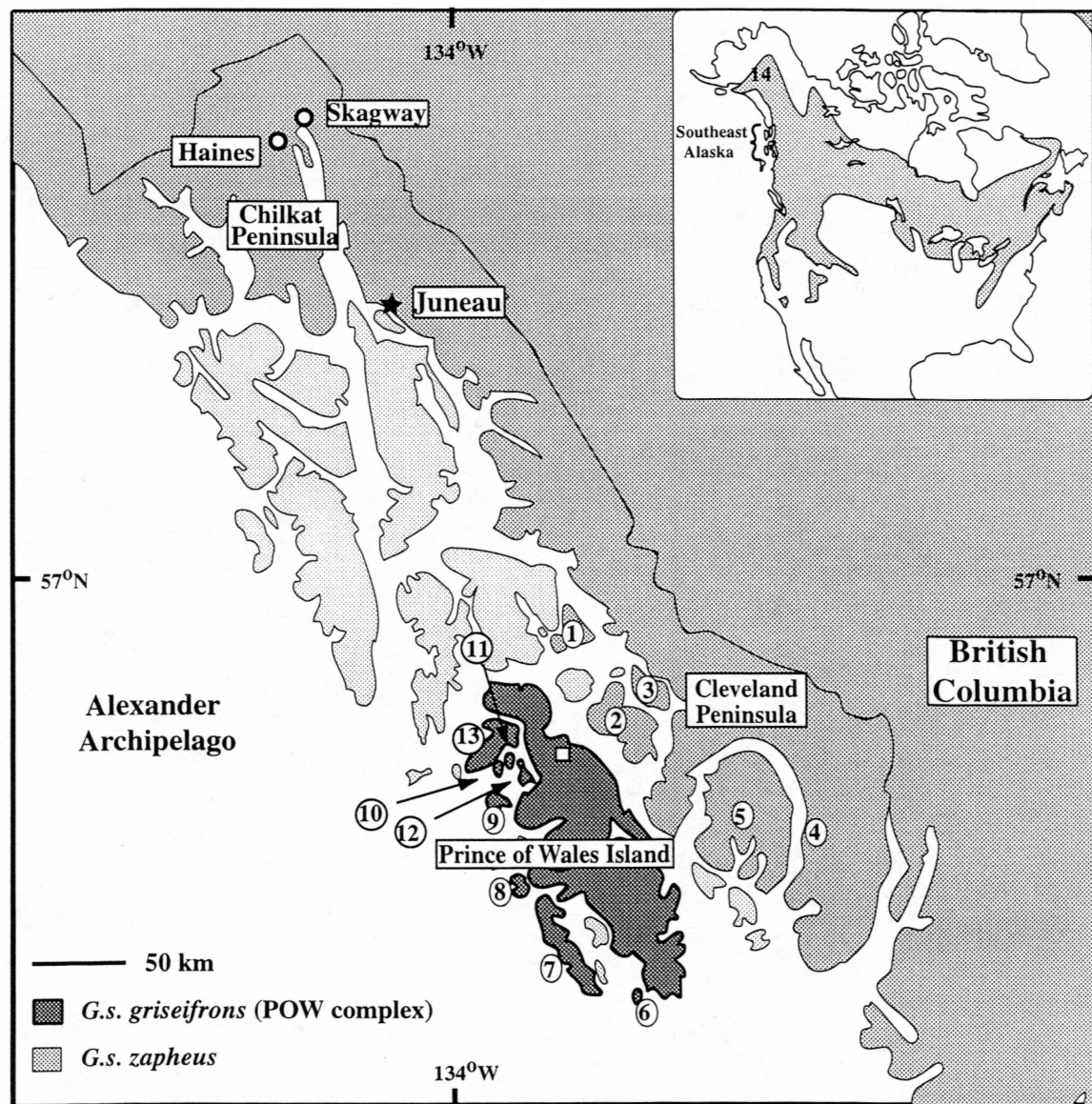
Figure 1. Distribution of flying squirrels in Southeast Alaska, including the type locality for *G. s. griseifrons* (□). Lightly shaded islands do not have flying squirrels or have not been inventoried. Inset map shows North American range of *G. sabrinus*. Numbers on map represent selected sample localities: (1) Mitkof I., (2) Etolin I., (3) Wrangell I., (4) Rudyerd Bay, (5) Revillagigedo I., (6) Barrier Island group, (7) Dall I., (8) Suemez I., (9) Heceta I., (10) Orr I., (11) El Capitan I., (12) Tuxekan I., (13) Kosciusko I., and (14) Interior Alaska. Other sampling localities indicated by name.

Table 1. Timber harvests* on POW complex islands with *G.s. griseifrons*; ROG, remaining old-growth habitat; H, harvested area; OGH, percentage of old-growth harvested to date; PTH, proposed timber harvest in million board feet (MMBF). Information is for USDA Forest Service land only.

	Island Area (ha)	ROG (ha)	H (ha)	OGH (%)	PTH (MMBF)
Barrier Islands	< 1000	498.4	0	0	0
Dall	65,820	26,046.6	1464.7	5.3	0
El Capitan	< 1000	484.2	27.3	5.3	0
Heceta	18,900	8497.0	6028.6	41.5	0
Kosciusko	48,220	20,420.3	7170.6	26.0	17
Orr	2335	1135.5	780.4	40.7	0
Prince of Wales	577,750	261,777.3	80,774.5	23.6	121-141
Suemez	15,012	8249.5	493.2	5.6	30
Tuxekan	8520	3572.1	3079.3	46.3	20

*as of April 2000

Table 2. Distribution of control region haplotypes in *G. sabrinus* in Southeast and Interior Alaska.

Subspecies	Sample Locations	Haplotypes																						Total
		A	B	C	D	E	F	G	H	I	J	K	L	M	N	O	P	Q	R	S	T	U	V	
G.s. griseifrons POW Complex	POW I.	6	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	6
	Dall I.	1	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	1
	El Capitan I.	2	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	2
	Heceta I.	2	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	2
	Kosiusko I.	2	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	2
	Orr I.	2	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	2
	Suemez I.	2	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	2
	Tuxekan I.	2	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	2
	Barrier Is.	2	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	2
G.s. zapheus	Mitkof I.	--	--	--	5	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	5
	Etolin I.	--	--	--	1	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	1
	Wrangell I.	--	--	1	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	1
	Revillagigedo I.	--	--	--	--	--	3	1	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	4
	Rudyerd Bay	--	1	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	1
	Juneau	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	3	3
	Chilkat Pen.	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	1	1	2
	Cleveland Pen.	--	--	1	6	4	--	--	--	--	--	3	3	2	--	--	--	--	1	--	--	--	--	20
	Skagway	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	1	--	--	1
	Haines	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	1	--	--	--	1
G.s. yukonensis	Interior AK	--	--	--	--	--	--	--	1	2	1	--	--	--	2	1	3	4	--	--	--	--	--	14
Total:		21	1	2	12	4	3	1	1	2	1	3	3	2	2	1	3	4	1	1	1	1	4	74

Chapter 2

Nuclear and mitochondrial perspectives on an island endemic of the Alexander Archipelago, Alaska: the Prince of Wales flying squirrel (*Glaucomys sabrinus griseifrons*)[2]

ABSTRACT

The Alexander Archipelago of Southeast Alaska has experienced intense anthropogenic disturbance in the form of timber harvesting, but there have been few investigations of the endemic fauna of the region. The Prince of Wales flying squirrel (*Glaucomys sabrinus griseifrons*) is an old-growth associated species endemic to the archipelago. Previous work showed this subspecific designation was consistent with a distinct mitochondrial lineage on eleven islands (the Prince of Wales [POW] complex). Individuals from this island complex were reciprocally monophyletic to those of the mainland and nearshore islands, and all individuals but one from the POW complex were identical. To obtain a nuclear perspective on this lineage and to investigate genetic diversity among the islands, we examined six microsatellite loci in 233 flying squirrels across Southeast and Interior Alaska. Individuals were sampled from nine populations from seven island and two mainland localities. Island populations showed lower heterozygosity and allelic diversity than mainland populations. Analyses of the microsatellite data revealed significant differences between the POW complex populations and those of the mainland and nearshore islands, congruent with the mitochondrial data. The six POW complex populations were genetically very similar, suggesting current or recent gene flow among these islands. Our data confirm

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mitochondrial DNA results indicating that *G. s. griseifrons* is genetically isolated from mainland populations and suggest that the flying squirrels of the POW complex may be diverging from those populations. To protect this evolutionary potential, knowledge of genetic variability and population structure in this subspecies should be used in planning future timber harvests.

INTRODUCTION

Northern flying squirrels (*Glaucomys sabrinus*) occur from interior Alaska down to California, and from the Washington coast to Maine, occupying temperate and boreal coniferous forests (Inset; Figure 2). Arbogast (1999) reported a major break (4.3 - 7.2%) between eastern (Alaska to Maine) and western (Washington to California) clades of *G. sabrinus* based on partial mitochondrial cytochrome b sequences. The contact zone between these two lineages is thought to be in northern Washington, possibly along the Cascade range (Arbogast pers comm.). Arbogast (1999) suggested that these lineages may have been separated in forested refugia during the Pleistocene full glacial advances. Phylogeography and population genetics of this species in the Pacific Northwest may provide insight into post-glacial ecosystem change, and genetic differentiation and incipient speciation due to founder events and isolation.

Demboski et al. (1998a) and Bidlack & Cook (submitted) focused on the colonization history of flying squirrels in Southeast Alaska (Figure 2). This region is biogeographically complex, consisting of over 2,000 named islands of the Alexander Archipelago stretching from Glacier Bay near Juneau to Misty Fiords National Monument just north of the Canadian border. Glacial cycles had a great impact on the flora and fauna of the region, with species turnover occurring as fluctuating temperature regimes caused faunal movement along the coast between habitats in Beringia and the lower latitudes of North America (Klein 1965; MacDonald & Cook 1996). The archipelago is a center of endemism for several taxa and the potential site of isolated glacial refugia (Klein 1965; Cook & MacDonald in press). This region of the north

Pacific coast is also part of the largest remaining expanse of temperate old-growth forest worldwide. Northern flying squirrels are thought to be dependent on old-growth forest in the Pacific Northwest because of their heavy dietary reliance on fungi and their use of mature trees and snags as nesting sites (Maser et al. 1986; Carey 1996). In the past fifty years this naturally fragmented system has experienced intense anthropogenic disturbance, mainly in the form of logging and associated road-building activities (Durbin 1999).

Cytochrome b gene and control region sequences identify a distinct lineage on 11 islands (the POW complex) in the Alexander Archipelago which share near genetic uniformity (43 of 44 individuals) for 1590 base pairs (Demboski et al. 1998a; Bidlack & Cook submitted). These individuals share unique base pair changes from squirrels of the mainland and nearshore islands. This reduction in genetic variation across populations of this island clade suggests they experienced a severe bottleneck, perhaps as the result of a founder event to the POW complex after the retreat of the glacial ice (around 13,000 yr. BP). This mitochondrial lineage coincides with the endemic Prince of Wales flying squirrel (*G. s. griseifrons*).

While the mitochondrial DNA (mtDNA) data suggest no recent gene flow between POW complex populations and mainland and nearshore island populations, these data reveal little about relationships among the POW islands. Further investigation using biparentally-inherited nuclear markers may give more insight into the colonization of the POW complex and levels of genetic differentiation on this group of islands. Microsatellite loci are thought to evolve more rapidly than mtDNA and often provide finer resolution of population-level dynamics (Bruford & Wayne 1993; Schlötterer & Pemberton 1994). These markers also provide an alternate view to the maternally inherited mitochondrial DNA; often, phylogenetic breaks are identified with mtDNA that do not show up in nuclear data because gene flow is male-mediated (e.g., Paetkau et al. 1998). We examined six microsatellite loci among animals from nine populations to investigate genetic diversity among populations in Southeast Alaska, and to compare

levels of genetic diversity between island and mainland populations. We investigate whether nuclear data provides an evolutionary signal similar to mtDNA in flying squirrels of the POW complex and whether microsatellites shed light on the colonization of this island complex. Lastly, we discuss our data in light of the management and conservation of this north Pacific coast endemic.

MATERIALS AND METHODS

Samples and DNA Extractions

We analyzed 233 animals from nine populations (Figure 2). Individuals were sampled from El Capitan, Heceta, Suemez, and Tuxekan islands (part of the POW complex), from Naukati Bay and Polk Inlet on POW, from two other Southeast localities, Mitkof Island and Helm Bay on the Cleveland Peninsula, and finally from Interior Alaska, around Fairbanks. Samples were the result of field inventories by the University of Alaska Museum or were obtained from marten trappers as incidental mortalities. Heart tissue associated with these voucher specimens, stored at $-70°$ C in the Alaska Frozen Tissue Collection, was used for DNA extraction. Whole DNA extraction followed a protocol modified from Miller et al. (1988).

Microsatellite Amplification

We used six polymorphic microsatellite loci specifically developed for northern flying squirrels: FS1, FS2, FS8, FS10, FS12 (Zittlau et al. 2000), and FLS6 (Wilson 2000; Table 3). Loci were amplified in 10 μL reactions containing ~50-100 ng DNA, 1.25X Perkin-Elmer PCR Buffer solution, 0.2 mM dNTPs, 1.0 unit Perkin-Elmer Taq polymerase, either 4.4 mM (FS1 and FS8) or 3.1 mM (FS2, FS10, FS12, FLS6) $MgCl_2$, and either 0.4 μM (FS1, FS2, FS8, FLS6) or 0.3 μM (FS10 and FS12) of each dye-labeled primer. All microsatellite loci were amplified on a Perkin-Elmer 9700 thermocycler; protocols are listed in the Appendix. PCR products and GS350 size standard (Perkin-Elmer) were electrophoresed through a 6% polyacrylamide gel on an

ABI 373 automated sequencer. Loci from a certain individual was run on each gel to standardize allele scoring. Samples were scored using ABI Genescan and Genotyper software.

Statistical Analyses

Descriptive statistics on number of alleles per population, percentage of polymorphic alleles, and expected (assuming Hardy-Weinberg equilibrium) and observed heterozygosity were obtained using GDA version 1.0 (Lewis & Zaykin 2000). Tests for Hardy-Weinberg equilibrium and genotypic linkage disequilibrium across loci were run in GENEPOP version 3.2 (Raymond & Rousset 1995a). For loci with four or fewer alleles, a complete enumeration method (Louis & Dempster 1987) was used to estimate P-values for each locus in each population to test for deviations from Hardy-Weinberg equilibrium. For loci with four or more alleles, P-values were estimated using a Markov-chain method with 1000 iterations, following the algorithm of Guo & Thompson (1992). Fisher exact tests for linkage disequilibrium per locus pair per population were performed using a Markov chain. Differences between island and mainland populations in expected and observed heterozygosity (H_e and H_o, respectively) were investigated using a one-way ANOVA, while differences in heterozygosity between all population pairs were tested using Tukey's test of honestly significant differences (HSD). Both analyses were performed in the software package STATISTICA (Statsoft Inc. 1995). Differences in microsatellite allele frequencies among all population pairs were tested in GENEPOP using an unbiased estimate of the P-value of the Fisher exact test for each locus (Raymond & Rousset 1995b). Significance across all multiple comparisons in this study was adjusted using a sequential Bonferroni correction (Rice, 1989) with an initial P-value of 0.05.

Analogs of F_{st} among population pairs were calculated using GENEPOP (weighted analysis of variance of allele frequencies, θ; Weir & Cockerham 1984) and differences from zero tested by bootstrapping across 1000 replicates using GDA. The α

level was set to 0.01 and was not corrected for multiple comparisons because a larger confidence interval could not be used reliably with six loci (P. Lewis pers. comm.). The program GeneClass version 1.0.02 (Cornuet et al. 1999) determined the probabilities of assigning individuals to populations using a Bayesian approach to detect immigrants using multi-locus genotypes (Rannala & Mountain 1997).

The program PHYLIP (version 3.57c; Felsenstein 1995) was used to run the next set of analyses. Allele frequencies were used to create 1000 replicate data sets in SEQBOOT. These data sets were then used to create 1000 genetic distance matrices (Nei's [1972] genetic distance [D_s] between populations) in GENDIST, which were imported into NEIGHBOR to create 1000 neighbor-joining trees. All trees were used in the program CONSENSE to produce a consensus tree with bootstrap values. PAUP* (Swofford 1999) was used to create a single unrooted D_s tree on which to represent bootstrap values.

Shared allele distance (D_{sa}) between individuals (Bowcock et al., 1994) were calculated using the program of J. Brzustowski (http://www.biology.ualberta.ca/jbrzusto/index.html) and distances were used to construct a distance phenogram in the Fitch program of PHYLIP.

RESULTS

The average number of alleles per locus ranged from 1.8 on Mitkof Island to 4.7 in Helm Bay, and all populations with the exception of Mitkof and Suemez islands had 100% allelic polymorphism (Table 4). The population from Heceta Island contained one unique allele for FS1, while Mitkof Island and Interior Alaska each had one unique allele for FS2. Interior Alaska also had unique alleles for FS10 (1), FS12 (3) and FLS6 (2). For locus FS10, all POW complex populations shared a 201-base pair allele not found in other populations (Table 5).

Probability tests for Hardy-Weinberg equilibrium indicated a significant departure from equilibrium in loci FS1 and FS12 in the population from Heceta Island and in locus FS2 from Mitkof Island ($P = 0.05$; 3 out of 54 cases, 5.6%). There was no linkage

disequilibrium detected among locus pairs across populations. One-way ANOVAs indicated that mainland populations (Helm Bay and Interior Alaska) had significantly higher observed and expected heterozygosities ($P \leq 0.001$) than island populations (Table 4; Figures 3 & 4). Mitkof Island showed a reduction in the number of alleles per locus, and was the only island to have significantly lower levels of observed heterozygosity ($P < 0.05$) than five other island populations with the Tukey HSD multiple comparisons test (Table 4; Figure 4). Although Mitkof Island is near the mainland and is relatively large compared to several other islands we examined (e.g., El Capitan Island), the small sample size (n = 17) for this island may nonetheless have resulted in lower measures of variability. The Tukey HSD test using expected heterozygosities resulted in no significant differences between population pairs. Across the POW complex, the most common alleles per locus were identical (Table 5).

Within the POW complex, the number of loci with significant differences in allele frequencies between populations (after sequential Bonferroni correction [$P \leq 0.0014$]) did not exceed two (out of six). However, between islands of the POW complex and other populations, and among the Mitkof I., Helm Bay and Interior populations, the number of loci with significant differences in allele frequencies between populations ranged from four to all six loci (Table 6). θ ranged from 0.003 to 0.111 and was not significantly different from zero in seven population pairs from the POW complex (after sequential Bonferroni correction [$P \leq 0.0014$]). Pairwise θ values between the POW complex and the other three populations were much higher, ranging from 0.185 to 0.702 (Table 6). Likewise, results from GeneClass indicate genotypic similarities among the POW complex populations, with the Mitkof I., Helm Bay and Interior populations being more clearly defined (Table 7). Individuals from these three latter populations had a low frequency of being misassigned. In contrast, individuals from the POW complex populations often had nearly equal probabilities of being assigned to other populations within the complex.

Phenograms constructed from D_{sa} and D_s show the POW complex populations clearly defined and separated from the other three populations (Figures 5 and 6). In Figure 5, all POW complex animals group together but do not form island-specific clades. The individuals from Mitkof Island form a monophyletic clade within the intermixed group of individuals from Interior Alaska and Helm Bay. A long branch separates the POW complex from the other three populations in Figure 6 with very high bootstrap support (99.9%); these island populations are clustered tightly together. A very long branch leads to the Mitkof Island population, indicating this population is highly differentiated from the others in allele frequencies.

DISCUSSION

The Alexander Archipelago experienced many cycles of glaciation during the Pleistocene, coupled with oceanic transgressions, isostatic rebound, and ecosystem change (Ager 1983; Mobley 1988; Mann & Hamilton 1995; Barrie & Conway 1999). These cycles had a great impact on the fauna of the region, and mammal species that occur in the archipelago today may be a mixture of neoendemics drawn from both Beringia and southern North America and paleoendemics that persisted in refugia through the last glacial maximum (Cook et al. in press). Flying squirrels are probable post-glacial colonizers of the archipelago; despite the numerous hypotheses concerning glacial refugia in Southeast Alaska (Worley & Jaques 1973; Heusser 1989; Heaton et al. 1996), no concrete evidence exists for the presence of forested refugia spanning the glacial maximum. Cytochrome b sequences show less than 3% divergence among animals from Maine to Alaska (Arbogast 1999), suggesting rapid post-glacial colonization across northern North America and into Southeast Alaska.

Colonization ability has primarily structured the distribution of mammals over the archipelago (Conroy et al. 1999) and new species appear to be colonizing islands of the archipelago (e.g., *Felis concolor*, *Alces alces*; MacDonald & Cook 1996). Flying squirrels are known from only 15 islands in the southern portion of the archipelago

(Figure 2), suggesting they may not be adept over-water dispersers. Our molecular data indicates that limited over-water dispersal ability and isolation on islands has shaped the genetic diversity of flying squirrel populations in Southeast Alaska.

POW Complex

The microsatellite distinctiveness of POW complex populations from other squirrels in Southeast Alaska coincides with the distinct mitochondrial haplotype present only on these islands, originally described by Demboski et al. (1998a) and further characterized by Bidlack & Cook (submitted). The POW clade apparently corresponds to the original subspecific designation of *G. s. griseifrons* (Howell 1934), which was based on the pelage coloration of two specimens from Prince of Wales Island. Congruence of mitochondrial and nuclear data suggests that the extremely low level of genetic diversity on these islands is not the result of either a selective sweep of the mitochondrial genome or lineage sorting of the mitochondrial haplotypes.

The agreement of the nuclear with the mitochondrial data also eliminates the possibility that this pattern of differentiation is an artifact of sex biased gene flow. Based on mitochondrial sequencing, Talbot & Shields (1996) reported an ancient lineage of brown bears (*Ursus arctos*) present on Admiralty, Baranof, and Chichagof islands of the Alexander Archipelago. They suggested that these bears had been isolated from the mainland for 550,000 - 700,000 years, and used this as support for a possible glacial refugium in Southeast Alaska. Paetkau et al. (1998) examined microsatellite variation in brown bears throughout Alaska and found that bears on these islands were not distinct from those of the mainland, nor did they cluster together as a genetically separate group. These authors suggested that the mitochondrial results did not reflect the current level of gene flow among the bears of Southeast Alaska, and that this was probably due to male-mediated gene flow undetectable using mtDNA alone. In contrast, our nuclear and mtDNA data for flying squirrels indicate the POW complex has been isolated from the mainland populations, with apparently no recent gene flow between them.

All six POW complex populations are highly related. Pairwise θ values are low (less than 0.15), indicating low to moderate levels of differentiation between populations (Table 6). Populations most closely related (according to pairwise θ values) are from El Capitan, Tuxekan, and Heceta islands and Naukati Bay on Prince of Wales Island. Polk Inlet and Naukati Bay are also closely related and, though 67 km apart, are both located on Prince of Wales Island. This may indicate substantial levels of gene flow across this island. Both males and females are promiscuous, and males travel long distances during the breeding season in the Pacific Northwest (Wilson 2000). Across all population pairs few loci have significant differences in allele frequencies. The two islands that differ most using these indices are Suemez and Heceta (Table 6), although the results from GeneClass (Table 7) and the pattern seen in the D_{as} phenogram (Figure 5) suggest that these islands are still highly associated with the others of the POW complex.

What could account for the extremely low level of mtDNA diversity on these islands, as well as the distinct microsatellite signature? The assumed low over-water dispersal capability of flying squirrels and the wide straits separating POW from nearshore islands and the mainland suggest that the POW population is the result of a single founder event. The fact that all squirrels from eleven islands share a distinct mitochondrial haplotype, with only one additional base pair change in one individual (Bidlack & Cook submitted), suggests a severe bottleneck in the POW population after the POW haplotype was established. The outer islands are not differentiated in microsatellite allele frequencies from POW itself, indicating that these islands may have been recently colonized. There is no way of conclusively determining if the squirrels of the outer islands are the result of recent colonizations, or if there is current gene flow among all the islands of the POW complex. The lowest pairwise θ value is 0.003 between the Naukati Bay and Tuxekan Island populations, which are separated by an oceanic strait less than 100 m wide. This strait freezes over in some winters and marten have been seen crossing the ice (S. McCoy pers.comm.), but it is not known if flying squirrels will cross open ice as well. We did not analyze gene flow (estimated Nm)

between populations because of the potential problems associated with estimating gene flow from estimates of F_{st} (θ) using small numbers of loci and individuals (Whitlock & McCauley 1999).

Our data, combined with that of Demboski et al. (1998a) and Bidlack & Cook (submitted), indicate that the POW complex lineage is isolated from the mainland and is diverging genetically from those populations. This clade may be on a separate evolutionary path and may represent an example of incipient speciation.

Levels of Genetic Diversity on Islands

Island populations have been extensively compared against mainland populations for differences in genetic diversity and level of inbreeding. Most studies of allozymes and DNA variation have found that island taxa have fewer alleles, lower heterozygosity, lower sequence diversity, fewer polymorphic alleles and a higher level of inbreeding than mainland taxa (e.g., Kilpatrick 1981; Gilbert et al. 1990; Lade et al. 1996; Estoup et al. 1996; Frankham 1997, 1998; Eldridge et al. 1999). Reduced genetic diversity in insular populations is generally attributed to an original founder event, which reduces the number of alleles coming from the source population, and subsequent bottleneck effects, caused by stochastic population fluctuations affecting small populations. Insular populations of flying squirrels along the north Pacific coast are consistent with other observations of lower genetic diversity in island populations, with fewer microsatellite alleles, fewer polymorphic alleles, and lower heterozygosity. Mitkof Island has a particularly striking lack of diversity, with two monomorphic loci and an observed heterozygosity of 0.128 (Table 5; Figure 4).

Mitkof Island

The population from Mitkof Island possesses little microsatellite variation compared with the other island populations we examined, and this low genetic diversity suggests a recent bottleneck or a series of bottlenecks following an older founder event.

All alleles found in the Mitkof Island population are found in the Southeast mainland population we studied (Helm Bay), except for the FS2-113 allele (Table 5) found in three individuals. Alleles for two loci are fixed, and two other loci show lower allelic diversity than in other populations. The Mitkof Island population is monophyletic and is allied with individuals from Helm Bay and Interior Alaska (Figure 5); however, this population is well differentiated (Figure 6) with pairwise θ values between Mitkof Island and other populations being relatively high, ranging from 0.604 to 0.702. Likewise, all six loci show significant differences in allele frequencies from other populations (Table 6). Bidlack & Cook (submitted) found no mitochondrial variation (1590 base pairs) in five individuals sequenced from this island, although unlike squirrels from the POW complex, the Mitkof Island haplotype was shared with mainland populations. Reduced microsatellite diversity and the shared mitochondrial haplotype with the mainland suggest a recent founder event. In contrast, the fixation of two microsatellite loci, the presence of a unique microsatellite allele, and significant allelic divergence from Helm Bay indicate an older event. Our Southeast Alaska mainland sampling is limited and the comparison of the Mitkof Island population with that of Helm Bay may not be valid. However, nuclear and mitochondrial data from all nine populations suggests that the colonization of Mitkof Island is probably more recent than the founding of the POW population.

Conservation Implications

Over 80% of the Alexander Archipelago is contained within the bounds of the Tongass National Forest. In the past fifty years this region has experienced intense anthropogenic disturbance, mainly in the form of logging and associated road-building activities. Up to 46% of the original old-growth forest has been cut from some islands, and further cuts are proposed that may impact forest-associated species (United States Department of Agriculture 2000). Until recently, there was little recognition of the potential impacts these activities may have on mammals of the region, especially on those primarily associated with old-growth forest. Additionally, very little inventory work had

been conducted across the archipelago to document species distributions. However, the new 10-year forest plan (USDA 1997) mandates the management and conservation of endemic taxa. Recently, *G. s. griseifrons* was listed by the IUCN as endangered, based on the projected rate of logging and the lack of significant areas of protected old-growth habitat within its limited range (Demboski et al. 1998b).

Our molecular data suggest that squirrels of the POW complex fit the definition of an evolutionarily significant unit (ESU; Ryder 1986), or a population (or set of populations) that has been historically (and reproductively) isolated from other such populations. An ESU is a lineage with distinct evolutionary potential, and reciprocal mitochondrial monophyly and significant allelic divergence at nuclear loci have been listed as criteria for determining ESU status (Waples 1991; Moritz 1994). The base pair changes seen in the mitochondrial sequences (Demboski et al. 1998; Bidlack & Cook submitted), unique microsatellite alleles, and changed microsatellite frequencies in the POW complex indicate past and continuing differentiation from mainland populations.

Island species apparently are more prone to extinction than their mainland counterparts (Diamond 1989), with nearly 80% of vertebrate extinctions since 1600 being island taxa (Reid & Miller 1989). Many island extinctions can be traced to the interaction of extrinsic factors (e.g., introduction of novel predators, overhunting, loss of habitat, and disease) with intrinsic factors such as small population size (Quammen 1996). However, the role that a lack of genetic variation plays in extinction probabilities may be substantial (Newman & Pilson 1997; Frankham 1997; Saccheri et al. 1998). All individuals from the POW complex share a common mitochondrial haplotype, and populations from these islands have lower heterozygosities than mainland populations for the six nuclear loci we examined. The increased risk of extinction on islands, combined with the low level of genetic variability in this subspecies and further habitat disturbance due to logging activities, may result in the loss of this evolutionarily distinct lineage.

The Prince of Wales flying squirrel has a distinctive history of colonization and isolation from other northern flying squirrels in Southeast Alaska. Our study provides a

preliminary account of the genetic diversity within and among island populations. Future work should focus on categorizing populations from more islands in the POW complex and from mainland British Columbia and Southeast Alaska to shed light on the origins of the POW complex populations. Patterns of population differentiation should be monitored to assess the impacts of habitat fragmentation and modification. Such a framework would provide managers with information on unique island populations that could be incorporated into the planning process for proposed timber harvests. Levels of variation of flying squirrel populations from other islands in the archipelago such as Revillagigedo, Wrangell, and Etolin islands should also be assessed, given the pronounced signal of a founder event on Mitkof Island. The characterization of a unique island lineage of flying squirrels in Southeast Alaska emphasizes the need for research into the population structure and genetic diversity of other old-growth associated endemics along the north Pacific coast. Patterns of endemism may emerge that current land use plans in Southeast Alaska do not adequately address.

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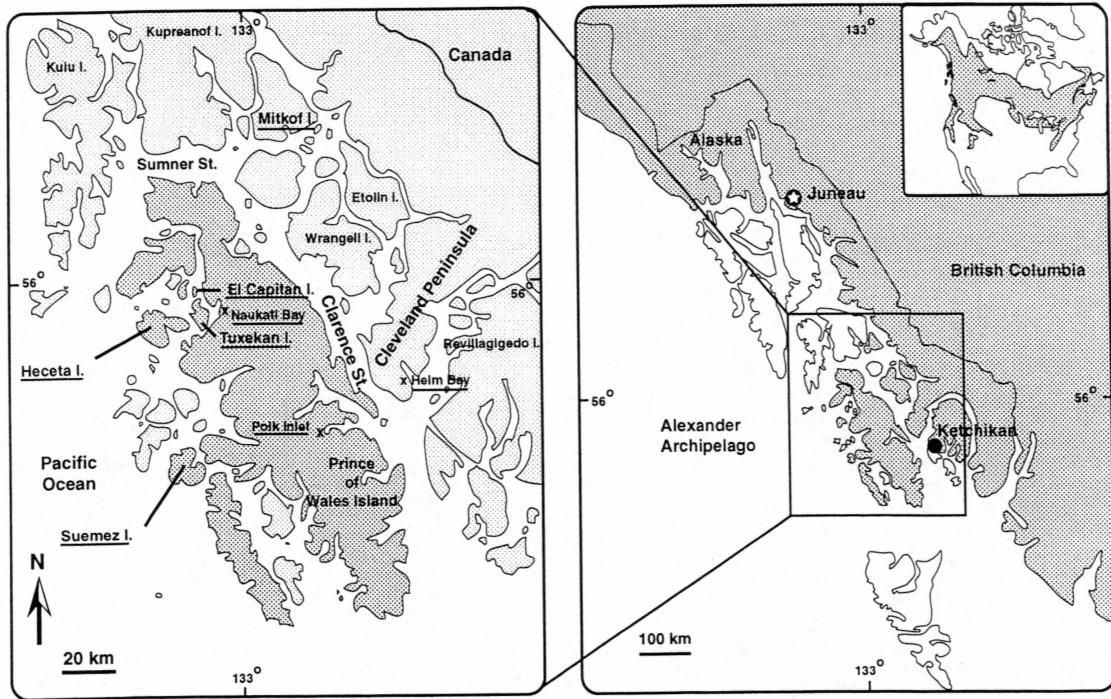
Figure 2. Maps of Southeast Alaska. Detailed map (left) includes sample localities (underlined) except for Interior Alaska; darker shaded area is distributional extent of *G. s. griseifrons* (POW Complex). Larger map (right) shows distribution of northern flying squirrels in the Alexander Archipelago and British Columbia (shaded); inset map shows range of *G. sabrinus*.
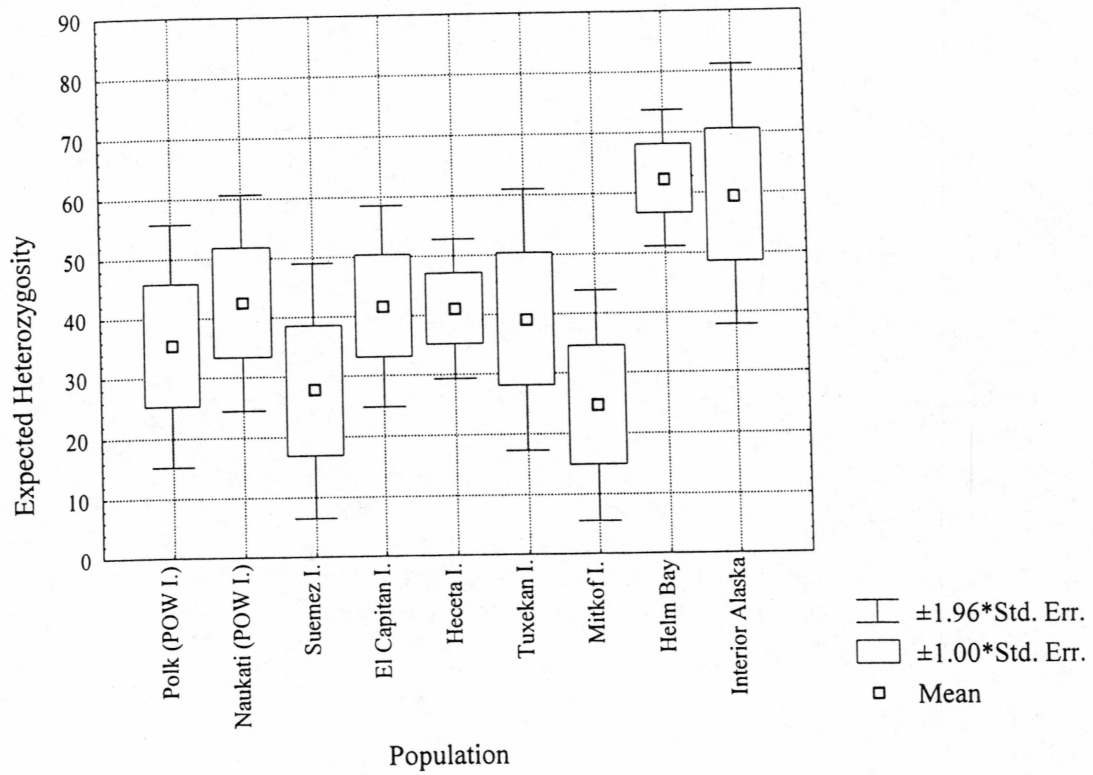
Figure 3. Plot of means and standard errors of expected heterozygosities of sampled populations.
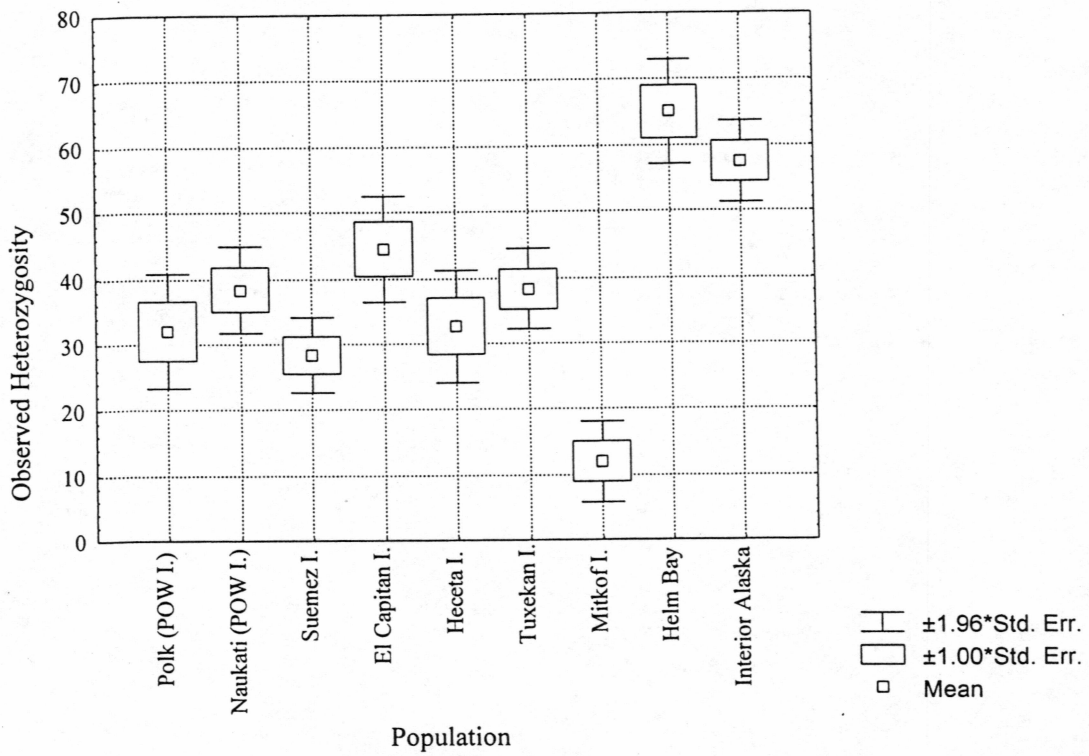
Figure 4. Plot of means and standard errors of observed heterozygosities of sampled populations.
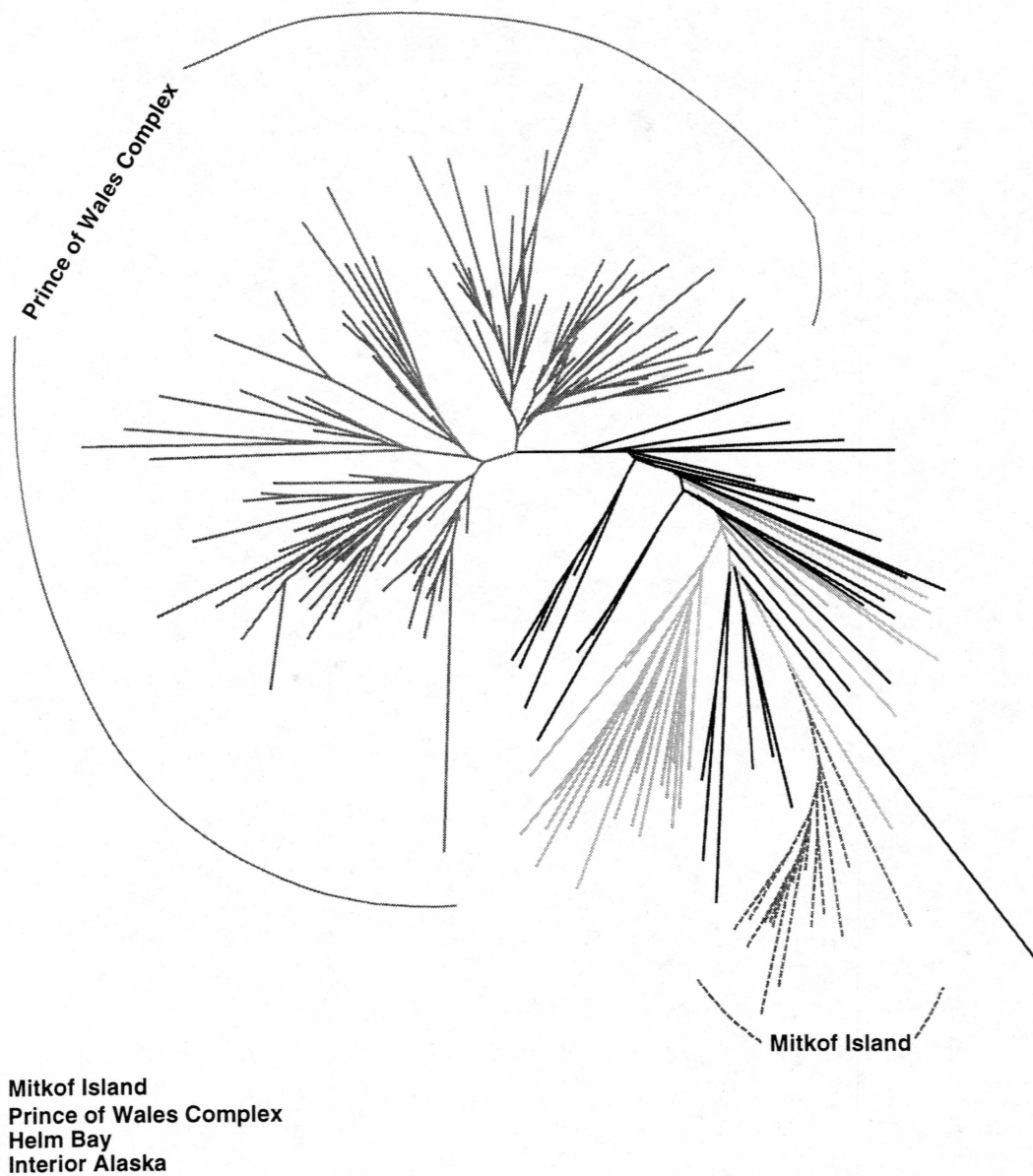
Figure 5. Phenogram based on shared allele distances (D_{sa}) among individuals.

44
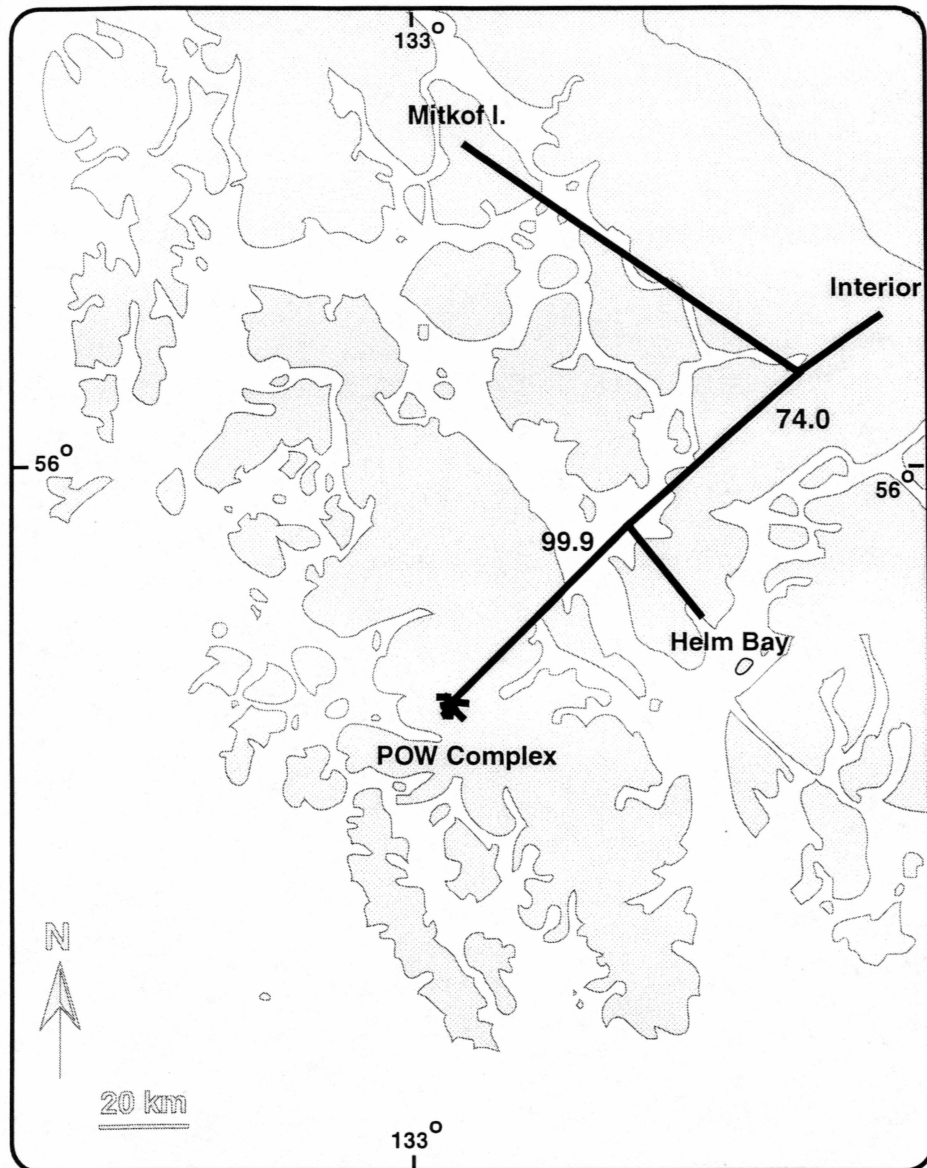
Figure 6. Unrooted neighbor-joining tree of Nei's (1972) D_s between populations superimposed on a map of Southeast Alaska. Numbers indicate bootstrap support (% out of 1000 replicates) of closest node.

Table 3. Microsatellite loci, repeat type, primer sequences, size range and number of alleles.

Locus	Repeat type	Primers	Size range in bp	Number of alleles
GS1	GT	F: GCTGCCCTCATTTTATCCCC	93-99	4
		R: GCTTCGTGTGTATATGTGTGTGTG		
GS2	GT	F: AACATTCTCGCCACATCTAA	101-113	7
		R: CTACACCCCCAGCCCTACAA		
GS8	GT	F: ATGCCATCTCCCCTCTC	214-222	5
		R: GCTGTGCTTCCAACCTGT		
GS10	GT	F: CTATGCTGAGGAGGAGTGGTG	191-201	4
		R: CGTTTATGTGAAGAGCCTTG		
GS12	GT	F: GTCTCTTGAGTTAGGTGCCC	104-116	7
		R: CCTTTCTTCTCTCCTCCCC		
FLS6	CCCT	F: TCGGACCTCTTGTTCGTCACC	152-196	12
		R: CAGCTTCCCATGGCCAAGGC		

Table 4. Descriptive statistics. Islands in boldface are part of POW Complex. Number of alleles per locus (A), proportion of polymorphic loci (%P), expected heterozygosity under Hardy-Weinberg equilibrium (H_e), observed heterozygosity (H_o) averaged over six loci.

Population	A	%P	H_e[†]	H_o[††]
Polk Inlet (POW I.)	3.167	100	0.355	0.320
Naukati (POW I.)	3.000	100	0.426	0.386
Suemez I.	3.000	83.3	0.278	0.286
El Capitan I.	3.667	100	0.417	0.444
Heceta I.	3.500	100	0.410	0.326
Tuxekan I.	3.167	100	0.391	0.383
Mitkof I.	1.833	66.7	0.247	0.128*
Helm Bay	4.667	100	0.624	0.644
Interior Alaska (Fairbanks)	4.5	100	0.595	0.580

[†] H_e of mainland populations significantly higher than island populations at $P = 0.001$.
[††] H_o of mainland populations significantly higher than island populations at $P < 0.001$.
* H_o of Mitkof Island significantly lower than all island populations except Suemez.

Table 5. Microsatellite allele frequencies. Table shows locus sizes in base pairs (first column) and number of individuals examined at each locus in each population.

Locus	Population Polk	Naukati	Suemez	El Cap	Heceta	Tuxekan	Mitkof	Helm	Int. AK
FS1	21	28	28	24	23	30	17	31	29
93	0.024	0.000	0.018	0.000	0.022	0.000	0.588	0.484	0.517
95	0.048	0.107	0.000	0.083	0.130	0.050	0.000	0.048	0.121
97	0.928	0.893	0.982	0.917	0.826	0.950	0.412	0.468	0.362
99	0.000	0.000	0.000	0.000	0.022	0.000	0.000	0.000	0.000
FS2	20	30	27	24	23	30	13	29	29
101	0.100	0.183	0.185	0.125	0.000	0.100	0.654	0.138	0.948
103	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.052
105	0.000	0.000	0.019	0.021	0.022	0.000	0.000	0.034	0.000
107	0.825	0.734	0.555	0.770	0.891	0.833	0.000	0.638	0.000
109	0.075	0.083	0.241	0.063	0.087	0.067	0.000	0.121	0.000
111	0.000	0.000	0.000	0.021	0.000	0.000	0.231	0.069	0.000
113	0.000	0.000	0.000	0.000	0.000	0.000	0.115	0.000	0.000
FS8	21	30	27	24	23	30	16	31	29
214	0.429	0.317	0.130	0.188	0.152	0.200	0.000	0.145	0.190
216	0.000	0.000	0.000	0.000	0.000	0.000	0.938	0.419	0.000
218	0.500	0.566	0.851	0.604	0.761	0.533	0.062	0.259	0.379
220	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.145	0.345
222	0.071	0.117	0.019	0.208	0.087	0.267	0.000	0.032	0.086

Table 5. (cont.)

Locus	Population Polk	Naukati	Suemez	El Cap	Heceta	Tuxekan	Mitkof	Helm	Int. AK
FS10	21	29	27	24	23	30	17	29	29
191	0.786	0.672	0.907	0.521	0.696	0.583	0.000	0.690	0.569
193	0.000	0.000	0.000	0.000	0.000	0.000	1.000	0.310	0.397
195	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.034
201	0.214	0.328	0.093	0.479	0.304	0.417	0.000	0.000	0.000
FS12	21	30	28	24	23	30	17	30	29
104	0.000	0.000	0.000	0.000	0.022	0.000	0.000	0.167	0.259
106	0.024	0.083	0.000	0.104	0.087	0.050	1.000	0.367	0.052
108	0.976	0.917	1.000	0.896	0.674	0.950	0.000	0.466	0.310
110	0.000	0.000	0.000	0.000	0.217	0.000	0.000	0.000	0.034
112	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.138
114	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.190
116	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.017
FLS6	21	29	28	24	23	30	17	31	29
152	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.138
156	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.121
160	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.032	0.190
164	0.000	0.000	0.000	0.000	0.000	0.000	0.794	0.016	0.276
168	0.024	0.207	0.054	0.083	0.109	0.183	0.000	0.048	0.103
172	0.048	0.000	0.000	0.042	0.000	0.000	0.000	0.258	0.069
176	0.262	0.138	0.214	0.063	0.326	0.267	0.206	0.210	0.086
180	0.214	0.121	0.054	0.021	0.022	0.067	0.000	0.194	0.017
184	0.000	0.017	0.000	0.063	0.000	0.017	0.000	0.145	0.000
188	0.024	0.103	0.018	0.125	0.022	0.067	0.000	0.048	0.000
192	0.428	0.414	0.606	0.540	0.521	0.382	0.000	0.017	0.000
196	0.000	0.000	0.054	0.063	0.000	0.017	0.000	0.032	0.000

Table 6. Pairwise measures of microsatellite differentiation. Above diagonal: analog of Fst (θ; Weir & Cockerham 1984) across all loci; values not significantly different from zero in bold face ($P < 0.01$). Below diagonal: number of loci (out of six) with significant differences in allele frequency distributions; all pairwise comparisons were adjusted by a sequential Bonferroni correction ($P \leq 0.0014$).

	1	2	3	4	5	6	7	8	9
1. Polk Inlet (POW)	-	**0.007**	0.084	**0.052**	0.056	**0.029**	0.661	0.193	0.355
2. Naukati (POW)	0	-	0.072	**0.007**	**0.038**	**0.003**	0.604	0.187	0.319
3. Suemez I.	1	2	-	0.106	0.093	0.111	0.702	0.269	0.384
4. El Capitan I.	1	0	2	-	0.042	**0.006**	0.618	0.212	0.345
5. Heceta I.	1	1	2	2	-	0.041	0.628	0.194	0.350
6. Tuxekan I.	0	0	2	0	1	-	0.630	0.212	0.360
7. Mitkof I.	6	6	6	6	6	6	-	0.357	0.375
8. Helm Bay	5	5	5	5	5	5	5	-	0.185
9. Interior Alaska	6	6	6	6	6	6	5	4	-

Table 7. Results from GeneClass program; number of individuals assigned to particular populations. Rows do not add up to number of specimens examined per population because some animals were assigned to more than one population, or assigned to none.

	1	2	3	4	5	6	7	8	9
1. Polk Inlet POW)	2	6	1	3	1	4	0	4	0
2. Naukati (POW)	5	11	0	7	2	7	0	0	1
3. Suemez I.	2	12	8	3	5	1	0	0	0
4. El Capitan I.	0	4	1	15	0	3	0	1	0
5. Heceta I.	1	4	0	6	8	1	0	1	0
6. Tuxekan I.	0	6	1	8	6	8	0	0	0
7. Mitkof I.	0	0	0	0	0	0	14	1	0
8. Helm Bay	0	0	0	0	0	0	0	28	0
9. Interior Alaska	0	0	0	0	0	0	0	0	28

Appendix. Microsatellite PCR protocols.

FS1, FS10 & FS12:	FS2 & FS8:	FLS6:
94° - 1:00	94° - 1:00	94° - 3:00
94° - 00:30	94° - 00:30	94° - 00:40
60° – 00:20	62° – 00:20	70, 68, 66, 64, 62° – 00:50
72° – 00:10	72° – 00:10	72° – 00:30
x2 cycles	x2 cycles	x2 cycles at each temp.
94° – 00:15	94° – 00:15	94° – 00:40
58° – 00:20	60° – 00:20	60° – 00:50
72° – 00:10	72° – 00:10	72° – 00:30
x33 cycles	x33 cycles	x38 cycles
72° – 30:00	72° – 30:00	72° – 30:00

CONCLUSIONS

Research concerning mammals on nearshore oceanic archipelagos may provide insight into evolution on islands, metapopulation dynamics, founder effects and incipient speciation. This research provides a preliminary assessment of genetic variation in northern flying squirrels (*Glaucomys sabrinus*) in Southeast Alaska. Mitochondrial sequences identify an distinct island lineage on Prince of Wales Island and 10 islands to the west (Chapter 1). This lineage coincides with the subspecific designation (*G. s. griseifrons*) originally based on morphology (Howell, 1934). Given the reciprocal mitochondrial monophyly of mainland and nearshore populations and POW complex populations, it is unlikely that there has been recent gene flow across the straits that separate these clades. The glacial history of Southeast Alaska and the level of divergence in the mitochondrial DNA suggests that the POW complex is the result of a founder event after the retreat of the Cordilleran ice sheet (~13,000 BP).

Microsatellite variation in nine populations in Southeast Alaska is investigated in Chapter 2. The data, like that from the mitochondrial sequences, indicate that POW complex populations are isolated from those of the mainland and nearshore islands. This suggests that the genetic signal we see in the mitochondrial DNA is not due to sex-biased gene flow, a selective sweep of the mitochondrial genome, or lineage sorting. Microsatellite allele frequencies also suggest the POW complex populations are closely related to each other. The mtDNA data shows that all individuals but one from the POW complex are identical for 1590 base pairs. This, combined with our microsatellite data, suggests a distant founder event to Prince of Wales Island, a severe population bottleneck (or bottlenecks), and subsequent colonization of the outer islands.

The POW complex is distinct and probably isolated from other populations in Southeast Alaska. The criteria for identifying an Evolutionarily Significant Unit (ESU) include reciprocal mitochondrial monophyly and significant nuclear allelic divergence (Moritz, 1994). This insular clade of flying squirrels qualifies as an ESU according to

these criteria, and may be on a separate evolutionary path. Documenting intraspecific diversity across nearshore archipelagos may be particularly valuable when these areas are affected by large scale anthropogenic disturbances. Northern flying squirrels are associated with old-growth forest in the Pacific Northwest (Carey, 1996), but the Alexander Archipelago has been highly impacted by timber harvesting activities over the past 50 years (Durbin, 1999). The characterization of the POW complex may impact the planning of future timber harvests on islands of the archipelago, as further habitat disturbance and increased risk of extinction on islands (Diamond, 1989) may result in the loss of this evolutionarily distinct lineage.

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