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A nuclear perspective on endemism in northern flying squirrels (*Glaucomys sabrinus*) of the Alexander Archipelago, Alaska

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Abstract

We used microsatellites to examine population structure and genetic diversity in northern flying squirrels in the Alexander Archipelago of Southeast Alaska, with an emphasis on the endemic Prince of Wales flying squirrel (*Glaucomys sabrinus griseifrons*). Previous work showed this subspecific designation coincided with a distinct mitochondrial lineage on eleven islands (the Prince of Wales [POW] complex). To obtain a nuclear perspective on this lineage and to further investigate genetic diversity among insular populations, we examined six microsatellite loci in 233 flying squirrels representing eight populations in Southeast Alaska and a population from interior Alaska (seven island and two mainland localities). Island populations have lower heterozygosity and allelic diversity than mainland populations. Overall, population pairs show a pattern of isolation by distance, indicating there is little long-distance gene flow across the archipelago. Analyses of microsatellite allele frequencies reveal significant differences between the POW complex populations and others we examined, a finding congruent with the mitochondrial data. The population from Mitkof Island, a non-POW complex island, also differs significantly from other populations in allele frequencies. The six POW complex populations are genetically very similar, suggesting current or recent gene flow among these islands, while there seems to be no gene flow between the POW complex and other populations in Southeast Alaska. Our data corroborate mitochondrial DNA results indicating that *G. s. griseifrons* is genetically distinct and suggest a general pattern of isolation of insular flying squirrels in Southeast Alaska.

Introduction

The Alexander Archipelago of Southeast Alaska (Figure 1) has been characterized as a center of endemism for several mammalian taxa and the potential site of isolated glacial refugia (Klein 1965; Cook and MacDonald 2001). Moreover, much of the largest remaining expanse of temperate old-growth forest worldwide is spread across this biogeographically complex archipelago. Recent evolutionary studies of the insular fauna along the north Pacific coast have identified distinctive lineages or surprising patterns of phylogeographic structure (e.g. Cook et al. 2001). The northern flying squirrel, *Glaucomys sabrinus*, occurs on fifteen southern islands of the archipelago (Figure 1). Preliminary work on flying squirrels using the cytochrome *b* gene and control region sequences identified 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 and Cook 2001). These individuals share unique base pair changes from squirrels of the mainland and Mitkof, Etolin, Wrangell, and Revillagigedo islands. This mitochondrial lineage coincides with the endemic Prince of Wales flying squirrel (*G. s. griseifrons*), originally described on morphological characters of two specimens (Howell 1934).

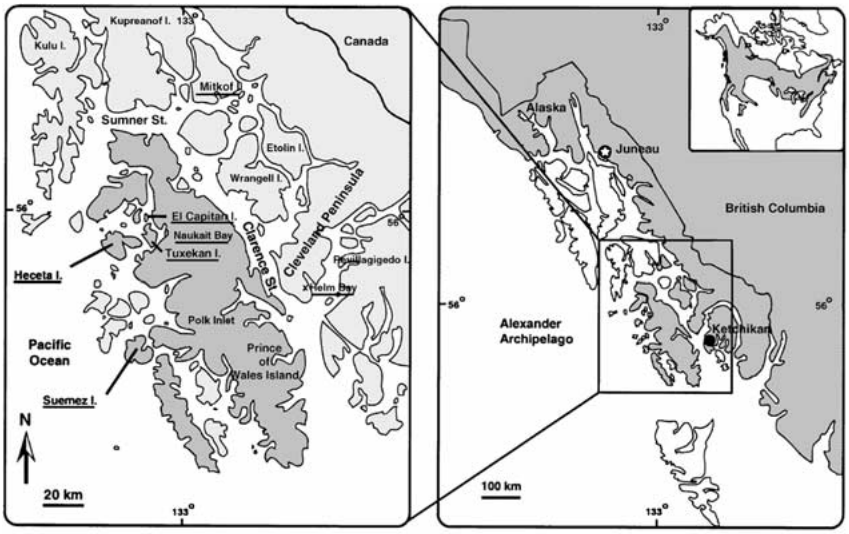

Figure 1. Maps of Southeast Alaska. Detailed map (left) includes sample localities (underlined) except for Interior Alaska; darker shaded area is distributional extent of *G. s. griseifrons* (POW Complex). Larger map (right) shows distribution of northern flying squirrels in the Alexander Archipelago and British Columbia (shaded); inset map shows range of *G. sabrinus*.

Mitochondrial DNA (mtDNA) data suggest no recent gene flow between POW complex populations and other populations in Southeast Alaska. We used biparentally-inherited nuclear markers to explore further the colonization of the POW complex and genetic differentiation among Southeast Alaska populations. Microsatellite loci provide an alternate view to the maternally inherited mitochondrial DNA. For example, phylogeographic breaks identified with mtDNA that do not show up in nuclear data may indicate that gene flow is male-mediated (e.g. Paetkau et al. 1998). These markers are also thought to evolve more rapidly than mtDNA and often provide finer resolution of population-level dynamics (Bruford and Wayne 1993; Schlötterer and Pemberton 1994). We examined six microsatellite loci among animals from nine populations to assess genetic diversity and gene flow among populations in Southeast Alaska, and whether microsatellites shed light on the colonization of the archipelago. We investigate whether nuclear data provide an evolutionary signal similar to mtDNA in flying squirrels of the POW complex and we compare levels of genetic diversity between island and mainland populations.

Materials and methods

Samples and DNA extractions

We analyzed 233 animals from nine populations (Figure 1). Individuals were sampled from El Capitan (n = 24), Heceta (n = 23), Suemez (n = 28), and Tuxekan (n = 30) islands (part of the POW complex), from Naukati Bay (n = 30) and Polk Inlet (n = 21) on POW, from two other Southeast localities, Mitkof Island (n = 17) and Helm Bay on the Cleveland Peninsula of the mainland (n = 31), and finally from Interior Alaska, near Fairbanks (n = 29; Appendix I). Each island was identified as a population with the exception of the large Prince of Wales Island, where we were able to assess genetic similarity among specimens from two distinctive localities (populations) separated by about 67 km. Approximately 50% of the individuals used in mitochondrial studies by Demboski et al. (1998a) and Bidlack and Cook (2001) were included in this study. Current sampling design focused on more intensive sampling of only nine populations, rather than geographically extensive sampling throughout Southeast Alaska. Fairbanks specimens were included to provide another mainland population for purposes of genetic diversity comparisons with island populations. Samples were the result of field inventories by the University of Alaska Museum and the USDA Forest Service, or were obtained from marten trappers as incidental mor-

talities. Voucher specimens and associated frozen tissues stored at –70 °C are archived in the University of Alaska Museum. Whole DNA extraction from heart tissue 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 1). 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 thermal cycler; protocols are listed in Appendix II. PCR products and GS350 size standard (Perkin-Elmer) were electrophoresed through a 6% polyacrylamide gel on an ABI 373 automated sequencer. Loci from the same individual were run on each gel to standardize allele scoring, with samples scored using ABI Genescan and Genotyper software.

Statistical analyses

Descriptive statistics on number of alleles per population, percentage of polymorphic loci, and expected (assuming Hardy-Weinberg equilibrium) and observed heterozygosity were obtained using GDA version 1.0 (Lewis and Zaykin 2000). Tests for Hardy-Weinberg equilibrium and genotypic linkage disequilibrium across loci were conducted with GENEPOP version 3.2 (Raymond and Rousset 1995a). For loci with four or fewer alleles, a complete enumeration method (Louis and 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 and 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, 1999 edition (Statsoft, Inc., Tulsa OK).

To investigate genetic diversity among populations, 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 and 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. To assess population subdivision, estimates of F_{st} among population pairs were calculated using GENEPOP (weighted analysis of variance of allele frequencies, θ; Weir and 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; bootstrapping over a small number of loci (e.g. six) leads to unreliable assessments of significance at low α levels (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 and Mountain 1997).

The program PHYLIP version 3.57c (Felsenstein 1995) was used to investigate the differences in allele frequencies among populations. 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 to represent allele frequency differences among populations and on which to represent bootstrap values. Shared allele distances (D_{sa}) between individuals (Bowcock et al. 1994) were calculated using the program of J. Brzustowski (http://www.biology.ualberta.ca/jbrzusto/sharedst.php) and distances were used to construct a distance phenogram in the FITCH program of PHYLIP. Lastly, to assess whether gene flow and genetic drift are at equilibrium among populations, Slatkin's (1993) isolation by distance program was used to plot M^ (a measure of gene flow) against geographic distance between population pairs.

Table 1. Microsatellite loci, repeat type, primer sequences, size range and number of alleles

Locus	Repeat type	Primers	Size range in bp	Number of alleles
FS1	GT	F: GCTGCCCTCATTTTATCCCC R: GCTTCGTGTGTATATGTGTGTGTG	93–99	4
FS2	GT	F: AACATTCTCGCCACATCTAA R: CTACACCCCCAGCCCTACAA	101–113	7
FS8	GT	F: ATGCCATCTCCCCTCTC R: GCTGTGCTTCCAACCTGT	214–222	5
FS10	GT	F: CTATGCTGAGGAGGAGTGGTG R: CGTTTATGTGAAGAGCCTTG	191–201	4
FS12	GT	F: GTCTCTTGAGTTAGGTGCCC R: CCTTTCTTCTCTCCTCCCC	104–116	7
FLS6	CCCT	F: TCGGACCTCTTGTTCGTCACC	152–196	12

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 2). The population from Heceta Island contained one unique allele for locus FS1, while Mitkof Island and Fairbanks each had one unique allele for locus FS2. Fairbanks 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 3).

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 Fairbanks) had significantly higher observed and expected heterozygosities ($P \leq 0.001$) than island populations (Table 2; Figures 2 and 3). 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 2; Figure 3). The Tukey HSD test using expected heterozygosities resulted in no significant differences between population pairs.

Allele frequencies indicate that the POW complex populations are distinct from other populations we examined. 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 4). Likewise, F_{st} (θ) ranged from 0.003 to 0.111 and was not significantly different from zero in seven population pairs from the POW complex. Pair-

Table 2. 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	N	A	%P	$H_e^{\dagger}$	$H_o^{\dagger\dagger}$
Polk Inlet (POW I.)	21	3.167	100	0.355	0.320
Naukati (POW I.)	30	3.000	100	0.426	0.386
Suemez I.	28	3.000	83.3	0.278	0.286
El Capitan I.	24	3.667	100	0.417	0.444
Heceta I.	23	3.500	100	0.410	0.326
Tuxekan I.	30	3.167	100	0.391	0.383
Mitkof I.	17	1.833	66.7	0.247	0.128*
Helm Bay	31	4.667	100	0.624	0.644
Interior Alaska (Fairbanks)	29	4.5	100	0.595	0.580

$^{\dagger}H_e$ of mainland populations significantly higher than island populations at $P = 0.001$; $^{\dagger\dagger}H_o$ 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

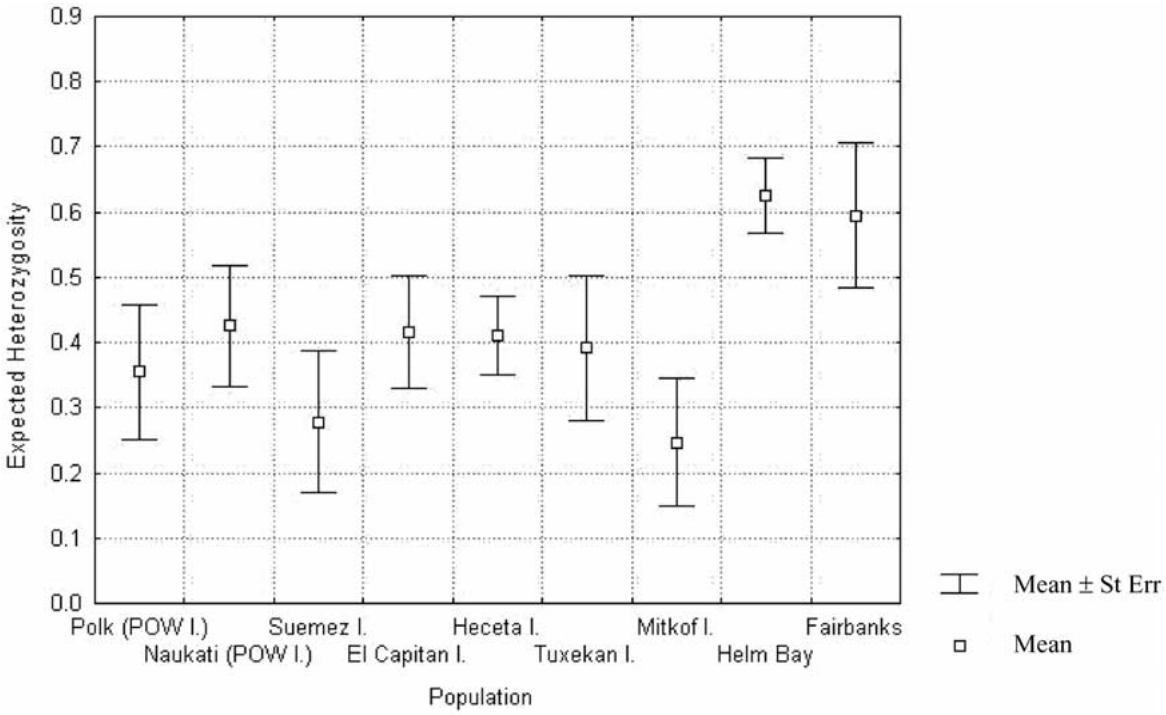

Figure 2. Plot of means, standard deviations, and standard errors of expected heterozygosities of sampled populations.

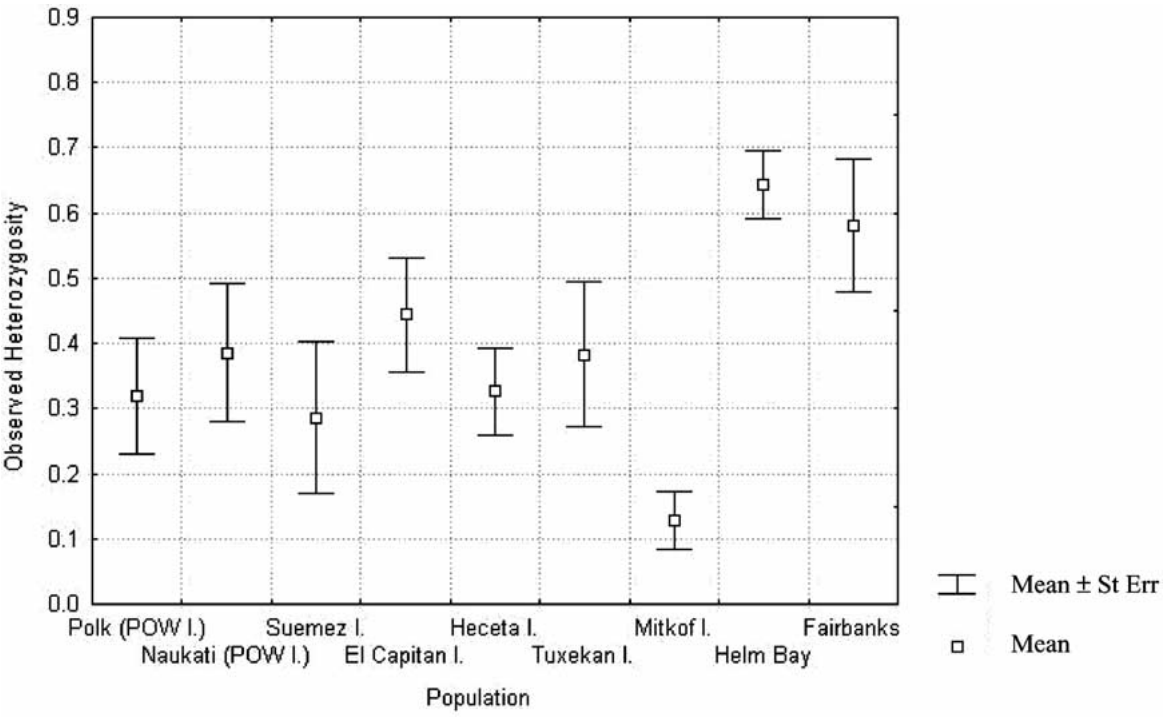

Figure 3. Plot of means, standard deviations, and standard errors of observed heterozygosities of sampled populations.

Table 3. Sizes of alleles in each population; alleles in boldface are unique to that population. Islands in boldface are part of POW complex

Population	N	FS1	FS2	FS8	FS10	FS12	FLS6
Polk Inlet	21	93,95,97	101,107,109	214,218,222	191,201	106,108	168,172,176,180,188,192
Naukati	30	95,97	101,107,109	214,218,222	191,201	106,108	168,176,180,184,188,192
Suemez I.	28	93,97	101,105,107,109	214,218,222	191,201	108	168,176,180,188,192,196
El Capitan I.	24	95,97	101,105,107,109,111	214,218,222	191,201	106,108	168,172,176,180,184,188,192,196
HecetaI.	23	93,95,97,**99**	105,107,109	214,218,222	191,201	104,106,108,110	168,176,180,188,192
Tuxekan I.	30	95,97	101,107,109	214,218,222	191,201	106,108	168,176,180,184,188,192,196
Mitkof I.	17	93,97	101,111,**113**	216,218	193	106	164,176
Helm Bay	31	93,95,97	101,105,107,109,111	214,216,218,220, 222	191,193	104,106,108	160,164,168,172,176,180,184, 188,192,196
Interior AK	29	93,95,97	101,**103**	214,218,220,222	191,193,**195**	104,106,108,110, **112**,**114**,**116**	**152**,**156**,160,164,168,172,176, 180

Table 4. Pairwise measures of microsatellite differentiation. Above diagonal: Fst (θ; Weir and 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 ($P \leq 0.0014$). Islands in boldface are part of POW complex

		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	0.0355
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	–

wise F_{st} (θ) values between the POW complex and the other three populations were higher, ranging from 0.187 to 0.702 (Table 4). Interestingly, F_{st} (θ) values between Mitkof Island and all other populations were very high, ranging from 0.357 to 0.702 (Table 4). Results from GENECLASS indicate genotypic similarities among the POW complex populations, with the Mitkof I., Helm Bay and Fairbanks populations being more clearly defined (Table 5). 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 4 and 5). All POW complex animals group together but

Table 5. 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. Islands in boldface are part of POW complex

		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

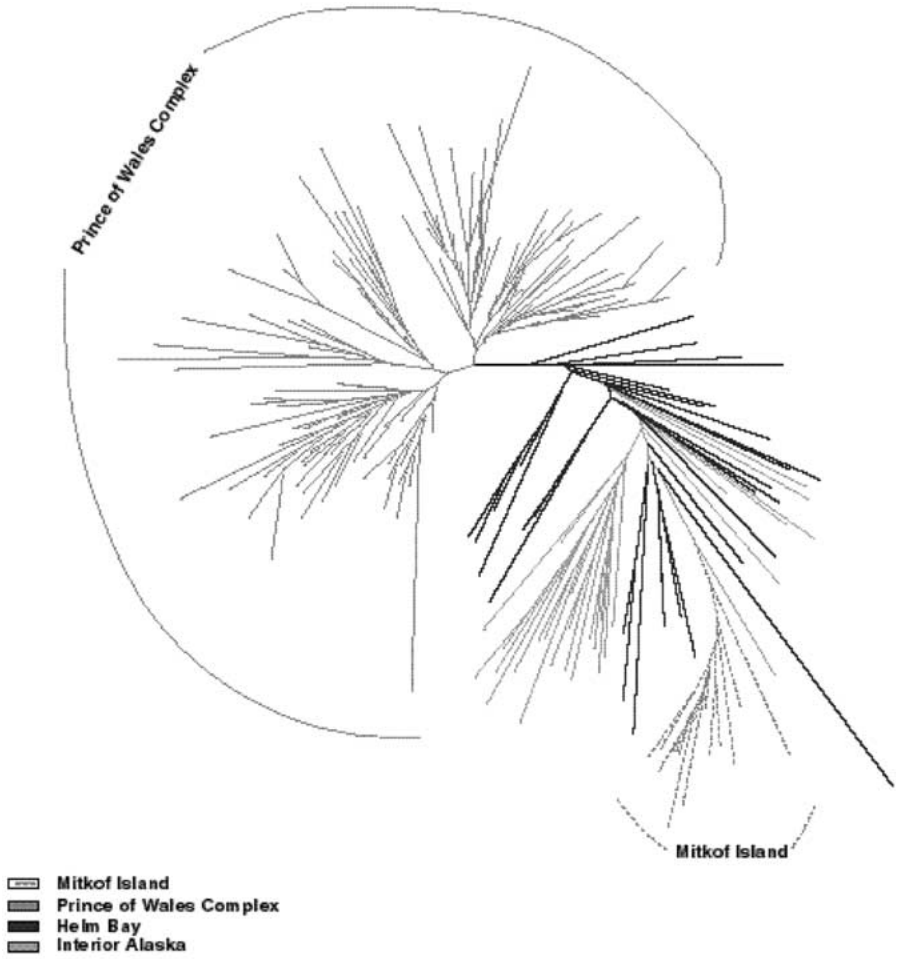

Figure 4. Phenogram based on shared allele distances (D_{sa}) among individuals.

do not form island-specific clades, while individuals from Mitkof Island form a monophyletic clade within the intermixed group of individuals from Fairbanks and Helm Bay (Figure 4). A long branch separates the POW complex from the other three populations (Figure 5) 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.

Our data indicate an apparent pattern of isolation by distance among island populations (Figure 6; r = –0.7955 for all population pairs; r = –0.7260 for POW complex pairs). Log M^ values near or below 0.0 indicate no gene flow between populations, and agree with our other analyses that separate the POW complex from other populations in Southeast Alaska. Pairwise comparisons involving Fairbanks samples were not included in the regression because of the much greater geographic distances involved. An examination of Cook's (1977) distance and deleted residuals vs. standardized residuals confirmed the large influence of these samples on the regression coefficient.

Discussion

Colonization history

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 and Hamilton 1995; Barrie and Conway 1999). These cycles had a great impact on the fauna of the region, and mammal species that occur in the

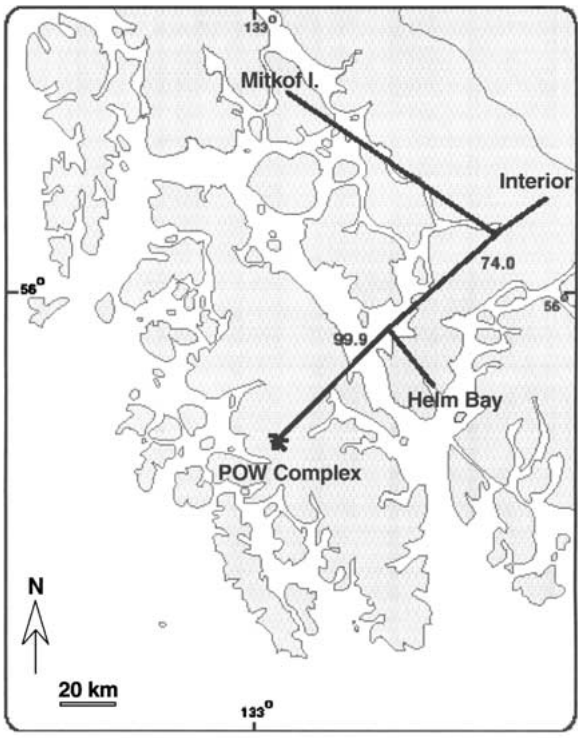

Figure 5. Unrooted neighbor-joining tree of Nei's (1972) D_S between populations superimposed on a map of Southeast Alaska. Numbers indicate bootstrap support (% out of 1000 replicates) of closest node.

archipelago today may be a mixture of neoendemics drawn from Beringia and southern North America and paleoendemics that persisted in refugia through the last glacial maximum (Cook et al. 2001). Flying squirrels are probable post-glacial colonizers of the archipelago. Although several hypotheses concerning glacial refugia in Southeast Alaska have been advanced (Worley and Jaques 1973; Heusser 1989; Heaton et al. 1996), no concrete evidence exists for the presence of forested refugia spanning the glacial maximum. A lack of forested areas during full glacial advances would probably preclude the persistence of flying squirrels. Phylogeographic studies based on mtDNA also point to close genetic relationships among flying squirrels from Southeast and Interior Alaska, British Columbia, Yukon and the lower 48 states of the United States, consistent with a post-Pleistocene expansion into the coastal region (Arbogast 1999; Demboski et al. 1998a; Bidlack and Cook 2001; Bidlack, unpublished data).

Colonization ability has played a primary role in structuring species composition of mammals across the archipelago (Conroy et al. 1999). Flying squirrels are known from only 15 islands in the southern portion of the archipelago (Figure 1). These distributional data indicate that they may not be adept over-water dispersers, or they have recently colonized the region. An understanding of how squirrels originally colonized these islands, and what factors have prevented them from colonizing others would provide a framework for their long-term conservation. It is possible that there is a water barrier threshold beyond which successful dispersal is unlikely. For example, Clarence Strait between POW and the mainland is 6 km at its narrowest point, while the distance among the POW complex islands is much less, often on the order of tens to hundreds of meters. There is apparently no gene flow between the POW complex and other Southeast Alaska populations we examined (Table 4, Figures 4 and 6). Microsatellite allele frequencies are very similar within the POW complex, but are quite different from other Southeast Alaska populations (Tables 4 and 5), perhaps reflecting the proximity of the POW islands to one another. A regression of genetic distances against geographic distances (Figure 6) also indicates a pattern of isolation by distance across Southeast Alaska among population pairs. This pattern suggests that these populations may currently be at equilibrium between genetic drift and gene flow. Therefore, it is reasonable to conclude that colonization of the islands has not occurred very recently, and there is little long-distance gene flow among them (Slatkin, 1993).

Microsatellite and mtDNA concordance on POW complex

D_{sa} and D_s phenograms suggest that the POW complex populations are clearly separated from the other three populations examined (Figures 4 and 5). 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 and Cook (2001). This POW clade 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

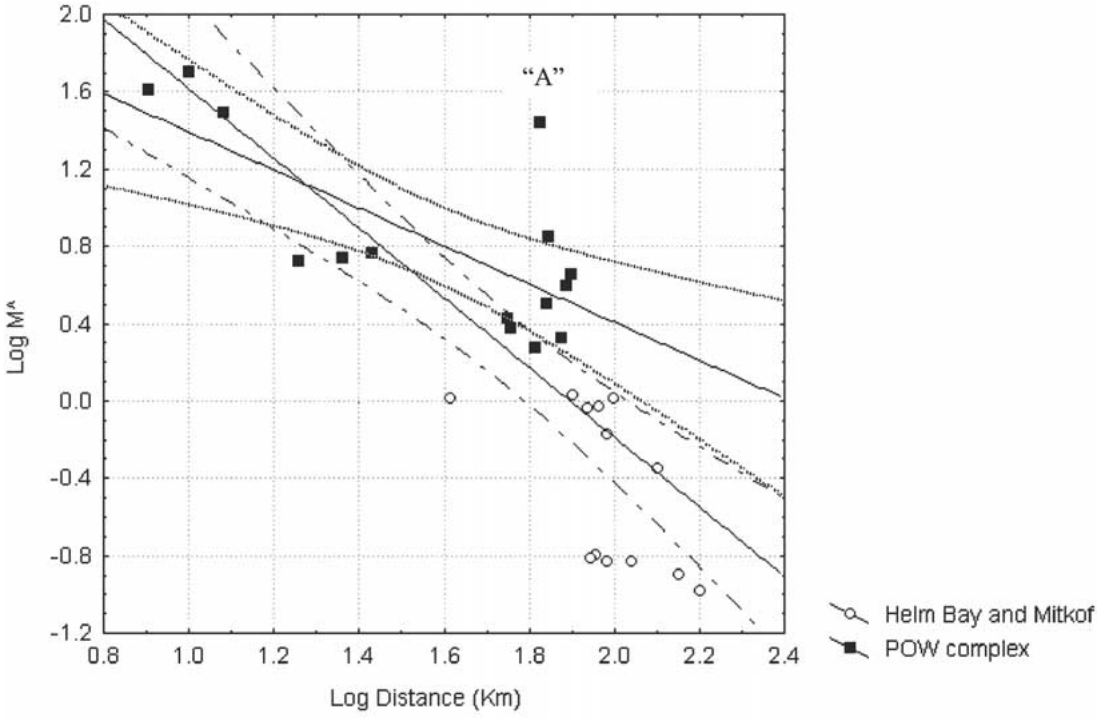

Figure 6. Isolation by distance regression of log M^ against log (distance) for population pairs. Darker line is regression of POW complex pairs (r = –0.7260); lighter line is regression of all population pairs (r = –0.7955); dashed lines are 95% confidence intervals. Point 'A' represents the Polk Inlet/Naukati Bay pair from POW.

sweep of the mitochondrial genome or lineage sorting of the mitochondrial haplotypes.

The agreement of nuclear and mitochondrial perspectives also eliminates the possibility that this pattern of differentiation is an artifact of sex-biased gene flow. Based on mitochondrial sequencing, Talbot and 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 invoked the possibility of a glacial refugium in Southeast Alaska (Heaton et al. 1996). 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 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 no recent genetic exchange between the POW complex and mainland populations.

All squirrels from eleven islands of the POW complex share a distinct mitochondrial haplotype, with only one additional base pair change in one individual (Bidlack and Cook 2001). Populations on these islands may have been separated from those of the mainland and other islands for a sufficient amount of time for reciprocal monophyly to occur. While the microsatellite alleles present in the POW complex are similar to those of Mitkof Island and the mainland, the allele frequencies are very different (Table 4), and there is a unique FS10 allele found only in the POW complex populations. This again suggests that the POW complex populations have been isolated for a period of time sufficient not only for a reduction in genetic variation (due to drift in a smaller population), but enough time for accumulation of new mutations to become fixed in the island populations.

All six POW complex populations are highly related. Pairwise F_{st} (θ) values are low (less than 0.15), indicating low to moderate levels of differentiation between populations (Table 4). However, some

isolation by distance across population pairs within the POW complex exists (Figure 6). The lowest pairwise F_{st} (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. Polk Inlet and Naukati Bay are also closely related and, though 67 km apart, are both located on Prince of Wales Island. The point representing the Naukati and Polk Inlet pair in the isolation by distance analysis ('A' in Figure 6) is above the regression line, and may indicate substantial levels of gene flow across this island. There are no published data concerning juvenile flying squirrel dispersal in the Pacific Northwest, but both males and females are promiscuous, and males travel long distances (> 1 km) during the breeding season (T. Wilson, pers comm.). Gene flow between populations may be affected not only by distance, but by habitat connectedness (on POW), width and depth of the water barrier between islands, and strength and direction of tidal flow, among other factors. We did not directly analyze gene flow (estimated Nm) because of potential problems associated with small numbers of loci and individuals (Whitlock and McCauley 1999). However, our data suggest that current gene flow exists among the six islands of the POW complex that we examined, and that this flow is primarily affected by distance between populations.

Levels of genetic diversity on islands

Variance in levels of inbreeding and genetic diversity of island populations have been extensively contrasted with mainland populations (e.g. Kilpatrick 1981; Gilbert et al. 1990; Lade et al. 1996; Estoup et al. 1996; Frankham 1997, 1998; Eldridge et al. 1999). Generally, insular populations have fewer alleles, lower heterozygosity, lower sequence diversity, fewer polymorphic loci and a higher level of inbreeding than mainland populations. Reduced genetic diversity is generally attributed to an original founder event on islands, and subsequent bottleneck effects caused by stochastic population fluctuations affecting small populations. Patterns of variation in insular flying squirrels along the north Pacific coast are consistent with these generalizations as they exhibit fewer microsatellite alleles, fewer polymorphic loci, and lower heterozygosity (Tables 2 and 3). Alternatively, these patterns in genetic variation among these nearshore islands may be more simply attributed to smaller effective population sizes on the islands as compared with the mainland.

The population from Mitkof Island possesses little microsatellite variation compared with other island populations we examined. This population has two monomorphic loci, two other loci that exhibit lower allelic diversity than in other populations (FS8 and FLS6; Table 3), and an observed heterozygosity of 0.128 (Tables 2 and 3; Figure 3). All alleles found in the Mitkof Island population are found at Helm Bay (Southeast mainland population), except for one unique allele (Table 3) found in three individuals. Bidlack and Cook (2001) found no variation in mitochondrial sequences (1590 base pairs) in five individuals from this island; however, unlike squirrels from the POW complex, the Mitkof Island haplotype was shared with mainland populations. The low microsatellite diversity and the single cytochrome *b* haplotype on Mitkof suggest that genetic drift in this small population has reduced or eliminated any preexisting variation. However, the fact that the cytochrome *b* haplotype is shared with the mainland indicates that a sufficient amount of time has not passed since separation from the mainland population for unique haplotypes to arise. The Mitkof Island population is monophyletic (Figure 4), and well differentiated (Figure 5; pairwise F_{st} (θ) values ranging from 0.604 to 0.702) from other populations in the archipelago. Likewise, all six loci show significant differences in allele frequencies from other populations (Table 4). Our Southeast Alaska mainland sampling remains limited and precludes an effective summary of the colonization history of Mitkof Island. However, nuclear and mitochondrial data from all nine populations suggest that the colonization of Mitkof Island is probably more recent than the founding of the POW complex populations, and that there is likely little gene flow between squirrels of this island and other populations in Southeast Alaska.

Conclusions

Over 80% of the Alexander Archipelago is managed by the Tongass National Forest, and 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 in Southeast Alaska, and further cuts are proposed that may impact forest-associated

species (USDA Forest Service, Tongass National Forest, unpublished data). Until recently, there was little recognition of the potential impacts these activities may have on forest-associated mammals of the region, and questions of phylogeographic structure and endemism were largely ignored. There is some uncertainty concerning the dependence of *G. sabrinus* on old-growth forest in the Pacific Northwest (Rosenberg and Anthony 1992), although there is evidence that flying squirrels are more abundant in old-growth forest (Witt 1992), and that abundance may be correlated with understory development, particularly ericaceous shrubs (Carey 1995). Although the current 10-year forest plan (USDA 1997) mandates the management and conservation of northern flying squirrels, there are no published studies of habitat use by northern flying squirrels in Southeast Alaska.

Recent studies of the insular fauna of Southeast Alaska have revealed surprising patterns of genetic structure relevant to the management of these taxa (e.g. Talbot and Shields 1996; Cook et al. 2001). Recently, the Prince of Wales flying squirrel (*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). Interestingly, an examination of mtDNA from North Pacific Coast ermine (*Mustela erminea*) has revealed an island lineage present on Prince of Wales, Suemez and Heceta islands, as well as on Graham Island, Haida Gwaii (Queen Charlotte Islands), British Columbia (Fleming and Cook, in press). Such concordant patterns of endemism highlight the need to incorporate phylogeographic information into management plans for the region.

G. s. griseifrons has a distinctive history of colonization and isolation from other northern flying squirrels in Southeast Alaska. Base pair changes seen in the mitochondrial sequences (Demboski et al. 1998a; Bidlack and Cook 2001), unique microsatellite alleles, and distinctive microsatellite frequencies in the POW complex indicate past and continuing differentiation from mainland populations. Future work should focus on unsampled islands in the POW complex and from mainland British Columbia and Southeast Alaska to shed light on the origins and relationships of the POW complex populations. The development of more microsatellite loci would also aid in the estimation of gene flow among populations.

Further work should be conducted on the Mitkof Island population, given the pronounced lack of genetic variation on this island. Levels of variation in flying squirrel populations from other islands in the archipelago such as Revillagigedo, Wrangell, and Etolin islands should also be assessed, and compared more extensively with mainland population genetic diversity.

Across the archipelago, patterns of population differentiation should be monitored to assess the impacts on this species of old-growth forest 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. The characterization of a distinctive 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. Concordant patterns of endemism, such as those identified for the POW complex, may emerge that current extractive land use paradigms do not adequately address.

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Appendix I

List of specimen Alaska Frozen Tissue Collection (AF) numbers

Polk Inlet, Prince of Wales I.
26303, 26304, 26305, 26306, 26307, 26308, 26309, 26310, 26311, 26312, 26313, 26321, 26322, 26324, 26325, 26326, 26329, 26340, 26345, 26346, 26349, 26350

Naukati Bay, Prince of Wales I.
5268, 5676, 12466, 14109, 14110, 28834, 28835, 28836, 28837, 28845, 28846, 28847, 28864, 28865, 28866, 28867, 28868, 28869, 28873, 28879, 28880, 28881, 28891, 28900, 28921, 28922, 28923,

28924, 28926, 28929

Suemez I.
16873, 26422, 26423, 26426, 26427, 26428, 26429, 26430, 26431, 26432, 26433, 26434, 26436, 26437, 26438, 26439, 26442, 26443, 26444, 26445, 26446, 26447, 26448, 26449, 26450, 26461, 26462, 26463

El Capitan I.
5195, 10411, 12463, 12467, 12485, 12486, 12499, 28902, 28903, 28917, 28918, 28919, 28936, 28953, 28954, 28960, 28961, 28962, 28963, 28964, 28969, 28970, 28971, 28972

Heceta I.
16874, 16879, 16894, 26404, 28991, 28992, 28993, 29004, 29005, 29006, 29016, 29022, 29023, 29024, 29025, 29031, 29032, 29033, 29034, 29035, 29036, 29053, 29054

Tuxekan I.
12498, 28853, 28854, 28860, 28861, 28874, 28888, 28904, 28914, 28915, 28934, 28935, 28940, 28944, 28955, 28956, 28973, 28975, 28976, 28977, 28978, 28979, 28980, 28981, 28982, 28985, 28988, 28989, 28990, 29015

Mitkof I.
5925, 5926, 5927, 14099, 14100, 14101, 14102, 17868, 19881, 19882, 20364, 21123, 21124, 21125, 24330, 24332, 27081

Cleveland Peninsula
5187, 5188, 5190, 14103, 14104, 14105, 14106, 14107, 25176, 25177, 25178, 25179, 25180, 25181, 27095, 27096, 27097, 27098, 27099, 27100, 27101, 27102, 27103, 27104, 27105, 22591, 22592, 22593, 22594, 22595, 22596, 25425

Fairbanks
5722, 11201, 11410, 14092, 14093, 14094, 14889, 14890, 15450, 17940, 18022, 18644, 18663, 18680, 18686, 18691, 18694, 18719, 19188, 22356, 24774, 24779, 24817, 24818, 24819, 24820, 24822, 24823, 24824

Appendix II

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

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