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Reduced genetic variation in insular northern flying squirrels (*Glaucomys sabrinus*) along the North Pacific Coast

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Abstract

Nearshore oceanic archipelagos are natural laboratories that could provide valuable insight into the role of evolutionary processes such as founder effects and incipient speciation in biotic conservation. The Alexander Archipelago of Southeast Alaska is an example of such a complex, yet few biological investigations have been conducted. For the past 50 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 corroborates the subspecific designation of the endemic Prince of Wales flying squirrel (*Glaucomys sabrinus griseifrons*). This island lineage exhibits severely reduced genetic variation and may be the result of an early Holocene founder event. Nearly all of the animals we examined on Prince of Wales Island and ten islands to the west had identical cytochrome *b* (52 of 53) and control region (21 of 21) sequences. In contrast, substantial polymorphism and little genetic structuring were found in comparable populations on the mainland 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.

INTRODUCTION

Archipelagos play a prominent role in conservation because they often harbour endemic species, which are especially susceptible to extinction (Diamond, 1989). 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 harbour endemic taxa and unique combinations of plants and animals not found on the mainland (e.g., Cook & MacDonald, 2001); 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 few exceptions (e.g., 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). Many of these endemics are spread over the Alexander Archipelago, which consists of over 2000 named islands (Fig. 1). This complex landscape has been further fragmented by extensive timber harvests and road building during the past 50 years (Durbin, 1999). Rudimentary inventories led to uncertainty over the distribution and abundance of endemic taxa; however, recent phylogeographic work highlights the need to incorporate information on endemics and evolutionary processes on islands into management plans for the region (Cook *et al*., 2001).

Because northern flying squirrels (*Glaucomys sabrinus*) generally are associated with old-growth forest in the Pacific Northwest (Carey, 1995, 1996; but see Rosenberg & Anthony, 1992), the Prince of Wales flying squirrel (*G. s. griseifrons*) has been the focus of conservation concern (Demboski, Cook & Kirkland, 1998*a*).

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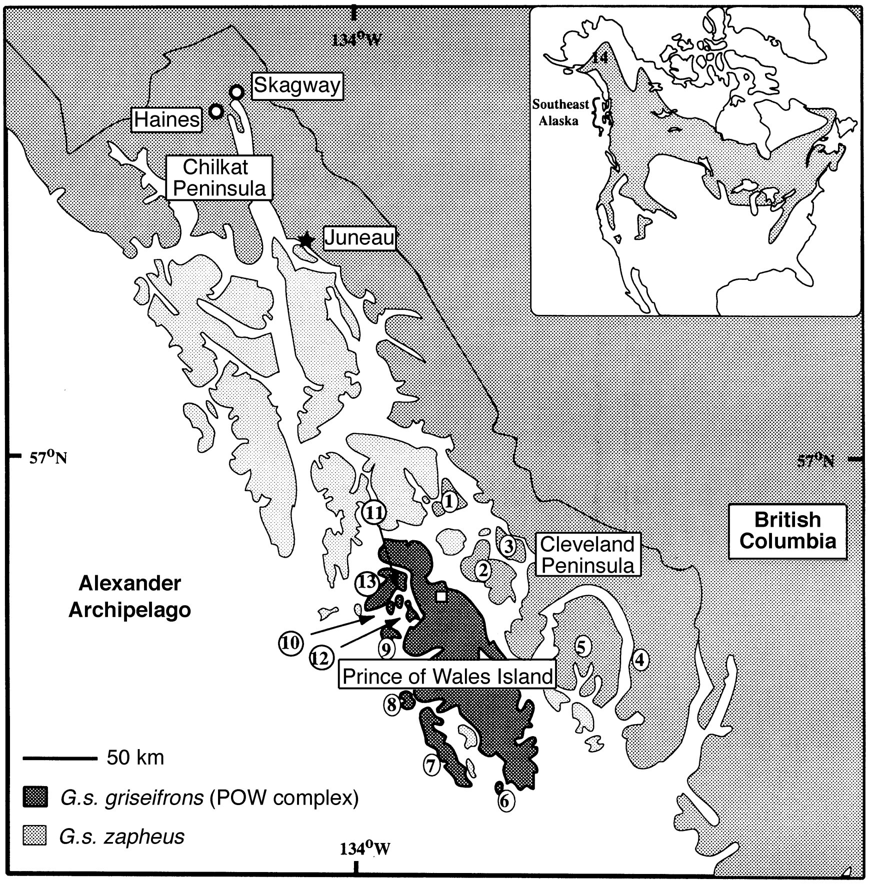

Fig. 1. Distribution of flying squirrels in Southeast Alaska, including the type locality for *Glaucomys sabrinus griseifrons* (☐). Lightly shaded islands do not have flying squirrels or have not been inventoried. Inset map shows North American range of *G. sabrinus*. Numbers on map represent selected 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., (14) Interior Alaska. Other sampling localities indicated by name.

Prince of Wales Island in the Alexander Archipelago has been heavily deforested and extensive future timber harvests are planned. Demboski, Jacobsen & Cook's (1998*b*) preliminary assessment of geographic variation in northern flying squirrels in Southeast Alaska used sequences of the mitochondrial cytochrome *b* gene. They characterized three subspecies from Alaska (*G. s. griseifrons, G. s. zapheus, G. s. yukonensis*), and found that animals from Prince of Wales, and eight nearby islands (POW complex), shared two diagnostic mutations across 1440 base pairs examined.

The mitochondrial control region evolves faster than cytochrome *b*, and 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 sequence variation and expanded Demboski *et al*.'s (1998*b*) cytochrome *b* data by adding complete sequences for animals from an additional four islands and three mainland areas. 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 disturbances may impact insular endemic populations.

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 8 mainland populations) was used for DNA extraction. Specimens, including many provided by marten trappers, were sampled from 22 locations (sample number 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 42 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 thermal cycler 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 under Accession numbers AF011738–AF011742, AF030389–AF030394, AF271669, AF271673, AF271668, AF359135–AF359213, AF359216–AF359218, AF359220–AF359233.

The program PAUP* (Swofford, 1999) was used to calculate genetic distances (both uncorrected 'p' and GR+I+Γ) among all individuals for which complete cytochrome *b* and 350 bp of control region was sequenced ($n = 32$). Parameters for maximum likelihood were estimated (Sullivan, Swofford & Naylor, 1999), and likelihood ratio tests were performed among them to determine the best evolutionary model to create a maximum-likelihood tree. Sequences from 32 individuals were used to construct a maximum-likelihood tree using the general time reversible model with invariable sites plus gamma (GTR+I+Γ). Fifty bootstrap replicates were performed across the tree to determine strength of relationships.

RESULTS

Cytochrome *b* and control region sequences show a division between the POW complex and other populations, reinforcing and clarifying the preliminary conclusions of Demboski *et al*. (1998*b*). All cytochrome *b* sequences ($n = 53$) from the POW complex are identical, except for one from the southern end of Prince of Wales Island which has one additional base pair transition. All 53 individuals from the POW complex are characterized by two synonymous base pair changes (one transition, one transversion) compared with all other individuals examined. 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 (Table 1).

Excluding the divergent Washington sequences, the 24 variable sites in the control region (of 350 base pairs; Table 2(a)) comprise 22 haplotypes. All control region sequences from the POW complex ($n = 21$) are identical and represent 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 monotypic POW complex samples, populations from the Cleveland Peninsula and Interior Alaska with large sample sizes ($n > 14$) include seven haplotypes each (Table 2(b)). Locations for which we sequenced only a few animals, such as Juneau, Revillagigedo Island and Chilkat Peninsula, were also variable. A single individual was examined for Etolin and Wrangell islands, Rudyerd Bay, Haines and Skagway.

Genetic distances (Table 3) between the POW complex and individuals from the mainland or nearshore islands (excluding Washington state) ranged from 0.33% to 0.61% (uncorrected 'p'). Differences among individuals from the mainland and nearshore islands ranged from 0.07% to 0.7%. GTR distances are similar to these uncorrected distances (Table 3). These low levels of divergence allow only two supported clades to be defined: Haines–Skagway (78%), and POW–Southern POW (80%; Fig. 2).

DISCUSSION

Mainland vs. island patterns of variation

Four minimally divergent cytochrome *b* haplotypes were found along the Southeast Alaska mainland. The level of variation among these haplotypes is consistent with cytochrome *b* diversity in other mammals sampled from the mainland (Cook *et al*., 2001). *Glaucomys sabrinus* is unique, however, in having so little cytochrome *b* diversity across 11 islands of the Alexander Archipelago. Similarly, while only one control region haplotype was found on the POW complex, four control region haplotypes on the nearshore islands of Revillagigedo, Wrangell, Mitkof and Etolin and 12 haplotypes on the mainland were identified. Many of these haplotypes are unique to a particular area (i.e., Rudyerd Bay, Haines, Skagway; Table 2(b)); however, small sample sizes ($n = 1$ for several localities) and incomplete geographic sampling preclude robust analysis of population genetic

Table 1. Cytochrome *b* haplotypes and nucleotide positions of substitutions

	Cytochrome *b* position and type of base pair substitution							
Haplotype	150	318	394	468	480	574	903	1131
Mainland	T	C	G	C	T	C	T	C
POW Complex	.	.	.	.	.	T	.	A
South POW	.	.	A	.	.	T	.	A
Haines & Skagway	.	.	.	T	.	.	.	.
Juneau & Chilkat Peninsula	.	.	.	.	C	.	.	.
Wrangell Island	.	T	.	.	.	.	.	.
Rudyerd Bay	C	.	.	.	.	.	.	.
Yukon Territory	.	.	.	.	.	.	C	.

Table 2(a). Control region haplotypes and positions of substitutions from 5′ end

Haplotype		77	96	106	114	115	149	150	151	173	174	178	230	252	255	258	259	272	277	305	308	312	315	322	341
A	POW Complex	C	C	G	C	G	T	C	C	C	T	C	T	A	T	C	T	T	T	A	A	A	A	T	C
B	Rudyerd Bay	T	.	.	.	.	.	.	T	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
C	Wrangell I. and Cleveland Pen.	.	.	.	.	A	.	.	.	.	C	.	C	.	.	.	C	.	.	.	.	G	.	.	.
D	Mitkof I., Etolin I. Cleveland Pen.	T	.	.	.	A	.	.	.	.	C	.	C	.	.	.	C	.	.	.	.	G	.	.	.
E	Cleveland Pen.	.	A	.	.	A	.	.	.	.	.	T	C	.	.	.	.	.	.	.	.	.	.	.	.
F	Revillagigedo I.	.	.	.	.	A	.	.	.	.	T	T	C	.	.	.	C	.	.	.	.	.	.	.	.
G	Revillagigedo I.	.	.	.	.	A	.	.	.	T	.	T	C	G	.	.	.	C	.	.	.	.	.	.	.
H	Cleveland Pen.	.	.	A	.	A	.	.	.	.	.	.	C	.	.	T	.	.	.	.	.	.	.	.	.
I	Cleveland Pen.	.	.	A	.	.	.	.	.	.	.	.	C	.	.	T	.	.	.	.	.	.	.	.	.
J	Cleveland Pen.	.	.	.	.	A	.	.	.	.	.	.	C	.	.	T	.	.	.	.	.	.	.	.	.
K	Cleveland Pen.	T	.	.	.	A	.	.	T	.	C	.	C	.	.	.	C	.	.	.	.	G	.	.	.
L	Haines	.	.	.	.	A	.	.	.	T	C	.	C	.	.	.	.	.	C	.	.	.	.	.	.
M	Skagway	.	.	.	.	A	.	.	.	T	C	.	C	.	.	T	.	.	C	.	.	.	.	.	.
N	Chilkat Pen.	.	.	A	.	A	.	.	.	.	.	.	C	.	.	.	.	.	.	.	.	.	G	.	.
O	Juneau & Chilkat Pen.	.	.	.	.	A	.	.	.	.	.	.	C	.	.	.	.	C	C	.	.	.	G	C	.
P	Interior Alaska	.	.	.	.	A	.	.	.	.	.	T	C	.	C	T	.	C	.	.	.	.	.	.	.
Q	Interior Alaska	.	.	.	.	A	.	.	.	.	.	T	C	.	.	T	.	.	.	.	.	.	.	.	T
R	Interior Alaska	.	.	.	.	A	.	.	.	.	.	T	C	.	.	T	.	.	.	.	.	.	.	.	.
S	Interior Alaska	.	.	.	.	A	C	.	.	.	.	T	C	.	.	T	.	.	.	.	.	.	.	.	.
T	Interior Alaska	.	.	A	.	A	.	.	.	.	.	.	C	.	.	T	.	.	.	.	G	.	.	.	T
U	Interior Alaska	.	.	A	T	A	.	T	.	.	.	.	C	.	.	T	.	.	.	G	G	.	.	.	T
V	Interior Alaska	.	.	.	T	A	.	.	.	.	.	.	C	.	.	T	.	.	.	G	.	.	.	.	T

Table 2(b). Distribution of control region haplotypes in *Glaucomys 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 I.	6	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	6
	Dall I.	1	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	1
	El Capitan I.	2	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	2
	Heceta I.	2	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	2
POW Complex	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	—	—	—	—	—	—	—	—	—	—	—	—	3	—	—	—	—	—	—	—	1
	Juneau	—	—	—	—	—	—	—	—	—	—	—	—	—	1	1	—	—	—	—	—	—	—	3
	Chilkat Pen.	—	—	1	6	4	—	—	3	3	2	1	—	—	—	—	—	—	—	—	—	—	—	2
	Cleveland Pen.	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	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	3	2	2	1	1	1	1	4	1	2	1	2	1	3	4	74

structure. A maximum-likelihood phylogeny based on cytochrome *b* and control region sequences is poorly resolved (Fig. 2). Only two clades are well supported (> 75%), one that includes Haines and Skagway, and the other representing the two haplotypes of the POW complex. This minimal geographic structuring along the mainland possibly reflects a rapid expansion into Southeast Alaska after the last glacial maximum (22,000–18,000 BP).

Colonization of islands may result in a loss of genetic diversity, with subsequent differentiation between the original and founder populations induced by genetic drift or differential selective regimes. Many insular mammal taxa exhibit lower genetic variation than their continental counterparts (e.g., Kilpatrick, 1981; Frankham, 1997, 1998). For example, the Channel Island fox, *Urocyon littoralis*, exhibits lower allozyme and mitochondrial haplotype diversity, and lower micro- and mini-satellite allelic diversity than mainland foxes (Gilbert *et al.*, 1990; Wayne *et al.*, 1991; Goldstein *et al.*, 1999). We suspect that the distinctive POW complex haplotypes may be due to a post-Pleistocene founder event to these islands, with the extremely low level of variation on the 11 POW complex islands (a single autapomorphic

Table 3. Genetic distance between combined cytochrome *b* and control region haplotypes; uncorrected ‘p’ below diagonal; GTR +Γ + I distances above diagonal

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20
1. POW		0.0007	0.0044	0.0044	0.0044	0.0060	0.0069	0.0045	0.0060	0.4053	0.3677	0.0060	0.0068	0.0052	0.0036	0.0060	0.0060	0.0036	0.0069	0.0052
2. Southern POW	0.0007		0.0052	0.0053	0.0053	0.0069	0.0078	0.0053	0.0069	0.4123	0.3745	0.0069	0.0077	0.0060	0.0044	0.0069	0.0070	0.0044	0.0078	0.0060
3. Revillagigedo I.	0.0040	0.0047		0.0014	0.0028	0.0014	0.0036	0.0029	0.0043	0.3795	0.3594	0.0014	0.0051	0.0021	0.0036	0.0044	0.0044	0.0051	0.0051	0.0021
4. Cleveland Pen.	0.0040	0.0047	0.0013		0.0029	0.0028	0.0052	0.0029	0.0043	0.3942	0.3652	0.0029	0.0051	0.0021	0.0037	0.0044	0.0044	0.0052	0.0052	0.0021
5. Cleveland Pen.	0.0040	0.0047	0.0027	0.0027		0.0028	0.0052	0.0014	0.0044	0.3688	0.3411	0.0044	0.0036	0.0021	0.0007	0.0044	0.0044	0.0052	0.0052	0.0021
6. Interior Alaska	0.0054	0.0060	0.0013	0.0027	0.0027		0.0051	0.0044	0.0059	0.3713	0.3514	0.0028	0.0051	0.0021	0.0036	0.0059	0.0059	0.0067	0.0067	0.0021
7. Juneau & Chilkat	0.0060	0.0067	0.0034	0.0047	0.0047	0.0047		0.0036	0.0051	0.3775	0.03651	0.0052	0.0059	0.0059	0.0061	0.0068	0.0068	0.0077	0.0077	0.0059
8. Chilkat	0.0040	0.0047	0.0027	0.0027	0.0013	0.0040	0.0034		0.0044	0.3789	0.3508	0.0045	0.0052	0.0036	0.0021	0.0045	0.0045	0.0053	0.0053	0.0036
9. Haines	0.0054	0.0060	0.0040	0.0040	0.0040	0.0054	0.0047	0.0040		0.3713	0.3514	0.0044	0.0007	0.0051	0.0052	0.0044	0.0044	0.0067	0.0051	0.0051
10. Washington	0.0848	0.0855	0.0821	0.0835	0.0808	0.0808	0.0815	0.0815	0.0808		0.0085	0.3754	0.3672	0.3754	0.3754	0.3847	0.3865	0.3942	0.3806	0.3796
11. Washington	0.0808	0.0815	0.0808	0.0808	0.0781	0.0795	0.0808	0.0788	0.0795	0.0074		0.3554	0.3474	0.3474	0.3474	0.3564	0.3580	0.3574	0.3524	0.3514
12. Revillagigedo I.	0.0054	0.0060	0.0013	0.0027	0.0040	0.0027	0.0047	0.0040	0.0040	0.0815	0.0801		0.0051	0.0036	0.0053	0.0060	0.0060	0.0068	0.0068	0.0036
13. Skagway	0.0060	0.0067	0.0047	0.0047	0.0034	0.0047	0.0054	0.0047	0.0007	0.0801	0.0788	0.0047		0.0043	0.0044	0.0051	0.0051	0.0076	0.0059	0.0043
14. Interior Alaska	0.0047	0.0054	0.0020	0.0020	0.0020	0.0020	0.0054	0.0034	0.0047	0.0815	0.0788	0.0034	0.0040		0.0029	0.0051	0.0051	0.0059	0.0059	0.0014
15. Cleveland Pen.	0.0034	0.0040	0.0034	0.0034	0.0007	0.0034	0.0054	0.0020	0.0047	0.0815	0.0788	0.0047	0.0040	0.0027		0.0053	0.0053	0.0044	0.0061	0.0029
16. Mitkof I., Etolin I. and Cleveland Pen.	0.0054	0.0060	0.0040	0.0040	0.0040	0.0054	0.0060	0.0040	0.0040	0.0828	0.0801	0.0054	0.0047	0.0047	0.0047		0.0014	0.0052	0.0007	0.0051
17. Wrangell I.	0.0054	0.0061	0.0040	0.0040	0.0040	0.0054	0.0061	0.0040	0.0040	0.0836	0.0809	0.0054	0.0047	0.0047	0.0047	0.0013		0.0068	0.0021	0.0051
18. Rudyerd Bay	0.0034	0.0040	0.0047	0.0047	0.0047	0.000	0.0067	0.0047	0.0060	0.0835	0.0795	0.0060	0.0067	0.0054	0.0040	0.0047	0.0061		0.0044	0.0059
19. Cleveland Pen.	0.0060	0.0067	0.0047	0.0047	0.0047	0.0060	0.0067	0.0047	0.0047	0.0047	0.0822	0.0795	0.0060	0.0054	0.0054	0.0054	0.0007	0.0020	0.0040	0.0059
20. Interior Alaska	0.0047	0.0054	0.0020	0.0020	0.0020	0.0020	0.0054	0.0034	0.0047	0.0821	0.0794	0.0034	0.0040	0.0013	0.0027	0.0047	0.0047	0.0054	0.0054	

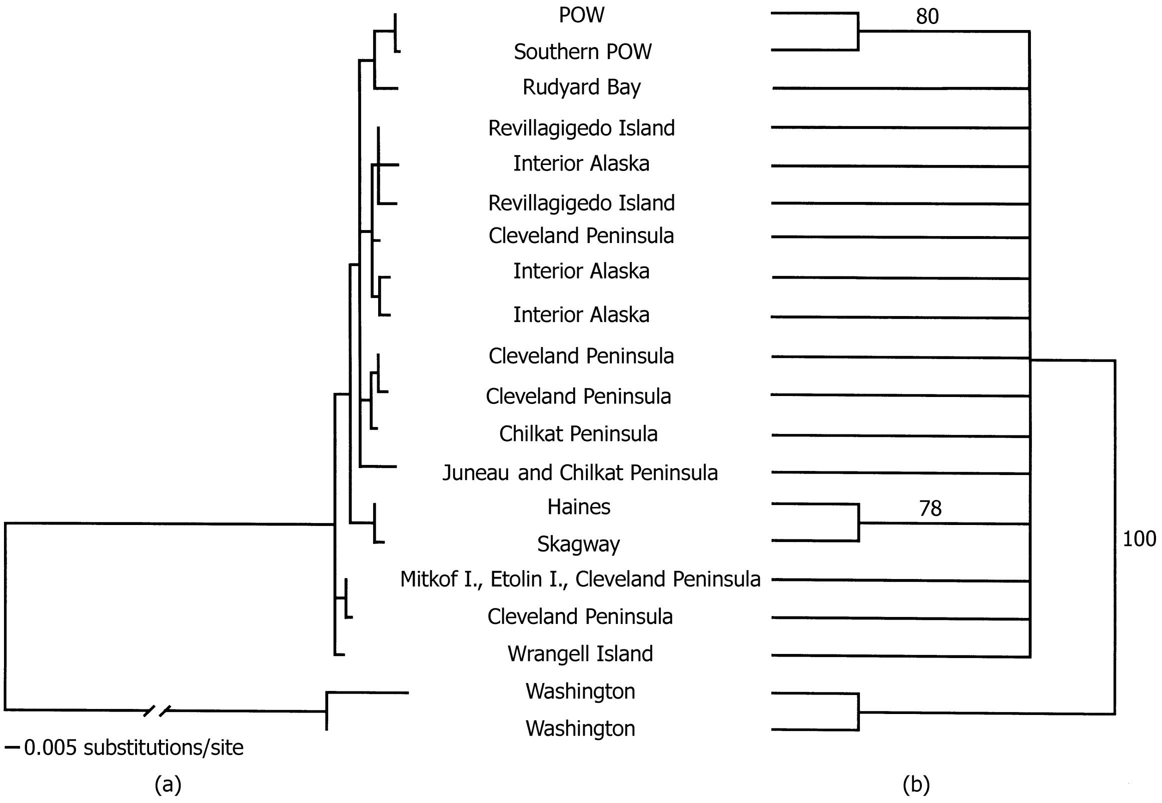

Fig. 2. Maximum-likelihood tree and bootstrapped ML tree of mitochondrial haplotypes of northern flying squirrels sampled from Southeast and Interior Alaska. Maximum-likelihood tree (a) shows minimal differentiation among haplotypes and little geographic structuring of populations. Two clades in Southeast Alaska are supported in the bootstrapped tree (b), with bootstrap percentages indicated above branches.

change in one individual) suggesting the possibility of a severe bottleneck in the ancestral population (Nei, Maruyama & Chakraborty, 1975). The apparent isolation of these islands, yet low level of genetic differentiation, indicates the Prince of Wales flying squirrel may offer an example of incipient speciation.

Phylogenetics and conservation

DNA sequencing and fingerprinting are commonly used to diagnose evolutionarily significant units (ESUs) and management units (MUs) because they provide efficient ways of gathering information about population subdivision in endangered or threatened taxa (Moritz, 1994). However, the prevailing dominance of genetic data over morphological or ecological information has raised concerns, including the possible misdiagnosis of populations (Paetkau, 1999), the potential dissociation between natural genetic markers and significant functional diversity (Pritchard, 1999; Crandall *et al.*, 2000), and the need to differentiate historical legacy from current dynamics (Crandall *et al.*, 2000). While these concerns are valid, genetic data may provide a first approximation of population subdivision, long before ecological or morphological differences can be assessed critically. Genetic data can also be used to assess diversity within a population, as limited genetic variability may be an important factor for the long-term viability of endangered taxa (Frankham, 1997; Newman & Pilson, 1997; Saccheri *et al.*, 1998). Our research is a first step towards quantifying population divergence in an insular endemic in Southeast Alaska because mitochondrial sequences can provide insight into both recent and historical genetic exchangeability (*sensu* Crandall *et al.*, 2000) among island and mainland populations.

Moritz's (1994) criteria for ESU designation were reciprocal mitochondrial monophyly and significant allele frequency divergence in nuclear alleles. MUs were recognized as populations with only significant allele frequency divergence at nuclear or mitochondrial loci. These criteria emphasized historical population structure and were based on theoretical rates of allele subdivision among populations. Moritz's criteria were not meant to be proscriptive, but rather meant to lay the groundwork for the entrance of genetic data into the ESU debate. Using Moritz's (1994) molecular criteria, mitochondrial sequences indicate that the POW flying squirrel should be minimally recognized as a MU because these populations have not experienced recent female-mediated gene flow with the mainland populations. Investigations of variability at nuclear loci may shed light on male-mediated gene flow between the POW complex and the mainland. Ecological characterization of these populations also would help evaluate functional diversity.

Because island systems tend to be simpler than continental ones, certain taxa may play different roles in these less complex systems. Both red squirrels (*Tamiasciurus*) and red-backed voles (*Clethrionomys*) are absent on the POW complex, and the effect of their absence on insular flying squirrels is unknown. All three species consume hypogeous fungi, and the two species of squirrel may compete for nesting space. In the absence of specific ecological studies, molecular research is providing a picture of historical population subdivision and limited genetic diversity in insular northern flying squirrels in the Tongass.

Conservation implications

The Tongass, covering 6.8 million coastal hectares, is the largest national forest in the United States. Unique challenges face managers across this complex landscape, and the current forest plan mandates the conservation of endemic taxa across the archipelago (United States Department of Agriculture, 1997). Mitochondrial sequences identify a unique and nearly monotypic clade of flying squirrels on the POW complex of islands, corroborating the original description of *G. s. griseifrons* (Howell, 1934) based on diagnostic morphological characters including a darker dorsal side, whiter ventral side, and greyer neck and head. To date, there have been few additional comparative morphological or ecological studies of subspecies of *G. sabrinus*.

Northern flying squirrels are associated with old-growth coniferous forests in the Pacific Northwest (Carey, 1995, 1996), where they utilize snags as nesting sites (Maser *et al*., 1986). They play an important role in boreal forests by consuming and disseminating hypogeous fungal spores which are required by conifers for the uptake of nutrients. Flying squirrels are also a major prey item of many avian and mustelid predators (Forsman, Otto & Carey, 1991; Wilson & Carey, 1996). Habitat favoured by flying squirrels has been heavily fragmented in Southeast Alaska, with up to 46% of the old-growth of the Tongass harvested on some islands (Table 4; United States Department of Agriculture, 2000). Private land (220,000 ha) also has been heavily logged (Durbin, 1999). An additional 200 million board feet of timber is currently scheduled to be harvested from four islands within the range of *G. s. griseifrons* between the years 2000 and 2010 (Table 4). Most old-growth forest in Southeast Alaska that is protected from timber harvests (e.g., Admiralty Island) is not within the range of the POW flying squirrel.

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 movement and differentiation in Southeast Alaska than previously thought, with both palaeoendemics and neoendemics possibly present (Demboski, Stone & Cook, 2000; Cook *et al*., 2001). We suggest that *G. s. griseifrons* is an example of the latter and, like several other island taxa, may be on a distinct evolutionary path. These studies emphasize the value of biogeographic studies of nearshore archipelagos; they may provide insight into incipient speciation and founder effects, and may reveal patterns relevant to conservation. Future land use should consider impacts on endemics along the North Pacific Coast. In particular, if the Prince of Wales flying squirrel is a subspecies endemic to some islands within the Tongass, as both morphologic and genetic data suggest, then the current logging pressure within its range may not be consistent with the management and conservation goals set forth in the new forest plan.

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REFERENCES

Carey, A. B. (1995). Sciurids in Pacific Northwest managed and old-growth forests. *Ecol. Appl.* **5**: 648–661.

Carey, A. B. (1996). Interactions of northwest forest canopies and arboreal mammals. *Northwest Sci.* **70**: 72–78.

Table 4. Timber harvests* on POW complex islands with *Glaucomys sabrinus 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. All areas are in hectares. Information is for USDA Forest Service land only.

	Island area	ROG	H	OGH	PTH
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	261,773.3	80,774.5	23.6	121–41
Suemez	15,012	8249.5	493.2	5.6	30
Tuxekan	8520	3572.1	3079.3	46.3	20

*as of April 2000

Conroy, C. J., Demboski, J. R. & Cook, J. A. (1999). Mammalian biogeography of the Alexander Archipelago of Alaska: a north temperate nested fauna. *J. Biogeogr.* **26**: 343–352.

Cook, J. A., Bidlack, A. L., Conroy, C. J., Demboski, J. R., Fleming, M. A., Runck, A. M., Stone, K. D. & MacDonald, S. O. (2001). A phylogeographic perspective on endemism in the Alexander Archipelago. *Biol. Conserv.* **97**: 215–227.

Cook, J. A. & MacDonald, S. O. (2001). Should endemism be a focus of conservation efforts along the North Pacific Coast of North America? *Biol. Conserv.* **97**: 207–213.

Crandall, K. A., Bininda-Edmonds, O. R. P., Mace, G. M. & Wayne, R. K. (2000). Considering evolutionary processes in conservation biology. *Trends Ecol. Evol.* **15**: 290–295.

Demboski, J., Cook, J. A. & Kirkland, G. (1998*a*). *Glaucomys sabrinus*. In *North American rodents: status survey and conservation action plan:* 37–39. Hafner, D., Yensen, E. & Kirkland, G. (Eds). Gland, Switzerland: IUCN.

Demboski, J. R., Jacobsen, B. K. & Cook, J. A. (1998*b*). Implications of cytochrome *b* sequence variation for biogeography and conservation of northern flying squirrels (*Glaucomys sabrinus*) of the Alexander Archipelago, Alaska. *Can. J. Zool.* **76**: 1771–1777.

Demboski, J. R., Stone, K. D. & Cook, J. A. (1999). Further perspectives on the Haida Gwaii glacial refugium. *Evolution* **53**: 2008–2012.

Diamond, J. M. (1989). The present, past and future of human-caused extinctions. *Phil. Trans. R. Soc. Lond. (B)* **325**: 469–477.

Durbin, K. (1999). *Tongass: pulp politics and the fight for the Alaska rain forest*. Corvallis, OR: Oregon State University.

Encalada, S. E., Lahanas, P. N., Bjorndal, K. A., Bolten, A. B., Miyamoto, M. M. & Bowens, B. W. (1996). Phylogeography and population structure of the Atlantic and Mediterranean green turtle *Chelonia mydas*: a mitochondria DNA control region sequence assessment. *Mol. Ecol.* **5**: 473–483.

Forsman, E. D., Otto, I. & Carey, A. B. (1991). Diets of spotted owls on the Olympic Peninsula, Washington, and the Roseburg District, Bureau of Land Management. In *Wildlife and vegetation of unmanaged Douglas-fir forests*, General Technical Report PNW-118. Ruggiery, L. F., Aubry, K. B., Carey, A. B. & Huff, M. H. (Eds). Portland, OR: United States Department of Agriculture, Forest Service.

Frankham, R. (1997). Do island populations have less genetic variation than mainland populations? *Heredity* **78**: 311–327.

Frankham, R. (1998). Inbreeding and extinction: island populations. *Conserv. Biol.* **12**: 665–675.

Gilbert, D. A., Lehman, N., O'Brien, S. J. & Wayne, R. K. (1990). Genetic fingerprinting reflects population differentiation in the California Channel Island fox. *Nature* **344**: 764–766.

Giles, B. E. & Goudet, J. (1997). Genetic differentiation in *Silene dioica* metapopulations: estimation of spatiotemporal effects in a successional plant species. *Am. Nat.* **149**: 507–526.

Goldstein, D. B., Roemer, G. W., Smith, D. A., Reich, D. E., Bergman, A. & Wayne, R. K. (1999). The use of microsatellite variation to infer population structure and demographic history in a natural model system. *Genetics* **151**: 797–801.

Good, S. V., Williams, D. F., Ralls, K. & Fleischer, R. C. (1997). Population structure of *Dipodomys ingens* (Heteromyidae): the role of spatial heterogeneity in maintaining genetic diversity. *Evolution* **51**: 1296–1310.

Howell, A. H. (1934). Description of a new race of flying squirrel from Alaska. *J. Mammal.* **15**: 64.

Kilpatrick, C. W. (1981). Genetic structure of insular populations. In *Mammalian population genetics:* 28–59. Smith, M. H. & Joule, J. (Eds). Athens, GA: University of Georgia.

Kocher, T. D., Conroy, J. A., McKaye, K. R. & Stauffer, J. R. (1983). Similar morphologies of cichlid fish in Lakes Tanganyika and Malawi are due to convergence. *Mol. Phylogen. Evol.* **2**: 158–165.

Lessa, E. P. & Cook, J. A. (1998). The molecular phylogenetics of tuco-tucos (genus *Ctenomys*, Rodentia: Octodontidae) suggests an early burst of speciation. *Mol. Phylogen. Evol.* **9**: 88–99.

Lomolino, M. V. (1994). Species richness of mammals inhabiting nearshore archipelagos: areas, isolation, and immigration. *J. Mammal.* **75**: 39–49.

MacDonald, S. O. & Cook, J. A. (1996). The land mammal fauna of southeast Alaska. *Can. Field-Nat.* **110**: 571–598.

Maser, C., Maser, Z., Witt, J. W. & Hunt, G. (1986). The northern flying squirrel: a mycophagist in southwestern Oregon. *Can. J. Zool.* **64**: 2086–2089.

Miller, S. A., Dykes, D. D. & Polesky, H. F. (1998). A simple salting out procedure for extracting DNA from human nucleated cells. *Nucleic Acids Res.* **16**: 215.

Moritz, C. (1994). Defining 'evolutionary significant units' for conservation. *Trends Ecol. Evol.* **9**: 373–375.

Nei, M., Maruyama, T. & Chakraborty, R. (1975). The bottleneck effect and genetic variability in populations. *Evolution* **29**: 1–10.

Newman, D. & Pilson, D. (1997). Increased probability of extinction due to decreased genetic effective population size: experimental populations of *Clarkia pulchella*. *Evolution* **51**: 354–362.

Paetkau, D. (1999). Using genetics to identify intraspecific conservation units: a critique of current methods. *Conserv. Biol.* **13**: 1507–1509.

Pope, L. C., Sharp, A. & Moritz, C. (1996). Population structure of the yellow-footed rock-wallaby *Petrogale xanthopus* (Gray, 1854), inferred from mtDNA sequences and miscrosatellite loci. *Mol. Ecol.* **5**: 629–640.

Pritchard, P. C. H. (1999). Status of the black turtle. *Conserv. Biol.* **13**: 1000–1003.

Ranta, P., Tanskanen, A., Niemalä, J. & Kurtto, A. (1999). Selection of islands for conservation in the urban archipelago of Helsinki, Finland. *Conserv. Biol.* **13**: 1293–1300.

Rosenberg, D. K. & Anthony, R. G. (1992). Characteristics of northern flying squirrel populations in young second- and old-growth forests in Western Oregon. *Can. J. Zool.* **70**, 161–166.

Saccheri, I., Kuusaari, M., Kankara, M., Vikman, P., Fortelius, W. & Hanski, I. (1998). Inbreeding and extinction in a butterfly metropopulation. *Nature* **392**: 491–494.

Smith, M. F. & Patton, J. L. (1993). The diversification of South American murid rodents: evidence from mitochondrial DNA sequence data for the akondontine tribe. *Biol. J. Linn. Soc.* **50**: 149–177.

Sullivan, J., Swofford, D. L. & Naylor, G. J. P. (1999). The effect of taxon sampling on estimating rate heterogeneity parameters of maximum-likelihood models. *Mol. Biol. Evol.* **16**: 1347–1356.

Swofford, D. L. (1999). PAUP*: Phylogenetic Analysis Using Parsimony (*and Other Methods), version 4.062a. Sunderland, MA: Sinauer Associates.

Thomas, W. K., Paabo, S., Villablanca, F. X. & Wilson, A. C. (1990). Spatial and temporal continuity of kangaroo rat populations shown by sequencing mitochondrial DNA from museum specimens. *J. Mol. Evol.* **31**: 101–112.

United States Department of Agriculture (1977). *Tongass Land and Resource Management Plan*. Juneau, AK: USDA Forest Service.

United States Department of Agriculture (2000). Geographic Information System database.

Wayne, R. K., George, S. B., Gilbert, D., Collins, P. W., Kovach, S. D., Girman, D. & Lehman, N. (1991). A morphologic and genetic study of the island fox, *Urocyon littoralis*. *Evolution* **45**: 1849–1868.

Wilson, T. M. & Carey, A. B. (1996). Observations of weasels in second-growth Douglas-fir forests in the Puget Trough, Washington. *Northwest Nat.* **77**: 35–39.