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GENETIC DIFFERENTIATION OF A SUBSPECIES OF SPRUCE GROUSE (*FALCIPENNIS CANADENSIS*) IN AN ENDEMISM HOTSPOT

PATRICK D. BARRY[1,3] AND DAVID A. TALLMON[1,2]

[1]*School of Fisheries and Ocean Sciences University of Alaska Fairbanks, 17101 Point Lena Loop Road, Juneau, Alaska 99801, USA; and*
[2]*Biology and Marine Biology Programs, University of Alaska Southeast, 11120 Glacier Highway, Juneau, Alaska 99801, USA*

ABSTRACT.—We examined the population genetics and phylogenetics of *Falcipennis canadensis isleibi*, a subspecies of Spruce Grouse from the Alexander Archipelago of southeast Alaska, which was recently given subspecies status on the basis of subtle differences in plumage coloration and its limited distribution on several islands. The taxonomic status of *F. c. isleibi* is particularly consequential, both because little is known about its evolutionary, demographic, and conservation status and because island endemics often face high extinction risks. Samples were collected from central Alaska, British Columbia, and Prince of Wales (POW) and Zarembo (ZAM) islands in the Alexander Archipelago and identified to subspecies using established morphological traits. We sequenced the cytochrome-*c* oxidase I (COI) subunit of the mitochondrial genome ($n = 62$) and genotyped each individual at six nuclear microsatellite loci ($n = 65$). Individuals from POW and ZAM shared a unique mitochondrial haplotype not observed in other populations of other subspecies (*F. c. franklinii and F. c. canadensis*), whereas haplotypes were shared by individuals identified as *franklinii* or *canadensis*. Microsatellite loci revealed significant divergence among all subspecies populations ($\bar{F}_{ST} = 0.352$) as well as divergence between POW and ZAM populations of *F. c. isleibi*. These data corroborate the morphological classification of *F. c. isleibi* as a separate subspecies. Spruce Grouse are not managed as a single species by the state of Alaska, but instead as an aggregate with other forest grouse species. Our results indicate that populations of *F. c. isleibi* warrant special management attention to maintain this distinct evolutionary lineage. *Received 16 July 2009, accepted 7 March 2010.*

Key words: Alexander Archipelago, *Falcipennis canadensis*, microsatellites, mtDNA, population structure, Spruce Grouse.

Diferenciación Genética de una Subespecie de *Falcipennis canadensis* en un Centro de Alto Endemismo

RESUMEN.—Examinamos la genética de poblaciones y la filogeografía de *Falcipennis canadensis isleibi*, una subespecie del archipiélago Alexander del sudeste de Alaska, a la cual se le confirió recientemente el estatus de subespecie con base en diferencias sutiles en la coloración del plumaje y en su distribución restringida en varias islas. El estatus taxonómico de *F. c. isleibi* es particularmente importante tanto porque se sabe poco sobre su estado evolutivo, demográfico y de conservación, como porque los endémicos insulares están generalmente sujetos a un alto riesgo de extinción. Recolectamos muestras en Alaska central, British Columbia y en las islas Prince of Wales (POW) y Zarembo (ZAM) en el archipiélago de Alexander, e identificamos las muestras hasta el nivel de subespecie utilizando caracteres morfológicos establecidos. Secuenciamos la subunidad I de la citocromo-c oxidasa (COI) del genoma mitocondrial ($n = 62$) y genotipificamos cada individuo con base en seis loci nucleares microsatelitales ($n = 65$). Los individuos de POW y ZAM compartieron un único haplotipo mitocondrial que no fue observado en otras poblaciones de otras subespecies (*F. c. franklinii* y *F. c. canadensis*), mientras que individuos identificados como *franklinii* o *canadensis* compartieron haplotipos. Los loci microsatelitales revelaron una divergencia significativa entre todas las poblaciones de subespecies ($\bar{F}_{ST} = 0.352$) y también divergencia entre las poblaciones de *F. c. isleibi* de POW y ZAM. Estos datos corroboran la clasificación morfológica de *F. c. isleibi* como una subespecie. *Falcipennis canadensis* no es manejada como una única especie por el estado de Alaska, sino como un agregado junto con otras especies de bosque del mismo género. Nuestros resultados sugieren que las poblaciones de *F. c. isleibi* requieren de una atención especial en el manejo para mantener ese linaje evolutivo distinto.

CONSERVATION OF BIODIVERSITY on islands has become an increasingly important concern because of high rates of endemism and extinction of island faunas (Steadman 1995, 2006). Insular endemics, particularly bird species, are at a disproportionately high risk of extinction as a result of both inbreeding and human-related causes such as overharvest and the introduction of invasive species (Diamond et al. 1989, Frankham 1995, Steadman 1995, Duncan and Blackburn 2007). In the Alexander Archipelago of the

[3]E-mail: pdbarry@alaska.edu

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Pacific Northwest, temporal and spatial separation of island populations has given rise to many endemic island species of conservation concern (Cook et al. 2001, 2006). Many endemic species in the Alexander Archipelago have been described morphologically (MacDonald and Cook 1996), but only recently have phylogenetic studies corroborated and refined some of these classifications (Bidlack and Cook 2002, Fleming and Cook 2002, Lucid and Cook 2004, Topp and Winker 2008).

The Alexander Archipelago is a potential hotspot of avian endemism because it is one of the largest temperate archipelagoes in the world, with >1,100 named islands. Phylogenetic studies have revealed that Prince of Wales Island (the largest in the Alexander Archipelago) supports a large number of endemics of other vertebrate taxa, including Ermine (*Mustela erminea*; Fleming and Cook 2002), Northern Flying Squirrel (*Glaucomys sabrinus*; Bidlack and Cook 2002), Keen's Mouse (*Peromyscus keeni*; Lucid and Cook 2004), and Chum Salmon (*Oncorhynchus keta*; Kondzela et al. 1994). Dickerman and Gustafson (1996) concluded from morphological differences observed in 7 birds (4 males and 3 females) that there is a subspecies of Spruce Grouse, *Falcipennis canadensis isleibi*, endemic to the Alexander Archipelago.

Spruce Grouse inhabit northern coniferous forests from the Atlantic coast in Canada across the interior, extending into parts of the northern United States and west to the Pacific coast of Alaska. Since Uttal's (1939) recognition of four subspecies of Spruce Grouse, the taxonomy of this species has undergone many revisions, in part because there is little morphological variation throughout their range, especially among females (Boag and Schroeder 1992). Traditionally, morphological characters (natal plumage, number of rectrices, and egg color) as well as behavioral characteristics have been used for taxonomy. However, recent molecular studies have been helpful in resolving higher-level classification when similar morphologies are the result of homoplasy rather than homology (Short 1967; Ellsworth et al. 1995, 1996). Currently, the males of both mainland and island Spruce Grouse subspecies are distinguished by subtle differences in plumage coloration, whereas females are distinguished by size. *Falcipennis c. franklinii* has white-tipped upper-tail coverts and lacks a terminal chestnut band on the tail, whereas *F. c. canadensis* lacks white tipping but has a chestnut band (Dickerman and Gustafson 1996). The newly described *F. c. isleibi* is most similar to *F. c. franklinii* but has shorter wings and a longer tail and, in males, narrower white tips (Dickerman and Gustafson 1996). The divisions between *F. c. franklinii*, *F. c. canadensis*, and *F. c. isleibi* rely on both morphological and behavioral traits whose expression may be attributable to genetic or environmental differences among subspecies.

The identification and description of demographically isolated populations and subspecies is extremely important for proper monitoring, management, and designation of the legal status of wildlife species (Waples and Gaggiotti 2006). However, it can be difficult to discern whether a group of animals is truly reproductively isolated in a given area on the basis of morphological traits alone (Weigel et al. 2002). Zink (2004) highlighted the difficulty in designating subspecies by showing that 97% of continental avian subspecies lacked the genetic divergence characteristic of distinct evolutionary units. The phylogenetic relationship of Spruce Grouse to other circumpolar members of the family Tetraonidae has been explored and described (Ellsworth et al. 1996, Gutiérrez et al. 2000). However, DNA techniques have not been used to confirm or refute subspecies' relationships and population structure in Spruce Grouse. Many populations of Spruce Grouse have received management attention because they are declining or rare along the southern boundary of their distribution (Williamson et al. 2008), primarily as a result of degradation, loss, and fragmentation of habitats (Storch 2007). If the geographically isolated populations of Spruce Grouse on the islands of the Alexander Archipelago are genetically and demographically independent populations on their own evolutionary trajectory, they may warrant special management and protection. Our goals were to use mitochondrial DNA (mtDNA) and microsatellite genetic markers to infer population differentiation and investigate the validity of the subspecies status of *F. c. isleibi*, which was previously based solely on morphological data.

Methods

Sample collection and DNA extraction.—From September 2005 to May 2007, blood samples were collected from trapped birds in British Columbia (n = 23) and wing muscle samples were collected from hunter-killed birds in southeast Alaska (n = 32) and central Alaska (n = 10; Fig. 1). Individual animals were identified to the subspecies level on the basis of morphology and geographic location.

We extracted DNA from blood and muscle samples using Qiagen DNeasy kits (Qiagen, Valencia, California) or using a protein kinase K and ammonium acetate procedure (Puregene DNA isolation protocol; Gentra Systems, Minneapolis, Minnesota). The extracted DNA was hydrated in either Qiagen AE buffer or Tris-EDTA solution and stored in 1.5-mL tubes at 3°C until analysis was complete and then permanently stored at –20°C.

Mitochondrial data.—We amplified the cytocrome-*c* oxidase I (COI) mtDNA gene, using the protocol described by Kerr et al. (2007), for 62 birds (British Columbia [BC] *F. c. canadensis*, n = 9; BC *F. c. franklinii*, n = 14; central Alaska [CA] *F. c. canadensis*, n = 8; Prince of Wales Island [POW] *F. c. isleibi*, n = 20; and Zarembo Island [ZAM] *F. c. isleibi*, n = 11). The final concentrations for each reagent in a 10-µL polymerase chain reaction (PCR) were as follows: 0.75 mM each primer, 2.5 mM $MgCl_2$, 1 mg mL^{-1} BSA, 0.2 mM deoxyribonucleotide triphosphates, 2 µL of 1:50 (DNA:MiliQ-H2O) diluted DNA template, 1X PCR buffer (Promega, San Luis Obispo, California), and 1 unit of *Taq* polymerase. To check for successful amplification, the PCR product was separated on a 1.5% agarose gel and stained with Gelred (Biotium, Hayward, California). The amplified gene product was sequenced at the High-Throughput Genomics Unit at the University of Washington, Seattle (GenBank accession nos. GQ375574–GQ375613).

Forward and reverse COI sequences were aligned, examined for congruence, and quality-trimmed using the program GENEIOUS PRO (Drummond et al. 2007). High-quality forward sequences were saved in FASTA file format and imported into the program CLUSTALX, version 2.0, in which infiles were created for use in other analysis programs (Thompson et al. 1997). Unique haplotypes were determined from a 531-base-pair (bp) segment of COI using the program DNACOLLAPSER (Villesen 2007). The median-joining network algorithm (Bandelt et al. 1999) in NETWORK (Fluxus Engineering, Clare, United Kingdom) with default

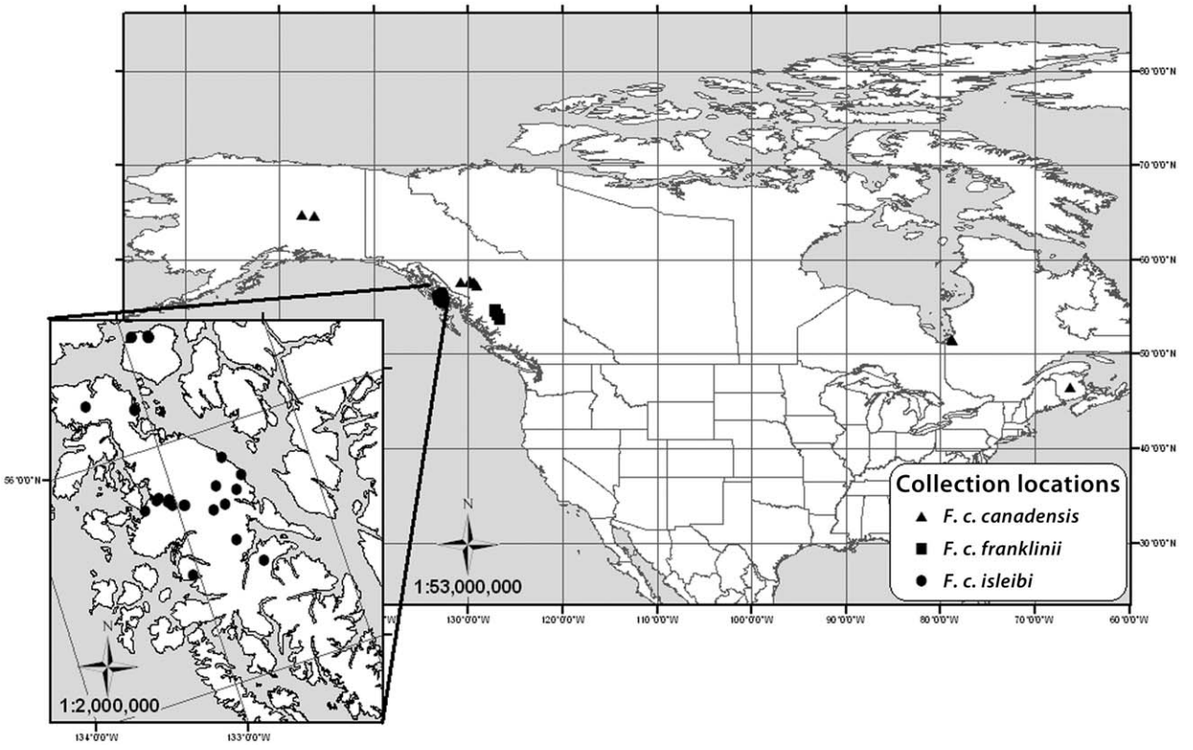

Fig. 1. Map of collection locations for blood samples collected from *Falcipennis canadensis canadensis* and *F. c. franklinii* in British Columbia, wing muscle samples from hunter-killed *F. c. canadensis* in central Alaska and *F. c. isleibi* in the Alexander Archipelago, and *F. c. canadensis* sequenced by Kerr et al. (2007) in central Alaska (DQ432923), New Brunswick (DQ433637), and Quebec (DQ433635 and DQ433636). One sampling location is given for all central Alaska samples as a result of difficulty in resolving hunter-reported collection locations.

settings (weights = 10, ε = 0) was used to create a minimum spanning network to investigate relationships between haplotypes. All individual sequences were compared with sequences from known species in GenBank (National Center for Biotechnology Information) using the BLAST function. Four COI sequences from individual *F. c. canadensis* previously reported by Kerr et al. (2007) were obtained from GenBank (DQ433635 [QB01], DQ433636 [QB02], DQ433637 [NB01], DQ432923 [AK01]). We ran MODELTEST (Posada and Crandall 1998) on the alignment of our sequences and those from GenBank using Akaike's information criterion (AIC) to identify the best-fit model of nucleotide evolution. In PAUP (Swofford 2003), we constructed, using the TrN model of nucleotide evolution (Tamura and Nei 1993), both a consensus bootstrapped tree using all individual samples and a maximum-likelihood tree using only haplotypes. A sequence from Dusky Grouse (*Dendragapus obscurus*) from Kerr et al. (2007) was used as an outgroup (GenBank assession number DQ432884). We applied Fu's method (Fu and Li 1993) to test the assumption of selective neutrality in ARLEQUIN (Excoffier et al. 2005). An analysis of molecular variance (AMOVA) was conducted using unweighted haplotype frequencies in ARLEQUIN (Excoffier et al. 2005) to partition genetic variance within and among populations. To test for population differentiation, we ran exact tests and calculated F_{ST} values in ARLEQUIN (Excoffier et al. 2005).

Nuclear data.—We genotyped 65 individuals at 6 microsatellite loci: BG15 and BG16 (Piertney and Höglund 2001), TUT1 and TUT2 (Segelbacher et al. 2000), LLST3 (Piertney et al. 1998), and LLSD3 (Piertney and Dallas 1997). Conditions for the PCR were changed from the original published protocols for two loci. For LLSD3 we added a 72°C extension to all cycles, and for LLST3, in addition to adding a 72°C extension, we reduced the total number of cycles from 35 to 22. The PCR reactions were performed using fluorescently labeled primers with an IRDye infrared dye (LI-COR, Lincoln, Nebraska) and analyzed using a LI-COR 4300 automated sequencer (LI-COR) on 7% acrylamide gels.

The microsatellite data were analyzed for null alleles, stuttering, and large-allele dropout in MICRO-CHECKER, version 2.2.3 (van Oosterhout et al. 2004), using Monte Carlo simulation with 95% confidence intervals. Allele counts and frequencies were tabulated in POPGENE (Yeh and Boyle 1997). The Markov-chain Monte Carlo (MCMC) probability test for conformance to Hardy-Weinberg expectations was conducted in ARLEQUIN (Excoffier et al. 2005) following Guo and Thompson (1992), at which time observed and expected heterozygosities were computed. Allelic richness per population per locus based on a minimum sample size of 8 individuals was calculated in FSTAT (Goudet 1995). We compared expected heterozygosity and allelic richness of the island population with the largest sample size (POW) to the nearest mainland

population using a Wilcoxon signed-rank test for reduced genetic variation in the island population (Luikart et al. 1998). The coefficient of inbreeding (F_{IS}) per population for each locus was calculated in ARLEQUIN (Excoffier et al. 2005). The permutation module in GENETIX (Belkhir et al. 2002) was used to estimate the probability of linkage disequilibrium for all locus pairs within each population. An AMOVA was performed using unweighted allele frequencies in ARLEQUIN (Excoffier et al. 2005) to partition genetic variance within and among populations. Genetic differentiation among populations was estimated with pairwise F_{ST} values computed in ARLEQUIN (Excoffier et al. 2005) and the exact *G* test in GENEPOP (Raymond and Rousset 1995) for each population pair across all loci. Population structure was also investigated without *a priori* assumptions about the population of origin for birds sampled using the program structure (Pritchard et al. 2000). We assumed an admixture model with correlated allele frequencies among populations and ran five iterations of $K = 1–8$ with 500,000 MCMC repetitions following a burn-in of 100,000 repetitions. To estimate the most likely number of subpopulations or clusters (K), we used the estimated log probability of the data $\Pr(X|K)$. Relationships among groups were examined graphically using multiple subprograms in PHYLIP (Felsenstein 1989); we used the subroutine SEQBOOT to create 1,000 bootstrap replicates from the data, GENDIST to calculate Cavalli-Sforza's chord distances (Cavalli-Sforza and Edwards 1967), FITCH to construct trees for each bootstrapped microsatellite data set by the method described by Fitch and Margoliash (1967), and CONSENSUS to create a majority-rule consensus tree. The program TREEVIEW (Page 1996) was used to visualize the tree.

Results

We observed consistent patterns of divergence in both mtDNA sequences and nuclear microsatellite allele frequencies for populations identified as *F. c. isleibi*. There was fairly low sequence divergence among Spruce Grouse subspecies, but the individuals from the Alexander Archipelago of southeast Alaska all shared a haplotype unique to this region that has two C→T transition mutations not found elsewhere (Fig. 2A). Nuclear data showed strong divergence between mainland and island populations and resolved subspecies differentiation between *canadensis* and *franklinii* groups.

Mitochondrial DNA.—Eight transitions occurred along the 531-bp fragment of the COI fragment that we examined, and this produced 8 haplotypes among the 66 birds sampled. All of the individuals sampled from POW and ZAM islands, classified as *F. c. isleibi*, shared one haplotype unique to southeast Alaska (haplotype 8; Fig. 2). Haplotype 8 has two mutations that distinguish it from haplotype 3, which is the majority haplotype for both *F. c. canadensis* and *F. c. franklinii* from BC (Fig. 2A) The two individuals from Quebec shared haplotype 5, which was unique to the region, and one individual sampled from New Brunswick had haplotype 2, which was a singleton. The animals morphologically classified as either *F. c. canadensis* or *F. c. franklinii* shared haplotype 3 and did not segregate into distinct groups based on COI sequences. All of the individuals, regardless of subspecies distinction, had <1% nucleotide sequence divergence from four birds classified as *F. c. canadensis* in GenBank. Not surprisingly, all of the individuals had >7% sequence divergence from Dusky Grouse.

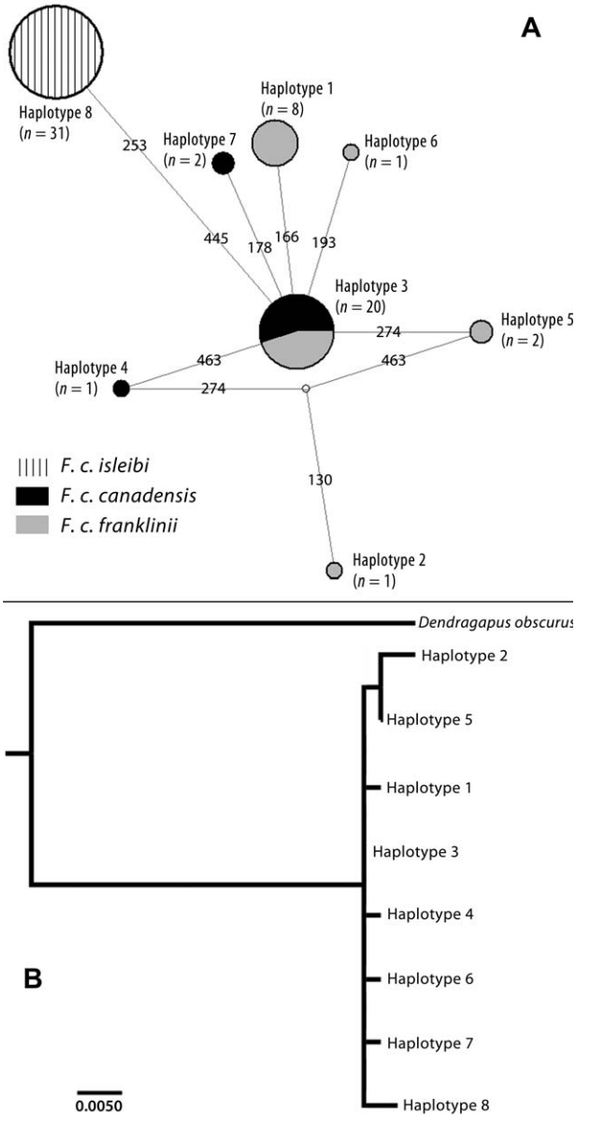

Fig. 2. (A) Minimum spanning tree network for eight mtDNA cytochrome-*c* oxidase I (COI) haplotypes. Each branch is labeled with the position of the mutation within the COI sequence that separates the two haplotypes. Four COI sequences from *Falcipennis canadensis canadensis* (Kerr et al. 2007) were obtained from GenBank (DQ433635 [QB01], DQ433636 [QB02], DQ433637 [NB01], and DQ432923 [AK01]). Sequences from eastern Canada represented two unique haplotypes; haplotype 5 consisted of QB01 and QB02, and haplotype 2 was represented by NB01. Haplotype 1 was observed only from birds in central Alaska (CA) including AK01. Haplotype 3 consisted primarily of birds collected from British Columbia but included one individual from CA. Haplotypes 4, 6, and 7 were all from birds from BC. Haplotype 8 included all birds from Prince of Wales and Zarembo islands. (B) A maximum-likelihood tree constructed using the TrN model of nucleotide evolution from mtDNA COI haplotypes. A sequence from Dusky Grouse (*Dendragapus obscurus*) was used as an outgroup (GenBank accession number DQ432884).

TABLE 1. Pairwise F_{ST} values between populations of Spruce Grouse (*Falcipennis canadensis*) for both mtDNA (half matrix above diagonal) and microsatellite genetic markers (below diagonal). Significance values are in parentheses. BC = British Columbia, CA = central Alaska, POW = Prince of Wales Island, and ZAM = Zarembo Island.

	BC (*F. c. canadensis*)	BC (*F. c. franklinii*)	CA (*F. c. canadensis*)	POW (*F. c. isleibi*)	ZAM (*F. c. isleibi*)
BC (*F. c. canadensis*)	—	0.021 (0.399)	0.761 (0.000)	0.968 (0.000)	0.953 (0.000)
BC (*F. c. franklinii*)	0.068 (0.001)	—	0.683 (0.000)	0.924 (0.000)	0.897 (0.000)
CA (*F. c. canadensis*)	0.027 (0.204)	0.093 (0.000)	—	0.976 (0.000)	0.964 (0.000)
POW (*F. c. isleibi*)	0.463 (0.000)	0.464 (0.000)	0.556 (0.000)	—	0.000 (0.999)
ZAM (*F. c. isleibi*)	0.379 (0.000)	0.388 (0.000)	0.490 (0.000)	0.224 (0.000)	—

We observed small genetic differences (<1%) among geographic locations. However, a few striking patterns arose from both bootstrapped consensus (data not shown) and maximum-likelihood trees. First, in the consensus dendrogram, all of the animals from southeast Alaska islands clustered together in the same clade, which was supported by 859 of 1,000 bootstrap replicates. Second, all but one of the birds sampled from central Alaska clustered together in the same group, which was supported by 611 of 1,000 bootstrap replicates. However, there was no segregation of *F. c. canadensis* from *F. c. franklinii* at the COI mtDNA locus, which is congruent with our results indicating that animals morphologically identified as *F. c. canadensis* and *F. c. franklinii* shared haplotypes. The maximum-likelihood tree shows a clustering of haplotypes 2 and 5, which comprised eastern Canada samples. With <1% sequence divergence, the branch lengths are not long; however, haplotype 8, which represents all POW and ZAM samples, had the longest branch (Fig. 2B).

Tests for population differentiation from mtDNA markers revealed strong divergence among populations. $\bar{F}_{ST}$ was extremely high (0.907), and the exact *G*-test for population differentiation was highly significant ($P = 0.000 \pm 0.000$). Pairwise F_{ST} values ranged from 0.000 to 0.976 (Table 1). Two pairwise comparisons, of POW with ZAM and of *F. c. canadensis* with *F. c. franklinii* from BC, had F_{ST} values of 0.000 and were not significant ($P = 0.999 \pm 0.001$, $P = 0.399 \pm 0.015$, respectively). AMOVA results indicate that 90.73% of the genetic variance is among populations, whereas 9.27% exists within populations. We did not detect any departures from neutrality in any of the populations ($\bar{F}_S = -0.26$).

Nuclear markers.—The number of polymorphic microsatellite loci ranged from 4 to 6 for each population, with 2–13 alleles per locus. The largest number of alleles per locus observed within any one population was 7, and the minimum was 1. Both of the island collections and one mainland collection showed no variation for at least one of the microsatellite loci. POW was fixed for a single allele at locus LLST3, ZAM was fixed at both LLST3 and LLST4, and the CA population was fixed at BG16. Allelic richness was low but did not differ substantially from the number of alleles observed within each population. Observed heterozygosity (H_O) per locus ranged from 0.048 to 0.786, both of which were for the same locus (LLSD3) in different populations (Table 2). Expected heterozygosity for *F. c. isleibi* collected from POW was significantly lower than that of the closest mainland population, *F. c. canadensis* from BC (Wilcoxon signed-rank test, $W = 21$, $P = 0.028$, $n = 6$). Similarly, allelic richness was significantly lower on POW ($W = 19$, $P = 0.046$, $n = 6$; Table 2).

Only 1 of the 25 population-by-locus tests departed significantly from Hardy-Weinberg expectations (BC–*F. c. canadensis* at one locus BG15, $P = 0.0342$; Table 2). This result was not significant after sequential Bonferroni correction ($\alpha = 0.01$). We detected evidence for linkage disequilibria in 3 of the 55 pairwise locus comparisons; only one pair in one population (TUT1–TUT2 in ZAM–*F. c. isleibi*) remained significant after sequential Bonferroni correction ($P = 0.005$, $\alpha = 0.008$).

Tree and cluster analyses showed strong divergence between island and mainland populations. The Fitch and Margoliash (1967) pairwise distance tree showed two main clusters (mainland and island populations) separated by a long branch (Fig. 3). The two island collections, ZAM and POW, clustered together, supported by 1,000 of 1,000 bootstrap replicates. Using the program STRUCTURE (Pritchard et al. 2000), the number of clusters with the greatest $\Pr(X|K)$ was $K = 2$, separating island from mainland samples. Analyses with $K > 2$ did not resolve differences between islands or among mainland samples and had lower likelihood values.

TABLE 2. Basic descriptive statistics (means ± SD) for five populations of Spruce Grouse (*Falcipennis canadensis*) averaged for six microsatellite loci. Values are given for sample size (n), observed heterozygosity (H_O), expected heterozygosity (H_E), number of alleles (A), allelic richness (R), and coefficient of inbreeding (F_{IS}). BC = British Columbia, CA = central Alaska, POW = Prince of Wales Island, and ZAM = Zarembo Island.

	n	H_O	H_E	A	R	F_{IS}
BC (*F. c. canadensis*)	8.7 ± 0.52	0.484 ± 0.229	0.641 ± 0.173	4.500 ± 1.871	4.425 ± 1.815	0.257
BC (*F. c. franklinii*)	13.7 ± 0.52	0.601 ± 0.229	0.606 ± 0.121	4.000 ± 1.095	3.574 ± 0.815	0.008
CA (*F. c. canadensis*)	8.8 ± 0.41	0.589 ± 0.134	0.674 ± 0.065	4.333 ± 1.751	4.200 ± 1.707	0.133
POW (*F. c. isleibi*)	20.7 ± 0.52	0.228 ± 0.152	0.312 ± 0.224	2.833 ± 0.1472	2.161 ± 0.997	0.274
ZAM (*F. c. isleibi*)	10.7 ± 0.52	0.257 ± 0.117	0.403 ± 0.214	2.000 ± 0.894	1.876 ± 0.794	0.374

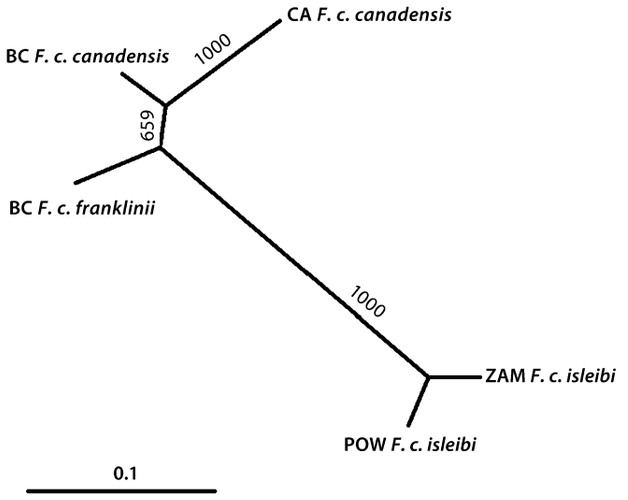

Fig. 3. A bootstrapped unrooted Fitch and Margoliash tree for five populations of Spruce Grouse, constructed using Cavalli-Sforza chord distances from microsatellite frequency data. Population labels contain the location of collection (BC = British Columbia, CA = central Alaska, POW = Prince of Wales Island, and ZAM = Zarembo Island) and subspecies based on morphology.

Nuclear markers revealed significant genetic differences both between geographically isolated populations of the same subspecies and between subspecies with overlapping ranges. Although within-population variation was larger than among-population variation (63.94% and 36.06%), $\bar{F}_{ST}$ among geographic locations was high (0.352) and highly significant ($P < 0.001$). Pairwise F_{ST} values ranged from 0.027 to 0.556. All pairwise F_{ST} values were significant except between the collections of *F. c. canadensis* from British Columbia and central Alaska (Table 1). The highest levels of divergence, as measured by pairwise F_{ST}, were found in comparisons between island and mainland populations followed by comparisons between populations of *F. c. canadensis* and *F. c. franklinii*. An exact *G*-test showed that all population pairs were significantly different from one another, except for collections of *F. c. canadensis* from British Columbia and central Alaska ($P = 0.054$).

Discussion

Intraspecific structure among island and mainland populations.—Our genetic data support Dickerman and Gustafson's (1996) classification of *F. c. isleibi* as a separate subspecies on the basis of morphology. All birds from both ZAM and POW islands shared a unique mtDNA haplotype, and pairwise F_{ST} values from both mtDNA and nuclear markers showed the highest levels of differentiation between island and mainland populations. Typical F_{ST} values for phylogenetic studies of other galliform birds range from 0.019 to 0.264 (Segelbacher et al. 2003, Oyler-McCance et al. 2005, Rodríguez-Muñoz et al. 2007), but the highest values that we obtained were closer to those observed between other endemic insular bird populations and mainland conspecifics (Topp and Winker 2008). The sequence divergence of *F. c. isleibi* from other Spruce Grouse does not meet the level (≥2.5%) suggested by Kerr et al. (2007) to warrant classification of *F. c. isleibi* as a separate species.

Given the history of repeated glaciation and biogeographic patterns of many species in southeast Alaska (Mann 1986, Heaton et al. 1996), it is probable that mainland and island populations of Spruce Grouse have been separated from one another for >10,000 years (Dickerman and Gustafson 1996). Spruce Grouse are known to be poor long-distance flyers, and POW and ZAM islands are separated from the mainland by ~6.4 km and ~3.2 km, respectively (Dickerman and Gustafson 1996). Both morphological and molecular data suggest that *F. c. isleibi* is a distinct subspecies that is reproductively isolated from the mainland populations and on its own evolutionary trajectory.

Subspecies differentiation between F. c. canadensis *and* F. c. franklinii.—We did not expect individuals that were morphologically described as either *F. c. canadensis* or *F. c. franklinii* to share the same mtDNA haplotype, because the distinction of two separate subspecies based on color morphology has been widely accepted. The shared haplotypes between these two subspecies may be a result of a putative hybrid zone in British Columbia across which haplotypes pass between the two subspecies (Jewett et al. 1953, Short 1967). It is worth noting, however, that Gutiérrez et al. (2000) observed just over 1% sequence divergence of 3,809 bp of five mtDNA genes (cytochrome *b*, cytochome oxidase-3, adenosine triphosphatase-8, adenosine triphosphatase-6, and NADH dehydrogenase-2) between individuals classified as *F. c. canadensis* and *F. c. franklinii*.

In contrast to the mtDNA sequences, nuclear markers corroborate morphological classification of both mainland subspecies. Although levels of allele frequency differences between *canadensis* and *franklinii* were lower than those found in island and mainland comparisons, they were still prevalent. This may be attributable to the greater length of time since isolation of the island population, or there may be or may have been hybridization of mainland subspecies. Interestingly, no significant allele frequency differences were observed between two geographically separated populations of *F. c. canadensis* (central Alaska and British Columbia).

Intraspecific structure between POW and ZAM populations.—Many insular endemics of the Alexander Archipelago, such as the Prince of Wales Flying Squirrel (*G. s. griseifrons*; Demboski et al. 1998, Bidlack and Cook 2001), Keen's Mouse (*P. k. oceanicus* and *P. k. sitkensis*; Lucid and Cook 2004), and Ermine (*M. e. celenda* and *M. e. seclusa*; Fleming and Cook 2002), show high divergence from mainland conspecifics, but both the Prince of Wales Flying Squirrel and the Ermine show little or no divergence among island populations. *Falcipennis c. isleibi* has been observed on eight islands in the archipelago (POW, ZAM, San Fernando, Warren, Kosciusko, Heceta, Suemez, and Mitkof; Dickerman and Gustafson 1996). Dickerman and Gustafson (1996) suggested that there may be moderate levels of gene flow among these island populations. Prince of Wales Island is the proposed core of the population (Dickerman and Gustafson 1996), and only Warren and Mitkof islands are separated from POW by >1.6 km. We observed high levels of allele frequency divergence between populations of *F. c. isleibi* populations on POW and ZAM islands, albeit from very small sample sizes. In order for indviduals to disperse from POW

to ZAM, they would have to travel ~3.2 km through a series of small islands. Although it is not inconceivable that gene flow occurs, at least in a stepping-stone fashion, the ability of these small islands to sustain populations of Spruce Grouse is unknown, and our results suggest that any influence of dispersal is extremely low. Genetic data from these islands will be needed to infer their evolutionary and demographic relationships.

Conservation recommendations.—The conservation of endemic bird species of the Alexander Archipelago is of concern because of the projected increase in human disturbance (Cook et al. 2006) and the susceptibility of these species to extinction following human arrival (Steadman 1995, 2006). Habitat degradation and small population sizes are the principal threats for Spruce Grouse populations throughout their range (Storch 2007). Frankham (1997) suggested that the reduced genetic variation typical of island populations may also increase their risk of extinction. Thus, the lower genetic variation observed in both POW and ZAM compared with mainland populations reflects small effective population sizes, which could diminish their capacity to respond to a changing environment. The potentially low level of dispersal into these insular populations indicates that there may be little opportunity for the demographic rescue that many mainland grouse populations rely on to persist (Storch 2007). Although no estimates of abundance or demographic trends of *F. c. isleibi* on POW or other islands are yet available, population sizes are considered low (Storch 2007). A minimum viable population size (Shaffer 1981) may exist below which grouse populations will not recover, regardless of conservation efforts. Despite major conservation efforts, populations of the closely related Black Grouse (*Tetrao tetrix*), Western Capercaillie (*T. urogallus*), and Greater Prairie Chicken (*Tympanuchus cupido pinnatus*) are either extinct or close to extinction (Klaus and Bergmann 1994, Westemeier et al. 1998, Storch 2007). Our results suggest that *F. c. isleibi* warrants increased management attention to determine the evolutionary and demographic status and relationships of the few island populations that compose this endemic subspecies of southeast Alaska.

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