



Examining the uncertain origin and management role of martens on Prince of Wales Island, Alaska

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Abstract: Conservation biologists are generally united in efforts to curtail the spread of non-native species globally. However, the colonization history of a species is not always certain, and whether a species is considered non-native or native depends on the conservation benchmark. Such ambiguities have led to inconsistent management. Within the Tongass National Forest of Alaska, the status of American marten (*Martes americana*) on the largest, most biologically diverse and deforested island, Prince of Wales (POW), is unclear. Ten martens were released to POW in the early 1930s, and it was generally believed to be the founding event, although this has been questioned. The uncertainty surrounding when and how martens colonized POW complicates management, especially because martens were selected as a design species for the Tongass. To explore the history of martens of POW we reviewed other plausible routes of colonization; genetically and isotopically analyzed putative marten fossils deposited in the late Pleistocene and early Holocene to verify marten occupancy of POW; and used contemporary genetic data from martens on POW and the mainland in coalescent simulations to identify the probable source of the present-day marten population on POW. We found evidence for multiple routes of colonization by forest-associated mammals beginning in the Holocene, which were likely used by American martens to naturally colonize POW. Although we cannot rule out human-assisted movement of martens by Alaskan Natives or fur trappers, we suggest that martens be managed for persistence on POW. More generally, our findings illustrate the difficulty of labeling species as non-native or native, even when genetic and paleo-ecological data are available, and support the notion that community resilience or species invasiveness should be prioritized when making management decisions rather than more subjective and less certain conservation benchmarks.

Keywords: aDNA, carbon, indigenous, invasive, *Martes caurina*, nitrogen, Pacific marten

Examen del Origen Incierto y el Papel del Manejo de las Martas en la Isla Príncipe de Gales, Alaska

Resumen: Los biólogos de la conservación están unidos generalmente en esfuerzos para acortar la expansión de especies no-nativas a nivel global. Sin embargo, la historia de colonización de una especie no siempre es certera, y si una especie es considerada no-nativa o nativa depende del punto de referencia de la conservación. Dichas ambigüedades han llevado a un manejo inconstante. Dentro del Bosque Nacional Tongass de Alaska, el estado de la marta americana (*Martes americana*) en la isla más grande, más biodiversa y más deforestada, Príncipe de Gales (PDG), no está claro. Se liberaron diez martas en la PDG temprano en la década de 1930, y se creyó generalmente que esto fue el evento fundador, aunque esto se ha cuestionado. La incertidumbre alrededor de cuándo y cómo las martas colonizaron PDG complica el manejo, especialmente porque las martas fueron seleccionadas como una especie de diseño para el Tongass. Para explorar la historia de las martas en PDG revisamos otras rutas posibles de colonización; analizamos fósiles putativos de marta, depositados en el Pleistoceno tardío y en el Holoceno temprano, para verificar la ocupación de las martas en PDG; y usamos datos genéticos contemporáneos de las martas en PDG y en el continente en simulaciones que se fusionan para poder identificar el origen probable de la población actual de martas en PDG. Encontramos evidencia de múltiples rutas de colonización de mamíferos asociados al bosque que comenzaron en el Holoceno, las

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cuales fueron usadas probablemente por las martas americanas para colonizar naturalmente PDG. Aunque no podemos descartar el movimiento asistido por humanos de las martas por nativos de Alaska o tramperos de piel, sugerimos que las martas sean manejadas para permanecer en PDG. Con una mayor generalidad, nuestros hallazgos ilustran la dificultad de etiquetar a las especies como nativas o no-nativas, incluso cuando hay datos genéticos y paleo-ecológicos disponibles, y apoyamos la noción de que la resiliencia de la comunidad o la capacidad de invadir de una especie deberían ser prioridades cuando se tomen decisiones de manejo en lugar de puntos de referencia de conservación más subjetivos y menos certeros.

Palabras Clave: aADN, carbono, invasivo, marta del Pacífico, *Martes caurina*, nativo, nitrógeno

Introduction

Non-native species (i.e., species introduced to a site intentionally or accidentally by humans) are nothing new to ecologists (Elton 1958), and their global impact on conservation has been well recognized for decades (Diamond 1984). The opportunity introduced species provide for ecological and evolutionary insights (Schlaepfer et al. 2012) and the conservation problem they often present have led to the development of a new field, invasion science (Richardson 2011; Simberloff et al. 2013). Although methods of quantifying, predicting, and minimizing the effects of non-native and invasive species have matured (Richardson 2011; Simberloff et al. 2013), the definition of what constitutes a non-native species is still debated (Lodge & Shradler-Frechette 2003; Donlan & Martin 2004). This lack of consensus has led to an apparent paradox among conservation strategies and on-the-ground management efforts.

It is widely accepted that the introduction of species should be eliminated and that already established populations of non-native species should be controlled (Lodge et al. 2006). However, there is also a strong interest in restoring altered communities, which in some cases could entail the propagation of non-native species. For example, Donlan et al. (2006) promoted rewilding western North America with surrogate species, including Old World lions (*Panthera leo*), elephants (*Elephas maximus*), and camelids, to replace related species that were driven to extinction during the Pleistocene. More recently, de-extinction, the revival of once extinct species via genomic techniques, has been proposed. Candidates include not only recently extinct species (e.g., Passenger Pigeons [*Ectopistes migratorius*] and Pyrenean ibexes [*Capra pyrenaica*]), but also long-extinct mammoths (*Mammuthus* spp.), mastodons (*Mammuth* spp.), and saber-toothed cats (*Smilodon* spp.) (Sandler 2013; Seddon et al. 2014). This paradox, between sharply limiting the spread of non-native species and introducing surrogate non-native or prehistoric species seems to be driven principally by differing views on benchmarks for conservation (Callicott 2011).

Many conservationists, particularly in North America, advocate a pre-Columbian benchmark for restoration (Callicott 2011); others argue that time of human arrival,

not European arrival, should be the target (Hunter 1996). This lack of consensus has implications for the status of species (non-native vs. native) that are relevant for theoretical conservation initiatives, such as Pleistocene rewilding or de-extinction, but also has led to inconsistencies in active management programs. For example, in the United States, agencies have invested heavily in the recovery of the endangered Channel Island fox (*Urocyon littoralis*) (introduced ~6 ka by Native Americans [Rick et al. 2009]), yet advocated for the removal of feral pigs (*Sus scrofa*) on the Hawaiian Islands (introduced by Polynesians ~2 ka [Nogueira-Filho et al. 2009]). At the same time, feral horses (*Equus* spp.) in the U.S. Intermountain West (introduced by Spanish in 1600s) are recognized as an “integral part of the natural system” (Public law 92-195), whereas foxes (*Alopex lagopus* and *Vulpes vulpes*) on the Aleutian Islands (introduced by Russians in the 1700s [Bailey 1993]) have been targeted for removal for >50 years. Although the assignment made to species as native or non-native is sometimes arbitrary, the ramifications are not trivial.

Tongass National Forest, the largest U.S. national forest, encompasses the Alexander Archipelago and the narrow mainland of southeastern Alaska. The Tongass has a series of old-growth reserves established to sustain a variety of design species (i.e., species that have been targeted to help guide management decisions [USFS 2008]). Due to its narrow habitat specialization and large spatial requirements, the American marten (*Martes americana*) was selected as one of these forest design species. Theoretically, forest management practices that allow marten populations to persist should afford sufficient habitat to sustain other vertebrate members of the ecological community (Buskirk 1992). Being the largest island in the chain (6700 km²), possessing the highest levels of endemism, and having the most roads and deforestation, Prince of Wales Island (POW) is a centerpiece in management discussions for the Tongass (Cook et al. 2006). Consequently, the viability of martens on POW has received particular attention. A complicating factor involves the uncertainty surrounding when martens arrived on POW and whether they should be considered native or non-native.

Because of a dynamic glacial history, the Alexander Archipelago possessed multiple routes of colonization (coastal refugia and both continental and Beringian

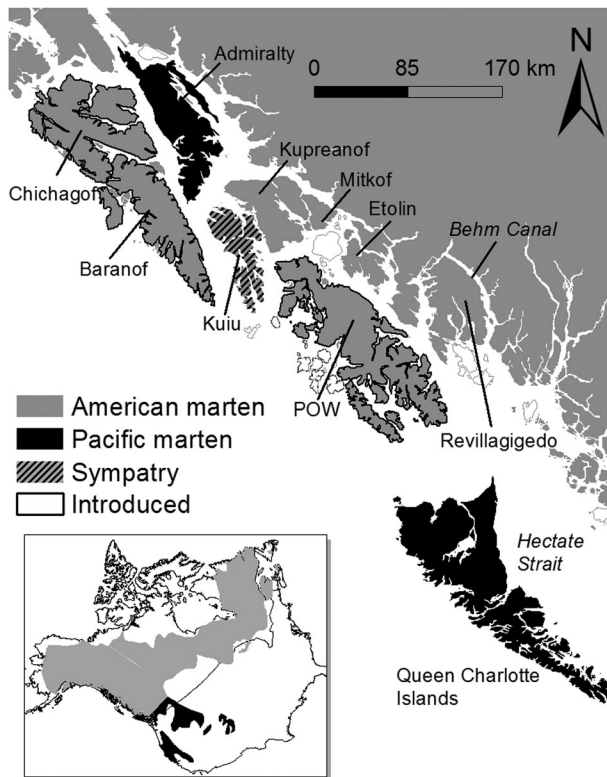


Figure 1. Current American (*Martes americana*) and Pacific marten (*M. caurina*) range-wide (inset map) and archipelagic distribution in the Pacific Northwest (United States).

routes) for terrestrial fauna (Cook et al. 2006). Consequently, levels of endemism are extremely high (Cook & MacDonald 2001) with multiple distinctive lineages occur across the islands (Cook et al. 2006). Two species of marten—American and Pacific (*M. caurina*) that originated from different refugia in North America colonized the region following different dispersal routes (Fig. 1). The American marten is native to several islands, including Etolin, Kuiu, Kupreanof, Mitkof, Revillagigedo, and Wrangell Islands, and the mainland, whereas the Pacific marten is native to Admiralty and Kuiu Islands and Haida Gwaii (Queen Charlotte Islands) of northern British Columbia (MacDonald & Cook 2007; Fig. 1). Beginning in the 1930s, the Alaska Game Commission began reintroducing American martens to the Alexander Islands. In 1934, martens were released to POW and Baranof Islands, and from 1949 to 1952 they were released on Chichagof Island (Fig. 1). Although the marten populations on Baranof and Chichagof appear to be derived from stocking, the origin of the POW population has been questioned (Giannico 1986), even soon after their original release (Elkins & Nelson 1954). This confusion surrounding when and how martens colonized POW—Pleistocene relicts, natural colonization during the Holocene, or human assisted dispersal by Alaskan

Natives, Russian-European fur trappers or, most recently, Alaskan state agencies or fur farms—has led to ambiguity in their status and role as a design species.

We used a multipronged approach to determine the historical plausibility of various routes of arrival of marten on POW. To examine what species of marten historically inhabited POW, we examined late Pleistocene and early Holocene specimens and analyzed stable isotopes and ancient DNA (aDNA). Finally, we used contemporary biological samples and analyzed multilocus genotypes from martens on POW and from the source population for the introduction to explore the origin of present-day martens on POW.

Methods

Potential Routes of Colonization

In 1934, 10 American martens (6 males and 4 females) were captured along Behm Canal and transplanted to POW (Fig. 1). This translocation is generally believed to have been the colonization event for marten on POW, although their rapid growth following such a limited release without protection from trapping has led some to suspect there was a cryptic population of martens already present on the island (Elkins & Nelson 1954; Giannico 1986). To determine the plausibility of a marten population inhabiting POW before 1934, we explored both natural and alternative human-assisted colonization events. Specifically, we assessed whether martens could be native to POW as Pleistocene relicts or Holocene colonizers by examining potential refugia, colonization routes, mammalian phylogeography, and bathymetry across the Alexander Archipelago. We also reviewed historical literature, expedition accounts, and harvest records to explore alternative scenarios of human-assisted dispersal: a pre-Colombian translocation by Native Alaskans; a historical introduction during the fur trade (1700–1800s), especially by Russian and European trappers; and recent (post 1890s) introduction by trappers or via escape from fur farms.

Pleistocene and Early Holocene Martens

Fossil remains of putative martens ($n = 36$) have been collected from On Your Knees Cave (site 49-PET-408) on POW and dated to the Pleistocene and Holocene via stratigraphic position (Heaton & Grady 2003). To validate morphological identification of martens, especially from American mink (*Neovison vison*), we first analyzed the stable isotopic signature ($\delta^{13}\text{C}$ and $\delta^{15}\text{N}$) of bone collagen from marten and mink fossils and compared them with contemporary samples of the 2 species. Because martens principally feed on terrestrial organisms and mink on marine and freshwater organisms (Ben-David 1996; Ben-David et al. 1997), their isotopic signatures should be distinct (Chisholm et al. 1982).

We extracted collagen (Supporting Information) from fossil bone samples of mink ($n = 4$) and marten ($n = 7$) and contemporary marten ($n = 4$) from POW following Tuross et al. (1988). Results are provided as per mil (parts per thousand [‰]) ratios relative to the international standards of Peedee Belemnite (PDB; $\delta^{13}\text{C}$) and atmospheric nitrogen (AIR; $\delta^{15}\text{N}$) and calibrated with internal laboratory standards. We corrected the carbon isotope values of fossils by -1.2‰ for Pleistocene samples and -1.5‰ for Holocene samples to account for the global decrease in the ^{13}C of atmospheric carbon dioxide (Chamberlain et al. 2005). We obtained $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ from blood and muscle of contemporary mink in southeastern Alaska ($n = 10$) from Ben-David et al. (1997), and we corrected these with tissue-specific discrimination factors to make them comparable to bone collagen (4.15‰ for ^{13}C and -1‰ for ^{15}N) (Ben-David et al. 2012). We employed a multivariate analysis of variance to compare contemporary and fossil samples of marten and mink and determined isotopic overlap between species with a convex hull polygon and a Bayesian estimate of standard ellipse areas corrected for small sample size in SIAR (Parnell et al. 2010; Jackson et al. 2011).

To determine species, we extracted aDNA from the dentine of 7 intact fossil teeth following standard laboratory procedures for aDNA (Supporting Information). We obtained American and Pacific marten sequences for the cytochrome b (cytb) region from GenBank to design 2 sets of primers: a 200 and a 100 base pair fragment (Supporting Information). Cytb was amplified using a nested polymerase chain reaction (PCR) (conditions in Supporting Information), and PCR products were visualized on an 8% non-denaturing polyacrylamide gel stained with ethidium bromide and 1 kb ladder. Bands that aligned to the correct position (100 or 200 base pair) were cut from the gel, and DNA was extracted by the crush and soak method. We sequenced DNA in both 5' and 3' directions on an ABI 3730xl capillary sequencer. All samples were run in 3 separate PCR reactions and sequenced 3 times. The PCR products were cloned using pGEM-T Easy Vector System II-cloning kit (Promega, Madison, Wisconsin). Positive colonies were sampled for a $25\mu\text{L}$ PCR reaction mixture and amplified using vector primers T7 and SP6. The PCR products from the cloned libraries were cleaned using ExoSapIT, and the products were sequenced (ABI 3730xl capillary sequencer) in both 5' and 3' directions.

We analyzed sequence chromatograms for cytb in FinchTV 1.4 (Geospiza) and manually aligned the forward and reverse sequences in MEGA 6.06 (Tamura et al. 2013). We aligned the original sequences and clones to cytb sequences of American and Pacific martens from GenBank. We calculated the Tamura-Nei (1993) nonsynonymous to synonymous substitution ratio (dN/dS) and base pair composition for all sequenced samples with MEGA 6.06 and tested for saturation in Dambe 5.3.109 (Xia 2013).

Contemporary Martens on POW

We obtained multilocus microsatellite genotypes (5 polymorphic microsatellite loci: Ma2, Ma5, Ma19, Tt1, Gg7 [Davis & Strobeck 1998]) of martens from a previous study conducted in southeastern Alaska (Pauli 2010; see Supporting Information for details). Marten samples were collected from POW ($n = 168$) and the mainland along Behm Canal ($n = 77$; source of individuals for the 1934 release) to compare the current level of genetic differentiation between marten populations with simulated levels of differentiation under the following 2 scenarios: the POW marten population derived solely from the 10 individuals introduced in 1934 from Behm Canal and a Holocene dispersal event from the mainland by martens colonized POW prior to the release.

We quantified genetic divergence between contemporary martens inhabiting POW and Behm Canal with pairwise F_{ST} in Arlequin 3.5 (Excoffier & Lischer 2010). We used coalescent simulations in the program SIMCOAL2 (Laval & Excoffier 2004) to determine if the observed divergence between POW and Behm Canal was plausible under the 2 colonization scenarios. SIMCOAL2 simulates coalescent events between 2 populations and generates genotype frequencies, which are then used to calculate F_{ST} values between modeled populations. In this case, we compared the observed F_{ST} (between POW and Behm Canal) with the simulated F_{ST} obtained from both scenarios. These simulations required a set of a priori parameters, including effective population size of demes, mutation rates, and generations since population split. We used a generation time of 5 years for martens (Buskirk et al. 2012), and simulated POW splitting from the mainland deme at 15 generations (introduction in 1934) and 2000 generations (Holocene colonization). Effective population size of POW ($N_e = 1400$) was estimated as a population average based on Alaska Department of Fish and Game trapping records from 1984 to 2009 and assuming a 60% marten harvest rate (Paul 2009). This parameter was held constant in all simulations. Because we did not know the size of the source population from the mainland, we simulated 2 possible effective population sizes for the mainland deme: an isolated, local population for Behm Canal ($N_e = 408$; estimated from local harvest records as described above for POW) and a panmictic continental population ($N_e = 10,000$), as suggested by Kyle and Strobeck (2003). For the contemporary introduction, we simulated an introduction of 10 individuals and, therefore, modeled a positive growth rate for POW to reach current size in 15 generations. For early Holocene colonization simulations, we modeled a founding population size of 1400 individuals, followed by a second, recent introduction of 10 individuals. In the early Holocene scenario, we used a constant population size. For both scenarios, we assumed colonization did not occur

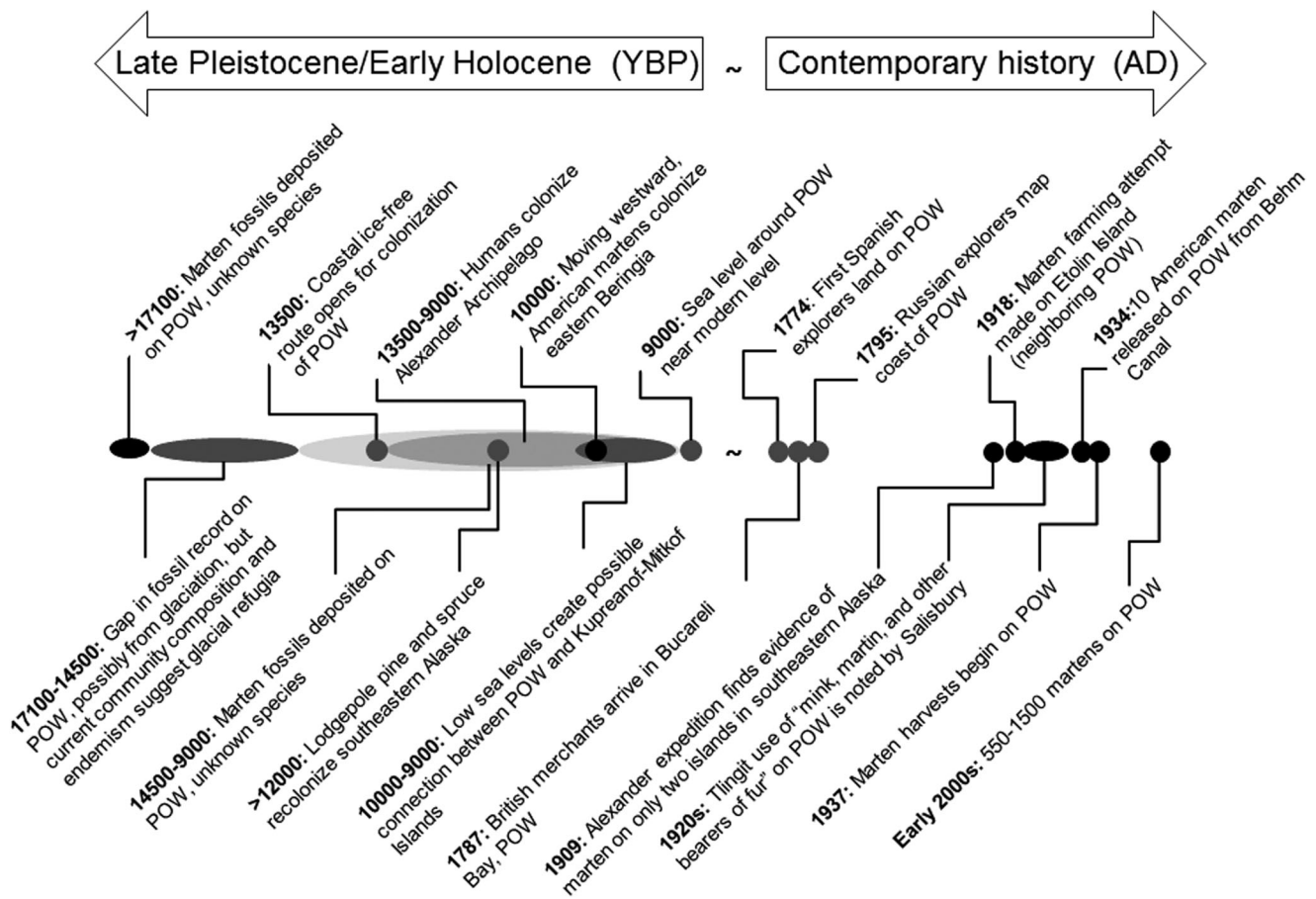


Figure 2. History of Prince of Wales Island (POW) related to possible colonization events by marten (*Martes spp.*) from the late Pleistocene to the present (gray ovals, relevant events on POW; black ovals, events specific to marten).

following the designated population split because POW is an island.

Finally, we estimated microsatellite mutation rate ($\mu = \theta/4N_e$) for martens using the average θ for our 5 microsatellite loci and our estimate of N_e for POW. Because coalescent simulations are sensitive to mutation rate, we also explored both colonization scenarios using 2 extreme mutation rates for mammals ($\mu = 8.1 \times 10^{-3}$ and 8.0×10^{-5} [Peery et al. 2012]). Each simulation performed 1000 iterations of a coalescent event, and the resultant genotypes were analyzed in Arlequin 3.5 to calculate F_{ST} values between simulated island and mainland populations. We generated P values by comparing the observed F_{ST} with the distribution of 1000 simulated F_{ST} values.

Results

Potential Routes of Colonization

There were multiple opportunities for martens to colonize POW prior to the 1934 release (Fig. 2). Fossil evidence from the late Pleistocene (>14 ka) and early Holocene (9–14 ka) suggested that martens had previ-

ously existed on the island and POW likely possessed ice free refugia during the last glacial maximum (Heaton et al. 1996; Carrara et al. 2007; Peacock et al. 2007). However, American martens currently inhabit POW, whereas the paleoendemic species in the archipelago are Pacific martens (Small et al. 2003). Further, American marten populations are not strongly diverged between islands within the Alexander Archipelago or from mainland populations (Stone et al. 2002). This is in contrast to other mammalian paleoendemics on the Alexander Islands, such as ermine (*Mustela erminea*) and brown bears (*Ursus arctos*), which show a deep genetic divergence from mainland populations (Cook et al 2001; Cook et al. 2006). Thus, the phylogeography of martens across the islands suggests that American martens on POW are not a Pleistocene relict.

A Holocene colonization appears to be more likely. Glaciers in southeastern Alaska reached their modern positions by 13.5 ka and the archipelago was forested by 12.5 ka (Mann & Hamilton 1995), whereas lodgepole pine colonized interior Canada at the latitude of POW >10 ka, providing a colonization route for forest carnivores, including martens (Strong & Hills 2013). Bathymetry maps indicated that POW was connected

with the mainland during this same time period via Kupreanof and Mitkof Islands (Small et al. 2003), both of which have native American marten populations that appear to have colonized during the Holocene (Stone et al. 2002). There is strong evidence that other forest-associated species, including flying squirrels, colonized POW from the mainland during this period (Bidlack & Cook 2002). Particularly high levels of endemism on POW, as well as several species with multiple lineages within the region (i.e., ermine, black bears), indicate that multiple colonization routes were available during the early Holocene (Cook et al. 2001, 2006; Fleming & Cook 2002; Weckworth et al. 2005; Peacock et al. 2007). However, a continental colonization route for American martens, rather than a coastal colonization by Pacific martens, would be the most likely path of a historic (i.e., prerelease) colonization for marten (Small et al. 2003).

Humans and martens coexisted in the archipelago soon after the most recent glacial maximum (Mandryk et al. 2001). Martens are of cultural significance to Tlingit and Haida, which have inhabited southeastern Alaska for thousands of years, which provides a potential incentive to move this species to unoccupied areas. A traditional story from a Tlingit community on POW mentions that “he tied the end of the thread to the tail of a little marten skin she was wearing,” indicating familiarity with the species (Velten 1939:69), although this story could have been adopted from elsewhere. Additionally, a schoolteacher living on POW in the 1920s noted that Tlingit trapped “mink, martin, and other bearers of fur” (Salisbury 1962:125). Although there is no direct evidence of Tlingit or Haida moving wildlife intentionally, it is now acknowledged that other pre-Colombian indigenous groups were capable of translocating culturally important wildlife (Grayson 2001).

Europeans arrived in the region around POW in 1774. The Spanish were first followed by British merchants, and finally Russians established settlements in the area. Fur trapping was the primary economic interest from the 1700s to early 1900s. Russian explorers moved other economically valuable species, such as red and arctic foxes to the islands of Alaska (Andrews 1916; Statham et al. 2011). Although martens were naturally distributed throughout the majority of the Alexander Archipelago (Fig. 1), the first formal biological inventory of southeastern Alaska, the Alexander Expedition in 1909, collected only 1 specimen from Kuiu Island, which they purchased from a Native Alaskan, and observed tracks of 1 marten on Kupreanof Island. Thus, the biologists lamented in their inability “to obtain very definite information as to the distribution of the species over these islands, where it does not appear to be abundant” (Swarth 1911, p. 140). This highlights the cryptic nature of marten populations and raises the possibility that native martens on POW went undetected prior to 1934.

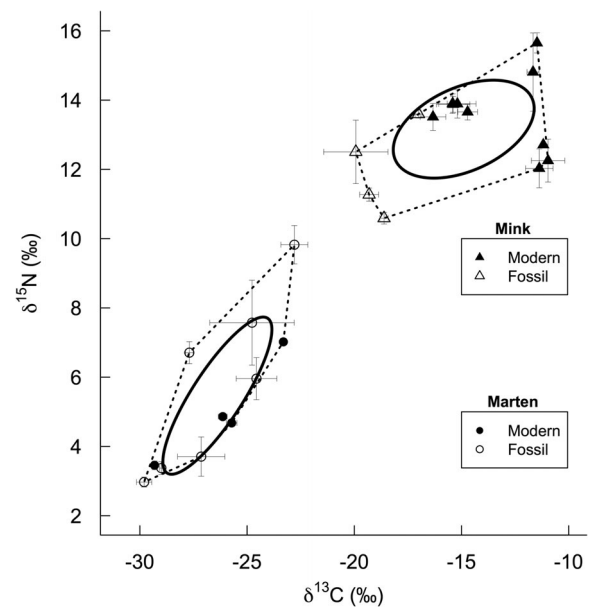


Figure 3. Isotopic signature ($\delta^{13}\text{C}$ and $\delta^{15}\text{N}$) of contemporary and fossil marten (*Martes spp.*) and mink (*Neovison vison*) as well as convex hulls (dotted lines) and isotopic ellipses (solid lines) for the 2 species.

In the early 20th century, there was interest in farming martens for pelts, but rearing martens in Alaska proved challenging (Isto 2012). Nevertheless, 32 permits were issued in the 1920s to capture 124 martens for farming. In particular, a marten farm on Etolin Island, adjacent to POW, was started in 1918 and successfully bred martens (Bower 1919). Trappers during that period also acknowledged opportunistically moving female martens to new islands with hopes of establishing populations (Paul 2009).

Thus, of the colonization paths we considered for martens to POW, several, starting in the Holocene, appear possible. A cryptic population of marten already present on POW would explain how 10 individual martens (only 4 of which were females) released to POW were surprisingly prolific (Elkins & Nelson 1954), even without trapping closures. In contrast, Baranof (7 animals released in 1934) and Chichagof (21 released 1949–1952) were closed to trapping after release, and their populations were augmented by trappers releasing an unknown number of animals (Johnson 1981; Paul 2009).

Pleistocene and Early Holocene Martens

Our isotopic analysis of fossil bones corroborated the morphological identification of marten on POW. As expected, the isotopic signature ($\delta^{13}\text{C}$ and $\delta^{15}\text{N}$) of marten and mink were significantly different (Tukey, $P < 0.01$) (Fig. 3), and the fossil mink and marten did not differ

isotopically from their contemporary conspecifics (Tukey, $P > 0.05$) (Fig. 3). Similarly, neither convex hulls nor Bayesian ellipses calculated for marten and mink overlapped between the 2 species (Fig. 3). Thus, martens of an unknown species were present on POW prior to the 1934 release either as a Pleistocene relict that was extirpated or as an early Holocene colonizer.

We obtained 3 independent PCR amplicons for a fragment of *cytb* (190 bp) for 2 samples (based on their stratigraphic positions, 1 from the Late Pleistocene and the other Early Holocene), and sequenced 3–5 clones for each. The other samples were not successfully sequenced, likely due to DNA degradation. Cloned sequences generally aligned well with published marten sequences (Supporting Information). Original sequences 5A, 5B, and clone 1 of 5C were removed from the analysis due to indels. No stop codons were found when we compared our sequences with the vertebrate mitochondrial protein code. Base pair composition (T = 22.7%, C = 35.5%, A = 32.0%, G = 9.7%) followed a pattern consistent with that of other mammals. In particular, the second position was richest in T, and the third position was depleted in G. The number of conserved sites was 172; 17 were variable sites and 16 of these sites were parsimoniously informative. Tests indicated that saturation had not been attained. Across the 61 amino acids in the contemporary sequences, there were 8 synonymous and 2 nonsynonymous changes (dN/dS = 0.16). In the aDNA samples, including clones, there was an equal number ($n = 11$) of synonymous to and non-synonymous substitutions (dN/dS = 0.34).

There was evidence of cytosine deamination in cloned sequences (18 C-T and 5 G-A transitions) and oxidative damage (11 transversions [A-C, G-T, C-G and T-A]), both of which are common in samples and likely explain the higher dN/dS ratio observed in aDNA sequences (Hofreiter et al. 2001; Gilbert et al. 2007; Lamers et al. 2009; Supporting Information). Nevertheless, we detected 3 additional G-A transitions when aligning the clones to American martens. There were an additional 2 C-T transitions and 3 G-A transitions as well as 2 A-C transversions in cloned sequences relative to Pacific marten sequences (Table 1).

Contemporary Martens on POW

Modern populations of martens on POW and Behm Canal possessed reasonably high levels of genetic diversity and equivalent levels of allelic richness and heterozygosity (Supporting Information). The observed genetic divergence between contemporary martens on POW and Behm Canal ($F_{ST} = 0.151$) suggested 2 structured and moderately differentiated populations. In contrast, coalescent simulations with the highest mutation rate ($\mu = 8 \times 10^{-3}$) generated populations with very little genetic differentiation, regardless of period or N_e . No simulations

Table 1. Consensus *cytb* sequences for 2 marten (*Martes* spp.) fossils (5, 6) collected from Prince of Wales Island, Alaska, cloned 3 times (A, B, C) and aligned against contemporary American (*M. americana*) and Pacific marten (*M. caurina*) at parsimoniously informative sites where they differ.*

Base pair position	24	33	51	54	82	84	138	151	159	162
American marten	A	G	C	A	T	A	G	A	G	T
Pacific marten	G	A	T	G	C	G	A	G	C	C
5A	.	A	.	G	.	.	A	.	A	A
5B	.	A	.	G	.	.	A	.	A	A
5C	.	A	.	G	.	.	A	.	A	A
6A	.	A	.	G	.	.	A	.	A	A
6B	.	A	.	G	.	.	A	.	A	A
6C	.	A	.	R	.	.	A	.	A	A

*Dots denote where clones match American martens. Sequences are boxed when clones match Pacific marten.

produced values as high as the observed F_{ST} (Supporting Information), and simulations with the lowest mutation rate were similar to those with the moderate mutation rate. Consequently, we focused on the simulation scenarios with the moderate and empirically derived mutation rate for martens.

Mean simulated F_{ST} values for recent colonization scenarios were low (maximum $F_{ST} = 0.052$) and were all significantly lower than observed under the moderate mutation rate, meaning that genetic differentiation would be low if POW was founded solely by the 1934 release (Figs. 4a & 4b). The only scenario in which expected F_{ST} was not different than observed F_{ST} was when the source population along Behm Canal was geographically isolated and small ($N_e = 408$) and the mutation rate was at the lower extreme (Supporting Information). Even in this case, only 6.5% of simulations yielded an expected F_{ST} of 0.15 or higher ($P = 0.065$). Altogether, our simulations suggested it was unlikely that the POW population was founded by a single introduction event of 10 martens in 1934.

Simulations of a natural colonization event 2000 generations ago yielded larger F_{ST} values (Supporting Information; Figs. 4c & 4d). Simulations under the moderate mutation rate with a small source population yielded F_{ST} values greater than observed ($F_{ST} = 0.314$; $P < 0.05$). However, with a large source population, mean expected F_{ST} did not differ from the observed ($F_{ST} = 0.151$; $P = 0.46$). Thus, it is feasible that POW was colonized 2000 generations ago by individuals from a large, panmictic, mainland population.

Discussion

The multiple lines of evidence we present indicate that it is unlikely the marten population on POW is solely derived from the 10 individuals released in 1934. This is not particularly surprising, given that martens possess a

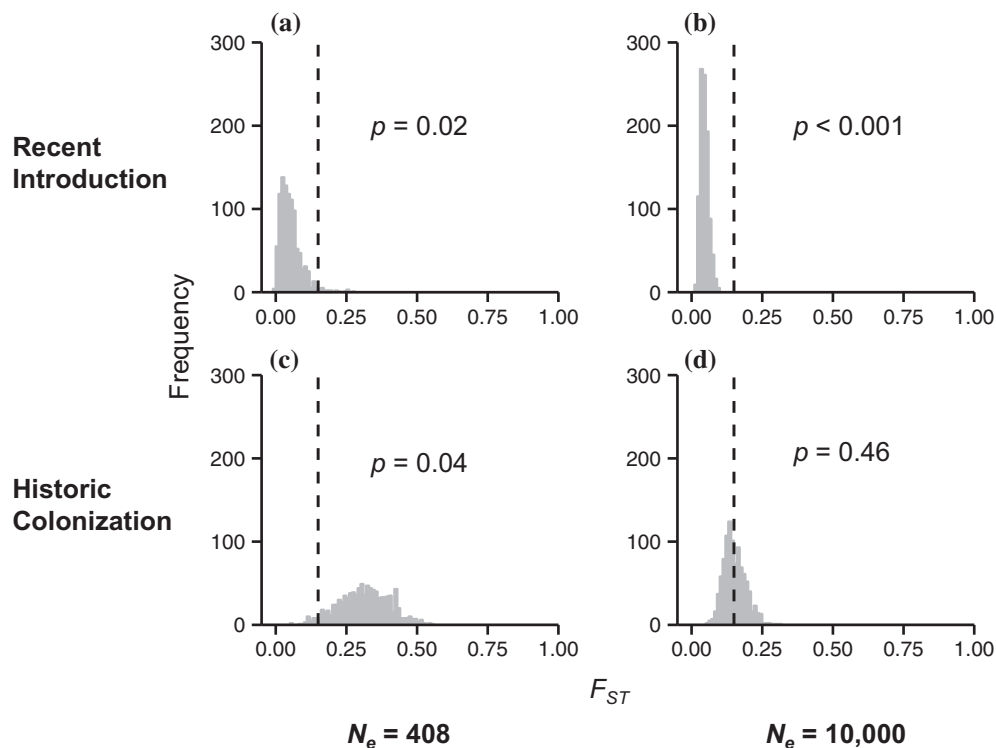


Figure 4. Distribution of F_{ST} values obtained from coalescent simulations under 4 different scenarios of American marten (*Martes americana*) colonization of Prince of Wales Island from Behm Canal: (a, b) a single reintroduction event in 1934 with 10 founding animals and (c, d) a historic (early Holocene) colonization event 2000 years ago featuring either a small or large effective source population size (N_e). Observed F_{ST} values (dotted vertical line) are compared with simulated ones for these 2 populations.

life-history strategy with a low invasion potential (e.g., slow life history strategy, habitat specialist, and wide ranging [Sakai et al. 2001]). In a review of documented marten releases, Powell et al. (2012) found that over half were unsuccessful, the majority were slow in recovery, and introduction success was related to the number of individuals released and total number of release sites. The 1934 POW release was very small both in terms of size and scale, yet, if one assumes no other martens were present on the island, it was successful in a remarkably short period (Powell et al. 2012).

Our results point to a natural colonization of POW by martens during the Holocene. First, a suitable land bridge connecting POW to intermediate islands and the mainland enabled forest associated mammals to colonize POW. This is evidenced by flying squirrels on POW, which appear to have colonized during the Holocene and exhibit a level of genetic differentiation from the mainland ($F_{ST} = 0.19$ [Bidlack & Cook 2002]) similar to what we observed for American marten. Further, our combined isotopic and aDNA analysis of fossil remains demonstrated that martens were present on POW during the Pleistocene and Holocene. Finally, cloned sequences from Holocene marten fossils were the most similar to American marten, but they shared nucleotide sequences

with Pacific marten. These sequencing results suggest that Holocene martens were either American martens for which there were sequencing misreads due to hydrolytic or oxidative damage or were a historical population of martens with incomplete sequence divergence from American and Pacific marten. Because the rate of sequencing errors across the 191 bp gene was <10%, it seems unlikely that these differences can be fully accounted for by oxidative damage or deamination. Thus, we suspect that fossils were of a historic population of martens that were genetically distinct from contemporary ones and that these differences were ultimately lost via extinction of this population or genetic swamping by the arrival of American martens in the Holocene. To better elucidate this deep history and definitively ascertain the taxonomic group of marten originally on POW, future researchers should examine more fragments of the cytb gene and perhaps other mitochondrial and nuclear genes. Regardless, our aDNA cloning suggests marten presence on POW during the Holocene.

Persistence of American martens through the Holocene to present day is supported by our coalescent simulation modeling that showed a Holocene colonization event from a large mainland population was plausible (whereas a 1934 release was generally not supported).

Our simulations were sensitive to mutation rate, which if misidentified could affect our results. Nevertheless, we are confident in the mutation rate we derived empirically from marten microsatellites, especially because the rate was between the 2 extremes observed in mammals (Peery et al. 2012). We also found that effective population size of the source was important in our simulations. A Holocene colonization from a large source population appeared a plausible source of POW martens, whereas a small source population was not. We believe that the large source population is the most realistic scenario because it has been shown that mainland populations of American martens are large and panmictic across much of the continent (Kyle & Strobeck 2003). A Holocene hybridization event on POW between colonizing American martens and some relictual population of martens would also influence our coalescent simulations. However, the presence of a relictual population would increase divergence estimates between POW and mainland and reinforce the conclusion that the introduction from 1934 was not the sole source of this population.

Although the 1934 release alone seems improbable and the Holocene colonization event most likely, it is impossible to rule out some combination of historic releases of martens to POW by Native Americans, from fur farms, or fur trappers. In general, the role of indigenous groups in relocating wildlife has been overlooked (but see Grayson 2001), even though early translocation of vertebrates, even seemingly improbable ones, have been documented. For example, Native Americans populated the Channel Islands with foxes (Rick et al. 2009); early Europeans released mustelids, including a species of marten (*Martes martes*), to islands of the Mediterranean (Alcover 1980) as well as the British Isles (Montgomery et al. 2014); Polynesians brought a myriad of vertebrates to the Hawaiian Islands (Kirch 1982); and Aztecs acquired large wildlife, even American bison (*Bison bison*), for their zoo-like collections in Mexico (Prescott 1843). Commercial fur trappers and farmers also relocated wildlife regionally and even augmented other island reintroduction sites for martens during the same period; however, there is no evidence that they moved marten to POW. Because of the complexity of multiple reintroduction events, we were unable to capture those scenarios with coalescent simulations. Ultimately, then, we cannot eliminate the possibility that a milieu of human-assisted introductions of unknown origin occurred in place of or in addition to a Holocene colonization.

At least in North America, the colonization histories of organisms are sometimes taken for granted, and whether a species is native or non-native has been prematurely assumed. Recently, Frey (2013) re-evaluated the status of red foxes across North America and found the claims that most foxes were of European origin from colonial-era releases were based on a few spurious accounts. Combined with genetic evidence (Statham et al. 2012), the

widely held notion that most North American foxes are non-native seems largely refuted, and most populations of foxes in North America appear to be an expanding native species. Martens on POW also seem to be one of these more nuanced colonization stories, one that would have been impossible to disentangle without the depth of paleo-ecological and genetic data that was available for this species and system (Willis & Birks 2006). Nevertheless, even in the face of some uncertainty around the role of human assistance in colonization, we advocate a cautious approach and one that promotes marten persistence on POW.

Finally, our findings illustrate some of the challenges in designating species as either native or non-native even in the rare cases where reasonably complete genetic, paleo-ecological, and historical information are available. The implicit uncertainty around the mode of a species' arrival, coupled with individual preferences on the manner and timing of arrival, appears to account for the inconsistencies in the identification and management of non-native species. Currently, whether or not a species is identified as non-native seems to be an amalgam of conservation benchmarks, institutional inertia, cultures responsible for translocations, degree of species invasiveness, and charisma of the species. More generally, then, a reevaluation of the very concept of non-native species is in order, especially one that prioritizes the invasiveness of the species and community resiliency rather than aesthetic values or subjective benchmarks. Such consensus on identifying non-native species would enhance the consistency of conservation and management.

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Supporting Information

Additional Information on molecular analyses of fossils (Appendix S1), the aDNA sequence results (Appendix S2), and coalescent simulation results (Appendix S3) are available online. The authors are solely responsible for the content and functionality of these materials. Queries (other than absence of the material) should be directed to the corresponding author.

Literature Cited

- Alcover JA. 1980. Note on the origin of the present mammalian fauna from the Balearic and Pityusic Islands. *Instituto Municipal de Ciencias Naturales Miscelanea Zoologica* 6:141–149.

- Andrews CL. 1916. Alaska under the Russians—industry, trade and social life. *The Washington Historical Quarterly* **7**:278–295.
- Bailey EP. 1993. Introduction of foxes to Alaskan Islands—history, effects on avifauna and eradication. Resource Publication 193. US Fish and Wildlife Service, Washington, D.C.
- Ben-David M. 1996. Seasonal diets of mink and martens: effects of spatial and temporal changes in resource abundance. Ph.D. thesis. University of Alaska, Fairbanks.
- Ben-David M, Hanley TA, Klein DR, Schell DM. 1997. Seasonal changes in diets of coastal and riverine mink: the role of spawning Pacific salmon. *Canadian Journal of Zoology* **75**:803–811.
- Ben-David M, Newsome SD, Whiteman JP. 2012. Lipid and amino acid composition influence incorporation and discrimination of ^{13}C and ^{15}N in mink. *Journal of Mammalogy* **93**:399–412.
- Bidlack AL, Cook JA. 2002. A nuclear perspective on endemism in northern flying squirrels (*Glaucomys sabrinus*) of the Alexander Archipelago, Alaska. *Conservation Genetics* **3**:247–259.
- Bower WT. 1919. Alaska fisheries and fur industries in 1918. U.S. Commissioner of Fisheries, Washington, D.C.
- Buskirk SW. 1992. Conserving circumboreal forests for martens and fishers. *Conservation Biology* **6**:318–320.
- Buskirk SW, Bowman J, Gilbert JH. 2012. Population biology and matrix demographic modeling of American martens and fishers. Pages 77–92 in Aubry KB, Zielinski WJ, Raphael MG, Proulx G, Buskirk SW, editors. *Biology and conservation of martens, sables, and fishers: a new synthesis*. Cornell University Press, Ithaca, New York.
- Callicott JB. 2011. Postmodern ecological restoration: choosing appropriate temporal and spatial scales. *Philosophy of Ecology* **11**:301–326.
- Carrara PE, Ager TA, Baichtal JF. 2007. Possible refugia in the Alexander Archipelago of southeastern Alaska during the late Wisconsin glaciation. *Canadian Journal of Earth Sciences* **44**:229–244.
- Chamberlain CP, Waldbauer JR, Fox-Dobbs K, Newsome SD, Koch PL, Smith DR, Risebrough R. 2005. Pleistocene to recent dietary shifts in California condors. *Proceedings of the National Academy of Sciences of the United States of America* **102**:16707–16711.
- Chisholm BS, Nelson DE, Schwarcz HP. 1982. Stable-carbon isotope ratios as measure of marine versus terrestrial protein in ancient diets. *Science* **216**:1131–1132.
- Cook JA, Bidlack AL, Conroy CJ, Demboski JR, Fleming MA, Runck AM, Stone KD, MacDonald SO. 2001. A phylogeographic perspective on endemism in the Alexander Archipelago of southeast Alaska. *Biological Conservation* **97**:215–227.
- Cook JA, Dawson NG, MacDonald SO. 2006. Conservation of highly fragmented systems: the north temperate Alexander Archipelago. *Biological Conservation* **133**:1–15.
- Cook JA, MacDonald SO. 2001. Should endemism be a focus of conservation efforts along the North Pacific Coast of North America? *Biological Conservation* **97**:207–213.
- Davis CS, Strobeck C. 1998. Isolation, variability, and cross-species amplification of polymorphic microsatellite loci in the family Mustelidae. *Molecular Ecology* **7**:1776–1778.
- Diamond JM. 1984. ‘Normal’ extinction of isolated populations. Pages 191–246 in Nitecki MH, editor. *Extinctions*. Chicago University Press, Chicago.
- Donlan CJ, Martin PS. 2004. Role of Ecological history in invasive species management and conservation. *Conservation Biology* **18**:267–269.
- Donlan CJ, et al. 2006. Pleistocene rewilding: an optimistic agenda for twenty-first century conservation. *The American Naturalist* **168**:660–681.
- Elkins WA, Nelson UC. 1954. Wildlife introductions and transplants in Alaska. *Proceedings of the 5th Alaska Science Conference*. U.S. Fish and Wildlife Service, Washington, D.C.
- Elton CS. 1958. *The ecology of invasions by animals and plants*. Methuen, London.
- Excoffier L, Lischer HEL. 2010. Arlequin suite ver 3.5: a new series of programs to perform population genetics analyses under Linux and Windows. *Molecular Ecology Resources* **10**:564–567.
- Fleming MA, Cook JA. 2002. Phylogeography of endemic ermine (*Mustela erminea*) in southeast Alaska. *Molecular Ecology* **11**:795–807.
- Frey JK. 2013. Re-evaluation of the evidence for the importation of red foxes from Europe to colonial America: origins of the southeastern red fox (*Vulpes vulpes fulva*). *Biological Conservation* **158**:74–79.
- Giannico GR. 1986. Geographic and sexual variation of the American pine marten (*Martes americana*) in the Pacific Northwest, with special reference to the Queen Charlotte Islands. MS thesis. University of Victoria, Victoria, British Columbia.
- Gilbert MTP, Binladen J, Miller W, Wiuf C, Willerslev E, Poinar H, Carlson JE, Leebens-Mack JH, Schuster SC. 2007. Recharacterization of ancient DNA miscoding lesions: insights in the era of sequencing-by-synthesis. *Nucleic Acids Research* **35**:1–10.
- Grayson DK. 2001. The archaeological record of human impacts on animal populations. *Journal of World Prehistory* **15**:1–68.
- Heaton TH, Grady F. 2003. The Late Wisconsin vertebrate history of Prince of Wales Island, Southeast Alaska. Pages 17–53 in Schubert BW, Mead JI, Graham RW, editors. *Ice Age cave faunas of North America*. Indiana University Press, Bloomington.
- Heaton TH, Talbot SL, Shields GF. 1996. An ice age refugium for large mammals in the Alexander Archipelago, Southeastern Alaska. *Quaternary Research* **46**:186–192.
- Hofreiter M, Jaenicke V, Serre D, Haeseler Av, Pääbo S. 2001. DNA sequences from multiple amplifications reveal artifacts induced by cytosine deamination in ancient DNA. *Nucleic Acids Research* **29**:4793–4799.
- Hunter MH Jr. 1996. Benchmarks for managing ecosystems: Are human activities natural? *Conservation Biology* **10**:695–697.
- Isto SC. 2012. *The fur farms of Alaska*. University of Alaska Press, Fairbanks.
- Jackson AL, Inger R, Parnell AC, Bearhop S. 2011. Comparing isotopic niche widths among and within communities: SIBER-Stable isotope Bayesian ellipses in R. *Journal of Animal Ecology* **80**:595–602.
- Johnson L. 1981. Otter and marten life history studies. Final report. Alaska Department of Fish and Game, Juneau.
- Kirch PV. 1982. The impact of prehistoric Polynesians on the Hawaiian ecosystem. *Pacific Science* **36**:1–14.
- Kyle CJ, Strobeck C. 2003. Genetic homogeneity of Canadian mainland marten populations underscores the distinctiveness of Newfoundland pine martens (*Martes americana atrata*). *Canadian Journal of Zoology* **81**:57–66.
- Lamers R, Hayter S, Matheson C. 2009. Postmortem miscoding lesions in sequence analysis of human ancient mitochondrial DNA. *Journal of Molecular Evolution* **68**:40–55.
- Laval G, Excoffier L. 2004. SIMCOAL 2.0: a program to simulate genomic diversity over large recombining regions in a subdivided population with a complex history. *Bioinformatics* **20**:2485–2487.
- Lodge DM, Shrader-Frechette K. 2003. Nonindigenous species: ecological explanation, environmental ethics, and public policy. *Conservation Biology* **17**:31–37.
- Lodge DM, et al. 2006. Biological invasions: recommendations for policy and management. *Ecological Applications* **16**:2035–2054.
- MacDonald SO, Cook JA. 2007. *Mammals and amphibians of southeast Alaska*. Special publication 8. Museum of Southwestern Biology, University of Mexico, Albuquerque.
- Mandryk CAS, Josenhans H, Fedje DW, Mathewes RW. 2001. Late Quaternary paleoenvironments of Northwestern North America: implications for inland versus coastal migration routes. *Quaternary Science Reviews* **20**:301–314.
- Mann DH, Hamilton TD. 1995. Late Pleistocene and Holocene paleoenvironments of the North Pacific coast. *Quaternary Science Reviews* **14**:449–471.

- Montgomery WI, Provan J, McCabe AM, Yalden DW. 2014. Origin of British and Irish mammals: disparate post-glacial colonization and species introductions. *Quaternary Science Reviews* **98**:144–165.
- Nogueira-Filho SLG, Nogueira SSC, Fragoso JMV. 2009. Ecological impacts of feral pigs on the Hawaiian Islands. *Biodiversity and Conservation* **18**:3677–3683.
- Parnell A, Inger R, Bearshop S, Jackson AL. 2010. Source partitioning using stable isotopes: coping with too much variation. *PLOS ONE* **5**(e9672) DOI: 10.1371/journal.pone0009672.
- Paul TW. 2009. Game transplants in Alaska. Technical bulletin 4. 2nd edition. Alaska Department of Fish and Game, Juneau.
- Pauli JN. 2010. Ecological studies of the American marten (*Martes americana*): quantifying cryptic processes in an elusive species. PhD thesis. University of Wyoming, Laramie.
- Peacock E, Peacock MM, Titus K. 2007. Black bears in Southeast Alaska: the fate of two ancient lineages in the face of contemporary movement. *Journal of Zoology* **271**:445–454.
- Peery MZ, Kirby R, Reid BN, Stoelting R, Doucet-Béer E, Robinson S, Vásquez-Carrillo C, Pauli JN, Palsbøll PJ. 2012. Reliability of genetic bottleneck tests for detecting recent population declines. *Molecular Ecology* **21**:3403–3418.
- Powell RA, Lewis JC, Slough BG, Brainerd SM, Jordan NR, Abramov AV, Monakhov V, Zollner PA, Murakami T. 2012. Evaluating translocations of martens, sables, and fishers: testing model predictions with field data. Pages 93–137 in Aubry KB, Zielinski WJ, Raphael MG, Proulx G, Buskirk SW, editors. *Biology and conservation of martens, sables, and fishers: a new synthesis*. Cornell University Press, Ithaca, New York.
- Prescott WH. 1843. *History of the conquest of Mexico*. Harper Brothers, New York.
- Richardson DM. 2011. Invasion science. The roads travelled and the road ahead. Pages 397–401 in Richardson DM, editor. *Fifty years of invasion ecology: the legacy of Charles Elton*. Blackwell Publishing, Oxford.
- Rick TC, Erlandson JM, Vellanoweth RL, Braje TJ, Collins PW, Guthrie DA, Stafford TW Jr. 2009. Origins and antiquity of the island fox (*Urocyon littoralis*) on California's Channel Islands. *Quaternary Research* **71**:93–98.
- Sakai AK, et al. 2001. The population biology of invasive species. *Annual Review of Ecology and Systematics* **32**:305–332.
- Salisbury OA. 1962. *The customs and legends of the Thlinget Indians of Alaska*. Bonanza Books, New York.
- Sandler R. 2013. The ethics of reviving long extinct species. *Conservation Biology* **28**:354–360.
- Schlaepfer MA, Sax DF, Olden JD. 2012. Toward a more balanced view of non-native species. *Conservation Biology* **26**:1156–1158.
- Simberloff D, et al. 2013. Impacts of biological invasions: what's what and the way forward. *Trends in Ecology & Evolution* **28**:58–66.
- Seddon PJ, Moehrenschrager A, Ewen J. 2014. Reintroducing resurrected species: selecting deextinction candidates. *Trends in Ecology & Evolution* **29**:140–147.
- Small MP, Stone KD, Cook JA. 2003. American marten (*Martes americana*) in the Pacific Northwest: population differentiation across a landscape. *Molecular Ecology* **12**:89–103.
- Statham MJ, Sacks BN, Aubry KB, Perrine JD, Wisely SM. 2012. The origin of recently established red fox populations in the United States: Translocations or natural range expansions? *Journal of Mammalogy* **93**:52–65.
- Statham MJ, Trut LN, Sacks BN, Kharlamova AV, Oskina IN, Gulevich RG, Johnson JL, Temnykh SV, Acland GM, Kukekova AV. 2011. On the origin of a domesticated species: identifying the parent population of Russian silver foxes (*Vulpes vulpes*). *Biological Journal of the Linnean Society* **103**:168–175.
- Stone KD, Flynn RW, Cook JA. 2002. Post-glacial colonization of northwestern North America by the forest-associated American marten (*Martes americana*, Mammalia: Carnivora: Mustelidae). *Molecular Ecology* **11**:2049–2063.
- Strong WL, Hills LV. 2013. Holocene migration of lodgepole pine (*Pinus contorta* var. *latifolia*) in southern Yukon, Canada. *The Holocene* **23**:1340–1349.
- Swarth HS. 1911. *Birds and mammals of the Alexander Expedition*. University of California in Zoology **7**:9–172.
- Tamura K, Nei M. 1993. Estimation of the number of nucleotide substitutions in the control region of mitochondrial DNA in humans and chimpanzees. *Molecular Biology and Evolution* **10**:512–526.
- Tamura K, Stecher G, Peterson D, Filipski A, Kumar S. 2013. MEGA6: molecular evolutionary genetics analysis. Version 6.0. *Molecular Biology and Evolution* **30**:2725–2729.
- Tuross N, Fogel ML, Hare PE. 1988. Variability in the preservation of the isotopic composition of collagen from fossil bone. *Geochimica et Cosmochimica Acta* **52**:929–935.
- USFS (U.S. Forest Service). 2008. *Tongass land and resource management plan: final environmental impact statement*. R10-MB-603a. USFS, Alaska Region, Juneau.
- Velten HV. 1939. Two southern tlingit tales. *International Journal of American Linguistics* **10**:65–74.
- Weckworth BV, Talbot S, Sage GK, Person DK, Cook J. 2005. A signal for independent coastal and continental histories among North American wolves. *Molecular Ecology* **14**:917–931.
- Willis KJ, Birks JB. 2006. What is natural? The need for a long-term perspective in biodiversity conservation. *Science* **314**:1261–1265.
- Xia X. 2013. DAMBE5: a comprehensive software package for data analysis in molecular biology and evolution. *Molecular Biology and Evolution* **30**:1720–1728.