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Boston University



Integrating archaeology and ancient DNA analysis to address invasive species colonization in the Gulf of Alaska

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Abstract: *The intentional and unintentional movement of plants and animals by humans has transformed ecosystems and landscapes globally. Assessing when and how a species was introduced are central to managing these transformed landscapes, particularly in island environments. In the Gulf of Alaska, there is considerable interest in the history of mammal introductions and rehabilitating Gulf of Alaska island environments by eradicating mammals classified as invasive species. The Arctic ground squirrel (*Urocitellus parryii*) is of concern because it affects vegetation and seabirds on Gulf of Alaska islands. This animal is assumed to have been introduced by historic settlers; however, ground squirrel remains in the prehistoric archaeological record of Chirikof Island, Alaska, challenge this timeline and suggest they colonized the islands long ago. We used 3 lines of evidence to address this problem: direct radiocarbon dating of archaeological squirrel remains; evidence of prehistoric human use of squirrels; and ancient DNA analysis of dated squirrel remains. Chirikof squirrels dated to at least 2000 years ago, and cut marks on squirrel bones suggested prehistoric use by people. Ancient squirrels also shared a mitochondrial haplotype with modern Chirikof squirrels. These results suggest that squirrels have been on Chirikof longer than previously assumed and that the current population of squirrels is closely related to the ancient population. Thus, it appears ground squirrels are not a recent, human-mediated introduction and may have colonized the island via a natural dispersal event or an ancient human translocation.*

Keywords: ancient translocation, ground squirrel, invasive species management, *Urocitellus parryii*, zooarchaeology

Integración de la Arqueología y el Análisis de ADN Antiguo para Abordar la Colonización de las Especies Invasoras en el Golfo de Alaska

Resumen: *El movimiento intencional y no intencional de plantas y animales causado por los humanos ha transformado a ecosistemas y paisajes a nivel mundial. Valorar cuándo y cómo una especie fue introducida es importante para el manejo de estos paisajes transformados, en particular en los ambientes isleños. En el Golfo de Alaska hay un interés considerable por la historia de las introducciones de mamíferos y la rehabilitación de los ambientes isleños del Golfo de Alaska por medio de la erradicación de mamíferos clasificados como especies invasoras. La ardilla terrestre del Ártico (*Urocitellus parryii*) es de interés porque afecta a la vegetación y las aves marinas en las islas del Golfo de Alaska. Se asume que este animal fue introducido por los colonizadores*

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históricos; sin embargo, los restos de ardillas terrestres en el registro arqueológico prehistórico de la Isla Chirikof, Alaska, cuestionan esta línea del tiempo y sugieren que las ardillas colonizaron la isla hace mucho tiempo. Utilizamos tres líneas de evidencia para abordar este problema: el fechado directo con radiocarbono de los restos arqueológicos de las ardillas, la evidencia del uso de las ardillas por humanos prehistóricos, y el análisis de ADN antiguo de los restos fechados de las ardillas. Las ardillas de Chirikof fueron fechadas a por lo menos 2000 años atrás, y las marcas de cortes en los huesos de las ardillas sugirieron un uso prehistórico por personas. Las ardillas antiguas también compartieron un haplotipo mitocondrial con las ardillas modernas de Chirikof. Estos resultados sugieren que las ardillas han estado en Chirikof mucho más tiempo del que se asumía previamente y que la población actual de ardillas está relacionada cercanamente con la población antigua. Así, parece que las ardillas terrestres no son una introducción reciente mediada por los humanos y podrían haber colonizado la isla por medio de eventos naturales de dispersión o translocación por humanos antiguos.

Palabras Clave: ardilla terrestre, manejo de especies invasoras, translocación antigua, *Urocyon parryi*, zooarqueología

Introduction

Landscapes and ecosystems around the globe have been transformed by the human-mediated introduction of domestic and wild plants and animals. Scientists and managers working to mitigate and manage these introductions face many challenges, including assessing when and how a species was introduced. For example, in the Gulf of Alaska, the U.S. Fish and Wildlife Service (USF&WS) manages the Alaska Maritime National Wildlife Refuge (hereafter the refuge) (Fig. 1a), which is a network of lands set aside to conserve natural diversity, especially for marine and migratory birds. Significant declines in bird populations on islands in the northeastern Pacific Ocean—particularly the Gulf of Alaska—have resulted from the introduction of exotic mammals to places that have few endemic mammal species (Bailey 1993; Ebbert & Byrd 2002; Towns et al. 2011). Given refuge goals, there is currently considerable interest in understanding the history of mammal introductions across this region and determining the potential for rehabilitating Gulf of Alaska island environments by eradicating those classified as invasive species. The refuge defines invasive species as a “species whose introduction into an ecosystem in which the species is not native causes, or is likely to cause, environmental or economic harm or harm to human health” (USF&WS 2003:2).

The Arctic ground squirrel (*Urocyon parryi*) is being considered for eradication because of its effect on vegetation and seabirds (Hatch & Hatch 1983; Ebbert & Byrd 2002; Barker & Derocher 2010). However, to classify a species as invasive and then eradicate that animal, managers must determine whether the species is native or introduced, and this status is not well documented for Arctic ground squirrels across much of the refuge. Ethnohistoric documents suggest the Alutiiq and Unangan peoples used the squirrel as a source of fur, although the antiquity of this practice is unknown (Clark 2010). Although squirrel remains have been recovered from prehistoric archaeological sites on Chirikof Island in the central Gulf of Alaska (Clark 2010) (Fig. 1), historic accounts

also document 19th and 20th century releases throughout the gulf as food for farmed foxes (Peterson 1967; Bailey 1993; Isto 2012). In studies of modern squirrel genetics, Cook et al. (2010) and Eddingsaas et al. (2004) suggest squirrel populations may be Pleistocene relicts on some Gulf of Alaska islands, but for other islands, how and when these populations were established remains unclear (MacDonald & Cook 2009; Cook et al. 2010).

The archaeological record has great potential to address this problem (Lyman 1998; Hofman et al. 2015a; West et al. 2017), and we applied archaeological data to investigate the origins, antiquity, and human use of the squirrel population on Chirikof Island, Alaska. We combined 3 lines of evidence: direct radiocarbon (accelerator mass spectrometry [AMS]) dating of archaeological squirrel remains; evidence of human use of squirrels (i.e., bone modification indicative of cooking or butchery); and ancient DNA analysis of dated squirrel remains. We used these data to assess the antiquity and genetic stability of Chirikof’s ground squirrel population and to consider the role of long-term data in the determination of native and indigenous species.

Complex Human–Environmental Interactions

For the USF&WS, the introduction history for this region is based on the historic record and the assumption that Russian and American settlers introduced a variety of mammal species beginning in the late 18th century. Ebbert and Byrd (2002) suggest that ancient native people did not translocate terrestrial mammals and that translocations likely only occurred following the arrival of Russian explorers in 1741. These suppositions are problematic. On the one hand, for some animals, such as cattle, the introduction history is clearly laid out in the historic record (Fields 2000; Ebbert & Byrd 2002; Black et al. 2005; Saltonstall & Steffian 2005). On the other hand, the history of Arctic ground squirrel introductions is less clear because they are found in both the prehistoric archaeological record and in recorded

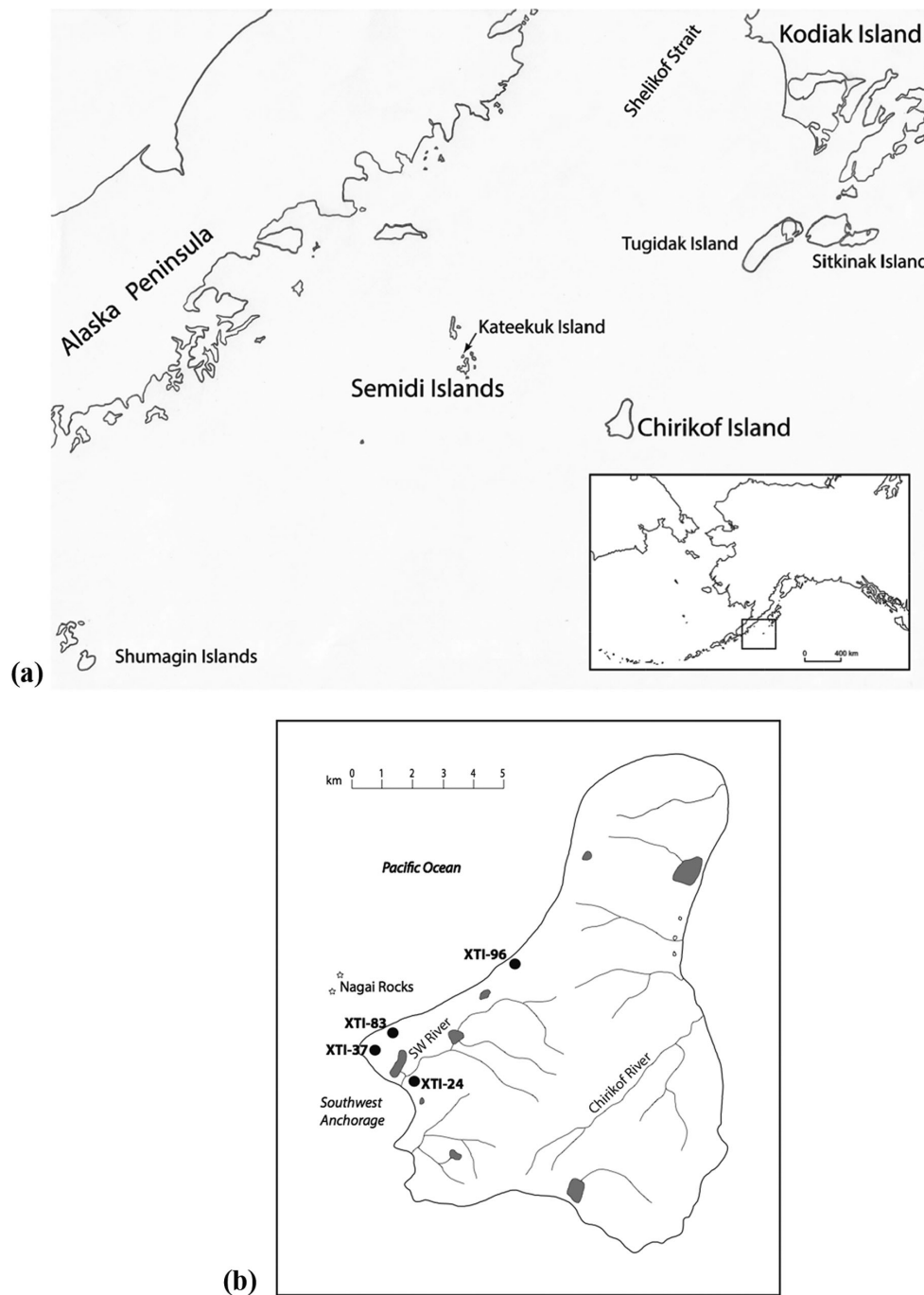


Figure 1. (a) The Gulf of Alaska and (b) locations of archaeological sites mentioned in text on Chirikof Island (gray polygons, freshwater lakes and streams). Maps courtesy of Jack Withrow and modified from Withrow (2015) and West et al. (2017).

historic introductions on other islands (e.g., Bailey 1993; Ebbert & Byrd 2002; Clark 2010).

A longer view of human settlement in the region is that native people moved among the islands in this region for thousands of years, carrying both raw materials and animals with them (e.g., Fitzhugh & Kennett 2010). Genetic data suggest Chirikof Island ground squirrels are closely

related to other populations on small gulf islands and to continental populations (Cook et al. 2010). These data demonstrate there is a complex pattern of movement across the region that in some cases suggests human transport rather than natural dispersal.

Despite the confounding factors that challenge understanding of this animal's history, this type

of long-term perspective from the archaeological or ethnohistoric records is rarely considered in a management context (Lyman 1996; Wolverton & Lyman 2012) (but see Grayson [2005]). Therefore, an interdisciplinary collaboration among archaeologists, geneticists, and the USF&WS has led us to the use of multiple lines of evidence to test the hypothesis that the ground squirrel population has inhabited Chirikof Island for a longer period than assumed previously.

Chirikof Island

Chirikof Island (~115 km²) is in the central Gulf of Alaska (135 km from the Alaska Peninsula and 150 km from the Kodiak archipelago) (Fig. 1), where it is exposed to open ocean and heavy surf that make access difficult. Based on archaeological data produced by surveys in 1963, 2005, and 2013, the earliest occupation of Chirikof was approximately 5000 years ago (Workman 1966, 1969; Saltonstall & Steffian 2005). The artifact assemblages suggest Alutiiq and Unangan people traveled to Chirikof from the Kodiak archipelago, the Alaska Peninsula, and the Aleutian Islands (Saltonstall & Steffian 2005).

Russian explorers spotted the island in 1741 and by the mid-nineteenth century the Russian American Company had established a trading post on the island, where ground squirrels were hunted and coats were made from their hides (Fig. 2a). By the late nineteenth century, Americans were farming Arctic fox (*Vulpes lagopus*) and cattle ranching on the island (Fields 2000; Black et al. 2005; Isto 2012). The last human occupants of the island left in the 1980s when the island became part of the refuge. They left behind the introduced foxes and a substantial cattle population.

Although the introduction history of both the cattle and fox is clear, the timing of ground squirrel introductions to Chirikof is much less clear (Dall 1874; Clark 2010; Cook et al. 2010). However, during fieldwork with the Alutiiq Museum in 2005, squirrel bones were recovered from several prehistoric sites, suggesting the squirrels may have been on Chirikof longer than previously assumed (Saltonstall & Steffian 2005). These sites include a Russian-era historic site (XTI-24), which was dated to before AD 1868 based on the collected artifacts and historic documents, and 3 prehistoric sites (XTI-37, XTI-83, and XTI-96), which contained well-preserved animal bones in middens (Saltonstall & Steffian 2005) (Fig. 1b). Based on a combination of radiocarbon dates obtained from archaeological charcoal and diagnostic artifacts, these prehistoric sites were 500–2000 years old (Saltonstall & Steffian 2005).

Arctic Ground Squirrel

Ground squirrels are a concern for the refuge because they cause erosion through grazing and burrowing

that alters landscapes and plant communities (Bailey & Faust 1981; Barker & Derocher 2010). These landscape changes affect not only the squirrels but also birds; nesting areas for many shorebirds may be diminished because of the erosion. Squirrels are also prey on the eggs and chicks of passerines and seabirds that burrow or nest on the ground (e.g., Bailey 1993). Ground squirrels have also been observed feeding on birds, lemmings, and other squirrels (e.g., Callahan 1993). On Chirikof the impact of ground squirrels on nesting birds is not well documented (but see Withrow [2015]), but observational data suggest burrow-nesting and surface-nesting species are rare on islands elsewhere in the Gulf of Alaska that are occupied by ground squirrels (Leschner & Burrell 1977; Hatch & Hatch 1983; Pollom et al. 2015). Unlike on mainland Alaska or on nearby Kodiak, Chirikof squirrels have few predators, although populations may be kept in check by predatory birds and the recently eradicated Arctic foxes.

Previous genetic analyses of Arctic ground squirrels in the Gulf of Alaska have explored the population history and evolutionary relationships with both nuclear (nDNA) and mitochondrial (mtDNA) markers (Eddingsaas et al. 2004; Cook et al. 2010; Galbreath et al. 2011; McLean et al. 2016). Phylogeographic analysis of the mitochondrial cytochrome *b* gene (*cyt b*) identified 4 nonoverlapping clades spanning Alaska (west Beringia, southwest, central, and north) (Eddingsaas et al. 2004). Focusing on the southwestern clade with *cyt b* and 8 nuclear microsatellite loci, Cook et al. (2010) hypothesize that insular Arctic ground squirrel populations should be genetically distinguishable if they were established before the Last Glacial Maximum (LGM), whereas if humans introduced them, they would show little genetic differentiation. Phylogenetic analysis of *cyt b* yielded 3 groups (southwestern Alaska mainland and islands, peninsular mainland, and island non-native species) with limited phylogenetic support, but differentiation suggests the possibility of multiple coastal refugia during the LGM. Microsatellite data were generally in concordance with mtDNA loci but the relationships between populations in the Kodiak and Semidi regions (including Chirikof) were not very clear (Cook et al. 2010).

Galbreath et al. (2011) expanded these data sets with additional nuclear markers (anonymous loci) to test range-wide bioclimatic envelope models associated with climate change. They found discordance between nuclear and mitochondrial data sets. In particular, nuclear data showed a close relationship between Beringia and the southwestern clades that was not identified in mitochondrial data sets. Galbreath et al. (2011) propose this discordance could be attributed to sex-biased dispersal, specifically natal philopatry in female squirrels. In the context of island introductions, the arrival of females is critical for the success of an introduction and, therefore, mtDNA provides important information on the history of invasion in island populations.

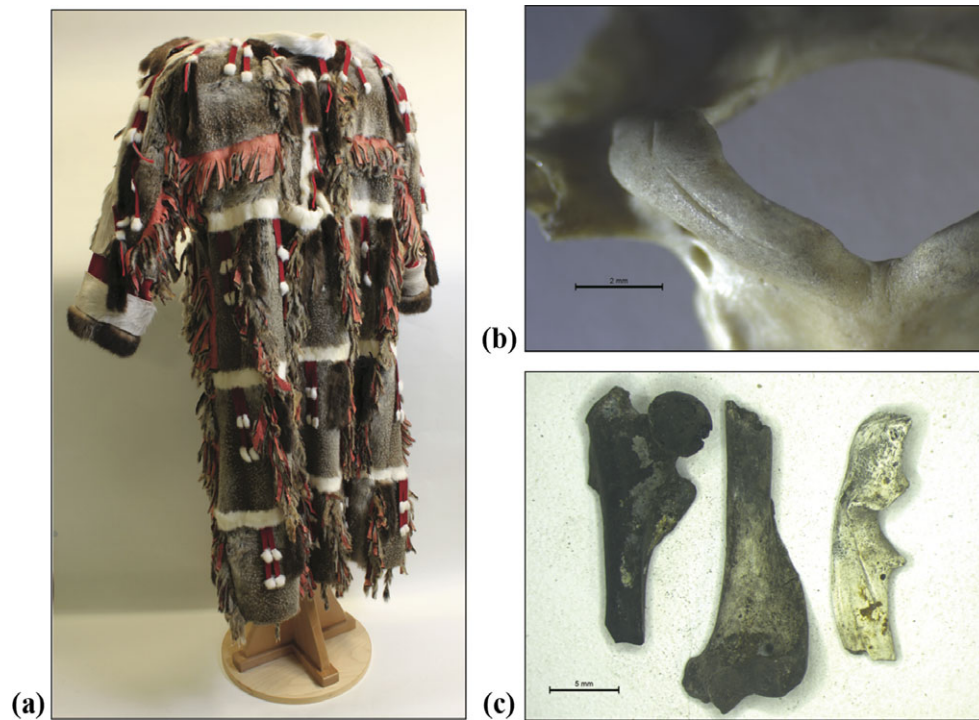


Figure 2. Evidence of human use of ground squirrels on Chirikof Island, Alaska: (a) parka created by Susan Malutin, Grace Harrod, and community volunteers for the Alutiiq Museum in 1999, with support from the Alaska State Museum grant-in-aid program (catalog number AM405; photo by Marnie Leist); (b) skeletal elements showing cut marks across the occipital condyle of the cranium; and (c) burning and calcification seen on a proximal femur, distal humerus, and proximal ulna.

Methods

AMS Dating

We directly dated squirrel bone to provide a strong temporal association between the AMS data and the ancient DNA analyses and to eliminate the possibility that the squirrels were intrusive in the archaeological sites. Material from the historic-era site (XTI-24) was not directly dated because of the large error associated with dates from this period. For dating of this site, we used previously described historic artifacts and documents (Saltonstall & Steffian 2005). From the other 3 sites (XTI-37, XTI-83, and XTI-96), we chose specimens from the top and bottom levels of each midden to establish temporal depth. Complete limb bones and mandibles were analyzed, and each bone was sampled with a new and sterile rotary blade. Approximately 400 mg of bone were sent to Oxford Radiocarbon Accelerator Unit for AMS dating and approximately 100 mg was used for ancient DNA analyses. We calibrated AMS results with the Oxcal calibration program version 4.2 (Bronk Ramsey et al. 2009) in IntCal13 (Reimer et al. 2013).

Zooarchaeology

To explore human use of the squirrel remains, we examined the archaeological ground squirrel remains

for modification. Human modification can include burning, breakage, and cut marks that result from skinning or butchering with stone tools (Binford 1981; Fisher 1995).

Although butchery and skinning of small mammals rarely results in cut marks on the bones (Lyman 1992; Stahl 1996), Grayson (1989) notes that marks on the cranium, mandible, and feet of archaeological yellow-bellied marmots (*Marmota flaviventris*) in the White Mountains of California suggest skinning. Binford (1981) suggests that cut marks elsewhere on the skeleton indicate butchery for meat. Because cut marks reflect the tool's cutting edge, those created by a stone tool are likely to be V shaped in cross section, narrow, and relatively straight, although natural processes may have a similar effect (Fisher 1995). Burning is a clearer mark of human activity. If small mammal carcasses were skinned and then cooked over a fire, one should see burning on the distal ends of long bones (Medina et al. 2012). The limbs are less meaty and, therefore, more likely to be exposed to direct fire and to exhibit burning in the form of brown, black, or white coloring, depending on temperature (Medina et al. 2012). At 3 sites (XTI-24, XTI-83, and XTI-96; sample sizes at XTI-37 were too small for robust analysis), we examined all skeletal elements (except vertebrae, ribs, and loose teeth) for burning and cut marks. We looked for burn marks with the naked eye and used an optical

Table 1. *Urocitellus parryi* bone samples analyzed and listed by archaeological site.^a

Site number	Sample number	Skeletal element	Radiocarbon age range, BP (¹⁴ C years)	δ13C (‰) ^b	Calibrated age range, cal BP; 2σ ^c (years)	Base pairs sequenced ^d
XTI-24	XTI24.1	humerus, left				968
XTI-24	XTI24.2	humerus, left				861
XTI-24	XTI24.3	humerus, left				861
XTI-37	AM513:05_1	mandible, right	248 ± 22	-21.9	270 ± 70	968
XTI-37	AM513:05_2	femur, right				968
XTI-96	XTI96.1	humerus, right	409 ± 22	-22.1	490 ± 40	968
XTI-96	XTI96.2	humerus, right	408 ± 23	-20.9	490 ± 40	968
XTI-83	XTI83.4	humerus, left	2045 ± 26	-21.1	2000 ± 40	968
XTI-83	XTI83.3	humerus, left	2093 ± 25	-21.1	2060 ± 40	968
XTI-83	AM513:17_1	mandible, right	2000 ± 24	-21.4	1950 ± 30	968
XTI-83	AM513:17_2	cranium				968
XTI-83	XTI83.5	mandible, left	2139 ± 24	-21.2	2130 ± 70	560

^aAccelerator mass spectrometry dates produced by the Oxford Radiocarbon Accelerator Unit (ORAU) are reported for bone as a radiocarbon age range and a calibrated age range for 3 sites on Chirikof Island (XTI), Alaska. The dates were calibrated using OxCal version 4.2 (Bronk Ramsey 2009) and the IntCal 13 calibration curve (Reimer et al. 2013). Samples from XTI-24 are historic (~ AD1868 and 1890) and were not dated using radiometric methods.

^bMeasured value.

^cMedian probability.

^dTotal base pairs sequenced of the cytochrome *b* gene from a consensus sequence of overlapping primer pairs.

stereomicroscope at 25× magnification to look for cut marks.

Ancient DNA

We extracted DNA with phenol–chloroform extraction protocols designed to minimize contamination, which included alternating species between extractions, using extraction blanks, and decontaminating reagents when possible (Wisely et al. 2004). All pre-polymerase chain reactions (PCR) protocols were conducted in an isolated ancient DNA laboratory located in a separate building from the primary genetics laboratory. DNA from Arctic ground squirrels was not previously extracted in our ancient DNA lab, and DNA from modern Arctic ground squirrels was never processed in the primary genetics lab.

A portion of the mitochondrial *cyt b* gene (968 base pairs [bp]) was amplified in 12 ancient ground squirrel samples (Table 1 & Supporting Information). Mitochondrial *Cyt b* is commonly used for examining phylogeographic relationships among mammals. Sequencing this gene from our ancient DNA samples allowed for direct comparison with published *cyt b* sequences from the phylogeographic studies of modern Arctic ground squirrels conducted by Cook et al. (2010), Eddingsaas et al. (2004), and Galbreath et al. (2011). Although Cook et al. (2010) and Galbreath et al. (2011) also present data from nuclear microsatellite loci, amplification of nuclear DNA in ancient samples can be more problematic than amplification of mtDNA due to the degraded nature and lower copy number of nuclear DNA in archaeological bone. Therefore, we targeted only mtDNA markers, which have

a high copy number and can be more effectively recovered in degraded DNA extracts.

All sequences from our ancient DNA samples were aligned to publically available *cyt b* sequences downloaded from GenBank from Eddingsaas et al. (2004), Cook et al. (2010), and Galbreath et al. (2011), including sequences from 5 Chirikof ground squirrel tissue samples. Three alignments were generated: all samples (total length = 968 bp); without XTI83.5, which was missing data (total length = 968 bp); and without XTI83.5 trimmed to minimize missing data (total length = 814 bp). All alignments were performed with MAFFT version 7.017 as implemented in Geneious version 7.1.4. To explore the genetic relationships among islands in the Gulf of Alaska, network analysis for this combined data set was conducted using the median joining algorithm as implemented in Network version 4.6.1.3 with default parameters. We could not use BEAST to date the divergence between Chirikof and other island samples because there was not enough sequence variability to confidently resolve the relationships in this clade (i.e., BEAST runs could not reach stationarity).

Results

Collagen extraction was successful in all samples; carbon to nitrogen ratios were from 3.4 to 3.5 (an indication of good preservation [DeNiro 1985; Ambrose 1990]). When calibrated, these 7 AMS dates ranged from 2130 ± 70 to 270 ± 70 cal BP (Table 1). Squirrels associated with XTI-24, the historic village, were estimated to date from the

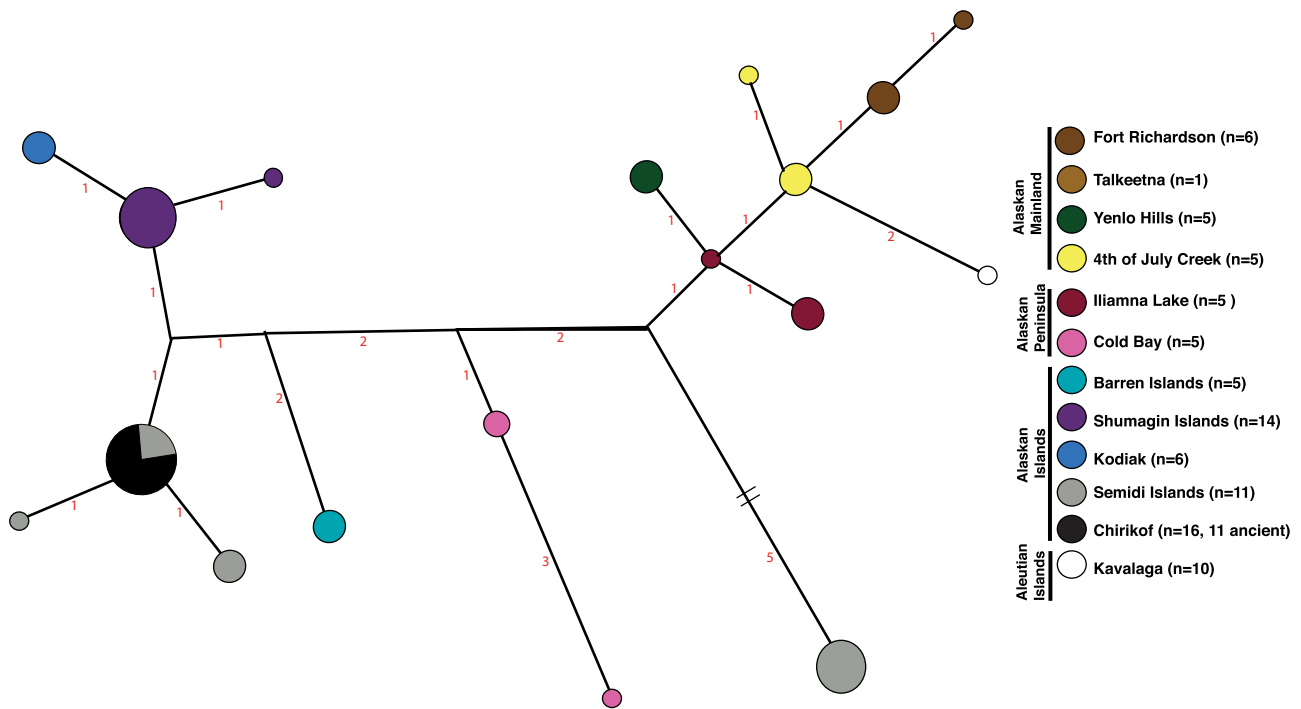


Figure 3. Median joining haplotype network of Southwest Alaskan ground squirrels. The size of the circles and branch lengths are proportional to number of individuals represented and the number of mutations between haplotypes (red), respectively. Hash marks indicate shortened branches. Chirikof squirrels from archaeological contexts share a haplotype with modern Chirikof squirrels and individuals from the Semidi Islands.

Russian period (before AD 1869) based on associated artifacts.

Substantial numbers of squirrels were deposited in the 3 archaeological sites, and both cut marks (Fig. 2b) and burning (Fig. 2c) were identified in the assemblage. The total number of identified specimens (NISP) of ground squirrel was 17,852 (449 in 0.6 m³ of excavated material from site XTI-24; 1240 in 0.2 m³ from XTI-83, and 16,163 in 0.4 m³ from XTI-96). Approximately 4% of the assemblage was burned (NISP = 705) or had cut marks (NISP = 20). Some bones were fully burned or calcined, whereas others are only partially burned (Fig. 2c). These elements were from both the appendicular and axial skeletons, including the cranium. We identified V-shaped, linear cut marks primarily on the occipital condyles of cranial specimens (NISP 11) (Fig. 2b) but also on long bones (NISP 7), innominate (NISP 1), and 1 foot bone (NISP = 1).

We successfully amplified ancient DNA from 12 ground squirrel samples that were historic to 2130 years old (Table 1). A total of 968 bp were generated for 9 of these samples, and at least 814 bp were generated for 11 of the 12 samples. The oldest sample (XTI83-5) did not amplify well for several primers, despite multiple replicate PCRs. Ancient and modern sequences from Chirikof had no

sequence variation across 968 bp in 9 samples and across the length of the recovered fragment in the additional 3 samples. The same *cyt b* haplotype was shared among all ancient samples and the 5 extant ground squirrel individuals from Chirikof. The Chirikof Island squirrels were most closely related to those from the Semidi Islands and shared a haplotype with Kateekuk Island specifically (Fig. 3). The squirrel populations from the Kodiak and the Shumagin Islands were also closely related; only 2–3 base pairs separated *cyt b* haplotypes.

Discussion

The results of the AMS analyses confirmed that squirrels were present on Chirikof Island at least 2000 years ago. The results of ancient DNA analyses suggest the contemporary population of squirrels is closely related to this ancient population.

These results are illustrative, but they are limited because we examined only 1 gene of the mitochondrial genome; *cyt b* is not expected to accumulate detectable changes over short periods (about 2000 years) due to its low mutation rate (Bradley & Baker 2001); and this region

may not reflect the evolutionary patterns in the larger ground squirrel mitogenome or nuclear genome. This difference has been documented elsewhere (e.g., Hofman et al. 2015b) and looking beyond this gene could illuminate the history of this ground squirrel population. For example, Cook et al. (2010) and Galbreath et al. (2011) used multiple markers, including microsatellites and mitochondrial DNA, that are generally in concordance with the nuclear data. However, the degraded nature of archaeological samples makes the use of microsatellite markers difficult and unreliable (Kuhn et al. 2010), and although our results support the presence of ground squirrel on Chirikof for at least 2 millennia, the precise timing of the colonization or introduction event is unclear.

Based on biological, archaeological, ethnohistoric, and historic data, Clark (2010) proposes 4 potential dispersal mechanisms of ground squirrels to Chirikof Island.

First, squirrels may have been introduced as part of the fox-farming industry in the late 19th or early 20th century. There is no clear documentation of squirrel releases on Chirikof to feed the fox farms, but there is a long-standing association between fox farming and squirrels as food sources in the Gulf of Alaska (Peterson 1967; Bailey 1993; Isto 2012). Although the presence of ground squirrel bones in prehistoric sites challenges this hypothesis, it is possible there were multiple introductions and the contemporary population is the result of a historic introduction. However, the lineage we sampled (Fig. 3) was identical to the lineage recovered in the extant individuals described in Cook et al. (2010), suggesting matrilineal continuity between ancient and modern Chirikof squirrels.

Second, squirrels could have dispersed overwater by swimming or rafting from the closest landmass, the Semidi Islands (about 56 km) (Fig. 1a). The ancient and modern Chirikof squirrels are most closely related to and share the same *cyt b* haplotype of a modern squirrel from the Semidi Islands to the northwest and more distantly to those from the Kodiak archipelago and Shumagin Islands (Figs. 1a & 3). Although a local extinction on Chirikof followed by a recolonization event or repeated colonizations from the Semidi Islands is possible, it may be improbable. Clark (2010) argues the aggressive social behavior among male squirrels may have driven losing males from the Semidis to Chirikof. Clark (2010) also notes there is little evidence of swimming among squirrels, and given the long distance and extreme climates, this seems like the least likely possibility. However, Murie (1959) observed squirrels swimming short distances in the Aleutian Islands. On the other hand, rafting may have occurred across this region, where the dominant currents and gyres move east to west. The limited diversity (only a single haplotype on Chirikof) and the shared haplotype found in Chirikof and Semidi squirrels (1 of 4 Semidi Island haplotypes) do not exclude rafting or swimming as a mechanism for squirrel dispersal to Chirikof.

Third, the Chirikof squirrels may be a Pleistocene relict population. Clark (2010) raises the possibility that the squirrels survived Pleistocene glaciation by living in or migrating from refugia (e.g., Karlstrom 1969). Geological data suggest the island was glaciated in the Late Wisconsin period and the southwest coast was ice free by 13,400 years ago (Nelson et al. 2015), whereas Kodiak Island supported a refugium through the Late Wisconsin glacial advance (Manley & Kaufman 2002). This scenario could be tested by further survey of the potential Pleistocene landscape and search for a fossil record on Chirikof that would link the squirrels to this era. However, Cook et al. (2010) hypothesized genetic differentiation in populations established before the LGM, which is not observed in Chirikof populations.

Finally, native people may have introduced the Chirikof squirrels. Ethnohistoric accounts and oral history establish that Alutiiq people traveled to Chirikof to hunt ground squirrel for parkas (Holmberg 1985; Gideon 1989; Khlebnikov 1994) and did so until 1798, when they were forbidden by the Russians to hunt the squirrels for personal use (Clark 2010). Furthermore, documentation of both cut marks and burning on the prehistoric squirrel bones confirmed that ancient people used the squirrels as a source of fur, if not food.

The zooarchaeological analyses establish human use of the squirrels through both cutting and burning (Fig. 2). The cut marks on the base of the cranial remains suggest the heads were removed during the skinning process, although the small number of marks elsewhere on the skeleton suggests the animals may have been butchered as well (Binford 1981; Grayson 1989; S. Haakanson, personal communication). Although the squirrels may have been used for food after skinning, the burned specimens do not show any distinct pattern of burning to provide convincing evidence of roasting, and boiling as a cooking process leaves almost no evidence on the bones (Roberts et al. 2002; Medina et al. 2012). Therefore, the bones were likely burned after they were discarded in the fire as refuse (Stahl 1996; Roberts et al. 2002). Three lines of evidence—the ethnohistoric data that identify squirrels as a fur source, the abundance and density of squirrel remains in the archaeological record, and the presence of bone modification—provide strong evidence for human use of squirrels on prehistoric Chirikof.

Cook et al. (2010) hypothesize that Kateekuk Island in the Semidi group may be the source of the Chirikof squirrel population via human introduction because the 2 populations share a *cyt b* haplotype. In his 1874 diary, Dall writes that the Semidi Islands were visible from the bluffs on the southwest side of Chirikof, and Partnow (2001) reports that Alutiiq people from the Alaska Peninsula regularly traveled to the Semidi Islands and beyond. Although we are not aware of archaeological data from the Semidi Islands themselves, the literature suggests that people moved widely across the Gulf of Alaska by boat

and that Chirikof was settled several times by people coming from different places. The types of stone and bone artifacts recovered from Chirikof's archaeological sites strongly suggest the island was occupied by groups moving from Kodiak and the Alaska Peninsula to Chirikof and perhaps by people from as far west as the Aleutian Islands (Workman 1966; Saltonstall & Steffian 2005). Further archeological work in the Semidi Islands could illuminate the likely cultural connection with Chirikof.

Our data confirm that native people interacted with squirrels on Chirikof for 2000 years before Russian and American occupations of Alaska and that there is persistence in matrilineal lines between ancient and modern Chirikof squirrels. This supports our hypothesis that squirrels have been on Chirikof Island longer than previously assumed. Additionally, the large sample size and high density of squirrel bones in just 3 sites suggests there was a substantial presence of ground squirrels on the island over the 2000 years we considered. However, distinguishing among the mechanisms responsible for the squirrel introduction requires additional data. To further test these hypotheses one could recover squirrel bones >2000 years old on Chirikof and use divergence dating between Chirikof and other island squirrel populations. These analyses would require additional sequence data from both ancient and modern populations.

Regardless of the mechanism of dispersal, the longevity of the Chirikof squirrel population complicates its classification as an invasive or native species (Robbins 2004; Warren 2007). Therefore, our data have implications for the squirrel's potential eradication, and our results challenge managers to select an appropriate target for restoration that may incorporate human influence. (See Callicott [2002] and Turner and Berkes [2006] for a discussion of historically appropriate baselines.) According to the refuge, it is important to know whether the squirrels colonized Chirikof naturally or if they were introduced and whether they have become "functionally native" (Warren 2007). To address this problem, one must separate the influence of the squirrel from the destructive effects of invasive fox and cattle in island ecosystems, which can be done as islands recover through both fox and cattle removal. Squirrel influence on bird populations and their nesting habitats can be examined through time by using archaeological and paleoenvironmental records, where floral, faunal, and climate records from the pre-fox and pre-cattle landscape can be reconstructed through chemical, zooarchaeological, and genetic means.

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Supporting Information

Polymerase chain reaction methods (Appendix S1) and primer information (Appendix S2) 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.

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