Whole-genome sequencing reveals a deeper history of dynamic biotic complexity along North America's North Pacific Coast

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Abstract

Shared phylogenetic breaks across taxa, syntopic clusters of endemics, and paleogeographic reconstruction of isostatic and vegetation change over time suggest the existence of one or more ice-free glacial refugia off of North America's North Pacific Coast. An incomplete fossil record, however, creates uncertainty over which species persisted in hypothesized refugia, obscuring interpretation of the timing, potential duration, and surrounding paleoenvironments. We use whole-genome resequencing to assess the historical biogeography of these complex northern landscapes that consist of multiple coastal archipelagos and mountain ranges. Discovery of distinct insular and continental clades within Pacific martens (M. caurina) is consistent with previous morphometric and parasitological studies and also with the Coastal Refugium Hypothesis, thereby supporting the persistence of diverse, potentially forested refugial communities along the western edges of the Alexander Archipelago. We found no evidence of admixture on islands that received translocations of American pine martens (M. americana) in the mid 1900s, but we detected introgression in two geographically distinct zones of secondary contact. Evidence of early-generational hybrids across multiple hybrid zones, each backcrossed with M. americana, is consistent with a history of genetic dilution of M. caurina through outbreeding with M. americana. Into the future, these hybrid zones will serve as iterative tests for the outcome of admixture, providing instructive natural experiments for forecasting outcomes of proactive measures such as genetic rescue by natural resource managers.

INTRODUCTION

Centers of endemism and geographically clustered hybrid zones are hypothesized to be the legacy of species persistence through the Pleistocene in multiple ice-free glacial refugia followed by population expansion and contact (Hultén 1937; Heusser 1989; Heaton & Grady 2003; Swenson & Howard 2005). Isostatic adjustment and lower sea-levels (Mann 1986) are credited with exposing areas of the continental shelf along North America's North Pacific Coast (NPC) and providing terrestrial sanctuary for insular and coastal species displaced by the expanding Cordilleran and Laurentide ice sheets (Heaton *et al.* 1996; Hewitt 2000; Lacourse *et al.* 2003; Mathewes & Clague 2017). Multiple refugia are hypothesized within southeast Alaska's contemporary Alexander Archipelago (e.g., Baranof, Chichagof, Dall, Heceta, and Prince of Wales islands; Foster 1965; Fedorov & Stenseth 2002; Carrara et al. 2003, 2007; Ager 2019) and British Columbia's Haida Gwaii Archipelago and surrounding areas (Heusser 1989; Mathewes & Clague 2017). Ice-free coastal refugia may have also played an integral role in human colonization of the Americas by opening a maritime migration corridor. New evidence shows the Pacific Northwest was inhabited by humans as early as > 15-16 thousand years ago (kya; Devièse*et al.* 2018; Davis *et al.* 2019) and the Alexander Archipelago at least > 10 kya, but likely more than 13 kya (Dixon et al. 2014; Carlson & Baichtal 2015; Lesnek *et al.* 2018; Mackie *et al.* 2018; McLaren *et al.* 2018), potentially predating the opening of an ice-free migration corridor through central Alberta, Canada (<=14.8 kya; Margold *et al.* 2019). An incomplete fossil record, however, creates uncertainty over which species persisted in hypothesized refugia, obscuring interpretation of their duration and paleoenvironments. Unlike humans with access to rudimentary sea-faring technologies (Erlandson *et al.* 2007), other coastal refugial mammals would have been essentially isolated from mainland populations, leading to a cessation in gene flow and divergence over time (Hewitt 2000). As such, the genomes of refugial descendants can provide clues to whether populations or species diverged in refugial isolation. Predicted variation includes high genetic differentiation from other refugial populations and the abundance of endemic or ancestral alleles, low genetic diversity as a consequences of small population sizes and genetic drift, and less spatial structure relative to recently colonized populations (Hewitt 2000).

In contrast to glacial isolation, post-glacial population expansion from multiple refugia can lead to secondary contact and the formation of hybrid zones between previously isolated taxa (Hewitt 2000; Swenson & Howard 2005). The rapid climatic oscillations of the Pleistocene (Williams 1998) led to recurrent opportunities for contact and gene flow between incompletely diverged taxa (Hewitt 2000, 2003). The consequences of genetic exchange are complex and range from homogenization to hybrid speciation (Arnold 1997; Harrison & Harrison 1993; Genovart 2009; Abbott *et al.* 2016), depending on the level of differentiation, but they can now be examined in detail using whole-genome sequences (Twyford & Ennos 2012).

North American martens are relatively small meso-carnivores, hypothesized to have diverged in at least two independent glacial refugia south of the Laurentide ice sheet (Stone *et al.* 2002): one refugium east of the Rocky Mountains or Mississippi River drainage giving rise to American pine martens (*Martes americana* , Turton 1806) and another to the west, presumably the cradle for Pacific martens (*Martes caurina* , Merriam 1890). However, the disjunct contemporary range of Pacific martens and occurrence of two natural hybrid zones between these species one occurring on near-coastal islands (Kuiu and Kupreanof, AK) along the NPC and another in the northern Rocky Mountains (Fig. 1) suggest Pacific martens may have a deeper evolutionary history along the NPC than previously thought (Pauli *et al.* 2015). The widespread coast-to-coast boreal distribution of *M. americana* directly contrasts with the fragmented distribution of *M. caurina,* found along the Pacific coast (CA, OR, WA), mountaintops of the American Southwest (NM, CO, UT) and northward into Montana and Idaho, and four islands within the putative refugial archipelagos of the NPC: Graham, Moresby, Kuiu, and Admiralty islands.

In addition to two natural hybrid zones, a series of intentional wildlife translocations in the mid-1900s introduced *M. americana*to multiple NPC islands without prior knowledge of the native marten species in the region (Powell *et al.* 2012). While these introductions may complicate the interpretation of genomic signals from this region, they also provide a framework for interpreting the consequence of natural versus anthropogenically-mediated gene flow on the evolution and persistence of species. Accidental introductions (Fenichel *et al.* 2008; Weber *et al.* 2017) or intentional, motivated by economics (McNeely 2001; Fenichel *et al.* 2008; Powell *et al.* 2012), public safety (Massei *et al.* 2010) or conservation (Powell *et al.* 2012), are increasingly common and can result in unanticipated consequences, including hybridization with native species (Todesco *et al.* 2016) and exchange of parasites (Prenter et al. 2004) with unknown evolutionary outcomes. Genetic management techniques, including genetic rescue (Whiteley *et al.*2015) or gene tweaking (Thomas *et al.* 2013), are increasingly proposed as viable mechanisms to boost diversity in small inbred populations. Application of these techniques can be informed through investigations of existing hybrid zones and historical wildlife translocations which can cumulatively inform a predictive framework for anticipating the evolutionary consequences of genetic exchange. In martens, hybridization may disproportionately impact Pacific martens through genetic dilution from outbreeding (Colella *et al.* 2018a). Low genetic variation in certain insular populations of *M. caurina* (Stone *et al.* 2002; Small *et al.* 2003) has led to further concerns over their persistence, which may be exacerbated by ongoing harvest of forests and extraction of minerals on those islands (Durbin 1999; USDA 2018).

We use whole-genome resequencing data to refine our understanding of the biogeographic history of NPC martens and place these results within the context of the Coastal Refugium Hypothesis (CRH; Heusser 1989; Scudder & Gessler 1989; Heaton *et al.* 1996; Demboski *et al.* 1999; Sawyer *et al.* 2019). With increased molecular resolution, we explore the evolutionary consequences of introgression in New World *Martes* to further inform natural resource management initiatives. We examine the distribution of geographic variation and estimate the timing and directionality of introgression to assess the role of natural hybridization, historic wildlife translocations, natural colonization events, and glacial cycling in shaping the evolution of New World martens.

MATERIALS AND METHODS

Genome sequencing, assembly, and post-processing

We generated 11 whole-genome sequences representing both New World marten species, including individuals collected in both known hybrid zones (Kuiu [KUI] and the northern Rocky Mountains [MTX]) and multiple translocated islands (Prince of Wales Island, POW; Chichagof Island, CHI), with an Old World sable (*Martes zibellina*) included as an outgroup (Table 1). Sequences were generated on an Illumina HiSeq X through the Beijing Genomics Institute (BGI Americas, Philadelphia, PA, USA) and NextSeq 500 through the Molecular Biology Facility at the University of New Mexico. Sampling was based on previous genetic (Dawson *et al.* 2017; Colella *et al.* 2018a) and morphological analyses (Colella *et al.* 2018b) that helped define species limits and refine hybrid zone locations through the identification of mixed mitochondrial and nuclear haplotypes. Subsamples of liver tissue were loaned from the University of New Mexico's Museum of Southwestern Biology (MSB) and the Burke Museum at the University of Washington (UWBM). DNA extractions followed a DNeasy Blood and Tissue Kit (Qiagen, Venlo, The Netherlands) protocol. Our assembly pipeline followed Colella *et al.* (2018c). Read quality was examined using FastQC (Andrews 2010) and adapter sequences and sex chromosomes removed by excluding those scaffolds from the reference (Trimmomatic v0.33; Bolger *et al.* 2014). The Burrows-Wheeler aligner (BWA, Li & Durbin 2010) was used to map reads to the domestic ferret genome (*Mustela putorius furo* ; Peng *et al.* 2014) and an additional BWA iteration extracted mitochondrial genomes using the same reference. Final depth of coverage ranged from 19 to 30X (Table 1). PCR duplicates were removed using Picard v1.9 (MarkDuplicates; http://broadinstitute.github.io/picard/) and nuclear and mitochondrial consensus sequences called using SAMtools (mpileup; Li *et al.* 2009). Single nucleotide polymorphisms (SNPs) were called with the Genomic Analysis Toolkit (GATK, Haplotypecaller; McKenna *et al.* 2010) for all North American marten and again against the *M. zibellina* outgroup. SNPs were filtered (Supplemental Information1) by minimum depth (minDP = 2, set to 1/3$^{\mathrm{rd}}$ the coverage of our lowest coverage sample, as recommended for PSMC analyses; Li & Durbin 2011), genotype quality (minGQ = 30), minimum minor allele frequency (MAF = 0.1), and scaffold size (1Mb). Private alleles and indels were removed using VCFtools (Danecek *et al.* 2011). A MAF of 0.1 removed singletons (e.g., individual-specific, rare mutations), which are not informative about allelic overlap among populations, to reduced potential sequencing errors more common in lower coverage genomes. Format conversions (vcf, ped, bed) were conducted in PLINK (Purcell *et al.* 2007). Missing data were removed (–max-missing, VCFtools) based on analysis specifications. Variants were spaced (1 per 100bp window) to account for linkage disequilibrium and sorted into 46 'pseudo-chromosomes' to enable the application of human-specific analyses to a non-model system with only 38 chromosomes using custom python scripts available online at https://github.com/jpcolella/.

Assessing genomic differentiation

We generated maximum-likelihood phylogenies for complete mitochondrial genomes and autosomal SNPs, with and without the inclusion of an outgroup, using RAxML (GTRCAT model, 10,000 bootstrap replicates, random starting seed; Stamatakis 2014). It should be noted that phylogeny inference using highly variable data (e.g., SNPs) can induce acquisition bias resulting in longer branch lengths (Leaché *et al.* 2015). Phylogenies were visualized in FigTree v1.2.2 (http://tree.bio.ed.ac.uk/software/figtree/).

Principal component analyses (PCA) were run and visualized using the SNPrelate (Zheng *et al.* 2012) R v3.3.4 library (R Development Core Team 2008). Diversity statistics (F_{IS} , $\mathrm{F_{ST}}$, $\mathrm{F_S}$ or relatedness2 statistics, nucleotide diversity (π), and Tajima's D) were calculated in VCFtools (Commands: –het, –weir-fst-pop, –relatedness2, –window-pi, –TajimaD, respectively), with π and D calculated in 100 bp window intervals. *F2* statistics were generated from the compute_moment_stats and compute_most_additive_trees functions in

MixMapper (Lipson *et al.* 2013) with 1000 bootstraps and SNP blocks of 100 (1 per 100 bp) in MatLab 2018 (The MathWorks, Inc., Natick, Massachusetts, USA).

Introgression analyses

For ADMIXTURE (Alexander *et al.* 2009) analyses, sites with >80% missing data were removed. The greatest delta (Δ) in cross-validation (cv) score identified the most appropriate number of populations (K) by iteratively leaving a sample out and reexamining the partitioning of genetic structure among the remaining samples. ADMIXTURE results were visualized in R v3.3.4. Populations identified by ADMIXTURE were used in *F*- statistics.

F -statistics were run in AdmixTools (Patterson *et al.*2012) using *M. zibellina* as an outgroup. *F3*- statistics [Target: Source-1, Source-2] explicitly test for admixture (3PopTest in AdmixTools) and considered all permutations where Source-1, Source-2, and the Target samples came from different populations. While a significantly negative *F3* score (Z < 5) denotes admixture in the Target sample, a positive *F3* value does not necessarily indicate the absence of admixture (Peter 2016). To determine the generational status (e.g., F1, F2, B1, B2, etc.) of each identified hybrid individual, we use R code available from Lavretsky *et al.*(2016) to simulate multi-generational hybrids based on unadmixed*M. americana* ('POP1') and *M. caurina* ('POP2'). We contrasted admixture proportions of empirical hybrids against proportions output for simulated multi-generational hybrids to estimate generational status. Last, Treemix (Pickrell & Pritchard 2012) was used to infer historical relationships among populations with 2, 3 or 4 mixture events.

To characterize the backcrossing history of each hybrid sample, we used*F4*- statistics, similar to D-statistics or ABBA/BABA (Kulathinal*et al.* 2009; Green *et al.* 2010; Durand *et al.*2011), in AdmixTools with block-jackknifing accommodating non-independence between loci. Although *F* -statistics alone cannot deduce the direction of gene flow in a system, admixture graph fitting can test whether a proposed evolutionary model fits the data well (Lipson *et al.* 2013; Martin *et al.* 2015). AdmixtureGraph (Mailund *et al.* 2016) iteratively fit hybrid individuals into two non-admixed tree topologies (RAxML topology and the RAxML topology collapsed into K = 6 populations; Supplemental Information 2-3) by estimating the minimal error placement from *F4* results. We tested all *F4* population permutations excluding hybrids identified through *F3*- statistics. We then tested hybrids against individuals from 'pure' populations (e.g., *F4* (Outgroup, Hybrid; continental*americana* , insular *caurina*)) to decipher the backcrossing histories of hybrid samples and characterize patterns of gene flow across populations. *F4* -statistics [W, X ; Y, Z] are negative (Z-score [?] -5) if there is more allelic overlap between X and Y than between X and Z, and positive (Z score [?] 5) if there has been more recent allele sharing between X and Z than between X and Y. To estimate the timing of introgressive events, we converted drift unit branch lengths (*D*) output from MixMapper to absolute time (years) using the formula *D [?]* 1- $e^{-t/2Ne}$ (solved for *t*generations; Puckett *et al.* 2015) and a generation time of 5 years (Buskirk *et al.* 2012). Small sample sizes and the absence of a *Martes* linkage map prevents linkage disequilibrium-based estimates of N_e and more refined dating of admixture events.

Historical demography

PSMC (Li & Durbin 2011) was used on consensus genomic sequence data to characterize historical demography by examining heterozygosity densities in 100bp sliding windows across the genome. PSMC was run twice for each individual, once utilizing all mapped sequence data and again on data down-sampled to 20X coverage (near the lowest coverage sample) using the DownsampleSam tool (Picard). Results were scaled by a general mammalian mutation rate (2.2 x 10ˆ-9 per base pair per year; Kumar & Subramanian 2002) and marten generation time (5 years), resulting in distributions of effective population size (N_e) through time. One hundred PSMC bootstrap replicates were performed and plotted for both full-coverage and down-sampled data to confirm consistent distributional shapes and enable comparison across individuals, as PSMC is sensitive to variation in coverage depth (ideal coverage >18X; Nadachowska-Brzyska *et al.* 2016).

RESULTS

Genomic differentiation

Mitochondrial and nuclear phylogenies (Fig. 2d) show strong support for two reciprocally monophyletic species: *M. americana* and *M. caurina.* Both trees also show support for an insular *M. caurina*clade (QCI, ADM). The placement of the two continental *M. caurina*(COL, PNW) differed in the two phylogenies, but they were strongly supported as distinct from the insular *M. caurina* , suggesting substantial genomic divergence between continental and insular *M. caurina* . Two individuals (KUI, MTX) exhibited cytonuclear discordance. PCA results (Fig. 2a-c) are consistent with our nuclear phylogeny, demonstrating substantial divergence not only between New World*Martes* species (PC1 = 46%; Fig. 2b), but also between insular and continental populations of *M. caurina* (PC1 = 39%; Fig. 2c). Within *M. caurina,* PC2 also separates mainland *M. caurina*populations (COL, PNW) and accounts for a notable 34% of the variation. PCA plots (Fig. 2a-c) highlight the intermediacy of putative hybrid individuals (KUI, MTX) and also their distinction from each other.

The inbreeding coefficient, F_{IS}, is highest for insular*M. caurina* : 0.89 for ADM and 0.77 for QCI (Supplemental Information 4). Mainland *M. caurina* also exhibit high F_{IS}, 0.65 for PNW and 0.62 for COL, followed by populations of *M. americana.* CHI has the lowest F_{IS} of all *M. americana* populations and the 2 putative hybrid individuals have an F_{IS} of essentially 0. F_{ST} was 0.438 between pooled *M. americana* and pooled *M. caurina* . Contrasting pooled *M. americana* against pooled (insular and mainland) populations of *M. caurina* found similar, high F_{ST} estimates (mean = 0.44-0.45, weighted mean = 0.68-0.70) with only moderate F_{ST} estimates between insular and continental populations of *M. caurina* (mean = 0.01, weighted mean = 0.11; Supplemental Information5).*F2*- statistics (Supplemental Information 6) and F_S(local inbreeding or relatedness2, Supplemental Information 7) demonstrate similar patterns of genetic differentiation between individuals and species, highlighting divergence within *M. caurina* . Nucleotide diversity (π) was similar across species, but analysis of insular and mainland *M. caurina* populations separately produced elevated π estimates, with the highest values for insular *M. caurina* (Supplemental Information 8). Insular *M. caurina* also exhibited the highest positive *D* value, with mainland *M. caurina* exhibiting only negative and the lowest median *D* value (Supplemental Information 8).

Tests for introgression

The most supported ADMIXTURE result parsed six populations (K = 6; Fig. 3a): two within each marten species and two putative hybrids, identified as independent populations (KUI, MTX). Although K6 exhibited the greatest Δcv (Supplemental Information 9), we also examined alternative values of K (Lawson *et al.* 2018; Supplemental Information 10) to thoroughly characterize genetic structure in North American*Martes* (Janes et al. 2017). The K2 model splits *M. caurina*from *M. americana* , while K3 through K5 models identify additional intraspecific structure. Insular and continental *M. caurina*populations were separated under K4 and K6 models, while K7 distinguished each *M. caurina* sample as an independent population (Supplementary Information 10-11). Under all values of K, only two individuals were identified as admixed: KUI and MTX. Negative*F3* -statistics (Table 2) also consistently identified these samples as hybrids between *M. americana* and *M. caurina* , while all other *F3* results were positive or insignificant (Supplemental Information 12-13). Consistent with geography and a K3 ADMIXTURE model, *F3* -statistics, among populations identified by K6, found the KUI hybrid to be the result of mixture between the insular populations of each species, while the continental hybrid appears to be a combination of the continental populations of each species (Table 2). Under K2 through K4 models, hybrid samples contain near 50% genetic proportions corresponding to each species, suggesting that these individuals may represent F1 hybrids or early generational backcrosses (Supplemental Information 11). Consistent with these results, hybrid-class simulations most support the KUI hybrid (empirical admixture proportions: 55% *M. americana* , 45% *M. caurina*) as an F1 or 48-54% assignment to each species. Empirical admixture proportions for the MTX hybrid (60% *M. americana* , 40% *M. caurina*) fall intermediate to simulated admixture proportions for an F1 hybrid and a single generation backcrossed with *M. americana*(73-76% assignment to the backcrossed species, 22-26% assignment to the other parental species). Results were identical for simulations on all *M. americana* and *M. caurina* and for insular and continental populations of each species, when run separately, indicating that both hybrids represent early generational stage crosses, with MTX potentially the result of a complex backcrossing history between both species. R simulations based on Lavretsky *et al.* (2016) demonstrate the loss of a signature of introgression after four generations of backcrossing into either species (Supplemental Information 14).

Consistent with those backcross estimates, Treemix phylogeny estimations rooted on *M. zibellina* placed both hybrids as separate sister lineages to the *M. americana* clade and identified the first most likely gene flow event between MTX and an *M. caurina* ancestor, followed by a migration event between KUI and *M. caurina*(Supplemental Information 15). Interestingly, the third most likely migration event was estimated between the outgroup *M. zibellina*and the KUI hybrid.

F4 statistics show the directionality and intensity of backcrossing is similar across the two hybrids (Supplemental Information 16-17). KUI shares more genetic overlap with insular *M. caurina*compared to the continental clade and MTX has similar proportions of insular and continental *M. caurina* alleles (Supplemental Information 16-17). For tests across all individuals, AdmixtureGraph identified KUI as a mix between CHI and POW (minimal error = 0) and MTX as a cross between ADM and the ancestor of continental *M. americana* (Supplemental Information 18-20). Interestingly, when considering only the populations identified by ADMIXTURE (e.g., pooled continental and insular populations of each species), AdmixtureGraph identified both hybrids as a mix between the continental populations of each species. However, notably there were multiple clustered minimal-error estimates (Supplemental Information 20) that we anticipate will be resolved with increased sample sizes. MixMapper found that the timing of admixture did not differ from the present (0) for either hybrid (Supplemental Information 21).

Demographic histories

PSMC identified three major demographic histories: distinct insular and continental histories within *M. caurina* and a single general demographic history within *M. americana* with greater variance relative to either *M. caurina* distribution (Fig. 3b). All*M. caurina* distributions are lower (N_e) than*M. americana* , with insular *M. caurina* exhibiting the lowest N_e of all groups examined. Of the populations examined, CHI has the highest historic peak in N_e, while MAK had a larger effective population size recently.

DISCUSSION

Ice-cover during the last glacial maximum (LGM; 26.5-19 kya) displaced most high-latitude species, forcing them into ice-free glacial refugia (Hultén 1937; Hewitt 2000, 2003; Bennett & Provan 2008; Clark *et al.* 2009). The largest documented LGM macro-refugium in North America was located south of the Laurentide and Cordilleran ice sheets in the contiguous U.S., evidenced by fossil data, climatic modeling, and phylogeographic signatures (Graham *et al.* 1996; Jackson *et al.* 2000; Holliday *et al.* 2002), although the extent and complexity of this refugium requires further resolution. Additional LGM refugia are hypothesized in Beringia, the area of exposed continental shelf connecting Alaska to eastern Siberia (Hultén 1937; Abbott & Brochmann 2003; Hope *et al.* 2013), and multiple smaller micro-refugia are proposed among the archipelagos of the North Pacific Coast (Heaton *et al.* 1996; Hewitt 2000; Carrara et al. 2003, 2007; Lacourse *et al.* 2003; Mathewes & Clague 2017), although the duration and possible cyclic recurrence of these refugia remains uncertain. Coastal refugia within the Alexander and Haida Gwaii archipelagos could explain high levels of endemism in this region (Cook & MacDonald 2001; Dawson *et al.* 2007) and clustered phylogenetic breaks separating insular and continental populations (Colella *et al.* 2018c; Sawyer *et al.* 2019) hypothesized to result from post-glacial refugial population expansion limited by secondary contact with closely-related, previously-allopatric taxa (Hewitt 2000). Paleoendemic refugial persistence also explains the rapid reestablishment of complex biotic communities so quickly following deglaciation (Lesnek et al. 2018; Ager 2019).

Significant geographic structure within Pacific martens is consistent with the Coastal Refugium Hypothesis (CRH) (Fig. 3, 4), suggesting the persistence of at least one insular *M. caurina* population in a North Pacific coastal refugium potentially located along the western fringe of the Alexander or Haida Gwaii archipelagos. The two clades within *M. caurina* are genetically distinct, parsing an insular and continental lineage, despite mitochondrial nesting (Fig. 2d; Supplemental Information 5). The two insular and continental *M. caurina* clades are geographically discontinuous (Fig. 1) and estimated to have diverged almost 1 million years ago (Fig. 3b), although PSMC date estimates are highly sensitive to scaling (mutation rate and generation time). Divergence predating the most recent interglacial suggests that insular *M. caurina* may have diverged from continental populations over multiple glacial cycles, perhaps initially in a coastal refugium and then subsequently on one or more NPC islands. Our genomic results initially contradict the fossil record, which shows a scarcity of fossils on POW Island during the LGM (~20-15 kya, Lesnek *et al.* 2018) and documents martens appearing on POW during the late Pleistocene (>14 kya) and early Holocene (9-14 kya, Heaton & Grady 2003; Pauli et al. 2015). Absence of martens and other mammals in the Southeast Alaskan fossil record during the LGM may reflect sampling bias, as most dated fossil materials from the region were collected from the Shuká Káa cave at the northern end of POW. Insular *M. caurina* have not been documented on POW and this site was likely ice-covered at the peak of the LGM (Lesnek *et al.* 2018). Even so, a number of meso-carnivore teeth from Shuká Káa cave morphologically identified as mink (*Mustela vison*) may instead mark the early presence of insular*M. caurina* (Heaton & Grady 2003), as these species have similar tooth morphology. Similar to misidentifications of Pleistocene coastal black bear (*Ursus americanus*) fossils from POW that were originally listed as brown bears (*Ursus arctos*) due to size differences over evolutionary timescales (*Lindqvist pers. obs.),* insular *M. caurina* are physically larger than both*M. americana* and continental *M. caurina* (Colella et al. 2018b) which may confound taxonomic assignment of dentition. The persistence of diverse communities of large terrestrial mammals, including caribou, bears, and foxes, evident in the fossil record both pre- and post-LGM (Lesnek *et al.* 2018), points to a higher potential for local refugial persistence through the LGM over the recolonization of these outer islands from mainland sources since the Holocene (Ager 2019).

The viability of a coastal migration route for human colonization of the Americas hinges on our understanding of glacial extent and biotic community composition along the NPC during the late Pleistocene. Geological investigations of southeast Alaska have produced mixed results. Bathymetry (Carrara et al. 2003, 2007) and palynology (Ager 2019) support the persistence of coastal refugia, while cosmogenic exposure dating has shed doubt on hypothesized refugial locations (Lesnek et al. 2018). Multiple signatures of refugial persistence across taxa (Foster 1965; Heaton et al. 1996; Hewitt 2000, 2003; Weckworth et al. 2005; Colella *et al.* 2018c; Sawyer *et al.* 2019) is detailing increasingly complex refugial communities along the coast.

For marten, the laterally dilated cranial shape of insular *M. caurina* hints at a dietary shift towards the consumption of marine prey items (Colella et al. 2018b), also documented in stomach contents (Giannico & Nagorsen 1989) and also reflected in insular wolves of the NPC (Darimont *et al.* 2009; Muñoz-Fuentes *et al.* 2010). Martens rely on deep persistent snow and complex forest structure (Proulx 1997; Pauli et al. 2013; Manlick et al. 2017; Martin et al. 2019) for predator avoidance, thermal management, and efficient locomotion, suggesting that refugial ecosystems would have contained forest community assemblages. Access to both marine and terrestrial prey items and timber resources along a NPC migration route, would have enhanced human survivorship during an early pulse of human migration into the Americas via the Pacific coast (Fladmark 1979; Dixon 1993).

The insular-continental biogeographic and phylogenetic break within*M. caurina* is largely consistent with signatures from numerous other NPC paleoendemics (bears, Heaton *et al.* 1996; deer, Latch*et al.* 2009; ermine, Colella *et al.* 2018c; shrews, Demboski & Cook 2001; deer mice, Sawyer *et al.* 2019) and also evident in the few associated parasites examined to date (*Soboliphyme baturini* , Koehler *et al.* 2007, 2009; Hoberg*et al.* 2012). Disparate distributions of insular lineages across these heterogeneous archipelagos suggests that the geographic pattern and duration of refugial isolation may vary across climate cycles, depending on the ecological plasticity and dispersal abilities of incumbent species. Insular 'ABC' brown bears (*Ursus arctos,*Heaton *et al.* 1996), for example, are currently geographically restricted to the three northern most islands of the Alexander Archipelago (Admiralty, Baranof, Chichagof), while the insular black bear lineage (Byun *et al.* 1997) has a more southerly distribution encompassing southern Alexander Archipelago islands, the Haida Gwaii Archipelago, Vancouver Island, and coastal British Columbia. Under the assumption of niche conservatism, this phylogeographic pattern suggests a cooler, northern refugium within the Alexander Archipelago and a slightly warmer, perhaps more heavily vegetated refugial ecosystem to the south, either in the southern Alexander Archipelago or Haida Gwaii. Early paleoclimatic models for NPC refugia hypothesized these areas to be primarily tundra and unable to support forest-associated taxa such as black bears and martens (Hansen & Engstrom 1996; Ager 2007). More recently however, palynological investigations and radiocarbon dating of postglacial peat and sediment cores indicate that coastal forests similar to today's forests existed in the Alexander Archipelago during the last interglacial (Ager 2019). Rapid colonization of the western-most islands by pine trees (*Picea*) immediately following glacial recession (~17 kya) hints at the potential refugial persistence of coniferous forests (Lesnek et al. 2018; Ager 2019) and parallels our hypothesis that refugial persistence of insular*M. caurina* is more likely than post-glacial recolonization.

Comparative demography also identified at least three major evolutionary trajectories: *M. americana,* continental *M. caurina* (Fig. 3b) and insular *M. caurina,* consistent with the CRH. *Martes americana* distributions of effective population size are overall higher than those of *M. caurina* clades, consistent with the contiguous contemporary range of this species and historical divergence in and subsequent expansion from a single, large eastern refugium (Stone*et al.* 2002). Within *M. americana* , Chichagof Island exhibits the highest effective population size (N_e), followed by central Alaska (Fig. 3b). Although high $N_e$is surprising for an insular population, Chichagof Island received iterative translocations of *M. americana* in the mid-1900s from multiple source populations, including four other islands in southeast Alaska (Baranof, Wrangell, Mitkof [Petersburg], and Revillagigedo [Ketchikan] islands) and one distant continental locality (Polly Creek, in central AK). These introductions may inflate population size estimates as a consequence of outbreeding (Paul 2009) and make the historical distribution of N_e for this individual resemble that of its source populations (e.g., MAK). In contrast, Prince of Wales Island (POW) received introductions from only two proximate sources: Revillagigedo and Mitkof islands (Burris & McKnight 1973). Revillagigedo Island shows a similar demographic history to POW, suggesting those translocations resulted in successful establishment (Elkins & Nelson 1954). Although our results hint at insular-continental structure within *M. americana* (Fig. 3), this signal is muddled by historical wildlife translocations and remains unresolved from a nuclear perspective (Fig. 2). Relative to *M. americana* , both continental and insular *M. caurina* have persistently smaller effective population sizes.

Among continental species, a common response to rising temperatures is the upward distributional shift in elevation (or latitude) to retain suitable environmental conditions (Hampe & Jump 2011). The fragmented contemporary distribution of continental *M. caurina* populations (U.S. west coast, Pacific Northwest forests, mountaintops of the American Southwest; Fig. 1) is consistent with the retention of a cooler paleoclimatic niche for species experiencing increasing fragmentation under current warming conditions (Brown 1971; Anderson *et al.*2000; Parmesan 2006; Hampe & Jump 2011; Meng *et al.* 2019). Relative to all continental taxa, insular *M. caurina* show a significantly depressed effective population size through time, with the highest overall inbreeding coefficients. Although likely a consequence of island life, small effective population sizes and high levels of inbreeding place insular martens at an elevated risk of extinction (Frankham 1998; Rybicki & Hanski 2013).

Refugial divergence along the NPC also explains the disjunct contemporary distribution of *M. caurina* (Fig. 1). Along the NPC,*M. caurina* inhabits at least three islands; however, Admiralty Island in Southeast Alaska is more than 300 km north of the two insular Canadian populations. Although geographic disjunction across three islands is substantial, the genetic similarly of these island populations points to historical divergence in a single coastal refugium and a potentially more widespread historical distribution of insular*M. caurina* throughout NPC islands. Higher density sampling across the NPC will be necessary to refine the geographic limits of insular and continental *M. caurina* clades. Refugial divergence of insular*M. caurina* is further supported by the persistence of the insular lineage in the Kuiu Island hybrid zone (Dawson *et al.* 2017), ~20km south of Admiralty Island, relictual signatures of*M. caurina* on POW (Pauli *et al.* 2015), and associated nematodes (*Soboliphyme baturini*) on Chichagof Island (Koehler*et al.* 2007, 2009; Hoberg *et al.* 2012). Chichagof martens harbor distinctive nematodes that are phylogenetically close to *S. baturini* found in other populations of *M. caurina* , suggesting*M. caurina* or a 'ghost' marten lineage may persist or have persisted on this island until relatively recently (Koehler *et al.* 2007, 2009; Hoberg *et al.* 2012). Similarly, POW is hypothesized to have been colonized by multiple natural sources (Pauli*et al.* 2015) and iterative translocations of *M. americana*to this island were surprisingly successful considering as few as 10 martens (4 females) were introduced to the island (Paul 2009). In contrast, our genomic analyses did not find *M. caurina* alleles in either of the individuals sequenced from islands that received translocations of *M. americana* . Instead, we found each island to be genetically aligned with *M. americana* and their translocation source populations: Chichagof Island with central Alaska and POW with Revillagigedo Island (Fig. 2 and 3). Overall our results suggest that*M. caurina* were either not present on these islands prior to translocations or were recently replaced or swamped by introduced or invading *M. americana* . Interspecific competition, outbreeding, or the introduction of foreign pathogens among other variables may have impacted native *M. caurina* (Plein *et al.* 2016; Colella*et al.* 2018b; Northover *et al.* 2018). Ultimately, until additional hybrids are sequenced, our results discourage the translocation of American marten for the genetic rescue or restoration of coastal martens due to potential swamping and emphasize the importance of careful source population selection, as the NPC harbors significant cryptic diversity and complex evolutionary histories.

We detected a hybrid individual collected from each natural mixing zone: Kuiu Island Alaska and western Montana in the northern Rocky Mountains (Table 2; Fig. 2-4; Supplemental Information 9-16). Both hybrids were female, had *M. americana* mitochondrial haplotypes (Fig. 2d), and mixed nuclear ancestry, with the Montana hybrid containing continental*M. caurina* alleles and the Kuiu hybrid containing insular*M. caurina* alleles (Table 2; Supplemental Information 10-13, 16-17). Both admixed individuals were identified as early generational-stage hybrids (e.g., F1's or a single generation backcrossed with *M. americana* , Supplemental Information 11-12) with introgression occurring recently (Supplemental Information 21). Although sample sizes are small, the absence of late-generational hybrids is surprising, especially for the Montana zone which has persisted for many generations (Wright 1953). Detection of only early-generational hybrids is consistent with the presence of hybrid incompatibilities, where F1 hybrids experience a temporary elevation in fitness (heterosis) compared to later generational-stage hybrids (e.g., F2 and beyond) that may suffer outbreeding depression as a result of disrupted co-adapted gene complexes (Todesco *et al.* 2016). The disruption of co-adapted gene complexes or genes involved in local adaptation via introgression, and particularly loci involved in disease and pathogen resistance (Alibert *et al.* 1994), may pose a particular challenge to naïve insular taxa. This hypothesis warrants further genomic investigation with fine-scale sampling from within hybrid zones and translocated islands.

Differentiation between insular and continental *M. caurina* was suggested previously based on reduced-representation genetic approaches (Demboski *et al.* 1999, 2001; Stone *et al.* 2002; Small*et al.* 2003; Dawson *et al.* 2017), but the extent of divergence was unknown. A genomic pattern of refugial divergence may be more widespread than previously suspected and additional forest-associated taxa, that are not well represented in the fossil record, may have persisted in NPC refugia. Our results underscore the importance of reevaluating work previously based on one or a few genes, as genomic resolution continues to provide unexpected insight into the evolutionary complexities of coastal refugia (Miller et al. 2012; Colella et al. 2018c) and complex landscapes and holds great promise to unravel complexity across time.

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DATA ACCESSIBILITY

Raw reads will be made available through NCBI's SRA upon manuscript acceptance (Accession number: #####). Custom python scripts are available at: https://github.com/jpcolella.

AUTHOR CONTRIBUTIONS

All authors conceived the project. JAC designed the project. JPC conducted bioinformatic analyses and wrote the first draft of the manuscript with scientific and editorial input from TL, SLT, JAC, and CL. TL designed bioinformatics pipeline and provided computational support. JAC collected and provided tissue

samples through the Museum of Southwestern Biology. JAC and JPC funded genome sequencing. CL and JAC provided access to computational resources.

TABLES & FIGURES

Figure 1. Range maps for *M. americana* (green) and*M. caurina* (blue) based on IUCN distributions (iucnredlist.org) and mitochondrial haplotype distributions (Dawson *et al.* 2017; Colella *et al.* 2018b) with hybrid zones (Kuiu and Kupreanof island and western Montana into southern British Columbia) denoted by gray hashing. Genome samples are indicated with points and 3-letter locality abbreviations that correspond to Table 1. Genome samples are colored based on ADMIXTURE K6 assignment (Fig. 3a): insular*M. caurina* (QCI, ADM), continental *M. caurina* (COL, PNW), continental (MAK, CHI*) and insular *M. americana* (POW*, REV). * indicates an island population that was received *M. americana*translocations from a mainland source population. Two hybrid individuals (KUI, MTX) are shown in grey with an X.

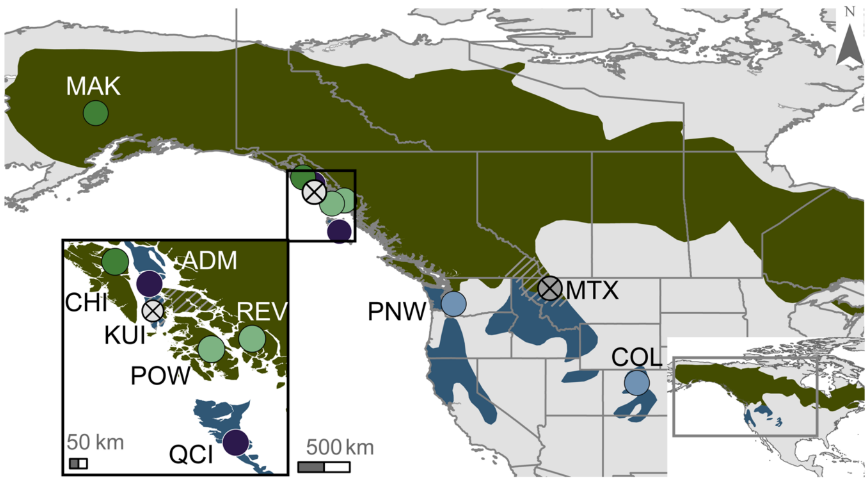


Figure 2. (a) Principal component analysis (PCA) of only*M. americana* samples; (b) All examined New World *Martes*samples, identifying 2 intermediate hybrids; and (c) PCA of *M. caurina* samples only, demonstrating significant differentiation between insular and continental populations (39% of variation, PC1), and also between distinct continental populations of *M. caurina* (34% of variation, PC2). (d) mirrored mitochondrial genome (mitogenome, left) and autosomal SNP (right) phylogenies, depicting 2 monophyletic marten species (*M. americana* green, *M. caurina* blue) and 2 unsorted, putative hybrids (MTX, KUI). Sample abbreviations are defined in Table 1.

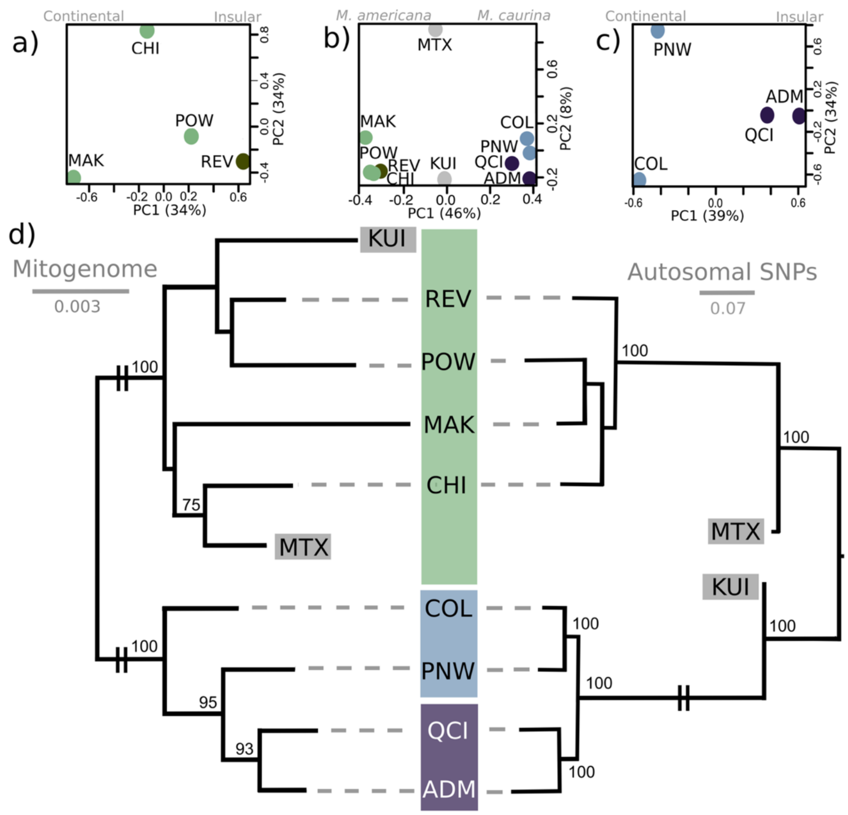


Figure 3. (a) Optimal ADMIXTURE population assignments (K6) and ancestry proportions for sampled New World *Martes* genomes, indicating 2 independent populations within each species (*M. caurina* blue and *M. americana* green) and two admixed individuals (KUI, MTX: gray; see also Supplemental Information 10). Specimen ID's correspond to abbreviations in Table 1; (b) PSMC distributions of historical demography for unadmixed samples scaled by a generation time of 5 years (g) and general mammalian mutation rate (mu = 2.2×10^{-9}) demonstrating 3 distinct demographic trajectories: one for continental (C) *M. caurina* , one for insular (Is.) *M. caurina* , and one for *M. americana* . Colors correspond to the admixture plot in (a).

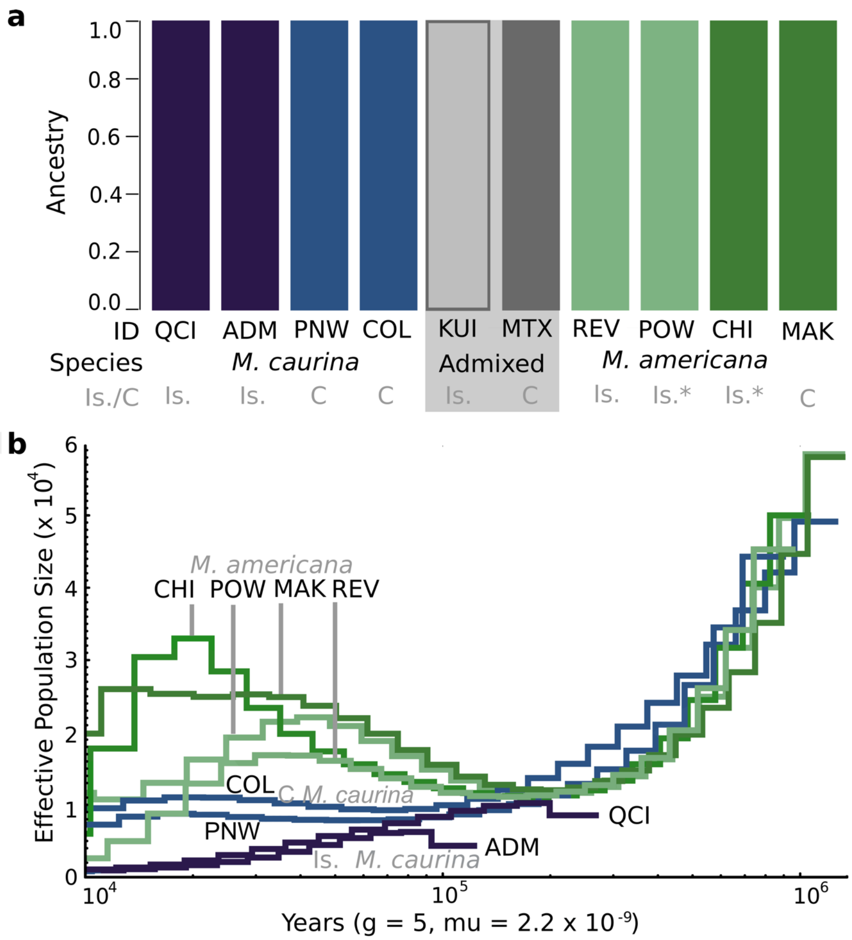
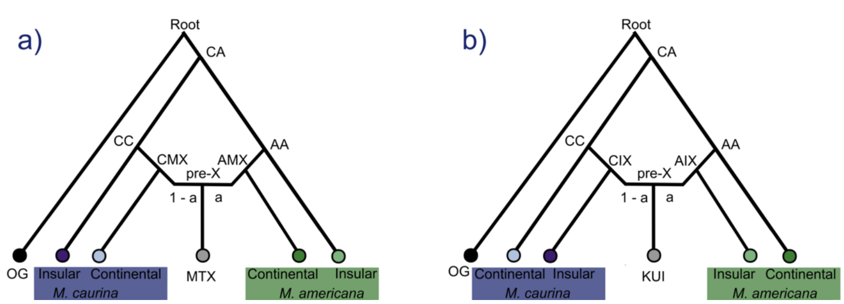


Figure 4. AdmixtureGraph results for each hybrid (KUI, MTX) based on *F4* -statistics of populations identified by ADMIXTURE (K = 6). Node abbreviations indicate: CA = coalescence between all *M. caurina* and *M. americana* , CC = coalescence between all *M. caurina* lineages, AA = coalescence between all *M. americana*lineages, CMX/CIX = coalescence between mainland/insular *M. caurina* and hybrids, AMX/AIX = coalescence between mainland/insular*M. americana* and hybrids. OG = outgroup (*Martes zibellina*).



TABLES

Table 1. Museum specimen catalog numbers (ID), collection localities, and corresponding abbreviations (Abbrev.), species assignment, genomic coverage, and year of collection. MSB = Museum of Southwestern Biology (MSB:Mamm), UWBM = University of Washington (UWBM:Mamm), Burke Museum, UAM =

University of Alaska Museum of the North (UAM:Mamm). IS = insular *M. caurina* clade, C = continental*M. caurina* clade

Museum ID	Abbrev.	Collection locality	Species	Coverage	Year Collected	Sequencing method
MSB197660	MAK	Central Alaska	*M. americana*	22.52	2005	NextSeq 500
MSB221158	POW	Prince of Wales Is.	*M. americana*	19.89	2004	HiSeq 10X
MSB34701	CHI	Chichagof Is.	*M. americana*	21.15	1994	NextSeq 500
MSB197227	REV	Revillagigedo Is.	*M. americana*	23.77	2003	HiSeq 10X
UWBM81779	PNW	Washington	*M. caurina* (C)	30.33	<2010	HiSeq 10X
MSB224027	COL	Colorado	*M. caurina* (C)	28.19	2008	NextSeq 500
MSB197762	ADM	Admiralty Is.	*M. caurina* (IS)	21.15	2004	NextSeq 500
MSB157350	QCI	Graham Is.	*M. caurina* (IS)	24.46	2005	HiSeq 10X
UAM69033	MTX	Montana	admixed	19.95	2004	NextSeq 500
UAM48762	KUI	Kuiu Is.	admixed	28.57	1997	HiSeq 10X
MSB192919	Mazi	Russia	*M. zibellina*	28.65	2005	HiSeq 10X

Table 2. Significantly (Z score [Z] > 5) negative F3 statistics for both hybrids examined and associated source populations, group according to ADMIXTURE K6 results (Is.*americana* = REV, POW; ML *americana* = CHI, MAK; Is.*caurina* = QCI, ADM; ML *caurina* = PNW, COL - where Is. indicated island and ML indicated mainland or continental localities). SE = standard error.

Target	Source2	Source2	F3	SE	Z	SNPs
KUI	Is. americana	Is. caurina	-0.341386	0.011619	-29.382	36782
KUI	ML *americana*	Is. *caurina*	-0.339249	0.01224	-27.716	37150
KUI	Is. *americana*	ML *caurina*	-0.32483	0.013937	-23.306	38129
KUI	ML *americana*	ML *caurina*	-0.322549	0.014368	-22.449	38495
MTX	ML americana	ML caurina	-0.304466	0.015845	-19.215	35448
MTX	ML *americana*	Is. *caurina*	-0.303897	0.015705	-19.35	34614
MTX	Is. *americana*	ML *caurina*	-0.298224	0.018026	-16.545	35550
MTX	Is. *americana*	Is. *caurina*	-0.297276	0.017801	-16.7	34681