Complete genomes of two extinct New Zealand passerines show responses to climate fluctuations but no evidence for genomic erosion prior to extinction

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Abstract
Direct human intervention, pre-human climate change (or a combination of both), as well as genetic effects, contribute to species extinctions. While many species from oceanic islands have gone extinct due to direct human impacts, the effects of pre-human climate change and early human settlement on the genomic diversity of insular species and the role that loss of genomic diversity played in their extinctions remains largely unexplored. To address this question, we sequenced whole genomes of two extinct New Zealand passerines, the huia (*Heteralocha acutirostris*) and South Island kōkako (*Callaeas cinereus*). Both species showed similar demographic trajectories throughout the Pleistocene. However, the South Island kōkako continued to decline after the last glaciation, while the huia experienced a slight recovery. Moreover, there was no indication of inbreeding resulting from recent mating among closely-related individuals in either species. This latter result indicates that population fragmentation associated with forest clearing by Maōri may not have been strong enough to lead to an increase in inbreeding and exposure to genomic erosion. While genomic erosion may not have directly contributed to their extinctions, further habitat fragmentation and the introduction of mammalian predators by Europeans may have been an important driver of extinction in huia and South Island kōkako.

Keywords: genetic erosion, glaciations, decline, extinction, ecological speciation
**Introduction**

Species declines and extinctions are complex and multifactorial [1,2]. Two paradigms have been proposed in conservation biology [3]. The first paradigm focuses on how extrinsic factors, such as climate fluctuations or human activities, contribute to population decline and extinction. While the role of humans in the extinction of species over the past 500 years is well-recognised in what is now referred to as the ‘sixth extinction wave’ [4], climate has also been shown to be a major driver of species demography and species extinctions [5,6]. However, the relative impact of human activities and climate on biodiversity are still intensely debated and these impacts may well vary among species [1,2].

While extrinsic factors are the primary cause of population decline, they will often expose declining population to additional threats that are intrinsic to small populations. This is why a second paradigm, which instead focuses on intrinsic processes such as demographic and genetic effects, is also central to conservation biology [3]. The role that detrimental genetic effects play in the long-term persistence of populations is now well-recognised [7,8]. Such detrimental effects can be referred to as genetic erosion, which reduces species viability through drift, inbreeding and increase in genetic load [7,9]. In fact, recent empirical data on extinct woolly mammoths (*Mammuthus primigenius*; [10,11]), endangered gorilla (*Gorilla beringei* sp.; [12]) and crested ibis (*Nipponia nippon*; [13]) have shown that severe population declines expose populations to genetic erosion. Moreover, species that have experienced long-term, pre-human decline in effective population size ($N_e$) may be more vulnerable to human-induced declines and to genetic erosion as was suggested for the critically endangered Sumatran rhinoceros (*Dicerorhinus sumatrensis*; [14]). Similarly, several avian species on the IUCN Red List of Threatened Species have been subject to long-term, pre-human population reductions in effective population size ($N_e$) [15], further highlighting the link between long-term population decline and higher exposure to genetic erosion.

Species from oceanic islands recently colonised by humans are particularly vulnerable to human disturbance due to their small census size and effective population size ($N_e$) and their limited ability to alter their range in response to human-induced pressures [9,16]. Moreover, consistent with theory on the genetics of small populations, island populations have experienced larger extinction rates compared to mainland species [17]. In fact, even though islands represent only 5.3% of the surface of the earth, they have hosted 75% of the known vertebrate extinctions.
over the past 500 years [18] due to both habitat modification, over-hunting and the introduction of non-native mammalian predators [19].

As a case in point, New Zealand has experienced two major decline and extinction events of its endemic fauna in association with Polynesian/Maōri (c.1360 CE; [20]) and European settlement (c. 1800 CE; [21]). A large number of these population declines and extinctions have been attributed to direct human impact via hunting (e.g. moa, [22]; Megadyptes waitaha [23]). Moreover, because New Zealand avian species evolved in absence of mammalian predators and because a large proportion of endemics are flightless, the accidental or deliberate introduction of mammals has been an important driver for species decline [21]. For many avian species, the colonisation of New Zealand from a small number of founders, their persistence in a confined geographic area as well as pre-human climate fluctuations may have reduced the genetic diversity of species well before human settlement (e.g. kea, Nestor notabilis; [15,24]). It is thus likely that species with historically low genetic diversity, such as New Zealand avian species, may have been even more vulnerable to genomic erosion following the human-induced declines over the last 800 years [25]. Yet, to date, the effect of pre-human climate fluctuations and of Polynesian/Maōri settlement on the genome-wide diversity of insular avian species in New Zealand remains largely unexplored. Understanding these effects would allow us to determine whether genomic erosion contributed to their extinction.

Here, we examine the long-term response to climate change and the recent effects of human settlement on the genome-wide diversity of two extinct New Zealand forest passerines from the Callaeidae family or New Zealand wattlebirds [26,27], the huia (Heteralocha acutirostris) and South Island kōkako (Callaeas cinereus). Huia were common throughout the North island but went extinct in 1907, whereas South Island kōkako were only found in the South Island and was officially declared extinct in the 1960s [28]. Using demographic reconstructions, we show that these species had similar responses to habitat change during the last glaciation. Moreover, inbreeding coefficients were no consistent with genomic erosion close to the time of extinction. Our data thus suggest that further habitat fragmentation and the introduction of mammalian predators may have been the main driver of the extinction of these two species.

Materials and Methods

Sample collection, DNA extraction, library preparation and sequencing
We extracted DNA from historical toepads for one Huia (*Heteralocha acutirostris*) and one South Island kōkako (*Callaeas cinereus*) collected in 1886 and 1849, respectively (Table S1). We then built deep-sequencing libraries (see supplementary material) following Meyer and Kircher [29]. In order to increase library complexity, we performed six independent PCR amplifications per bird. After pooling of libraries in equimolar ratios, we sequenced each bird library on a single HiseqX lane. All laboratory procedures were conducted in a dedicated ancient/historical DNA lab, and we took appropriate precautions to minimize the risk of contamination in historical samples [30].

**Bioinformatics data processing**

After trimming adapters, we mapped the raw data for the two historical specimens to a *de novo* assembly for the North Island kōkako (*Callaeas wilsoni*; https://b10k.genomics.cn/) using BWA 0.7.13 aln [31]. We then removed duplicates, realigned bam files around indels and filtered them for mapping quality (see supplementary material). We then called variants for each bird using bcftools mpileup (v. 1.3) [32], filtered them for base quality, depth and removed SNPs within 5 bp of indels. Finally, we masked repeats and CpG sites from bam and vcf files using BEDtools [33] (see supplementary material).

**Data analysis**

We first used the Pairwise Sequentially Markovian Coalescent (PSMC 0.6.5) [34] model to infer changes in the effective population sizes (*N*<sub>e</sub>) of huia and South Island kōkako over time. Secondly, we used mlRho v.2.7 [35] to estimate population mutation rate (∂θ), which approximates expected heterozygosity under the infinite sites model. Finally, we identified runs of homozygosity (ROH) and estimated individual inbreeding coefficients (*F*<sub>ROH</sub>) using the sliding-window approach implemented in PLINK [36] (see supplementary material).

**Results**

Demographic reconstruction using the PSMC based on 10× and 14× coverage genomes (Fig. S1, Table S1) and using various correction rates for low coverage showed broadly similar *N*<sub>e</sub> trajectories in huia and South Island kōkako (Fig. 2, S2-3). However, while huia showed a nearly stable *N*<sub>e</sub> between 1my and 100ky BP, South Island kōkako experienced a severe decline dating
back to ~400 ky BP. Both species experienced a 2- to 10-fold decline in \( N_e \) coinciding with the last glaciation some 60-70 ky BP (Fig. 2). Moreover, while the \( N_e \) of both species was estimated at ~4,000-5,000 at the end of the Last Glacial Maximum (LGM) some 15 ky BP, huia \( N_e \) seems to have increased slightly to ~8,000 after the LGM. Conversely, South Island kōkako seemed to have continued to decline to a \( N_e \) of ~2,000 (Fig. 2).

Both species showed similar levels of genome-wide heterozygosity, estimated at 0.94-1 SNPs per thousand base pairs (Table 1). While the inbreeding coefficient was higher in South Island kōkako (\( F_{ROH}=0.32 \)) compared to huia (\( F_{ROH}=0.19 \)), the majority of the ROH identified were < 1Mb in both species (Fig. 1).

**Discussion**

Using complete genomes, we examined the long-term response to climate change and tested the hypothesis that habitat modification associated with Maōri settlement impacted the genome-wide diversity of huia and South Island kōkako prior to their extinction.

Demographic reconstructions indicated very similar responses to glaciations with a reduction in \( N_e \) for huia and South Island kōkako shortly after the onset of the last glaciation and little to no recovery at the end of the Last Glacial Maximum (LGM) some 14-22 ky BP [37]. This overall pattern is very similar to another forest passerine, the rifleman (\( Acanthisitta chloris \), [15]). However, the rifleman had a much higher \( N_e \) of ~40,000 at the end of the LGM [15], compared to that of huia and South Island kōkako, respectively. While the signal of long-term population decline could indicate limited migration between subpopulations, this decline in \( N_e \) is consistent with a severe reduction in forest cover in the southern North Island and the South Island [38,39]. With the exception of extensive forest tracts mostly confined to the northern parts of the North Island and some smaller isolated forest patches in the South Island (Fig. 1; [52,55]), most of New Zealand’s vegetation was characterised by extensive grassland and shrublands at the LGM [37–39]. Being both forest species, Huia and South Island kōkako were thus most likely restricted to such forest refugia, as was the case for several other forest species [41–43]. Yet, it is unclear why both species had a similar \( N_e \) at the LGM while the forest refugium was smaller in the South Island compared to the North Island [38]. Moreover, it is surprising that South Island kōkako had a lower \( N_e \) compared to huia after the LGM while both species should have experienced a relatively similar population expansion.
An abundance of fossils from the early to late Holocene deposits of forest species (e.g., kaka, *N. meridionalis*; pigeon, *H. novaeseelandiae*; parakeets, *Cyanoramphus* spp; [44,45]) suggests that demographic expansion did occur as species tracked their habitat after the LGM [37–39]. However, open-habitat species like the alpine kea (*N. notabilis*) seem to have experienced a decline in *N_e* or at least lack of post-glacial demographic recovery, as their range became restricted to alpine areas [15,24]. Because both huia and South Island kōkako were forest dwellers, they should have also experienced population expansion after the LGM. In fact, *N_e* estimates of ~30-80,000 birds prior to human arrival in New Zealand based on rapidly evolving mitochondrial sequences suggest that post-glacial recovery could have occurred in both species [46]. However, because the number of recombination events is limited over the recent past and because of the lag time between demographic expansion and increase in *N_e*, PSMC lacks the power to detect recent population fluctuations [34,47]. Moreover, the reliability *N_e* estimates can be affected by coverage, proportion of missing data and the uncertainty about substitution rates (Fig. S2-3; [34,48]). These estimates should be thus interpreted with caution. Nevertheless, in spite of these limitations, the overall long-term decrease in *N_e* in both species is consistent with that of extant endangered species classified as endangered on the IUCN Red List of Threatened Species [15,49]. Conversely, the rifleman had a higher heterozygosity [50] and *N_e*, which is consistent with their least concern conservation status [49].

While the relatively important declines in *N_e* through time in both species could have made them more vulnerable to genomic erosion, inbreeding (*F_{ROH}* was low in both species and mostly comprised of fragments < 1Mb, indicating that the observed inbreeding was the result of shared ancestral relatedness and not of recent mating among related individuals [51]. While 40% of forest had been cleared by Maōri between the 13th and 19th century [52–54], our result suggests that habitat fragmentation prior to the 1850s may not have been severe enough to reduce gene flow among populations and did not increase inbreeding in huia and South Island kōkako populations. Because European settlement had just started at the time of sampling of these museum skins c. 1860-1880 [52], forest habitat may still have allowed large populations to thrive. In fact, previous results based on historical microsatellite data did not show evidence for population subdivision in huia [46].

Although our data does not show evidence for genetic erosion, future temporal comparison of historical genomes spanning the time of European settlement to the extinction of these species
(i.e. huia: 1907; South Island kōkako: mid 1960s) could indicate whether genetic erosion associated with human-induced bottlenecks contributed to their extinction [55]. This may be especially relevant to South Island kōkako, which went extinct in the 1960s. Assuming a generation time of six years [46,56], a period of 100 years corresponds to c. 17 kōkako generations, which may have been enough for small and fragmented populations to accumulate genetic load. For instance, a population decline dating back to 20 and 100 years ago for the endangered Grauer’s gorilla [12] and crested ibis [13], respectively, led to increases in inbreeding and genetic load. Moreover, numerous extant avian species in New Zealand have lost a large proportion of their historical genetic diversity and may also have accumulated genetic load, with severe consequences for their viability (e.g. kākāpō, *Strigops habroptilus*; [57,58]; saddleback, *Philsturnus sp.* and South Island robin, *Petroica australis* [59,60]). Conversely, huia went extinct in 1907 [28], c. 20 years after the study skin was sampled, which corresponds to c. three generations [46,56]. It is thus quite possible that huia experienced a rapid decline and extinction resulting mostly from further forest clearance and the introduction of mammalian predators by Europeans, without genetic erosion contributing markedly to their extinction [61].

Our results indicate a severe reduction in $N_e$ as a result of long-term climate change. While our data did not allow to detect very recent bottlenecks associated with humans, low inbreeding levels close to extinction suggests that Maōri settlement did not lead to an increase in inbreeding in huia and South Island kokako. Consequently, in spite of a low post-glacial $N_e$ which could have made them more vulnerable to genomic erosion, both species do not seem to have been exposed to genomic erosion at the time of European arrival. While temporal comparison of historical genomes in South Island kōkako are required to properly examine the role of genetic erosion in the extinction of the species, it seems likely that huia went extinct rapidly through the combined effects of forest clearance and mammalian predation.

**Figures and Tables**
Figure 1. Sampling locations of the huia and South Island kōkako museum skins. Green-shaded areas depict forest refugia during the Last Glacial Maximum (LGM) c. 22,000 years BP, after Alloway et al. [37]. Barplots depict the distribution of ROHs > 100kb in huia and South Island kōkako.
Figure 2. PSMC for huia (blue) and South Island kōkako (purple) assuming a generation time of 6 years [46,56] and using a uniform False Negative Rate (uFNR) correction rate of 40%. The x axis corresponds to time before present in years on a log scale, assuming a substitution rate of $1.38 \times 10^{-8}$ substitution/site/generation inferred from [62]. The y axis corresponds to the effective population size.

Table 1. Heterozygosity per 1,000bp estimated as $\theta$ and inbreeding estimated as the proportion of the genomes in Runs of Homozygosity ($F_{ROH}$).

<table>
<thead>
<tr>
<th>Species</th>
<th>$\theta$</th>
<th>$\theta$ (95% CI)</th>
<th>$F_{ROH} &lt; 100$Kb</th>
<th>$F_{ROH} &gt; 1$Mb</th>
</tr>
</thead>
<tbody>
<tr>
<td>Huia</td>
<td>0.944</td>
<td>0.942-0.947</td>
<td>0.187</td>
<td>0</td>
</tr>
<tr>
<td>South Island kōkako</td>
<td>1</td>
<td>0.998-1</td>
<td>0.319</td>
<td>0</td>
</tr>
<tr>
<td>Rifleman</td>
<td>1.67*</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
</tr>
</tbody>
</table>

$\theta =$ population mutation rate which approximates heterozygosity under the infinite sites model
*estimated as SNP rate per $10^3$ bases [50]

Data accessibility. Raw fastq reads are deposited at the NCBI Sequence Read Archive (SRA), accession number (pending).

Acknowledgements. We thank Anita Gamauf (Vienna Museum, Austria), Mark Adams (Natural History Museum), Alan Tennyson (Te Papa, New Zealand), Emma Burns (Otago Museum, New Zealand) and Matt Rayner (Auckland Museum, Auckland, New Zealand) for lending museum
skins. We also thank Guojie Zhang and the Bird 10,000 Genome Project (B10K) (https://b10k.genomics.cn/) for access to the genome assembly. We acknowledge support from the Uppsala Multidisciplinary Centre for Advanced Computational Science for assistance with massively parallel sequencing and access to the UPPMAX computational infrastructure. Sequencing was performed by the Swedish National Genomics Infrastructure (NGI) at the Science for Life Laboratory, which is supported by the Swedish Research Council and the Knut and Alice Wallenberg Foundation.

**Funding.** This work was supported by FORMAS (2015-676) to L. D.; the Swiss National Science Foundation to N. D. (P2SKP3_165031 and P300PA_177845); University of Otago PBRF grants to N.D., M. K. and B.C.R.

**References**


44. Worthy TH, Holdaway RN. 1996 Quaternary fossil faunas, overlapping taphonomies, and


