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Demographic decline and lineage-specific adaptations characterize New Zealand kiwi

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Small and fragmented populations may become rapidly differentiated due to genetic drift, making it difficult to distinguish whether neutral genetic structure is a signature of recent demographic events, or of long-term evolutionary processes that could have allowed populations to adaptively diverge. We sequenced 52 whole genomes to examine Holocene demographic history and patterns of adaptation in kiwi (*Apteryx*), and recovered 11 strongly differentiated genetic clusters corresponding to previously recognized lineages. Demographic models suggest that all 11 lineages experienced dramatic population crashes relative to early- or mid-Holocene levels. Small population size is associated with low genetic diversity and elevated genetic differentiation (F_{ST}), suggesting that population declines have strengthened genetic structure and led to the loss of genetic diversity. However, population size is not correlated with inbreeding rates. Eight lineages show signatures of lineage-specific selective sweeps (284 sweeps total) that are unlikely to have been caused by demographic stochasticity. Overall, these results suggest that despite strong genetic drift associated with recent bottlenecks, most kiwi lineages possess unique adaptations and should be recognized as separate adaptive units in conservation contexts. Our work highlights how whole-genome datasets can address longstanding uncertainty about the evolutionary and conservation significance of small and fragmented populations of threatened species.

1. Introduction

Evolutionary biologists and conservation geneticists have long examined population genetic structure to aid inferences about the evolutionary histories of populations [1,2] and to delineate conservation units [3]. However, in species with small and fragmented populations, genetic drift may cause rapid shifts in allele frequencies [4], potentially leading to a strengthening of pre-existing genetic structure [5] or the evolution of novel genetic structure, sometimes within only a few generations [6–8]. For example, brief periods of isolation and strong drift may drive increased F_{ST} [5–8], increased divergence along with principal component axes of genetic variation [5] and formation of novel clusters in genetic clustering analyses [7]. Similarly, monophyletic mitochondrial lineages are often assumed to indicate deep evolutionary divergence [3], but even monophyly could arise from standing ancestral variation over moderate time scales during severe bottlenecks (effective population size $N_e \ll 100$) [3,9].

This decoupling between the strength of population genetic structure and the timing of isolation in small and fragmented populations has the potential to confound evolutionary and conservation inferences. Although strong neutral genetic structure is often assumed to indicate the likely existence of local adaptation [10,11], local adaptation can also evolve under scenarios of shallow divergence and high gene flow [12,13]. Furthermore, hybrids could be favoured in

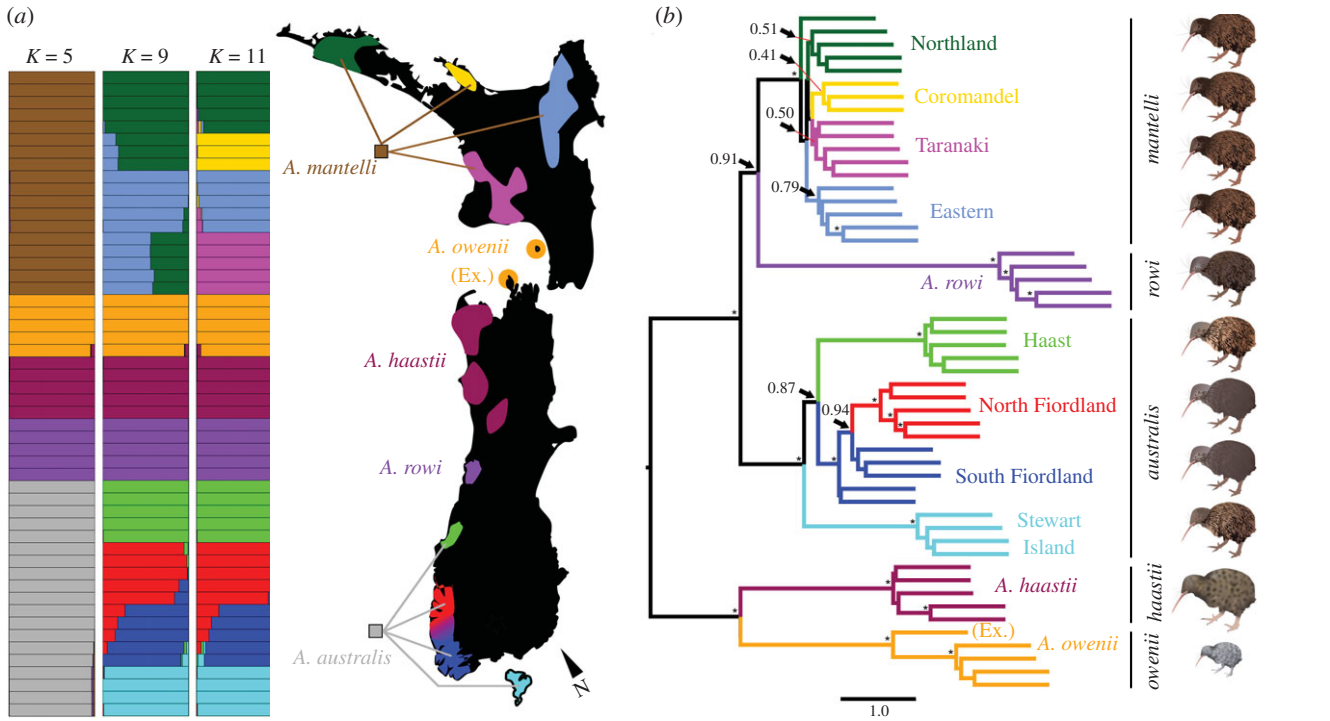

Figure 1. Genetic structure and phylogeographic relationships. (*a*) Genetic clusters and admixture proportions with $K = 5$, 9 and 11 clusters, representing the five species, the optimal number of clusters detected by *PCAngsd* and the 11 lineages, respectively. Each bar represents one individual and colours represent the proportion of ancestry belonging to each genetic cluster. The approximate contemporary geographic distribution of each lineage is shown in the map of New Zealand. The D'Urville Island population of *A. owenii* ($n = 1$) has recently gone extinct (Ex.). (*b*) Species tree constructed in *Astral-III* from 4260 separate ML gene trees of UCE loci. Branch support values (local posterior probability) greater than or equal to 0.95 are indicated with an asterisk, with branch support of additional key nodes labelled with arrows. Internal branch lengths are in coalescent units. Terminal branch lengths were not estimated and an arbitrary value was chosen for visualization. Kiwi illustrations were generated by Uriel Garcilazo Cruz and authors E.K.M. and J.T.W. (Online version in colour.)

intermediate environments [14] or experience heterosis (hybrid vigour) under certain genomic architectures [15,16]. Nonetheless, the general assumption that populations strongly differentiated at neutral loci are likely to also be adaptively differentiated has fuelled conservation guidelines that advise against the mixture of individuals from such populations [3,10,11]. Genetic mixing of adaptively differentiated populations could erode their evolutionary uniqueness through gene swamping [17], promote maladaptation to local conditions [18] and lead to outbreeding depression [10,19]. On the other hand, restoring genetic connectivity between populations that are not locally adapted could help re-establish historical ecological and evolutionary processes, and may have beneficial effects such as increasing within-population genetic diversity [20], reducing inbreeding depression through genetic rescue [21] and reducing extinction risk through heightened metapopulation connectivity [22]. Which of these approaches is more desirable is especially difficult to evaluate in small, fragmented and neutrally differentiated populations, as drift during range fragmentation and population bottlenecks may contribute to strong genetic structure [5–7], in which case populations may not necessarily deserve any special recognition as separate taxa or conservation units.

Kiwi (*Apteryx*) represents a classic genus of conservation concern that is divided into small and fragmented populations. While formerly widespread throughout New Zealand [23], kiwi geographic ranges and population sizes have been dramatically reduced by introduced predators, habitat degradation and historical hunting pressures [24–27]. There are five recognized species (figure 1): three species of brown kiwi (*A. australis*, *A. mantelli* and *A. rowi*) that show only minor morphological and ecological differences and were until recently treated as a single species [28], and two species of spotted kiwi (*A. haastii* and *A. owenii*). Within these five species, 11 extant genetic lineages have been previously identified on the basis of mitochondrial sequences and reduced-representation nuclear SNP datasets [29]. Divergence among extant intraspecific lineages of *A. australis* and *A. mantellii* (both possess four lineages) has previously been dated to the late Pleistocene (0.11–0.26 Ma) and lineages probably experienced bottlenecks during the last glacial period [29], yet some individuals are genetically admixed, suggesting recent genetic contact [29]. In these lineages, it remains unknown whether strong neutral genetic structure primarily reflects deep (and potentially adaptive) evolutionary divergence and isolation, or whether pre-existing minor and non-adaptive differentiation has been exacerbated by drift during range fragmentation and population declines.

Here, we sequence the whole genomes of 52 individuals from the 11 kiwi lineages to investigate their recent demographic history and test for signatures of adaptation. Specifically, we model changes in population size of each lineage throughout the Holocene and test whether small population size is associated with increased genetic differentiation and common genetic threats such as increased inbreeding and the loss of genetic diversity. We also compare signatures of selection at two different taxonomic depths: selective sweeps in extant lineages and genes under positive selection in the most recent common ancestor (MRCA) of all kiwi. Our goals are twofold: (i) to understand how demographic events and selection fundamentally shaped the evolution of small and fragmented kiwi populations and (ii) to inform the conservation genomic management of these charismatic and threatened birds.

2. Methods

Further details of all methods are given in the electronic supplementary material, Methods.

(a) DNA sequencing and SNP discovery

We sequenced 52 whole genomes (150 bp paired-end reads; three to five individuals per lineage, electronic supplementary material, table S1) on an *Illumina* HiSeq X to a mean depth of 15.4 x. Reference-aligned reads were used to identify 27.5 million SNPs (electronic supplementary material, table S2) in *ANGSD* v. 0.930 [30].

(b) Genetic structure

Using *ANGSD*, we estimated pairwise genetic differentiation (Hudson's F_{ST}) [31] and genetic divergence (d_{XY}) between lineages, and nucleotide diversity (π), observed heterozygosity (H_o), and Tajima's D within lineages. F_{ST}, d_{XY} and π were calculated both globally and in 50 kbp windows. Estimates of d_{XY} and π were adjusted relative to the total number of available genomic sites, including monomorphic sites (electronic supplementary material, table S2). We identified genetic clusters using *PCAngsd* v. 0.982 [32], both with automatic detection of the number of clusters, and with five and 11 clusters manually selected.

(c) Phylogeny construction

Phylogenetic relationships were inferred from 4260 ultra-conserved element (UCE) loci identified with *PHYLUCE* v. 1.7.1 [33]. SNP genotypes at UCEs plus an additional 1 kbp flanking region on either side were called in *ANGSD* and used to construct maximum-likelihood (ML) trees for each individual locus using *RAxML-NG* v. 1.0.2 [34]. A single species tree was then estimated from the UCE trees using *Astral-III* v. 5.7.7 [35]. Divergence times between lineages were estimated in *SNAPPER* v. 1.0.1 [36] using 1000 randomly selected SNPs from one individual per lineage (electronic supplementary material, table S2), with divergence of the kiwi basal split calibrated using a previous estimate of 5.96 Ma [29].

(d) Inbreeding

We calculated inbreeding coefficients (F_{ROH}) for each individual from runs of homozygosity (ROHs) using *ROHan* [37] with minimum lengths of 1.5 and 5 Mbp and background tolerance for heterozygosity in true ROHs (--rohmu) of 5×10^{-5}. Longer ROHs represent inbreeding between individuals sharing ancestors in more recent generations, as they have not yet been broken down by recombination [37].

(e) Demographic history

We estimated changes in effective population size (N_e) from the beginning of the Holocene (11.9 ka, or 622 generations ago) until the present using *PopSizeABC* [38]. *PopSizeABC* uses coalescent simulations to model a single population experiencing changes in N_e over time and employs the site-frequency spectrum and linkage disequilibrium between SNPs at different physical distances as summary statistics in an approximate Bayesian computation (ABC) framework. In each simulation, log-uniform priors on N_e ranged from 20 to 2 000 000 individuals and N_e was constrained to vary no more than 100-fold between adjacent time bins. We used a recombination rate (2.1×10^{-8}) reported for *Rhea* [39], and a substitution rate (1.35×10^{-8}) we estimated for kiwi from d_{XY}. We performed 500 000 simulations (for each of $n = 3$–5 individuals, corresponding to empirical population sizes, with the individual from the extinct D'Urville Island population of *A. owenii* excluded; electronic supplementary material, table S1) and retained the 0.1% of simulations with summary statistics most closely matching the empirical data to estimate posterior distributions of N_e. To test the ability of the ABC method to recover known demographic histories, we performed parameter cross-validation using 200 pseudo-observed datasets.

To estimate pre-Holocene demographic history and to confirm *PopSizeABC* Holocene results, we also estimated changes in N_e from the present to *ca* 2 Ma using *SMC++* v. 1.15.2 [40]. For each lineage, we ran *SMC++* with 10-fold cross-validation, using called genotypes from all 314 autosomal scaffolds ≥ 1 Mbp in length.

(f) Loci under selection

To test for lineage-specific adaptations, we examined signatures of selective sweeps in each lineage using *RAiSD* [41], which calculates a composite statistic μ based on multiple sweep signatures, including reduced genetic diversity levels, shifts in the site-frequency spectrum towards rare alleles and increased linkage disequilibrium. We ran *RAiSD* from called genotypes at lineage-specific SNPs (electronic supplementary material, table S2) using default parameters. To interpret *RAiSD* output, it is necessary to set a threshold μ value for detecting true sweeps: because demographic stochasticity associated with population bottlenecks can mimic sweep signatures [41], we simulated neutral genomic data based on each lineage's inferred demographic history (figure 2*a*) to set conservative thresholds for distinguishing putative true sweeps from signatures that could alternatively be explained by demographic bottlenecks. Lineage-specific thresholds were adjusted relative to our simulation effort and genome size to allow an expectation of 1.144 false positives per lineage due to demographic stochastitcity. These thresholds were set by calculating the 99th percentile of the maximum μ values observed across 10 000 independent neutral chromosomal simulations (10 Mbp each) using parameters from the 500 retained demographic scenarios from *PopSizeABC*, with each scenario simulated 20 times. However, we excluded the 5% of scenarios with the most severe simulated bottleneck effects (highest mean $\mu_{maximum}$ among the 20 replicates) to account for demographic uncertainty, as these extreme retained scenarios are unlikely to represent the true demographic history.

We also tested for genes showing evidence of positive selection (rate of non-synonymous to synonymous substitutions $\omega < 1$) [42] in the common ancestor of kiwi. We inferred 9381 individual gene trees of one individual from each kiwi lineage (electronic supplementary material, table S1) plus four outgroups using *IQ-TREE* [43] and tested for elevated ω using the branch-site model implemented by *aBSREL* in *HyPhy* [42]. We then tested for enrichment in gene ontology (GO) terms using over-representation analysis of non-redundant biological processes in *WebGestalt 2019* [44], both for genes near selective sweeps (less than or equal to 10 kbp) in extant lineages (*RAiSD*), and for genes under positive selection in the common ancestor (*aBSREL*).

3. Results

(a) Genetic structure and phylogenetic relationships

The optimal number of genetic clusters selected by PCAngsd was $K = 9$; however, subtle intraspecific structure within *A. mantelli* was not resolved. Instead, genetic clustering with $K = 11$ successfully recovered the 11 previously recognized [29] lineages (figure 1*a*; electronic supplementary material, figures S1 and S2). Within *A. australis* and *A. mantelli*, admixture among intraspecific lineages was frequently detected (figure 1*a*). Admixture among the four *A. mantelli* lineages (up to 14.3%) varied greatly among individuals, suggesting recent hybridization events affecting specific individuals.

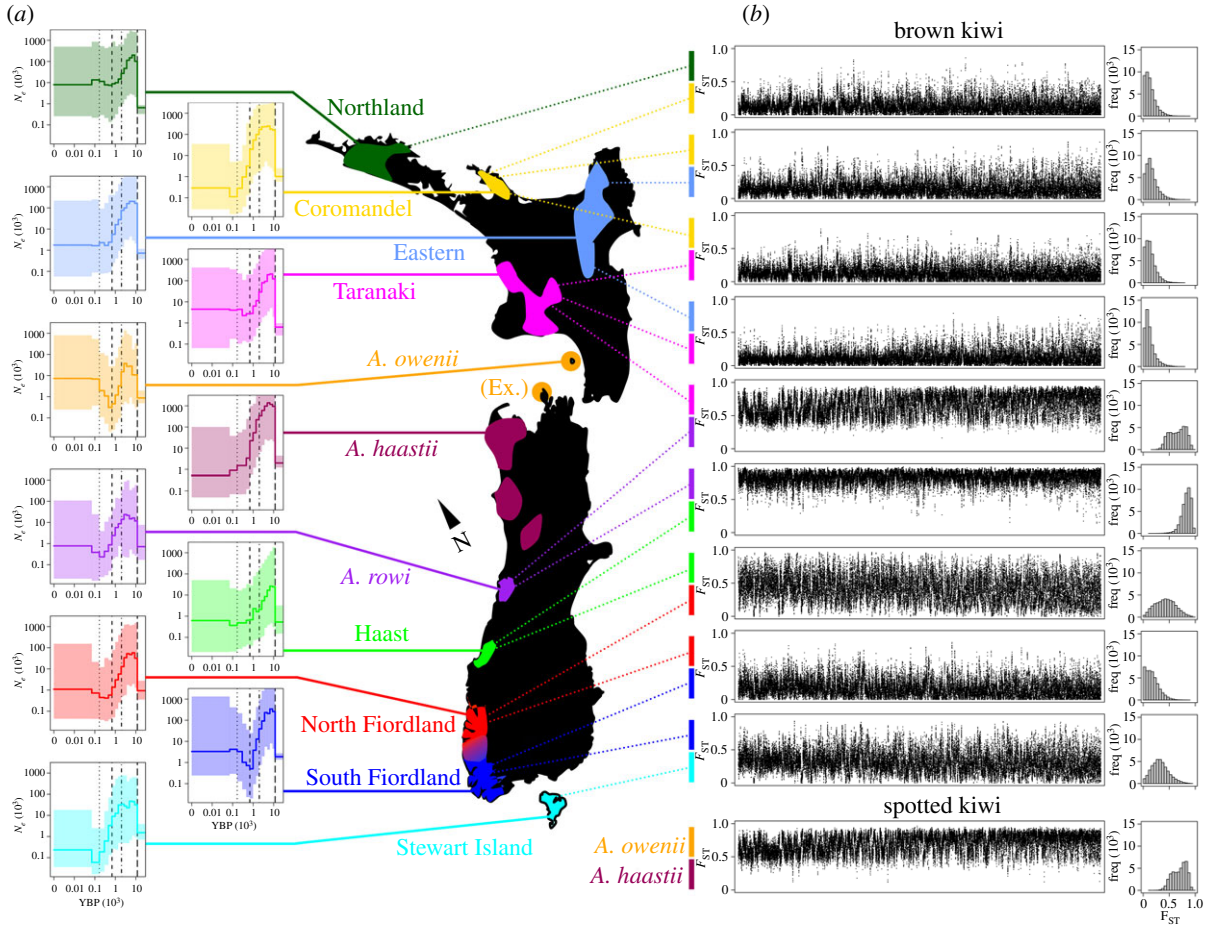

Figure 2. Holocene demographic history and population differentiation. (*a*) Holocene changes in effective population size (N_e) inferred in *PopSizeABC*, for time bins ranging from the present (0 years before present, YBP) to 11 900 YBP, with a final time bin representing pre-Holocene ancestral population size. Thick coloured line: median posterior estimate; shaded colours: 90% confidence interval. Vertical lines represent the timing of major Holocene events in New Zealand's history, from left to right: arrival of Europeans (dotted line); arrival of Maori (dashed line); Taupo volcanic eruption (dashed-dotted line); onset of Holocene climatic conditions (long-dashed line). (*b*) Population genetic differentiation (F_{ST}) in 50 kbp windows of the genome, between historically geographically adjacent lineages of brown and spotted kiwi (lineages indicated with coloured bars). Scaffolds are ordered from largest to smallest. Frequency histograms of windowed F_{ST} are shown in inset plots (far right). (Online version in colour.)

Admixture proportions showed strong geographic structure within *A. australis* (electronic supplementary material, figure S2), which could reflect either recent or ancient hybridization, or retention of an ancestral isolation-by-distance pattern. Overall, genetic clustering results strongly support the existence of 11 lineages but suggest that they have not evolved in continuous geographic isolation and that intraspecific lineages are not strongly reproductively isolated.

Phylogenetic analysis in *Astral-III* generally supported the existence of 11 lineages (figure 1*b*). All five species were reciprocally monophyletic, but relationships among lineages within *A. mantelli* were poorly resolved. Each of the Coromandel, Eastern and Taranaki lineages were inferred to be monophyletic with low to moderate support (PP = 0.41–0.79), but the Northland lineage was not monophyletic. By contrast, relationships among *A. australis* lineages generally had stronger support (PP ≥ 0.87) and the Haast, Stewart Island, and combined Fiordland lineages were each reciprocally monophyletic (PP ≥ 0.95), but with North Fiordland nested within South Fiordland. *SNAPPER* recovered an identical topology for relationships among the five species, but among intraspecific lineages the topology differed and had low support (electronic supplementary material, figure S3). Divergence times for the basal split among lineages within *A. australis* and *A. mantelli* were estimated to be 0.65 Ma (95% high probability density interval [HPD]: 0.26–1.12 Ma) and 0.54 Ma (95% HPD: 0.20–0.91 Ma), respectively (electronic supplementary material, figure S3). These estimates overlapped with 95% credible intervals (CIs) from a previous coalescent dating analysis [29], for both *A. australis* (mean: 0.24 Ma; 95% CI: 0.20–0.32 Ma) and *A. mantelli* (mean: 0.26 Ma; 95% CI: 0.22–0.32 Ma).

Despite evidence of admixture, the 11 lineages are strongly genetically differentiated (figure 2*b*; electronic supplementary material, table S3). Pairwise genetic differentiation (F_{ST}) between intraspecific lineages ranged from 0.20 to 0.57 in *A. australis* and from 0.11 to 0.16 in *A. mantelli* (electronic supplementary material, table S3). For comparison, F_{ST} between species ranged from 0.61 to 0.92 (electronic supplementary material, table S3). Nucleotide divergence (d_{XY}) ranged from *ca* 0.0020 across several of the shallowest intraspecific splits within *A. australis* and *A. mantelli*, to a maximum of 0.0086 across the kiwi basal split (electronic supplementary material, table S3). F_{ST} was strongly predicted by d_{XY} (figure 3*b*; electronic supplementary material, figure S4*a*,*b*). F_{ST} was also negatively correlated with pairwise mean census population size after accounting for d_{XY} (multiple linear regression of 5000 permuted datasets: adjusted $p = 0.0118$; electronic supplementary material, figure S4*c*,*d*), indicating that small population sizes are associated with elevated genetic differentiation.

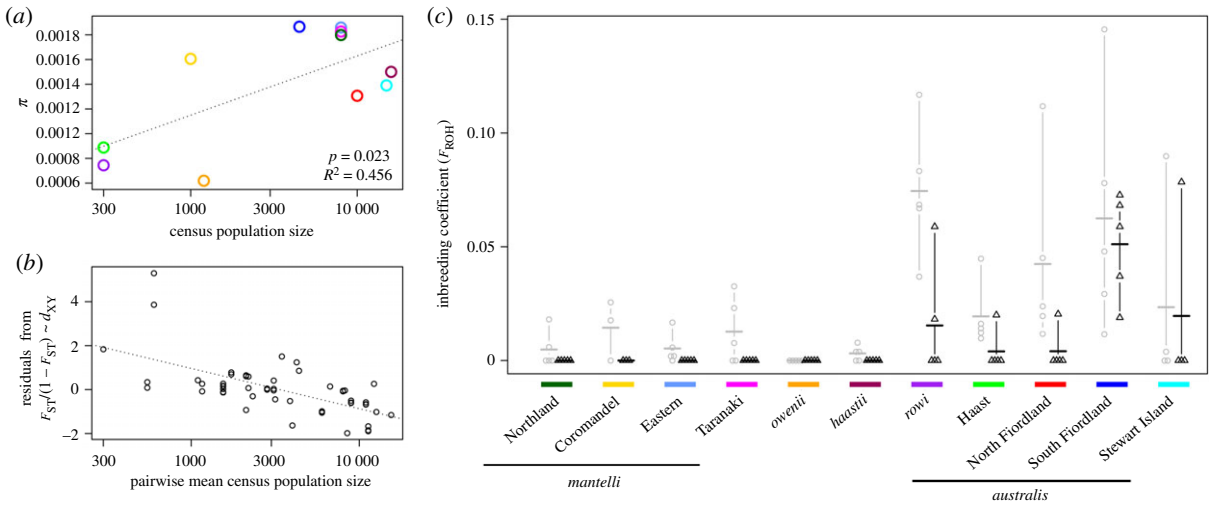

Figure 3. Genetic parameters potentially influenced by small population size. (*a*) Nucleotide diversity (π) versus census population size. A linear regression line is plotted (dotted grey line) and colours represent lineage identity. (*b*) Residuals from a regression of linearized F_{ST} versus d_{XY}, plotted against pairwise mean census population size, illustrating how small populations show higher F_{ST} than expected after accounting for d_{XY}. This relationship is plotted for visualization purposes only and a *p*-value cannot be calculated due to statistical non-independence of data points; see electronic supplementary material, figure S9 for a formal analysis. (*c*) Distribution of individual inbreeding coefficients (F_{ROH}) in each lineage, calculated from ROHs of at least 1.5 Mbp (grey circles) and 5.0 Mbp (black triangles). Thick horizontal lines represent mean F_{ROH}. For confidence intervals on individual estimates, see electronic supplementary material, table S5. (Online version in colour.)

(b) Demographic history

With the exception of *A. owenii*, *PopSizeABC* inferred dramatic population declines between the early Holocene and the present in all lineages (figure 2*a*). *A. owenii* also showed an initial population decline, but N_e subsequently recovered such that the median estimate of N_e near the present was only slightly lower than that in the early Holocene (figure 2*a*). This trend partly reflects the known history of *A. owenii* on Kapiti Island, which recently expanded from a severe population bottleneck [45,46]. Confidence intervals on N_e were broad, reflecting the substantial uncertainty detected in cross-validation analyses (electronic supplementary material, figures S5–S7), yet declines between the early Holocene (4882–11902 YBP) and the recent past (1–292 YBP) were statistically significant ($p < 0.001$) in all lineages (electronic supplementary material, figure S8).

Population trends estimated for the Holocene in *SMC++* (electronic supplementary material, figure S9) were qualitatively similar to those from *PopSizeABC*, showing peak N_e near the early Holocene and a sharp decline towards the present. All lineages also showed reduced N_e during the last glacial period relative to early Holocene levels, as has previously been inferred [29].

(c) Genetic health in small populations

Nucleotide diversity (π) ranged from 0.0006 to 0.0019 (electronic supplementary material, table S4) and was positively correlated with census population size [25] ($R^2 = 0.456$, $p = 0.023$; figure 3*a*), suggesting that recent population crashes are associated with a loss of genetic diversity. The three lineages with the lowest genetic diversity (*A. owenii*, *A. rowi* and Haast [*A. australis*]) are all small, spatially restricted populations (figure 1). Nucleotide diversity was strongly correlated with mean H_o ($R^2 = 0.93$, $p = 2.0 \times 10^{-6}$).

Individual inbreeding coefficients (F_{ROH}) were low across all lineages except the four *A. australis* lineages and *A. rowi* (figure 3*c*; electronic supplementary material, table S5). In *A. rowi*, $F_{ROH\ 1.5}$ was high but $F_{ROH\ 5.0}$ was relatively low, suggesting that *A. rowi* successfully avoids mating with extremely close kin such as siblings and cousins (low $F_{ROH\ 5.0}$), but mating individuals nonetheless share multiple instances of identical ancestors at more moderate depths within their pedigree (high $F_{ROH\ 1.5}$). Similar but less extreme patterns were uncovered for Haast and North Fiordland (*A. australis*). By contrast, elevated $F_{ROH\ 5.0}$ that is not substantially lower than $F_{ROH\ 1.5}$ in South Fiordland and Stewart Island suggests inbreeding between close relatives. Mean F_{ROH} was not correlated with census population size [25] ($p = 0.34$ and 0.98 for $F_{ROH\ 1.5}$ and $F_{ROH\ 5.0}$, respectively; electronic supplementary material, figure S10), suggesting that population crashes have not led to an increase in inbreeding.

(d) Loci under selection

We detected at least one lineage-specific sweep in eight of the 11 lineages (table 1). Importantly, selective sweeps were detected in three of four intraspecific lineages within *A. australis* and all four lineages within *A. mantelli* (though only one sweep in Coromandel, approximating the expected false positive rate), suggesting that selective sweeps differentiate not only separate species but also intraspecific lineages. The number of sweeps varied widely among lineages; this variation does not necessarily imply that some lineages experienced more selection than others, but could reflect our different abilities in each lineage to distinguish sweeps from the effects of bottlenecks, depending on each lineage's specific demographic history. We may have also detected fewer sweeps in lineages with smaller sample size (electronic supplementary material, table S2) where subtle sweep signatures are more difficult to distinguish from the genomic background [47].

Many genomic regions exhibited sweep signatures in more than one lineage (table 1; electronic supplementary material, table S6). Although shared signatures could reflect parallel selection or sweeps inherited from a common ancestor, they could also correspond to false positives repeatedly arising in centromeres or low-complexity regions [41] that mimic the

Table 1. Selective sweeps detected in *RAiSD*. Sweeps may be either shared among multiple lineages or lineage-specific (LS). GO term enrichment (false discovery rate FDR < 0.10) of genes near outlier peaks is reported for lineage-specific sweeps only, then across all sweeps total (final row). Totals across all lineages may not equal their respective column sums because shared sweeps are reported in multiple rows.

lineage	total	shared	LS	enriched GO terms (FDR)
A. australis				
Haast	8	0	8	—
North Fiordland	71	10	61	—
South Fiordland	0	0	0	—
Stewart Island	4	2	2	—
A. haastii	17	6	11	osteoclast differentiation (0.0122), hepatitis B (0.0122), human immunodeficiency virus 1 infection (0.0307), human cytomegalovirus infection (0.0456), insulin resistance (0.0465), leishmaniasis (0.0491), kaposi sarcoma-associated herpesvirus infection (0.0902), glycosaminoglycan biosynthesis (0.0962)
A. mantelli				
Coromandel	1	0	1	—
Eastern	179	48	131	—
Northland	39	24	15	—
Taranaki	102	47	55	—
A. owenii	0	0	0	—
A. rowi	0	0	0	—
all lineages	355	71	284	regulation of cell size (0.0160), regulation of anatomical structure size (0.0283)

effect of sweeps. Shared sweeps were not systematically located in low-diversity regions of the genome (electronic supplementary material, figures S11–S13), suggesting that *RAiSD* was not strongly biased by genome architecture. However, given this possibility, we consider sweeps detected in only a single lineage to be the least likely to be false positives. Migration between lineages may also generate false positives, but methods to distinguish migration from sweeps do not currently exist [41]. An excess of false positives generated by migration and admixture might explain why large numbers of sweeps were inferred in some of the most heavily admixed lineages (e.g. Eastern and Taranaki [*A. mantelli*], North Fiordland [*A. australis*]), but we also detected sweep signatures in unadmixed lineages (figure 1 and table 1), suggesting that migration cannot account for our key result that unique sweeps are consistently detected in most lineages.

Gene ontology (GO term) enrichment for genes near sweeps (less than or equal to 10 kbp) revealed significant enrichment in the regulation of cell size (FDR = 0.016) and regulation of anatomical structure size (FDR = 0.028) (table 1). Although these GO terms do not suggest any clear hypothesis regarding the source of selection on genes, it is noteworthy that kiwi have previously been identified to have unusual anatomical proportions including larger brain : body size ratios than other Palaeognaths [48] and disproportionately large egg : body size ratios relative to other birds [49]. There was significant GO term enrichment (FDR < 0.05) of genes near lineage-specific sweeps in *A. haastii* only, in osteoclast differentiation, insulin resistance, and several terms related to immune system response (table 1).

Our *aBSREL* analyses detected a total of 111 genes under positive selection in the MRCA of kiwi (FDR < 0.10). Four of these genes overlapped with the previously identified genes near selective sweeps in the 11 lineages, but this level of overlap was not significantly greater than expected by chance ($p = 0.13$ from 10^4 resampling replicates), suggesting that different suites of genes were under selection during the early and recent stages of kiwi diversification. There was no significant GO term enrichment for genes under positive selection in the MRCA.

4. Discussion

We examined Holocene demography and signatures of adaptation in order to characterize the evolutionary nature of New Zealand's 11 kiwi lineages. Our results reveal that most lineages are adaptively differentiated. All lineages experienced population bottlenecks in the most recent generations relative to early- or mid-Holocene population sizes. Drift associated with these bottlenecks is likely to have contributed to strong genetic structure, possibly enhancing weaker population genetic differentiation initiated during Pleistocene glacial cycles [29]. In particular, a small population size is associated with increased genetic differentiation (F_{ST}) and genome-wide loss of genetic diversity, but not increased rates of inbreeding. Despite the possibility that genetic drift in small populations has had a major impact on genetic structure, we find that eight of 11 lineages also possess signatures of lineage-specific selective sweeps, including seven of eight intraspecific lineages. This suggests that the 11 lineages should be recognized as adaptive units (AUs). We emphasize the need for caution regarding management decisions that might promote contact between AUs. Overall, our work highlights how small, fragmented populations of threatened species—likely to have been strongly impacted by genetic drift—may nonetheless possess unique adaptations.

(a) Holocene decline and drift

Holocene events have profoundly impacted kiwi. While currently restricted to small and fragmented populations (figure 1), several kiwi species (*A. australis*, *A. owenii* and *A. rowi*) were formerly much more widely distributed across New Zealand [23]. Five to six distinct mitochondrial lineages of these species went extinct during the Holocene [29]. Our demographic models demonstrate that even lineages that did not go extinct have experienced major Holocene population crashes (figure 2*a*). It is not possible to determine exactly when populations first began to decline because autocorrelation between adjacent time bins in our *PopSizeABC* models could cause estimates of population size (N_e) in time bins prior to a true crash to be downwardly biased. In other words, small early Holocene dips in our results may not be biologically meaningful. However, the sharpest declines in N_e (corresponding to the strongest signal for rapid changes in population size) often occur in the late Holocene (figure 2*a*), a time period marked by major events such as the cataclysmic Taupo volcanic eruption (*ca* 1.8 ka) that devastated habitats over the central North Island [50], and human colonization of New Zealand by Maori (*ca* 700 YBP [51]) and Europeans (*ca* 200 YBP).

Population crashes have probably increased genetic differentiation among lineages and led to genetic health concerns in some kiwi lineages. In particular, the negative association between F_{ST} and pairwise mean census population size after accounting for d_{XY} (figure 3*b*; electronic supplementary material, figure S4) suggests that population crashes have led to increased genetic differentiation. The positive association between nucleotide diversity and census population size (figure 3*a*) suggests that population crashes have led to a genome-wide loss of genetic diversity, with the most severe effects in the smallest populations. Loss of genetic diversity may also have affected larger populations, as π in all lineages ($0.6–1.9 \times 10^{-3}$) is either below or at the low end of the range of π typically recovered in other wild birds (10^{-3} to 10^{-2}) [52,53]. Low genetic diversity is often hypothesized to increase extinction risk as populations possessing little adaptive variation may be unable to respond to rapid environmental change [54], although the generality of this relationship has been questioned [55].

While small populations may also face the risk of inbreeding depression [21], inbreeding rates were not correlated with population size (electronic supplementary material, figure S10). Of the three lineages with the smallest population size (*A. owenii*, *A. rowi* and Haast [*A. australis*]), high inbreeding was only detected in *A. rowi* (figure 3*c*). The lack of inbreeding in *A. owenii* is especially remarkable, given that all Kapiti Island individuals are believed to be descended from five individuals translocated to the island in 1912 [45], whose offspring must have experienced inbreeding by the third generation (i.e. when $2^3 = 8$ unrelated ancestors would be required to remain fully outbred). As our samples were collected in 1989 (77 years post-bottleneck; electronic supplementary material, table S1) and the mean lifespan of *A. owenii* is roughly 45 years [56], it is possible (but unlikely) that we by chance only sequenced outbred first- and second-generation descendants of translocated individuals. Alternatively, an unknown native population of *A. owenii* may have existed on Kapiti Island [57]—but this interpretation has been challenged by genetic data [45,46]—or additional undocumented translocations may have taken place, in which case third generation and higher descendants of translocated individuals could remain entirely outbred. Sampling individuals born more recently—in both *A. owenii* and other small populations—could aid in determining whether inbreeding levels are stable or increasing over time.

In contrast with low inbreeding rates in most populations, moderate inbreeding rates were observed in South Fiordland and Stewart Island [*A. australis*], and in *A. rowi* (figure 3*c*). It has previously been suggested [58] that many New Zealand bird species may have purged deleterious alleles during bottlenecks associated with Pleistocene glacial cycles, in which case moderate inbreeding may not be a major genetic health concern. However, inbreeding depression has previously been documented in a very small kiwi population not included in the present study [59], highlighting the need for population-specific case studies to explicitly investigate the fitness consequences of inbreeding.

(b) Lineage-specific adaptations

Although range fragmentation and decline have probably increased genetic drift in kiwi, resulting in reduced genetic diversity and increased genetic differentiation (figure 3*a*,*b*), it is difficult to predict from neutral genetic structure whether intraspecific lineages are likely to be adaptively differentiated, and if they are not, whether conservation programmes should maintain strict separation of lineages. Here, we show that eight of 11 lineages possess signatures of lineage-specific selective sweeps (table 1) that are unlikely to be alternatively explained by demographic stochasticity associated with bottlenecks, suggesting that the kiwi lineages are not only neutrally but also adaptively differentiated.

It has been argued that small, fragmented populations of threatened species are among the least likely to possess adaptive differences as their genomes have probably been overwhelmed by genetic drift [60]. We show that this is not necessarily the case, and even small, fragmented populations may possess unique adaptations. Because larger population sizes allow for more efficient selection [61], some selective sweeps we detected may have occurred prior to the most recent bottlenecks. However, a recent meta-analysis found little evidence that genetic drift systematically overwhelms selection in small populations [62]. Thus, some sweeps could also have occurred in bottlenecked populations, perhaps even as a response to novel selective pressures associated with the factors leading to population decline. Further studies will be needed to understand the functional and ecological significance of genes under selection.

Given the presence of lineage-specific sweeps, we recommend that intraspecific lineages of kiwi be recognized as separate AUs [63]. Although identifying AUs has been recognized as an important goal in conservation genomics [60,63], conservation units may be delineated using various criteria and decisions to prevent or facilitate admixture between AUs will depend on context-specific goals. In species where there is little evidence of immediate genetic threats to survival (as the lack of increased inbreeding in AUs with smaller population size suggests for kiwi; figure 3*c*; electronic supplementary material, figure S10), then maintaining strict separation of AUs represents a prudent general management approach. Preventing contact between AUs helps to facilitate the preservation of small and fragmented populations with

unique neutral and adaptive evolutionary histories [3,17], and to avoid the genetic risks of admixture [3,10], such as disruption of locally adapted gene complexes [18] and reductions in fitness due to outbreeding depression [10]. However, in other contexts, promoting admixture could be an important tool to consider for genetic rescue of tiny populations of species that have low genetic diversity or experience strong inbreeding depression [21]. In kiwi, mixing AUs might not lead to severe fitness reductions as some hybridization probably occurs naturally (figure 1*a*), but future studies are needed [24]. Restoring some connectivity between AUs that formerly hybridized may also better mimic long-term species-level evolutionary processes, rather than encouraging further genetic drift and loss of diversity associated with more recent processes of fragmentation and decline. In general, when faced with small, fragmented and adaptively differentiated populations, conservation managers should carefully consider whether it is more desirable to preserve adaptive differences between populations at the risk of exposing them to inbreeding depression and loss of genetic diversity, or whether sacrificing the adaptive distinctiveness of such populations is more urgently needed to promote the genetic health and conservation of a species as a whole.

Data accessibility. DNA sequences: NCBI SRA: PRJNA745563 [64]. Example commands to run programs: https://github.com/jordanbemmels/kiwi-holocene (doi:10.5281/zenodo.5708723) [65].

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