The evolutionary history of the extinct ratite moa and New Zealand Neogene paleogeography

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The ratite moa (Aves: Dinornithiformes) were a speciose group of massive graviportal avian herbivores that dominated the New Zealand (NZ) ecosystem until their extinction ≈600 years ago. The phylogeny and evolutionary history of this morphologically diverse order has remained controversial since their initial description in 1839. We synthesize mitochondrial phylogenetic information from 263 subfossil moa specimens from across NZ with morphological, ecological, and new geological data to create the first comprehensive phylogeny, taxonomy, and evolutionary timeframe for all of the species of an extinct order. We also present an important new geological/paleogeographical model of late Cenozoic NZ, which suggests that terrestrial biota on the North and South Island landmasses were isolated for most of the past 20–30 Ma. The data reveal that the patterns of genetic diversity within and between different moa clades reflect a complex history following a major marine transgression in the Oligocene, affected by marine barriers, tectonic activity, and glacial cycles. Surprisingly, the remarkable morphological radiation of moa appears to have occurred much more recently than previous early Miocene (*ca.* 15 Ma) estimates, and was coincident with the accelerated uplift of the Southern Alps just *ca.* 5–8.5 Ma. Together with recent fossil evidence, these data suggest that the recent evolutionary history of nearly all of the iconic NZ terrestrial biota occurred principally on just the South Island.

ancient DNA | Oligocene Drowning | Dinornithiformes | phylogeny | taxonomy

The prolonged geographic isolation of New Zealand (80–60 million years) and the paucity of terrestrial mammals created a unique ecosystem dominated by an estimated 245 species of birds (1), providing an unparalleled opportunity to observe evolutionary processes. The most striking of the recent avian radiations is that of the extinct ratite moa (Aves: Dinornithiformes), a speciose order ranging in size from a large turkey to the 3 m tall *Dinornis* weighing up to 300 kg. Ratites are a basal lineage of birds that are hypothesized to have had a common ancestor *ca.* 80 million years ago on the Cretaceous southern supercontinent of Gondwana, which subsequently underwent either vicarious speciation as the landmass fragmented (2), and/or flighted dispersal (3). The extant members of the ratite lineage include the ostrich (Africa), emu, cassowary (Australia, New Guinea), rhea (South America), and kiwi (NZ). Extinct ratites include the giant elephant birds (Madagascar) and moa (NZ).

Since the first description in 1839 (4) the taxonomy of moa has remained contentious with up to 64 different species and 20 genera assigned at various times (1). The complex geological history of NZ, and significant regional variations in climate, diet, and sexual dimorphism (SD) have resulted in moa being highly variable morphologically, complicating attempts to define species limits. Morphometric and osteological studies have generally recognized two families, Emeidae and Dinornithidae (Table S1), which most recently have been considered to contain eight and three species respectively (1, 5–7). The recovery, amplification, and sequencing of ancient DNA from fossil bone has provided new insights into moa systematics, and revealed extreme cases of SD (8–14). Fig. 1 depicts the current taxonomy of moa (including changes proposed in this article), as well as summarizing species distributions, dimensions, and ecology.

In this study we use mitochondrial DNA sequences isolated from 119 specimens, in addition to previously published data (9–13), to study the mode and tempo of moa evolution, phylogeography, and taxonomy, and relate this to the geological and ecological history of NZ.

Results and Discussion

The accuracy of studies employing molecular clocks to date speciation events is becoming increasingly scrutinized. Problems associated with calibration points, substitution saturation, base composition bias, model selection, and more recently with the time dependency of molecular clocks, can all distort the temporal accuracy of phylogenetic reconstructions (15–18). We used two contrasting approaches in an attempt to establish timeframes for the divergence events within and between moa species: an externally (fossil) calibrated analysis of avian mitochondrial protein-coding sequences, and an internally calibrated Bayesian analysis of radiocarbon-dated moa mitochondrial control region sequences.

For the externally calibrated approach, two datasets (designated mtg-10,692 and mtg-2,153) were generated from existing moa GenBank data (9–13, 19) (Dataset S1 and Dataset S2). We followed the procedure of Baker et al. (9) and dated the branching events at the base of the moa phylogeny using a 10,692 bp dataset (mtg-10,692) comprising mitochondrial coding region sequences from 26 avian taxa (including 9 ratites), which was externally calibrated from the fossil record (*SI Text* and Table S2). We used a variety of methods to deal with biases introduced by substitution saturation and phylogenetic artifacts (*SI Text*), and estimated divergence dates using BEAST v.1.4.8 (20) as described in *Methods*.

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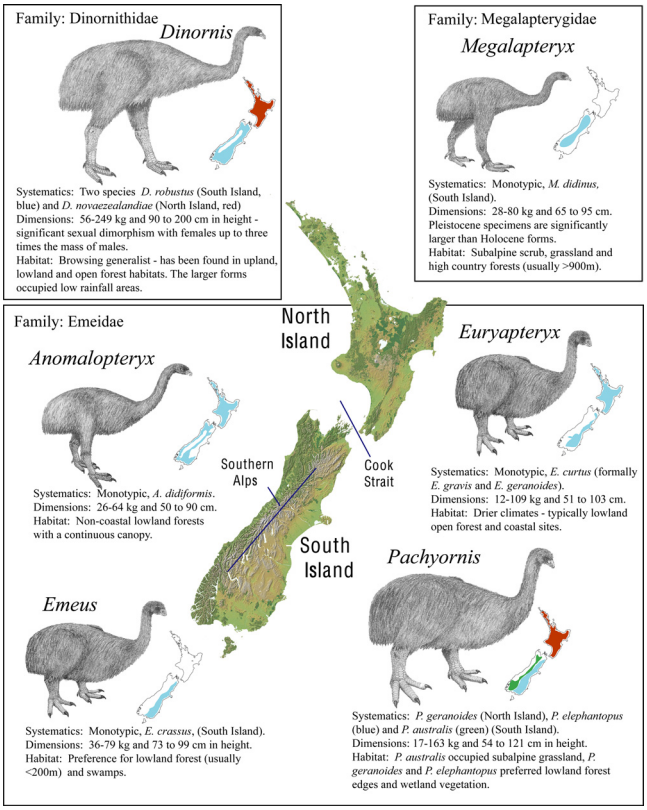

Fig. 1. Systematics, dimensions, and approximate distributions of moa in the three family, six genera, nine species taxonomy advocated in this study. Estimates of body size and habitat were generated from information contained in Worthy and Holdaway (2002) and are discussed in more detail in the *SI Text*. A comparison with the generally accepted two family, 11 species classification used from the 1980's to 2002 is presented in Table S1.

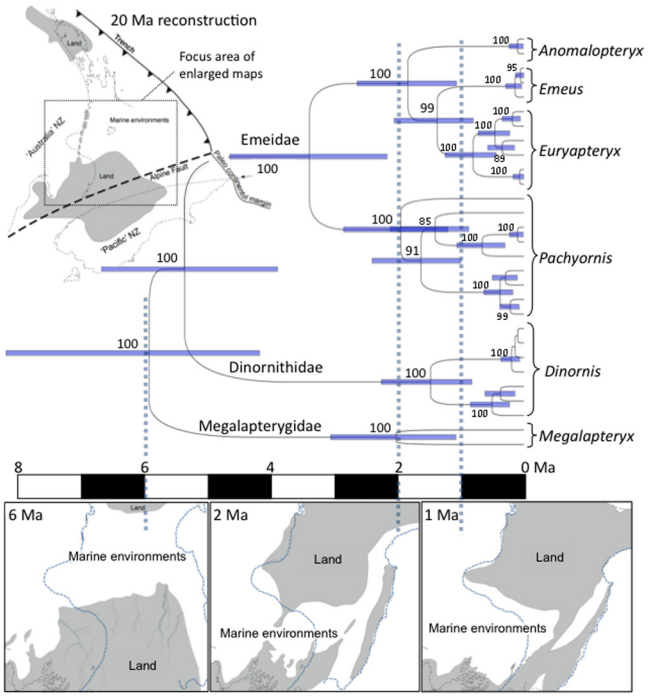

Fig. 2. A spatial and temporal context for the evolution of moa. Molecular phylogeny and date estimates of the moa radiation generated from the mtg-2,153 dataset (see *Methods*), compared with the new paleogeographic model of Neogene New Zealand. A Bayesian Inference tree is shown with Bayesian posterior probability values (>80%) indicated on the nodes with support, whereas the node bars correspond to the 95% HPD. A series of four paleogeographic maps, based on extensive geological mapping of the area (see *Methods* and *SI Text*), show different time horizons during the Neogene and the presence/absence of North and South Island landmasses in central New Zealand. The uplift of the Southern Alps (*ca.* 5–8.5 million years) and periodic bridging to the North Island in the Pleistocene (<2 million years) appear to be instrumental in moa speciation. The absence of deep (*ca.* 20 Million years) splits in the moa phylogeny suggest that all recent moa species originated from the southern landmass (see main text), consistent with the phylogeographic distributions (Fig. 3, Fig. 4*B*, and *SI Text*).

The resulting posterior distribution of the molecular date estimate for the basal moa branch was then used to calibrate analyses within a shorter dataset of 2153 bp (9) of 29 moa mitochondrial protein coding sequences (mtg-2,153, Table S2) to obtain date estimates for species-level divergences within the moa phylogeny.

For the internally calibrated approach, an alignment of 80 *Megalapteryx*, *Dinornis*, and *Pachyornis* control region sequences (389 bp) from radiocarbon-dated moa bones (Table S3) were used as calibration points (or "time-stamped" data) to temporally calibrate a Bayesian framework using BEAST (21).

Moa Systematics at Family and Genus Level. Bayesian analysis of the mtg-2,153 mitochondrial coding region dataset (2,153 bp for 29 moa) yielded the maximum *a posteriori* (MAP) tree shown in Fig. 2. Similar analysis of the control region data from 263 moa control region sequences (389 bp) yielded the higher resolution tree shown in Fig. 3 (presented in detail in Fig. S1), and more detailed analyses with restricted taxonomic coverage shown in Fig. 4. The phylogenetic arrangement in Figs. 2 and 3 has a number of discrepancies with current taxonomic arrangements (Table S1) in which just two families (Dinornithidae and Emeidae) are recognized, with two subfamilies within Emeidae (Megalapteryginae and Anomalopteryginae). Although these three main lineages are recovered in analyses of the mtg-2153 mitochondrial coding region dataset (Fig. 3), *Megalapteryx* is clearly the basal moa lineage. This arrangement is consistent with previous genetic studies (9, 11, 12), and recent analyses of morphological characters (1, 22), where *Megalapteryx didinus* falls as the basal moa lineage, and is outside Emeidae. Consequently, we propose that moa taxonomy be revised so that three families are recognized in the Dinornithiformes (Table S1): Dinornithidae (*Dinornis*); Emeidae (*Pachyornis*, *Emeus*, *Anomalopteryx*, *Euryapteryx*), and Megalapterygidae (new family; type and only genus *Megalapteryx*). Neither of the presently recognized subfamilies (Anomalopteryginae, Emeinae) is supported by either morphological cladistics (1, 22) or mtDNA analyses (9, 13), and should be discarded. At the genus level, there is strong support (posterior probabilities of 100%) (Fig. 3) for all six moa genera outlined by Cracraft (7) and refined by Worthy and Holdaway (1).

The suggestion that species can be recognized solely from genetic distances has previously been identified as problematic (23, 24). Suggestions that this approach can also be applied to the systematic studies of closely related extinct taxa (9) are particularly concerning given the lack of behavioral and ecological data. This problem is exemplified by the strong phylogeographic pattern exhibited within the alpine moa *M. didinus* (Fig. 4 and Fig. S1) where control region genetic distances of 4.7% separate mtDNA "clades". This distance is greater than that between well recognized genera such as *Emeus* and *Euryapteryx* (4.5%), and reinforces the idea that such genetic diversity measures are a product of population longevity and niche persistence, as well as other population parameters. Keeping this observation in mind, and integrating recent morphological studies and moa palaeoecological and phylogeographical data, we review the taxonomic status of all moa genera (*SI Text*) and propose the

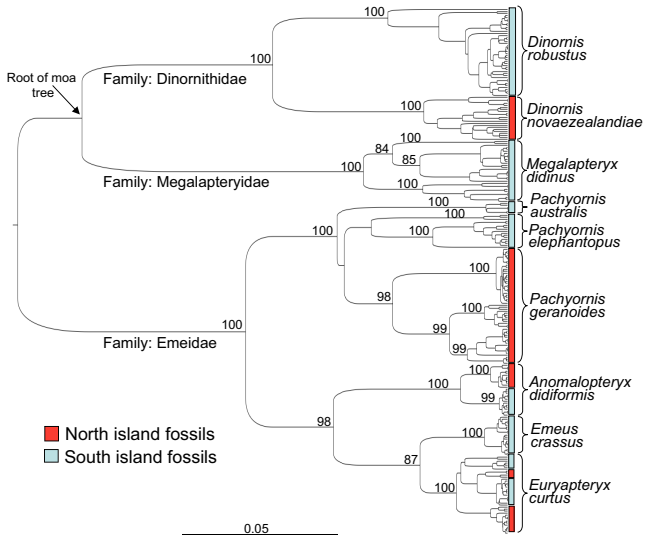

Fig. 3. Phylogenetic reconstruction of all moa species. The maximum *a posteriori* (MAP) tree of 263 mitochondrial control region sequences of each of the moa genera as generated in BEAST (*Methods*) using a HKY+G+I model on a 389 bp alignment. A more detailed tree in which the tips are visible is presented in Fig. S1. DNA sequences obtained from North and South Island fossils are colored red and blue respectively, and suggest that North Island specimens tend to occupy derived phylogenetic positions (see also Fig. 4*B* and *SI Text*). For clarity, posterior probabilities are shown only on basal nodes. Saturation effects in the control region data have resulted in the root of the moa tree being misplaced in this MAP tree, and the mitochondrial protein coding data (Fig. 2) is predicted to give a better estimate of the branching topology at the base of the moa radiation.

new taxonomic arrangement shown in Fig. 1 and Table S1. Overall, we have adopted a conservative approach to species designations and regard mtDNA diversity alone as insufficient to define species limits, especially in the context of recognized barriers to gene flow (discussed later in *Island Allopatry and Pleistocene Cycles*). As new DNA profiling approaches are devised (25) there may be minor future alterations to the taxonomic framework (3 families, 6 genera) proposed here.

Establishing a Timeframe for Moa Evolution. ***Reanalysis of existing mtDNA data.*** The mean divergence date between Dinornithidae and Emeidae was estimated at just 5.27 Ma [95% highest probability density (HPD): 3.1–9.0] in the BEAST analysis of the mtg-10,692 dataset, with 3rd codon positions RY-coded, and using two ratite and three nonratite calibration priors (*SI Text* and Table 1). This date is less than half the age of the 15 Ma (95% CI 14.5–15.6 Ma) date inferred using penalized likelihood in r8s used by Baker et al. (9), which we also recovered in r8s analyses of the mtg-10,692 dataset using standard nucleotide coding (15.8 Ma). Importantly although, this estimate drops to 8.1 Ma when RY-coding is used to reduce the impact of saturated 3rd codon positions (*SI Text*). The estimate drops further, to 7.6 Ma, if the problematic calibration date of 82 Ma for the beginning of rafting between New Zealand and Australia/Antarctica is discarded in favor of other, more secure, avian calibrations (*SI Text*). Because this geological event would not prevent the dispersal of flying paleognaths, it is now questionable (3).

It appears that the differences between our analysis and that of Baker et al. (9) are not primarily with the programs used, but rather the use of the saturated 3rd codon positions and the problematic 82 Ma calibration. When these issues are dealt with, r8s infers a date of 7.6 Ma, well within the 95% HPD of the BEAST analysis. Overall, we prefer the BEAST estimate to those of r8s because the latter assumes autocorrelated rate changes among branches, which

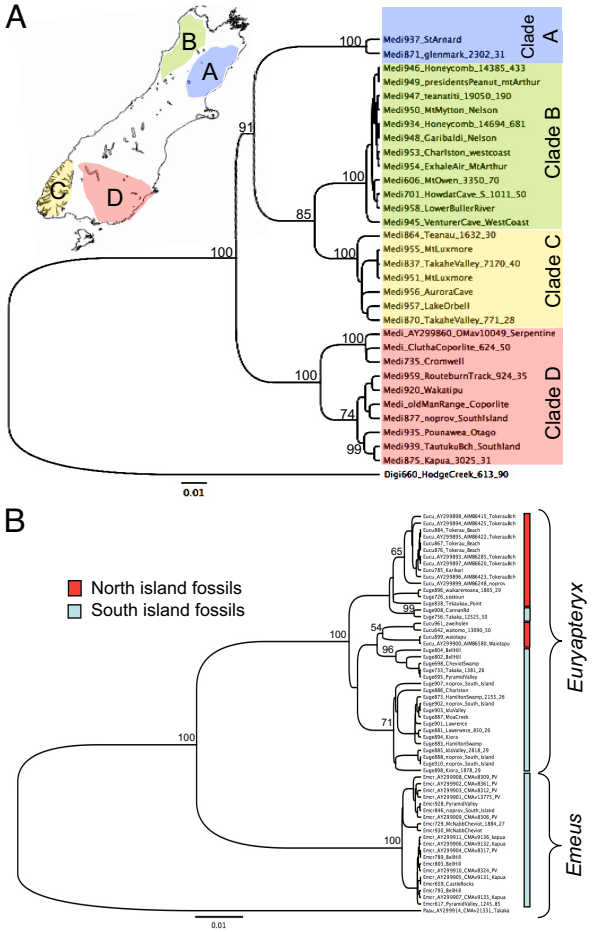

Fig. 4. Phylogenetic reconstruction of *Megalapteryx* (*A*) and *Emeus*/*Euryapteryx* (*B*) using mtDNA control region data. Localities of the moa fossils are indicated on the tip labels together with ages of fossils (AMS ^{14}C dates and error) where known. The original morphological identifications are given as a 4 letter abbreviation, with sample, museum, and GenBank numbers where relevant. The trees shown here are MAP trees generated in BEAST (*Methods*) using *Dinornis* or *Pachyornis*, respectively, as outgroups. Posterior probabilities are shown on nodes with support. Deep phylogenetic splits in *Megalapteryx* (*A*) are caused by prolonged periods of geological isolation resulting in four distinct South Island clades (A-D). The lack of diversity in *Emeus* and lack of North-South monophyly in *Euryapteryx* demonstrates a complex evolutionary history involving bottlenecks and periodic gene flow between the North and South Islands in the Pleistocene. The phylogenetic position of North Island *Euryapteryx* specimens supports a southern origin for this species.

is not supported by covariance analysis within our BEAST analyses. Consequently, we calibrated the larger moa mtg-2,153 bp dataset with the lognormal approximation of the posterior distribution for the Dinornithidae/Emeidae split from the mtg-10,692 analysis, and used this to estimate a variety of divergence dates within moa evolution (Table 1). In line with the above results, the dating estimates described in Table 1 are considerably more recent than previously suggested (11, 19). Interestingly, the new 5.8 Ma estimate for the basal divergence of *Megalapteryx* correlates closely with the rapid phase of mountain uplift during the Miocene-Pliocene. It is also notable that there is no moa fossil evidence (26) that contradicts the timeframe proposed here.

Internally calibrated time-stamped data. BEAST analysis of the radiocarbon-dated *Megalapteryx*, *Dinornis*, and *Pachyornis* control region sequences (see *Methods* and *SI Text*) provided an estimated mutation rate of 8.7% per million years (95% HPD 2.34–20.4%). The

Table 1. Estimated dates for moa speciation events

Dataset	Taxon split	Date, Mya	
		Mean	95% CI
mtg-2,153	*Megalapteryx*/others (moa root)	5.81	4.10–8.04
mtg-10,692 (12n3ry)	Dinornithidae/Emeidae (MRCA)	5.27	3.82–7.02
mtg-2,153	Emeidae (MRCA)	3.33	2.12–4.57
mtg-2,153	*Megalapteryx* (MRCA of clades)	1.98 (1.28)	1.05–3.00 (0.218–2.6)
mtg-2,153	*Dinornis* (MRCA)	1.45 (1.20)	0.81–2.21 (0.34–2.16)
mtg-2,153	*Pachyornis* (MRCA)	1.91 (0.93)	1.18–2.80 (0.14–1.95)
mtg-2,153	*Anomalopteryx*/*Emeus* + *Euryapteryx*	1.80	1.04–2.59
mtg-2,153	*Emeus*/*Euryapteryx*	1.35	0.79–2.02

BEAST analyses of mitochondrial protein coding data (mtg-2,153 bp) were calibrated with the posterior distribution of the Dinornithidae/Emeidae split generated from the avian mtg-10,692 dataset as described in *Methods* and *SI Text*. Divergence estimates obtained from BEAST analysis of control region sequences from radiocarbon-dated bones of *Pachyornis*, *Dinornis*, and *Megalapteryx* are shown in brackets. Mya, million years ago; SI, South Island; NI, North Island; CI, confidence interval; MRCA, most recent common ancestor.

large error surrounding this estimate results largely from the small temporal range of the moa specimens analyzed (1,000 to 19,000 years). Analyses using a relaxed molecular clock did not markedly alter the estimates or confidence intervals.

A short-term mutation rate estimate for the control region of ≈8.7% per million years is lower than estimated for other bird species, but may reflect both the longevity and low mass-specific metabolism expected from such large birds. However, the temporal dependency of rate calculations within this time frame make accurate dating particularly difficult (17, 18). Within these limitations, the BEAST control region divergence estimates are similar to the mtg-2,153 analysis (see Table 1). Clearly, a better understanding of the temporal dependency of the control region mutation rate will require sequences from many more Pleistocene-aged specimens from all moa genera. Therefore, the 8.7% CR mutation rate estimate must be used with caution, although it is a useful starting point for temporal reconstructions and estimates of effective population size.

New Zealand Biogeography and Moa Phylogeography. A new model of the paleogeographic development of central NZ through the Neogene (*ca.* the last 23 Ma) is depicted in a series of simplified maps in Fig. 2 and Figs. S2–S4, outlining the relationship between land and marine environments. These maps are based on new geological mapping of central North Island (King Country, Taranaki, and central Hawke's Bay) and the analysis and interpretation of sedimentary facies of mostly marine stratigraphic units exposed onshore, or encountered in hydrocarbon exploration drill holes in both onshore and offshore areas (*SI Text*). As a result of extensive Pliocene-Pleistocene domal uplift of central North Island (27) and associated erosion (28), marine units of Neogene age, particularly those of Early and Middle Miocene age, were formerly more extensive. The drawing of the paleogeographic maps required a certain amount of interpretation and extrapolation, based partly on the location of marine basins through time. The paleogeographic maps are also based on a new understanding of the Neogene tectonic evolution of NZ (29). The shifting location of the eastern North Island (present shoreline shown for reference) is partly schematic as there is considerable uncertainty about the relative positions of its various tectonic blocks through the Neogene, although the extent of marine basins is well established (30). A more detailed discussion of the Neogene paleogeography can be found in the *SI Text*.

The revised temporal framework for moa evolution permits a reevaluation of the biological impacts of the geological and ecological history of NZ, especially during the Neogene (last 23 Ma). The "Oligocene Drowning" hypothesis (31) suggests that a pronounced marine transgression in the Oligocene (*ca.* 30–21 Ma) reduced the mitochondrial diversity of moa (and many other biota) potentially down to a single lineage. During the transgressive high sea stand around 23 Ma, NZ is suggested to have been reduced to a few scattered islands, including a limited landmass in present day Northland and a larger southern island (Fig. 2, Fig. S3, and *SI Text*). The survival of many endemic vertebrates preserved as early Miocene fossils strongly argues against total submergence (32), and eggshell and bone remains from the lower-middle Miocene (19–16 Ma) Manuherikia Group Formation indicate the presence of at least two large flightless ratite taxa within a few million years of the drowning event (33). Analysis of the subsequent reemergence of the landmass reveals several major, and previously undetected, implications for the evolution of NZ's terrestrial biota. Paleogeographic reconstructions show that as the North Island reemerged in a progressively southward direction from modern day Northland, it was continuously separated from the larger South Island landmass by a major marine seaway (the Manawatu Strait) from *ca.* 30 Ma until ≈1.5–2 Ma (Fig. 2, Fig. S3, and *SI Text*). Importantly, this implies that endemic terrestrial biota incapable of dispersing across a major marine barrier would have been geographically isolated for >25 Ma. Furthermore, the endemic North Island biota would presumably descend from taxa that had survived the marine transgression on the limited emergent Northland landmass. Recently detected phylogeographic patterns in NZ skinks are consistent with this model and indicate an early Miocene vertebrate fauna on the North Island (34).

Within the moa phylogeny there are no genetic splits that might correspond to a >25 Ma separation (Table 2), indicating that the Quaternary moa lineages appear to have been descended from populations occupying only one island. In light of the molecular dates, the South Island Manuherikia Group fossils (33) suggest that the progenitors of the three moa families probably derive from a single South Island ancestral moa lineage which had survived the Oligocene Drowning event. A South Island ancestral moa lineage is also supported by the phylogeographic distributions of species and specimens in Fig. 3 and Fig. 4*B* (and *SI Text*) where the majority of North Island taxa and clades occupy derived phylogenetic positions. Discoveries of tuatara (35), acanthisittid wrens (36), and kauri (37) from similar South Island fossil deposits, and a parallel lack of deep genetic splits in these taxa imply that the recent evolutionary history of many iconic NZ endemics was also on the South Island. This latter observation raises important questions about the nature of terrestrial biotic diversity on the North Island from >25 Ma to *ca.* 1.5–2 Ma, at which point a major biotic interchange is likely to have taken place.

The Origin of Diversity within the Dinornithiformes. The rapid morphological radiation of the moa is matched by other island endemics

such as the moa-nalos of Hawaii (38), and stands in contrast to the fossil record of many volant species in NZ and Australia (33). The timing of the moa radiation appears to correlate closely with the formation of the Southern Alps, and concomitant increase in habitat diversity across NZ, *ca.* 5 Ma (39). Although the transpressional Alpine Fault became active in the early Miocene (*ca.* 20 Ma), the uplift resulting in the Southern Alps only began to accelerate *ca.* 5–8.5 Ma (see discussion in *SI Text*). The Alps act as a barrier to the circumpolar (westerly) airflow creating wet rainforests to the west, and dryer, warmer climate to the east. Therefore, accelerated uplift generated both upland versus lowland environments, and wet versus dry habitats, forming a mix of forest, shrubland, and grasslands. In this regard, it is important to note that NZ did not experience the pronounced Miocene shift toward aridification detected elsewhere in the world (40, 41). If the niche specialization of the recent species reflects ancestral states, it is possible that the first divergence in the moa tree (*Megalapteryx ca.* 5.8 Ma) was because of the evolution of upland (*Megalapteryx*) and lowland (*Dinornis*/Emeid) ecotypes, whereas the subsequent increase in habitat diversity was the trigger for speciation within Emeidae.

Island Allopatry and Pleistocene Cycles. Following the initiation of moa family-level diversity in the early Pliocene (*ca.* 3.3–5.8 Ma), phylogeographic analyses (Figs. 3 and 4 and Fig. S1) suggest that a further cycle of intrageneric diversity was created by the movement of southern endemics into the North Island *ca.* 1.5–2 Ma, as the Manawatu Strait started to close and land links first became possible (Fig. 2). The subsequent formation of the seaway now known as Cook Strait is a geologically young event that first formed in a mid-Pleistocene interglacial period (*ca.* 450 kyr) (42) and prevented gene flow between each island apart from during periods of lowered sea levels during major glaciations (*SI Text*).

An additional consequence of the mountainous topography and rain-shadow zones in both islands is that NZ was able to sustain diverse habitats and refugia throughout the Pleistocene glacial and interglacial periods, despite its comparatively small size (43). The Pleistocene was dominated by long (*ca.* 100 kyr), cold climatic periods during which grasslands and shrublands were the main vegetation, interspersed with shorter (10 kyr), warm, forest-dominated phases (44). The divergence date estimates in Table 1 suggest that the Pleistocene climate shifts were instrumental in shaping the distribution of moa species and moa clades within NZ. To explore this issue further, we examine three genera in detail to illustrate the role of Pleistocene niche stability in the maintenance of genetic diversity.

Emeus. A striking feature of Fig. 4*B* is the lack of significant mtDNA phylogeographic diversity or structure in *Emeus crassus*, a species that is abundant in Holocene fossil deposits. This observation potentially relates to the drastic reduction of *Emeus'* favored habitat of wet forests during glaciations, potentially to a single geographic region somewhere in the south-eastern South Island during the last glaciation (Otiran) (*ca.* 75 to 14 kyr). During the Holocene, a rapid increase in this habitat is likely to have led to a rapid population expansion. This model of a severe bottleneck, followed by a rapid expansion would account for the abundance of fossil remains, but an extremely restricted genetic diversity compared with the other moa genera.

Euryapteryx. The phylogeographic patterns seen in *Euryapteryx* are more complex than observed in other moa species, and probably reflect several dispersal events between the North and South Islands. *Euryapteryx curtus* is the only moa taxon found on both islands that does not exhibit North/South monophyly, suggesting a relatively recent interchange between the islands. The largely coastal lowland habitat of this species makes it one of the more likely to have utilized Pleistocene land bridges. The date estimates and tree topologies in Table 1 and Figs. 3 and 4 suggest that the common ancestor of *Emeus* and *Euryapteryx* diverged in the South Island shortly after the initial island connections (*ca.* 1.5–2.0 Mya), with *Euryapteryx* subsequently diversifying into several major clades. During two different glacial low sea stands (very roughly 450 kyr and 100 kyr), *Euryapteryx* haplotypes appear to have dispersed to the North Island, with the phylogeographic distribution suggesting that in the penultimate glacial this movement occurred via a land bridge on the western side of Cook Strait.

Megalapteryx. The evolutionary history of the South Island upland specialist species *Megalapteryx didinus* is not well understood, with only two specimens included in a previous genetic study of moa taxonomy (9). Fig. 3 and Fig. 4*A* reveal large amounts of genetic diversity in *Megalapteryx* relative to other moa genera, which likely relates to the persistence of upland/montane habitat during both glacial and interglacial periods. Despite the deep genetic diversity, there is presently no known parallel morphological diversity, and we consider all four clades members of a single species (see *SI Text*). The geographic boundaries separating the four clades (A-D, Fig. 4*A*) correspond to the presence of known barriers which effectively split the uplands of the South Island into four regions during the Pleistocene (*SI Text*). During glacial cycles, extensive ice fields covered the Southern Alps and glaciers flowed outwards from the mountains at numerous points. In central South Island the narrow West Coast was split by glaciers extending to near sea level, while on the eastern side of the Alps major glaciers extended to the lowlands and were accompanied by extensive glacial outwash plains. Interestingly, comparison of the *Megalapteryx* phylogeny with that of the endemic giant invertebrate alpine scree weta (43) demonstrates striking similarities in the phylogeographic structure of the clades, suggesting common barriers to geneflow between these two upland species.

Clearly the complexity of the genetic patterns seen in the different moa taxa provide strong evidence that the use of universal genetic distance(s) to define meaningful taxonomic units is not viable, and moa should not be used as an example of the effectiveness of DNA barcoding (45) (*SI Text*). Although COI mtDNA signatures clearly discriminate some taxa (as do all mtDNA genes), no single genetic distance can define moa taxa because the genetic diversity of each genus reflects a differing palaeoecological history involving niche persistence, ecology, and population longevity. An accurate model of species diversity is only possible through the integration of a range of data from mitochondrial and nuclear DNA, stable isotopic and ecological signatures, osteology, and morphometrics.

This is the first detailed genetic study of all members of an entire extinct order, and demonstrates the influential role of tectonic uplift, mountain building, and glacial cycles on the evolution and genetic diversity of NZ terrestrial endemics. The data clarifies moa taxonomic relationships that have remained problematic for >160 years, and suggests that the glacial landscape allowed the rapid diversification of a single moa lineage that survived the Oligocene Drowning. This pattern appears likely to be widespread within other extant NZ endemics. The important new geological model of Neogene NZ emphasizes our current lack of knowledge about the pre-Pliocene landscape, and raises important questions about the role of marine barriers and the biotic diversity of the north and south islands. The combined geological and genetic data suggests that the NZ Neogene terrestrial record is likely to have been marked by the significant loss of terrestrial endemics from a highly unusual environment, which is only just beginning to be characterized.

Materials and Methods

Samples, DNA Extractions, PCR, and Sequencing. A detailed list of the samples used in this study (location, museum numbers, ^{14}C dates) and extraction methods [after (11)] can be found in Table S3 or in previous publications (9–13, 19). PCR was used to amplify two overlapping sections of mitochondrial control region (totaling ≈389 bp, depending on genus) from the moa samples, and sequencing was performed using an ABI 310 or 3730. Strict ancient DNA (aDNA) guidelines were followed to minimize contamination of samples with exogenous DNA, including

multiple negative extraction and amplification controls to detect contamination. All DNA extractions and PCRs were set-up and performed in a geographically isolated, specialist aDNA laboratory. Three bone subsamples were sent to Copenhagen for independent ''blind'' replication, and the resulting sequences matched those previously generated.

Sequence Analysis. The 263 aligned moa control region sequences (389 bp, FASTA file in *SI Text*) were used to jointly estimate a phylogenetic tree and substitution model parameters using Metropolis-Hastings Markov chain Monte Carlo (MCMC) integration. ModelTest 3.06 (46) recommended an HKY+G+I model of substitution for the control region sequences under the Akaike Information Criterion. The tree presented in Fig. 3 (also see Fig. 4 and Fig. S1) is the MAP tree (assuming a ''strict'' molecular clock) obtained from the MCMC analysis (two independent runs each comprising 50 million generations) using BEAST (20). No explicitly informative priors were used in the analysis, so the tree will correspond closely to a maximum likelihood (ML) estimate of the tree under the same model of substitution. Detailed phylogenies for three moa genera shown in Fig. 4 were generated as described above, using just the sequences for these taxa plus an outgroup, as detailed.

Timescales for moa evolution were estimated with BEAST v.1.4.8 (20), using 40 million generations. To compare our date estimate for the Dinornithidae/Emeidae divergence with those from Baker et al. (9), we also inferred dates from the mtg-10,692 dataset using r8s (47) under penalized likelihood (see *SI Text*).

For the internally calibrated approach, 80 *Megalapteryx*, *Dinornis*, and *Pachyornis* control region sequences (389 bp) with associated radiocarbon ages (Table S3) were used as calibration points (or time-stamped data) and analyzed with BEAST using priors for the mutation rate (2% to 50% per million years), and the maximum (oldest) age of each genus based on the mtg-2,153 analysis (*SI Text*). The results are presented in Table 1.

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