

# Moa were many

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**Until a few hundred years ago, New Zealand hosted several species of flightless ratite birds, collectively known as the moa. We have used mitochondrial sequence data for *Dinornis*, the largest of the moa, and new coalescent approaches to estimate its population size prior to human arrival *ca.* 1000–6000 years BP. We show that, as little as 1000 years ago, *Dinornis* numbered between 300 000 and 1.4 million and that the standing population of all moa species was *ca.* 3–12 million. This estimate is an order of magnitude larger than the accepted population estimate (*ca.* 159 000) for all moa species at the arrival of humans and suggests that moa numbers had already declined prior to human settlement.**

**Keywords:** *Dinornis*; mtDNA; coalescent; population size; extinction

## 1. INTRODUCTION

New Zealand's moa (Aves; Dinornithiformes) have been the subject of much scientific scrutiny since being described by Owen in 1839 (Owen 1840). Such has been the interest in these lost giants that we now know more about the biology of these extinct ratites than we do about many extant taxa (Anderson 1989; Worthy & Holdaway 2002). Recently, two studies of ancient DNA (Bunce *et al.* 2003; Huynen *et al.* 2003) have shown that there were distinctly different species of moa on the North and South islands of New Zealand as little as 1000 years ago, and that three previously recognized *Dinornis* species were sexually dimorphic forms of the same species—the females very much larger than the males. These papers dealt with the controversy surrounding the number of moa species, which has seen the number of recognized species progressively reduced from 38 to 11 (Cracraft 1976), and now to 10 (Bunce *et al.* 2003; Huynen *et al.* 2003). However, an unresolved controversy remains, which is the size of the moa population before humans arrived—an important question to address when trying to gauge whether moa were already in decline prior to their subsequent rapid extinction by hunting and habitat loss (Anderson 1989; Holdaway & Jacomb 2000) following the arrival of humans (Anderson 1989; Worthy & Holdaway 2002).

Population geneticists have long recognized that genetic variation increases with population size (Wright 1931) and that, under neutral models of evolution, levels of genetic variation can track population trends across deep

ecological time (Kimura 1983). For the maternally inherited mitochondrial DNA (mtDNA), the relation between genetic diversity,  $\theta$ , and the long-term effective female population size,  $N_{e(f)}$ , is  $\theta = 2\mu N_{e(f)}$ , where  $\mu$  is the mutation rate per generation. Migration, fluctuations in population size, selection and population structure all affect levels of genetic variation, but now a maximum-likelihood method exists that simultaneously estimates  $\theta$  and migration rates for multiple populations (Beerli & Felsenstein 1999, 2001). This tool has been employed to estimate historic population sizes for a range of species, including whales in the North Atlantic (Roman & Palumbi 2003).

We have used mtDNA sequence data for *Dinornis* (Bunce *et al.* 2003; Huynen *et al.* 2003), the largest of the moa, and new coalescent approaches (Beerli & Felsenstein 2001) to estimate its population size prior to human arrival *ca.* 1000–6000 years BP (Bunce *et al.* 2003; Huynen *et al.* 2003). We show that, as little as 1000 years ago, *Dinornis* species could have numbered in the hundreds of thousands and that the standing population of all moa species could have been in the millions. This estimate is more than an order of magnitude larger than the currently accepted population estimate (*ca.* 159 000) (Holdaway & Jacomb 2000) for all moa species at the arrival of humans and adds support to the idea that moa numbers had already declined prior to human settlement.

## 2. MATERIAL AND METHODS

Moa control region sequences for *Dinornis* spp. were obtained from GenBank submissions AY326127–57 (Bunce *et al.* 2003) and AY299861–92 (Huynen *et al.* 2003). These sequence data are derived from the conserved central region of the control region, and do not contain any indels that might otherwise bias analyses dependent on an infinite-sites nucleotide-site mutation model, such as the Beerli-Felsenstein method implemented in MIGRATE (Beerli & Felsenstein 2001). These data were aligned and tested for neutrality in MEGA v. 2.1 (Kumar *et al.* 2001) using Tajima's neutrality test (Tajima 1989). Next, we estimated genetic diversity,  $\theta$ , from our data for North and South Island populations using MIGRATE v. 1.7.3, which uses a maximum-likelihood-based coalescent approach to estimate  $\theta$  and gene flow, together with 95% confidence limits (Beerli & Felsenstein 2001). Start parameters were generated from initial  $F_{ST}$ -calculations undertaken in MIGRATE under the default settings. MIGRATE was then run several times using the parameter estimates derived from the previous run, and different random number seeds to determine whether our results were consistent between runs. This consistency was established, so we then used our approximation of  $\theta$  to calculate the effective female population size ( $N_{e(f)}$ ) by solving  $\theta = 2\mu N_{e(f)}$  for  $N_{e(f)}$ . We employed two estimates of mutation rate ( $\mu$ ) for the mitochondrial control region in our calculations of  $N_{e(f)}$ . The first,  $2.08 \times 10^{-7} \text{ bp}^{-1} \text{ yr}^{-1}$ , was derived from a phylogenetic study (Quinn 1992), while the second,  $9.6 \times 10^{-7} \text{ bp}^{-1} \text{ yr}^{-1}$ , was obtained from a study of ancient DNA (Lambert *et al.* 2002). Both rates are very high, being, respectively, 10 and 50 times greater than the widely accepted mutation rate of  $2 \times 10^{-8} \text{ bp}^{-1} \text{ yr}^{-1}$  for mtDNA-encoded protein-coding genes (Lovette 2004). However, we employed these because the use of a high  $\mu$  results in conservatively lower estimates of  $N_e$ . Estimates of  $N_e$  were then converted to  $N_c$ , total census size, using a three-step approach described in § 3.

## 3. RESULTS AND DISCUSSION

We used mtDNA control-region sequences published previously (Bunce *et al.* 2003; Huynen *et al.* 2003) to calculate values of  $\theta$  for North Island ( $n = 21$ ) and South Island ( $n = 36$ ) forms of the largest, most widely distributed and best represented of the moa, *Dinornis* spp. (Worthy & Holdaway 2002). We have estimated effective female population numbers,  $N_{e(f)}$ , for *Dinornis* spp. on each island, using our values of  $\theta$  together with estimates of mutation

Table 1. Historical population size estimates based on genetic diversity. CI, confidence interval.

population	$n$	$\mu$ (bp <sup>-1</sup> yr <sup>-1</sup> )	$\theta$ mean (95% CI)	$N_{e(f)}$ ( $\times 10^3$ ) (95% CI)	$N_C$ census population ( $\times 10^3$ ) (95% CI)
North Island	21	$2.08 \times 10^{-7}$	0.094 (0.0734–0.1700)	227 (176–409)	930 (720–1668)
North Island	21	$9.8 \times 10^{-7}$	0.0944 (0.0734–0.1700)	48 (37–87)	197 (153–354)
South Island	36	$2.08 \times 10^{-7}$	0.0488 (0.0328–0.0707)	117 (79–170)	479 (321–694)
South Island	36	$9.8 \times 10^{-7}$	0.0488 (0.0328–0.0707)	25 (17–36)	102 (68–147)

rates,  $\mu$ , derived from phylogenetic studies (Quinn 1992) and studies of ancient DNA (Lambert *et al.* 2002; table 1). From these data we have determined  $N_C$  from  $N_{e(f)}$  using three conversions described previously (Roman & Palumbi 2003; table 1). First, we converted  $N_{e(f)}$  to total effective population size,  $N_e$ , using an average male-to-female sex ratio for *Dinornis* of 1:1.4 (Bunce *et al.* 2003). Next, we converted  $N_e$  to the number of breeding adults,  $N_A$ , using the observation that the  $N_A:N_e$  ratio approaches 2.0 in most populations with a constant population size (Nunney & Campbell 1993). Finally, we used estimates of the proportion of adults and juveniles derived from the fossil record to convert  $N_A$  to  $N_C$ , the total census population size. To convert  $N_A$  to  $N_C$  we used age-structure data for 55 *Dinornis* skeletons found at Pyramid Valley, which suggest that ca. 84% of *Dinornis* populations were adult, with the remaining 16% consisting of sub-adults (2%) and juveniles (14%) (Worthy & Holdaway 2002).

The genetic diversity,  $\theta$ , observed in *Dinornis* suggests a population size of up to 479 000 on South Island, with almost twice as many, 930 000, on North Island (table 1). These data indicate a standing population for *Dinornis*, across both islands, of between 299 000 and 1 409 000, much larger than the previous upper bound for all moa species of 159 000 at human arrival, which was based on a regional analysis of distribution and abundance (Holdaway & Jacomb 2000).

This result has major implications for our understanding of the processes leading to the extinction of the moa because it implies that *Dinornis*, and presumably moa in general, declined from a collective population size in the millions, as little as 1–6000 years BP, to ca. 159 000 at human settlement ca. 1280 AD (Holdaway & Jacomb 2000).

However, given both that our estimated population size is so much higher than previous estimates (Anderson 1989; Holdaway & Jacomb 2000) and our estimate of long-term population numbers is based on measurements of genetic diversity,  $\theta$ , the factors that might influence  $\theta$  need to be carefully assessed to ensure the veracity of our estimates of population size before further discussion of its implications. Population structure can increase  $\theta$ , so several strongly differentiated populations, mistakenly analysed together, may result in a higher cumulative  $\theta$  than if analysed separately (Roman & Palumbi 2003). Phylogenetic analyses of the *Dinornis* control region data (Bunce *et al.* 2003; Huynen *et al.* 2003) indicate modest levels of population structure and haplotype diversity within islands but significant genetic structure between islands. Combined analysis of the North Island and South Island moa data in MIGRATE also reveals strong genetic structure and no sig-

nificant gene flow between the two islands. Consequently, we have taken a conservative approach and analysed the North and South Island data separately to minimize the value of  $\theta$  obtained.

In our case, the sampling of populations across a temporal time frame of 1–6000 years BP may also produce high estimates of  $\theta$ , leading to an inflated estimate of population size. However, given the effective neutrality of the data (according to Tajima's neutrality test; Tajima 1989), the probability of significant lineage sorting within a temporal frame of 5000 years is low, so our estimate of population size for *Dinornis* is probably valid.

Another potential problem is that the choice of a low mutation rate will tend to overestimate population size from genetic diversity. This is particularly problematic for our study, as recent work suggests that extracts of ancient DNA can have an inhibitory and mutagenic effect under PCR (Pusch & Bachmann 2004), which implies that our estimates of genetic diversity may be artificially high just by the nature of the DNA template used. Here, again, we have reduced the influence of this parameter by being conservative and choosing a range of mutation rates,  $\mu$ , for the mitochondrial control region of moa of  $2.08$ – $9.6 \times 10^{-7}$  bp<sup>-1</sup> yr<sup>-1</sup> (Quinn 1992; Lambert *et al.* 2002); these rates are respectively 10–50 times greater than the widely accepted  $2 \times 10^{-8}$  bp<sup>-1</sup> yr<sup>-1</sup> for protein-coding genes (Lovette 2004).

Assuming that our estimate of population size for *Dinornis* is accurate, and that the population sizes of the other nine smaller and more common moa species are, on average, at least the same as *Dinornis*, we estimate that the long-term population numbers for moa in New Zealand were somewhere in the vicinity of 3–12 million. *Dinornis* spp., while ubiquitous, is far from the most abundant of the moa species found in Holocene fossil sites (making up 4–17% of moa fossils; Worthy & Holdaway 2002), so our estimate for the total pre-human moa population is probably cautiously low.

The obvious question we ask is what drove moa populations down from 3–12 million to ca. 159 000. The demise of the moa has been the subject of much debate. Some have blamed climate change, whereas others have suggested that moa were in an inexplicable 'terminal decline', owing to some intrinsic flaw, prior to the arrival of humans (Pimm 2002). Neither argument is overly compelling. Mounting evidence shows that all of the common moa species persisted until first contact with humans (Worthy & Holdaway 2002), which thwarts the generally unscientific notion of a 'terminal decline', while the appealing notion of extinction owing to long-term climate change seems improbable since climatic fluctuations in New Zealand over the past

1000 years have been relatively minor in comparison with past events (Worthy & Holdaway 2002).

Volcanism may have had regular and dramatic impacts on moa populations, particularly in North Island. Lake Taupo is known to have had particularly destructive eruptions ca. 3300 and 1850 years BP (Sparks *et al.* 1995). The later event is known to have completely destroyed forest in an 80 km radius and undoubtedly had much wider effects, which may have altered available habitat and food supply for New Zealand's largest terrestrial herbivores over a period of several years, leading to dramatic declines in moa numbers through habitat destruction or through interactions with disease spread.

Disease is a more persuasive explanation for the decline in moa populations prior to human arrival, with a plethora of diseases (e.g. influenza, salmonellosis and tuberculosis) known to occur in ratites (Arnall & Keymer 1975), and epizootics from cross-species transmission now well documented (Friend *et al.* 2001). While as yet mostly unexplored, given the prevalence of epizootics globally, it is likely that New Zealand's avi-fauna were regularly challenged with new pathogens brought in from Australia and elsewhere by migrant birds (Baker 1991) and that many New Zealand bird populations experienced regular population crashes. Some, or all, of the moa species may have been affected by a major epizootic that lowered population numbers drastically prior to, or in conjunction with, the arrival of humans, and it is possible that without the pressure of exploitation and habitat loss moa numbers would have rebounded after such an event. If such epizootics occurred, they might have left a detectable signature in the genomic architecture of affected species and as techniques improve perhaps future studies of ancient DNA will be able to answer this question.

The demise of the moa is complex and veiled by time. No one can deny the dramatic effect of human settlement and the exploitation and habitat changes that followed on New Zealand's fauna (Worthy & Holdaway 2002). However, if our new estimates of moa numbers are correct then we need to reconsider the factors that might have influenced these populations prior to the arrival of humans, perhaps gaining greater insight into modern conservation problems from the lessons of the past.

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