Disentangling the impacts of climate and human colonization on the flora and fauna of the Australian arid zone over the past 100 ka using stable isotopes in avian eggshell

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Abstract

Throughout the Quaternary, the flora and fauna of Australia evolved and adapted to the high-amplitude, low- and high-frequency climate changes that characterized the ice-age cycles. However, during the last glacial cycle, between ~120 and 15 ka, unprecedented irreversible changes in flora and fauna occurred, and in that same interval modern humans established their first firm presence in the landscape. Disentangling the impacts of the first-order trend toward a colder, drier planet through the Late Quaternary from the impacts of human colonization has been challenging, from both the chronological and paleoenvironmental perspectives. We utilize the stable isotopes of carbon and oxygen preserved in near-continuous time series of *Dromaius* (emu) eggshell from five regions across Australia to provide independent reconstructions of ecosystem status and climate over the past 100 ka. Carbon isotopes are determined by the diet consumed by the female bird, whereas oxygen isotopes record the status of local moisture balance in the months prior to breeding. Together, $\delta^{13}\text{C}$ and $\delta^{18}\text{O}$ provide ecosystem status and climate from the same dated sample, reducing correlation uncertainties between proxies. Combined with recent improvements in the chronologies of Late Quaternary shorelines fringing inland lake basins and deflation during arid times, these data collectively reaffirm that Australia generally became increasingly, albeit irregularly, drier from the last interglaciation through to the last glacial maximum. *Dromaius* eggshell $\delta^{18}\text{O}$ documents peak aridity between 30 and 15 ka, but shows no evidence of exceptional climate change between 60 and 40 ka. In contrast, *Dromaius* $\delta^{13}\text{C}_{\text{diet}}$ documents an irreversible loss of the majority of palatable summer-rainfall-related C_4 grasses across the Australian arid zone between 50 and 45 ka, about the same time that the giant megafaunal bird, *Genyornis*, became extinct, and coincident with human dispersal across the continent. Our data indicate that changes unique to Australia occurred between 50 and 45 ka that led to a new climate-vegetation relationship and an overall reduction in effective moisture across much of the continent. The large summer-rainfall-dominated lakes of interior Australia failed to re-fill subsequently, despite a wide range of global climate states. A full explanation for the mechanisms behind these changes remains elusive, but they are almost certainly related to human agency. Plausible explanations include a change in fire regime resulting from human-lit fires, a change in fire regime following extinction of megafaunal browsers, and/or a threshold response to increasing aridity. Of these, the climate change explanation is least likely, given the lack of evidence for unprecedented aridity between 60 and 40 ka, and the successful adaptation of Australian ecosystems to 2.5 Ma of similar changes.

1. Introduction

Australia represents a natural laboratory in which the impacts of climate change and human activity may be evaluated in near isolation from other continents. As mammals began their ascendancy at the beginning of the Cenozoic, the marsupial lineage expanded in South America, with at least one marsupial line migrating across a forested Antarctica and on to the Australian continent by ~65 Ma (Woodburne and Case, 1996). Soon after, continental drift severed Australia from Antarctica, initiating the continent's long isolated drift northward, carrying only marsupial mammals and a remnant Gondwanan biota. Since the early Eocene thermal maximum (~50 Ma) Earth has been gradually, although irregularly cooling (Zachos, 2008). Planetary cooling lowered ocean surface-water temperatures, resulting in less atmospheric water vapor, hence less rainfall on a planetary average. As the planet dried, Australia evolved an increasingly open-habitat-adapted fauna in response to the retraction of wet, closed forests and tropical rainforests, and the expansion of grasslands (Martin, 2006; Price, 2012). Australia's drift trajectory eventually brought the continent into the subtropical high-pressure zone, where the descending limb of the Hadley Cell results in reduced precipitation receipts relative to adjacent latitudes. Together, these effects placed additional moisture stress on Australia's biota, with evolutionary adaptations that resulted in a diverse arid-adapted flora and fauna capable of adjusting to reduced surface waters and increased climate variability on millennial and shorter timescales (Byrne et al., 2008).

By the Quaternary, many Gondwanan elements of the flora were limited to relict wet forests in coastal regions, while the flora and fauna of the expanded arid zone adapted to rare heavy rains, frequent long drought, and an erratic fire regime resulting from lightning strikes, most frequently in the build up to the austral summer wet season (Prideaux, 2004; Prideaux et al., 2007; Byrne et al., 2008). As Australia drifted closer to Asia, a few opportunistic small placental rodents arrived, presumably by chance on rafts from the north, as did placental bats that reached Australia through the skies. But well into the last glacial cycle marsupial mammals and a diverse array of reptiles dominated the terrestrial fauna of Australia. Australia also hosted a rich avian fauna, including three large, flightless birds that all were present at 100 ka¹. Two were closely related members of the ratite family of birds, the emu, *Dromaius novaehollandiae*, occupying a wide range of habitats across most of the continent, and the cassowary, *Casuarius casuarius*, restricted to relict rainforest habitats in NE Australia, southern New Guinea and the Aru Islands. The third species, *Genyornis newtoni* (Rich, 1979; Murray and Vickers-Rich, 2004), was the sole remaining representative of the Dromornithidae, a family of non-ratite giant birds endemic to Australia.

In this paper we utilize isotopic proxies for diet and climate archived in the eggshells of these giant birds that document an abrupt and irreversible shift in the structure and function of ecosystems across the Australian arid zone ~50 ka, and that provide independent evidence eliminating climate as a plausible cause of that shift. We utilize the stable isotopes

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¹ We use "ka" to express ages in thousands of years before present (calibrated years when derived from ¹⁴C ages). "Late Quaternary" includes the past 130 ka. Uncertainties, unless identified differently, are $\pm 1\sigma$. Elevations are expressed in Australian Height Datum (AHD), effectively mean sea level.

of carbon and oxygen in eggshell calcite and of carbon and nitrogen isolated from intracrystalline organic compounds to disentangle the impacts of natural climate change from those derived from human agency over the past 100 ka. As we show below, five 100-ka-timeseries of *Dromaius* $\delta^{13}\text{C}_{\text{diet}}$ from discrete regions across the Australian arid zone document a dramatic reduction of C_4 plant elements in *Dromaius* diet in each region between 50 and 45 ka. Complementary records of $\delta^{13}\text{C}_{\text{diet}}$ preserved in eggshell of co-habiting *Genyornis* show no loss of C_4 plants in their diets prior to the species' extinction 47.5 ± 2.5 ka, emphasizing the abruptness of the change. The evolution of climate provided by $\delta^{18}\text{O}$ in the same eggshell fragments used for dietary reconstructions records strong changes in moisture balance over the past 100 ka, but the timings of ecosystem collapse and peak aridity do not coincide in any region. The coincidence in time of a dramatic change in ecosystem status, megafaunal extinction, and human dispersal across Australia, coupled with the lack of evidence for unprecedented climate change during this interval, strongly suggests that the consequences of human colonization of the Australian continent include a dramatic reduction in ecosystem diversity, and potentially a persistent reduction in moisture availability across the arid zone.

2. Background

Through most of the past 100 ka sea levels were low enough that Australia was connected by land with Papua New Guinea in a continent known as Sahul. However, the data in this paper are restricted to within the current coastline of the continent, and we use the term "Australia" in that restricted sense. The dry Australian interior is described in many different ways. In this paper we refer to the broad desert regions that occupy 70% of the continent as the Australian arid zone (Fig. 1), where mean annual precipitation (MAP) is <400 mm, but interannual variability is high, and prolonged droughts are common. This arid zone incorporates regions conventionally defined as both arid and semi-arid. Primary productivity is low across the arid zone, in part because of infrequent and highly variable rainfall, but also because the interior lacks significant nutrient reserves. Arid-zone soil inventories of plant-available phosphorus (<250 kg ha $^{-1}$) and nitrogen (<2 kg ha $^{-1}$) are among the lowest recorded on any continent (Raupach et al., 2001). Deep weathering earlier in the Cenozoic and the lack of significant geologic uplift has removed most nutrients from the underlying regolith accessible to plants. The combination of low and unpredictable rainfall amounts, exceptionally low soil nutrients, and little chance of nutrient replenishment from the substrate or from atmospheric transport, results in a landscape unusually sensitive to disturbance.

From an ecosystem perspective, climate constraints on the Australian biota are more closely linked to changes in moisture availability than to changes in temperature, with the two most important moisture sources related to the summer monsoon (northern Australia), and precipitation derived from the westerly circulation (southern Australia). Short-term variability is linked to changes in ENSO, as the western Pacific Warm pool and throughflow into the eastern Indian Ocean influence moisture transfer to the atmosphere across northern Australia.

The most obvious change to the Late Quaternary Australian fauna was the disappearance of its megafauna, dominantly browsers, but also most large carnivores. Dating

megafaunal extinction has been challenging, especially for those taxa rarely preserved in the fossil record. The timing of extinction for megafauna taxa infrequently preserved in the geological record (Wroe and Field, 2005) is not explored here. Rather, we accept that for those megafaunal elements most common in the fossil record and with clear extinction timelines, all disappeared sometime between 60 and 40 ka (Miller et al., 1999; Roberts et al., 2001; Prideaux, 2010; Roberts and Brook, 2010; Johnson et al., 2016; Miller et al., 2016a; Saltr et al., 2016). The reduction in foraging pressure that occurred following the loss of large browsing herbivores likely resulted in complex impacts, most logically beginning with the expansion of trees and shrubs, but possibly evolving into changes in the natural fire regime (Flannery, 1990; Bond and Keeley, 2005; Lopes dos Santos et al., 2013). The reduction of large predators and coexisting megafaunal herbivores may have impacted the distribution and density of animals that remained.

The arrival of humans on the Australian continent represents the first time a large placental mammal established a significant population across Australia. More recently, human colonization of New Zealand and Madagascar impacted their flora and fauna in complex and often unpredictable ways (Barnosky et al., 2004). In the Americas, where Late Pleistocene megafaunal extinction is broadly synchronous with human dispersal, it is also synchronous with a major climate perturbation, complicating assignment of cause despite precise geochronologies and unequivocal evidence of predation. In Australia, where the timing of extinction is far earlier, the task of separating the footprint of human colonization on the flora and fauna of Australia from that of climate change remains challenging (e.g. Bird et al., 2013). A resolution of these challenges is the focus of this paper.

3. The Late Quaternary of Australia

Continuous reconstructions of Australia's terrestrial environment over the past 130 ka come from relatively few sedimentary archives that are restricted to the better-watered peripheral regions of the continent, and from marine records recovered on adjacent continental shelves. The lack of continuous sediment accumulations and the poor preservation of organic remains, including pollen, across most of the arid zone leaves large gaps in any attempt to reconstruct how Late Quaternary climate and environments changed across 70% of the continent.

Pollen-derived reconstructions of vegetation change are most consistent with a primary climate driver linked to orbital variability and changes in Northern Hemisphere (NH) continental ice sheets. The 230 ka pollen-based record from Lynch's Crater, in the humid tropics of northeastern Queensland, shows low-frequency variability that closely matches tilt and precession frequencies of ice-age cycles (Kershaw et al., 2007). Pollen preserved in marine sediment cores recovered around northern Australia that span the past 150 ka to 300 ka similarly show a dominant correspondence with orbitally paced ice-volume changes, with wetter conditions during interglaciations and drier times when ice volumes increased (Kershaw et al., 2003; Kershaw et al., 2006; Van der Kaars et al., 2006). The strong correlation between ecosystem change from sites in and around Australia with orbitally driven ice-volume change suggests that the ice-volume curve can be used as a first-order template for the evolution of Australia's climate through the Late Quaternary.

Sub-orbital variability ($<10^4$ years) is likely linked to changes in ocean circulation and variations in monsoon flow and westerly circulation operating at lower amplitudes than those

on precessional time scales and longer. Abrupt climate oscillations observed in Greenland ice cores during the last glacial cycle were generally muted in the Southern Hemisphere (Stocker, 1998), but with clear impacts on the Indo-Australian summer monsoon (Muller et al., 2012; Ayliffe et al., 2013; Denniston et al., 2013b).

Across the arid zone numerous basins that have been dry or ephemeral during the historic period, preserve well developed shorelines, often at multiple levels, that document past times of more effective delivery of moisture to the continental interior. Although they are discontinuous, shorelines, along with thick sequences of lake sediment and sedimentary evidence of more active inflowing rivers, provide unambiguous signals of wetter climate states throughout the arid zone during the Late Quaternary. Hesse et al. (2004) suggest that during MIS 4-2 reduced monsoon rainfall characterized northern Australia records, whereas changes in temperature and moisture content of the westerly circulation dominate southern Australia records. Magee et al. (2004) and Cohen et al. (2015) show that Kati Thanda-Lake Eyre (Fig. 2), which receives its moisture from the monsoon-watered north, was a deep, permanent lake in MIS 5, but became intermittently dry subsequently, with the last high-level permanent lake about 48 ka, and has remained at low levels or an ephemeral salt lake through the Holocene. In contrast, Bowler et al. (2003; 2012) found that the Willandra Lakes of the Murray Basin remained full from before 60 ka until 40 ka, with moisture delivered by westerly storms over the southeastern highlands of Australia, but began to dry after 40 ka, and have been dry through the Holocene.

Summarizing the published records, Australia was irregularly drying from the end of MIS 5 through to MIS 2, but there is little evidence for large-scale fluctuations in climate across Australia during MIS 3 (57-30 ka). And none of the reconstructions provide evidence to suggest that climate variability in MIS 3 was more extreme than during previous stadials over the past 300 ka.

3.1 The Lake Eyre Basin (LEB) We use the stable isotopes of C, O, and N preserved in dated eggshell of *Dromaius* and *Genyornis* to reconstruct diet and climate over the past 100 ka. Our largest dataset comes from the southern part of the LEB, which also provides the best opportunity for capturing variability in the penetration of monsoon moisture into the continental interior. The LEB catchment (Fig. 2) is one of the largest endorheric basins in the world, covering 1.14×10^6 km², and is fed dominantly by summer-monsoon rainfall in its northeast portion (Habeck-Fardy, 2014). The main LEB depocenter, Kati Thanda-Lake Eyre in the driest region of the continent, is currently an ephemeral playa, with its lowest point below -15 m AHD.

3.1.1 Kati Thanda-Lake Eyre (KT-LE) Although the LEB has been hydrologically closed since at least the Neogene, KT-LE contains <10 m of sediment in the main depocenter (Magee et al., 1995). This lack of sediment reflects the efficiency of sediment deflation by aeolian processes rather than low sedimentation rates. Optically stimulated luminescence (OSL) provides time-of-deposition ages for quartz grains in fluvial, beach, nearshore lacustrine, and aeolian sediment extending back >200 ka. These OSL dates have resulted in a comprehensive, although still evolving, palaeohydrologic record of KT-LE for the past ~150 ka (Magee and Miller, 1998; Nanson, 1998; 2008; Magee et al., 2004; Cohen et al., 2015).

Magee et al. (2004) combined multiple dating methods from lacustrine, fluvial, beach ridge and aeolian sediment to reconstruct a stratigraphically defined lake-level curve for KT-LE. Subsequently, Cohen et al. (2015) derived a lake-level curve based exclusively on OSL dates from high-level beach ridges at the southern margin of the lake. Despite differences in these strategies, both studies document high lake levels in MIS 5, lower lake levels in MIS 4 and 3, and no significant high lake level after ~45 ka

Evidence of KT-LE high lake levels pre-dating MIS 5 are known from terrain east and northeast of the modern playa. A mostly lacustrine transgressive sedimentary sequence capped by a gypseous playa-deflation unit ~12 km east of KT-LE is almost identical in relative elevation, sediment composition, and stratigraphy to a MIS 5 sequence cropping out at the shore of KT-LE (Magee, 1997). However, OSL dating reveals the lacustrine units to have been deposited in MIS 7 (Fig. S1), demonstrating a similar LEB response to repeated 100 ka glacial/interglacial cycles.

The intensity of regional aridity between lake high-stands is reflected in the depth of aeolian deflation of KT-LE lacustrine sediment. Deflation of sediment from the depocenter is limited by the regional groundwater level, which in turn is a reflection of changes in the relative balance between rainfall and evaporation on millennial timescales. Prior to the MIS 5 lacustrine episode, older KT-LE lacustrine sediment was excavated by aeolian deflation to the underlying [silicified Miocene sediment](#) (Magee et al., 1995; Magee et al., 2004). The lack of weathering products or pedogenesis indicative of prolonged exposure at the base of the sediment fill suggests all sediment was deflated from the basin during MIS 6. Despite uncertainty on the timing of this deep deflation event, it demonstrates that the maximum aridity of the past 130 ka (MIS 5-1) was not as intense as in earlier Quaternary arid intervals.

3.1.2 Lake Frome East and north of the Flinders Ranges lie a chain of large playa lakes in a sub-basin of the LEB, of which Lake Frome is largest (Habeck-Fardy and Nanson, 2014). The Frome depocenter receives monsoonal water via streamflow during wet phases, with the highest shorelines at 15 m AHD (Cohen et al., 2011; Cohen et al., 2015), at which point it decants into KT-LE (Nanson, 1998; DeVogel et al., 2004; Leon and Cohen, 2012), which has maximum MIS 5 shorelines that are slightly lower. OSL ages of beach sediment associated with the overflow level range from ~110 ka through to ~48 ka, coincident with the final high lake at KT-LE (Cohen et al., 2015). After 48 ka Lake Frome ceased to overflow; a drying trend is recorded by lower shorelines, with the Holocene represented by briefly occupied shorelines between 0 and +5 m AHD (Cohen et al., 2015).

3.2 Human dispersal across Australia The exact date humans first reached the Australian continent may never be precisely known, but what is more important, and is now much better known than previously, is the timing of human dispersal around the continent. A recent review of the evidence concludes that humans occupied most of the continent before 47 ka (O'Connell and Allen, 2015). Evidence for occupation of the arid zone is hampered by the lack of optimal preservation sites, but at least opportunistic human forays into what is now the arid zone as early as 50 ka is apparent (Bowler et al., 2012; Smith, 2013; Miller et al., 2016a).

3.3 Megafaunal extinction Late Cenozoic vertebrate evolution produced large body sizes within numerous marsupial lineages in Australia, with maximum sizes peaking during the Pleistocene. The Late Quaternary extinctions in Australia, as in the Americas and elsewhere, were dominantly of large-body forms (>45 kg), the megafauna. In a debate now more than 150 years old, the cause of megafaunal extinctions, climate or human agency, or a combination of the two, has yet to be conclusively resolved, despite increasingly precise geochronologies and the continuous addition of fossil records. Several recent reviews summarize the arguments (Barnosky et al., 2004; Wroe and Field, 2005; Gillespie et al., 2006; Johnson, 2006; Byrne et al., 2008; Roberts and Brook, 2010; Braje and Erlandson, 2013; Bird et al., 2013; Stuart, 2014; Johnson et al., 2016) and are not repeated here. From the perspective of this paper the key information in the published literature is that the low temperatures and aridity of MIS 6 had no lasting impact on regional faunal diversity (Prideaux, 2010), suggesting most megafauna were adapted to the climatic extremes that characterized the Quaternary, an interpretation consistent with a 300 ka faunal sequence from South Australia (Prideaux et al., 2007). For those megafaunal taxa most common in the Pleistocene fossil record, most, if not all, were present on the continent when humans first arrived, but all were extinct before 40 ka (Roberts et al., 2001; Johnson, 2006; Prideaux, 2010; Saltr et al., 2016), well before the cold, arid phases of MIS 2.

4. Australia's giant flightless birds: *Genyornis*, *Dromaius* and *Casuarius*

At nearly 2 m height and up to 55 kg, the emu (*Dromaius novaehollandiae*) is the world's second largest extant bird (Fowler, 1991). It is a member of the ratite family of birds, and can be found over most of the Australian continent. *Dromaius* are generalist feeders. Their diet consists of almost anything palatable, although they target optimal food sources when foraging (Davies, 1978). Throughout Australia the birds dominantly eat a variety of fruits, seeds, and green herbage of annual and perennial plants, including grasses, and when available, insects and small lizards or snakes are occasionally consumed. Typically, non-plant sources constitute no more than 5% of emu diet (Davies, 1978; Dawson, 1984). Water makes up 65 to 70% of *Dromaius* body mass (Dawson, 1984). To maintain water balance the birds drink free water in considerable quantities daily when it is available, although in extreme drought they can satisfy a significant fraction of their required water from plant-derived water. *Dromaius* travel widely, exploiting seasonal food resources, and efficiently move long distances to locate new food supplies when necessary. *Dromaius* breed at about the same time of year throughout the country, with the female typically laying 8 to 12 large (130 x 90 mm) dark green eggs in a crude nest as early as April, but most commonly in May or June, although occasionally as late as July (Malecki et al., 1998). Eggshell calcification occurs over 1 to 2 days. The male exclusively tends the nest, with incubation lasting 50 to 60 days (Fowler, 1991). Defense of the nest is based on solitary locations and inconspicuous settings, but the male will generally flee if disturbed. The brood, accompanied by the male, leaves the nest shortly after hatching. Traditionally, Aboriginal Australians utilized emu meat and eggs as food sources (Cane, 1987). The southern cassowary, *Casuarius casuarius*, is slightly smaller than *Dromaius*, and is restricted to the rainforests of NE Australia. It is extant.

Although not much taller than *Dromaius*, *Genyornis newtoni* was a more robust bird (Fig. S2), with an estimated body mass of at least 160 kg, and possibly as much as 275 kg (Murray and Vickers-Rich, 2004). The species was first described in detail from skeletal remains embedded in lake-floor sediment of Lake Callabonna, South Australia (Stirling and Zeitz, 1900).

4.1 *Genyornis* and *Dromaius* eggshell This study relies primarily on geochronologic and isotopic data derived from fragments of *Genyornis* and *Dromaius* eggshell. Eggshell were targeted because, unlike bone, eggshell calcite faithfully preserves organic compounds that are tightly held within its dense crystalline matrix (Brooks et al., 1990), and they occur commonly in sand dunes across the arid zone. The combination of a stable low-Mg calcite mineralogy and the intra-crystalline protection of organic components provides optimal preservation potential. Reliable isotopic values can be derived from both organic and mineral phases, and eggshell can be directly dated by amino acid racemization (AAR), ^{14}C , and U/Th, and indirectly from the enclosing sediment dated by OSL (Magee et al., 2009).

Genyornis eggshell are the most common form of megafaunal remains found in Australia. They were first recognized by Williams (1981) who described diagnostic morphological differences between *Genyornis* and *Dromaius* eggshell, with egg dimensions estimated from curvature measurements to be 155 x 125 mm, larger and rounder than *Dromaius* eggshell; average thicknesses are 1.15 mm, similar to *Dromaius* eggshell. A nearly complete but crushed egg of *Genyornis* found by our research team north of Port Augusta and reconstructed by N. Schroeder of the South Australia Museum is smaller (126 x 97 mm), but represents a single sample. Based on the size of the reconstructed egg, Grellet-Tinner et al. (2016) questioned its taxonomic identity, suggesting instead an extinct 5 kg megapode may be the bird responsible for the eggs. However, Miller et al. (2016b) show that the arguments for a megapode origin of the eggshell are inconsistent when considering all of the available evidence, and as there is no other extant or extinct bird known from the Australia arid zone of sufficient size other than *Genyornis*, we remain confident that the taxonomic identification by Williams (1981) as *Genyornis* is correct, and that terminology is retained. A search for DNA within the eggshell matrix to establish phylogenetic relationship is ongoing. Importantly, the taxonomic attribution of the eggshell is not particularly relevant to this paper, which seeks to evaluate ecosystem and climate change utilizing isotopic data from the eggshell of two avian taxa that nested together across the arid zone – one now extinct and the other extant.

Genyornis eggshell often occur locally in great numbers in recently deflated sand dunes that were their ancient nesting grounds. While it is unlikely that eggshell will be found in direct association with either skeletal remains of the parent bird or identifiable developing embryos, we have found isolated *Genyornis* bones in the same deflation hollows that contain common *Genyornis* eggshell fragments (Fig. S2), and numerous complete *Genyornis* skeletons have been recovered from Lake Callabonna, located between lakes KT-LE and Frome, confirming that the genus occupied the same landscape in which their putative eggs are preserved.

Of the three giant flightless birds extant in Australia when humans arrived, only *Genyornis* became extinct. The initial estimate for the timing of *Genyornis* extinction, 50 ± 5

ka (Miller et al., 1999), and the mechanisms that led to its extinction can now be better constrained. Miller et al. (2016a) argued that variably burnt *Genyornis* eggshell fragments were diagnostic of humans gathering eggs from a nest, cooking them, then discarding eggshell fragments in and around their transient cooking fires. Burn patterns on eggshell fragments diagnostic of extreme thermal gradients, as well as both unburnt and heavily burnt fragments in the same small cluster cannot be explained by regional bushfires, and only occur in *Genyornis* eggshell within a narrow OSL-constrained time window that defines an estimated age range for human predation, and for *Genyornis* extinction, of 47.5 ± 2.5 ka (Miller et al., 2016a).

4.2 Nesting and egg-laying behavior Both *Genyornis* and *Dromaius* nested on the ground. Eggshell of both genera are commonly found in the same deflation hollows in dunefields, lunettes, and coastal dunes. Nesting co-location ensures that both birds had access to the same local food sources, albeit their diets will be biased by niche differences. For the extinct *Genyornis*, behavioral information is largely unknown, although *Genyornis* eggshell occur in a more clustered pattern across the landscape than for *Dromaius*. This interpretation is supported by the discovery of a site near Port Augusta where a group of 6 nests, several with >1kg of fragmented eggshell, were located at the same apparent depositional horizon, with individual nests separated by 10 to 20 m and all lying within a 100 m radius. AAR dating suggests that the nests were coeval and the total mass of eggshell collected from each nest indicated minimum clutch sizes ranging from 4 to 17 eggs. This find suggests that *Genyornis* either nested in small groups or that individual birds or pairs returned repeatedly to the same site for consecutive nestings. Colonial nesting is a common characteristic in waterfowl, the bird group to which the Dromornithids are thought to be related (Murray and Vickers-Rich, 2004).

4.3 Eggshell preservation and discovery Shortly after *Dromaius* chicks hatch, they leave the nest. The abandoned nest retains hatched egg fragments that may be consumed by other animals, either for the post-hatching organic residues or as a source of calcium. Remnant unconsumed pieces are soon destroyed by abrasion from wind-blown quartz sand or widely dispersed by wind and/or other natural processes. Preservation is most likely if eggshell is rapidly buried, especially where sand is sourced from carbonate-rich sediment. Because rain is slightly acidic, eggshell buried in sediment lacking the buffering effect of carbonate is slowly dissolved and rarely survives on millennial timescales. Discovery is most likely in recent deflation hollows where eggshell may be found in dense concentrations, often with larger pieces that can be refitted (Fig. 3). In other instances eggshell fragments occur in lower densities scattered over wider areas. Although the thicknesses of their eggshell are similar, *Dromaius* eggshell have a more complex internal structure and are more susceptible to wind abrasion, giving *Genyornis* eggshell a slightly higher probability of preservation.

In regions with carbonate-buffered soils, the abundance of eggshell in the landscape is determined by three factors.

- Rapid burial. Eggshell burial and preservation is most likely when aeolian processes are actively redistributing mobile sand. During episodes of dune formation, eggshell can be rapidly buried by several meters of sand. Burial rate is an abiotic factor dominantly

influenced by geomorphology and climate. Hence, the abundance of preserved eggshell is rarely correlated to the number of nesting birds.

- **Re-exposure.** Landscape instability is required to re-expose sparsely distributed buried eggshell. Sediment instability in dunes often occurs when stabilizing vegetation cover is lost, resulting in wind erosion that excavates hollows leaving heavier objects, including eggshell fragments, exposed as a residual lag. Frequently, our collection coincides with freshly exposed eggshell *in situ* in sands at the deflation surface (Fig. 3). Fragments with sharp edges and clusters with refittable fragments are indicators of recent, first-time exposure.

- **Population abundance.** The number of nesting or egg-laying birds can be important for eggshell abundance, controlled by biotic factors including population dynamics, competition, resource availability, and predator pressure. However, the abundance of nesting birds is usually the least important factor influencing eggshell abundance in the fossil record.

4.4 Egg predation Eggshell fragments of both taxa commonly contain distinctive holes, often in pairs, attributed to the canine teeth of predators (Fig. 3). We interpret these fragments to be from an egg that was removed from its nest by a toothed predator and taken to a nearby location for breaking and consumption. The relatively high abundance of predated eggs suggests that predators excavated a shallow hole into which the egg was placed as part of the opening process, and this greatly increased the probability of subsequent burial and preservation.

There is also evidence of human predation on the eggs of both *Dromaius* and *Genyornis*. Their eggs were apparently harvested and cooked, often in small transient fires, beginning about 50 ka for both taxa. For *Genyornis*, predation terminated with the birds' extinction by ~47 ka, whereas burnt eggshell fragments of *Dromaius* are common from 50 ka to modern times (Miller et al., 2016a).

4.5 Eggshell collection bias Eggshell fragments that provide the primary data on which this paper is based are from ~3000 collections made over three decades. These collections have not been random. Initially, eggshell was collected from sites that had the potential to date Quaternary palaeoenvironments. Subsequently, as the research extended to questions of *Genyornis* extinction and the birds' dietary reconstructions, collecting strategies targeted a wider range of environments and regions, and specific time periods to fill identified temporal gaps. Because of the evolving collection efforts, our data are not randomly distributed in time or space, and some modest biases are likely introduced by the non-random nature of the compiled datasets. A primary goal has been to derive region-specific near-continuous time-series of dated eggshell spanning the past ~100 ka. The non-random distributions of eggshell through time in the graphs that follow demonstrate the variable efficiencies of preservation and re-exposure agents more than variations in bird population densities.

4.6 The extinction of *Genyornis newtoni* Of the three giant flightless birds extant in Australia when humans arrived, only *Genyornis* became extinct. The initial estimate for the timing of *Genyornis* extinction, 50 ± 5 ka (Miller et al., 1999), can now be better constrained. Miller et al. (2016a) used OSL ^{14}C and AAR to develop a thermal isochron for human-burnt

Genyornis eggshell fragments in 10 regions across Australia (Fig. 4B). Unburnt fragments in these collections have the lowest A/I recorded for each region. The seven OSL ages on sediment enclosing burnt *Genyornis* eggshell have a pooled mean age of 46.5 ± 1.0 ka, and an arithmetic mean age of 49.0 ± 4.4 ka. Considering both results, Miller et al. (2016a) place *Genyornis* extinction at 47.5 ± 2.5 ka.

5. Materials and Methods

Our datasets are derived from measurements of carbon, oxygen, and for some comparisons, nitrogen isotope ratios in individually dated eggshells of *Genyornis newtoni* and *Dromaius novaehollandiae*. Eggshell of both species are found across the Australian arid zone. Initial investigations into light stable isotopes in eggshell focused on ostrich eggshell in archaeological sites (von Schirnding et al., 1982; Johnson et al., 1998), and in long time series extending back to the Miocene (Stern et al., 1994; Segalen et al., 2002). Subsequently, with the recognition that eggshell of the two large Australian birds were commonly exposed in aeolian sediment spanning much of the past 100 ka, the potential of stable isotopes from those archives has been explored.

5.1 Carbon isotopes Eggshell contains two sources of carbon. Inorganic crystals of calcium carbonate constitute 97% of eggshell mass. The balance is mostly organic compounds, primarily protein and lesser amounts of carbohydrate and lipid (Burley and Vadehra, 1989), that form a complex organic web within the calcite crystals (Fig. S3). Because the organic compounds are intracrystalline they are relatively immune to diffusional loss over time, which occurs commonly in biominerals such as mollusk shells, where most organic compounds are intercrystalline (Miller et al., 2000; Penkman et al., 2008). In both organic and mineral components $\delta^{13}\text{C}$ is set by the bird's diet, offset by fixed biological fractionation factors (BFF). For herbivorous birds $\delta^{13}\text{C}$ primarily reflects the proportions of C₃ and C₄ plants that contributed to the female bird's diet (von Schirnding et al., 1982; Johnson et al., 1998). Eggshell calcite carbon ($\delta^{13}\text{C}_{\text{carb}}$) is primarily set by dissolved bicarbonate in the blood, which has a rapid turnover period (days to weeks). Carbon in the organic matrix ($\delta^{13}\text{C}_{\text{org}}$) is set by protein reserves in the body and reflects dietary intake in the weeks to months prior to egg laying. The different time constants for the two carbon sources allow us to consider them as independent dietary proxies.

Controlled feeding experiments show that BFF result in $\delta^{13}\text{C}_{\text{org}}$ enriched by $\sim 2\text{‰}$ relative to diet in eggshell of the African ostrich (von Schirnding et al., 1982). Additional BFF related to calcification further fractionate $\delta^{13}\text{C}_{\text{carb}}$. We estimate the difference between $\delta^{13}\text{C}_{\text{carb}}$ and $\delta^{13}\text{C}_{\text{diet}}$ empirically. For *Dromaius*, 808 paired measurements of $\delta^{13}\text{C}_{\text{org}}$ and $\delta^{13}\text{C}_{\text{carb}}$ in eggshell fragments that span most of the past 150 ka show that $\delta^{13}\text{C}_{\text{carb}}$ is $10.13 \pm 0.06\text{‰}$ (SE_{mean}) more positive, on average, than $\delta^{13}\text{C}_{\text{org}}$, and exhibits no trend with either sample age (diagenesis) or with the proportion of C₄ elements in the birds' diet. For *Genyornis*, 345 paired measurements of $\delta^{13}\text{C}_{\text{org}}$ and $\delta^{13}\text{C}_{\text{carb}}$ that range in age from ~ 50 to ~ 200 ka reveal $\delta^{13}\text{C}_{\text{carb}}$ averages 12.1‰ more positive than $\delta^{13}\text{C}_{\text{org}}$. The conversion of $\delta^{13}\text{C}_{\text{org}}$ and $\delta^{13}\text{C}_{\text{carb}}$ to $\delta^{13}\text{C}_{\text{diet}}$ is described in Table 1.

Plants utilizing C₃ and C₄ photosynthetic pathways fractionate atmospheric CO₂ differently during their synthesis of organic compounds utilized in plant growth. Most trees, shrubs, and winter grasses in our field areas utilize the C₃ photosynthetic pathway, whereas summer-rainfall-dependent grasses almost exclusively utilize the C₄ photosynthetic pathway in the most arid regions, but C₄ grasses are less abundant in cooler, wetter regions (Hattersley, 1983). A number of plant taxa in Australia utilize intermediate C₃ and C₄ photosynthetic pathways, typically plants in the Chenopodiaceae family. We sampled a wide range of plant types across the arid zone, measured $\delta^{13}\text{C}$ in their leaves, stems, and seeds. All trees and shrubs sampled have average $\delta^{13}\text{C}$ similar to the global average for C₃ plants ($-26 \pm 2\text{‰}$), and most sampled grasses utilize the C₄ photosynthetic pathway, with an average $\delta^{13}\text{C}$ similar to the global average ($-12 \pm 1\text{‰}$; Fig. 5), with chenopods having intermediate values ($-21 \pm 6\text{‰}$; Table S1).

Eggshell $\delta^{13}\text{C}$ is a palaeodietary record. From a paleovegetation perspective, $\delta^{13}\text{C}_{\text{diet}}$ is not directly comparable to pollen, which provides a measure of what was pollenating in the landscape. Eggshell $\delta^{13}\text{C}_{\text{diet}}$ provides an index of the proportion of C₃ and C₄ plants with high forage value that were accessible to, and selected for consumption, by the female bird preceeding and during the egg-laying season. A striking difference between diet and vegetation is illustrated by $\delta^{13}\text{C}_{\text{diet}}$ in sub-modern (<3 ka) *Dromaius* eggshell from KT-LE (Fig. 1), where dune vegetation currently is dominated by C₄ sandhill canegrass (*Zygochloa*), but *Dromaius* $\delta^{13}\text{C}_{\text{diet}}$ averages $-22.4 \pm 1.9\text{‰}$ ($n = 44$), demonstrating that the birds are eating dominantly C₃ plants (Fig. 5), presumably because *Zygochloa* as well as species of spinifex grasses, *Triodia* and *Plectrachne*, have little or no forage value.

5.2 Oxygen isotopes The oxygen in eggshell calcite is in isotopic equilibrium with body water (Nagy, 1987). Depending on the species and environmental conditions, body water is derived primarily from ingested water, which for *Dromaius* is dominated by free-water sources, but also includes variable amounts of plant leaf-water, with additional input from metabolic water produced by oxidation of food (Luz et al., 1984). Johnson et al. (1998) found that the $\delta^{18}\text{O}$ of ostrich eggshell raised in a controlled setting tracked the $\delta^{18}\text{O}$ of drinking water linearly, when birds were provided with ample drinking water. Although body water $\delta^{18}\text{O}$ values can vary between individuals at the time of egg-laying, isotopic fractionation during secretion of the calcite crystals can be considered a constant because the birds' maintain an internal body temperature of 38°C (Maloney and Dawson, 1993).

The $\delta^{18}\text{O}$ of environmental source water is set by the $\delta^{18}\text{O}$ of precipitation, modified by evaporative processes that occur before the birds access the water by drinking or from water in plant tissue. Across our field areas current weighted annual mean $\delta^{18}\text{O}$ of precipitation exhibits little variability, averaging $-5 \pm 1\text{‰}$ (SMOW; IAEA/WMO, 2015). Because calcification of the *Dromaius* egg occurs in autumn, free-water sources available at that time are likely to be isotopically enriched by evaporative processes in late summer. If leaves provide a significant portion of the bird's required water, then body water will be even more enriched in ^{18}O (Förstel, 1978). In the LEB rare mound springs provide access to relatively depleted ^{18}O Great Artesian Basin water, which has become much more accessible from bore-derived stock watering points under European pastoral activities.

Each eggshell provides a single-year snapshot reflecting the average isotopic composition of source water for females in the days to weeks prior to egg laying. Because of high interannual rainfall variability, single-season “snapshots” of source water evaporation provided by $\delta^{18}\text{O}$ are unlikely to reflect the mean state of climate. Evaluating large datasets of $\delta^{18}\text{O}$ from the most arid region of Australia showed that averaging $\delta^{18}\text{O}$ from ~20 eggshell calcified in different, but closely spaced years provides an estimate of $\delta^{18}\text{O}$ within $\pm 1\%$ of the mean (Miller and Fogel, 2016). The average $\delta^{18}\text{O}$ in sub-modern *Dromaius* eggshell is highly correlated with the contemporary point-potential evapotranspiration (PPET) for February plus March, the late summer months preceding egg calcification (Fig. 6). PPET is the evaporation and transpiration that would theoretically occur from a small water body and surrounding region assuming unlimited water supply and no other large moisture sources in the vicinity (http://www.bom.gov.au/climate/averages/climatology/gridded-data-info/metadata/md_ave_et_1961-90.shtml); it is measured in mm water equivalent (mm w.e.). To reconstruct a time-series of PPET we measure $\delta^{18}\text{O}$ in a dense age range of eggshell, then compute 20-point running means of $\delta^{18}\text{O}$, and convert that value to PPET. With the time-density of eggshell samples available to us, PPET provides an estimate of the mean climate state averaged over several hundred to several thousand years. Because $\delta^{18}\text{O}$ is measured in the same eggshell fragment used to reconstruct $\delta^{13}\text{C}_{\text{diet}}$, correlation uncertainties are minimized; absolute ages of paired PPET- $\delta^{13}\text{C}_{\text{diet}}$ have inherent uncertainties resulting from age-model calibrations, but the phasing between the two proxies is only weakly dependent on dating uncertainties.

Training sets for $\delta^{18}\text{O}$ and climate for the extinct *Genyornis* are not possible. Instead, we compare $\delta^{18}\text{O}$ in *Genyornis* and *Dromaius* from KT-LE, our largest dataset. The average $\delta^{18}\text{O}$ from 418 KT-LE *Genyornis* is more positive (+5.6‰) than in 261 coexisting *Dromaius* (+3.7‰). The most likely explanation for this isotopic difference is that *Genyornis* calcified their eggs during summer rather than autumn, and the calculated PPET reflects conditions during December plus January, the two hottest months of the year (<http://www.bom.gov.au/climate/data/>), assuming *Genyornis* body temperature was similar to that of *Dromaius*. While we confidently convert *Dromaius* eggshell $\delta^{18}\text{O}$ to the corresponding PPET for Feb+Mar, we consider the PPET axis for *Genyornis* plots to reflect “summer PPET”, with greater emphasis for interpretation based on changes in PPET, rather than the actual values. We interpret regional differences in PPET derived from *Genyornis* eggshell $\delta^{18}\text{O}$ to correctly classify regions into greater and lesser breeding-season aridity (Table 2), assuming the bred at about the same time across their range.

To utilize the relationship between $\delta^{18}\text{O}$ and PPET as a paleoaridity proxy through the Late Quaternary, during which time the global climate system varied significantly, four assumptions must be met:

- 1) *The birds do not change their seasonality of breeding.* This is likely correct. *Dromaius* currently breed at about the same time across the continent despite strong climate contrasts. Breeding time is hormonally driven and unlikely to have changed on millennial timescales. $\delta^{18}\text{O}$ of precipitation currently has a ~3‰ seasonal range, underscoring the importance of this assumption.

2) *The birds breed every year regardless of climate state.* During extreme drought and limited dietary sources, female birds may remain in an area but not lay eggs. This may reduce the magnitude of an isotope-derived aridity signal during extreme drought.

3) *Changes in $\delta^{18}\text{O}$ of precipitation are small relative to changes in eggshell $\delta^{18}\text{O}$ over the past 100 ka.* $\delta^{18}\text{O}$ of precipitation currently exhibits little variability across Australia, but model simulations suggest $\delta^{18}\text{O}$ of precipitation during peak global cold times in Australia may have been as much as 2‰ more negative than currently (Lee et al., 2008). Changes in air mass trajectories may also influence $\delta^{18}\text{O}$ of precipitation, with both maritime air masses and monsoon incursions delivering 1 to 2‰ more negative $\delta^{18}\text{O}$ precipitation (Denniston et al., 2013a; Risi et al., 2013; Srivastava et al., 2015). Eggshell $\delta^{18}\text{O}$ values are also affected by the 1‰ shift in seawater $\delta^{18}\text{O}$ during peak ice volumes, but this is partially offset by lower LGM temperatures. On the other hand, the range of individual eggshell $\delta^{18}\text{O}$ across the arid zone during the Late Quaternary is from -6 to +20‰ (Tables S8 – S16), much larger than isotopic changes unrelated to evaporative enrichment.

4) *The bird's source water is dominantly from free-water sources.* This is likely to be correct except for the karst terrain in the North West Cape. There, water is efficiently removed from the ground surface limiting the potential for evaporative isotopic enrichment. If a significant proportion of the birds' water requirements are met by leaf water, particularly during drought, this would amplify the aridity signal, although breeding is less likely in such times. In recent times pastoral activities have provided access to relatively negative $\delta^{18}\text{O}$ groundwater that birds likely utilize.

Although we cannot directly evaluate how the extinct *Genyornis* met its water needs, the close correlation between changes in eggshell $\delta^{18}\text{O}$ and $\delta^{18}\text{O}$ of source water for ostrich suggests that *Genyornis* eggshell $\delta^{18}\text{O}$ is also set by the bird's source water. Seasonal variability in $\delta^{18}\text{O}$ of precipitation across Australia is typically ~3‰, so if *Genyornis* calcified eggshell at a different time of year than *Dromaius*, the $\delta^{18}\text{O}$ of source water might differ from that of *Dromaius* by a up to similar amount.

5.3 Nitrogen isotopes A variety of factors determine the $\delta^{15}\text{N}$ in eggshell. The dominant control is the $\delta^{15}\text{N}$ in the bird's food source. For plant sources, $\delta^{15}\text{N}$ is increasingly enriched in more arid climates (Johnson et al., 1993; Handley et al., 1999; Amundson et al., 2003; Newsome et al., 2011). Additionally, $\delta^{15}\text{N}$ increases by 3 to 5‰ with each trophic level in the food chain (Minagawa and Wada, 1984; Michener and Schell, 1994; Kelly, 2000). Newsome et al. (2011) have shown that for *Dromaius*, which feeds principally on plants, the $\delta^{15}\text{N}$ in organic matter within the birds' eggshell serves as a proxy for paleoprecipitation. Here we use eggshell $\delta^{15}\text{N}$ primarily to compare feeding strategies between co-habiting *Dromaius* and *Genyornis*.

5.4 Analyzing eggshell isotopes Prior to isotopic analyses eggshell fragments are mechanically cleaned by grinding to remove secondary surface contaminants, and for

Dromaius eggshell which has a tripartite structure, to isolate the prismatic layer. The remaining eggshell is then reduced in mass by one third with the stoichiometric addition of 2N HCl *in vacuo* following procedures outlined in Miller et al. (2005a). For $\delta^{15}\text{N}$ and $\delta^{13}\text{C}_{\text{org}}$ a ~5-mg subsample was placed in a silver capsule and dissolved with three sequential 20-microliter aliquots of 6 N HCl-sequal grade. The residual acid was evaporated at room temperature under a fume hood for approximately 24 hr; remaining water was removed in an oven at 85°C for 24 to 36 hr and stored at 50°C under N₂ until analysis. $\delta^{15}\text{N}$ and $\delta^{13}\text{C}_{\text{org}}$ were determined using an elemental analyzer (NC 2500; CE Elantech, Lakewood, NJ) interfaced with Thermo Finnigan (San Jose, CA) Delta Plus XL or Delta V Plus mass spectrometers (Carnegie Institution of Washington, Washington, DC and University of California, Merced).

For $\delta^{13}\text{C}_{\text{carb}}$ and $\delta^{18}\text{O}$ measured at the Geophysical Laboratory, Carnegie Institution of Washington, USA, and the University of California, Merced, USA, ~0.1 to 0.2 mg of powdered eggshell was placed in an exetainer vial and flushed with helium to remove air in a Thermo Fisher Gas Bench. Six drops of 100% H₃PO₄ were added to each vial manually, then placed into a heating block at 70°C. For each batch of 40 to 80 eggshell powders we analyzed several blank vials and multiple standards, including internal lab standards along with NBS-18, NBS-19, and RO-22 (Isoanalytical). Evolved CO₂ was analyzed on Thermo Finnigan Delta XL Plus or Delta V Plus mass spectrometer. The $\delta^{18}\text{O}$ is measured in ‰ with respect to Standard Mean Ocean Water (SMOW) and converted to PDB; an internal carbonate standard analyzed six times had standard deviations of $\pm 0.15\text{‰}$ for $\delta^{18}\text{O}$ and ± 0.25 for $\delta^{13}\text{C}_{\text{carb}}$. At the Research School of Earth Sciences, Australian National University $\delta^{13}\text{C}_{\text{carb}}$ and $\delta^{18}\text{O}$ were determined on an automated individual-carbonate reaction Kiel device coupled to a MAT-251 IRMS. Approximately 0.2 mg of each powdered sample was reacted with 2 drops of 103% H₃PO₄ at 90°C to liberate CO₂. The $\delta^{18}\text{O}$ and $\delta^{13}\text{C}$ values are reported in ‰ deviations relative to V-PDB. The results have been normalized such that the NBS-19 standard yields $\delta^{18}\text{O}_{\text{VPDB}}$ (-2.20‰), $\delta^{13}\text{C}_{\text{VPDB}}$ (+1.95‰), and NBS-18 yields $\delta^{18}\text{O}_{\text{VPDB}}$ (-23.0‰), $\delta^{13}\text{C}_{\text{VPDB}}$ (-5.0‰). The standard deviations for in-run measurements of NBS-19 were $\pm 0.04\text{‰}$ for $\delta^{18}\text{O}$ and $\pm 0.02\text{‰}$ for $\delta^{13}\text{C}$.

Throughout this paper eggshell $\delta^{13}\text{C}_{\text{org}}$, $\delta^{13}\text{C}_{\text{carb}}$ and $\delta^{18}\text{O}$ are expressed in ‰ with respect to the PDB standard; $\delta^{15}\text{N}$ is reported in ‰ with respect to $\delta^{15}\text{N}_{\text{air}}$.

5.5 Evaluating the effects of burning on isotopic ratios Many of our collections from Western Australia <50 ka contain variably burnt eggshell of both *Genyornis* and *Dromaius*, often heated enough to alter AAR, but not reaching temperatures sufficiently high to blacken the eggshell (Miller et al., 2016a). To test whether heating eggshell in a fire compromises their isotopic signature we measured $\delta^{13}\text{C}_{\text{org}}$ and $\delta^{15}\text{N}$ in heavily burnt, partially burnt, and visually unburnt fragments from two isolated *Genyornis* eggshell clusters that we presume to be single clutches. The results (Table S2) demonstrate that even in fully blackened eggshell fragments neither $\delta^{13}\text{C}_{\text{org}}$ nor $\delta^{15}\text{N}$ are statistically different from the unheated fragments. However, N is lost preferentially to C in response to heating; C/N may provide a reliable means to separate unburnt from lightly burnt eggshell fragments.

5.6 Geochronology Most paleoenvironmental time series are developed from continuously deposited sediment accumulations that contain paleoenvironmental proxies. No known single sediment accumulation provides such a time series for eggshell. Alternatively, if each eggshell is dated, and the distribution of dates across a limited geographic region is sufficiently dense, a composite time-series can be reconstructed. Dating of eggshell has been undertaken successfully through ^{14}C , AAR and OSL, and with mixed success with U/Th (Magee et al., 2009).

5.6.1 Radiocarbon (^{14}C) *Dromaius* eggshell may be dated reliably by radiocarbon. *Dromaius* eggshell collected immediately after hatching in 1991 and 1995 at KT-LE (Fig. 2) returned ^{14}C concentrations of 1.186 ± 0.007 and 1.160 ± 0.007 fraction modern respectively (Table S3), consistent with Southern Hemisphere atmospheric $^{14}\text{CO}_2$ levels during the years of their calcification. Like other carbonate media, eggshell calcite is subject to slow exchange with younger carbon from its surroundings. To minimize effects of contamination we routinely remove the outer 70% of each fragment by stoichiometric addition of 2N HCl *in vacuo*. Calibrated radiocarbon ages ≤ 40 ka are deemed reliable. However, even with this rigorous pretreatment we obtained finite calibrated ^{14}C ages ≤ 46 ka on samples known to be at least 70 ka. Consequently, we consider all ^{14}C dates >40 ka to represent minimum ages. All radiocarbon dates are reported as calibrated years BP or as ka, thousands of calibrated years BP.

We provide 228 ^{14}C dates on *Dromaius* eggshell ranging from post-nuclear bomb testing to >50 ka (Table S3)². Dates ≤ 40 ka are calibrated using Calib 7.1 and SHcal13 (Stuiver, 2005). We include 45 ^{14}C dates on *Genyornis* eggshell (Table S4), including multiple dates on single fragments or multiple fragments of the same collections to test whether apparent finite ages are secure absolute dates or whether all dates on *Genyornis* should be considered minimum ages. Re-dating of *Genyornis* eggshell fragments for which early AMS ^{14}C dates were <40 ka using more rigorous pretreatment and graphitization procedures have in all cases produced calibrated ages >43 ka. We conclude that all reliable radiocarbon dates on *Genyornis* eggshell are >43 ka and all should be regarded as minimum ages (Miller et al., 2016a). For *Dromaius* eggshell we consider all ^{14}C ages >40 ka to be minimum dates.

5.6.2 Optically Stimulated Luminescence (OSL) For sites with eggshell ^{14}C ages >40 ka we consider OSL ages of the enclosing sediment to be the most reliable chronometric method. OSL requires eggshell to be in a stratigraphic context, where burial occurred shortly after the egg was laid. This criterion is usually met if a concentration of several tens of fragments is located within a narrow vertical zone, especially if many fragments of a larger section of eggshell are found in place (e.g. Fig. 3). We report 36 OSL dates for sites where *Genyornis* and/or *Dromaius* eggshell meet these criteria (Table S6). All but seven of these have been published previously. As a test of the technique, Magee et al. (2009) report an

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² The numbers of ^{14}C dates used to derive age models may differ from the number of ^{14}C -dated samples in the $\delta^{13}\text{C}_{\text{diet}}$ and/or PPET reconstructions because not all ^{14}C -dated samples were analyzed for $\delta^{13}\text{C}_{\text{org}}$, $\delta^{13}\text{C}_{\text{carb}}$, and/or $\delta^{18}\text{O}$.

OSL age on quartz grains within an intact *Dromaius* egg, which agreed with direct ^{14}C , U/Th, and AAR dating of the eggshell within $\pm 1\sigma$.

5.6.3 Uranium series (U/Th) The potential of U/Th dating for eggshell calcite has been explored in several studies, with mixed success (Lundberg, 1990; Schwarcz and Morawska, 1993; Miller et al., 1999; Magee et al., 2009; Sharp et al., 2015). Neither uranium nor thorium are incorporated into the eggshell during calcification. Uranium is water-soluble, and is assumed to be taken up soon after burial, whereas thorium is insoluble. For U/Th ages to reflect eggshell age, the eggshell must be closed both to further U-uptake shortly after deposition and to loss of the daughter product, ^{230}Th . A potential complication derives from eggshell pores, which provide a conduit through the eggshell that allows the developing embryo to exchange gases with the atmosphere. After burial, pores are often infilled with fine sediment that may contain thorium, which adsorbs strongly to clay minerals, including both common thorium (^{232}Th) and ^{230}Th . To identify samples with significant concentrations of unsupported ^{230}Th , hence erroneous U/Th dates, Miller et al. (1999) exclude all U/Th dates with $^{230}/^{232}\text{Th}$ activity ratios <30 ; lower values are indicative of significant secondary ^{230}Th . We utilize U/Th ages that meet this criterion (Table S7) in developing age models (Section 7).

5.6.4 Amino acid racemization (AAR) The utility of avian eggshell for AAR has been recognized since the work of Brooks et al. (1990). Unlike molluscan biominerals, in which the majority of the protein residues are preserved in intercrystalline locations where they are subject to slow diffusional loss, eggshell protein is encapsulated within the calcite crystals and effectively immune from diffusional processes (Miller et al., 2000). We rely on the extent of isoleucine epimerization, in which the protein amino acid L-isoleucine racemizes about one of its two central carbon atoms to form the non-protein diastereomer D-alloisoleucine at a rate dependent on ambient temperature. The ratio of the two enantiomers (A/I) is measured by ion-exchange high-pressure liquid chromatography; A/I reflects time and the integrated thermal history experienced by the sample. Quality control is monitored with a laboratory standard, ILC-G (Wehmiller, 2013); 383 A/I analyses of the ILC-G standard in our lab average 0.457 ± 0.012 .

For eggshell buried continuously beneath ≥ 2 meters of sediment and not subjected to any other heat source, the effective diagenetic temperature (EDT) is set by the integrated sediment temperature, which is approximated by the time-integrated mean annual air temperature (MAT). For such samples collected in limited geographic regions over which the MAT varies by no more than 1°C , A/I can be used directly as an index of relative age (aminostratigraphy; Miller et al., 2000).

Eggshell buried <2 m for a significant portion of its history will yield A/I higher than in more deeply buried same-age eggshells due to the higher-amplitude annual temperature cycle and the exponential dependency of racemization rate on temperature. An extreme example of shallow burial is an abandoned nest of unhatched *Dromaius* eggs found at the ground surface close to KT-LE. Based on regional patterns of sand deposition it is unlikely that the samples were ever beneath more than a few tens of cm of sediment. A/I in eggshell from this nest is 0.74 ± 0.02 and the eggshell have a ^{14}C age of 31.3 ± 0.4 ka (AA-19014;

Table S3), but an AAR apparent age of 67 ka. Five other KT-LE *Dromaius* eggshell with ^{14}C ages between 30.4 and 31.7 ka have an average A/I of 0.52 ± 0.02 , requiring the shallowly buried nest to have experienced an EDT 2.5 °C higher than the more deeply buried samples to explain the difference. The higher EDT is explained by the high-amplitude temperature cycle for shallowly buried samples, even though all samples experienced the same arithmetic mean temperature.

To minimize the effects of shallow burial, we derive an age model for each region, using a conservative approach that closely follows the lowest A/I for each ^{14}C age (Section 7). As a result, shallowly buried samples are likely to have AAR apparent ages that are too old, but rarely will a sample return an AAR age that is too young. Magee et al. (2009) provide a detailed analysis of the impact of shallow burial and the consequent asymmetric dating uncertainties.

5.6.5 Converting AAR from relative to absolute ages To place the relative ages derived from A/I into an absolute time frame requires estimates for the evolution of MAT in each region and application of quantitative reaction kinetics. Alternatively, a series of independently dated samples over a wide temporal range from a limited region can be used to derive an empirically based age model. The latter method is employed here.

The key temporal focus of this paper is the interval between 60 and 40 ka, during which humans first spread widely across the continent, most megafauna disappeared, and ecosystems apparently changed. The thermal isochron for 47.5 ± 2.5 ka (Fig. 4B) predicts the average A/I in *Genyornis* eggshell calcified between 50 and 45 ka based on the current MAT for the region of collection. We utilize this isochron to derive a same-age thermal isochron for *Dromaius* eggshell. Because isoleucine racemizes slightly faster in *Dromaius* eggshell than in *Genyornis* eggshell, we constrain that difference using a laboratory experiment in which fragments of ancient *Dromaius* and *Genyornis* eggshell, each with a natural A/I of 0.4, were incrementally heated at 143 °C (Fig. 4A). The rate of increase in A/I is, on average, 10% higher in *Dromaius* than in *Genyornis*. We use this difference to define the equivalent A/I for a *Dromaius* sample 47.5 ka, and allow a $\pm 4\%$ uncertainty to incorporate the likely range of A/I for the interval from 50 to 45 ka. This curve allows the reliable separation of *Dromaius* eggshell calcified >50 ka from eggshell calcified <45 ka in each region, based on their A/I and regional MAT.

KT-LE has the highest number of associated ^{14}C , OSL, and U/Th ages, and consequently provides the most securely constrained age model (Section 7.1.2). We derive age models for the other regions using the thermal isochron for 47.5 ± 2.5 ka (Fig. 4B) and other available independent age constraints, then by following the overall form of the KT-LE age model, modified by the MAT differences between regions. *Genyornis* age models are derived by decreasing the *Dromaius* age models in each region by $\sim 10\%$ to accommodate *Genyornis*' slightly lower racemization rate.

6. Results: *Genyornis* was an herbivore Little is known of the dietary preferences of *Genyornis* or of its life cycle. Based on bill morphology, *Genyornis* is frequently considered a browser (Murray and Vickers-Rich, 2004). However, *Genyornis* $\delta^{13}\text{C}_{\text{diet}}$ (Section 7) indicates the female bird always consumed a significant proportion of C_4 food sources,

although never a purely C₄ diet as found in co-habiting *Dromaius*. *Genyornis* may have satisfied a significant portion of its dietary needs with chenopods or other mixed C₃-C₄ photosynthetic plants, which are common in the arid zone. We measured $\delta^{13}\text{C}$ in 100 chenopod samples from the South Australia transect and found an average of $-21 \pm 6\text{‰}$ (Table S1; range: -29 to -12‰). However, the dominant chenopod around KT-LE is *Atriplex*, a C₄ genus. Although contemporary *Dromaius* do not consume *Atriplex* in significant quantities, it is possible that *Atriplex* was a significant component of *Genyornis* diet.

Whether *Genyornis* was an herbivore or carnivore can be evaluated with nitrogen isotopes. $\delta^{15}\text{N}$ is enriched by 3 to 5‰ in each trophic level (Section 5.3). If *Genyornis* diet contained higher proportions of animal-based food sources than did *Dromaius*, which typically has no more than ~5% animal in its diet (Davies, 1978; Dawson, 1984), then *Genyornis* eggshell $\delta^{15}\text{N}$ should be more positive than in eggshell of co-habiting *Dromaius*. The average $\delta^{15}\text{N}$ in 114 >50 ka KT-LE *Dromaius* is $+11.1\text{‰}$ (S.E. $\pm 0.2\text{‰}$), statistically indistinguishable from the average $\delta^{15}\text{N}$ in 174 >50 ka co-habiting KT-LE *Genyornis* ($+10.7\text{‰}$; S.E. $\pm 0.2\text{‰}$; Table 3; data are in Tables S8, S9). We conclude, therefore, that *Genyornis* was primarily an herbivore.

7. Results: Paired reconstructions of ecosystem status and moisture balance for the past 100 ka from five regions across arid Australia

Dromaius is a generalist feeder (Dunstan et al., 2013). Quantifying *Dromaius* $\delta^{13}\text{C}_{\text{diet}}$ establishes the proportion of C₃ and C₄ forage consumed in the weeks to months before egg laying. Miller et al. (2005a) showed that prior to 50 ka *Dromaius* had an isotopically variable diet, sometime eating almost exclusively C₄ grasses, other times almost exclusively C₃ trees and shrubs, and more frequently, variable mixtures of the two. However, the C₄ grass dietary component was abruptly reduced 50 ± 5 ka, which we interpreted as a major loss of biodiversity at the base of the food chain. Because the timing of the dietary shift is indistinguishable from the appearance of humans in the landscape (O'Connell and Allen, 2015), and the disappearance of the remaining megafauna (Johnson, 2006), a dramatic dietary shift raises compelling questions about the roles of climate, megafauna, and humans in determining the status of ecosystems across the arid zone.

The conclusion of an abrupt change in KT-LE *Dromaius* diet was challenged by Murphy et al. (2012), who argued that the $\delta^{13}\text{C}_{\text{diet}}$ running mean suggested that the central tendency of the data showed a significant decline well before 50 ka, and that a better explanation would be the general drying of the climate as the global ice-age cycle developed. However, Murphy et al. (2012) failed to recognize that a change in mean $\delta^{13}\text{C}_{\text{diet}}$ was not the basis for the interpretation of an abrupt ecosystem change. Rather, the primary evidence is the dramatic decline in the proportion of C₄ plants in *Dromaius* diet. Furthermore, a running mean obscures abrupt changes in any time-series.

To re-evaluate whether the loss of C₄ plants in *Dromaius* diet was abrupt or gradual, we have expanded our datasets of $\delta^{13}\text{C}_{\text{diet}}$ by collecting and analyzing additional *Dromaius* and *Genyornis* eggshell from the three regions described in Miller et al. (2005a), and by adding two new regions, Lake Frome, South Australia, and the North West Cape in Western Australia (Fig. 1). This paper incorporates over 3,400 individually dated reconstructions of

$\delta^{13}\text{C}_{\text{diet}}$ from which we can more securely evaluate whether ecosystem transformation was abrupt or gradual.

The dominant constraint climate exerts on ecosystems across the Australian arid zone is moisture availability, with temperature a secondary determinant except at high elevations. In the following sections we use time-series of both $\delta^{13}\text{C}_{\text{diet}}$ and $\delta^{18}\text{O}$ converted to PPET to derive estimates of the mean climate state over the past ~100 ka, which enables us to evaluate the role of climate as a possible driver for observed shifts in *Dromaius* $\delta^{13}\text{C}_{\text{diet}}$. We use reconstructed $\delta^{13}\text{C}_{\text{diet}}$ and PPET in co-habiting *Genyornis* to independently evaluate climate and ecosystem status from ~100 ka until *Genyornis* extinction ~47 ka. The data on which the plots are derived is tabulated in Supplemental Tables S8-S16; composite figures of the actual $\delta^{18}\text{O}$ measurements expressed as the 20-point running mean for *Dromaius* from all five sites are shown in Figure S9 and for all *Genyornis* from the four sites with sufficient data in Figure S10.

7.1 The South Australia Transect: Kati Thanda-Lake Eyre (KT-LE) - Lake Frome - Port Augusta The South Australia transect includes an expanded KT-LE dataset, a new dataset from around Lake Frome, and an expanded dataset from sites around Port Augusta. Mean monthly and annual precipitation received at stations along the transect (Table S5) document variations in rainfall amount and seasonality. KT-LE is the driest (MAP 140 to 145 mm) with weak summer (DJF) dominance (S:W = 1.5 to 2.3). Lake Frome is wetter (MAP 185 to 195 mm), also with weak summer dominance (S:W = 1.6 to 2.2). In contrast, Port Augusta is wettest (MAP 260 mm) with slight winter dominance (S:W = 0.8). Hattersley (1983) found that currently 87% of native grass species around KT-LE are C₄, decreasing regularly to the south to about 50% C₄ around Port Augusta. However, as noted previously, the majority of C₄ grasses growing in the region currently are not of sufficiently high forage value to be consumed by *Dromaius* (Fig. 5).

Several predictions from our primary hypothesis can be tested with the South Australia transect. If our hypothesis of an abrupt reduction of C₄ grass across the transect between 50 and 45 ka (Miller et al., 2005a) is correct, and given the strong correlation between C₄ grass abundance and warm, summer-rain conditions, we then predict greatly reduced variance and a modest shift to more negative *Dromaius* $\delta^{13}\text{C}_{\text{diet}}$ after 45 ka in all three regions. The north-to-south transect should reveal steadily smaller proportions of C₄ plants and less variance in $\delta^{13}\text{C}_{\text{diet}}$ for >50 ka *Dromaius*, but little gradient in *Dromaius* $\delta^{13}\text{C}_{\text{diet}}$ mean or variance after 45 ka. For *Genyornis*, a more specialized feeder, the transect should reveal less variance and more negative mean $\delta^{13}\text{C}_{\text{diet}}$ both along the transect and between the two taxa in each region. Similarly, PPET, reconstructed from $\delta^{18}\text{O}$, should reveal a strong north-to-south decrease for both >50 ka *Dromaius* and *Genyornis*. The derivation of the datasets that provide the information to test these predictions are given below, with a summary of those results shown in Figure 7.

7.1.1 KT-LE *Dromaius* diet on a relative age scale Because we previously found the greatest change in *Dromaius* diet over the past 140 ka to be in the loss of C₄ dietary components (Miller et al., 2005a), we expect the clearest isotopic signal to be acquired from

regions dominated by C4 (summer-wet) grasses and C3 trees and shrubs. KT-LE, where virtually all grasses presently are C4 and all trees and shrubs are C3, serves as our type-site to evaluate whether abrupt ecosystem change occurred between 50 and 45 ka. Around KT-LE eggshell of both bird taxa are common in deflation hollows, and span a wide range of ages.

From the KT-LE region 552 *Dromaius* eggshell fragments have been analyzed for the extent of isoleucine epimerization (A/I) and for $\delta^{13}\text{C}_{\text{org}}$ ($n=297$) and/or $\delta^{13}\text{C}_{\text{carb}}$ ($n=516$), a 50% increase in $\delta^{13}\text{C}_{\text{diet}}$ estimates from our earlier study (Miller et al., 2005a). Because $\delta^{13}\text{C}_{\text{org}}$ and $\delta^{13}\text{C}_{\text{carb}}$ derive their carbon from different reserves in the female bird, each with a different time-integration, we treat them as independent proxies of $\delta^{13}\text{C}_{\text{diet}}$, even when measured in the same fragment. We convert $\delta^{13}\text{C}_{\text{org}}$ and $\delta^{13}\text{C}_{\text{carb}}$ to $\delta^{13}\text{C}_{\text{diet}}$ with the linear transformation described in Table 1, providing 813 dated estimates of *Dromaius* $\delta^{13}\text{C}_{\text{diet}}$.

Although we have developed age models that allow A/I to be converted to absolute age using independent dates, we initially evaluate the KT-LE dietary reconstructions on the relative age scale provided by AAR (Fig. 8), to stay as close to the original data as possible. Although the x-axis represents time, it is not linear, due to Late Quaternary variations in MAT. The A/I for *Dromaius* eggshell calcified between 50 and 45 ka around KT-LE is between 0.53 and 0.57 (Fig. 4B) a time interval represented by unfilled circles in Figure 8. Samples with A/I >0.57 exhibit widely variable $\delta^{13}\text{C}_{\text{diet}}$, ranging from nearly pure C4 to pure C3, and more commonly anywhere in between those two extremes. In contrast, samples with A/I <0.53 rarely contain >50% C4 components in their reconstructed diet.

There is a slight tendency for $\delta^{13}\text{C}_{\text{carb}}$ to have a greater range of values, likely because $\delta^{13}\text{C}_{\text{carb}}$ is set by a shorter time-averaged dietary signal than $\delta^{13}\text{C}_{\text{org}}$, and is therefore more likely to record short-term dietary extremes. However, the two populations have similar means and variance (Fig. 8 caption). Accordingly, for the other regions $\delta^{13}\text{C}_{\text{diet}}$ is plotted without carbon source differentiation to simplify the plots. Tables of all data are provided in the supplemental documents.

7.1.2 KT-LE *Dromaius* diet on an absolute age scale The age model for KT-LE *Dromaius* is based on 100 radiocarbon-dated eggshell <40 ka (Table S3), and is extended from 50 ka back to 150 ka with 18 OSL dates and 3 U/Th dates (Tables S6, S7). We use first-order reversible kinetics to convert A/I into its linear relation with time (Miller et al., 2000), then develop an empirical fit to the dated controls generally following the oldest age for each range of A/I, where the slope is proportional to the integrated thermal history (Fig. 9).

Uncertainties inherent in the KT-LE age model are illustrated by plotting the average difference between model age and calibrated ^{14}C age over the past 40 ka (Fig. 10). The age model has high potential to produce ages too old for samples between 35 and 15 ka, illustrating the combined effects of the low rate of racemization during the cold of the LGM (Miller et al., 1997) and variably shallow burial histories. Most samples from this time interval were found within the Shelly Island Unit, which is usually <2 m thick, and often thinner, whereas the preceding Williams Point Aeolian Unit was much thicker with consequent deeper burial of eggshell (Magee and Miller, 1998). Aligning the age model with the oldest dates for each A/I provides a conservative result that has asymmetric uncertainties. A consequence of the asymmetric age uncertainties is that samples with true ages <45 ka may

be incorrectly placed with samples >50 ka, whereas almost no sample with a true age >50 ka will be placed with the <45 ka samples.

The 813 KT-LE *Dromaius* $\delta^{13}\text{C}_{\text{diet}}$ reconstructions are plotted against their model age in Figure 11, except that fragments ^{14}C -dated <40 ka (158 measurements of $\delta^{13}\text{C}_{\text{diet}}$) are plotted by their calibrated age, and identified by a darker fill. The abrupt loss of palatable C4 plants in *Dromaius* diet between 50 and 45 ka is illustrated by the change in the fraction of all samples within representative time intervals for which $\delta^{13}\text{C}_{\text{diet}}$ exceeds 50% C4 plants. We define 50% C4 plants in *Dromaius* $\delta^{13}\text{C}_{\text{diet}}$ as the average of the ten isotopically lightest (C3) and ten heaviest (C4) samples in all 383 KT-LE *Dromaius* samples >50 ka, which is -17.8‰. Eggshell fragments with $\delta^{13}\text{C}_{\text{diet}}$ more positive than -17.8‰ have >50% C4 plants in the female bird's diet. We group the data into consecutive 30-sample clusters, calculate the mean age and the percentage of samples with $\delta^{13}\text{C}_{\text{diet}}$ more positive than -17.8‰ for each cluster and plot those results in Figure 12. In all 13 clusters >50 ka at least half of each cluster records >50% C4 plants in *Dromaius* diet, whereas only 4 out of 388 samples <45 ka record a $\delta^{13}\text{C}_{\text{diet}}$ containing more than 50% C4 elements. This pattern confirms an abrupt loss of high-nutrient-value C4 grasses on the landscape some time between 50 and 45 ka. The variance in $\delta^{13}\text{C}_{\text{diet}}$ for all samples >50 ka (var: 12.6‰; $n = 383$) is five times the variance of all samples <45 ka (var: 2.6‰; $n = 388$).

We conclude that the larger dataset of $\delta^{13}\text{C}_{\text{diet}}$ reconstructed from KT-LE *Dromaius* eggshell provides irrefutable evidence that reinforces the original conclusion of Miller et al. (2005a), demonstrating a dramatic reduction in the proportion of palatable C4 grass in the diet of KT-LE *Dromaius* between 50 and 45 ka, and that the claim by Murphy et al. (2012) of a gradual shift in diet was an artifact of relying on mean $\delta^{13}\text{C}_{\text{diet}}$ rather than the proportion of C4 plants in *Dromaius* diet, and using a running mean, which inherently smooths transitions.

7.1.3. KT-LE *Dromaius* paired climate and dietary reconstructions The evolution of *Dromaius* $\delta^{13}\text{C}_{\text{diet}}$ and climate (PPET) reveals a prolonged relatively moist interval between 130 and 55 ka, during which $\delta^{13}\text{C}_{\text{diet}}$ remains highly variable (Fig. 13). A 5 ka increase in aridity (55 - 50 ka) predates the abrupt reduction of C4 plants in *Dromaius* diet, but the moisture balance recovers to a mean state only slightly drier than before 55 ka by 50 ka. KT-LE PPET appears to remain relatively moist until ~25 ka, after which it becomes steadily more arid, reaching peak aridity by 15 ka. Dry conditions characterize the early Holocene, followed by a mid Holocene peak in wetness before returning to an arid state in the late Holocene. The 20th Century PPET decrease is considered an artifact of the birds utilizing expanded sources of relatively negative $\delta^{18}\text{O}$ bore-water provided by pastoral stock-watering points, rather than a decrease in aridity.

The most arid region of Australia is centered on KT-LE. Multiyear droughts have occurred during the instrumental period, and presumably also occurred for even longer periods during MIS 2. Although *Dromaius* are adapted to subsisting on a low energy, low protein diet (Dawson and Herd, 1983), when food and/or water sources are severely restricted, they may refrain from breeding or leave the region.

The KT-LE PPET record differs from the other regions in the transect that indicate generally arid conditions between 50 ka and the Holocene (see below). We suggest KT/LE *Dromaius* likely produced eggs during MIS 3 and 2 only during uncommon wet

years/decades when dietary resources were sufficient to sustain such investments, resulting in a strong PPET bias against arid years. We interpret the trend toward apparently wetter conditions from 50 to 25 ka to reflect the birds breeding increasingly only during rare wet years during this interval, based on the opposite trends from Lake Frome and Port Augusta.

The shift toward higher PPET after 25 ka likely reflects consistently lower rainfall, rather than increased evaporation rates. KT-LE MIS 2 temperatures were $\sim 9^{\circ}\text{C}$ lower than present (Miller et al., 1997), which would have decreased evaporation. There are no coherent trends in *Dromaius* diet after 45 ka despite substantial changes in both precipitation and temperature.

This interpretation is supported by the frequency distribution of the 100 eggshell ^{14}C dates <40 ka from the region (Fig. 14). There is at least one date for almost every millennium of the past 40 ka, confirming that even during the peak cold/aridity of MIS 2 some years received enough rainfall that forage was sufficient for the birds to breed there. But the sparsity of ages between 28 and 14 ka relative to other time intervals suggests this was the least conducive interval for *Dromaius* breeding of the past 40 ka. Alternatively, conditions favorable for eggshell preservation were at their minimum then, although this is not likely because arid times maximize the potential for aeolian redistribution of sediment, promoting eggshell preservation.

While the interpretation of PPET for the KT-LE region is somewhat ambiguous, more straightforward evidence in support of ecosystem change comes from the PPET/ $\delta^{13}\text{C}_{\text{diet}}$ biplot (Fig. 15), in which two widely separated clusters emerge, linked by samples with model ages between 55 and 43 ka. Very likely many samples with model ages between 55 and 50 ka have authentic ages <50 ka, but shallow burial resulted in an elevated A/I. The <43 ka cluster encompasses climates of late MIS 3, the cold, dry MIS 2, and the relatively warm, wet middle Holocene, without eliciting a dietary response. The PPET/ $\delta^{13}\text{C}_{\text{diet}}$ biplot indicates a major restructuring of the ecosystem-climate relation at KT-LE between 50 and 45 ka.

7.1.4 KT-LE *Genyornis* paired diet and climate reconstructions *Genyornis* $\delta^{13}\text{C}_{\text{diet}}$ derived from 424 carbonate (mean: -19.5‰ ; var: 6.8‰) and 174 organic (mean: -19.2‰ ; var: 6.0‰) $\delta^{13}\text{C}$ measurements are plotted against their model age in Figure 16. The $\delta^{13}\text{C}_{\text{diet}}$ in KT-LE *Genyornis* has a slightly more negative mean, but half the variance as in co-habiting *Dromaius* (mean: -19.4‰ vs. -17.9‰ ; var: 6.6‰ vs. 12.9‰ , respectively), supporting our contention that *Genyornis* was a more specialized feeder than *Dromaius*. Although a running mean is an unsuitable statistic for *Dromaius* $\delta^{13}\text{C}_{\text{diet}}$ because of the abrupt change in dietary resources, we plot the running mean for *Genyornis* $\delta^{13}\text{C}_{\text{diet}}$ to evaluate whether there is a trend toward reduced C4 dietary component as *Genyornis* approached extinction. The dietary reconstruction indicates a modest trend toward a more C4-rich diet after ~ 120 ka, peaking about 75ka, but no apparent decrease in C4 plants as *Genyornis* approached extinction. Unlike the *Dromaius* time series (Fig. 13), in which the age model likely places some samples <45 ka in the >50 ka interval, *Genyornis* is extinct by ~ 47 ka so its time series provides a more robust measure of possible ecosystem change approaching 50 ka. The lack of a $\delta^{13}\text{C}_{\text{diet}}$ trend after 80 ka provides compelling evidence against a gradual reduction of C4 plants on the landscape.

7.1.5 Frome paired diet and climate reconstructions Carbonate-rich dunes, beach ridges, and lunettes in close proximity to Lake Frome (Fig. 2) commonly preserve both *Dromaius* and *Genyornis* eggshell. A/I for bracketing ages of 50 ka (A/I = 0.55) and 45 ka (A/I = 0.51) derived from Figure 4 are consistent with a 1°C lower MAT than at KT-LE. Development of an independent age model utilizes eleven ^{14}C dates and the KT-LE age model adjusted for a 1°C lower temperature (Fig. S4).

The evolution of *Dromaius* $\delta^{13}\text{C}_{\text{diet}}$ (Fig. 17) includes 90 samples >50 ka and 109 samples <45 ka. Variance in >50 ka *Dromaius* (9.2‰) is almost five times the variance in the <45 ka samples (1.9‰), and the mean is more positive (-19.4‰ vs -23.2‰) similar to differences in the *Dromaius* KT-LE dataset (Table 2). Compared to KT-LE *Dromaius* >50 ka, Frome *Dromaius* >50 ka are more negative (-19.4‰ vs -17.9‰) and less variable (9.2‰ vs 12.9‰); the <45 ka samples show similar differences, but smaller magnitudes (Table 2).

To evaluate whether a changing climate played a dominant role in the observed dietary shift in Frome *Dromaius*, we reconstructed PPET from the running mean of $\delta^{18}\text{O}$ in 187 dated *Dromaius* eggshell fragments (Fig. 17). The PPET time-series indicates relatively wet conditions prior to 70 ka, after which there is a gradual increase in aridity, and the onset of a major arid shift began after 40 ka. Significant C4 components remain in *Dromaius* $\delta^{13}\text{C}_{\text{diet}}$ until ~50 ka, but are greatly reduced by 45 ka, preceding the main PPET transition toward a more arid state by ~10 ka. The lack of concordance between the two proxies makes a climate explanation for the dietary shift untenable. The peak in aridity during MIS 2 contrasts sharply with high water levels in Lake Frome ~18 ka as proposed by Cohen et al. (2011), although the wetter Holocene may reflect a local increase in westerly-derived precipitation that could explain their reconstructed low-level shorelines.

The biplot of PPET and *Dromaius* $\delta^{13}\text{C}_{\text{diet}}$ (Fig. 18) produces discrete clusters for samples >50 ka and <45 ka, despite both relatively wet and dry conditions after 45 ka. Increased moisture during the Holocene indicated by PPET is similar in value to conditions prior to 50 ka, but vegetation did not respond in the same manner. The difference in ecosystem responses to similar wetness is indicative of a fundamental shift in the climate-vegetation relation sometime between 50 and 45 ka.

The 172 Frome *Genyornis* $\delta^{13}\text{C}_{\text{diet}}$ (Fig. 19) are more negative on average than at KT-LE (-21.0‰ vs -19.4‰) and exhibit less variance (4.7‰ vs 6.6‰; Table 2), consistent with the *Dromaius* $\delta^{13}\text{C}_{\text{diet}}$ record and with the lower MAT and less abundant C4 grass around Lake Frome. The $\delta^{13}\text{C}_{\text{diet}}$ running mean exhibits little change in the proportion of C4 elements, providing additional evidence against a gradual climate-driven reduction in C4 vegetation approaching 50 ka.

7.1.6 Port Augusta paired diet and climate reconstructions The Port Augusta region has a MAT 2 to 3°C lower and receives more rain in all seasons than at KT-LE, with a slight winter-rain dominance, resulting in a lower proportion of C4 grasses. *Dromaius* and *Genyornis* eggshell were collected from deflation hollows excavated in aeolian sand derived from playa lakes and in longitudinal dunes north of Port Augusta (Fig. 2). A/I values in *Dromaius* between 0.44 and 0.40 separate eggshell >50 ka from those <45 ka (Fig. 4). The Port Augusta age model (Fig. S5) includes 22 radiocarbon dated *Dromaius* eggshell <40 ka and seven OSL dates associated with *Dromaius* and/or *Genyornis* eggshell. From 307 dated

Dromaius eggshell fragments 504 $\delta^{13}\text{C}_{\text{diet}}$ estimates were determined, nearly equally distributed between $\delta^{13}\text{C}_{\text{org}}$ and $\delta^{13}\text{C}_{\text{carb}}$ (Fig. 20). The $\delta^{13}\text{C}_{\text{diet}}$ in samples >50 ka is more variable than <45 ka (var: 7.3‰ vs. 2.0‰), although the variance is only one-half of that in the >50 ka series at KT-LE (Table 2). Port Augusta *Dromaius* $\delta^{13}\text{C}_{\text{diet}}$ >50 ka has lower proportions of C4 plants than at KT-LE, averaging 3‰ more negative (Table 2). Palatable grasses growing in the Port Augusta region before 50 ka were likely a mixture of C4 and C3 grasses; alternatively, *Dromaius* may never have consumed a pure grass diet there. The sudden shift toward a more negative $\delta^{13}\text{C}_{\text{diet}}$ over the past century likely reflects the complete loss of C4 grasses due to the arrival of rabbits that rapidly decimate fresh grass sprouts (Johnson et al., 2005).

The evolution of PPET (Fig. 20) is based on 295 dated *Dromaius* eggshell fragments. Variably moist conditions characterize the older portion of the record with a slight trend toward increasing wetness. Increasingly arid conditions beginning shortly after 65 ka reach peak aridity ~20 ka. Aridity persisted until ~15 ka, before becoming wetter through the early/middle Holocene, followed by steady drying. The abrupt loss of C4 plants in *Dromaius* diet ~50 ka occurred during a trend toward more arid conditions, which may have contributed to that loss. But, the absence of an increase in C4 plants in response to the mid Holocene peak in moisture suggests that climate is unlikely to have been a significant factor in the ecosystem reorganization ~50 ka. Access to relatively negative $\delta^{18}\text{O}$ bore water under European land-management practices produced an acclimatic trend toward lower PPET values over the past century.

The climate-diet relation provided by the $\delta^{13}\text{C}_{\text{diet}}$ -PPET biplot (Fig. 21) defines two distinct clusters for Port Augusta *Dromaius*, one composed exclusively of >50 ka samples and the other samples <45 ka, further reinforcing our interpretation of a change in the climate-vegetation relationship shortly after 50 ka.

The $\delta^{13}\text{C}_{\text{diet}}$ recorded by 221 Port Augusta *Genyornis* eggshell reveals no significant trend approaching 50 ka (Fig. 22), reinforcing the evidence from KT-LE and Frome that no gradual reduction in C4 plants occurred prior to 50 ka. The mean $\delta^{13}\text{C}_{\text{diet}}$ in Port Augusta *Genyornis* is about 1‰ more negative than Frome and nearly 3‰ more negative than around KT-LE, a pattern consistent with decreasing proportions of C4 grasses in cooler, more winter-rainfall dominant regions.

7.1.7 Implications from the South Australia transect The regional $\delta^{13}\text{C}_{\text{diet}}$ and PPET reconstructions along the climate gradient of the South Australia transect show patterns consisted with our initial predictions (Fig. 7). The close correspondence of $\delta^{13}\text{C}_{\text{diet}}$ mean and variance and PPET both within and between *Dromaius* and *Genyornis* eggshell across the transect bolsters the integrity of the isotopic proxies and the strength of our proposed interpretations (Section 7.1). The high correlation between regional MAT and average PPET for both taxa >50 ka and for *Dromaius* at peak LGM aridity (Fig. 7) reinforces the integrity of $\delta^{18}\text{O}$ and its conversion to PPET as a paleoaridity index.

7.2 Darling region, New South Wales A large number of ephemeral flood-out lakes exist along the lower Darling River extending upstream more than 200 km from its confluence with the Murray River (Fig. 2). All of these lakes have sandy lunettes on their downwind

margin, on which wind-deflated hollows frequently expose *Dromaius* eggshell, and in sediment >50 ka, also *Genyornis* eggshell. Additional samples come from around Lake Victoria on the Murray River 50 km downstream from its confluence with the Darling River, and from the currently dry Willandra lakes, ~ 120 km NE of the Darling-Murray confluence. Mean annual temperatures vary by ~ 1 °C across the region and are 1 to 2 °C lower than Port Augusta, indicating even fewer C4 grasses in this region. The age-model for the Darling samples (Fig. S6) is derived from 39 ^{14}C dated *Dromaius* eggshell <40 ka, nine ^{14}C dates >40 ka that provide minimum limiting ages, and two OSL dates on sediment enclosing *Dromaius* or *Genyornis* eggshell. A/I of 0.41 and 0.38 bracket the 50 to 45 ka time window (Fig. 4).

Dromaius $\delta^{13}\text{C}_{\text{diet}}$ reconstructed from 240 dated fragments (Fig. 23) defines a pattern broadly similar to those in the South Australia transect. Samples >50 ka exhibit more than three times the variance and a mean $\delta^{13}\text{C}_{\text{diet}} \sim 2\text{‰}$ more positive than samples <45 ka (Table 2). However, mean $\delta^{13}\text{C}_{\text{diet}} >50$ ka is 5‰ more negative than $\delta^{13}\text{C}_{\text{diet}}$ in KT-LE samples, consistent with the predicted smaller percentage of C4 grasses.

The evolution of PPET, derived from 155 dated *Dromaius* eggshell, describes a variably moist climate until ~ 40 ka, after which climate gradually became more arid, with the most arid interval between 25 and 14 ka (Fig. 23). Effective moisture then increased through the early Holocene, before becoming more arid again in the Late Holocene. This reconstruction is consistent with recent summaries of climate change since 60 ka for the Willandra lakes based on dustiness, lake deflation, and lake-level changes (Bowler et al., 2003; 2012). Willandra lake desiccation is documented beginning ~ 40 ka, with greatly increased dust fluxes and local lake deflation subsequently as climate became more arid. Maximum aridity occurred between 30 and 15 ka. Brief high lake-level phases in this period reflect catchment/runoff processes in the Eastern Highlands catchment rather than local relative wetness, whereas *Dromaius* $\delta^{13}\text{C}_{\text{diet}}$ and PPET reflect precipitation in close proximity to the nesting sites.

Although there are large uncertainties in the conversion from A/I to age, no conversion can align the onset of aridity with the shift in *Dromaius* diet because the dietary and climate proxies come from the same samples. Climate change is an unlikely explanation for the loss of *Dromaius* dietary diversity.

The *Dromaius* $\delta^{13}\text{C}_{\text{diet}}$ /PPET biplot yields distinct clusters for samples >53 ka and <45 ka (Fig. 24). The bimodal distribution reinforces our conclusion that a permanent transformation of the climate-vegetation relationship in the Darling region occurred ~ 50 ka. Insufficient *Genyornis* samples are available to evaluate changes in diet or climate over time (Fig. S7).

7.3 North West Cape, Western Australia (WA) Extensive linear sand dunes in the North West Cape region, Western Australia (Fig. 1) were produced by strong southerly winds mobilizing shelly beach sand throughout the Late Quaternary (Hesse, 2010). The high biogenic carbonate content of the sand provides an optimal environment for eggshell preservation. Recent aeolian deflation has exposed significant concentrations of both *Genyornis* and *Dromaius* eggshell. We aggregate samples over a 200 km north-south region with similar MAT ($\sim 23.5 \pm 1^\circ\text{C}$) to obtain sufficient density of dietary reconstructions to evaluate potential shifts in ecosystem status over the past 100 ka. The age model for the WA

samples (Fig. S8) is derived from 19 radiocarbon dates on *Dromaius* eggshell, and eight OSL dates on quartz in sediment enclosing either *Genyornis* and/or *Dromaius* eggshell. We further constrain the age model by calculating that *Dromaius* A/I corresponding to 45 and 50 ka at 23.5°C should be 0.649 and 0.705, respectively (Fig. 4).

The WA region contains the greatest concentration of burnt eggshell yet discovered in Australia. Miller et al. (2016a) conclude that the patterns of burning are diagnostic of humans harvesting eggs of both *Genyornis* and *Dromaius* and discarding eggshell fragments in and around their cooking fires. Blackened eggshell diagnostic of transient high-heat fluxes, which greatly accelerates racemization, is visually apparent. However, we also find human-discarded eggshell fragments with less extreme heating, insufficient to produce blackening but sufficient to accelerate racemization. Heated, but unblackened eggshell complicates the utility of AAR as a dating tool because of the temperature dependency of the rate constant. The possibility of unintentionally analyzing heated eggshell for AAR results in a larger window of uncertainty for the WA age model than for other regions. Consequently, we consider *Dromaius* with A/I between 0.705 and 0.741 (50 to 54 ka, $n = 18$) also as indeterminate in age, and designate those as neutral shaded symbols in Figure 25. It is also possible that humans colonized the WA region before our other regions and the AAR dates of 54 to 50 ka reflect human predation of *Dromaius* eggs. Undoubtedly, the ages for some samples in this group are correctly assigned, but many with true ages <45 ka return model ages >50 ka due to transient heating after being discarded near a campfire.

$\delta^{13}\text{C}_{\text{diet}}$ is derived from 449 dated *Dromaius* eggshell fragments (Fig. 25). Samples >54 ka exhibit greater variability (var = 15.8‰; $n = 87$) than those <45 ka (var = 2.3‰, $n = 294$), consistent with a severe reduction in the proportion of C4 palatable grasses between 55 and 45 ka. For the >54 ka samples $\delta^{13}\text{C}_{\text{diet}}$ from $\delta^{13}\text{C}_{\text{carb}}$ is more variable (var = 18.3‰) than from $\delta^{13}\text{C}_{\text{org}}$ (var = 13.0‰), presumably a result of its shorter integration time, but the two $\delta^{13}\text{C}_{\text{diet}}$ sources have indistinguishable mean and variance for samples <45 ka. The NW Cape has abundant grass cover currently, dominated by spinifex grasses (C4), which are of low forage value and rarely eaten.

PPET plotted at the same scale as other regions exhibits little structure (Fig. 25). The $\delta^{18}\text{O}$ signal in the NW Cape region had the poorest fit in our “modern” calibration dataset when regressed against all climate terms related to aridity (Fig. 6). This is likely a consequence of the karst system on which the sand dunes of the NW Cape are superimposed. Rainwater is efficiently removed from the surface and carried by interconnected subterranean conduits where little evaporation occurs. Drinking water is mostly accessed by birds and other animals where the ground surface intercepts the water table in springs, soaks, or caves. There is no apparent isotopic enrichment during MIS 2 even though aridity increased substantially across Australia. However, the average $\delta^{15}\text{N}$ in *Dromaius* eggshell (+10‰, both <45 ka and >54 ka) is similar to KT-LE, indicative of an arid landscape, despite the lack of evaporative ^{18}O -enrichment of source water. The $\delta^{18}\text{O}$ of *Dromaius* source water <45 ka averages 1‰ less positive than in >54 ka *Dromaius*. We suggest that the observed shift in $\delta^{18}\text{O}$ represents a change in $\delta^{18}\text{O}$ of precipitation, rather than a lessening of aridity in this region. Nevertheless, the $\delta^{13}\text{C}_{\text{diet}}$ /PPET biplot (Fig. 26) separates samples >54 ka from those

<45 ka, based mainly on differences in $\delta^{13}\text{C}_{\text{diet}}$, suggesting a change in the climate-vegetation relationship.

The mean $\delta^{13}\text{C}_{\text{diet}}$ from 137 dated *Genyornis* eggshell (-19.5‰; Fig. 27) is indistinguishable from that for co-existing *Dromaius* (-19.9‰), but the variance (7.6‰) is half the variance in *Dromaius* eggshell (15.9‰; Table 2), reinforcing the more restricted dietary range of *Genyornis*.

7.4. Climate change was not the primary cause of the abrupt reduction in *Dromaius* diet

~50 ka Our ~100 ka time-series of $\delta^{13}\text{C}_{\text{diet}}$ in *Dromaius* eggshell derived from five discrete regions across the Australian arid zone are consistent with an abrupt loss of C4 dietary resources, and consequent decrease in $\delta^{13}\text{C}_{\text{diet}}$ variance between 50 and 45 ka. Because *Dromaius* is a generalist feeder consuming a wide range of palatable plants, eggshell $\delta^{13}\text{C}_{\text{diet}}$ serves as a reliable proxy for the isotopic range of high-value forage in the landscape through time. The abrupt reduction in $\delta^{13}\text{C}_{\text{diet}}$ variability and loss of most C4 plants signifies a significant restructuring of arid land ecosystems across Australia between 50 and 45 ka.

$\delta^{13}\text{C}_{\text{diet}}$ variance decreases with lower MAT and greater MAP (Table 2). Those regions with high MAT and low MAP also have the highest inter-annual rainfall variability; summer rains are commonly associated with cyclones and/or transient monsoon-related depressions, separated by longer intervals of little rainfall. C4 grasses respond quickly to summer rain, and the high percentage of *Dromaius* eggshell with $\delta^{13}\text{C}_{\text{diet}}$ dominated by C4 plant components prior to 50 ka likely reflects a high frequency of significant summer rain, followed by the appearance of abundant nutritious C4 grasses, with *Dromaius* capitalizing on their high value nutrients. The lack of C4 elements in *Dromaius* diets after 45 ka could be related to a lack of summer rain or other impacts that reduced floral diversity.

A composite of all five PPET time-series (Fig. 28) demonstrates that unlike the dietary shifts, which occur in the same time window across the arid zone, climate evolution over the past 100 ka was diachronous. Except for the NW Cape, which lacks surface water evaporation, the other four regions share three primary features: generally moist conditions prior to 60 ka, peak aridity between 30 and 10 ka, long after the dietary shift, and a brief return to wetter conditions at some point during the Holocene, before returning to relative aridity in the latest Holocene. However, across the critical 60 to 40 ka time period there is little shared variability. The Darling and Frome regions remained relatively moist more than 5 ka after the dietary shift, whereas Port Augusta and KT-LE both display an onset of drier conditions prior to the dietary shift, but peak aridity occurs much later in all four regions. The lack of coincidence of a change in moisture balance with the change in *Dromaius* diet confirms that the dietary shift is not a response to climate change.

8. Explaining the changes in *Dromaius* diet

At the time of European colonization the flora and fauna of Australia had been manipulated by humans over the preceding 50 ka, at least to some extent through frequent use of fire on the landscape (e.g. Jones, 1969; Johnson, 2006; Bird et al., 2013). Consequently, the geographic pattern of flora and fauna documented by early Europeans had developed a quasi-equilibrium with Aboriginal activities (Gammage, 2011). Our $\delta^{13}\text{C}_{\text{diet}}$ reconstructions confirm a shift in ecosystem structure and function, between 50 and 45 ka. Biplots of $\delta^{13}\text{C}_{\text{diet}}$

and PPET show that the dietary shift represents a fundamental change in the relationship between climate and vegetation across the Australian arid zone. The timing of this shift is coincident with human dispersal across the continent and megafaunal extinction. Continuous, datable sedimentary records spanning the Late Quaternary with secure environmental proxies would aid in evaluating the roles of humans and climate in explaining this shift, but such depositional systems have not yet been identified from the vast continental interior. The most likely records with appropriate proxies and achievable chronological precision may be in marine cores strategically placed to sample large terrestrial regions (van der Kaars and DeDeckker, 2002; Van der Kaars et al., 2006; Lopes dos Santos et al., 2013). In the following sections we explore the most likely mechanisms that could produce the observed changes (also see review by Bird et al., 2013).

8.1 Climate change Global climate change is one of the constants throughout Earth history. Climate change during the Quaternary was both frequent and of high amplitude, with the highest magnitude of change on millennial timescales after the mid-Pleistocene transition (MPT, ~0.7 Ma), when glacial cycles shifted from 40 ka to 100 ka cyclicities. The LEB lake-level records demonstrate that oscillations between regular monsoon rains sufficient to sustain permanent lakes in its depocenters, and aridity, when sediment delivered to depocenters by streamflow in wet times was deflated to the regional groundwater table. This alternating wet/dry pattern apparently occurred at least since the MPT. Ecosystems adapted to this range of Quaternary climatic stressors long before humans colonized the continent. If climate change is a likely explanation for ecosystem restructuring 50 ka, then the magnitude of change would have to be outside the range of variability to which ecosystems were already adapted.

For the most recent glacial-interglacial cycle, the past 120 ka, river discharge records and lake shorelines show a wetter arid-zone climate early in the cycle, with variably more arid conditions beginning close to the MIS 5/4 boundary, spatially variable increases in aridity between 60 and 40 ka, followed by the driest, coldest times during the LGM, 30-15 ka (Hesse, 2010). This pattern is coincident with the generally expanding volume of Northern Hemisphere ice sheets through the same interval, and the consequential reduction in ocean surface-water temperatures. A postulated climate cause of the observed dietary shift is a gradual response to the continued weakening of the Australian summer monsoon between 60 and 40 ka (Murphy et al., 2012; Cohen et al., 2015). Our data allow us to test that proposition. The evolution of PPET over the past 100 ka is consistent with significant changes in moisture balance, including generally wetter conditions through the early portion of the last glacial cycle, irregular drying through MIS 3 and the driest conditions between 30 and 15 ka. However, in none of the five regional time-series of paired PPET/ $\delta^{13}\text{C}_{\text{diet}}$ is the observed change in $\delta^{13}\text{C}_{\text{diet}}$ coincident with a dramatic increase in aridity, or with the extinction of *Genyornis*.

Our results also contrast with the conclusions of Cohen et al. (2015) who argued that increased aridity ~50 ka caused *Genyornis* extinction. Cohen et al. (2015) base their argument on the KT-LE water-level record, which actually reflects monsoon runoff from the northern reaches of the LEB catchment rather than effective moisture around KT-LE; consequently any connection between lake level and *Genyornis* survivability is tenuous. In

contrast, eggshell PPET directly records the status of moisture where the birds are breeding. In the Frome and Darling regions ecosystem change preceded aridity by 5 to 10 ka, whereas it post-dated by 10 ka the onset of a trend toward greater aridity at Port Augusta.

The discordance between the timing of the abrupt shift in ecosystem structure and climate change, coupled with the lack of evidence for unprecedented aridity shift 60-40 ka in Australian records from outside the arid zone, challenge a climate explanation for the dietary shift. Long speleothem $\delta^{18}\text{O}$ records from terrestrial sites elsewhere in the Southern Hemisphere (Carolin et al., 2013 (Borneo); Cruz et al., 2005 (Brazil)) show no exceptional changes during the 60-40 ka interval, and even longer climate records spanning the Middle and Late Quaternary from ice and marine sediment cores similarly fail to identify exceptional climate change between 60 and 40 ka. The shift in climate-vegetation relation revealed through biplots of PPET and $\delta^{13}\text{C}_{\text{diet}}$ in all five regions requires the ecosystem shift to persist through the cold/dry LGM and the warmer/wetter Holocene, a pattern inconsistent with a primary climate driver.

8.2 A changed fire regime Australia is fire prone (Russell-Smith et al., 2007). Natural fires commonly result from lightning strikes during convective storms throughout the arid zone, and in the build up to the wet season in the tropical north. Today, many plant taxa require fire for their seeds to germinate, and the wide range of post-fire recovery strategies invoked by plants testifies to their long exposure to burning.

8.2.1 A changed fire regime following human colonization Humans have had controlled use of fire for at least 400 ka (Karkanas et al., 2007; Bowman et al., 2009), so we can be confident that humans colonizing Australia were capable of making fire as needed. Humans burn for many reasons, including to clear vegetation allowing easier travel, to hunt game along the fire front, to promote the growth of new seedlings of a desired food source, and to communicate with distant populations. For human burning to impact ecosystem structure it must differ in important ways from the natural fire regime. The two primary differences between human and natural fire regimes are that humans can initiate fires at any time, and human burning can be accomplished at lower fuel loads. In contrast, lightning strikes exhibit high seasonality and require significant fuel loads, dry conditions, and sustained winds to produce large fires. An ancillary factor that might allow a changed fire regime to impact Australian ecosystems in unexpected ways is the exceptionally low nutrient reserves in soils throughout the arid zone (Raupach et al., 2001).

For infrequently burnt landscapes, nutrient availability may increase in soils following a fire. However, if insufficient time elapses between fires to build up a pool of soil organic matter, nutrient levels after subsequent fires may drop. Burning releases nutrients to the atmosphere as ash where they can be transported by wind far from their source. For example, significant losses of N, P, and K following seasonal burning in all ecosystem types in Ghana resulted in a net depletion of N and P in soils, contributing to a decline in soil fertility despite replenishment from distant sources through wet and dry atmospheric deposition (Kugbe et al., 2012). Most of the vast arid zone of central Australia is far from potential nutrient replenishment sources from either atmospheric deposition or substrate weathering, suggesting that an increase in fire frequency would likely result in significant reductions in soil nutrients.

A common ecosystem response to increased frequency of burning is the expansion of grass at the expense of trees and shrubs (Bond, 2008). If human colonization resulted in more frequent burning across the arid zone, replacement of C₃ trees and shrubs by C₄ plants is expected. Carbon isotopic records of bulk soil organic matter from a sedimentary sequence in NW Australia show a shift from mixed C₃/C₄ to dominantly C₄ vegetation some time after 120 ka (Pack et al., 2003), consistent with an expansion of C₄ vegetation at some time after 120 ka. In apparent contrast is the reduction in C₄ grasses in *Dromaius* diet that characterizes the dietary shift at ~50 ka. In both the KT-LE and NW Cape regions C₄ grasses currently dominate the landscapes but the percentage of C₄ plants in the diets of *Dromaius* breeding there is typically <25%, whereas prior to 50 ka, it was >60% on average. This apparent contradiction is resolved by the observation that around KT-LE and the NW Cape the C₄ grasses, mostly species of spinifex (*Triodia* and *Plectrachne*) and cane grass (*Zygochloa*) have little or no forage value. Some spinifex sprouts are palatable, but subsequent to sprouting none of the plant is palatable (Pyne, 1991). The prediction that more frequent fires after 50 ka would have promoted the expansion of grasses is met, despite the marked decrease in C₄ plants in *Dromaius* $\delta^{13}\text{C}_{\text{diet}}$ after 45 ka.

A more difficult question is whether more frequent burning would produce more or less charcoal, and whether such a change would be recorded in proximal archives. Charcoal results from incomplete combustion, primarily of woody tissue, with finer charcoal derived from incomplete oxidation of leaves. The quantity of charcoal generated by a fire and the efficiency at which it may be transferred to a distant depocenter are poorly understood. Large pieces of charcoal from wood can be relocated only locally, but smaller, fine particles can be transported easily by wind. Whether more frequent, smaller fires that result from human ignition under limited fuel loads would generate on average more or less charcoal than the natural fire regime remains ambiguous. Mooney et al. (2011) synthesized 223 sedimentary charcoal records from Australasia and concluded “*There is no distinct change in fire regime corresponding to the arrival of humans in Australia at 50 ± 10 ka and no correlation between archaeological evidence of increased human activity during the past 40 ka and the history of biomass burning*”. However, none of their 223 records that record charcoal prior to 40 ka is from the Australian arid zone, and Mooney et al. (2011) do not provide an argument that predicts either an increase or decrease in charcoal frequency under a human fire regime compared to an earlier natural fire regime in a fire-prone system. Rather the strongest correlation of the charcoal frequency record in the Mooney et al. (2011) dataset is to climate; warm, wet interglacial/interstadial climates produce greater biomass accumulations than do cold, dry glacial/stadial climates. The mass of charcoal is, unsurprisingly, highly correlated with the standing biomass available to burn, regardless of whether a natural or human fire regime is the responsible ignition agent. We consider the evidence from charcoal abundance patterns to be ineffective as a predictor of the shift to a human fire regime across arid Australia 50 to 45 ka; Bird et al. (2013) reached a similar conclusion.

We offer no direct evidence in support of a human fire regime being responsible for the documented change in ecosystem status, but do conclude that the consequences of a changed fire regime provides a mechanism consistent with the observed shift in *Dromaius* $\delta^{13}\text{C}_{\text{diet}}$ and with the expansion of non-palatable but fire-promoted C₄ grasses throughout

Australia's arid zone. The geographic distribution of C₄ spinifex taxa, the most common grasses across the arid zone and of limited forage value, has expanded recently (Latz, 2007), consistent with our evidence of ecosystem restructuring after 45 ka. The exceptionally low nutrient reserves characteristic of the Australian arid zone (Raupach et al., 2001) potentially result in the region's ecosystems unusual sensitivity to a changed fire regime.

8.2.2 A changed fire regime following reduced browsing pressure A change in fire regime is also a plausible consequence of megafaunal extinction. Regardless of causal mechanism, the available evidence suggests that the Australian megafauna went from a dominant presence across the arid zone prior to 60 ka to ecological insignificance by 40 ka. The loss of megafaunal browsers very likely resulted in ecological impacts from reduced browsing pressure (Bond and Keeley, 2005; Johnson, 2009; Staver et al., 2009). One consequence, the probability of increased densities of shrubs and woody vegetation with fewer browsers, led Flannery (1990) to suggest that megafaunal extinction on its own was a likely cause for a changed fire regime (Lopes dos Santos et al., 2013).

There are likely to be strong differences between the introduction of a human fire regime and a new fire regime resulting from reduced browse pressure. The new fire regime following human colonization could be maintained continuously, whereas ecosystem adjustment to reduced browse pressure would likely produce a new equilibrium fire regime on millennial timescales. The persistence of the dietary shift exhibited by the *Dromaius* $\delta^{13}\text{C}_{\text{diet}}$ reconstructions over 50 ka makes human ignition a more plausible explanation for the abrupt reduction of palatable C₄ grasses throughout the Australian arid zone than a reduction in browse pressure.

8.3 Ecosystem restructuring and the anomalous Holocene monsoon Possibly the single most perplexing climate question from the Australian Quaternary is why the summer-monsoon-fed arid-zone lakes failed to return to high perennial levels during the Holocene. The available evidence documents permanent megalakes during MIS 5, which were similar to levels attained during other earlier wet intervals. By late MIS 5 or perhaps as late as the MIS 5/4 transition, arid-zone lake levels were variably lower, mixed with intervals of playa stages, and by mid MIS 3 arid-zone megalakes were dominantly in a dry playa state, with occasional shallow ephemeral fillings during unusually wet years or decades. This pattern is consistent with the overall trend toward a drier planet between 115 and 20 ka, as continental ice sheets expanded, sea level lowered, and ocean surface waters cooled. However, the Northern Hemisphere Indian, Asian and African monsoons were reinvigorated in the early Holocene, as boreal summer insolation peaked, sea level rose, sea-surface temperatures increased, and vegetation expanded (Kutzbach et al., 1996; Dykoski et al., 2005; Fleitmann et al., 2007; Wang et al., 2007; Cai et al., 2015). There are fewer strong Southern Hemisphere monsoonal systems, but they are in general antiphase at precessional timescales (Cruz et al., 2005; Wang et al., 2007; Ayliffe et al., 2013; Denniston et al., 2013a), and most show a Holocene increase in monsoon precipitation (e.g. Strikis et al., 2011). The dominant control on the strength of the Australian summer monsoon remains unclear, with local insolation, sea level, and the strength of the Asian winter monsoon all apparently important (Miller et al., 2005b; Tierney et al., 2008; Griffiths et al., 2009). What is clear is that while the Australian summer

monsoon was partially reinvigorated at ~14 ka (Wyrwoll and Miller, 2001) and delivered low-level fillings of KT-LE (Magee et al., 2004), it never delivered as much moisture to the arid zone lakes at any time in the Holocene as it did in MIS 3, let alone MIS 5, despite a Late Holocene austral summer insolation (30°S) maximum comparable to any peak in the past 90 ka, as well as high sea levels and warm ocean surface waters. Explanations for this discrepancy remain inconclusive, but large-scale re-ordering of ecosystems (McPherson, 2007; Bonan, 2008, among others) across the Australian arid zone may have played a role (Miller et al., 2005b). Bird et al., (2013) provides a detailed summary of the arguments both for and against this hypothesis, concluding that insufficient evidence is currently available to rigorously test whether the hypothesis is valid.

9. Summary and Conclusions

The Australian arid zone has its origins in the Pliocene with the widespread progressive development of salt lakes, stony deserts, and dunefields. The onset of the Quaternary resulted in additional cooling, reduced precipitation, and higher-amplitude climate shifts across much of Australia, especially after the mid-Pleistocene transition, when the amplitude of glacial-interglacial cycles increased. As the continent became more arid, the Australian biota evolved and adapted. Some extinctions may have occurred in response to these stressors, but by the beginning of the Late Quaternary, ~130 ka, a significant fraction of the Australian megafauna were still extant. There is no evidence that the climate changes of the Late Quaternary were any more extreme than those of previous glacial-interglacial cycles. Despite the lack of evidence for Late Quaternary climate extremes, two unprecedented events occurred: a growing body of work has revealed that all remaining megafauna disappeared from mainland Australia between about 50 and 40 ka, and in this paper we provide evidence for a fundamental restructuring of arid zone ecosystems between 50 and 45 ka. Over 2200 estimates of *Dromaius* $\delta^{13}\text{C}_{\text{diet}}$ determined from individually dated eggshell fragments in 5 regions across the Australian arid zone indicate that prior to 50 ka *Dromaius* diet included a large proportion of C4 grass, frequently more than 50%, and that the proportion of C4 dietary resources dropped dramatically between 50 and 45 ka in all regions, and remained low through to modern times. To further test whether the dietary change was abrupt or gradual, we determined $\delta^{13}\text{C}_{\text{diet}}$ in over 1200 eggshell fragments of co-habiting *Genyornis* that span the time interval from >100 ka to ~50 ka. *Genyornis* always includes a significant fraction of C4 plants in its diet. We find no trend in *Genyornis* $\delta^{13}\text{C}_{\text{diet}}$ that would indicate a reduction in C4 plants in the landscape between 100 and 50 ka, reinforcing the evidence of an abrupt change in ecosystem structure after 50 ka.

Throughout the last glacial cycle (115 to 15 ka) global climate irregularly cooled and dried, and the shoreline records of Australia's large arid-zone lakes reflect that drying. But the lack of firm evidence demonstrating that the extent of aridity during this interval exceeded the magnitude of arid periods during previous cycles weakens any argument that climate change was responsible for the loss of megafauna or reduction in palatable C4 plants. To more rigorously evaluate the possibility of a climate explanation for ecosystem restructuring, we use the $\delta^{18}\text{O}$ of eggshell calcite, a paleoaridity proxy (point-potential evapotranspiration; PPET) to compare the evolution of climate and ecosystem structure over

the past ~100 ka in the same five regions showing abrupt ecosystem restructuring ~50 ka. These data indicate that the abrupt shift in $\delta^{13}\text{C}_{\text{diet}}$ does not coincide with a change in climate; the onset of Late Quaternary aridity lags the dietary shift by 10 ka in the Darling region, and peak aridity post-dates the dietary shift by 10 to 20 ka in all areas. Biplots of *Dromaius* $\delta^{13}\text{C}_{\text{diet}}$ and PPET from all five regions suggest a permanent shift in the climate-ecosystem relation occurred between 50 and 45 ka, but this shift is not associated with a significant change in climate.

Changes in *Dromaius* diet occurred during the same time interval that early human colonizers dispersed across Australia. Therefore, if climate is an unlikely driver for ecosystem restructuring, the question arises as to what humans might have done that could have resulted in the dramatic change to high-value forage. A definitive answer remains elusive, but the generalist feeder, *Dromaius*, was able to adjust its feeding strategies to the changes in palatable plant supply. *Genyornis*, a more selective feeder, shows no hint of a dietary adjustment prior to the bird's extinction. For both *Genyornis* and *Dromaius*, the harvesting of the birds' eggs by humans (Miller et al., 2016a), would have reduced reproductive success. However, only *Genyornis* became extinct. Plausibly, egg harvesting, coupled with reduced diversity of high-value forage and possibly enhanced by hunting, produced a combination of stressors that explain *Genyornis* and other megafaunal extinctions. The timing of *Genyornis* extinction, 47.5 ± 2.5 ka, is now more securely defined than previously.

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Table 1

Taxon	$\delta^{13}\text{C}_{\text{org}}$	From $\delta^{13}\text{C}_{\text{org}}$ to diet	$\delta^{13}\text{C}_{\text{carb}}$	From $\delta^{13}\text{C}_{\text{carb}}$ to $\delta^{13}\text{C}_{\text{org}}$	From $\delta^{13}\text{C}_{\text{carb}}$ to diet
<i>Dromaius</i>	Measured	-2‰	Measured	$-10.1 \pm 1.7\text{‰}$ $n = 806$	-12.1‰
<i>Genyornis</i>	Measured	-2‰	Measured	$-12.1 \pm 1.6\text{‰}$ $n = 346$	-14.1‰

Table 2

<45 ka							<i>Dromaius</i>							
<i>Dromaius</i>	MAT (°C)	MAP (mm w.e.)	A/I (max)	<i>n</i>	Mean $\delta^{13}\text{C}_{\text{diet}}$ <45 ka (‰)	Variance (‰)		A/I (min)	<i>n</i>	Mean $\delta^{13}\text{C}_{\text{diet}}$ >50 ka (‰)	Variance (‰)	PPET Peak aridity MIS 2 (mm w.e.)	Δ aridity (MIS2-1)	PPET mean >50 ka (mm w.e.)
NW Cape	23.4	250	0.65	336	-22.6	2.4		0.75	111	-19.2	15.9	NA	NA	NA
KT-LE	21.5	120	0.53	379	-22.7	2.8		0.57	397	-17.9	12.9	710	240	500
Frome	20.7	180	0.51	109	-23.2	1.9		0.55	90	-19.4	9.2	670	190	475
Pt Augusta	19.2	300	0.40	293	-24.7	2.0		0.44	204	-21.6	7.3	540	160	410
Darling	17.8	255	0.38	113	-25.1	2.6		0.41	117	-22.9	9.5	535	135	400
<i>Genyornis</i>														
<i>Genyornis</i>	MAT	MAP	extinct					A/I	<i>n</i>	Mean (‰)	Variance (‰)	PPET mean	PPET variance	
NW Cape	23.4	250	-	-	-	-		0.53	224	-19.5	7.6	NA	NA	
		120	-	-	-	-		0.47	593	-19.4	6.6	595	1120	
Frome	20.7	180	-	-	-	-		0.47	172	-21.0	4.7	550	650	
		300	-	-	-	-		0.36	221	-22.0	3.2	440	20	
Darling	17.8	255	-	-	-	-		0.33	57	-20.2	8.2	NA	NA	

Table 3

Taxon	<i>Genyornis</i>			<i>Dromaius</i> >50 ka		
Region	$\delta^{15}\text{N}$	S.E.	<i>n</i>	$\delta^{15}\text{N}$	S.E.	<i>n</i>
KT-LE	10.7‰	0.2‰	174	11.1‰	0.2‰	114

Figure 1

Figure 2

Figure 3

Figure 4

Figure 5

Figure 6

Figure 7

Figure 8

Figure 9

Figure 10

Figure 11

Figure 12

Figure 13

Figure 14

Figure 15

Figure 16

Figure 17

Figure 18

Figure 19

Figure 20

Figure 21

Figure 22

Figure 23

Figure 24

Figure 25

Figure 26

Figure 27

Figure 28