Quaternary record of aridity and mean annual precipitation based on δ^{15}N in ratite and dromornithid eggshells from Lake Eyre, Australia

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Abstract The cause(s) of the late Pleistocene megafauna extinction on the Australian continent remains largely unresolved. Unraveling climatic forcing mechanisms from direct or indirect human agents of ecosystem alteration has proven to be extremely difficult in Australia due to the lack of (1) well-dated vertebrate fossils and (2) paleo-environmental and -ecological records spanning the past approximately 100 ka when regional climatic conditions are known to have significantly varied. We have examined the nitrogen isotope composition (δ^{15}N) of modern emu (*Dromaius novaehollandiae*) eggshells collected along a precipitation gradient in Australia, along with modern climatological data and dietary δ^{15}N values. We then used modern patterns to interpret an approximately 130-ka record of δ^{15}N values in extant *Dromaius* and extinct *Genyornis newtoni* eggshells from Lake Eyre to obtain a novel mean annual precipitation (MAP) record for central Australia spanning the extinction interval. Our data also provide the first detailed information on the trophic ecology and environmental preferences of two closely related taxa, one extant and one extinct. *Dromaius* eggshell δ^{15}N values show a significant shift to higher values during the Last Glacial Maximum and Holocene, which we interpret to indicate more frequent arid conditions (<200 mm MAP), relative to δ^{15}N from samples just prior to the megafauna extinction. *Genyornis* eggshells had δ^{15}N values reflecting wetter nesting conditions overall relative to those of coeval *Dromaius*, perhaps indicating that *Genyornis* was more reliant on mesic conditions. Lastly, the *Dromaius* eggshell record shows a significant decrease in δ^{13}C values prior to the extinction, whereas the *Genyornis* record does not. Neither species showed a concomitant change in δ^{15}N prior to the extinction, which suggests that a significant change in vegetation surrounding Lake Eyre occurred prior to an increase in local aridity.

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Introduction

The late Pleistocene extinction on the Australian continent claimed approximately 60 taxa and appears to have selectively impacted large browsing herbivores (Burney and Flannery 2005). Since the extinction occurred prior to severe climatic change associated with the Last Glacial Maximum (LGM; approx. 30–15 ka) but after human colonization (approx. 60–50 ka), human activities are believed to have contributed to the megafauna's demise (Roberts et al. 2001; Johnson and

Prideaux 2004; Burney and Flannery 2005; Miller et al. 2005; Prideaux et al. 2007). For reasons related to poor fossil preservation and inherent difficulty in precisely dating bone material in this time range, there is ongoing debate about exactly when most of the large Pleistocene fauna went extinct (Trueman et al. 2005). The most reliable chronology for an extinct taxon is derived from eggshell fragments of the flightless dromornithid bird *Genyornis newtoni*, which retain organic material in depositional environments where the preservation of organics is otherwise marginal (Miller et al. 1999, 2005). In addition to providing a reliable substrate for age determination via amino acid racemization (Miller et al. 1999), stable carbon isotope values (δ^{13}C) in eggshells of *Genyornis* and extant emu (*Dromaius novaehollandiae*) supply a unique paleoecological record of environmental change in semiarid Australia over the late Pleistocene (Johnson et al. 1999; Miller et al. 2005).

Previously published *Dromaius* eggshell δ^{13}C records from the Lake Eyre (LE) basin and other localities in south central Australia suggest a dramatic change in vegetation structure and composition over the extinction interval (approx. 45–50 ka), namely a significant reduction in palatable C_4 plant biomass on the landscape (Johnson et al. 1999; Miller et al. 2005). In conjunction with sedimentological records (Magee et al. 2004), temporal shifts in *Dromaius* eggshell δ^{13}C values support a "bottom–up" environmental cause (Miller et al. 2005) rather than a direct anthropogenic extinction agent, such as overhunting (Martin 1984) or disease (MacPhee and Marx 1997). Whether the shift in vegetation occurred in response to human alteration of the landscape, such as a change in fire regime, or to a natural decrease in monsoon intensity over interior Australia remains a contentious issue. Continuous pollen or macrofossil records from lacustrine archives are virtually nonexistent owing to the alkaline conditions combined with drying and deflation events that define most interior playa lake basins. Some evidence for a human-induced change in fire regime comes from marine and terrestrial sediment cores (Kershaw 1988; Kershaw and Nanson 1993), but these sites are located in more mesic habitats on the periphery of the continent.

A useful approach to addressing these questions may be a closer look at extant species that survived the extinction (e.g., Johnson et al. 1999; Johnson and Prideaux 2004; Miller et al. 2005). These data help characterize past environmental change at the landscape level, providing constraints for hypotheses regarding potential cause(s) of the extinction. Unlike sedimentological records from the LE basin (Magee et al. 2004), which are primarily influenced by regional climatic conditions operating in the monsoon-watered catchment, eggshell isotope values record changes in vegetation and soil conditions that are controlled by local climatic and precipitation regimes.

Dromaius are generalist foragers known to consume a wide variety of vegetation types, insects, and small animals (Dawson and Herd 1983; Perrin and Middleton 1985). As such, *Dromaius* and the related African ostrich have provided reliable biological proxies for assessing vegetation change at the landscape level through analyses of the carbon isotopic composition (δ^{13}C) of both the organic and inorganic fractions contained in modern and fossil eggshells (von Schirnding et al. 1982; Johnson et al. 1993, 1998, 1999). Based on what is known about extant species, interpretation of the landscape has also been characterized by the δ^{13}C in fossil eggshells of *Dromaius* and *Genyornis* in Australia (Miller et al. 2005) and *Aepyornis* spp. in Madagascar (Clarke et al. 2006).

The nitrogen isotopic record contained in these eggshells has been more difficult to interpret, because there are multiple factors influencing the δ^{15}N values of an omnivorous bird. An increase in δ^{15}N value of +3 to +5‰ with each trophic step in a food web has been recognized across taxonomic groups and ecosystems (Minagawa and Wada 1984; Kelly 2000). For *Dromaius*, which feeds principally on plants, the δ^{15}N in dietary vegetation is the primary determinant for the δ^{15}N in the organic constituents of its eggshells (Johnson et al. 1993). The δ^{15}N of plants in arid environments has been correlated to mean annual precipitation (Handley et al. 1999; Amundson et al. 2003); therefore, we predicted that the δ^{15}N of *Dromaius* and *Genyornis* eggshells collected in a restricted basin should provide a long-term record of local climate and precipitation.

Animals living in arid environments also are subject to physiological stresses that result in nitrogen conservation strategies and enhanced isotopic discrimination relative to their diets (Ambrose and DeNiro 1986; but see Murphy and Bowman 2006). In addition to physiological effects, *Dromaius* are known omnivores, which will increase the δ^{15}N value of their tissues relative to the nitrogen isotope composition of primary producers at the base of the food web (i.e., plant δ^{15}N). All of these factors are important in utilizing the δ^{15}N record to provide a reliable proxy for annual precipitation and hence aridity.

In this paper, the nitrogen isotope composition of a suite of modern *Dromaius* eggshells collected along a precipitation gradient in continental Australia is interpreted based on modern climatological data, as well as modern plant and prey (e.g. insect) δ^{15}N values. These data are used to interpret an approximately 130-ka paleoecological record of *Dromaius* and *Genyornis* eggshell δ^{15}N and δ^{13}C values from the LE basin in central Australia. Our goal was to examine temporal changes in the diet of a widely distributed, generalist omnivore that survived the late Pleistocene megafauna extinction and which is still a common component of the arid Australian interior today. We specifically

address changes in the eggshell $\delta^{15}N$ record, but we also rely on sedimentological data from the LE basin, isotopic data collected from the modern environment, and coeval shifts in fossil eggshell $\delta^{13}C$ values to provide context and constrain our interpretations.

Materials and methods

Field collection and precipitation estimates

Fossil *Dromaius* and *Genyornis* eggshells were collected within the LE basin during repeated field seasons between 1992 and 2008, where they are commonly found in deflated dune deposits (i.e., blow-outs) in a variety of dune types adjacent to modern and paleo shorelines (Johnson et al. 1999; Miller et al. 1999, 2005). An eggshell sample collection area was approximately 5–50 m² in size. Within this area, we often found eggshell fragments of different ages and from both species. Similarly, a collection might have consisted of a single species within a narrow age range. Modern *Dromaius* eggshells were collected opportunistically during field seasons throughout south-central Australia over the same time period.

Plants were collected during seven field trips made to Australia from 1994–2007 during June and July. Many plants were collected within 100 m of major highways, but a substantial number were sampled in remote locations corresponding to sites where modern and fossil *Dromaius* eggshells were found. At each site of collection, the latitude and longitude were recorded, along with the general landscape characteristics. The strategy for plant collection was twofold. First, plants that comprised the dominant vegetation within a region were sampled regardless of whether they were considered to be *Dromaius* or *Genyornis* potential food sources. For example, eucalypts are typically abundant in regions with >200 mm annual precipitation, but very few animals consume their leaves so we did not collect them. Depending on location, grass specimens were collected regardless of whether or not they contained seeds or flowers. Second, plants, including seeds and flowers, were sampled that constituted potential *Dromaius* (or *Genyornis*) dietary items. Based on the literature (Dawson and Herd 1983; Perrin and Middleton 1985) and personal observation, small herbaceous plants, flowers/seeds of *Acacia*, and grass flowers/seeds were particularly targeted. Chenopods were especially targeted even though it is not known whether they were a part of the extinct *Genyornis* diet or not, although *Dromaius* are known to consume them.

In 1994, plants were collected and preserved in two different ways. First, 20- to 30-cm-long terminal leaves

with branches were dried in a plant press, mounted without glue on herbarium sheets, and then sub-sampled for isotopic analysis in the laboratory. In addition, 100- to 200-mg samples of leaf, flower, or seeds were placed into small tubes and frozen in liquid nitrogen within a few hours of collection. They were kept frozen at $-186°C$ until they were freeze-dried at a laboratory in Australia prior to shipment back to the USA. A comparison of $\delta^{13}C$ and $\delta^{15}N$ values of plants preserved using the two preservation methods was found to be within analytical precision. Therefore, in subsequent years plants were dried and preserved in plant presses as described above.

Insects were collected with nets or captured individually in 2007 from locations in South Australia ranging from the Nullarbor Plain to the west of Alice Springs. They were killed in jars with naphthalene within approximately 24–36 h after collection. After they had expired, insects were pinned and air-dried. For isotopic analysis, abdominal tissue was sampled from larger insects. For smaller ones, the entire organism was combusted for isotopic analysis. *Dromaius* are known to be omnivorous, and the insect effort, while limited in its extent, provides at least some data for interpreting fossil and extant bird diets from the Australian outback.

The mean annual precipitation value assigned to each modern eggshell or plant sample was determined using mean annual rainfall data for the period 1970–2000 obtained from the Australian Bureau of Meteorology (http://www.bom.gov.au), by interpolation to the station closest to the point of collection and from continental and state-wide climate maps.

Direct dating and isotopic analysis

Direct dating of the organic fraction of eggshell fragments in this study was accomplished via accelerator mass spectrometry (AMS) ^{14}C and/or amino acid racemization [see Johnson et al. (1999) and Miller et al. (1999, 2005) for details on sample preparation methods, laboratory protocols, age calibration and associated error among radiocarbon, amino acid racemization, and other dating techniques]. Specifically, Johnson et al. (1999) describes the use of the *D*-alloisoleucine to *L*-isoleucine ratios (D/L) to age *Dromaius* eggshells. Radiocarbon dates were corrected for isotopic fraction using $\delta^{13}C$ values and converted to calendar years using the CALIB 4.3 software program (Stuiver and Reimer 1993) for samples with raw ^{14}C ages younger than 20 ka and the equation in Bard (1998) for samples older than 20 ka but younger than 50 ka. A hierarchal approach was used to bin individual fragments into age groups. We used calendar-corrected radiocarbon ages if available for samples younger than 50 ka. For all other samples we converted the D/L ratio to calendar years using

taxon-specific equations developed for the LE basin (Miller et al. 2005).

For $\delta^{15}N$ and $\delta^{13}C$ analysis of the organic fraction of (fossil and modern) *Dromaius* and (fossil) *Genyornis* eggshells, a small (approx. 10 mg) fragment of each eggshell was cleaned as described in Miller et al. (2005). For isotopic analysis of the organic fraction, an approximately 5-mg cleaned fragment was placed in a large (9 × 5 mm) silver capsule and dissolved with three sequential 20-μl aliquots of 6 N hydrochloric acid-sequanal grade. The residual acid was evaporated at room temperature under a fume hood for approximately 24 h; remaining water was removed in an oven at 85°C for 24–36 h and stored at 50°C under N_2 until analysis.

Nitrogen ($\delta^{15}N$) and carbon ($\delta^{13}C$) isotope values were determined using an elemental analyzer (NC 2500; CE Elantech, Lakewood, NJ) interfaced with a Thermo Finnigan (San Jose, CA) Delta Plus XL mass spectrometer (Carnegie Institution of Washington, Washington, DC). Isotopic results are expressed as δ values, $\delta^{13}C$ or $\delta^{15}N = 1000 \times [(R_{sample}/R_{standard}) - 1]$, where R_{sample} and $R_{standard}$ are the $^{13}C/^{12}C$ or $^{15}N/^{14}N$ of the sample and standard, respectively. The standards are Vienna–Pee Dee Belemnite (V-PDB) limestone for carbon and atmospheric N_2 for nitrogen. The units are expressed as parts per thousand, or per mil (‰). The within-run standard deviation of an acetalinide standard was ≤0.3‰ for $\delta^{13}C$ and ≤0.4‰ for $\delta^{15}N$ values for modern and fossil eggshell analyses and ≤0.2‰ for both isotope systems during modern plant and insect analyses.

For the $\delta^{13}C$ and $\delta^{15}N$ analysis of modern plants, field specimens were identified to the lowest possible taxonomic classification and grouped into general plant type (e.g., grass vs. chenopod). A small sample was removed from the dried edible portion, which included the leaf and flower if present, of each specimen that showed no evidence of herbivory, mold, or other discoloration. Approximately 1 mg of each sample was weighed into a small (3 × 5 mm) tin capsule and analyzed using the isotope ratio mass spectrometer system described above.

All statistical tests were calculated using the software program JMP (v7.0). Differences in mean $\delta^{13}C$ and $\delta^{15}N$ values among age groups were determined via a one-way analysis of variance (ANOVA) followed by a post-hoc Tukey pairwise comparison test. A multivariate analysis of variance (MANOVA) model of $\delta^{13}C$ and $\delta^{15}N$ values and an identity response function were also used to characterize changes in mean values of both $\delta^{13}C$ and $\delta^{15}N$ through time. Post-hoc Wilks' lambda and Pillai's trace multivariate tests were used to assess differences in the C and N isotopic composition of eggshells from different time periods. Statistical significance was tested at the $\alpha = 0.05$ level.

Results

Modern plants, insects, small vertebrates, and *Dromaius*

While we were successful in collecting both plants and modern *Dromaius* eggshells over a wide range of precipitation regimes (Tables 1, 2), the number of sites for which we were able to collect data for both plants and eggshells was limited due to the opportunistic recovery of modern *Dromaius* eggshells. As others have noted for Australia (Handley et al. 1999; Schulze et al. 1998, 1999), the $\delta^{15}N$ of plants varies nonlinearly as a function of mean annual precipitation (Fig. 1). At higher precipitation amounts, rainfall has less of an effect on the $\delta^{15}N$ of both *Dromaius* and plants. In the six locations where both *Dromaius* and plant data were collected, the average nitrogen isotope discrimination ($\Delta^{15}N_{Dromaius\text{-plants}}$) between *Dromaius* and plants was 6.3 ± 2.0‰ (range 3.0–8.9‰), which is well above the mean trophic discrimination factors (i.e., $\Delta^{15}N_{consumer\text{-diet}}$) reported for other birds; see review by Vanderklift and Ponsard (2003).

Plants segregated on the basis of general type (e.g., grass vs. chenopod) provide additional information for the interpretation of *Dromaius* eggshell $\delta^{15}N$ values (Fig. 2). Grasses (particularly seeds) and herbaceous dicots are common dietary items for *Dromaius* (Dawson and Herd 1983; Perrin and Middleton 1985). The mean [±standard error (SE)] of $\delta^{15}N$ in all grasses ($n = 252$) was 2.8 ± 0.3‰, whereas the herbs averaged 4.6 ± 0.4‰ ($n = 126$). *Dromaius* also consume the seeds and flowers of *Acacia*. *Acacia* have the capability for nitrogen fixation, and their $\delta^{15}N$ values are typically lower (3.3 ± 0.3‰; $n = 162$) in comparison to other sympatric plants, even though *Acacia* are present in even the most arid regions of Australia. In the arid regions of South Australia and New South Wales, however, *Dromaius* may rely on two general prey items that have higher mean $\delta^{15}N$ values than grasses and *Acacia*. During the breeding (i.e., egg-laying) season, insects can be important sources of protein (nitrogen) for *Dromaius* (Dawson and Herd 1983). Second, chenopods (saltbushes) often dominate the floral landscape of arid interior Australia, the seeds and flowers of which are consumed by *Dromaius*. These two groups of organisms were found to have higher $\delta^{15}N$, with a mean [±standard deviation (SD)] value of 8.4 ± 0.5‰ ($n = 61$) for insects and small invertebrates and of 9.0 ± 0.4‰ ($n = 100$) for chenopods.

Modern *Dromaius* eggshells $\delta^{15}N$ values grouped by rainfall regime, namely, <200 mm, 200–300 mm, and >300 mm MAP, have mean (±SE) $\delta^{15}N$ values of 12.7 ± 0.2‰ ($n = 84$), 11.1 ± 0.2‰ ($n = 69$), and 6.6 ± 0.4‰ ($n = 28$), respectively. Plants from localities

Table 1 Locality information, mean $\delta^{15}N$ values with associated SE, sample sizes, collection year, and mean annual precipitation (mm) of modern plant collections from Australia

Location	State	Year	n	$\delta^{15}N$	SE	MAP (mm)
Wilkatana	SA	2002	17	10.1	2.1	90
Lake Eyre	SA	1994	85	5.5	0.3	140
Darling Lakes	NSW	2002	44	5.2	0.7	150
Great Victorian Desert	SA	2001	15	4.1	1.0	150
Kangaroo Lake	SA	2006	30	4.8	0.8	150
Lake Frome	SA	2006	70	7.3	0.4	175
Darling Lakes	SA	2006	59	5.5	0.5	175
Port Augusta	SA	2002	21	6.3	0.6	210
Nullarbor	SA	2007	92	6.9	0.4	240
Alice Springs	NT	1994	35	6.2	0.7	300
Fink Gorge	NT	1994	17	5.8	0.9	300
Lake Gregory	WA	1998	35	3.4	0.7	350
Brookfield Conservation Park	SA	2007	180	4.7	0.2	350
Wolfe Creek Crater	WA	2001	87	2.4	0.5	400
Halls Creek (Tanami Track)	WA	2001	34	5.4	0.7	450
Ord River	NT	2001	35	−0.1	0.4	600
Great Northern Highway	NT	2001	23	1.1	0.6	650
Daly Waters	NT	1994	45	5.8	0.6	700
Great Northern Highway	NT	2001	21	3.2	0.6	750
Northern Kimberly	WA	2001	15	0.7	0.5	1050
Katherine	NT	1994	42	1.7	0.4	1100

SE Standard error, *MAP* mean annual precipitation

With the exception of the 2002 Wilkatana collection, MAP values were calculated using mean annual rainfall data for the period 1970–2000 obtained from the Australian Bureau of Meteorology (http://www.bom.gov.au) by interpolation to the station closest to the point of collection and from continental and state-wide climate maps
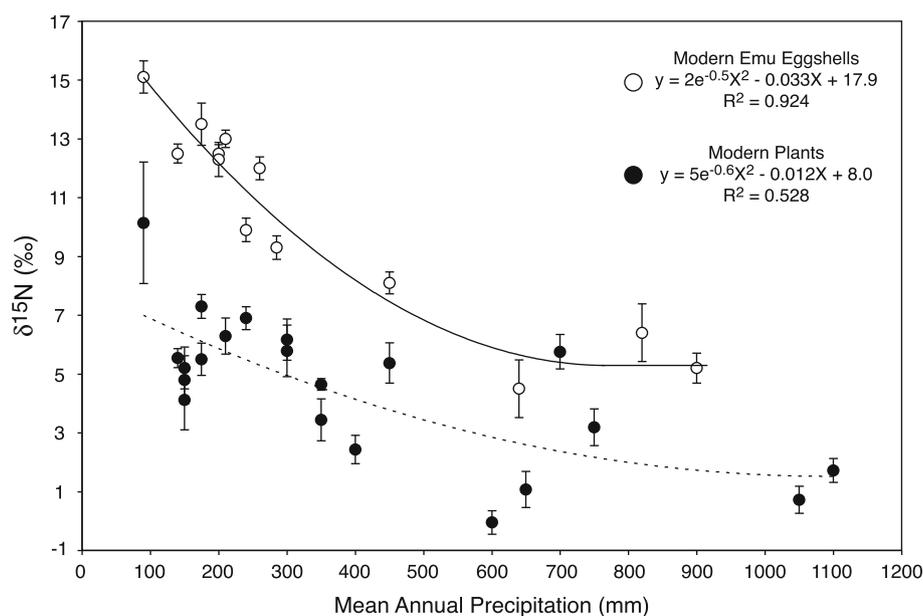
Table 2 Locality information, mean $\delta^{15}N$ values with associated SE, sample sizes, and mean annual precipitation (mm) of modern *Dromaius* eggshell collections from Australia

Location	State	n	$\delta^{15}N$	SE	MAP (mm)
Wilkatana (2002)[a]	SA	4	15.1	0.6	90
Wilkatana	SA	5	13.5	0.7	175
Lake Eyre	SA	46	12.5	0.3	140
Lake Tandou	SA	13	12.5	0.3	200
Darling Lakes	SA	13	12.3	0.6	200
Port Augusta	SA	22	13.0	0.3	210
Nullarbor	SA	35	9.9	0.4	240
Lake Victoria	SA	8	12.0	0.4	260
Lake Mungo	SA	4	9.3	0.4	285
Yorke Peninsula	SA	12	8.1	0.4	450
Kangaroo Island	SA	3	4.5	1.0	640
Western Australia	WA	3	6.4	1.0	820
Tasmania	TAS	11	5.2	0.5	900

See "Materials and methods" section in text for information as to how mean annual precipitation values were determined

[a] The Wilkatana eggshells collected in 2002 were separated from other Wilkatana collections because 2002 was an exceptionally dry year in South Australia, receiving approximately half of the normal precipitation amount

Fig. 1 Trends in δ^{15}N values of modern *Dromaius* eggshells (*open circles*) and plants (*filled circles*) collected along a precipitation gradient in Australia; *error bars* represent standard error (SE). Each series has been fitted with a second order polynomial relationship. See "Materials and methods" section in text for information as to how mean annual precipitation values were determined

Modern Emu Eggshells
$y = 2e^{-0.5}X^2 - 0.033X + 17.9$
$R^2 = 0.924$

Modern Plants
$y = 5e^{-0.6}X^2 - 0.012X + 8.0$
$R^2 = 0.528$

grouped by these rainfall regimes have mean ($\pm$SE) δ^{15}N values of 6.1 $\pm$ 0.8‰, 6.3 $\pm$ 0.2‰, and 2.8 $\pm$ 0.6‰, respectively. Based on these mean plant δ^{15}N values, mean ($\pm$SE) *Dromaius* $\Delta^{15}N_{\text{eggshell-diet}}$ values are 6.6 $\pm$ 0.8‰ at <200 mm MAP, 4.8 $\pm$ 0.3‰ at 200–300 mm MAP, and 3.8 $\pm$ 0.7‰ at >300 mm MAP. The Δ^{15}N at < 200 mm MAP is significantly higher than that measured for almost all animal species for which such information is available. Because *Dromaius* diets are often omnivorous in arid regions (<200 mm MAP), and owing to the preponderance of chenopods in these areas, a Δ^{15}N calculated with the mean ($\pm$SE) dietary δ^{15}N value (8.8 $\pm$ 0.3‰) of these potential food sources yields a Δ^{15}N of 3.9 $\pm$ 0.4‰, which is a more typical $\Delta^{15}N_{\text{tissue-diet}}$ value.

At Lake Eyre, modern *Dromaius* eggshells ranged in δ^{15}N from 8.1‰ to 16.2‰. Mean annual rainfall at Marree in the Lake Eyre Basin was 197 mm for the time period 1971–2000 (range 78–387 mm). Large rainfall events typically occurred during the summer months (January and February), especially in extremely high rainfall years. During average years, winter rainfall (June–August) is approximately 10–20 mm per month, which corresponds to the *Dromaius* breeding (egg-laying) season.

Late Pleistocene eggshell δ^{15}N

Mean *Dromaius* eggshell δ^{15}N values from the 75- to 50- and 130- to 75-ka age groups at Lake Eyre were statistically indistinguishable and also not significantly different from the δ^{15}N values of *Genyornis* eggshells of similar age (Table 3; Figs. 4, 5). Mean *Dromaius* eggshell δ^{15}N values for the 30- to 15- and 15- to 1-ka age groups, and for the modern time period, however, were significantly higher

than those for the pre-extinction (75–50 and 130–75 ka) age groups (Table 3; Fig. 3a); we found no significant changes in variance across age groups. More interesting than the mean values were the ranges in δ^{15}N values for each of the time periods. From 15 to 1 ka, δ^{15}N ranged from a minimum of 8.2‰ to a maximum of 17.8‰, which is almost identical to the δ^{15}N values of the modern samples. From 30 to 15 ka, δ^{15}N were distributed over a wider range, with values of 6.8 to 17.7‰. The distribution, means, and variance of the two groups are statistically similar. For the 75- to 50-ka group, however, δ^{15}N values ranged from 6.5 to 13.9‰, whereas for the 130- to 75-ka group, eggshell δ^{15}N values ranged from 6.0 to 16.3‰.

Multivariate tests revealed a significant difference between *Dromaius* eggshells from the 130–75 ka and 75–50 ka age groups that is primarily due to differences in mean δ^{13}C values (Figs. 4, 5). MANOVA results also suggested that *Genyornis* eggshells from both of the pre-extinction age groups are significantly different than their *Dromaius* counterparts, but differences between the species appear to be primarily driven by differences in mean δ^{13}C (not δ^{15}N) values (Fig. 4). Mean isotope values for the *Dromaius* eggshells from the post-extinction age groups (30–15, 15–1 ka, and modern) were not significantly different from one another.

Based on the data for modern *Dromaius* eggshells (Table 2; Fig. 1), the δ^{15}N for fossil *Dromaius* and *Genyornis* eggshells in each time period they occur was assigned a rainfall regime. Individual eggshells with δ^{15}N values of <10‰ were assigned to the >300-mm regime; eggshells with δ^{15}N values in the range 10–13‰ were placed in the 200- to 300-mm regime, while those with δ^{15}N >13‰ were designated to the <200-mm regime
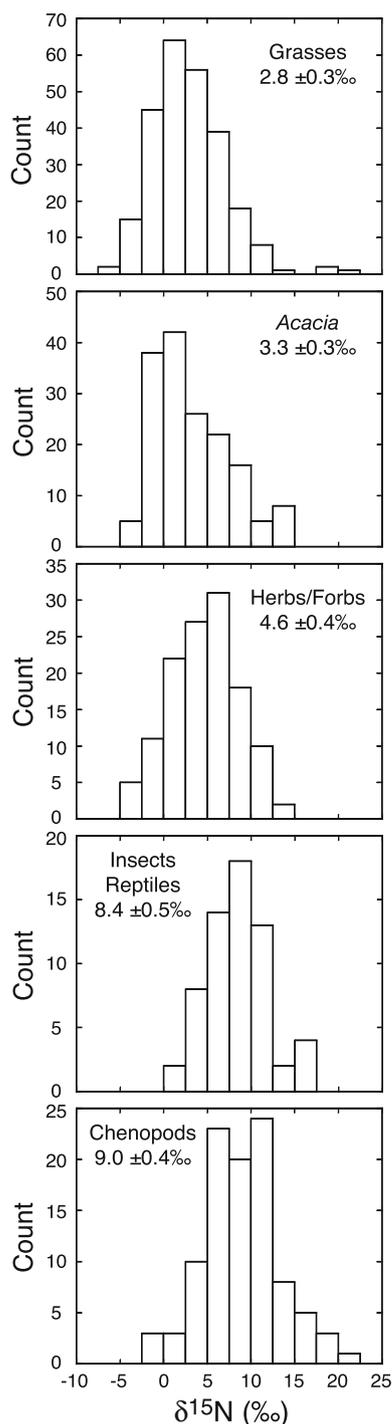
Fig. 2 Histograms of foliar $\delta^{15}N$ values grouped by plant type. Plants were collected from across central Australia (see Table 1 for list of collection localities). *Numbers* represent mean $\delta^{15}N$ ($\pm$SE). See text for sample sizes

(Table 3 and Fig. 5). The *Dromaius* eggshells dating to 15–1 ka were laid under environmental conditions similar to present conditions, but the 30- to 15-ka *Dromaius* were subjected to a greater proportion of very low MAP. In contrast, just prior to the megafaunal extinction event, the

Dromaius breeding season (austral winter) corresponded with higher rainfall, including conditions with >300 mm of MAP for approximately 30% of the time. During the larger 130- to 75-ka age group, climatic conditions in terms of MAP were the most moderate, with the majority of *Dromaius* producing eggshells at 200–300 mm of rainfall per year.

Discussion

The significant increase of approximately 2‰ in *Dromaius* eggshell $\delta^{15}N$ values from 130–50 ka to 30 ka–present may be primarily driven by two factors: (1) a change in *Dromaius* trophic level and/or physiological conditions and/or (2) shifts in the isotopic composition of vegetation. In our study, we relied on isotopic data for modern *Dromaius* eggshells and plants collected throughout Australia (Figs. 2, 3), coeval shifts in fossil eggshell $\delta^{13}C$ values (Figs. 4, 5), and previously published sedimentological records from the LE basin (Fig. 6; Magee et al. 2004) to provide constraints on these possibilities.

Temporal shifts in *Dromaius* trophic level and/or changes in the diet-to-eggshell trophic discrimination factor could explain the increase in *Dromaius* $\delta^{15}N$ values over time. *Dromaius* are not strict herbivores and opportunistically consume insects and lizards in albeit low proportions (Dawson and Herd 1983; Perrin and Middleton 1985). The nitrogen isotope data suggest that *Dromaius* are indeed omnivorous since mean modern eggshell $\delta^{15}N$ values are on average approx. 6‰ greater than mean modern plant $\delta^{15}N$ values collected from similar habitats (Fig. 1), which is a difference larger than the +3–5‰ diet–tissue trophic discrimination factor commonly found in controlled feeding experiments with avian subjects (Vanderklift and Ponsard 2003). A study of ostrich, another ratite related to *Dromaius*, found that eggshell $\delta^{15}N$ values are approximately 3.0–3.5‰ higher than those in the diet; however, this study was conducted on captive birds able to eat and drink ad libitum (Johnson et al. 1998).

Wild *Dromaius* living in semiarid central Australia are subject to a variety of physiological demands known to affect $\delta^{15}N$ trophic discrimination factors in birds and mammals, including diet quality (i.e., protein content), nutritional status, and water-stress. A captive feeding experiment on a small passerine (Pearson et al. 2003) showed that $\delta^{15}N$ trophic discrimination factors are positively correlated with dietary protein content, but the maximum observed increase of approximately 0.5‰ in $\delta^{15}N$ trophic discrimination factors from a diet comprising 20–100% protein is small in relation to the approximately 2‰ increase in LE *Dromaius* eggshell values over time. Since *Dromaius* are primarily herbivorous, a systematic

Table 3 Mean $\delta^{13}C$ and $\delta^{15}N$ values of the organic fraction of *Dromaius* and *Genyornis* eggshells from Lake Eyre, South Australia

Genera	Age (ka)	n	$\delta^{13}C$ (‰)[a]	$\delta^{15}N$ (‰)[a]
Dromaius	Modern	46	−19.9 (0.3)[b]	12.5 (0.3)
	15–1	104	−20.3 (0.3)	12.8 (0.2)
	30–15	29	−20.2 (0.5)	12.7 (0.4)
	75–50	28	−17.4 (0.6)	11.0 (0.4)
	130–75	80	−15.6 (0.3)	10.9 (0.3)
Genyornis	75–50	74	−18.4 (0.2)	10.2 (0.3)
	130–75	56	−18.2 (0.3)	10.8 (0.3)

[a] $\delta^{13}C$ and $\delta^{15}N$ values are presented as the mean, with the standard error (SE) in parenthesis

[b] A −1‰ correction was applied to the mean $\delta^{13}C$ values of modern *Dromaius* eggshells to account for the global decrease in the ^{13}C content of atmospheric carbon dioxide (i.e., "Suess Effect"), due largely to fossil fuel burning over the last 150 years
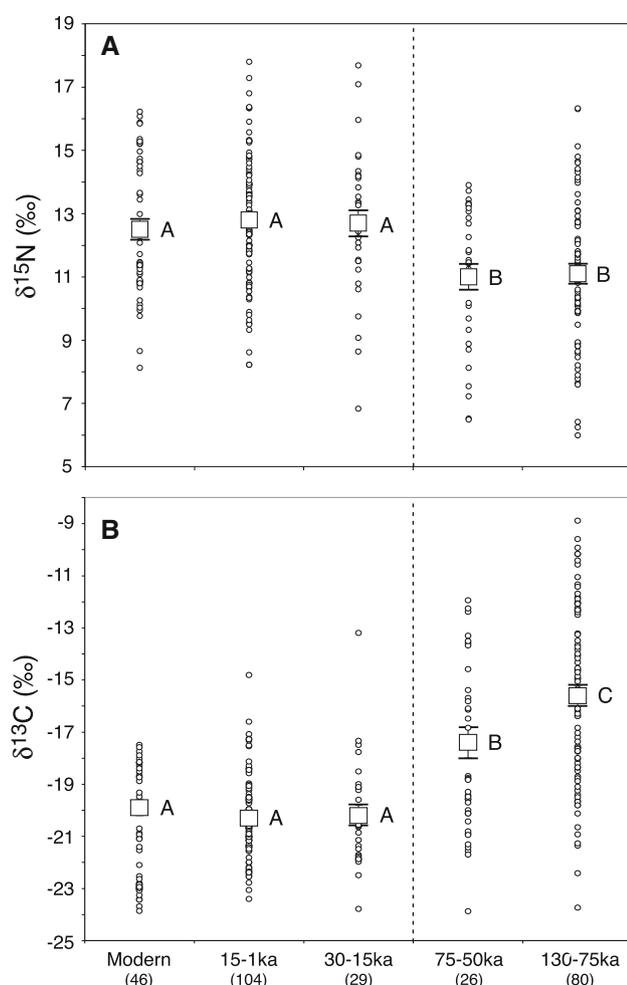

Fig. 3 $\delta^{15}N$ (**a**) and $\delta^{13}C$ (**b**) values of *Dromaius* eggshells grouped into two pre-extinction (50–75, 75–130 ka) and three post-extinction (modern, 1–15 ka, 15–30 ka) time periods. *Dashed vertical line* represents timing of the Australian megafauna extinction at approx. 50 ka, *large white boxes* represent mean isotope values of each group, *error bars* represent SE. *Different uppercase letters* denote significant differences in isotope values among groups as determined by a one-way analysis of variance (ANOVA) followed by a post-hoc Tukey pairwise comparison test (see "Materials and methods" section for details)
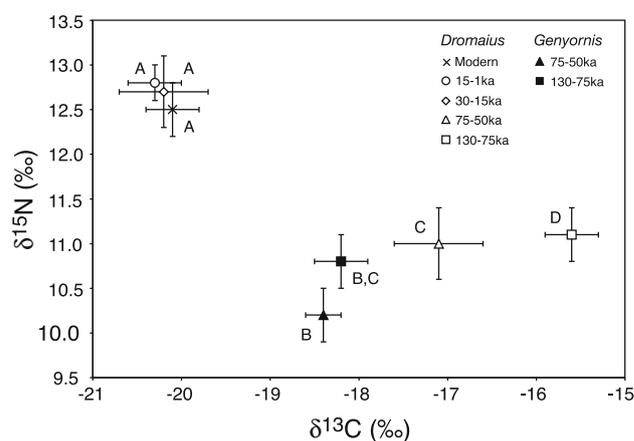

Fig. 4 $\delta^{13}C$ versus $\delta^{15}N$ plot showing the isotopic evolution in mean isotope values of *Dromaius* and *Genyornis* eggshells through time at Lake Eyre. *Different uppercase letters* denote significant differences in isotope values among groups as determined by a multivariate analysis of variance (MANOVA) model followed by post-hoc Wilks' lambda and Pillai's trace tests (see "Materials and methods" section for details)

increase in insectivory from 20 to 100% is unlikely, and thus a significant increase in diet quality over time is probably not a major contributor to the observed increase in eggshell $\delta^{15}N$ values.

A second physiological mechanism that could contribute to the trend in eggshell $\delta^{15}N$ values is related to changes in nitrogen cycling experienced during water-stress. Uric acid and urea, the major form of nitrogenous waste in birds and mammals, respectively, is significantly ^{15}N-depleted (up to 10‰) relative to body nitrogen pools (Steele and Daniel 1978; Castillo and Hatch 2007). Mammals experiencing water-stress produce urine highly concentrated in urea (Levinsky and Berliner 1959; Maloiy 1972, 1973), but unfortunately, experimental manipulations examining birds are non-existent. An increase in urea production thus yields an increase in body pool $\delta^{15}N$ values, a mechanism commonly used to explain differences in $\delta^{15}N$ values among

Fig. 5 Frequency distribution of local precipitation in the Lake Eyre basin over time assuming that fossil *Dromaius* and *Genyornis* eggshell $\delta^{15}N$ values were primarily driven by the nitrogen isotope composition of local vegetation as they appear to be in the modern scenario. We used the negative polynomial relationship (*open circles*, Fig. 1) to bin modern and fossil eggshells into three precipitation regimes (<200, 200–300, >300 mm). See "Results" section in text for information as to how fossil eggshells were designated into precipitation regimes
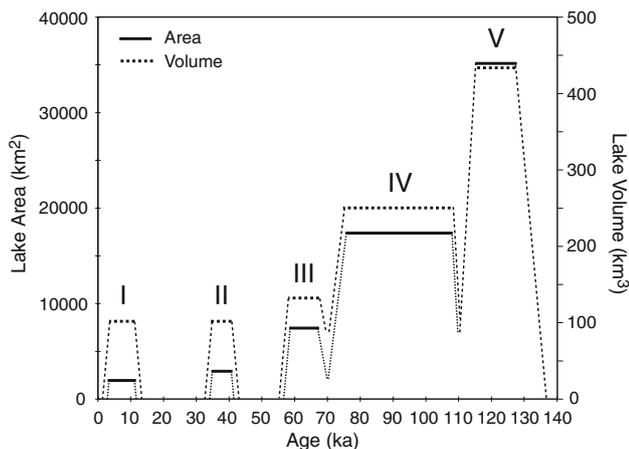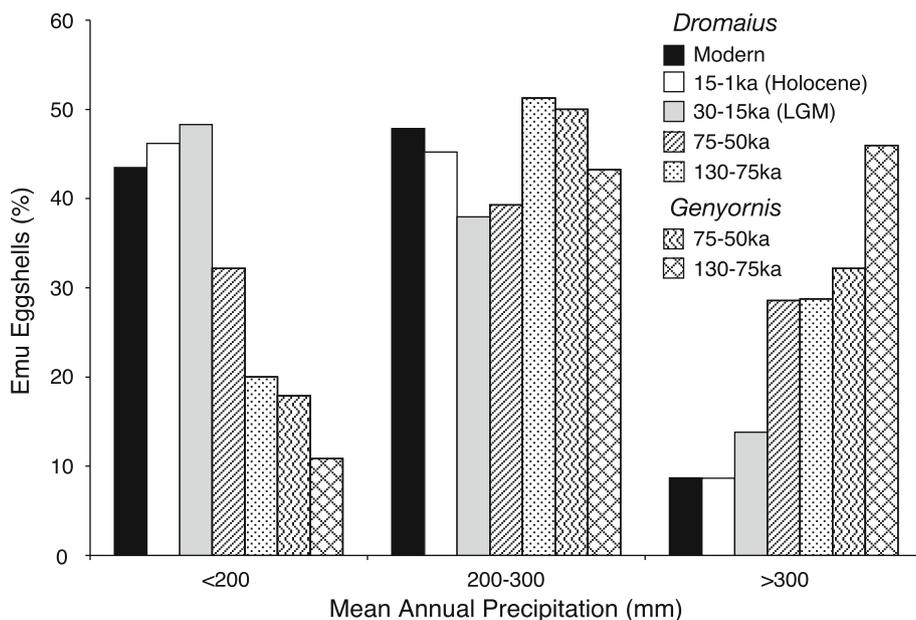

Fig. 6 Lake level and volume curve for Lake Eyre over the past 140 ka (modified from Magee et al. 2004). The deepest perennial lake (Phase V) formed at the beginning of the last interglacial (approx. 130 ka) and continued until approx. 105 ka with sustained lake levels >25 m above the modern playa, suggesting an enhanced and consistent summer monsoon during this time interval. After a brief dry episode at approx. 105 ka, the lake refilled to a depth of approx. 15 m above the modern playa and persisted for approx. 25 ka (Phase IV, 75–100 ka). Another brief period of aridity occurred between the end of Phase IV and the beginning of Phase III (approx. 65 ka). At the end of Phase III (approx. 60 ka), a permanent decrease in the water table occurred when significant deflation and aeolian redistribution excised the modern Lake Eyre playa basin to a height approx. 15 m below sea level. Temporary and shallow lakes (<5 m) occurred again at approx. 40 ka (Phase II) and approx. 10 ka (Phase I), but these only persisted for a few millennia

water-dependent and drought-tolerant mammalian herbivores living in xeric habitats (Ambrose and DeNiro 1986; Cormie and Schwarcz 1996). *Dromaius*, however, do not produce concentrated urine during water-stress (Dawson

et al. 1985); therefore, this mechanism is most likely not an important factor in the observed trends in $\delta^{15}N$ values over time.

Sedimentological records from the LE basin, isotopic data for modern *Dromaius* eggshells and plants collected throughout Australia, and temporal shifts in fossil *Dromaius* eggshells $\delta^{13}C$ values provide support that the approximately 2‰ increase in fossil *Dromaius* eggshell $\delta^{15}N$ values over time is primarily driven by changes in environmental—rather than physiological—factors. LE is the terminus of one of Australia's largest internal drainage catchments that extends far to the north of the basin and essentially acts as a rain gauge for the Australian monsoon. LE level/volume records show five periods (I–V) of permanent lacustrine conditions over the past 130 ka (Fig. 6; Magee et al. 2004). The most striking aspect of the record occurred at the end of Phase III (approx. 60 ka), when a significant lowering of the water table resulted in the excavation by deflation of the modern LE playa that now lies approximately 15 m below sea level. Short-lived perennial but shallow lakes (<5 m) occurred again at approximately 40 ka (Phase II) and approximately 10 ka (Phase I), but these only persisted for a few millennia.

There also exists a strong negative relationship between MAP and plant $\delta^{15}N$ values in Australia (Table 1; Fig. 1; Handley et al. 1999; Schulze et al. 1998, 1999). Mean plant $\delta^{15}N$ values decrease by approximately 6‰ from 100 to 600 mm of MAP. In regions where MAP was >600 mm per year, there was little change in the $\delta^{15}N$ as a function of rainfall. Other regions around the world show a similar trend in plant $\delta^{15}N$ values with increasing MAP, but the slope of the relationship varies depending on local temperature, elevation, and soil properties (Heaton 1987;

Schulze et al. 1991; Austin and Vitousek 1998; Handley et al. 1999; Amundson et al. 2003). Plants from localities that receive <200 mm of MAP (Wilkitana, SA in 2002) have extremely high $\delta^{15}N$ values in comparison to plants from more mesic habitats, probably because these regions contain higher proportions of xeric adapted vegetation (e.g., chenopods). Consideration of plant type is more important for assessing the *Dromaius* eggshell $\delta^{15}N$ values (Fig. 2) than is the mean $\delta^{15}N$ for all plants within a specific collection area.

A negative trend was also observed in the relationship between modern *Dromaius* eggshell $\delta^{15}N$ values and MAP (Fig. 2). Mammalian herbivores show similar trends in tissue $\delta^{15}N$ values versus annual rainfall, and it is assumed that these relationships are primarily driven by the rainfall-related variations in plant $\delta^{15}N$ values discussed above (Ambrose and DeNiro 1986; Sealy et al. 1987; Cormie and Schwarcz 1996). The slope of the relationship between MAP and eggshell $\delta^{15}N$ values, however, is much steeper than the negative slope observed in the modern plant data (Fig. 1). As a result, the mean ($\pm$SE) $\delta^{15}N$ values for *Dromaius* eggshells collected from sites that receive between 200 and 300 mm of MAP are statistically distinguishable from eggshells collected from sites receiving $\leq$200 mm of annual rainfall. Furthermore, mean $\delta^{15}N$ values of eggshells collected from areas that receive <200 mm of MAP are >6‰ higher than plants collected from similar environments. If discriminations are calculated based on an omnivorous diet with significant input from chenopod species, however, discrimination values for *Dromaius* growing in the most arid regions have values within the 3–5‰ range typically found for birds (Vanderklift and Ponsard 2003).

We can use the relationship between modern *Dromaius* eggshell $\delta^{15}N$ values and MAP as a coarse proxy for precipitation in the LE basin over time, assuming that fossil *Dromaius* eggshell $\delta^{15}N$ values were primarily driven by the nitrogen isotope composition of local vegetation as they appear to be in the modern scenario. An approximately 2‰ decrease in mean eggshell $\delta^{15}N$ values from today to before the extinction (approx. 130–50 ka) suggests that the LE basin received higher amounts of mean annual precipitation prior to the extinction. A greater percentage of pre-extinction eggshells lie within the 200- to 300- and >300-mm precipitation regimes, while most post-extinction eggshells fall within the <200- and 200- to 300-mm regimes (Fig. 4). A shift to drier, more arid conditions is supported by the LE sedimentological record, which suggests a major and permanent change in precipitation regime from pre-extinction (130–50 ka) to 30- to 15-ka time periods. In contrast to expectations based on paleoenvironmental records from previous interglacial periods, which suggest relatively wetter conditions in comparison to

glacial periods, the arid isotopic pattern of the LGM continues through the Holocene and into the present. The present interglacial period (Holocene) is an unusually arid time period in the LE basin in comparison to either interval prior to the extinction (75–50 or 130–75 ka).

Genyornis eggshell mean $\delta^{15}N$ values are statistically indistinguishable from mean $\delta^{15}N$ values of coeval *Dromaius*, but *Genyornis* have significantly higher mean $\delta^{13}C$ values than *Dromaius*, as noted in previous studies (Miller et al. 2005). In Fig. 4, the significant difference between *Dromaius* and *Genyornis* mean isotope values for the 75–50-ka age group results from an interaction in both $\delta^{13}C$ and $\delta^{15}N$ isotope values evaluated by the MANOVA test. Assuming that diet–eggshell discrimination values are comparable between the two species, the similarity in mean $\delta^{15}N$ values between the two taxa suggests that *Dromaius* and *Genyornis* occupied similar trophic levels—primarily herbivorous with periods of opportunistic omnivory.

In Australia, the extinct Dromithidae include species with a full range of foraging strategies, from obligate herbivores to strict carnivores. Paleontological evidence suggests that *Genyornis* was most likely a browser (Rich 1979). Based on the similarity in mean $\delta^{15}N$ values and its variance in these two species, we propose treating *Dromaius* and *Genyorni*s as omnivores. Using our calculated bins for determining MAP in which *Genyornis* was breeding, we find significant differences in their environmental settings (Fig. 4). A greater proportion of *Genyornis* reproduced during wetter seasons than did coeval *Dromaius*, suggesting that *Genyornis* relied on available water or vegetation that flourished during wet years, thus providing an environmental factor that may have played a role in its extinction. Based on modeling, Brook and Bowman (2002) argue that overhunting was the most likely cause for the Australian megafaunal extinction, while Johnson and Prideaux (2004) note that there is scant evidence for environmental change during the extinction interval. Our data provide further convincing evidence to the contrary: the $\delta^{15}N$ values of *Genyornis* eggshells demonstrate that these birds preferred breeding during wetter periods than did the *Dromaius*.

Our modern *Dromaius* and plant analysis along a fairly extensive MAP range was instrumental in estimating MAP levels during the Pleistocene and Holocene of the Lake Eyre basin, which co-varies almost exactly with the sedimentological lake level record (Magee et al. 2004). Our $\delta^{15}N$ record of two flightless birds represents the first detailed data on the trophic ecology of closely related taxa—one extinct and the other extant—as well as a MAP record for central Australia that spans more than 100 ka. Other than the earlier work by Grocke (1997) and recent work by Prideaux et al. (2007), paleontologists have been at a loss to adequately compare the forging ecology of a

species that adapted and survived relative to one that succumbed to the megafauna extinction from either environmental or other pressures.

While the organic eggshell $\delta^{13}C$ data show that a significant decrease in mean values occurred prior to the extinction, the carbon isotopic shifts were *not* associated with coeval changes in mean $\delta^{15}N$ values (Figs. 4a, 5). This implies that a significant change in vegetation surrounding LE occurred prior to an increase in local aridity and that the processes driving the shifts in the *Dromaius* eggshell carbon and nitrogen isotope records may be decoupled. A change in the seasonal timing and frequency of fires, which in turn affected vegetation, is perhaps the only other plausible mechanism that could have driven the observed decrease in $\delta^{13}C$ values while having no effect on $\delta^{15}N$ values (Kershaw 1986; Miller et al. 2005). Whether or not Australia's megafauna went extinct within a short time window, as shown by Miller et al. (1999, 2005), remains to be proven in other regions of the continent. Future isotopic analysis of carefully dated eggshell fragments from different regions would help address the controversy by providing additional environmental information (e.g., C_3–C_4 vegetation and MAP) that persisted at the time of megafaunal extinction.

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