

Paleoecological analysis of elephant bird (*Aepyornithidae*) remains from the Late Pleistocene and Holocene formations of southern Madagascar

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Abstract

The Faux Cap site is a Late Pleistocene to Holocene dunal fossil locality situated at the extreme southern end of Madagascar. Stratigraphic sections were taken from eight different points at the site from which 11 samples of *Aepyornithidae* (elephant bird) eggshells were collected from the Older Sand Dunes (OSD) and 20 samples from the Younger Sand Dunes (YSD) formations. Radiocarbon analysis yielded two dates from associated gastropod remains: 42,055 ± 805 BP to 32,605 ± 300 BP (Late Pleistocene) from the OSD deposits, and one date of 1135 ± 45 BP (Holocene) from the YSD deposits. Based on C and N isotopes analysis, a negative correlation between moisture and $\delta^{15}\text{N}$ values provides evidence that an aridification process occurred from Late Pleistocene (average of 10.62‰) to Holocene (average of 14.83‰). To examine aspects of elephant bird diet, we use eggshell and bone collagen proteins. To evaluate fractionation between food and tissue formation, we used modern chicken eggs, and as the difference of $\delta^{13}\text{C}_{\text{PDB}}$ values was about 1‰, this indicates that eggshell protein can be used for dietary analysis. Correction of $\delta^{13}\text{C}_{\text{PDB}}$ values for eggshell protein and bone collagen derived from the OSD and YSD deposits, provides inference that elephant bird food resources were poor in C_3 and more in C_3 type of CAM plants (e.g. Euphorbiaceae and Crassulaceae). Our research at the Faux Cap site shows that paleoenvironmental systems changed between the Late Pleistocene and Holocene probably due to a

paleoclimatic change dominated by an aridification process.

Key words: *Aepyornithidae*, Pleistocene, Holocene, C and N isotopes, CAM plants

Résumé détaillé

Dans le site de Faux Cap, des coupes stratigraphiques ont été effectuées en huit points où 11 échantillons de coquilles d'*Aepyornithidae* ont été collectés à partir des anciennes dunes de sable (« Old Sand Dunes » - OSD) et 20 échantillons des récentes dunes de sable (« Young Sand Dunes » - YSD). Une série de datations radioactives au C^{14} utilisant des gastéropodes comme faune associée a donné les résultats suivants : 42 055 ± 805 BP à 32 605 ± 300 BP pour l'OSD (Pléistocène supérieur) et 1135 ± 45 BP pour l'YSD (Holocène).

Comme les isotopes radioactifs stables sont des bons indicateurs environnementaux, des mesures d'isotopes stables des C et N ont été faites sur les protéines des débris d'œufs et les collagènes d'ossements d'*Aepyornithidae*. La corrélation négative entre l'humidité et la valeur de $\delta^{15}\text{N}$ prouve qu'un processus d'assèchement s'est opéré à partir de la fin du Pléistocène (la valeur moyenne du $\delta^{15}\text{N}$ est de 10,62 ‰) à l'Holocène (valeur moyenne de $\delta^{15}\text{N}$ de 14,83 ‰), ces valeurs ne tiennent pas encore compte de la différence des valeurs (fractionnement) entre la source d'alimentation et la formation des tissus. En effet, une des équations à résoudre pour les études paléoenvironnementales et celles du régime alimentaire ancien est la différence des teneurs isotopiques des éléments physico-chimiques entre la source d'alimentation et les tissus formés. De plus, il est nécessaire de déterminer le tissu et la molécule sur lesquels il va falloir travailler afin d'établir un modèle. Pour le régime alimentaire, nous avons menés une expérience sur des œufs de poules modernes en utilisant des valeurs isotopiques du C ($\delta^{13}\text{C}_{\text{PDB}}$) pour pouvoir évaluer ce fractionnement et pour servir de modèle, ce qui nous a permis d'enregistrer une moyenne de fractionnement du $\delta^{13}\text{C}_{\text{PDB}}$ de l'ordre de 1 ‰, et ce entre les protéines des coquilles et les albumines. Nous pouvons donc utiliser les protéines des coquilles pour l'analyse du régime alimentaire, tout en considérant aussi ce

changement de valeur de 1 ‰, pour les collagènes d'ossements. Le choix des protéines résulte du fait que les éléments organiques présentent une variabilité des valeurs (à l'exemple du C) faible, ce qui indique une stabilité plus élevée par rapport à celle des éléments non organiques (le CaCO₃ de la coquille par exemple). Comme nous travaillons sur des subfossiles, il pourrait y avoir des échanges de C entre le sol et le subfossile, ce qui pourrait fausser les valeurs. Ce risque est plus grand chez les éléments non organiques que chez les autres organiques.

Etant de l'ordre des Ratites, les Aepyornithidae ont un régime alimentaire omnivore, avec une forte tendance végétale. Aussi, il est nécessaire de savoir à quel type photosynthétique (C₃ ou C₄ ou CAM) la principale source d'alimentation des Aepyornithidae appartient, et une mesure isotopique du C permet cette analyse. Pour pouvoir disposer d'une palette de résultats comparables, nous avons pris les valeurs isotopiques de C des échantillons provenant des huit points de Faux Cap, ainsi que celles provenant d'autres échantillons additifs :

- 12 autres coquilles de Faux Cap dont leur appartenance stratigraphique n'est pas précise,
- six coquilles de Toliara et
- huit ossements provenant de Beloha, appartenant au laboratoire de Paléontologie de l'Université d'Antananarivo.

Aussi bien pour les protéines des coquilles que pour les collagènes des ossements, les valeurs obtenues permettent de mentionner que la principale source d'alimentation est légèrement du type C₃, majoritairement des plantes CAM du type C₃ (ex : les Euphorbiaceae et les Crassulaceae) ; et ce de la fin du Pléistocène jusqu'à l'Holocène. La végétation de la région est donc restée pratiquement la même depuis la fin du Pléistocène jusqu'à actuel, ce qui déculpabilise l'humain en tant que responsable direct de la disparition des Aepyornithidae, du moins de la destruction irréversible de leur habitat naturel.

Mots-clés : Aepyornithidae, Pléistocène, Holocène, isotope stable du carbone et de l'azote, plantes CAM du type C₃.

Introduction

The extinct genus *Aepyornis*, commonly known as elephant birds, was discovered in the southern part of Madagascar. The members of this genus, all flightless, are characterized by body masses estimated at 330-570 kg and reaching a height 2.5-3 m (Amadon, 1947; Silyn-Roberts & Sharp, 1986).

Elephant birds have been extinct for several hundred years and may still have been present after European colonization of the island in the early 16th-century (de Flacourt, 1658). Several different studies of *Aepyornis* subfossil remains (eggshells and bones) have been conducted based on specimens collected in the western and southern portions of the island (Chassaniol, 1879; Grandidier, 1903; Bradbury, 1919; Caudey, 1931; Amadon, 1947; MacPhee *et al.*, 1985; MacPhee, 1986; Yoshida & Kondo, 1992; Burney *et al.*, 2004; Clarke *et al.*, 2006; Bond & Silander, 2007).

Using stable isotope analyses of carbon (¹³C) and nitrogen (¹⁵N), numerous authors have clarified changes during the Quaternary in local environments and shifts in the diet of herbivores and carnivores (Johnson *et al.*, 1993, 1997; Lajtha & Michener, 1994; Hobson, 1995; Johnson *et al.*, 1998; Palmqvist *et al.*, 2003; Michener & Latjha, 2007; Pérez-Crespo *et al.*, 2012). In terrestrial ecosystems, the dominant control of δ¹³C plant values is via photosynthetic pathways (Koch, 2007). Thus, one of the aims of this current study is to examine aspects of Aepyornithidae diet and to document possible paleoecological shifts that would have impacted these birds during the Late Pleistocene – Holocene of extreme southern Madagascar. During the latter portion of this period, humans colonized the island and have been proposed as a driving factor in the extinction of the megafauna (Burney *et al.*, 2004; Crowley, 2010).

Materials and methods

Under the direction of Professor Nagaoka of Nagasaki University, excavations were conducted in the Quaternary dunes of Faux Cap (Late Pleistocene-Holocene). However, eggshell collectors frequently visit the site, and the stratigraphic integrity of several zones is disturbed. In cases of non-compromised areas, the samples need to be excavated from packed soil, in original deposition position, rather than deposits reworked by collectors.

Study site

The village of Faux Cap occurs in a rocky area interspersed with sand and partially engulfed by huge Aeolian dunes (Figure 1). From a southeastern direction and passing towards the north, seven sites were excavated (Table 1), and considerable quantities of elephant bird eggshells were collected from *in situ* deposits horizontally aligned from the bottom to the upper dune deposits (Figure 2); all belong to

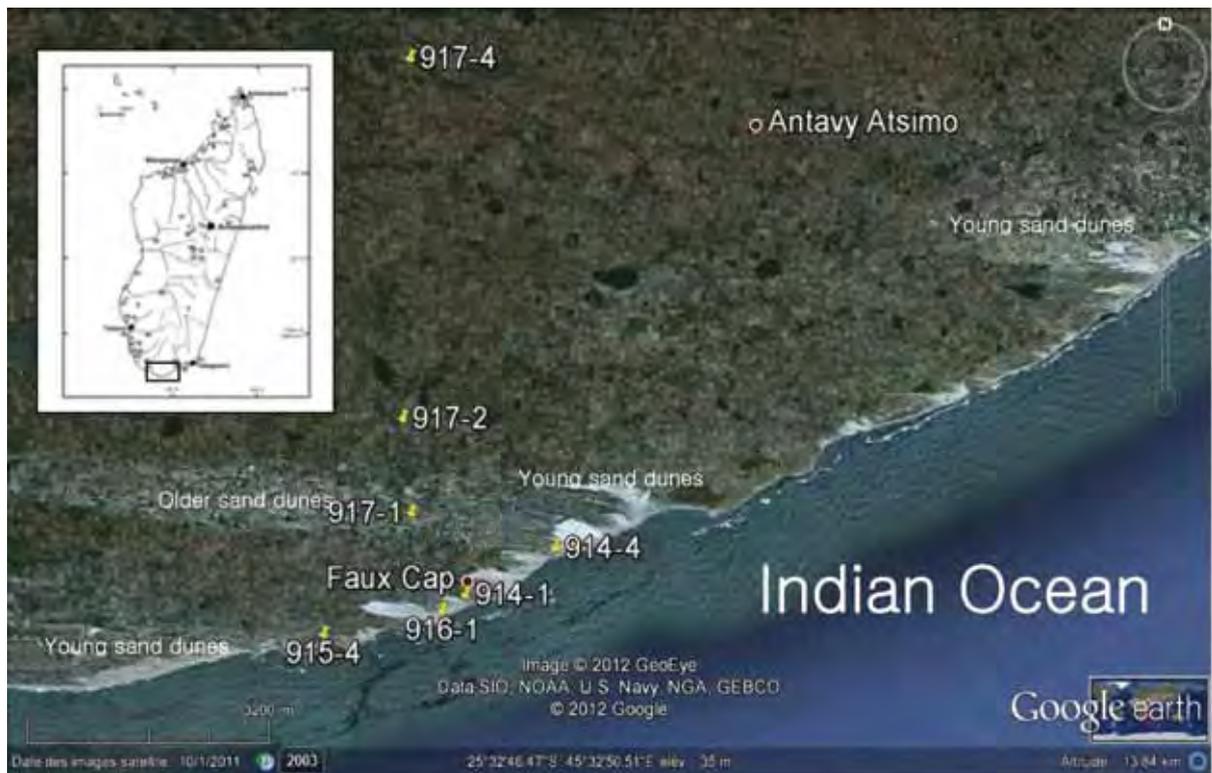


Figure 1. Detailed location of studied samples and photo-geological interpretation based on satellite imagery (Google Earth, 2011).

Aepyornian Quaternary sediment deposits (ancient and recent) (Battistini, 1964). During this period, there were three maximum oceanic transgressions (Tatsimian, Karimbolian, and Flandrian), followed by

three important regressions, which in turn formed the typical Aepyornian dune formations (Mahé & Sourdat, 1972).

Dating references

To confirm the age of the *in situ* Aepyornithidae eggshells samples from the Faux Cap sites (Figure 2A), we conducted radiocarbon dating. *In situ* gastropod samples were collected from different strata as associated fauna (Figure 2B), and submitted for radiocarbon dating. Dates are presented in BP, with 1954 as the base year. Radiocarbon ages were measured by Professor Yoshida at the University Museum - University of Tokyo, Japan.

Table 1. Coordinates of the observed sections during this study in Faux Cap area as shown in Figure 1.

Points	Latitude	Longitude
914-1	S25°34'10.9"	E45°31'41.0"
914-4	S25°33'52.3"	E45°32'23.5"
915-4	S25°34'26.6"	E45°30'33.1"
916-1	S25°34'17.4"	E45°31'29.6"
917-1	S25°33'37.8"	E45°31'15.3"
917-2	S25°32'59.9"	E45°31'11.7"
917-4	S25°30'36.8"	E45°31'16.8"

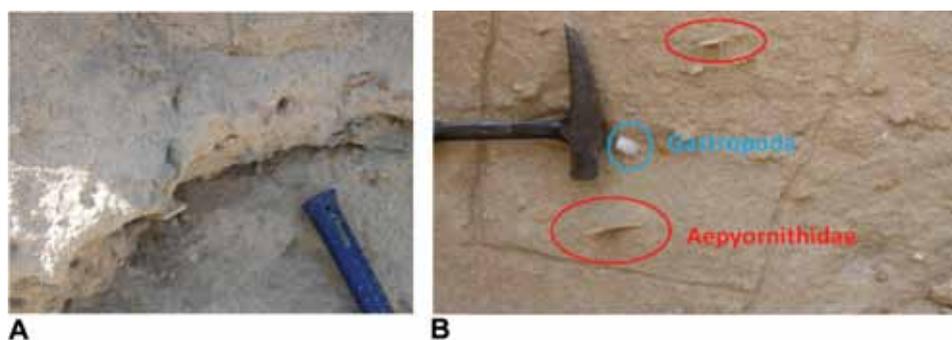


Figure 2. Photographs showing: A) the eggshells samples in the bottom level formed by whitish compacted dunes and B) gastropod and eggshells samples in the upper level formed by reddish dunes.

Sample preparation and protein extraction of modern chicken eggs

Using eggshell and bone collagen, we examined in an integrated fashion, the dietary history over the lifetime of the individual bird; this is an approach used by different researchers (Lajtha & Michener, 1994; Hobson, 1999; Kelly 2000; Michener & Lajtha, 2007).

The use of stable isotope analysis in avian dietary studies has certain limitations: it is not well understood how stable isotope fractionate or change once they are incorporated into tissues, and the precise turnover rates of isotopes in birds tissues is not well documented (Hobson, 1999). To help understand how this fractionation takes place, we used eggs of domestic chickens as our model. We analyzed albumin, membrane, and eggshells protein to determine fractionation between different tissues.

Sample preparation, bone collagen, and eggshell protein extraction

The main purpose of purifying the samples was to increase the quality of recovered insoluble protein/collagen. Extractions (Figure 3) were performed in Professor Koike's laboratory, Ropponmatsu Campus, Kyushu University. Protein and collagen were extracted from eggshells and bones following the protocol outlined in Figure 4.

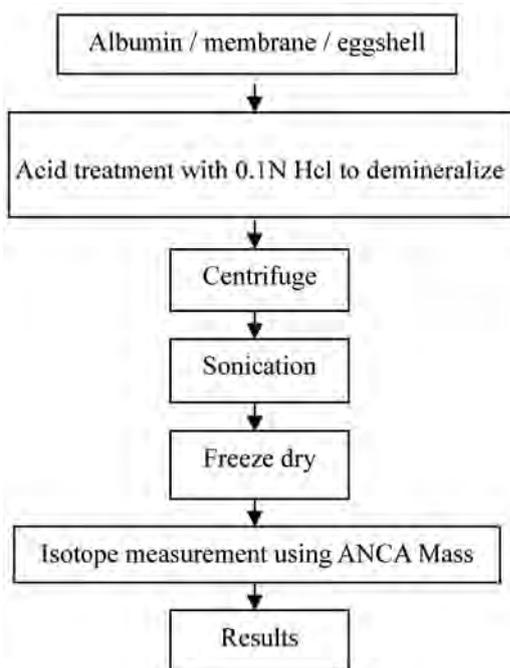


Figure 3. Protein extraction process for domestic chicken eggs (Koike *et al.*, pers. comm.).

Isotopes measurements

Two tin capsules per tissue sample, each $0.80 \text{ mg} \pm 0.02$, were needed to make these measurements. Carbon ($\delta^{13}\text{C}$) and nitrogen ($\delta^{15}\text{N}$) content values were measured using a continuous flow isotope ratio mass spectrometer (ANCA-mass 20-20, Europe Scientific Instruments, UK) housed at the Lyceum, Hakozaki Campus, Kyushu University. On average, two measurements were obtained for each sample.

Isotopic results are expressed as δ values, $\delta^{13}\text{C}$ or $\delta^{15}\text{N}$ (‰) = $[(R_{\text{sample}} / R_{\text{standard}}) - 1] \times 1000$, where R_{sample} and R_{standard} are the ratio $^{13}\text{C}/^{12}\text{C}$ or $^{15}\text{N}/^{14}\text{N}$ of the sample and standard, respectively. The standards are Vienna-Pee Dee Belemnite (V-PDB) limestone for carbon and atmospheric N_2 for nitrogen. Isotope measurement values are influenced by the deposition history of the specimens. Isotopic

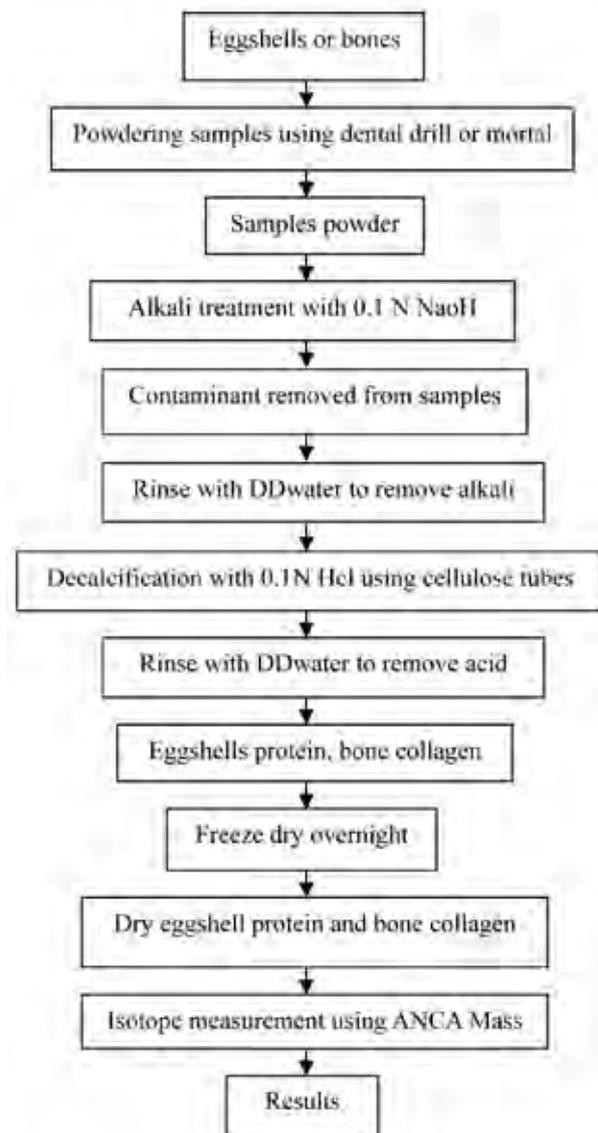


Figure 4. Chart of bone collagen and eggshell protein extraction (Koike *et al.*, pers. comm.).

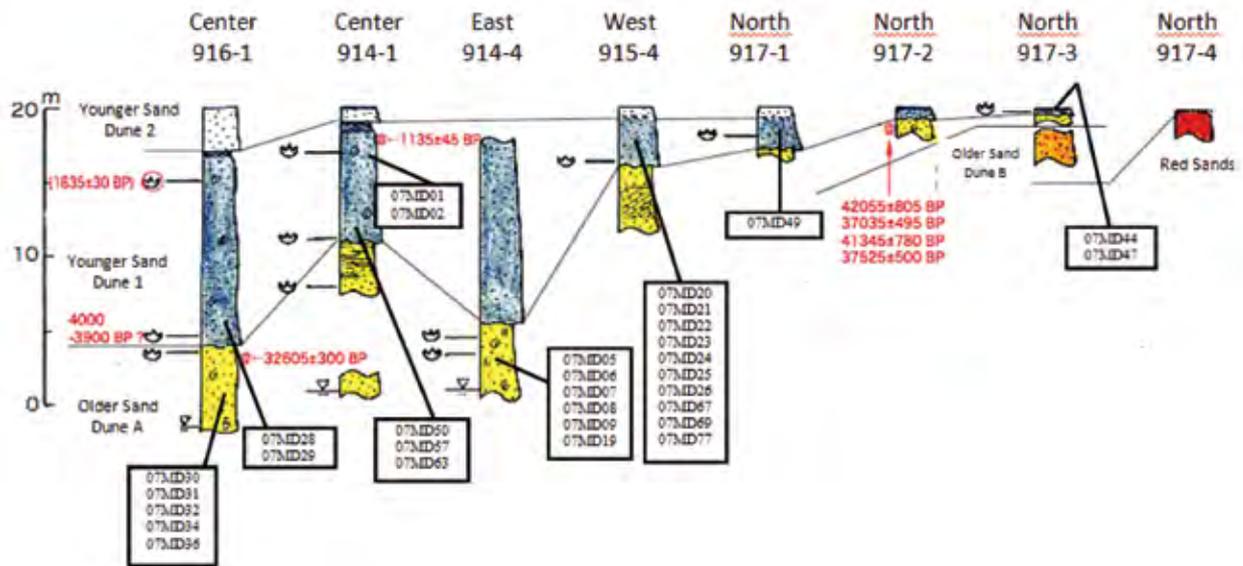


Figure 5. Locality and stratigraphic provenience of *Aepyornis* eggshell fragments from the Faux Cap site, radiometric data and references of collected *in situ* samples used in this study (Nagaoka, pers. comm., 2007).

alteration during diagenesis (Johnson *et al.*, 1997) and exogenous carbon contamination from the soil (Shin, 2010) can compromise the values. The C/N ratio can be used as an indicator of sample quality, particularly for bone collagen (Shin, 2010). We establish a range between 2.8 and 3.3 for C/N. However, as with ostrich eggshell, the C/N ratio from our collected *Aepyornithidae* eggshell did not have optimum fossilization conditions (Johnson *et al.*, 1997). Thus, we use organic fraction instead of inorganic, as the former is more stable and, hence, less variable (Clarke *et al.*, 2006).

Results

Biostratigraphy

The stratigraphy and geochronology of the Faux Cap area sedimentary sequence is well documented (Battistini, 1964; Mahé & Sourdat, 1972; Nagaoka, pers. comm.) (Figure 5). These Quaternary formations are more than 20 m thick and are divisible into three lithological units (lower to upper): 1) towards the north, red sand outcrops and along the littoral, 2) Older Sands Dunes (OSD) and 3) Younger Sands Dunes (YSD). The two last units are aeolian in nature and not necessarily physically continuous. The YSD is subdivided into two subunits: YSD1 and YSD2, which are not always continuous. From the south to north, the thickness of the formations decreases from 20 m to 5 m, and in the latter section the YSD2 disappears. Two different genera of *Aepyornithidae*, *Mullerornis* and *Aepyornis*, occur in the Faux Cap area deposits (Tovondrafale, 1994).

Description of aepyornithid eggshell remains

The eggs and eggshell fragments recovered from the Faux Cap region vary in the quality of preservation and, in some cases, exhibit some moderate weathering at certain localities (Figures 2 & 6). The size of the *Aepyornis* eggs is about 39 x 23 cm (Figure 6A). In the immediate area of Faux Cap, eggshell coloration is variable, in part associated with weathering – when exposed to air the remains are brownish (Figure 6B), and those excavated from soil are whitish with blackish small spots associated with weathered organic matter (Figure 6C).

In the case of smooth eggshell fragments exposed to the air, the external pore morphology is dominated by long grooves (3-6 pores per cm²), along with shorter groove pores, and small ovate to round pores (5-9 pores per cm²) (Figure 6B). Some specimens exhibit a predominance of small ovate to round pores with certain areas devoid of the long pores. The internal surface of eggshell fragments does not have the same pore types.

In the case of eggshell still held within sediments, the external surface is not notably rough, but with a whitish brown coloration (Figure 6C) and relatively uncommon ovate pores less than 1 mm in diameter. The internal surface is distinctly smoother than the external surface and without pores. Eggshells specimens generally show a prismatic structure (Figure 7A) and vary in thickness (Figures 7B), which is presumably related to generic (*Mullerornis* versus *Aepyornis*) and interspecific (within *Aepyornis*) differences.

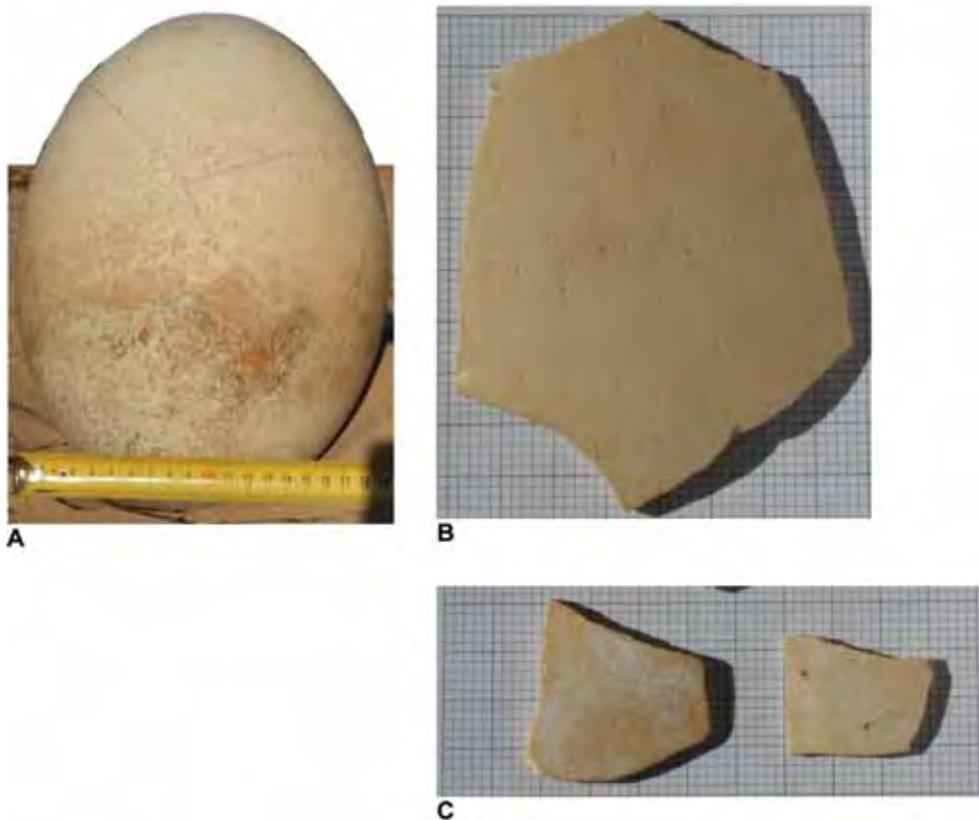


Figure 6. A) Complete egg of *Aepyornis* (Razakamanana's private collection); B) external surface of eggshell exposed to air; C) external surface of eggshell protected in the sand.

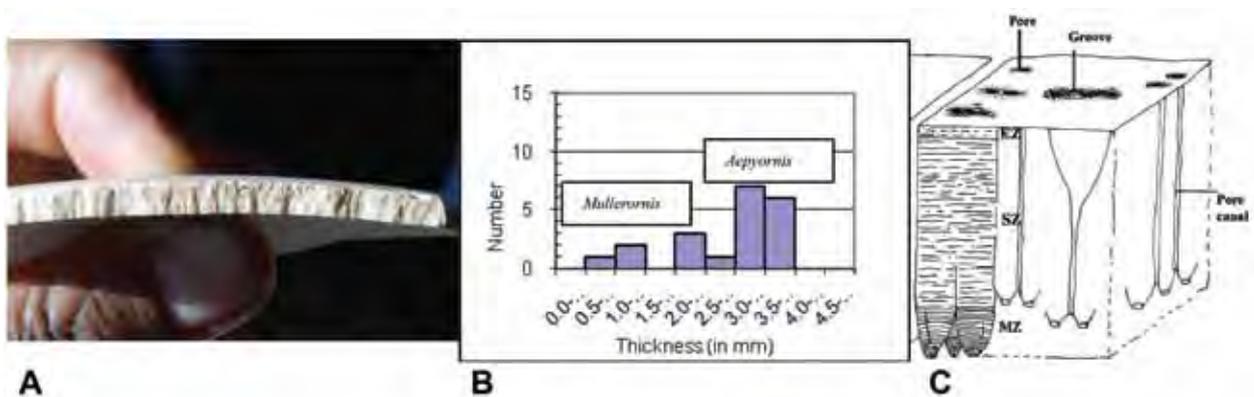


Figure 7. A) Thickness structure of the eggshell of the Aepyornithidae genera; B) two different genera (*Mullerornis* and *Aepyornis*) of Aepyornithidae showing distinct differences in eggshell thickness (Tovondrafale, 1994); C) structure of *Aepyornis* eggshell (modified after Sauer, 1966; Patnaik *et al.*, 2009).

Tovondrafale (1994) noted across an assortment of fragments collected in southern Madagascar that there is a statistically significant gap between thickness measurements from 1.7 to 2.0 mm and two different groups occur: [0-1.7] mm for the genus *Mullerornis* only found in the YSD and [2.0-6.6] mm for the genus *Aepyornis* genus in the OSD (Figure 7B). The *Aepyornis* eggshell shows typical dagger-point pore structures and a pore canal pattern (Figure 7A), which is formed by three layered zones (from the outer to the inner): external zone (EZ), spongy

zone (SZ), and mammillary zone (MZ) (Sauer, 1966; Patnaik *et al.*, 2009) (Figure 7C).

Gastropod dating

Six gastropod samples were submitted for radiocarbon age dating, five from OSD and one from YSD (Table 2). Two different ranges of dates were obtained: between $32,605 \pm 300$ yrs BP and $42,055 \pm 805$ yrs BP from the OSD samples and 1135 ± 45 yrs BP from the YSD sample.

Table 2. List of measured samples with radiocarbon ages.

Area	Strata	Lab No.	Locality	Material	Date (yrs BP)
Faux Cap	OSD (Late Pleistocene)	06MD25	916-1	Gastropod	32,605 ± 300
		06MD33	917-2	Gastropod	42,055 ± 805
	06MD34	917-2	Gastropod	37,035 ± 495	
	06MD35	917-2	Gastropod	41,345 ± 780	
	06MD36	917-2	Gastropod	37,525 ± 500	
	YSD (Holocene)	06MD20	914-1	Gastropod	1135 ± 45

Isotopes analysis of modern chicken egg to evaluate stable carbon isotopic fractionation between diet and tissues

Patterns of isotope fractionation are often not well known within the tissues of a given individual (Hobson, 1999), but in the context of this study it was important to understand these patterns between Aepyornithidae tissue (eggshell and bone) and inference associated with the diet of these birds. To provide greater insight into these patterns, we analyzed modern chicken eggs and used the results as a model for elephant birds (Table 3).

For chickens, we obtain the average $\delta^{13}\text{C}$ for the egg albumin (-15.51‰) and protein from the egg white (-14.31‰) with a difference of 1.20‰, and values of -15.59‰ and -14.52‰ from the egg yellow with a difference of 1.07‰. Thus, the average value of approximately 1‰ is carbon fractionation enrichment between what is obtained in the diet as compared to assimilation during tissue formation. The $\delta^{13}\text{C}$ values of eggshell protein from the modern chicken differed from those of the egg albumin by -1‰, indicating that

eggshell protein can be used for dietary analysis after correcting for +1‰. In Figure 8, we plot the ratios of C/N and $\delta^{13}\text{C}/\delta^{15}\text{N}$.

Isotopes analysis of Aepyornithidae eggshell and bone

For $\delta^{15}\text{N}$, enrichment is about 3‰ between diet and tissues (Thorp & Sponheimer, 2007), due to biochemical fractionation during tissue synthesis (Johnson *et al.*, 1997). Bone collagen carbon fractionation is about 1‰ (chicken model in Hobson, 1999).

Paleoenvironmental reconstruction

On the basis of radiocarbon dating, material recovered from the OSD is Late Pleistocene in age (42,055 ± 805 yrs BP to 32,605 ± 300 yrs BP) and from the YSD is Holocene in age (1135 ± 45 yrs BP) (Table 4). This information, combined with the isotope analysis, provides important insight into environmental shifts across these two periods of the Quaternary.

Table 3. Stable N and C isotopes obtained from domestic chickens.

Samples	Lab No.	% C	% N	%C / %N	$\delta^{13}\text{C}$	$\delta^{15}\text{N}$
Albumin white egg 1	07EG01A	48.08	13.85	3.5	-14.85	4.85
Albumin white egg 2	07EG02A	47.15	13.70	3.4	-16.17	4.27
	Average	47.61	13.77	3.5	-15.51	4.56
Albumin red egg 1	07EG03A	45.84	13.07	3.5	-15.73	3.39
Albumin red egg 2	07EG04A	40.93	11.83	3.5	-15.44	3.32
	Average	43.38	12.45	3.5	-15.59	3.35
Membrane white egg 1	07EG01B	47.27	15.71	3.0	-13.91	5.27
Membrane white egg 2	07EG02B	45.59	14.95	3.1	-15.07	4.37
	Average	46.43	15.33	3.0	-14.49	4.82
Membrane red egg 1	07EG03B	48.26	15.97	3.0	-14.52	3.78
Membrane red egg 2	07EG04B	46.90	15.11	3.1	-14.63	3.67
	Average	47.58	15.54	3.1	-14.58	3.72
Eggshell protein white egg 1	07EG01C	47.95	14.53	3.3	-13.44	5.07
Eggshell protein white egg 2	07EG02C	46.55	13.85	3.4	-15.17	4.21
	Average	47.25	14.19	3.33	-14.31	4.64
Eggshell protein red egg 1	07EG03C	43.50	13.17	3.3	-14.45	3.35
Eggshell protein red egg 2	07EG04C	47.47	14.29	3.3	-14.59	3.78
	Average	45.48	13.73	3.31	-14.52	3.57

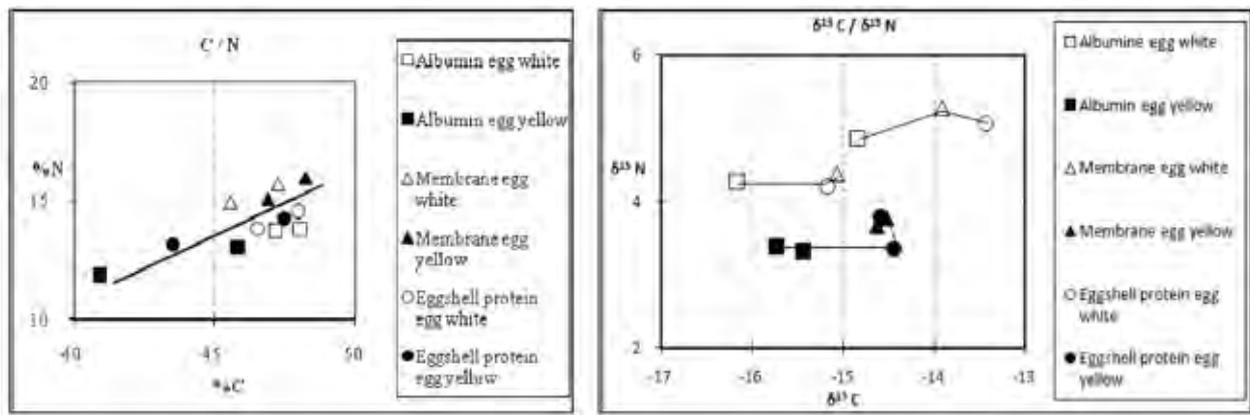


Figure 8. Isotope analysis of modern chicken: A) C/N ratio of tissues and B) $\delta^{13}\text{C}/\delta^{15}\text{N}$.

Table 4. Proteins isotopes analysis of Aepyornithidae eggshell samples from Faux Cap stratigraphic sections (without fractionation correction).

Lithostratigraphy	Locality	Lab No.	Thickness (mm)	Strata age	%C	%N	C/N	$\delta^{13}\text{C}$	$\delta^{15}\text{N}$
Old Sand Dunes (OSD)	916-1	07MD30	3.02	32605 ± 300 BP	48.20	4.79	10.06	-24.00	9.76
	916-1	07MD31	3.25		57.10	8.50	6.71	-22.80	12.00
	916-1	07MD32	3.45		57.80	8.30	6.96	-24.10	9.70
	916-1	07MD34	2.65		49.20	7.50	6.56	-25.30	12.40
	916-1	07MD36	2.90		51.30	7.30	7.02	-23.20	12.60
	914-4	07MD05	3.02		20.70	4.55	4.54	-23.21	8.99
	914-4	07MD06	3.10		38.15	3.53	10.80	-23.00	9.83
	914-4	07MD07	2.95		55.10	5.64	9.76	-25.00	10.50
	914-4	07MD08	3.15		37.64	4.20	8.96	-24.72	11.40
	914-4	07MD09	3.06		18.57	3.16	5.87	-22.01	8.69
914-4	07MD19	3.00	45.60	6.80	6.70	-24.90	11.00		
Young sand dunes (YSD)	916-1	07MD28	3.90	1135 ± 45 BP	41.72	11.03	3.78	-23.34	15.96
	916-1	07MD29	0.90		45.85	10.70	4.28	-21.90	12.21
	917-3	07MD44	2.76		33.50	8.80	3.80	-21.90	16.0
	917-3	07MD47	3.50		46.72	12.34	3.78	-22.40	16.39
	917-1	07MD49	3.70		45.70	12.60	3.62	-23.50	13.00
	91-4	07MD20	3.55		45.75	10.93	4.18	-22.69	15.79
	915-4	07MD21	2.20		49.42	12.82	3.85	-22.23	15.79
	915-4	07MD22	3.05		49.40	13.79	3.58	-22.76	16.32
	915-4	07MD23	3.35		48.96	13.91	3.51	-22.91	17.60
	915-4	07MD24	2.50		48.18	10.06	4.78	-23.18	14.69
	915-4	07MD25	2.20		46.10	9.80	4.70	-22.94	15.61
	915-4	07MD26	3.05		41.09	7.38	5.56	-23.42	12.68
	915-4	07MD67	3.60		49.50	13.50	3.70	-22.00	16.30
	915-4	07MD69	1.15		41.80	10.50	4.00	-22.40	12.60
	915-4	07MD77	3.05		52.20	13.40	3.90	-21.60	15.50
	914-1	07MD50	3.40		50.50	13.80	3.70	-23.50	15.20
	914-1	07MD57	3.75		49.30	13.40	3.70	-22.00	15.90
	914-1	07MD63	1.10		50.90	11.60	4.40	-21.80	13.40
	914-1	07MD01	3.30		54.52	12.18	4.47	-22.81	14.19
	914-1	07MD02	3.55		53.18	14.13	3.76	-21.40	11.54

Aepyornithidae eggshells from the Faux Cap stratigraphic sections were corrected for $\delta^{13}\text{C}$ by 1‰ and $\delta^{15}\text{N}$ by 3‰ and bone remains from Beloha were corrected for $\delta^{13}\text{C}$ by 1‰ and $\delta^{15}\text{N}$ by 3‰);

this information provides interesting inferences into the diet of these extinct birds. Although the form of deposition was not optimal in certain cases, on the basis of C and N composition ratio there is

a separation between samples dated to the Late Pleistocene and those from the Holocene. Values of $\delta^{15}\text{N}$ from the YSD are higher than from the OSD,

providing a clear indication that across these periods there was a pattern of aridification (Figure 9a). $\delta^{13}\text{C}$ corrected by 1‰ and $\delta^{15}\text{N}$ corrected by 3‰:

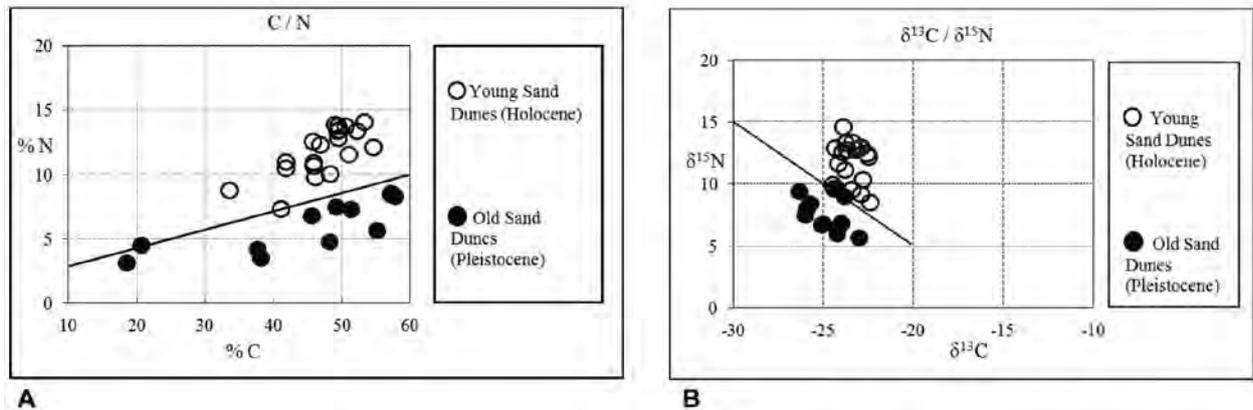


Figure 9. Isotope analysis of Aepyornithidae eggshells: A) C/N ratio and B) $\delta^{13}\text{C}/\delta^{15}\text{N}$.

Table 5. Isotopes analysis of additional Aepyornithidae eggshell proteins and bones collagen samples (without fractionation correction).

Lab No.	Samples	Site	%C	%N	C/N	$\delta^{13}\text{C}$	$\delta^{15}\text{N}$
04EG01	Thick eggshell	Faux Cap	49.4	11.7	4.2	-22.70	16.19
04EG02	Thick eggshell	Faux Cap	54.9	11.3	4.9	-23.73	14.92
04EG03	Thick eggshell	Faux Cap	48.8	10.8	4.5	-22.95	15.41
04EG04	Thick eggshell	Faux Cap	51.2	12.7	4.0	-22.80	14.41
04EG05	Thick eggshell	Faux Cap	35.3	8.7	4.1	-22.61	15.88
04EG06	Thick eggshell	Faux Cap	36.0	7.6	4.8	-22.25	16.27
04EG07	Thick eggshell	Faux Cap	43.8	8.7	5.0	-23.19	15.78
04EG08	Thick eggshell	Faux Cap	37.2	8.2	4.5	-24.19	13.57
04EG09	Thick eggshell	Faux Cap	39.6	8.7	4.6	-23.16	15.94
02EG01	Thick eggshell	Faux Cap	30.7	7.7	4.0	-23.08	15.44
02EG02	Thick eggshell	Faux Cap	42.3	10.4	4.1	-22.68	15.88
02EG05	Thick eggshell	Faux Cap	43.1	11.1	3.9	-22.39	14.01
07AEP01	Bone	Beloha	44.7	15.7	2.9	-19.7	11.8
07AEP02	Bone	Beloha	45.5	15.9	2.84	-19.62	14.1
07AEP03	Bone	Beloha	47.6	16.2	3.1	-21.1	13.2
07AEP04	Bone	Beloha	43.5	12.9	3.4	-22.7	12.7
07AEP06	Bone	Beloha	44.2	15.3	2.9	-19.8	14.3
07AEP08	Bone	Beloha	46.0	16.0	2.9	-19.6	15.8
07AEP09	Bone	Beloha	41.3	14.3	2.9	-19.6	11.7
07AEP10	Bone	Beloha	40.0	13.2	3.0	-21.7	15.3
06EG02	Thick eggshell	Toliara	41.5	8.9	4.7	-24.00	15.56
06EG03	Thick eggshell	Toliara	41.8	7.9	5.3	-21.62	14.47
06EG04	Thick eggshell	Toliara	42.1	8.8	4.8	-21.58	15.05
06EG05	Thick eggshell	Toliara	44.2	8.9	5.0	-22.16	13.73
06EG06	Thin eggshell	Toliara	48.8	9.8	5.0	-22.31	11.64
06EG07	Thin eggshell	Toliara	44.6	9.0	5.0	-22.56	13.05

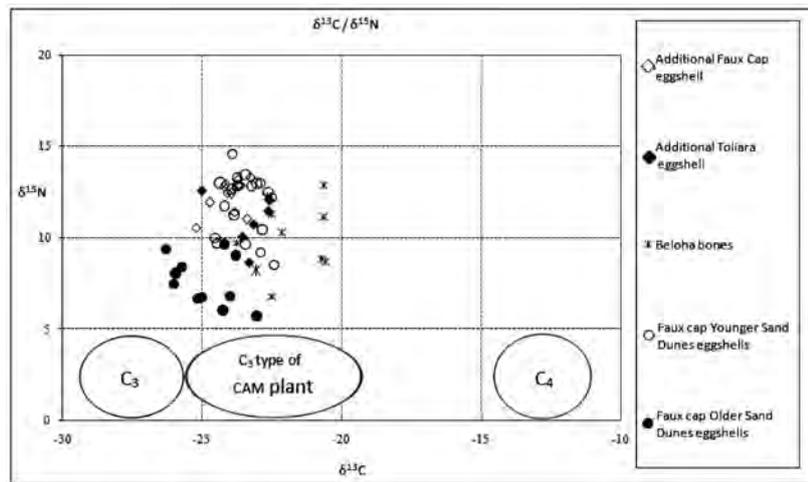


Figure 10. $\delta^{13}\text{C}/\delta^{15}\text{N}$ of Aepyornithidae eggshell and bone samples from different sites in southern Madagascar.

- For the OSD, $\delta^{15}\text{N}$ values fall between 5‰ and 10‰, $\delta^{13}\text{C}$ values are between -26‰ and -22.5‰.
- For the YSD, $\delta^{15}\text{N}$ values fall between 7.5‰ and 15‰, $\delta^{13}\text{C}$ values are between -25‰ and -22‰.

Using information from stable isotopes (Figure 9b), it is evident that amongst the OSD material, some $\delta^{13}\text{C}$ values are low, under -25‰. This can be best explained in combination with the $\delta^{15}\text{N}$ values and indicate moisture environmental conditions.

Diet analysis

An additional 12 eggshell samples from Faux Cap and six from Toliara collected from surface deposits were analyzed to provide further data for diet analysis (Table 5). The exposed surfaces of the Toliara samples were similar to those from Faux Cap collected in the YSD. Further, bone material of elephant birds held in the collections of the Department of Biological Anthropology and Paleontology, The University of Antananarivo, and collected at Beloha in 1932 were also employed. These bone samples were not dated, but only used in the diet analysis.

After correcting $\delta^{13}\text{C}$ by 1‰ and $\delta^{15}\text{N}$ by 3‰ the additional eggshell samples from Toliara yielded $\delta^{15}\text{N}$ values between 8‰ and 13‰ and $\delta^{13}\text{C}$ values between -25‰ and -22‰. The additional Faux Cap specimens produced $\delta^{15}\text{N}$ values between 10‰ and 14‰ and $\delta^{13}\text{C}$ values between -25‰ and -23‰. These values are closer to the Faux Cap samples from the YDS. Further, the $\delta^{15}\text{N}$ values of Toliara material tend to be lower (more humid conditions) than those from Faux Cap (drier conditions), suggesting climatic differences between these two sites. In the case of the bone remains from Beloha,

$\delta^{15}\text{N}$ values are between 7‰ and 13‰ and $\delta^{13}\text{C}$ values range from -24‰ to -20‰; these values are similar to those from the YSD of Faux Cap. In a graphic representation associated with our dietary analysis, we combine different sources of data, all of which have $\delta^{13}\text{C}$ values corrected by 1‰ and $\delta^{15}\text{N}$ values corrected by 3‰ (Figure 10).

Discussion

The study area in the extreme south

Today, extreme southern Madagascar holds notably dry ecological conditions, a bushland thicket vegetation, and 300 to 600 mm of annual rainfall (Donque, 1972). This zone has a considerable number of known subfossil sites (Goodman & Jungers, 2013). Human presence in the extreme south is relatively late and one of the closest archeological sites to Faux Cap, known as Talaky, dates from 840 ± 80 yrs BP (Burney *et al.*, 2004).

Environmental controls on nitrogen

Loss of moisture can be observed through enrichment of $\delta^{15}\text{N}$ as a response to aridification (Johnson *et al.*, 1997), particularly in arid regions with mean annual rainfall < 400 mm (Thorp & Sponheimer, 2007), which is the case today in southern Madagascar. Based on stratigraphic sections excavated at Faux Cap, after considering the 3‰ fractionation in $\delta^{15}\text{N}$ values, the average $\delta^{15}\text{N}$ values of OSD samples is 7.62‰ and of YSD samples is 11.83‰. Hence, by extrapolation, the Faux Cap OSD formations dated to the Late Pleistocene ($32,605 \pm 300$ yrs BP) showed more humid conditions than those dated to the Holocene (1135 ± 45 yrs BP) (Figure 9b).

Diet

Plants (C_3 Calvin or C_4 Hatch-Slack photosynthetic pathways) fractionate atmospheric carbon for carbohydrate synthesis. Carbon isotopes of animal tissue (such as eggshell, bone, and muscle) reflect the isotope composition of the food a given species or individual ingested (Johnson *et al.*, 1997). C_3 plants include trees, shrubs, and forbs, as well as temperate or shade-adapted grasses, while C_4 plants are mainly tropical grasses. Between these extremes are succulent plants, with Crassulacean Acid Metabolism (CAM); alternating C_3 and C_4 pathways by night and day depending on local environmental conditions. Carbon isotope values, relative to the international standard V-PDB are: $C_3 = -26\text{‰}$ and $C_4 = -12\text{‰}$ (Thorp & Sponheimer, 2007). After correcting $\delta^{13}\text{C}$ values by 1‰ , our results are (Figure 10):

- Beloha bones: -24‰ to -20‰ ,
- Additional eggshell Faux Cap: -25‰ to -23‰ ,
- Additional eggshell Toliara: -25‰ to -22‰ ,
- Faux Cap OSD (Late Pleistocene): -26‰ to -23‰ ,
- Faux Cap YSD (Holocene): -25‰ to -22‰ .

$\delta^{13}\text{C}$ values for some of the OSD samples approach the C_3 standard, but most indicate predominantly C_3 types of CAM plants as dietary sources. Most importantly, these values did not change across the Late Pleistocene ($42,055 \pm 805$ yrs BP to $32,605 \pm 300$ yrs BP) to Holocene (1135 ± 45 yrs BP) transition, which by inference indicates little in the way of dietary shifts at the Faux Cap site during these periods.

Extinction and environment change

In the context of environmental change, Clarke *et al.* (2006) propose that in the latter portion of the Holocene of western and southern Madagascar there was an expansion of xerophilous thicket and contraction of woodland vegetation (CAM plants). Crowley (2010) noted "Large extinct taxa persisted in the Spiny Thicket and Succulent Woodland until at least ca 1000 Cal BP. Population declines in these species likely resulted from a combination of continued hunting pressure, habitat reduction and transformation, and climatic desiccation". In the case of extreme southern Madagascar, based on data presented herein, natural climatic change is presumed to have had a very important influence on the range contraction and final disappearance of elephant birds. Based on current information, the human element, specifically associated with hunting, gathering of eggs, and habitat destruction

is presumed to have been of less importance (Goodman & Jungers, 2013).

Conclusion

From our study, levels of relative moisture between Late Pleistocene ($42,055 \pm 805$ yrs BP to $32,605 \pm 300$ yrs BP) and Holocene (1135 ± 45 yrs BP) shows a progressive decline (aridification). However, these shifts did not result in changes in the dietary regime of elephant birds, which consisted of C_3 and C_4 types of CAM plants across this period.

In the case of grassland fires, which involves herbaceous vegetation (C_4 type), in extreme southern Madagascar, Poaceae ground cover is sparse. This helps to explain why fire is an uncommon aspect in this portion of the island and when present are short-lived (MacPhee, 1986). On the basis of the results presented herein, with C_4 plants not being notably present in this region for something approaching 40,000 years, notable changes took place in local environmental conditions before humans colonized this area. The closest archeological site to Faux Cap is Talaky, dated to 840 ± 80 yrs BP (Burney *et al.*, 2004).

Future studies associated with elephant bird ecology in southern Madagascar should focus on an expanded geographical coverage for the types of analyses presented herein, including sites such as Talaky, Cap Ste. Marie, Lavanono, and Maroaloke. More detailed dating and isotope analyses are needed from these sites to provide greater precision in paleoenvironment changes and dietary shifts in elephant birds at a regional scale.

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References

- Amadon, D. 1947.** An estimated weight of the largest known bird. *The Condor*, 49: 159-163.
- Battistini, R. 1964.** *Etude géomorphologique de l'extrême sud de Madagascar*. Cujas, Paris.
- Bond, W. J. & Silander, J. A. 2007.** Springs and wire plants: Anachronistic defenses against Madagascar's extinct elephant birds. *Proceedings of the Royal Society B*, 274: 1985-1992.
- Bradbury, W. M. C. 1919.** Some notes on the egg of *Aepyornis maximus*. *The Condor*, 21: 97-101.
- Burney, D. A., Burney, L. P., Godfrey, L. R., Jungers, W. L., Goodman, S. M., Wright, H. T. & Jull, T. A. J. 2004.** A chronology for late prehistoric Madagascar. *Journal of Human Evolution*, 47: 26-63.
- Cauderay, H. 1931.** Etude sur l'*Aepyornis*. *L'Oiseau et la Revue Française d'Ornithologie*, 1: 625-641.
- Chassaniol. 1879.** Note sur un œuf d'*Aepyornis* apporté de Madagascar par M. Léger. *Bulletin de la Société Académique de Brest*, 2: 233-244.
- Clarke, S. J., Miller, G. H., Fogel, M. L., Chivas, A. R. & Murray-Wallace, C. V. 2006.** The amino acid and stable isotope biochemistry of elephant bird (*Aepyornis*) eggshells from southern Madagascar. *Quaternary Science Review*, 25: 2343-2356.
- Crowley, B. E. 2010.** A refined chronology of prehistoric Madagascar and the demise of the megafauna. *Quaternary Science Reviews*, 29: 2597-2601.
- de Flacourt, E. 1658.** *Histoire de la Grande Isle Madagascar*. Edition présentée et annotée par Claude Allibert. INALCO-Karthala, Paris.
- Donque, G. 1972.** The climatology of Madagascar. In *Biogeography and ecology of Madagascar*, eds. R. Battistini & G. Richard-Vindard, pp. 87-144. The Hague, Junk.
- Goodman, S. M. & Jungers, W. L. 2013.** *Les animaux et écosystèmes de l'Holocène disparus de Madagascar*. Association Vahatra, Antananarivo.
- Grandidier, G. 1903.** Note au sujet du squelette de l'*Aepyornis ingens*. *Bulletin du Muséum national d'Histoire naturelle*, 6: 318-323.
- Hobson, K. A. 1995.** Reconstructing avian diets using stable carbon and nitrogen isotope analysis of egg components: Patterns of isotopic fractionation and turnover. *The Condor*, 97: 752-761.
- Hobson, K. A. 1999.** Tracing diet and origins of migratory birds using stable isotope techniques. *Society of Canadian Ornithologists Special Publication*, 1: 5-6.
- Johnson, B. J., Fogel, M. L. & Miller, G. H. 1993.** Paleocological reconstructions in southern Egypt based on the stable carbon and nitrogen isotopes in the organic fraction and stable carbon isotopes in the individual amino acids of fossil ostrich eggshell. *Chemical Geology*, 107: 493-497.
- Johnson, B. J., Miller, G. H., Fogel, M. L. & Beaumont, P. B. 1997.** The determination of late Quaternary paleoenvironments at Equus Cave, South Africa, using stable isotopes and amino acid racemization in ostrich eggshell. *Palaeogeography, Palaeoclimatology, Palaeoecology*, 136: 121-137.
- Johnson, B. J., Fogel, M. L. & Miller, G. H. 1998.** Stable isotopes in modern ostrich eggshell: A calibration for paleoenvironmental applications in semi-arid regions of southern Africa. *Geochimica et Cosmochimica Acta*, 62: 2451-2461.
- Kelly, J. F. 2000.** Stable isotopes of carbon and nitrogen in the study of avian and mammalian trophic ecology. *Canadian Journal of Zoology*, 78: 1-27.
- Koch, P. L. 2007.** Isotopic study of the biology of modern and fossil vertebrates. In *Stable isotopes in ecology and environmental science*, eds. R. H. Michener & K. Lajtha, pp. 99-154. Blackwell Scientific, Oxford.
- Lajtha, K. & Michener, R. H. 1994.** *Stable isotopes in ecology and environmental science*. Blackwell Scientific Publications, Oxford.
- MacPhee, R. D. E. 1986.** Environment, extinction, and Holocene vertebrate localities in southern Madagascar. *National Geographic Research*, 2: 444-455.
- MacPhee, R. D. E., Burney, D. A. & Wells N. A. 1985.** Early Holocene chronology and environment of Ampasambazimba, a Malagasy subfossil lemur site. *International Journal of Primatology*, 6: 463-489.
- Mahé, J. & Sourdat, M. 1972.** Sur l'extinction des vertébrés subfossiles et l'aridification du climat dans le Sud-ouest de Madagascar. *Bulletin de la Société Géologique de France*, 14: 295-309.
- Michener, R. H. & Lajtha, K. 2007.** *Stable isotopes in ecology and environmental science*. Blackwell Scientific, Oxford.
- Palmqvist, P., Gröcke, D. R., Arribas, A. & Fariña, R. A. 2003.** Paleocological reconstruction of a lower Pleistocene large mammal community using biogeochemical ($\delta^{13}\text{C}$, $\delta^{15}\text{N}$, $\delta^{18}\text{O}$, Sr:Zn) and ecomorphological approaches. *Paleobiology*, 29: 205-229.
- Patnaik, R., Sahni, A., Cameron, D., Pillans, B., Chatrath, P., Simons, E., Williams, M. & Bibi, F. 2009.** Ostrich-like eggshells from a 10.1 million-yr-old Miocene ape locality, Haritalyangar, Himachal Pradesh, India. *Current Science*, 96: 1485-1495.
- Pérez-Crespo, V. A., Arroyo-Cabrales, J., Benammi, M., Johnson, E., Polaco, O. J., Santos-Moreno, A., Morales-Puente, P. & Cienfuegos-Alvarado, E. 2012.** Geographic variation of diet and habitat of the Mexican populations of Columbian Mammoth (*Mammuthus columbi*). *Quaternary International*, 276-277: 8-16.
- Sauer, E. G. F. 1966.** Fossil eggshell fragments of a giant struthious bird (*Struthio oshanai* sp. nov) from Etosha Pan, South West Africa. *Cimbebasia*, 14: 3-51.
- Shin, J. Y. 2010.** Modified bone density fractionation method for palaeodietary studies. *Bulletin Korean Chemical Society*, 32: 83-87.
- Silyn-Roberts, H. & Sharp, R. M. 1986.** Preferred orientation of calcite in the *Aepyornis* eggshell. *Journal of Zoology*, 208: 475-478.

Thorp, J. L. & Sponheimer, M. 2007. Contribution of stable light isotope to paleoenvironmental reconstruction. In *Handbook of paleoanthropology*, eds., W. Henke & I. Tattersall, pp. 289-306. Springer-Verlag, Berlin.

Tovondrafale, T. 1994. Contribution à l'étude des Aepyornithidae, causes des disparitions. Mémoire de Diplôme d'Etudes Approfondies, Université d'Antananarivo, Antananarivo.

Yoshida, A. & Kondo, N. 1992. Concerning the duplication of whole skeleton structure of extinct giant flightless bird *Aepyornis maximus* Is. Geoffr. from Madagascar. *Research Institute of Evolutionary Biology Science Report*, 7: 35-36.