

Isotopic and anatomical evidence of an herbivorous diet in the Early Tertiary giant bird *Gastornis*. Implications for the structure of Paleocene terrestrial ecosystems

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Received: 13 January 2014 / Revised: 28 January 2014 / Accepted: 31 January 2014 / Published online: 22 February 2014
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Abstract The mode of life of the early Tertiary giant bird *Gastornis* has long been a matter of controversy. Although it has often been reconstructed as an apex predator feeding on small mammals, according to other interpretations, it was in fact a large herbivore. To determine the diet of this bird, we analyze here the carbon isotope composition of the bone apatite from *Gastornis* and contemporaneous herbivorous mammals. Based on ^{13}C -enrichment measured between carbonate and diet of carnivorous and herbivorous modern birds, the carbonate $\delta^{13}\text{C}$ values of *Gastornis* bone remains, recovered from four Paleocene and Eocene French localities, indicate that this bird fed on plants. This is confirmed by a morphofunctional study showing that the reconstructed jaw

musculature of *Gastornis* was similar to that of living herbivorous birds and unlike that of carnivorous forms. The herbivorous *Gastornis* was the largest terrestrial tetrapod in the Paleocene biota of Europe, unlike the situation in North America and Asia, where *Gastornis* is first recorded in the early Eocene, and the largest Paleocene animals were herbivorous mammals. The structure of the Paleocene terrestrial ecosystems of Europe may have been similar to that of some large islands, notably Madagascar, prior to the arrival of humans.

Keywords Diet · Bird · Anatomy · Paleontology · Geochemistry

Communicated by: Sven Thatje

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Introduction

Gastornis, a giant early Tertiary ground bird from Europe, North America, and Asia, was the largest (up to 175 kg) (Andors 1995) terrestrial animal in the Paleocene ecosystems of Europe. This bird, also known as *Diatryma*, has been known since the 1850s (Buffetaut 1997; Buffetaut and Angst 2013). Although numerous well-preserved fossils have been discovered, its ecology remains poorly understood, and its diet, in particular, has remained a matter of debate (Witmer and Rose 1991; Andors 1992). The very peculiar anatomy of the massive skull and mandible has no real equivalent among modern birds, which makes comparative anatomical interpretations difficult and has led to conflicting conclusions. Whereas some authors accept the traditional interpretation of *Gastornis* as a large carnivore (Witmer and Rose 1991), others consider it as an herbivore (Andors 1992; Tütken et al. 2013).

Here, we have investigated the diet of *Gastornis* using two complementary approaches. The first approach is an analysis of the carbon isotope composition of the bone apatite of

Gastornis, based on samples recovered from various Paleocene and Eocene localities in France. Carbon isotope compositions of apatite from land vertebrates primarily reflect their diets, with a ^{13}C -enrichment relative to ^{12}C between apatite and diet that varies among animals (Koch 2007). Whereas this enrichment is well documented for carnivorous and herbivorous mammals (e.g., Cerling and Harris 1999; Koch 1998; Lee-Thorp and Van der Merwe 1987; Passey et al. 2005), only one herbivorous bird (ostrich) has been studied so far (Johnson et al. 1998). Therefore, in order to interpret the $\delta^{13}\text{C}$ values of *Gastornis* apatite in terms of diet, we have measured carbonate and food $\delta^{13}\text{C}$ values of various modern carnivorous and herbivorous birds in order to determine their carbonate-diet ^{13}C -enrichments. Independently, the feeding behavior of *Gastornis* has been tested using a morphofunctional comparison between its jaw musculature and that of modern birds having known diets. Finally, we discuss the inferred diet of *Gastornis* in terms of macroecology of early Tertiary terrestrial vertebrate communities.

Material and methods

Material

Carbon isotope compositions ($\delta^{13}\text{C}$) of the bone apatite from *Gastornis* and contemporaneous herbivorous mammals were used to determine the diet of *Gastornis*. Samples from four French localities were used (Fig. 1). Two of them, Cernay-les-Reims and Mont-de-Berru, are late Paleocene (Thanetian) and are located in the eastern Paris Basin. The other two, Monthelon in the eastern Paris Basin and Saint-Papoul in southwestern France, are early Eocene (Ypresian).

The proper values of ^{13}C enrichment between carbonate and food ($\Delta^{13}\text{C}_{\text{c-d}}$) of the modern carnivorous and herbivorous birds have to be determined in order to infer the diet of *Gastornis* from its apatite $\delta^{13}\text{C}$ value (Table 1). We sampled eggshells and bones as well as the food used to feed both carnivorous (vultures) and herbivorous (ostriches) birds raised in captivity (Parc aux Oiseaux des Dombes, Villars-les-Dombes, France and Ferme de l'Autruche Drômeoise, Livron, France) and measured their respective carbon isotope compositions.

Our morphofunctional analysis included a variety of birds with different ecologies. For comparison, we included grazers such as Muscovy duck (*Carina moschata*), pecking birds such as guineafowl (*Numida meleagris*) and chicken (*Gallus gallus*), and seed-cracking birds such as Darwin's finches (*Geospiza fortis*, *Geospiza magnirostris*, and *Platyspiza crassirostris*) (Table 2). Carnivores included broad-winged hawks (*Buteo platypterus*) and Eurasian sparrowhawks (*Accipiter nisus*). The development of jaw muscles in

Gastornis was inferred from a well-preserved mandible from the Thanetian of Mont-de-Berru, France (Angst and Buffetaut 2013).

Methods

Carbon isotope composition of bone and diagenesis

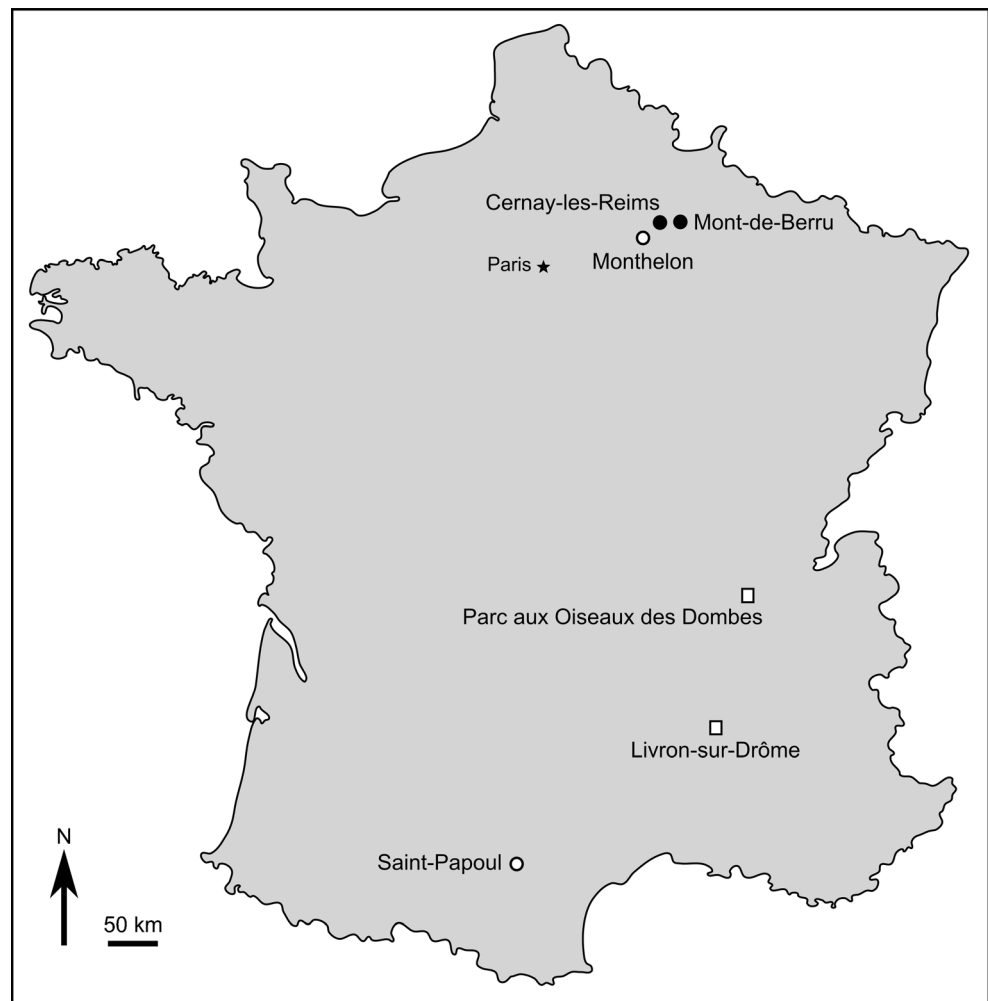
Postmortem changes in the carbon isotope composition of the bone may occur depending on the depositional environment, its geology (e.g., folding, fracturation and fluid circulation, and metamorphism), and chemical properties (T, pH, and Eh). Yet, these are independent of the geological age of the sedimentary deposit. Diagenetic alteration of the bones mainly takes place through mechanisms of dissolution–reprecipitation that may be either microbially mediated or induced by mineral-fluid interactions (Blake et al. 1997; Lécuyer et al. 1999; Zazzo et al. 2004).

Modifications of the pristine carbon isotope compositions may occur as a result of either isotopic exchange between the apatite and a percolating aqueous phase or by addition of secondary apatite. There is no definitive combination of mineralogical or geochemical arguments known so far that allows the demonstration of a fully preserved stable carbon isotope composition of bones. In the framework of this study, however, two observations suggest that the original carbon isotope compositions of the bones sampled from *Gastornis* and contemporaneous herbivorous mammals were not significantly modified during their burial history. The first argument is provided by the amounts of carbonate measured in bone apatite, which range from 3.5 to 7.6 wt%, matching those known in living vertebrates (Le Geros and Le Geros 1984; Michel et al. 1995), thus suggesting that the addition of secondary apatite was negligible. The second argument is based on the observation of a negative offset of -1.95 ± 0.88 between the $\delta^{13}\text{C}$ of *Gastornis* and the cooccurring herbivorous mammals considering the four studied sites as a whole (Table 3). This observation means that the interpretation of differences in carbon isotope ratios between coexisting taxa, which are used to infer the $\delta^{13}\text{C}$ of their diet, does not depend on either the geological age or the history of the studied sedimentary deposits.

Carbon isotope analysis of organic matter

Samples of bird food (about 50 μg) were combusted in an elemental analyzer (Vario Pyro CubeTM) to produce carbon dioxide (CO_2) that was connected online in continuous flow mode to an IsoPrimeTM Isotopic Ratio Spectrometer (IRMS) for the determination of $\delta^{13}\text{C}$ values (‰ V-PDB). Laboratory reference materials calibrated against international standards were routinely measured along with the samples. Precision for $\delta^{13}\text{C}$ values are ± 0.1 ‰ (2σ).

Fig. 1 Location map of the geographical localization of the modern and fossil sites. *Black dot* Paleocene fossil site (Thanetian), *white dot* Eocene fossil site (Ypresian), and *white square* modern site (bird park or farm)



Carbon isotope analysis of eggshell carbonate

Stable carbon isotope ratios were determined by using an automated preparation system called MultiPrepTM coupled to a dual-

inlet IsoPrimeTM IRMS. For each sample, an aliquot of about 350 µg of calcium carbonate was reacted with anhydrous oversaturated phosphoric acid at 90 °C for 15 min. Isotopic compositions are quoted in the delta notation in ‰ relative to

Table 1 Measured carbon isotope fractionations between carbonate and food ($\Delta^{13}\text{C}_{\text{c-d}}$) for modern birds and herbivorous mammals taken from the literature and determined in this study

$\Delta^{13}\text{C}_{\text{c-d}}$ (‰)	Animal studied	Group	Diet	References
+8.3	Raptor	Bird	Carnivorous	This study
+13.4	Ostrich	Bird	Herbivorous	This study
+16.2±0.5	Ostrich	Bird	Herbivorous	Johnson et al. 1998
+12	Theoretical model	Mammal	Herbivorous	Krueger and Sullivan 1984
+11.5±0.3	Vole	Mammal	Herbivorous	Passey et al. 2005
+12to +14	Ungulate	Mammal	Herbivorous	Koch 1998
+12to +14	Ungulate	Mammal	Herbivorous	Lee-Thorp and Van der Merwe 1987
+12.8±0.7	Rabbit	Mammal	Herbivorous	Passey et al. 2005
+13.3±0.3	Pig	Mammal	Herbivorous	Passey et al. 2005
+13.63	Horse	Mammal	Herbivorous	Cerling and Harris 1999
+14.3	Rhino	Mammal	Herbivorous	Cerling and Harris 1999
+14.43	Zebra	Mammal	Herbivorous	Cerling and Harris 1999
+14.6±0.3	Bovine	Mammal	Herbivorous	Passey et al. 2005

Table 2 Summary of the muscle property data include in the analysis

Genus	Species	MAME (mm ²)	Head length (mm)	Diet
<i>Geospiza</i>	<i>magnirostris</i>	13.8	40.00	Seed-cracker
<i>Geospiza</i>	<i>fortis</i>	7.0	33.71	Seed-cracker
<i>Geospiza</i>	<i>fortis</i>	4.5	33.03	Seed-cracker
<i>Geospiza</i>	<i>fortis</i>	4.0	31.59	Seed-cracker
<i>Platyspiza</i>	<i>crassirostris</i>	4.3	32.78	Folivore
<i>Gallus</i>	<i>gallus</i>	6.1	74.59	Pecker
<i>Gallus</i>	<i>gallus</i>	7.6	75.59	Pecker
<i>Gallus</i>	<i>gallus</i>	6.3	75.00	Pecker
<i>Gallus</i>	<i>gallus</i>	9.7	75.00	Pecker
<i>Numida</i>	<i>meleagris</i>	8.3	63.66	Pecker
<i>Numida</i>	<i>meleagris</i>	4.9	63.45	Pecker
<i>Carinia</i>	<i>moschata</i>	22.9	140.16	Grazer
<i>Accipiter</i>	<i>nisus</i>	1.0	42.04	Carnivore
<i>Accipiter</i>	<i>nisus</i>	3.5	47.00	Carnivore
<i>Buteo</i>	<i>platypterus</i>	3.1	53.93	Carnivore

The carnivores have a much lower muscle cross-sectional area of the external adductor (MAME), and the seed-cracking finches have a much greater muscle cross-sectional area of the external adductor for a given head length

V–PDB. All sample measurements were duplicated and adjusted to the international references NIST NBS19 and NBS18. External reproducibility is ± 0.1 for $\delta^{13}\text{C}$ values (1σ).

Carbon isotope analysis of apatite carbonate

Forty-two apatite remains consisting of herbivorous mammal teeth and *Gastornis* bones from four French fossil localities and eight eggshell samples of ostrich from two French modern localities were recovered (Table 3). Samples were collected as isolated specimens, cleaned and analyzed for the carbon isotope compositions of their carbonate. For each sample, the most mineralized apatite part was selected, specifically mammalian tooth enamel, dense bone layers from *Gastornis* (Table 3), and eggshells.

For the carbon isotope analysis of the carbonate of fossil bones, about 10 mg of enamel, dentine, or bone powder was pretreated according to the procedure of Koch et al. (1997). Powders were washed with a 2 % NaOCl solution to remove organic matter, followed by a 0.1 M acetic acid solution to remove diagenetic carbonates. The powder/solution ratio was kept constant to 0.04 g.mL^{-1} for both treatments. Each treatment lasted for 24 h, and the samples were rinsed five times with distilled water.

In order to avoid contamination from chemicals used to preserve fossil samples, we decided to perform our carbonate isotopic analyses with a continuous flow automated inlet. The system we used is a MultiFlow Geo online with an IsoPrime IRMS from IsoPrime UK Ltd. The inlet consists of an automated phosphoric acid delivery system, a temperature-regulated sample tray kept at 70°C , a packed GC column to separate the CO_2 from unwanted compounds, and a heart-cut mechanism to specifically introduce the gas pulse of interest in

the source of the IRMS. The specifications of such a system are 0.15‰ for $\delta^{13}\text{C}$.

Morphofunctional study

To test whether species with different diets differ in the development of the external adductor muscle, we dissected the heads of one *Geospiza magnirostris*, three *Geospiza fortis*, one *Platyspiza crassirostris*, four *Gallus gallus*, two *Numida meleagris*, and one *Carina moschata* as representative of herbivores and two *Accipiter nisus* and one *Buteo platypterus* as representative of carnivores (Table 2). Dissections were done using a binocular microscope (Olympus SZX7). For all specimens, the jaw muscles were dissected and all the jaw muscle bundles were removed individually. Muscles were blotted dry and weighted on a microbalance ($\pm 0.01 \text{ mg}$). Muscles were transferred to a 30 % aqueous nitric acid solution for 24–48 h to digest the connective tissue and transferred to a 50 % aqueous glycerol solution (Loeb and Gans 1986). Fibers were teased apart using blunt-tipped glass needles and drawn using a dissecting scope with camera lucida. Next, drawings of the fibers were scanned, and fiber lengths were determined using NIH image. Muscle physiological cross-sectional area (PCSA) of each muscle bundle was calculated by dividing muscle mass (g), multiplied by muscle density (1.065 g.cm^{-3}) (Mendez et al. 1960), and by fiber length (cm). The cross-sectional areas of the external adductor are listed in Table 2. We summed up the cross-sectional areas of all the muscle bundles of the external adductor for each individual. Next, data on cross-sectional area and head length for each specimen were Log_{10} -transformed and used as input for a regression of cross-sectional area on head length. Unstandardized residuals were extracted and used as input

Table 3 $\delta^{13}\text{C}$ of bone apatite carbonate from *Gastornis* and contemporaneous herbivores from the four studied fossil sites and modern ostrich measurements from two French sites

Sample number	Sites	Taxa	Diet	Material	$\delta^{13}\text{C}$ (‰ V-PDB)	$\% \text{CO}_3^{2-}$ (wt%)
BRm01ed	Mont-de-Berru	<i>Pleurospirotherium</i>	Herbivorous	Enamel+dentin	-5.73	
BRm02ed	Mont-de-Berru	<i>Pleurospirotherium</i>	Herbivorous	Enamel+dentin	-8.09	
BRm03e	Mont-de-Berru	<i>Pleurospirotherium</i>	Herbivorous	Enamel	-6.58	
BRm06ed	Mont-de-Berru	<i>Plesiadapis</i>	Herbivorous	Enamel+dentin	-7.94	
BRm07ed	Mont-de-Berru	<i>Plesiadapis</i>	Herbivorous	Enamel+dentin	-7.89	
BRm08ed	Mont-de-Berru	<i>Plesiadapis</i>	Herbivorous	Enamel+dentin	-8.34	
BRm09ed	Mont-de-Berru	<i>Plesiadapis</i>	Herbivorous	Enamel+dentin	-8.63	
BRm10ed	Mont-de-Berru	<i>Pleurospirotherium</i>	Herbivorous	Enamel+dentin	-8.91	
BRm11ed	Mont-de-Berru	<i>Pleurospirotherium</i>	Herbivorous	Enamel+dentin	-7.53	
BRg1b	Mont-de-Berru	<i>Gastornis</i>	Indeterminate	Bone	-6.14	
BRg2b	Mont-de-Berru	<i>Gastornis</i>	Indeterminate	Bone	-5.93	7.6
BRg3b	Mont-de-Berru	<i>Gastornis</i>	Indeterminate	Bone	-6.14	5.9
BRg4b	Mont-de-Berru	<i>Gastornis</i>	Indeterminate	Bone	-6.77	3.5
BRg5b	Mont-de-Berru	<i>Gastornis</i>	Indeterminate	Bone	-9.30	
BRg6b	Mont-de-Berru	<i>Gastornis</i>	Indeterminate	Bone	-8.29	
BRg8b	Mont-de-Berru	<i>Gastornis</i>	Indeterminate	Bone	-7.23	
BRg9b	Mont-de-Berru	<i>Gastornis</i>	Indeterminate	Bone	-6.56	
CRm01ed	Cemay-les-Reims	<i>Neoplagiaulax</i>	Herbivorous	Enamel+dentin	-7.41	5.6
CRm02ed	Cemay-les-Reims	<i>Pleurospirotherium</i>	Herbivorous	Enamel+dentin	-8.46	6.3
CRm04ed	Cemay-les-Reims	Multituberculata	Herbivorous	Enamel+dentin	-8.45	
CRm07ed	Cemay-les-Reims	<i>Orthospirotherium</i>	Herbivorous	Enamel+dentin	-8.85	
CRm08ed	Cemay-les-Reims	<i>Plesiadapis</i>	Herbivorous	Enamel+dentin	-8.90	
CRm09ed	Cemay-les-Reims	<i>Chiromyoides</i>	Herbivorous	Enamel+dentin	-7.07	
CRm11ed	Cemay-les-Reims	<i>Pleurospirotherium</i>	Herbivorous	Enamel+dentin	-8.36	
CRm13ed	Cemay-les-Reims	<i>Arctocyonides</i>	Herbivorous	Enamel+dentin	-7.98	
CRg1b	Cemay-les-Reims	<i>Gastornis</i>	Indeterminate	Bone	-5.23	5.9
CRg3b	Cemay-les-Reims	<i>Gastornis</i>	Indeterminate	Bone	-2.50	
CRg4b	Cemay-les-Reims	<i>Gastornis</i>	Indeterminate	Bone	-8.62	
SPm1e	Saint-Papoul	Lophiodontidae	Herbivorous	Enamel	-11.73	
SPg02b	Saint-Papoul	<i>Gastornis</i>	Indeterminate	Bone	-8.87	
SPg03b	Saint-Papoul	<i>Gastornis</i>	Indeterminate	Bone	-9.86	
SPg04b	Saint-Papoul	<i>Gastornis</i>	Indeterminate	Bone	-8.03	
SPg05b	Saint-Papoul	<i>Gastornis</i>	Indeterminate	Bone	-9.10	
SPg06b	Saint-Papoul	<i>Gastornis</i>	Indeterminate	Bone	-9.34	
SPg07b	Saint-Papoul	<i>Gastornis</i>	Indeterminate	Bone	-11.51	
SPg09b	Saint-Papoul	<i>Gastornis</i>	Indeterminate	Bone	-10.13	
SPg10b	Saint-Papoul	<i>Gastornis</i>	Indeterminate	Bone	-9.43	
SPg11b	Saint-Papoul	<i>Gastornis</i>	Indeterminate	Bone	-10.00	
MTm1e	Monthelon	<i>Lophiodon</i>	Herbivorous	Enamel	-12.50	
MTm2ed	Monthelon	<i>Lophiodon</i>	Herbivorous	Enamel+dentin	-9.68	
MTg1b	Monthelon	<i>Gastornis</i>	Indeterminate	Bone	-8.86	
A1	Ferme de l'Autruche Drômoise	<i>Struthio camelus</i>	Herbivorous	Eggshell	-8.32	
A2	Ferme de l'Autruche Drômoise	<i>Struthio camelus</i>	Herbivorous	Eggshell	-12.04	
A3	Ferme de l'Autruche Drômoise	<i>Struthio camelus</i>	Herbivorous	Eggshell	-10.03	
A4	Ferme de l'Autruche Drômoise	<i>Struthio camelus</i>	Herbivorous	Eggshell	-8.80	
A5	Parc aux Oiseaux des Dombes	<i>Struthio camelus</i>	Herbivorous	Eggshell	-10.84	
A6	Parc aux Oiseaux des Dombes	<i>Struthio camelus</i>	Herbivorous	Eggshell	-11.06	
A7	Parc aux Oiseaux des Dombes	<i>Struthio camelus</i>	Herbivorous	Eggshell	-11.63	
A8	Parc aux Oiseaux des Dombes	<i>Struthio camelus</i>	Herbivorous	Eggshell	-10.34	

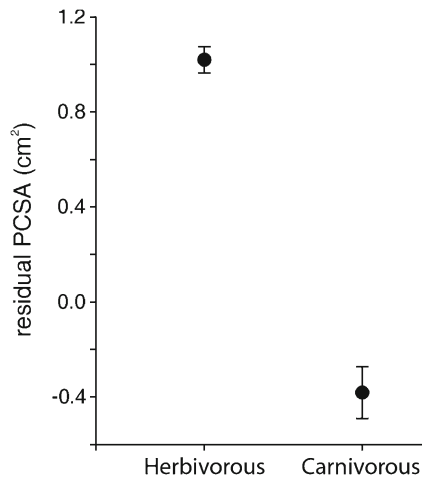


Fig. 2 Scatter plot representing mean (\pm SD) size-corrected physiological cross-sectional areas (PCSA) of the external adductor in herbivorous and carnivorous extant birds. Herbivores have a much greater cross-sectional area and thus can apply greater force than carnivores

Our results show that the external muscle mass is strongly dependent on head size ($r=0.59$; $P=0.021$). An ANOVA conducted on the residual (size-free) data detected highly significant differences between herbivores and carnivores with herbivores having a significantly greater external adductor for their head length than carnivores ($F_{1,13}=17.34$; $P=0.001$; Fig. 2).

Contrast stains, sample preparation, and micro-CT imaging system

Animals were dissected on one side while the other side was left intact. Heads were submerged in a 5 % phosphomolybdic acid solution (Metscher 2009) for 3 weeks before scanning. The specimens were subsequently scanned at the micro-CT imaging of the platform at the National Museum of Natural History in Paris and at the Center for X-ray Tomography (UGCT) at the University of Ghent (see Genbrugge et al 2011). Voxel sizes ranged between 123 and 123 μ m and scans were performed at 175 kV and 500 μ A.

for an ANOVA testing for differences in the external adductor mass between herbivores and carnivores.

Fig. 3 $\delta^{13}C$ of the diet of *Gastornis* and contemporaneous herbivores estimated from the $\delta^{13}C$ of apatite carbonate for the four studied sites. The bars correspond to the minimum and maximum values while the black triangle, black diamond, and white diamond illustrate the mean value. The hatched areas separate the different sites

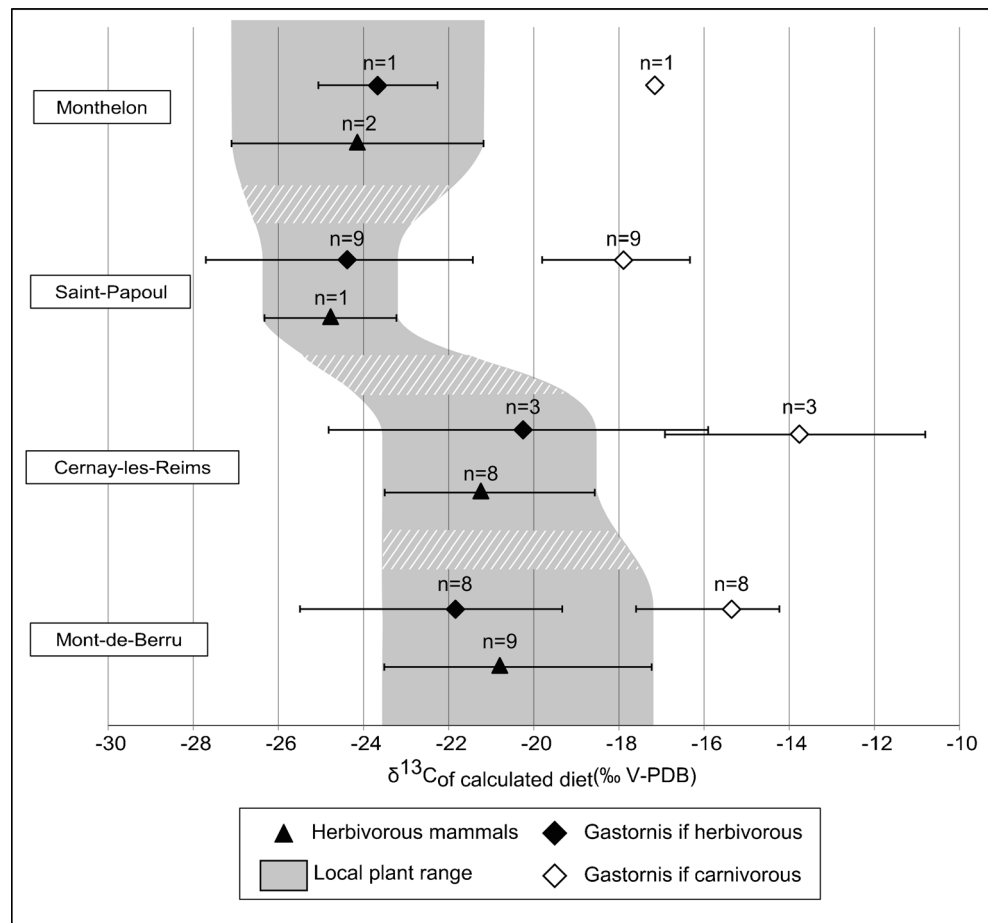


Table 4 Results of the Kruskal–Wallis statistical test performed for calculated $\delta^{13}\text{C}$ of diets between either a hypothetical carnivorous *Gastornis* or a hypothetical herbivorous *Gastornis* and the associated contemporaneous herbivorous mammals for each studied site

Mont-de-Berru		
P -value= 7.704×10^{-5}	<i>Gastornis</i> carnivorous	<i>Gastornis</i> herbivorous
	<i>Gastornis</i> herbivorous	–
	Mammals herbivorous	0.9176
Cemay-les-Reims		
P -value=0.04298	<i>Gastornis</i> carnivorous	<i>Gastornis</i> herbivorous
	<i>Gastornis</i> herbivorous	–
	Mammals herbivorous	1
Saint-Papoul		
P -value=0.0001229	<i>Gastornis</i> carnivorous	<i>Gastornis</i> herbivorous
	<i>Gastornis</i> herbivorous	–
	Mammals herbivorous	0.8997
Monthelon		
P -value=NA	<i>Gastornis</i> carnivorous	<i>Gastornis</i> herbivorous
	<i>Gastornis</i> herbivorous	–
	Mammals herbivorous	0.6434

The test is significant for P -value < 0.005 . These statistical tests show that the calculated $\delta^{13}\text{C}$ of the diet of a hypothetical carnivorous *Gastornis* is statistically different from the $\delta^{13}\text{C}$ of the diet inferred from the known herbivorous mammals for each studied site. In addition, calculated $\delta^{13}\text{C}$ of the diet of these herbivorous mammals is not statistically different from the calculated $\delta^{13}\text{C}$ of the diet of a hypothetical herbivorous *Gastornis* for each site

Results

Carbon isotopic evidence

Carbon isotope compositions of vertebrate-calcified tissues (bones, teeth, and eggshells) are related to those of their food with a ^{13}C -enrichment which depends on digestive physiology (De Niro and Epstein 1978; Kelly 2000; Passey et al. 2005). Therefore, the observed ^{13}C -enrichment between carbonate and food of one carnivorous bird is $+8.3\text{‰}$, much lower than values measured for ostriches which are $+13.4$ to $+16.2\text{‰}$ (Table 1). Assuming that fossils of *Gastornis* and associated faunas preserved their initial carbon isotope compositions (see “Methods” section), a hypothetical carnivorous *Gastornis* with a mean $\delta^{13}\text{C}$ value of $-8.0 \pm 2.1\text{‰}$ would have consumed food items having average $\delta^{13}\text{C}$ values of $-16.3 \pm 2.1\text{‰}$ (Fig. 3). Such an isotopic ratio would require that the preys of *Gastornis* were ingesting almost exclusively C_4 plants; their modern equivalents having $\delta^{13}\text{C}$ values in the -19 to -10‰ range. As the earliest record of C_4 plants dates back to the Eocene/Oligocene boundary (Kohn 2010), about 14 million years after the existence of the sampled

Gastornis individuals (Buffetaut and Angst 2013), the hypothesis of a carnivorous *Gastornis* can be dismissed. On the other hand, a putatively herbivorous *Gastornis* would have ingested plants having $\delta^{13}\text{C}$ values ranging from -24.2 ± 2.1 to $-21.4 \pm 2.1\text{‰}$ (Fig. 3), which are typical of C_3 plants. Using published herbivorous mammal carbonate-diet fractionation values ranging from 11.5 to 14.6‰ (Table 1), herbivorous mammals found associated with *Gastornis* consumed plants having comparable $\delta^{13}\text{C}$ values ranging from -23.1 ± 1.5 to $-20.0 \pm 1.5\text{‰}$ (Fig. 3). A Kruskal–Wallis test for significance confirms that the range of $\delta^{13}\text{C}$ values of the plant diet estimated from herbivorous mammals is the same as that estimated from *Gastornis* if it was herbivorous and significantly different from that of *Gastornis* if it was carnivorous (Table 4). Our isotopic results are in agreement with the conclusions of some authors who consider *Gastornis* as herbivorous on the basis of osteological evidence (Andors 1992) and preliminary studies on calcium isotopes (Tütken et al. 2013) but conflict with the widespread interpretation of this giant bird as a large carnivore (Witmer and Rose 1991).

Anatomical evidence

To check these geochemical results, we investigated the possible diet of *Gastornis* using a completely independent approach. We reconsidered the available anatomical evidence concerning the diet of *Gastornis*, based on comparisons of the jaw musculature among living birds. Our data show that the development of the jaw adductor musculature is closely related to diet. Dissections and micro-CT scans of contrast-enhanced skulls of modern birds having different diets were performed. Our analyses show that the musculus adductor mandibulae externus (MAME) (Baumel et al. 1993) is very well developed in herbivorous modern birds, reaching its greatest development in the seed-cracking Darwin’s finches. The insertion area of the superficial external adductor is markedly visible in lateral view on the mandible and is very large (Fig. 4b). Conversely, among the carnivorous birds of prey, the muscle is less developed, and its insertion area is much reduced (Fig. 4a). The development of the adductor musculature is directly linked to diet, with herbivores (*sensu lato*; i.e., including duck, chicken, guinea fowl, and the finches) having a much greater physiological cross-sectional area than carnivores ($F_{1,13} = 17.34$; $P = 0.001$; Fig. 2). An herbivorous diet requires a massive and strong musculature to close the jaw in order, for instance, to crush seeds (in ground finches from the Galápagos) or to browse and shear leaves as in the vegetarian finch or grazing ducks (Clabaut et al. 2009; Genbrugge et al. 2011). In contrast, carnivorous birds typically rip the flesh of their prey using their upper beak and tear off bite-sized pieces which are then swallowed whole. As this action is more strongly dependent on upper beak retraction than on pure adduction, the development of the external adductor and its insertion area is much reduced (Baumel et al. 1993). The insertion area of the

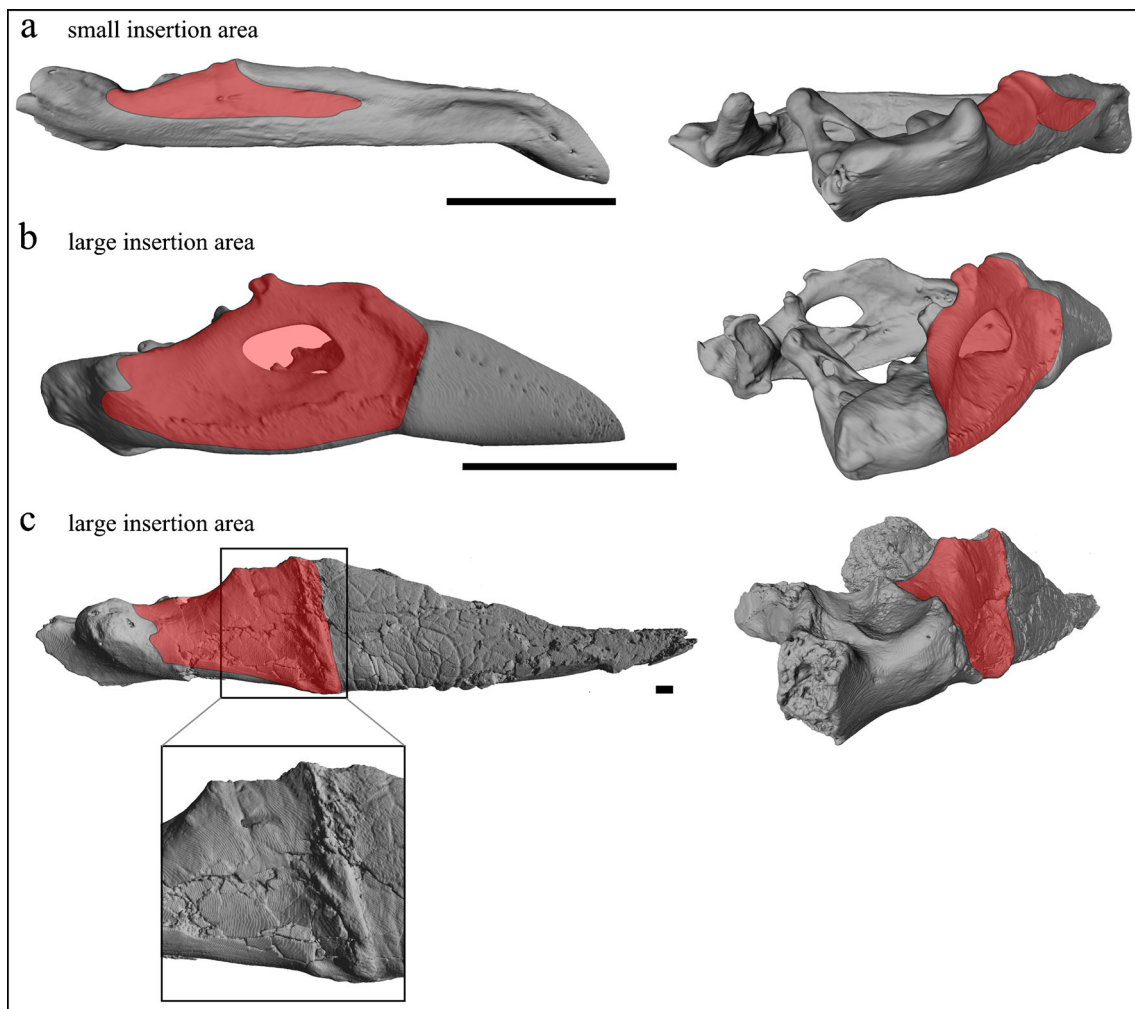


Fig. 4 Comparisons between the insertion area of the musculus adductor mandibulae externus superficialis (MAMES) (red area) on the mandible of modern birds and *Gastornis* in lateral view (left part) and in oblique

posterior view. **a** Carnivorous modern bird (*Buteo platypterus*), **b** seed-crushing passeriform (*Geospiza magnirostris*), and **c** *Gastornis* mandible and photo of the insertion area. Scale bar 1 cm

MAME of *Gastornis* is large and extensively developed as indicated by a well-developed bony ridge, illustrating the importance of this muscle (Fig. 4c; Angst and Buffetaut 2013). This suggests a crushing or shearing function compatible with an herbivorous and potentially seed-cracking diet.

Discussion

During the early Tertiary, three families of large ground birds radiated on the continents: Gastornithidae, Dromornithidae, and Phorusrhacidae. The phorusrhacids, a group of extinct flightless carnivorous birds, which *Gastornis* has sometimes been compared with (Bourdon and Cracraft 2011), display a jaw morphology more similar to that of the birds of prey examined here. Anatomically, *Gastornis* is reminiscent in many respects of the Dromornithidae, a group of giant Anseriformes from the Cenozoic of Australia, for which an

herbivorous diet has been proposed (Murray and Vickers-Rich 2004), a conclusion supported by isotopic evidence (Miller et al. 1999). Murray and Vickers-Rich (2004) have suggested that dromornithids were browsers feeding on tough plant material, including hard fruits and seeds. *Gastornis* may have had a similar diet. Interestingly, the fossil flora from the middle Eocene German site of Messel, where *Gastornis* remains have been found, includes a large proportion of plants with hard-shelled fruits and seeds (Collinson et al 2012). The fact that gastornithids were plant-eating birds sheds a new light on the structure of terrestrial ecosystems after the K/Pg crisis. The major mass extinction at the end of the Cretaceous wiped out all terrestrial animals with a body mass above 25 kg, consisting almost exclusively of nonavian dinosaurs (Archibald 1996), leaving many terrestrial niches vacant at the beginning of the Paleogene. Following this major perturbation, the Paleocene ecosystems of Europe recovered rapidly with a structure characterized by the largest animal, *Gastornis*,

being an herbivorous bird much larger than the contemporary top carnivores such as mesonychids (jackal to coyote size). On the contrary, the pattern in North America and Asia is different, as *Gastornis* was not present during the Paleocene and the largest herbivores were pantodont mammals (Rose 2009). Our results show that in the late Paleocene terrestrial ecosystems of Europe, the largest animal was thus an herbivore, a pattern similar to that prevalent today. However, the fact that this largest animal was the giant bird *Gastornis*, rather than a mammal, makes this Paleocene ecosystem surprisingly different from those of today's continents and somewhat similar to recently vanished ecosystems on large islands, such as New Zealand and Madagascar. In Madagascar, in particular, before the arrival of humans, the largest herbivores were aepyornithid birds and the largest local mammals (dwarf hippopotami) were smaller than the giant flightless birds. The peculiar structure of the European Paleocene ecosystems may reflect the fact that at that time, Europe was an "island continent", separated by seaways from the larger North American and Asian land masses, which hosted more usual ecosystems in which the largest herbivores were mammals. In the early Eocene, faunal evolution and dispersal resulted in a different situation where *Gastornis* was present not only in Europe but also in North America and Asia and coexisted with various large herbivorous mammals, such as *Coryphodon*. Interestingly, the only place where *Gastornis* survived beyond the early Eocene was in Europe.

Conclusion

Both morphological analysis and stable isotope geochemistry unambiguously show that *Gastornis* was an herbivorous giant terrestrial bird. The morphofunctional comparison with extant birds shows that *Gastornis* had a jaw musculature similar to that of modern browsing or granivorous birds. *Gastornis* was among the first land vertebrates to reach a large size after the mass extinction of the Cretaceous–Paleogene boundary, which eliminated large terrestrial forms mainly consisting of nonavian dinosaurs. Our results provide a new picture of the early Tertiary terrestrial ecosystems by showing that during this period of biotic recovery, in the Paleocene, an herbivorous bird was the largest vertebrate in Europe, unlike the situation in North America and Asia, where the earliest records of *Gastornis* are early Eocene and the large herbivores of the Paleocene were mammals.

Acknowledgments We thank Florent Goussard, Patricia Wills, and Alexis Dollion for their help with CT scanning and segmentation of bird mandibles. Thanks to Ronan Allain and Christine Argot (MNHN, Paris), Loïc Costeur (NHM, Basel), Yves Laurent (MHN, Toulouse), Emmanuel Robert (UCBL, Lyon), and Alain Phelizon (Châlons-en-Champagne) for the permission to sample fossils for the geochemical analysis. Thanks to Marie and Pascal Grussenmeyer from the Ferme de l'Autruche Drômoise (Livron, Drôme) and Géraldine Blanchon-Pothet from the Parc aux

Oiseaux des Dombes (Villars-les-Dombes, Ain) for the access to living birds, their eggshells, and their food. Thanks to Paul Wheeler from IsoPrims UK Ltd for the access to their IRMS facility in Manchester. This work was supported by a grant from the INTERRVIE program of the Institut National des Sciences de l'Univers.

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