

PERSPECTIVES

High C/N ratio (not low-energy content) of vegetation may have driven gigantism in sauropod dinosaurs and perhaps omnivory and/or endothermy in their juveniles

David M. Wilkinson^{*1} and Graeme D. Ruxton²

¹Natural Science and Psychology, Liverpool John Moores University, Liverpool, L33AF UK; and ²School of Biology, University of St Andrews, St Andrews, Fife, KY16 9HT, UK

Summary

1. Sauropod dinosaurs were the largest terrestrial animals ever, and the combination of selective pressures that might have led to such extraordinary sizes has long been discussed.

2. Here, we argue that a previous suggestion that large size may be a response to unusually high C/N ratios in available plant foods has been prematurely discarded. C/N ratios were likely to be high during much of the Mesozoic, and C/N ratio is entirely different from gross energy density as a measure of the value of a plant as food. In addition, we use recently published allometric equations for herbivore nitrogen and carbon use to make tentative calculations which suggest that if Mesozoic C/N ratios were greater than extant ones, this would have selected for one of two strategies: gigantism in ectothermic herbivores or endothermy (and selective foraging on high N material) in very small herbivores.

3. We speculate that smaller-bodied juvenile sauropods might have had a broader omnivorous diet and/or had higher mass-specific metabolic rates than adults. The former is potentially testable by changes in dentition; the latter matches evidence of high growth rates of juvenile sauropods.

Key-words: C/N, dinosaur, plant biochemistry, sauropod, stoichiometry

Introduction

Sauropod dinosaurs were the largest terrestrial animals ever – with estimated maximum body mass of up to at least 80 metric tons (Sander & Clauss 2008). In comparison, the largest known terrestrial mammal, *Indricotherium* (*Paraceratherium*), was closer to 15 tons, and a large modern elephant is only around half that mass (Turner & Antón 2004). Burness, Diamond & Flannery (2001) showed that the body mass of the largest species increased with available land area in the geologically recent past. When they applied their statistical relationships to more ancient animals, they predicted the correct size for *Indricotherium* but not the largest dinosaurs – which were substantially larger than this approach predicted. This raises the question: why were sauropods so large?

One potential explanation for the large size of sauropods is based on the nature of plant food during the Mesozoic (Midgley, Midgley & Bond 2002). Unfortunately, there is

no direct uncontroversial fossil evidence for the nature of sauropod food (Sander *et al.* 2011); however, the generally made assumption is that they eat a range of plants including conifers, ginkgos, cycads, ferns and horsetails (Gee 2011; Tütken 2011). The suggestion is that, compared to modern angiosperms, these taxa have low nitrogen concentrations and so high carbon/nitrogen (C/N) ratios (Midgley, Midgley & Bond 2002). In addition, higher atmospheric CO₂ concentrations during much of the Mesozoic (Berner 2004) would have had the effect of lowering plant nitrogen content even further because of the well-established relationship between high CO₂ and a decrease in the C/N ratio (Körner 2004; Midgley 2005). Therefore, Midgley, Midgley & Bond (2002) suggested that the large size of sauropods was an adaptation to low food quality, pointing out that present day megaherbivores are associated with plants of low food quality (following Owen-Smith (1988)). There is a view amongst many sauropod researchers that sauropods must have relied heavily on gut microbes for fermentative digestion of their plant food (Hummel & Clauss 2011; Sander *et al.* 2011). So,

*Correspondence author. E-mail: D.M.Wilkinson@ljmu.ac.uk

large body size effectively gives a much larger microbial fermentor – which has a range of interesting implications, including that sauropods may have produced enough methane to have a measurable effect on the Mesozoic climate (Wilkinson, Nisbet & Ruxton 2012).

The view amongst many important sauropod biologists appears to be that Midgley *et al.*'s idea has been shown to be wrong (e.g. Gee 2011; Hummel & Clauss 2011; Sander *et al.* 2011). We believe this conclusion to be premature. Sander *et al.* (2011) cite the work of Hummel *et al.* (2008) as showing 'that many pre-angiosperm plants are no less nutritious than angiosperms'. However, Hummel *et al.* (2008) measured energy content (by *in vitro* fermentation) – not C/N ratios – although they claimed that this falsifies the ideas of Midgley, Midgley & Bond (2002). Hummel *et al.* (2008) wrote: 'We note that the hypothesis that particularly low-quality forage in Mesozoic ecosystems led directly to gigantism in dinosaurs is not substantiated by our data because the energy yield from many potential sauropod food sources is comparable to that measured in extant browse species'. However, we feel that this misrepresents Midgley *et al.*'s argument, which was based on stoichiometry (C/N ratio) not energy content. That is, a key but apparently misunderstood prediction of Midgley *et al.*'s argument is that a sauropod may have needed to consume more vegetation than was necessary to provide its energy requirements to acquire enough nitrogen. Indeed, there is a reasonable consensus in plant ecology that plant quality (as measured both through stoichiometry and the presence of secondary plant compounds as anti-feedents) makes plants difficult for current herbivores to eat (Polis 1999; Sherratt & Wilkinson 2009), so an increased C/N ratio in the past may have produced unusual adaptations – such as sauropod gigantism.

Two additional problems have been raised with the ideas of Midgley, Midgley & Bond (2002), which have been claimed to help falsify the hypothesis (Sander *et al.* 2011). Firstly, it has been noted that there was no decrease in sauropod size associated with the rise of angiosperms in the Late-Cretaceous; however, angiosperms are unlikely to have formed any substantial part of sauropod diets until the very end of the Cretaceous, if at all (Barrett & Willis 2001; Gee 2011; Tütken 2011). In addition, we can envisage a situation in which the food quality problem has contributed to initial selection for the large size of sauropods but where new selection pressures prevent a size decrease in response to any later potential increase in food quality. An obvious possibility is that the evolution of very large predators such as *Tyrannosaurus* (as a response to large herbivores as potential food) made a decrease in herbivore size maladaptive – large size is very successful as an anti-predator adaptation in extant megaherbivores (Sinclair, Mduma & Brashares 2003). In addition, the role of potential pack hunting by smaller carnivorous dinosaurs may have also been important.

The second objection to Midgley *et al.*'s proposal is that large animals have a more efficient digestion because of

the increased retention times of food in their digestive system. This, however, is not fully supported by more recent studies (Sander *et al.* 2011). In fact, Midgley, Midgley & Bond (2002) simply note that most modern megaherbivores tend to specialize in low-quality food without specifying a mechanism of digestion. However, there is more to the digestive advantages of large size than just overall digestive efficiency. In a review of mammalian data, Clauss & Hummel (2005) suggested that a change in the focus of the digestive process, rather than just energy efficiency, may be the key adaptation that large size provides to herbivores. That is, Clauss & Hummel (2005) argue that large size does not lead to more energetically efficient digestion, but does lead to switch in 'digestive priorities' in favour of fibre. Indeed, it has been observed that larger extant herbivores have a higher fibre diet (Owen-Smith 1988) so managing to subsist on a particularly nitrogen poor diet. In the context of Midgley, Midgley & Bond (2002) ideas, the key issue is the extraction of nitrogen compounds from the plant food – not the overall efficiency of energy extraction.

More recently, Klaassen & Nolet (2008) reiterated the point that plant material provided a challenging diet because of its low nitrogen content compared to carbon. They went on to argue that endotherms are more effective than (same-sized) ectotherms at expulsion of carbon when C/N ratios in food are high and that this may have been an important selection pressure on the evolution of endothermy. Endotherms effectively 'burn off' excess carbon in the raised metabolism used to maintain body temperature. Clearly dinosaur thermoregulation has been a significant area of dispute since the 1970s (Desmond 1975). However, the consensus now seems to be that while younger sauropods may have had higher mass-specific basal metabolic rates (BMR), the adults may well have had lower mass-specific BMRs. Large adult size likely created potentially serious overheating problems, and adults no longer needed to sustain rapid growth with the additional requirements of building new tissue – above and beyond the BMR needed for maintenance of existing biomass (Eagle *et al.* 2011; Sander *et al.* 2011). So, these large dinosaurs are probably best thought of as inertial homeotherms because their size greatly restricts heat loss, but not endotherms (Clarke & Pörtner 2010). If so, the admittedly limited ($n = 3$ and we have been unable to find any additional data) modern reptile data of Klaassen & Nolet (2008) are probably reasonable first approximations to adult sauropod metabolism (but mammals may be a better match for the behaviour of body temperature). Klaassen and Nolet's data also suggest that the disadvantage faced by herbivorous reptiles declines with size, and thus (just as Midgley *et al.* argued), it may have been the high C/N ratio of plant matter, and not its energy density that – at least in part – drove sauropod gigantism. Although birds are closer relatives to sauropods than modern reptiles are, we feel the latter are appropriate physiological analogues, because of the unusually high body temperatures and metabolisms of birds, and their generally low body size: the largest

extant bird, the ostrich, weighs around 100 kg (Folch 1992), and even the extinct elephant bird was thought to have weighed less than 800 kg (Davies 2003). We do, however, consider data from mammals as well as reptiles to explore the effect of different metabolic rates. However, McNab (2009) makes a plausible case that dinosaur metabolism may be more like that of varanid lizards (somewhat higher than most modern reptiles, but much lower than mammals), so we feel that adult sauropods would in this respect be better modelled as reptiles than mammals.

Klaassen & Nolet (2008) derive allometric equations for maintenance nitrogen requirement (MNR; mgN day⁻¹) and field metabolic rate (FMR; kJ day⁻¹): see their table 1.

For reptiles,

$$\text{MNR} = 108M^{0.473},$$

and

$$\text{FMR} = 91M^{0.889}.$$

For mammals,

$$\text{MNR} = 411M^{0.863},$$

and

$$\text{FMR} = 872M^{0.772}.$$

In both cases, M is body mass in kilogram.

Dividing the field metabolic rate by the maintenance nitrogen requirement (in combination with Klaassen and Nolet's assumed conversion of carbon to energy (1kJ = 22.1 mgC)) allows us to obtain an allometric equation for the ratio of carbon to nitrogen (C/N) required in the diet: this comes out as

$$\text{C:N} = 18 \cdot 6M^{0.416}$$

for reptiles

and

$$\text{C:N} = 46 \cdot 9M^{-0.091}$$

for mammals.

We can use these equations to make a 'first go' at predicting the critical body mass (M_c) at which food of a given C/N ratio would perfectly match requirements.

For reptiles,

$$M_c = (\text{C} : \text{N}/18 \cdot 6)^{2.4}$$

For mammals,

$$M_c = (\text{C} : \text{N}/46 \cdot 9)^{-11}$$

Elser *et al.* (2000) measured the C/N ratio for 406 different modern terrestrial primary producers. They obtained a mean of 36 and standard deviation of 23. This mean value equates to a predicted 'break-even' body mass of 4.9 kg for reptiles and 18.3 kg for mammals (from the allometric equations above). For a C/N ratio equal to this mean plus

one standard deviation (C : N = 59), this becomes 16.0 kg for reptiles and 0.08 kg for mammals; and for the mean plus two standard deviations (C : N = 82), these values become 35.1 kg for reptiles and 0.002 kg for mammals.

From these arguments, we can see that if Mesozoic C/N ratio was greater than extant ones, then this would have selected for one of two strategies: gigantism in ectothermic herbivores or endothermy (and selective foraging on high N material) in very small herbivores. Many of the plant groups most relevant to the Mesozoic (e.g. Cycads, Horse-tails and Ginkgo) are missing from the data set used by Elser *et al.* (2000). However, there are data on conifers, and the mean \pm SD value for 17 species of *Picea* and *Pinus* is 54.6 ± 12.7 , suggesting a higher C/N ratio is plausible in the Mesozoic. Clearly, our calculations represent a substantial simplification of biological reality, ignoring many other aspects of the organisms' biology (we calculate 'ideal' weights for a particular C/N ratio – not predicted actual weights); however, we believe that our exploration does quantify one important selection pressure potentially acting on sauropods.

An interesting consequence of the arguments above is that a diet that might have been acceptable to adult sauropods would not have been so for juveniles. Compared to extant herbivorous birds and mammals, sauropod hatchlings were particularly small relative to their parents. The largest discovered dinosaur eggs are around 20cm in diameter and 60cm in length, suggesting a newborn mass of 1–10 kg (Horner 2000), for something that might ultimately grow to 30–70T. In contrast, newborn African elephants weight is 70–120 kg (Dale 2010), and they are unlikely to grow beyond 10T. Hence, it seems reasonable to speculate that diet might have changed over ontogeny (see also Fiorillo 1991; Barrett 2000; Gee 2011), with smaller individuals exploiting foods that have a lower C/N ratio than vegetation. Such a food might most obviously be animal flesh (perhaps from freshwater molluscs) – a similar speculation was made by Midgley, Midgley & Bond (2002). Such a hypothesis is testable, as it predicts that if jaws of juvenile sauropods are found, these might be expected to have more substantial dental batteries than those found in adults, and more particularly have teeth characteristic of an omnivorous diet. Alternatively or additionally, the stoichiometric problem of small size may have been solved in juvenile sauropods by a raised metabolism (closer to that of extant endotherms) – indeed, this seems the more plausible hypothesis. Compared to other dinosaurs, histological growth marks in bone structure are rare in sauropods and confined to later life (Sander *et al.* 2011), and this may be indicative of continuous growth in early life characteristic of extant terrestrial endotherms. It has previously been argued that high BMR in young sauropods allowed them to grow quickly and so become too large for their predators to attack (Heeren 2011; Sander *et al.* 2011). However, it may be the selection for raised metabolism was at least in part used to combat the challenges of an herbivorous diet with a high C/N ratio. These

arguments for the potential advantage of raised metabolisms in small and/or young sauropods follow directly from the need to process a large amount of carbon to extract the necessary amount of nitrogen and are insensitive to the details of the calculations above – which we stress (while being based on the best available data) have to be considered very tentative because so little reptile data are available in the literature.

The subject of how herbivores should best exploit a food source that has a ratio of macronutrients very different from the ideal for that animal's metabolism is the subject of very active current research (Raubenheimer & Simpson 1999; Behmer 2009). In a situation like that considered here, where the ratio of carbon to nitrogen in most available food types is likely to be higher than optimal for the herbivore, balance must be achieved by post-intensive regulation rather than by selective feeding. The two most commonly discussed methods of dealing with 'excess' ingested carbon are conversion to fat stores or 'venting off' via raised metabolism. The second of these seems very unlikely for adult sauropod dinosaurs, because their large body size may have made metabolic heat dissipation a considerable challenge that would not be helped by any raising on metabolism. Fat storage could also present thermal costs, because fat deposits can often provide an effective thermal insulator that would hinder shedding of metabolic heat. However, camels present an interesting extant example of herbivores that store fat in a concentrated position of the body so as to allow effective shedding of body heat. Consideration of whether adult sauropods might have had similar concentrated fat stores may repay closer investigation. Such stores might explain why such animals were able to survive in polar regions where there would have been continuous darkness (likely greatly curtailing feeding) for months on end (Sander *et al.* 2011).

There is potentially one further implication of these stoichiometric ideas for dinosaur ecology. Recently, several workers have put forward very reasonable suggestions – based on energetic considerations – that dinosaur biomass may have been much higher than is currently seen in mammal communities. This is in part because the lower suggested metabolic rates for dinosaurs (compared with mammals) mean that a given biomass of vegetation could support a higher biomass of animals (e.g. McNab 2009; Farlow, Coroian & Foster 2010; Trammer 2011). However, if these animals need to consume more vegetation that required for purely energetic reasons, this may make the highest suggested biomasses less likely; though, we consider that this effect is very unlikely to be large enough to undermine the basic logic behind these estimates of high dinosaur biomass.

There is a growing realization that the foraging decisions of extant animals are better predicted by foraging models based on the regulation of multiple nutrients rather than those based on maximization of energetic intake (Simpson *et al.* 2004) and that this has far reaching effects on our understanding of trophic interactions across ecosystems

(Simpson *et al.* 2004, 2009; Raubenheimer, Simpson & Mayntz 2009). We feel there is no logical reason why the same reasoning should not apply to previous ecosystems in general, and sauropod feeding in particular. We hope that this short article, with its tentative first attempt at making these ideas quantitative, will provoke further endeavours in this field.

Acknowledgements

We thank Paul Barrett for useful discussions and three referees for useful comments on an earlier version of this paper.

References

- Barrett, P.M. (2000) Prosauropod dinosaurs and iguanas: speculation on diets of extreme reptiles. *Evolution of Herbivory in Terrestrial Vertebrates. Perspective from the Fossil Record* (ed H.-D. Sues), pp. 42–78. Cambridge University Press, Cambridge.
- Barrett, P.M. & Willis, K.J. (2001) Did dinosaurs invent flowers? Dinosaur-angiosperm coevolution revisited. *Biological Reviews*, **76**, 411–447.
- Behmer, S.T. (2009) Insect Herbivore Nutrient Regulation. *Annual Review of Entomology*, **54**, 165–187.
- Berner, R.A. (2004) *The Phanerozoic Carbon Cycle*. Oxford University Press, Oxford.
- Burness, G.P., Diamond, J. & Flannery, T. (2001) Dinosaurs, dragons, and dwarfs: the evolution of maximal body size. *Proceedings of the National Academy of Sciences of the United States of America*, **98**, 14518–14523.
- Clarke, A. & Pörtner, H.-O. (2010) Temperature, metabolic power and the evolution of endothermy. *Biological Reviews*, **85**, 703–727.
- Clauss, M. & Hummel, J. (2005) The digestive performance of mammalian herbivores: why big may not be that much better. *Mammal Review*, **35**, 174–187.
- Dale, R.H.I. (2010) Birth statistics for African (*Loxodonta africana*) and Asian (*Elephas maximus*) elephants in Human Care: history and implications for Elephant welfare. *Zoo Biology*, **29**, 87–103.
- Davies, S.J.J.F.. (2003). "Elephant birds". *Grzimek's Animal Life Encyclopedia. 8 Birds I Tinamous and Ratites to Hoatzins*, 2 edn (ed M. Hutchins), pp. 103–104, Gale Group, Farmington Hills, Michigan.
- Desmond, A.J.. (1975) *The Hot-Blooded Dinosaurs*. Blond & Briggs, London.
- Eagle, R.A., Tutken, T., Martin, T.S., Tripathi, A.K., Fricke, H.C., Connely, M., Cifelli, R.L. & Eiler, J.M. (2011) Dinosaur body temperatures determined from isotopic (^{13}C - ^{18}O) ordering in fossil biominerals. *Science*, **333**, 443–445.
- Elser, J.J., Fagan, W.F., Denno, R.F., Dobberfuhl, D.R., Folarin, A., Interlandi, S., Kilham, S.S., McCauley, E.M., Schulz, K.L., Siemann, E.H. & Sterner, R.W.L. (2000) Nutritional constraints in terrestrial and freshwater food webs. *Nature*, **408**, 578–580.
- Farlow, J.O., Coroian, I.D. & Foster, J.R. (2010) Giants on the landscape: modelling the abundance of megaherbivorous dinosaurs of the Morrison Formation (Late Jurassic, western USA). *Historical Biology*, **32**, 403–429.
- Fiorillo, A.R.. (1991) Dental microwear of sauropod dinosaurs *Camarasaurus* and *Diplodocus*: implications for sauropod paleoecology. Contributions of the Paleontological Museum, University of Oslo, 364, 23–24.
- Folch, A.. (1992). "Family Struthionidae (Ostrich)". *Handbook of the Birds of the World. 1, Ostrich to Ducks* (eds Jd. Hoya & J. Sargatal), pp. 76–83. Lynx Edicions, Barcelona.
- Gee, C.T.. (2011) Dietary options for sauropod dinosaurs from an integrated botanical and paleobotanical perspective. *Biology of the Sauropod Dinosaurs: Understanding the Life of Giants* (eds N. Kelvin, K. Remes, C.T. Gee & P. Sander), pp. 34–56, Indiana University Press, Bloomington.
- Heeren, F. (2011) Rise of the titans. *Nature*, **475**, 159–161.
- Horner, J.R. (2000) Dinosaur reproduction and parenting. *Annual Reviews in Earth and Planetary Sciences*, **28**, 19–45.
- Hummel, J. & Clauss, M.. (2011) Sauropod feeding and digestive physiology. *Biology of the Sauropod Dinosaurs: Understanding the Life of Giants*

- (eds N. Klein, K. Remes, C.T. Gee & M.P. Sander), 11–33. Indiana University Press, Bloomington.
- Hummel, J., Gee, C.T., Südekum, K.-H., Sander, P.M., Nogge, G. & Clauss, M. (2008) *In vitro* digestibility of fern and gymnosperm foliage: implications for sauropod feeding ecology and diet selection. *Proceedings of the Royal Society B*, **275**, 1015–1021.
- Klaassen, M. & Nolet, B.A. (2008) Stoichiometry of endothermy: shifting the question from nitrogen to carbon. *Ecology Letters*, **11**, 785–792.
- Korner, C. (2004) Through enhanced tree dynamics carbon dioxide enrichment may cause tropical forests to lose carbon. *Philosophical transactions of the Royal Society B*, **359**, 493–498.
- McNab, B.K. (2009) Resources and energetics determined dinosaur maximal size. *Proceedings of the National Academy of Sciences of the United States of America*, **106**, 12184–12188.
- Midgley, J.J. (2005) Why don't leaf-eating animals prevent the formation of vegetation? Relative vs absolute dietary requirements. *New Phytologist*, **168**, 271–273.
- Midgley, J.J., Midgley, G. & Bond, W.J. (2002) Why were dinosaurs so large? A food quality hypothesis. *Evolutionary Ecology Research*, **4**, 1093–1095.
- Owen-Smith, N. (1988) *Megaherbivores: The Influence of Large Body Size in Ecology*. Cambridge University Press, Cambridge.
- Polis, G.A. (1999) Why are some parts of the world green? Multiple factors control productivity and the distribution of biomass. *Oikos*, **86**, 3–15.
- Raubenheimer, D. & Simpson, S.J. (1999) Integrating nutrition: a geometrical approach. *Entomologia Experimentalis et Applicata*, **91**, 67–82.
- Raubenheimer, D., Simpson, S.J. & Mayntz, D. (2009) Nutrition, ecology and nutritional ecology: towards an integrated framework. *Functional Ecology*, **23**, 4–16.
- Sander, P.M. & Clauss, M. (2008) Sauropod gigantism. *Science*, **322**, 200–201.
- Sander, P.M., Christian, A., Clauss, M., Fechner, R., Gee, C.T., Griebeler, E.-M., Gunga, H.-C., Hummel, J., Mallison, H., Perry, S.F., Preuschoft, H., Rauhut, O.W.M., Remes, K., Tutken, T., Wings, O. & Witzel, U. (2011) Biology of sauropod dinosaurs: the evolution of gigantism. *Biological Reviews*, **86**, 117–155.
- Sherratt, T.N. & Wilkinson, D.M. (2009) *Big Questions in Ecology and Evolution*. Oxford University Press, Oxford.
- Simpson, S.J., Sibly, R.M., Lee, K.P., Behmer, S.T. & Raubenheimer, D. (2004) Optimal foraging when regulating intake of multiple nutrients. *Animal Behaviour*, **68**, 1299–1311.
- Simpson, S.J., Raubenheimer, D., Charleston, M.A. & Clissold, F.J. (2009) Modelling nutritional interactions from individuals to communities. *Trends in Ecology and Evolution*, **25**, 53–60.
- Sinclair, A.R.E., Mduma, S. & Brashares, J.S. (2003) Patterns of predation in a diverse predator-prey community. *Nature*, **425**, 288–290.
- Trammer, J. (2011) Differences in global biomass and energy use between dinosaurs and mammals. *Acta Geologica Polonica*, **61**, 125–132.
- Turner, A. & Antón, M. (2004) *Prehistoric Mammals*. National Geographic, Washington, District of Columbia.
- Tütken, T. (2011) The diet of sauropod dinosaurs: implications of carbon isotope analysis on teeth, bones and plants. *Biology of the Sauropod Dinosaurs: Understanding the Life of Giants* (eds N. Klein, K. Remes, C. T. Gee & P.M. Sander), pp. 57–79. Indiana University Press, Bloomington.
- Wilkinson, D.M., Nisbet, E.G. & Ruxton, G.D. (2012) Could methane produced by sauropod dinosaurs have helped drive Mesozoic climate warmth? *Current Biology*, **22**, R292–R293.

Received 15 June 2012; accepted 17 October 2012

Handling Editor: Charles Fox