Peer Community In Ecology

Is vertebral count in mammals influenced by developmental temperature? A study with Dasypus novemcinctus

Mar Sobral *based on peer reviews by and* ***Darin Croft***

Frank Knight, Cristin Connor, Ramji Venkataramanan, Robert J. Asher (2020) Body temperatures, life history, and skeletal morphology in the nine-banded armadillo (Dasypus novemcinctus). Missing preprint_server, ver. Missing article_version, peer-reviewed and recommended by Peer Community in Ecology. 10.17863/CAM.50971

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Mammals show a very low level of variation in vertebral count, both among and within species, in comparison to other vertebrates [1]. Jordan's rule for fishes states that the vertebral number among species increases with latitude, due to ambient temperatures during development [2]. Temperature has also been shown to influence vertebral count within species in fish [3], amphibians [4], and birds [5]. However, in mammals the count appears to be constrained, on the one hand, by a possible relationship between the development of the skeleton and the proliferations of cell lines with associated costs (neural malformations, cancer etc., [6]), and on the other by the cervical origin of the diaphragm [7]. Knight *et al.* [8] investigate the effect of intrauterine temperature variation on skeletal morphology during development, and focus on a particular mammal, *Dasypus novemcinctus*, or nine-banded armadillo. Armadillos (Xenarthra) and are characterized by relatively low body temperatures and low basal rates of metabolism. *Dasypus novemcinctus* is the only xenarthran mammal to have naturally expanded its range into the middle latitudes of the U.S., and one of the few mammals that invaded North America from South America. It is one of few placentals that withstand considerable decrease of body temperature without torpor. It presents a resting body temperature that is low and variable for a placental mammal of its size [9] and is the only vertebrate that gives birth to monozygotic quadruplets. Among 42 monotreme, marsupial and placental genera, *Dasypus novemcinctus* shows the highest variation of thoracolumbar vertebral count [10]. The particularities of *Dasypus novemcinctus* regarding vertebral count variation and ability to withstand variable temperature qualify it as a target organism for study of the relationship between skeleton morphology and temperature in mammals. Knight *et al.* [8] explored variability in vertebral count within *Dasypus novemcinctus* to understand whether temperature during

development determines skeleton morphology. To this end they experimented with 22 armadillos (19 with data) and litters from 12 pregnant females, in two environments, for three years — an impressive effort and experimental setup. Moreover, they used a wide variety of advanced experimental and analytical techniques. For example, they implanted intra-abdominal, long-term temperature recorders, which recorded data every 6 to 120 minutes for up to several months. They analysed body temperature periodicity by approximation of the recordings with Fourier series, and they CT-scanned fetuses. All 19 individuals (from which data could be gathered) exhibited substantial daily variation in body temperature. Several intriguing results emerged such as the counter-intuitive finding that the mammals' body temperature fluctuates more indoors than outdoors. Furthermore, three females (out of 12) were found to have offspring with atypical skeletons, and two of these mothers presented an extremely low internal temperature early in pregnancy. Additionally, genetically identical quadruplets differed skeletally among themselves within two litters. Results are not yet definitive about the relationship of temperature during development and vertebral count in *Dasypus novemcinctus*. However, Knight et al. [8] demonstrated that nine-banded armadillos survive with high daily internal temperature fluctuations and successfully bring to term offspring which vary in skeletal morphology among and within genetically identical litters despite major temperature extremes.

References:

[1] Hautier L, Weisbecker V, Sánchez-Villagra MR, Goswami A, Asher RJ (2010) Skeletal development in sloths and the evolution of mammalian vertebral patterning. Proceedings of the National Academy of Sciences, 107, 18903–18908. doi: [10.1073/pnas.1010335107](https://dx.doi.org/10.1073/pnas.1010335107)

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[8] Knight F, Connor C, Venkataramanan R, Asher RJ. (2020). Body temperatures, life history, and skeletal morphology in the nine-banded armadillo (*Dasypus novemcinctus*). PCI-Ecology. doi: [10.17863/CAM.50971](https://dx.doi.org/10.17863/CAM.50971)

[9] McNab BK (1980) Energetics and the limits to a temperate distribution in armadillos. Journal of Mammalogy, 61, 606–627. doi: [10.2307/1380307](https://dx.doi.org/10.2307/1380307)

[10] Asher RJ, Lin KH, Kardjilov N, Hautier L (2011) Variability and constraint in the mammalian vertebral column. Journal of Evolutionary Biology, 24, 1080–1090. doi: [10.1111/j.1420-9101.2011.02240.x](https://dx.doi.org/10.1111/j.1420-9101.2011.02240.x)

Reviews

Evaluation round #2

DOI or URL of the preprint: https://doi.org/10.17863/CAM.50971
Version of the preprint: rev1

Authors' reply, 11 March 2020

10 March 2020

Dear Dr Sobral,

Many thanks for your time in evaluating our manuscript "Body temperatures, life history, and skeletal morphology in the nine-banded armadillo (Dasypus novemcinctus)" for peer-review in PCI-Ecology. Following our revised manuscript which addressed the reviewer and editor (your) concerns in the first revision (dated 22 Jan 2020 and available at https://osf.io/dpw69/), we have now revised the manuscript to account for your comments on the second revision, available here: https://osf.io/693kq/

The main text and figures are in the file "knight-asher*rev2.pdf". The changes since the previous version are shown in "knight-asher-postReview-trackChanges.doc". Our responses to your recommendations are shown in the file "knight-asher-reviewResponse.doc" in bold. Our supplementary data are in three appendices ("appendS1, S2, S3") and one .doc file ("knight-asher*supplementaryTables.doc").

We are pleased that the reviews of our original manuscript were so positive, and hope these further edits will enable our manuscript to be formally accepted as a peer-reviewed paper in PCI-Ecology without further delay.

Sincere regards, and on behalf of all coauthors,

Robert J. Asher Department of Zoology University of Cambridge, UK

Decision by Mar Sobral, posted 13 February 2020

Thanks for you interesting preprint

Dear Professor Asher and collaborators

I am definitely interested in recommending your preprint, which I really enjoyed.

However before I do so, I would like you to ask you for three main changes. A conceptual one, a formal one and a technic one. The conceptual change is very important to me.

Firstly, The technical issue which is in my opinion improvable is the use and description of statistical analyses troughput the paper. I believe the paper would be improved if stats are used systematically for all results and not only for some.

Secondly, there are (in my opinion) too many tables and even figures which are not necessary in the main body of the manuscript, please remove those which are not necessary to understand the message to suplemmmentary materials so that the manuscript bcames easier to digest and more atractive. Also try to cut a bit on superfluos text whenever possible.

Thirdly, the conceptual change; I completely agree with you in that (as you explained to one of the referees) the fact that two dams had genetically indentycal quadruplets with polymorphic skeletons is potentially very important. I wonder whether different skeletons have similar rates of survival and reproduction on the first hand and if we dont know this, what can we speculate about it?. On the second, I would like a deeper consideration of this fact troughput the paper. Which could be the evolutionary causes and consequences of this fact? How genetically determined are phenotypes in the wild? What do we know about other mammals regarding phenotypic lability or plasticity? Can we expect different epigenetic processes going on the different offsprings from the same dam? consider if including something about this also in the title.

I hope this is enough information for you to perform a last revision before recommendation.

Best regards

Evaluation round #1

DOI or URL of the preprint: https://osf.io/q8ndp

Authors' reply, 22 January 2020

Download author's reply
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Decision by Mar Sobral, posted 08 January 2020

Thanks for you interesting preprint

Two reviewers and myself have thought the manuscript is clear and interesting.

Some comments have been made by reviewers which are easy to implement and will improve the manuscript.

Please send a revision having into account the comments made by the two reviewers.

Best regards!

Reviewed by ?, 28 December 2019

On the manuscript
«Body temperatures, life history, and skeletal morphology in the nine-banded armadillo»
by Frank Knight, Cristin Connor, Ramji Venkataramanan, Robert J. Asher

Very interesting animal: belongs to one of the earliest-diverged placental lineages, is one of the few mammals that invaded North America from South America, is one of the few placentals withstanding considerable decrease of body temperature without torpor stance, is the only mammal (and not only mammal) which normally gives birth to monozygotic quadruplets.

Very important problem: the body temperature fluctuations during gestation, their inherent periodism, association with ambient temperature fluctuations, gestation. The separate task was checking an ad hoc hypothesis that the gestation temperature influences the vertebral numbers.

Shortcoming of the subject which greatly complicates solution of the problem is delayed implantation The duration of the delay is unknown. Therefore the implantation date is derived from the birth day based on an approximate estimate of 4.5 month gestation. This time is subtracted back from the date of birth which is, in turn, not known outdoors since the birth occurs under ground. However, the authors did all possible precautions in their analysis.

Methods. A wide variety of advanced experimental and analytical techniqus is employed. They include intra-abdominal implantation of long-term temperature recorders, analysis of body temperature periodism by approximation of the recordings with Fourier series, and CT-scanning of fetuses. Results. Pronounced 12 and/or 24 periods in body temperature fluctuations. Counter-intuitive finding that the body temperature

fluctuates in the 9-banded armadillo as if inversely proportional to the ambient temperature — less outdoors than indoors. Gestation is accompanied with a not much pronounced reduction of the range of body temperature fluctuations. BUT... nothing important on the vertebral variation in pups: the variation is inside the range known from the earlier research and does not depend from any factor taken into account — neither from genetic identity, nor from gestation temperature. In general, the manuscript supplies very thoroughly collected, very rare, interesting and important data on temperatures which should be definitely published as a preprint. However, the vertebrologic part is premature. I suggest to remove it from the main text and possibly include the CT results as supplementary material but without any theoretic considerations which look too huge relative to the facts.
If the authors will continue the vertebrology research, I would advice to take into account two sources which they missed:
Jenkins, Farish A., Jr. 1970. Anatomy and Function of Expanded Ribs in Certain Edentates and Primates. Journal of Mammalogy, Voi. 51, No. 2 (May,), pp. 288-301 http://www.jstor.org/stable/1378479?origin=JSTOR-pdf
Kuznetsov, Alexander N. 2007. The Definitive Variation of the Vertebral Column in Rhea americana L.: the Concept of Morphogenesis Precision. (Conference Poster) https://www.researchgate.net/publication/337732771_Precision_of_morphogenesis_concept

Reviewed by Darin Croft, 16 December 2019

This is a clearly-written, well-crafted, hypothesis-driven manuscript. With the caveat that my expertise lies in anatomy rather than physiology, I found no major issues with the manuscript. The experimental design and execution strike me as reasonable, and the authors are forthright about the potential shortcomings in their data and interpretations. The result is new information that is of clear value to the scientific community.

The only minor issue I found is one that can easily be resolved: information should be provided in the methods section about how different vertebral categories are recognized. Perhaps the criteria of Asher et al. (2011) were used; if so, that should be stated explicitly. I suspect some modification of those methods was used, as the counts of Asher et al. (2011) did not deal with “half vertebrate” (i.e., those with single ribs).

The only typographical modification to the manuscript I would suggest is clarifying that the values listed on line 352 represent hours.

There are a few typos in the figure captions. In Figure 1,. D. novemcinctus should be italicized (though this may be an artifact of how captions are uploaded) and scalebars should be two words (this also applies to Figures 9-10). Also in Figure 10, the initial word should be capitalized.