

Reporting Summary

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Statistical parameters

When statistical analyses are reported, confirm that the following items are present in the relevant location (e.g. figure legend, table legend, main text, or Methods section).

n/a Confirmed

- ☐ ☒ The exact sample size (*n*) for each experimental group/condition, given as a discrete number and unit of measurement
- ☐ ☒ An indication of whether measurements were taken from distinct samples or whether the same sample was measured repeatedly
- ☐ ☒ The statistical test(s) used AND whether they are one- or two-sided
Only common tests should be described solely by name; describe more complex techniques in the Methods section.
- ☐ ☒ A description of all covariates tested
- ☐ ☒ A description of any assumptions or corrections, such as tests of normality and adjustment for multiple comparisons
- ☒ ☐ A full description of the statistics including central tendency (e.g. means) or other basic estimates (e.g. regression coefficient) AND variation (e.g. standard deviation) or associated estimates of uncertainty (e.g. confidence intervals)
- ☒ ☐ For null hypothesis testing, the test statistic (e.g. *F*, *t*, *r*) with confidence intervals, effect sizes, degrees of freedom and *P* value noted
Give P values as exact values whenever suitable.
- ☒ ☐ For Bayesian analysis, information on the choice of priors and Markov chain Monte Carlo settings
- ☐ ☒ For hierarchical and complex designs, identification of the appropriate level for tests and full reporting of outcomes
- ☒ ☐ Estimates of effect sizes (e.g. Cohen's *d*, Pearson's *r*), indicating how they were calculated
- ☒ ☐ Clearly defined error bars
State explicitly what error bars represent (e.g. SD, SE, CI)

Our web collection on [statistics for biologists](#) may be useful.

Software and code

Policy information about [availability of computer code](#)

Data collection

Raman spectroscopic data were collected using LabSpec 5 software. Sample photographs were obtained using Leica LAS Core microscopic software.

Data analysis

Raman spectra were baselined and normalized in SpectraGryph 1.2, exported into Microsoft Excel and replotted. A Principal Component Analysis based on Raman data was performed in PAST 3. An ancestral state reconstruction based on a manually re-drawn composite phylogeny (pruned tree) including the *n*=19 biologically independent eggshell samples was performed in Mesquite 3.40. Compound figures were created in Photoshop CS5.

For manuscripts utilizing custom algorithms or software that are central to the research but not yet described in published literature, software must be made available to editors/reviewers upon request. We strongly encourage code deposition in a community repository (e.g. GitHub). See the Nature Research [guidelines for submitting code & software](#) for further information.

Data

Policy information about [availability of data](#)

All manuscripts must include a [data availability statement](#). This statement should provide the following information, where applicable:

- Accession codes, unique identifiers, or web links for publicly available datasets
- A list of figures that have associated raw data
- A description of any restrictions on data availability

The authors declare that all Raman data supporting the findings of this study are available within the paper (Fig. 2: pigment maps and depth profiles) and its supplementary information files (Source data for Fig. 1, Extended data Figs. 1, 2, 3, 6: Raman raw data). Additional PAST 3 files represent the raw data for the Extended data Figs. 4, and 5.

Field-specific reporting

Please select the best fit for your research. If you are not sure, read the appropriate sections before making your selection.

☐ Life sciences ☐ Behavioural & social sciences ☒ Ecological, evolutionary & environmental sciences

For a reference copy of the document with all sections, see nature.com/authors/policies/ReportingSummary-flat.pdf

Ecological, evolutionary & environmental sciences study design

All studies must disclose on these points even when the disclosure is negative.

Study description

Our study investigates the evolution of egg colours in non-avian dinosaurs: Using overall 19 eggshell samples (including Alligator, non-avian dinosaur eggshell specimens, extinct and modern birds), we present the first phylogenetic assessment of non-avian dinosaur egg colour. Applying high-resolution Raman microspectroscopy point measurements to eggshells that represent all the major clades of dinosaurs, and the sediment control samples, we tested for preservation and endogeneity of the eggshell pigments biliverdin and protoporphyrin. Every sample was surface cleaned, and run at 6 accumulations over 20 s exposure time. Pigment samples were blanked against other organic compounds that commonly preserve in fossil eggshells. We found egg colour pigments preserved in all eumaniraptoran eggshells (except from a North American Troodontid), but none in the sediment control samples. Raman pigment surface maps (2 accumulations, 5 s exposure) revealed that the coloured eggs were spotted and speckled. Raman pigment depth profiles demonstrated an identical mechanism of pigment deposition in pigmented nonavian and avian dinosaur eggs. Absence of colour in ornithischian and sauropod eggs represents a true signal rather than a taphonomic artefact. An ancestral state reconstruction using the obtained pigment data on a pruned tree that includes all sampled taxa shows that egg colour had a single evolutionary origin in non-avian theropod dinosaurs. The diversity in colour and pattern of non-avian eumaniraptoran eggshells challenges that known from modern birds, indicating far more complex reproductive behaviours in non-avian dinosaurs than previously known. Even though long thought, crown birds did not evolve egg colours, but inherited them from their nonavian dinosaur ancestors.

Research sample

A total of nineteen eggshell specimens and twelve adhering sediment samples were included in the eggshell pigment Raman point measurements. All samples represent museum specimens: Alligator mississippiensis eggshell (YPM uncat), Maiasaura peeblesorum eggshell (YPM VP PU 023464) and (YPM PU 22523), Saltasaurid eggshell (STIPB E360), Hypselosaurus priscus eggshell (YPM PU 21158), Heyuannia huangi eggshell (NMNS CYN-2004-DINO-05), Microtroodontid eggshell (IGM 11/1323) and (MAE 14-40), Troodontid eggshell (YPM VP PU 023259), and (PFMM-0014003517), and (FARB 6631), and (IGM 100/1003), Deinonychus antirrhopus eggshell (AMNH 3015), Enantiornithine eggshell (IGM 100/1339), Psammornis rothschildi eggshell (ZFMK 23a:III/c/23), Rhea americana eggshell (YPM ORN 24444), Ornithoid ratite eggshells (YPM PU 22594), Dromaius novaehollandiae eggshells (YPM ORN 24447), and Gallus domesticus eggshells (YPM ORN 23146). All of these specimens are housed either at the Yale Peabody Museum, at the American Museum of Natural History, at the Steinmann Institute Bonn, or the Zoological Research Museum Koenig Bonn. One additional tern eggshell was used to calibrate the Raman mapping routine (YPM uncat), shown in Fig. S2. Further five egg specimens from the YPM ornithological collection were photographed to illustrate pigment color changes through time. These eggs represent the taxa Dromaius novaehollandiae and Casuarius casuarius (YPM 1994, YPM 133667, YPM 141992, YPM 141998, YPM 142001, YPM 142002) and are shown in Fig. 2c.

Sampling strategy

No sample size calculation was performed. The nineteen eggshell samples, and the twelve sediment samples were chosen based on available coverage of the cladogram. We generally sampled one specimen per genus, except from Maiasaura which is covered by two biologically independent eggshell samples per genus. The sampled genera offer multiple representatives for every major branch of nonavian dinosaurs and allow to draw reliable conclusions about general evolutionary trends in nonavian dinosaur egg color evolution: Two samples cover ornithischian dinosaurs, two additional samples cover sauropod dinosaurs, nine samples cover nonavian eumaniraptoran dinosaurs, and five samples cover modern birds. Eggshells of Maiasaura represent the only ornithischian eggshells which are taxonomically assigned based on embryonic remains in their eggs. Within sauropods we sampled based on specimen availability. Since previous research suggests egg color in oviraptorids, we compiled a comprehensive theropod eggshell sample. We cover one oviraptorid, two microtroodontids, four troodontids, the only discovered deinonychosaurian eggshell, and one enantiornithine eggshell, as well as two extinct ratites, and two modern birds. Our samples offer also multiple representatives per "taphonomic environment", to rule out false negative, taphonomy-based pigment assignments. Six samples cover limestone host rocks (Maiasaura (n=2), South American Saltasaur, North American Troodontid, Deinonychus, ornithoid ratite eggshell), while other nine samples cover sand/siltstones (French Titanosaur, Heyuannia, Microtroodontids (n=2), Troodontids (n=3), Enantiornithine, Psammornis). Based on the sampling of comparable taphonomic environments, qualitative conclusions of pigmentation are backed up against potential taphonomic biases: Both host rock types cover eggshells with and without eggshell pigments, what suggests that

pigments can survive in both taphonomic environments, and corroborates that eggshells without evidence for pigmentation were originally unpigmented, and did not lose their pigments through taphonomic processes.

Data collection

Mark Norell and Tzu-Ruei Yang provided all eggshell and sediment specimens which are not part of the Yale Peabody collection (YPM). Jasmina Wiemann cleaned all eggshells with ethanol prior to any measurements. She also imaged all specimens and sediments (Leica MZ16 dissecting microscope with Optronics camera, LAS Core Software), and performed the Raman spectroscopy high-resolution point measurements, pigment surface maps, and depth profiles (recorded using LabSpec 5 and a Horiba LabRam 800) at Yale University. Jasmina Wiemann baselined and normalized all acquired spectra in SpectraGryph 1.2. Tzu-Ruei Yang corroborated Raman-based findings for some of the samples at the Steinmann Institute. Jasmina Wiemann, Tzu-Ruei Yang, and Mark Norell evaluated the data, and Jasmina Wiemann performed the statistical analyses in PAST 3, and the ancestral state reconstruction based on a manually re-drawn composite phylogeny including all nineteen sampled taxa in Mesquite 3.40. Jasmina Wiemann created the composite figures in Photoshop CS5. All authors interpreted the findings and wrote the manuscript.

Timing and spatial scale

Our eggshell and sediment museum specimens cover localities from all over the world (please see Supplementary Table 2 for details). The sample set was built from 2015 to 2017. All samples were imaged, and measured consecutively, from August 2017 to January 2018. Since all investigated specimens are accessioned in museums, not active sample collection was conducted in this study.

Data exclusions

All Raman point measurements for the total of 31 sample specimens are included in the manuscript and its Supplementary Information. Nineteen eggshell samples were included in the Raman point measurements. From this sample pool 8 samples fulfilled the criteria of an exposed, smooth surface area, and a substantial protoporphyrin pigment signal, necessary for eggshell pigment surface mapping (spots and speckles are based only on protoporphyrin). All of these samples were included into the eggshell pigment depth profiles, in addition to one unpigmented Alligator sample as control sample. All eggshell and sediment samples are included in the Principal Component Analysis shown in Extended data Fig. 4a, while only fossil eggshells are included in the second Principal Components Analysis shown in Extended data Fig. 4b. Reasoning for the exclusion of extant and "subfossil" eggshells here is the reduction of taphonomic noise in the chemospace to extract the pigment signal.

Reproducibility

Every Raman measurement presented in the main text Figs. and the Extended data Figs., point/map/depth profile, was repeated three times (technical replicates) independently and yielded similar, if not identical results. None of the sediment samples showed evidence for eggshell pigments, while all tested eumamiraptoran eggshells, except from the North American Troodontid, yielded evidence for pigmentation. All attempts at replication of these results were successful.

Randomization

Randomization cannot be applied to this study, because Raman spectra are characteristic for every sample, and can be taxonomically assigned at a glance based on their unique and distinctive taphonomic and compositional signatures. Also, we do not use any statistics that would require randomization. Samples were grouped together based on their identity (eggshell versus sediment), and their age (extant versus fossil). The Principal Component Analysis shown in the Extended data Fig. 4b suggests that fossil eggshells group as unpigmented eggshells, and pigmented eggshells.

Blinding

Blinding is not relevant to this study, because Raman spectra are characteristic for every sample, and can be taxonomically assigned at a glance based on their unique and distinctive taphonomic and compositional signatures. However, all eggshell and sediment spectra were analysed "blind" without any preliminary expectation towards the analytical outcome of pigment detection.

Did the study involve field work? ☐ Yes ☒ No

Reporting for specific materials, systems and methods

Materials & experimental systems

- n/a ☐ Involved in the study
- ☒ ☐ Unique biological materials
- ☒ ☐ Antibodies
- ☒ ☐ Eukaryotic cell lines
- ☐ ☒ Palaeontology
- ☒ ☐ Animals and other organisms
- ☒ ☐ Human research participants

Methods

- n/a ☐ Involved in the study
- ☒ ☐ ChIP-seq
- ☒ ☐ Flow cytometry
- ☒ ☐ MRI-based neuroimaging

Palaeontology

Specimen provenance

Alligator (extant, FL, US), Maiasaura, North American Troodontid, ornithoid ratite egg (Cretaceous, MT, US), Saltasaurid (Cretaceous, Argentina), French titanosaur (Cretaceous, France), Heyuannia and Chinese Troodontid (Cretaceous, China), Microtroodontids, Mongolian Troodontids, Enantiornithine (Cretaceous, Mongolia), Deinonychus (Cretaceous, MT, US), Psammornis (Quaternary, Algeria), Rhea and Dromaius (extant, MT, US), Gallus (extant, CT, US).

Specimen deposition

All unique fossil materials presented in our study are stored in the collections of the Yale Peabody Museum, the American Museum of Natural History, the Steinmann Institute for Paleontology, and the Zoological Research Museum Koenig, and are readily available for examination. Please see "Research sample" in this Reporting Summary for the catalogue numbers.

Dating methods

No new dates are provided.

☒ Tick this box to confirm that the raw and calibrated dates are available in the paper or in Supplementary Information.