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Guinea fowl eggshell structural organization and particular organic matrix protein patterns to decipher its exceptional biomechanical properties

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► To cite this version:

Joël Gautron. Guinea fowl eggshell structural organization and particular organic matrix protein patterns to decipher its exceptional biomechanical properties. World Poultry Conference, WG4 Symposium, WPSA French Branch, Nov 2021, Digital, France. <hal-04217230>

HAL Id: hal-04217230

<https://hal.inrae.fr/hal-04217230v1>

Submitted on 25 Sep 2023

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GUINEA FOWL EGG SHELL STRUCTURAL ORGANIZATION AND PARTICULAR ORGANIC MATRIX PROTEIN PATTERNS TO DECIPHER ITS EXCEPTIONAL BIOMECHANICAL PROPERTIES



Joël Gautron - project lead (INRA)

Nathalie Le Roy - post-doctoral researcher (INRA)

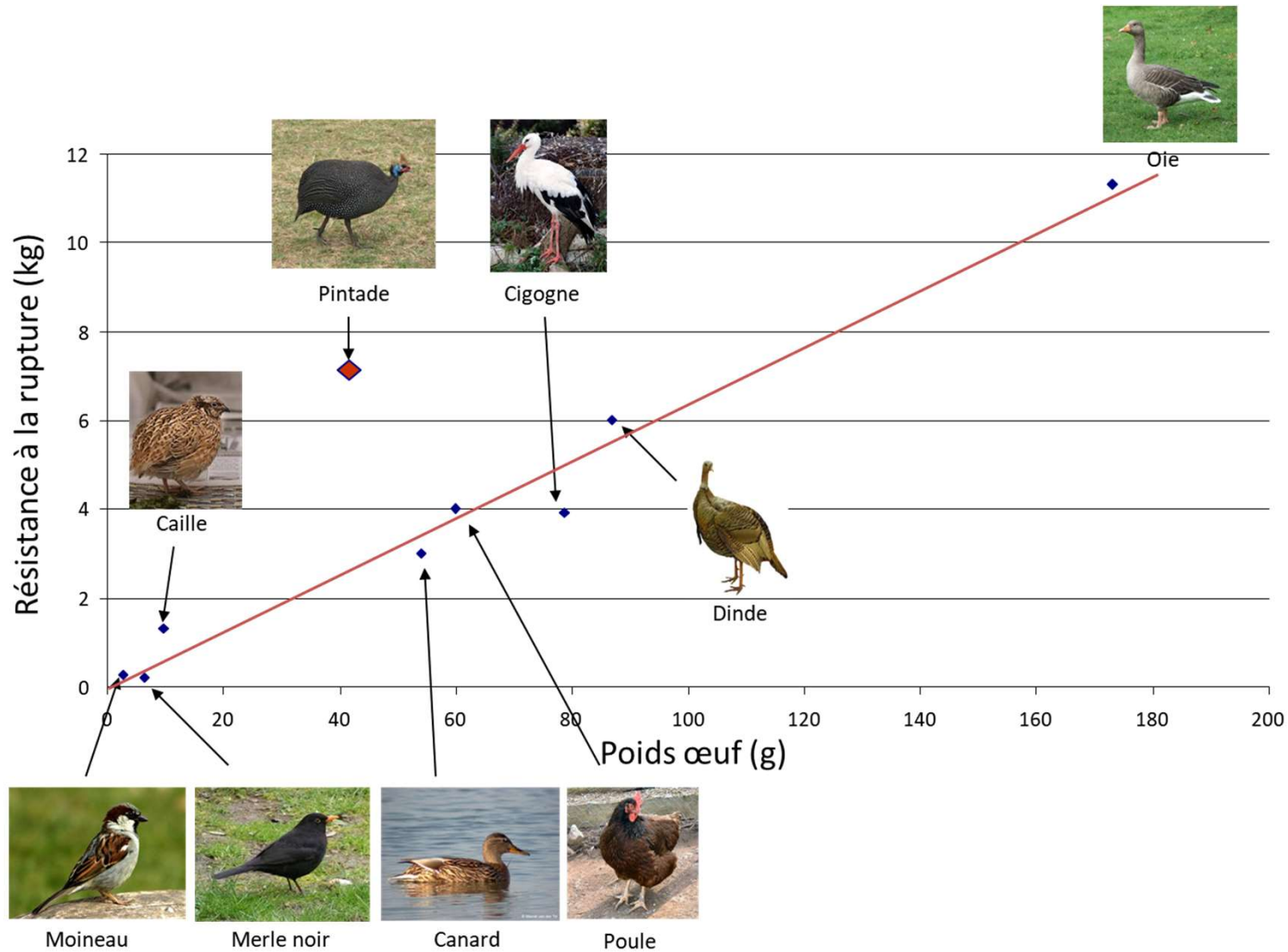
Aurélien Brionne - bioinformatics support (INRA)

Valérie Labas and Lucie Combes-Soia - proteomic platform PAIB2 (INRA)

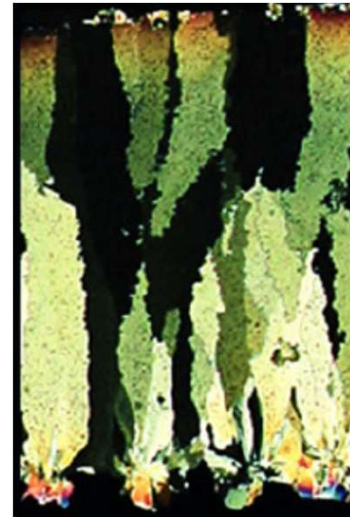
Alejandro B. Rodriguez-Navarro- Microstructure of the shell (University Granada, Spain)

Max Hincke (University of Ottawa, Canada)

The exceptional mechanical properties of Guinea fowl eggshells



The exceptional mechanical properties of Guinea fowl eggshells



Gallus gallus



Meleagris gallopavo

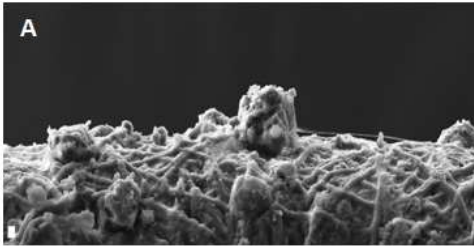


Sankofa pyrenaica



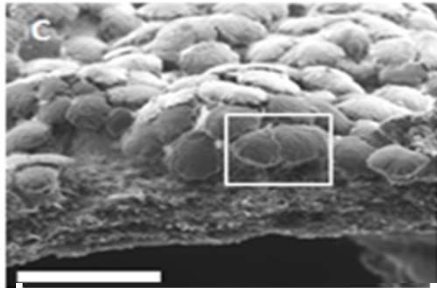
Numida meleagris

The outer layer, comprising two thirds of the eggshell thickness, has a more complex microstructural arrangement formed by smaller calcite microcrystals with diffuse/interlocking boundaries



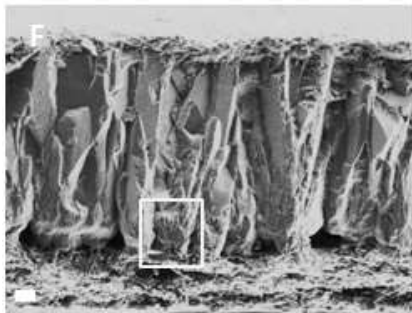
4 hours post ovulation :

Onset of mineralisation with deposition of « seeding sites »



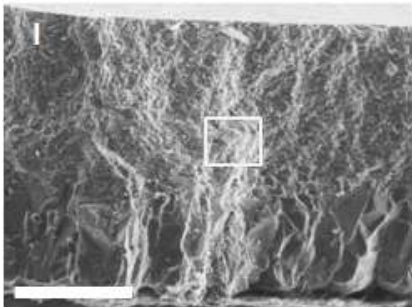
5 Hours P.O.:

One hour later, the eggshell membrane is almost fully covered by semi-spherical aggregates of calcite crystals



11 hours P.O.:

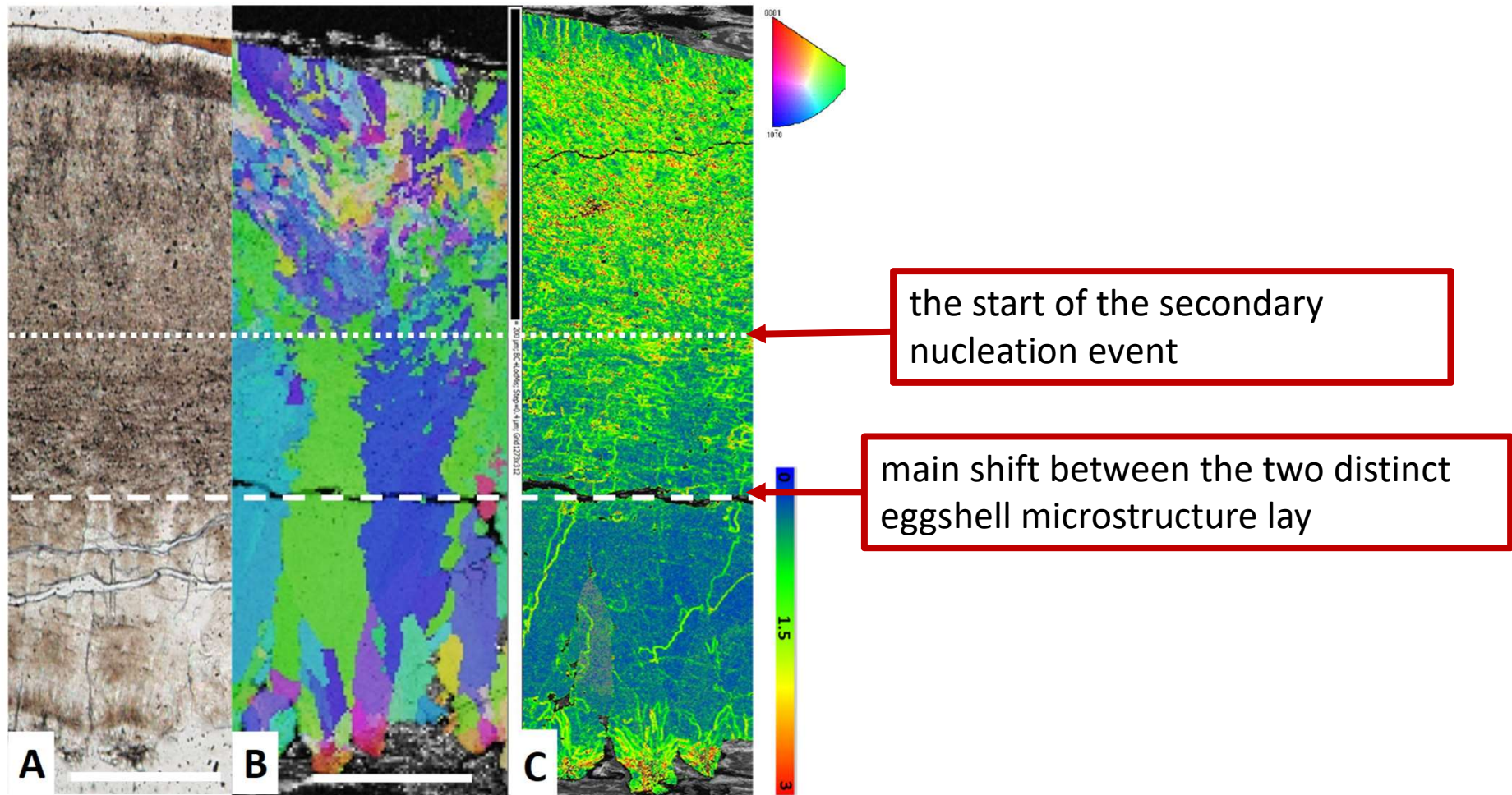
Cross-section view of an eggshell collected at 11 hrs p.o., showing a sharp microstructure change from large columnar calcite crystal units to calcite microcrystals



24 hours P.O.:

A fully formed eggshell with the lower part formed by large columnar units (lower part) and the top part formed by smaller calcite crystal units arranged with a brick-wall microstructure

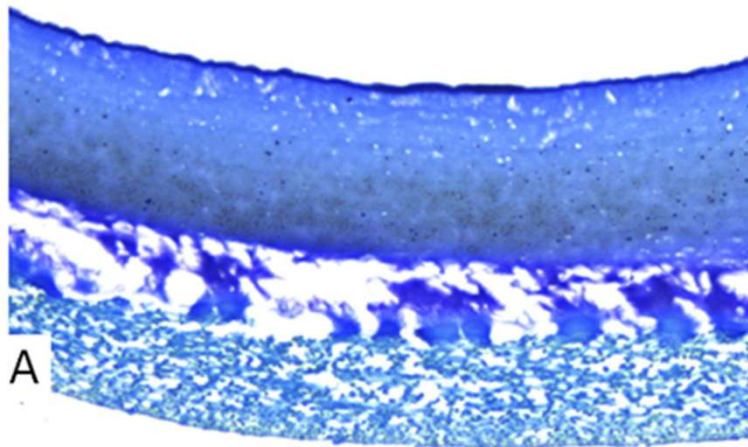
Particularities of the Guinea fowl eggshell ultrastructure



A) Optical microscopy view of the cross-section of a fully formed eggshell showing the non-homogenous distribution of occluded organic matter. B-C) EBSD crystal orientation and local misorientation maps of the eggshell cross-section showing the constituting calcite crystal units

The exceptional mechanical properties of Guinea fowl eggshells

- The Guinea fowl eggshell is a bilayer microstructure
- The shift in eggshell fabric (texture) between these layers is accompanied by changes in the distribution and amount of intra-crystalline organic matter



Second layer

First crystalline layer

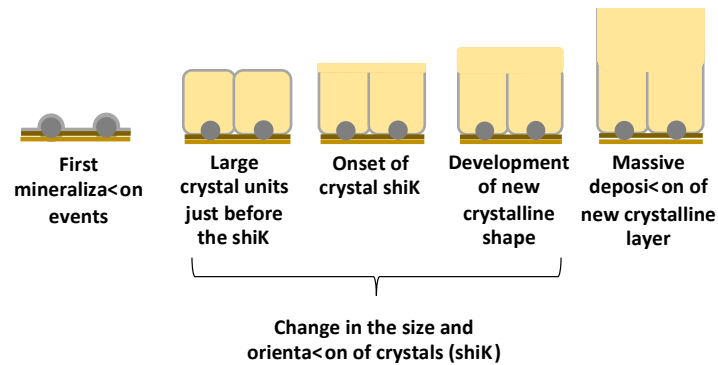
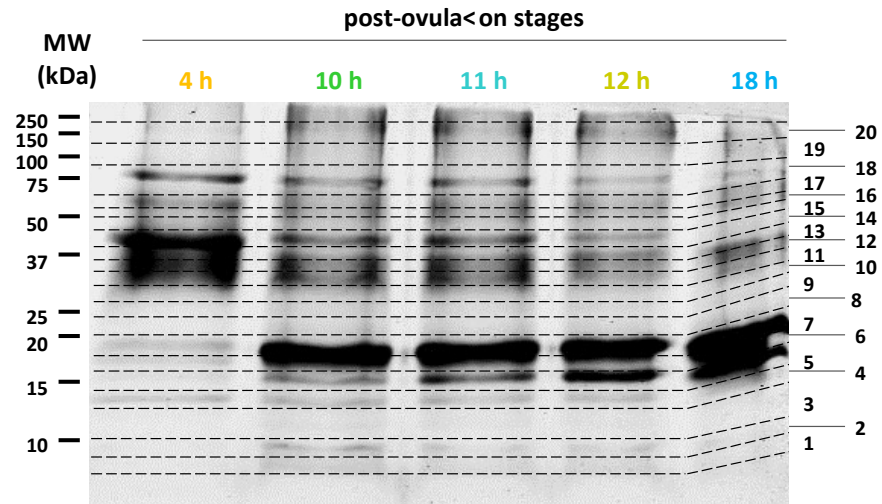
Shell membranes

The organic matrix is predicted to firstly induce the initial structural shift between these layers, followed by a secondary nucleation event involving smaller crystals with increasing misorientation.

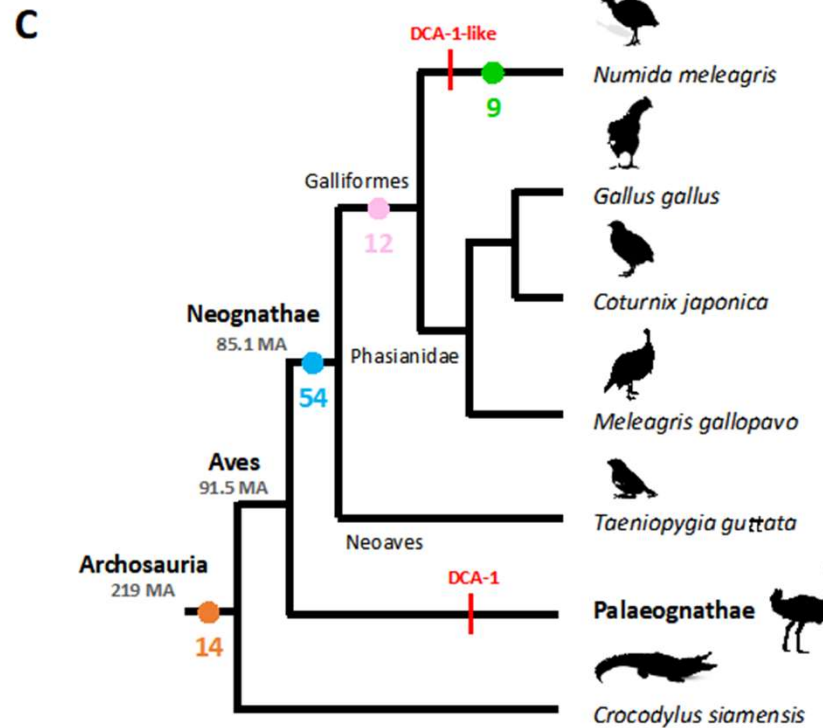
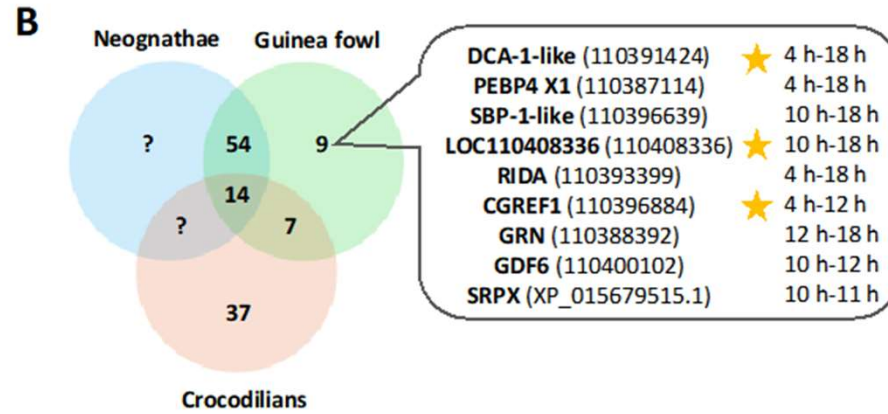
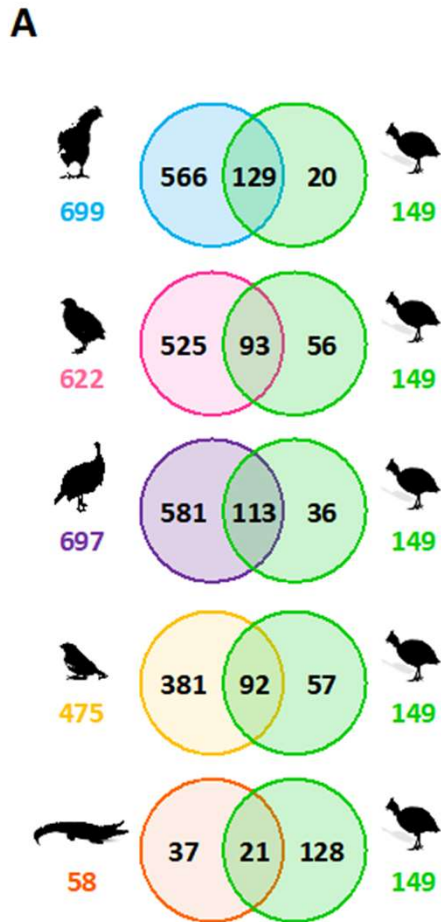


This particular organization is responsible for the exceptional mechanical properties of Guinea fowl eggshell by comparison to that of other birds.

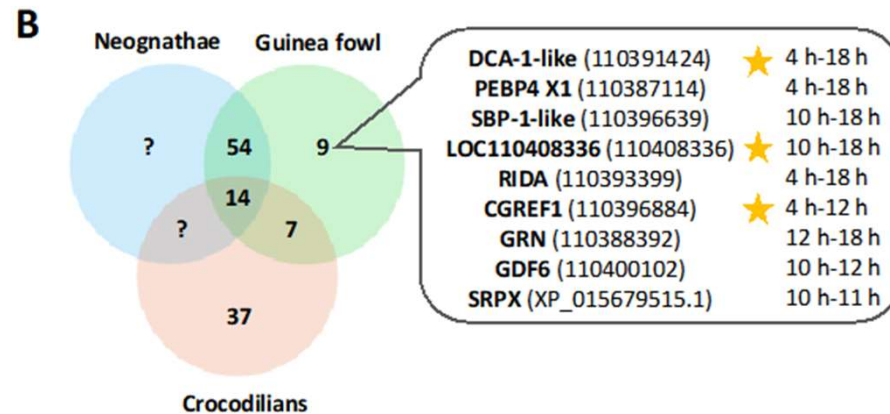
The exceptional mechanical properties of Guinea fowl eggshells



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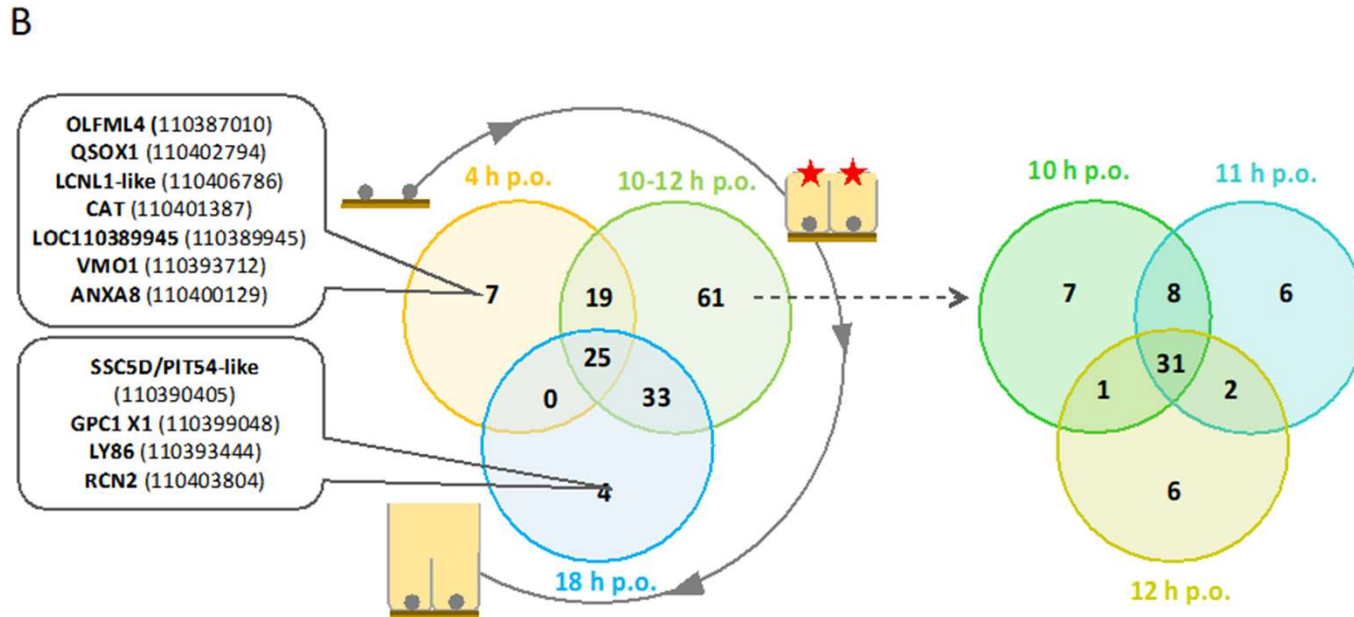
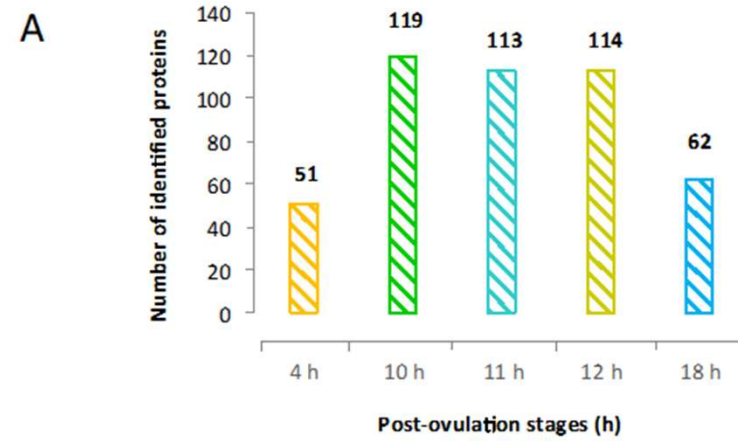


The exceptional mechanical properties of Guinea fowl eggshells



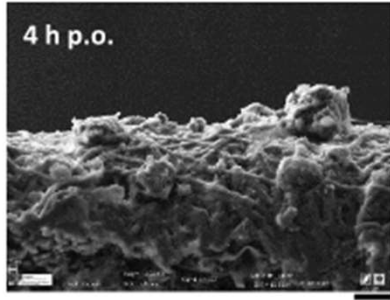
| Identified Proteins (9) | Protein short name | Accession N° (NCBI) | Gene ID | MW (kDa) | Mean WS | | | | |
|---|---------------------|---------------------|-----------|----------|---------|--------|--------|--------|--------|
| | | | | | 4 h | 10 h | 11 h | 12 h | 18 h |
| dromaiocalcin-1-like | DCA-1-like | XP_021238988.1 | 110391424 | 21 | 354.6 | 2854.7 | 1656.0 | 2860.0 | 3604.5 |
| phosphatidylethanolamine-binding protein 4 isoform X1 | PEBP4 X1 | XP_021230598.1 | 110387114 | 31 | 2.4 | 29.9 | 33.1 | 27.9 | 21.8 |
| small basic protein 1-like | SBP-1-like | XP_021248101.1 | 110396639 | 7 | 0.0 | 2.6 | 3.4 | 2.4 | 5.1 |
| uncharacterized protein At5g39570-like | LOC110408336 | XP_021272630.1 | 110408336 | 30 | 0.5 | 1.2 | 2.0 | 5.1 | 12.2 |
| ribonuclease UK114 | RIDA | XP_021241925.1 | 110393399 | 14 | 3.5 | 26.6 | 13.8 | 12.6 | 1.4 |
| cell growth regulator with EF-hand domain protein 1 | CGREF1 | XP_021248455.1 | 110396884 | 26 | 10.6 | 5.0 | 0.6 | 1.3 | 0.0 |
| Granulin precursor | GRN | XP_021233282.1 | 110388392 | 29 | 0.0 | 1.8 | 3.1 | 4.5 | 8.8 |
| Growth/differentiation factor 6 | GDF6 | OWK61448.1 | 110400102 | 43 | 0.0 | 7.7 | 6.4 | 6.2 | 0.0 |
| sushi repeat-containing protein SRPX isoform X1 | SRPX | XP_015679515.1 | | 51 | 0.0 | 2.0 | 3.1 | 0.6 | 0.0 |

The exceptional mechanical properties of Guinea fowl eggshells



The exceptional mechanical properties of Guinea fowl eggshells

First mineralization events



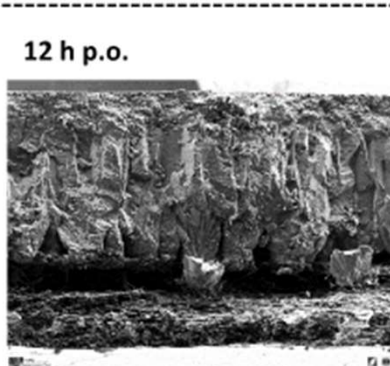
10 μm



4 h p.o.

OVALX2, **ExFAB**, **OVM**, **OVOT**, **HPX**, **LYZ C**, **DCA-1-like**, **OC-17-like**, **OVALY**, **HAPLN3**, **AVD-like**, **AGP-like**, **CST3**, **HBAAs**, **CLU**

Change in the size and orientation of crystals (shift)



10 μm



10 h p.o.

DCA-1-like, **ExFAB**, **OC-17-like**, **OVALX2**, **LYZ C**, **OVM**, **OC-116-like/MEPE-like**, **OVOT**, **OCX-32-like/RARRES1-like**, **AGP-like**, **EDIL3**, **HPX**, **AVD-like**, **CST3**, **CLU**



11 h p.o.

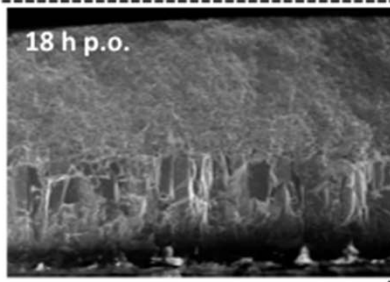
DCA-1-like, **OC-17-like**, **OVALX2**, **OC-116-like/MEPE-like**, **OVM**, **ExFAB**, **LYZ C**, **OVOT**, **OCX-32-like/RARRES1-like**, **AVD-like**, **AGP-like**, **EDIL3**, **CLU**, **HPX**, **CST3**



12 h p.o.

DCA-1-like, **OC-17-like**, **ExFAB**, **OC-116-like/MEPE-like**, **OVM**, **LYZ C**, **OVALX2**, **OVOT**, **CLU**, **OCX-32-like/RARRES1-like**, **ALB**, **EDIL3**, **CST3**, **AGP-like**, **HPX**

Massive deposition of new crystalline layer



20 μm



18 h p.o.

DCA-1-like, **OC-17-like**, **ExFAB**, **OC-116-like/MEPE-like**, **LYZ C**, **OVOT**, **Meleagrins-like**, **ALB**, **SBP-1-like**, **CST3**, **CLU**, **B2M**, **OVAL X2**, **OVM**, **AGP-like**

- NPNT** ★
- CALB1** ★
- ANXA2** ★
- S100-A6** ★
- ANXA1** ★
- LOC104050214**
- TSKU**
- SERPINF1**
- PTN**
- PTPRS**
- VTN**
- FSTL1**