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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)

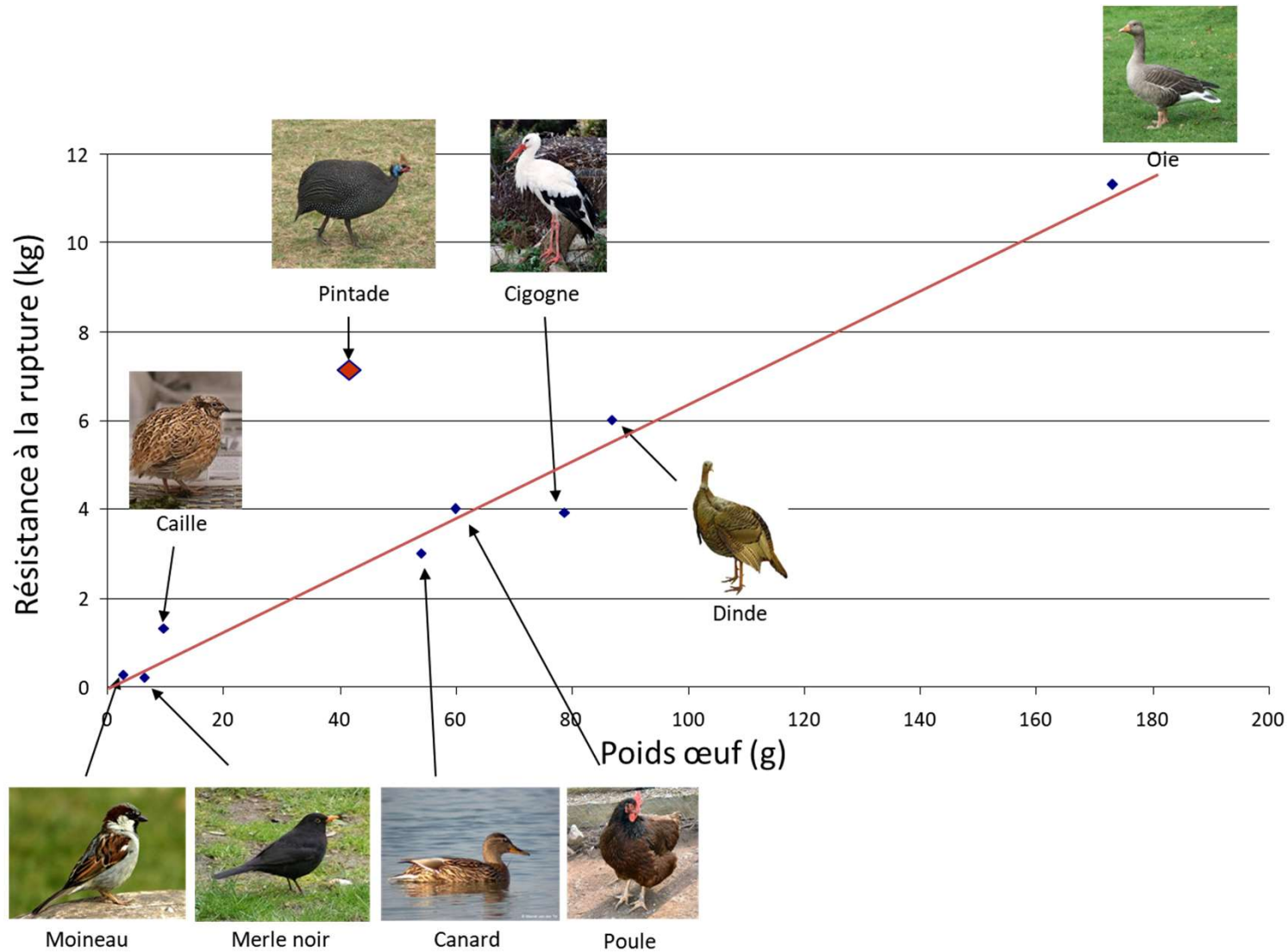
Valérie Labas and Lucie Combes-Soia - proteomic platform PAIB2 (INRA)

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



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Gallus gallus



Meleagris gallopavo

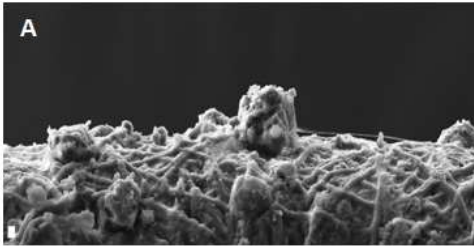


Sankofa pyrenaica



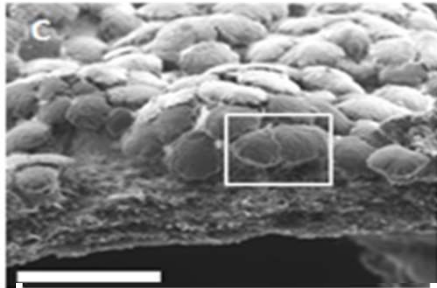
Numida meleagris

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



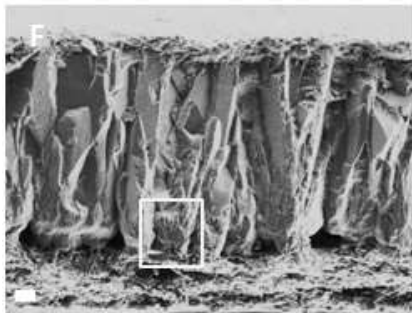
4 hours post ovulation :

Onset of mineralisation with deposition of « seeding sites »



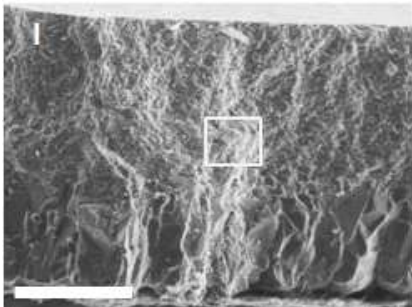
5 Hours P.O.:

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



11 hours P.O.:

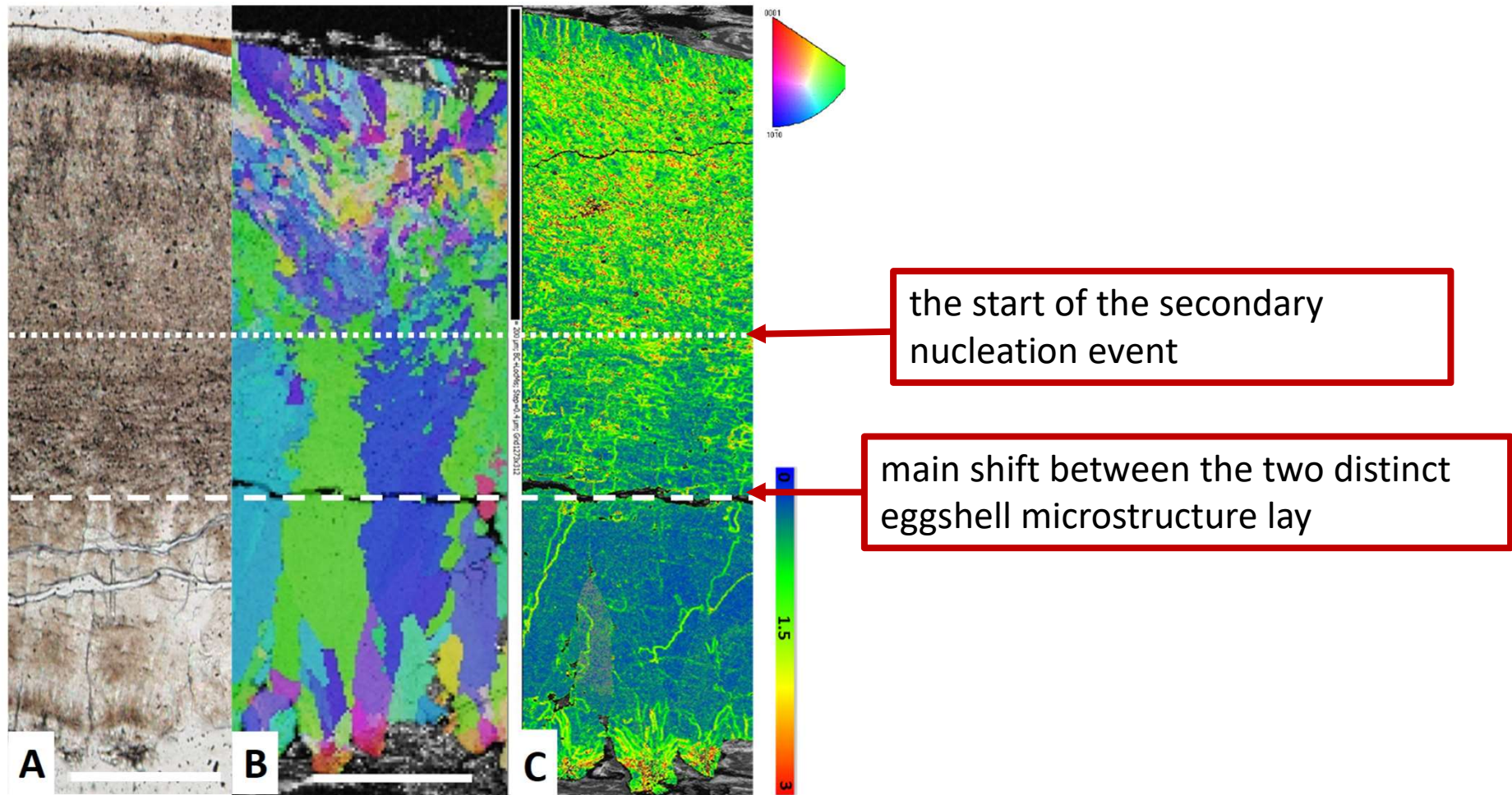
Cross-section view of an eggshell collected at 11 hrs p.o., showing a sharp microstructure change from large columnar calcite crystal units to calcite microcrystals



24 hours P.O.:

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

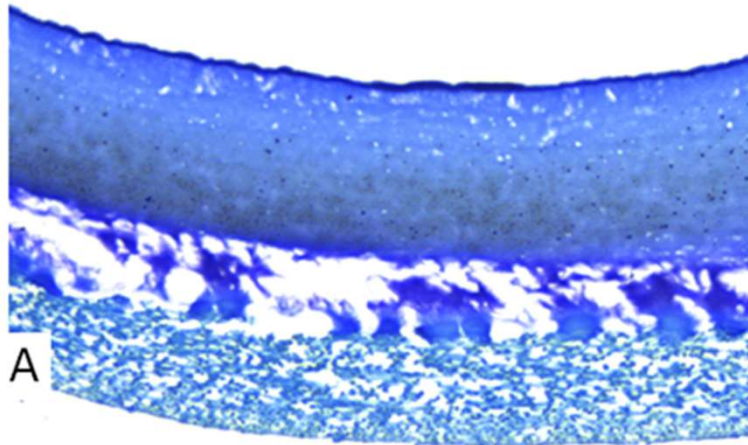
Particularities of the Guinea fowl eggshell ultrastructure



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

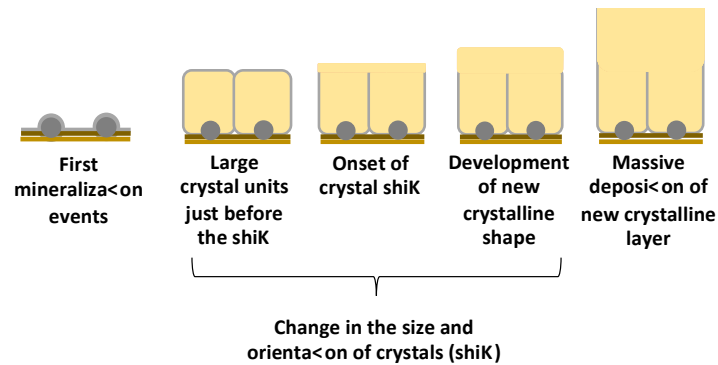
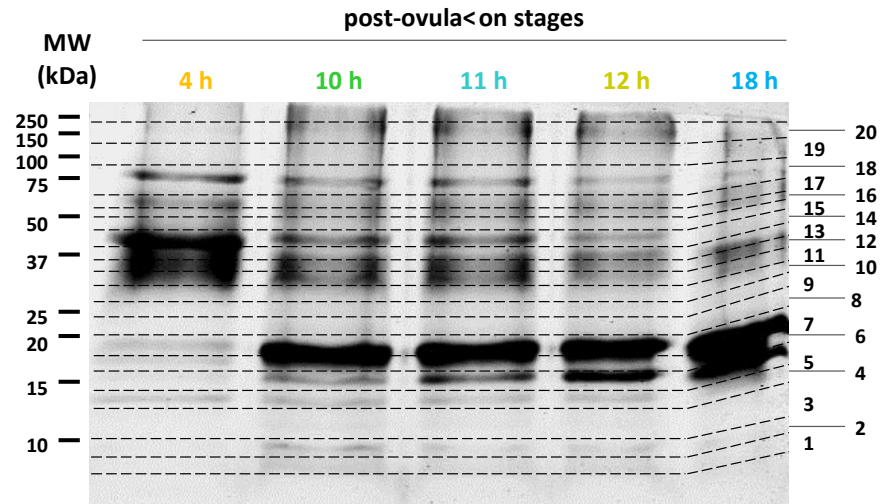
Shell membranes

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.

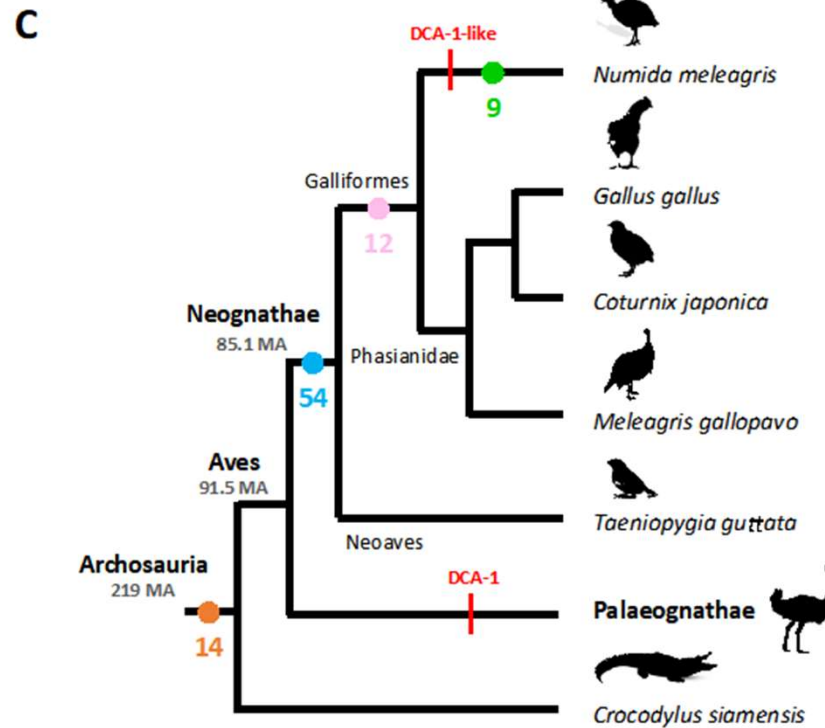
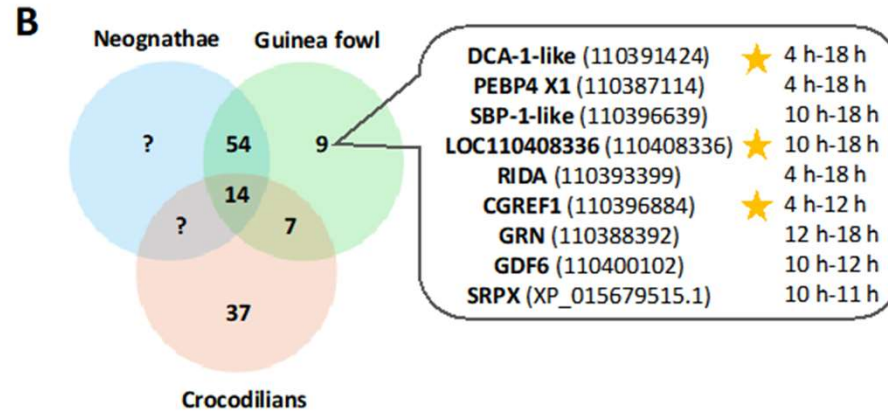
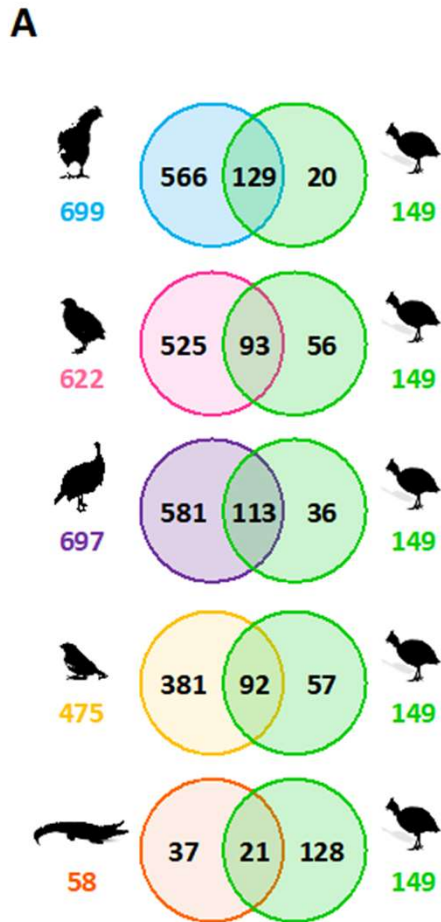


This particular organization is responsible for the exceptional mechanical properties of Guinea fowl eggshell by comparison to that of other birds.

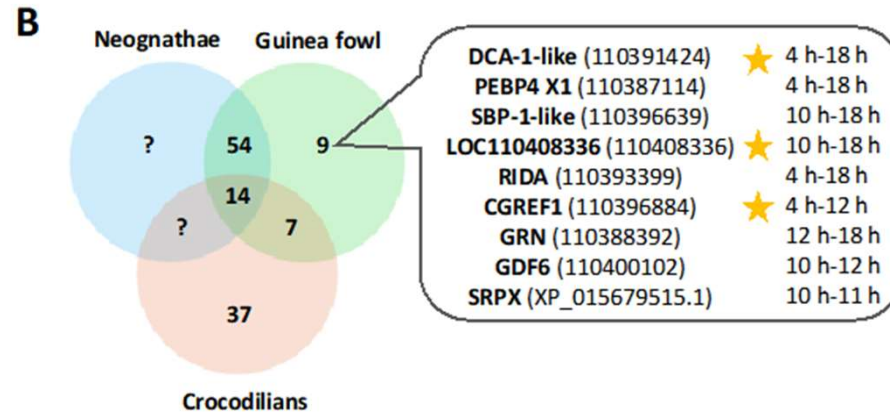
The exceptional mechanical properties of Guinea fowl eggshells



The exceptional mechanical properties of Guinea fowl eggshells

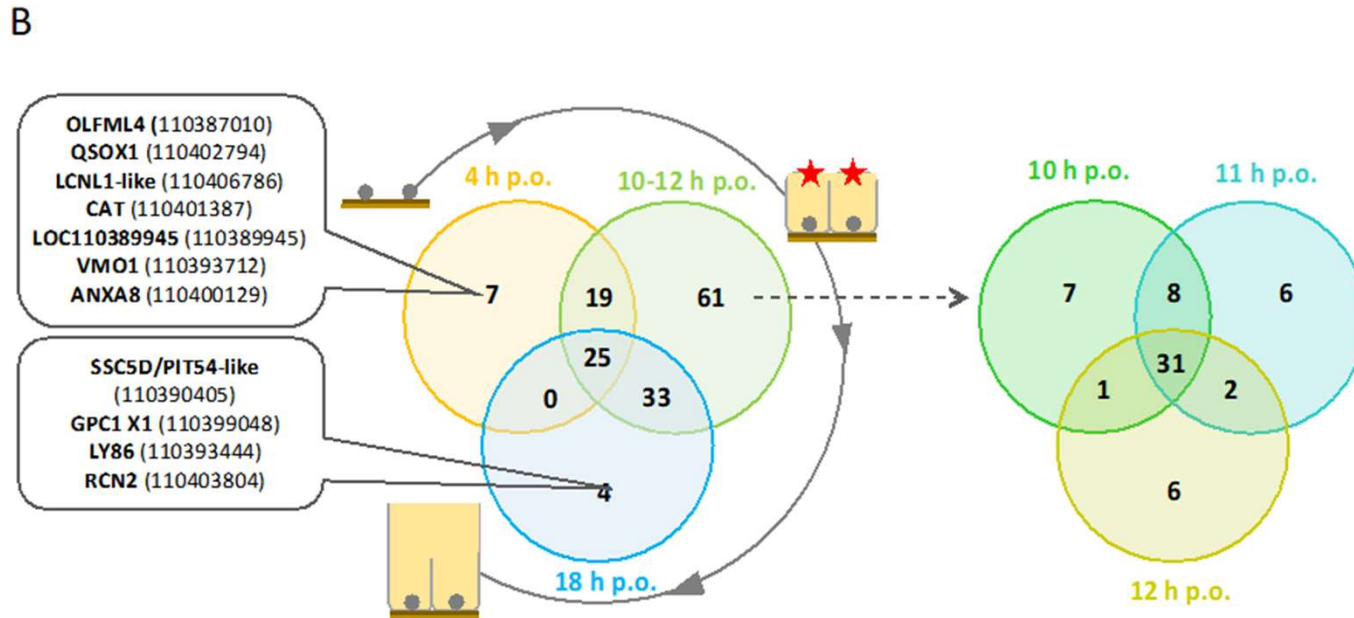
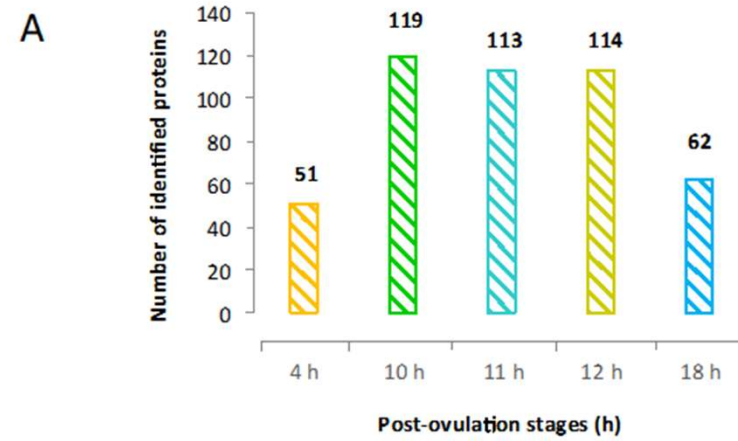


The exceptional mechanical properties of Guinea fowl eggshells



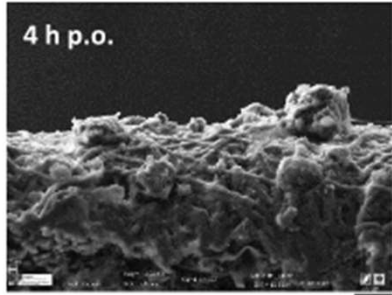
Identified Proteins (9)	Protein short name	Accession N° (NCBI)	Gene ID	MW (kDa)	Mean WS				
					4 h	10 h	11 h	12 h	18 h
dromaiocalcin-1-like	DCA-1-like	XP_021238988.1	110391424	21	354.6	2854.7	1656.0	2860.0	3604.5
phosphatidylethanolamine-binding protein 4 isoform X1	PEBP4 X1	XP_021230598.1	110387114	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



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First mineralization events



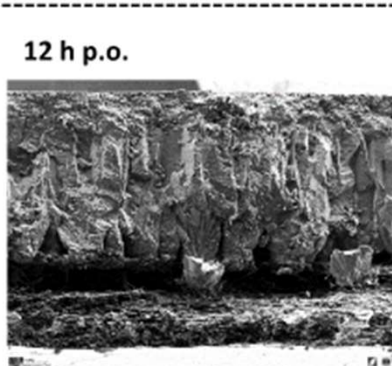
10 μm



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.

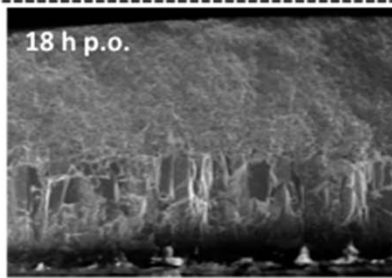
DCA-1-like, **OC-17-like**, **OVALX2**, **OC-116-like/MEPE-like**, **OVM**, **ExFAB**, **LYZ C**, **OVOT**, **OCX-32-like/RARRES1-like**, **AVD-like**, **AGP-like**, **EDIL3**, **CLU**, **HPX**, **CST3**



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**