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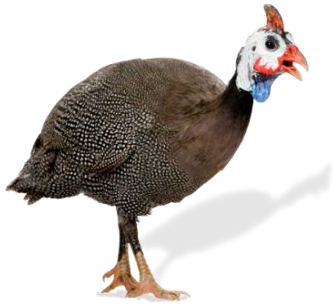
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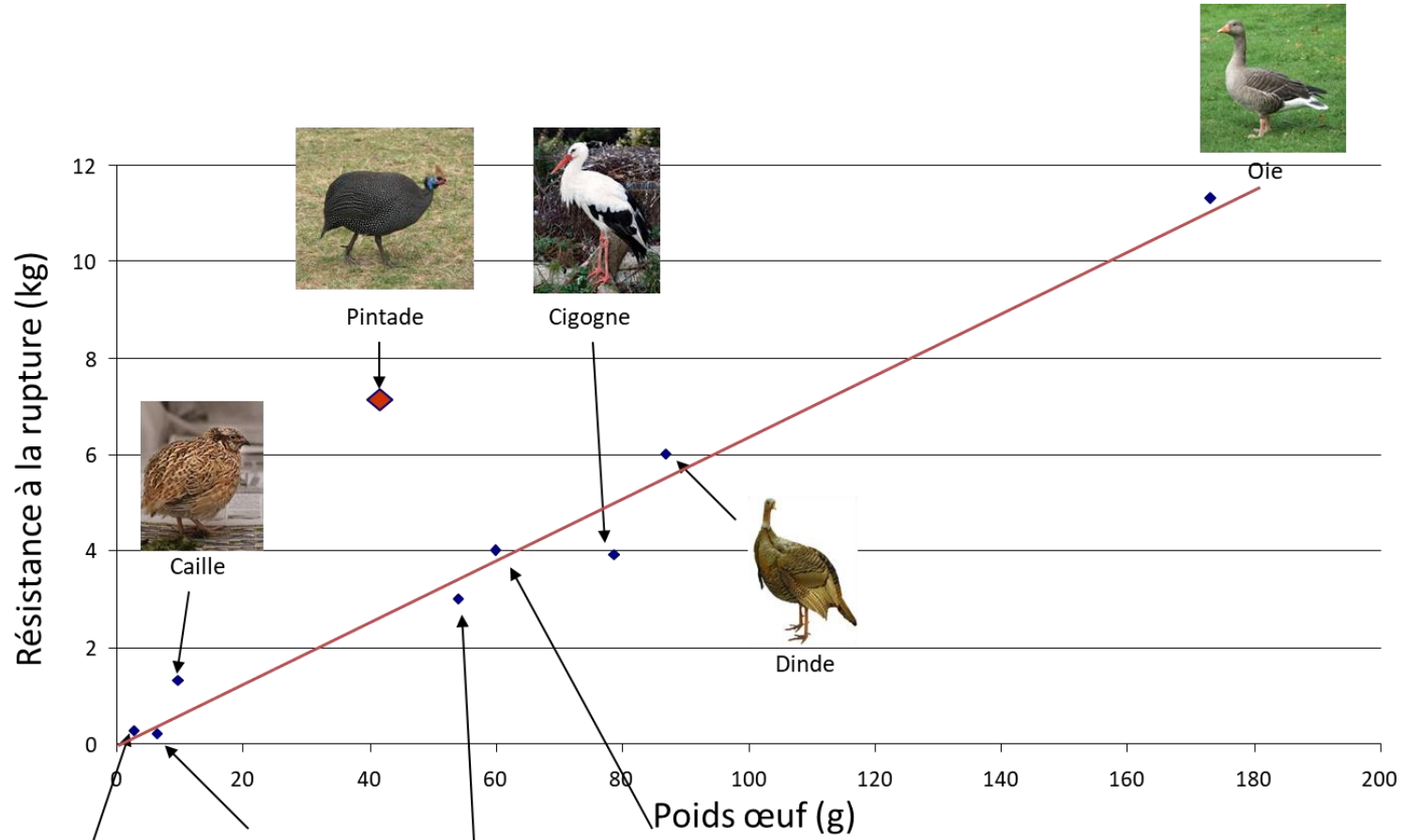
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# GUINEA FOWL EGGSHELL STRUCTURAL ORGANIZATION AND PARTICULAR ORGANIC MATRIX PROTEIN PATTERNS TO DECIPHER ITS EXCEPTIONAL BIOMECHANICAL PROPERTIES



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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



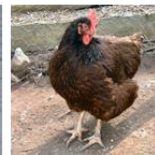
Moineau



Merle noir



Canard



Poule

# The exceptional mechanical properties of Guinea fowl eggshells



*Gallus gallus*



*Meleagris gallopavo*

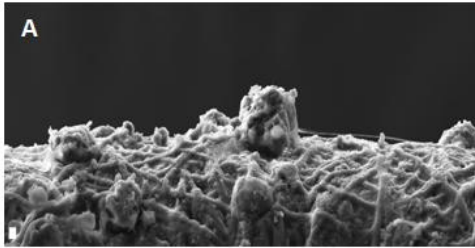


*Sankofa pyrenaica*



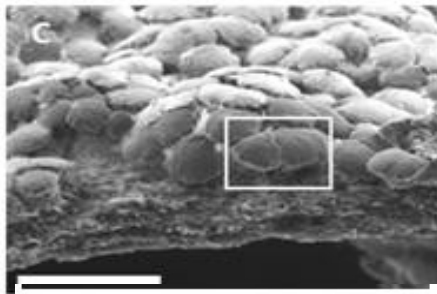
*Numida meleagris*

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



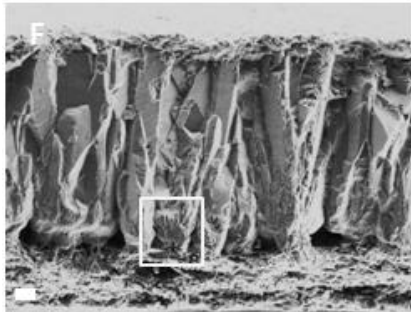
4 hours post ovulation :

Onset of mineralisation with deposition of « seeding sites »



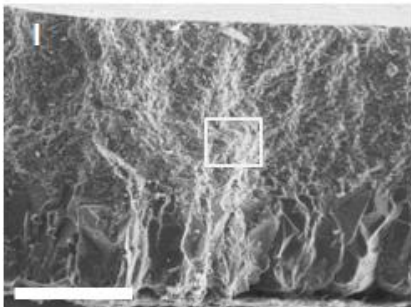
5 Hours P.O.:

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



11 hours P.O.:

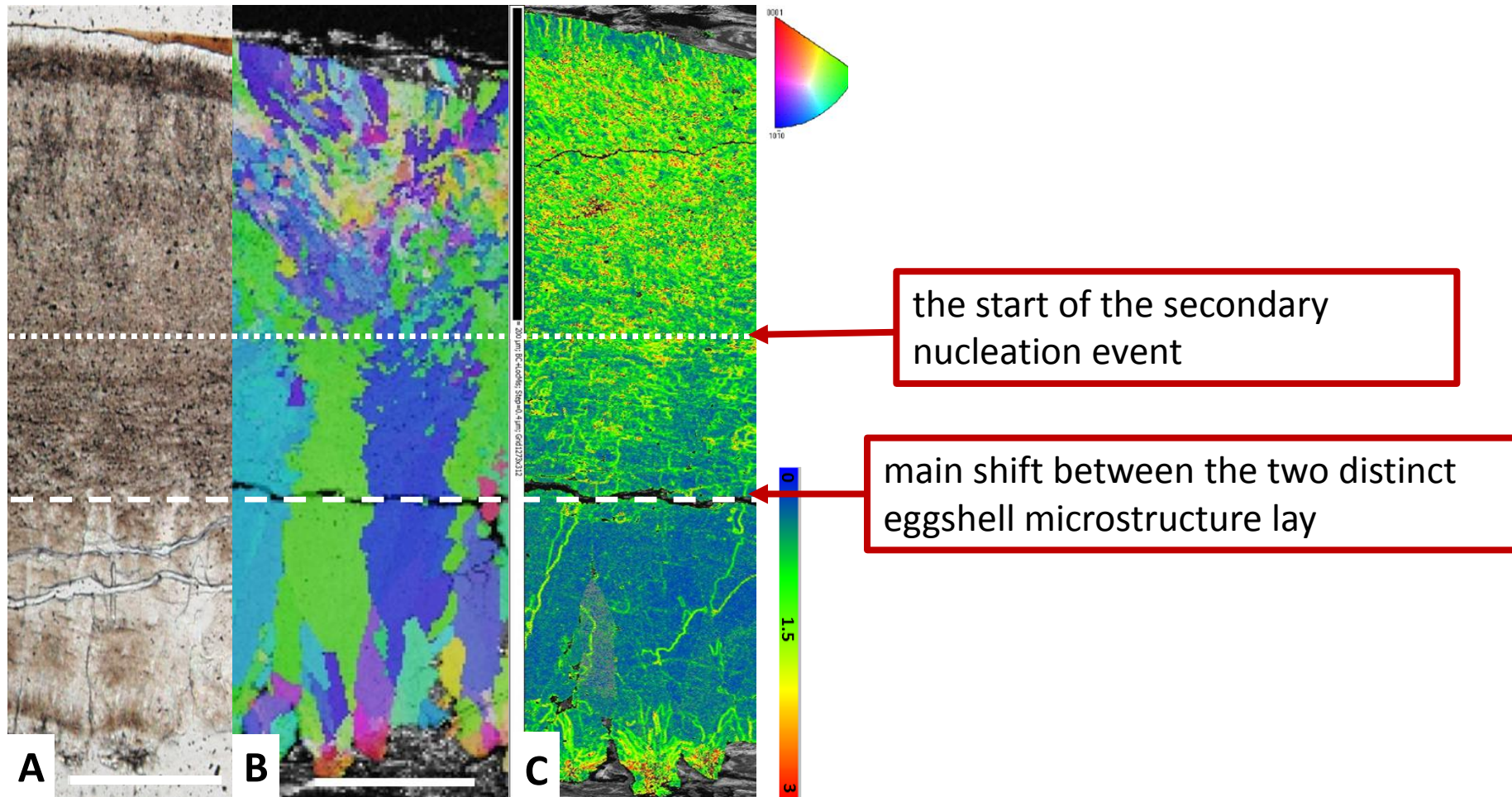
Cross-section view of an eggshell collected at 11 hrs p.o., showing a sharp microstructure change from large columnar calcite crystal units to calcite microcrystals



24 hours P.O.:

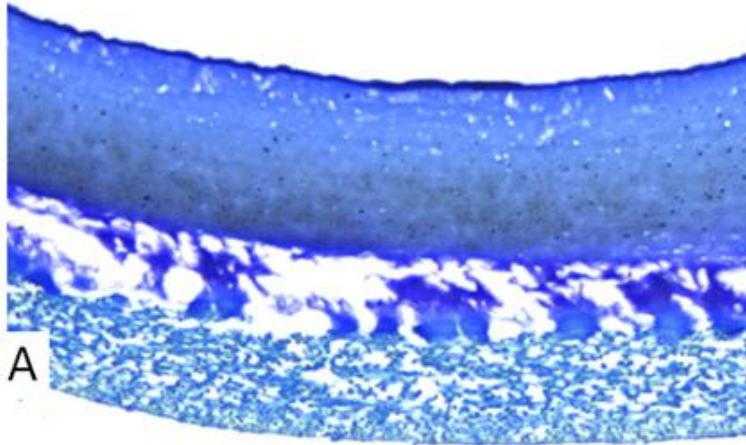
A fully formed eggshell with the lower part formed by large columnar units (lower part) and the top part form by smaller calcite crystal units arranged with a brick-wall microstructure





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 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

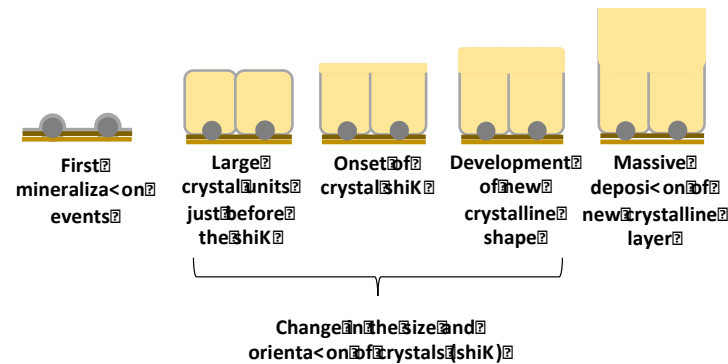
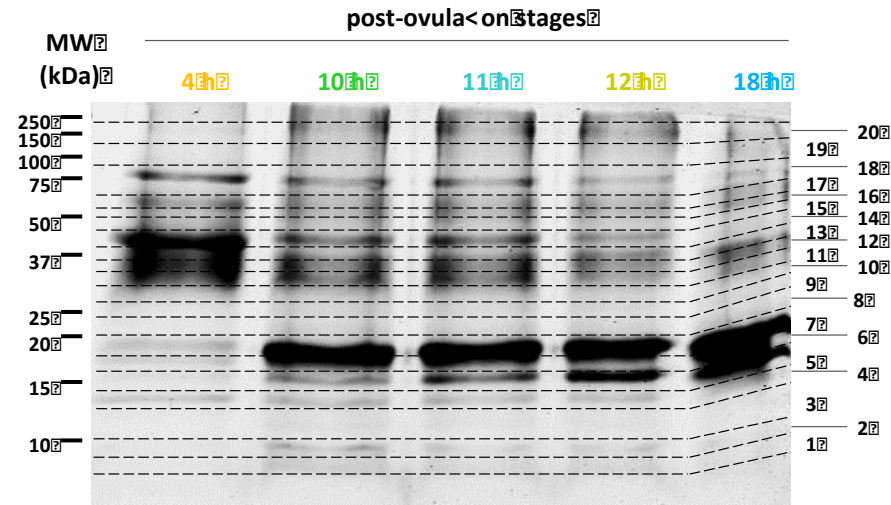
Shell membranes

**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.**



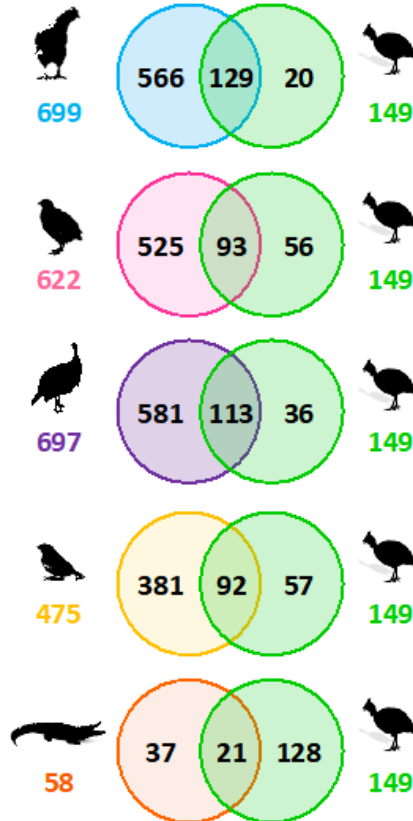
**This particular organization is responsible for the exceptional mechanical properties of Guinea fowl eggshell by comparison to that of other birds.**

# The exceptional mechanical properties of Guinea fowl eggshells

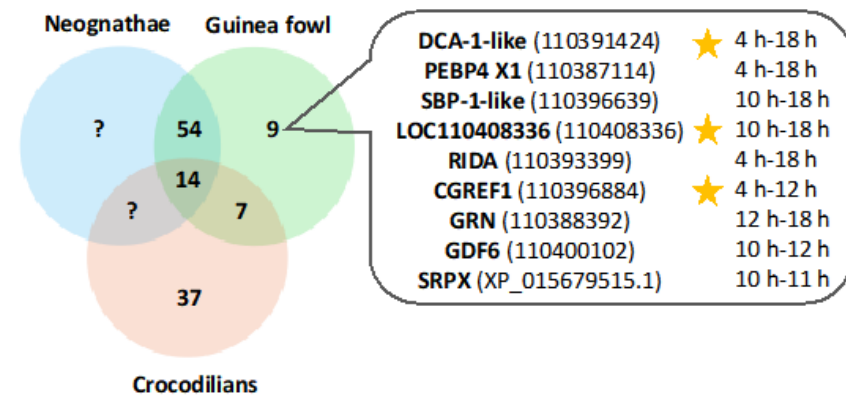




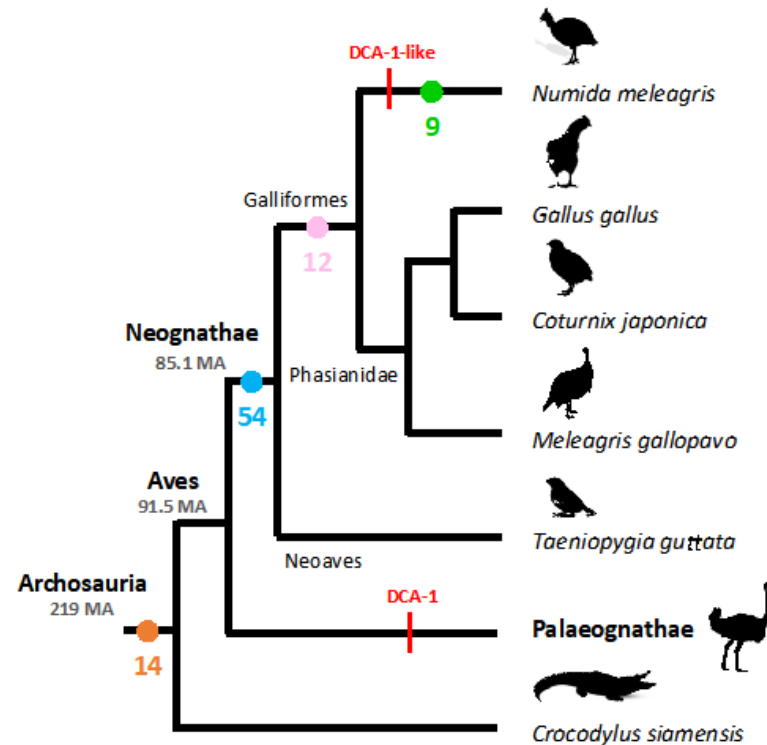
**A**

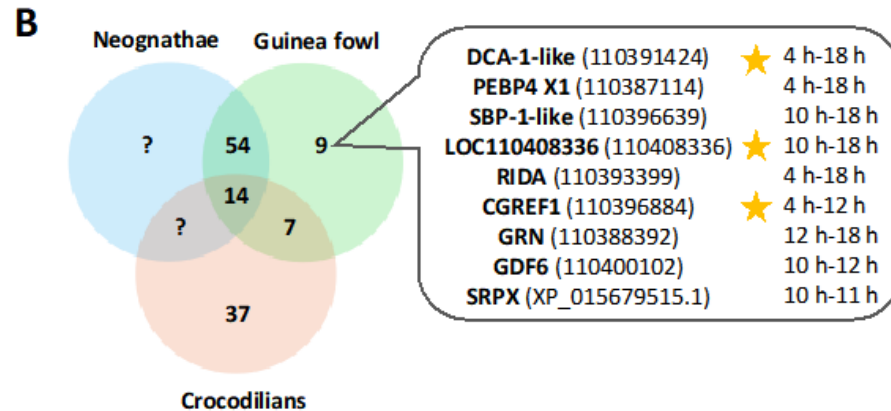


**B**

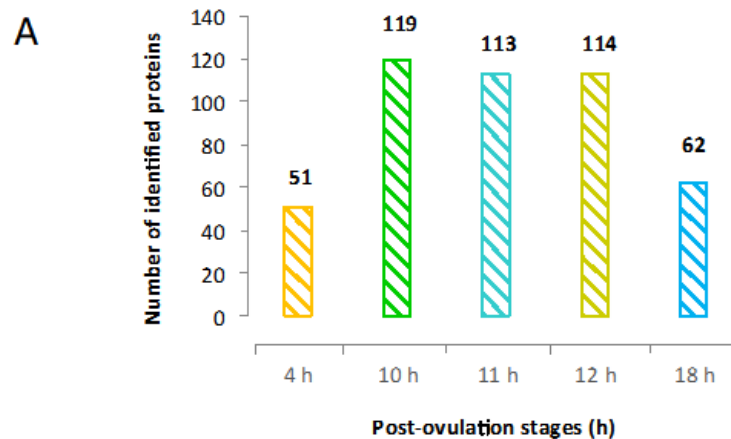


**C**

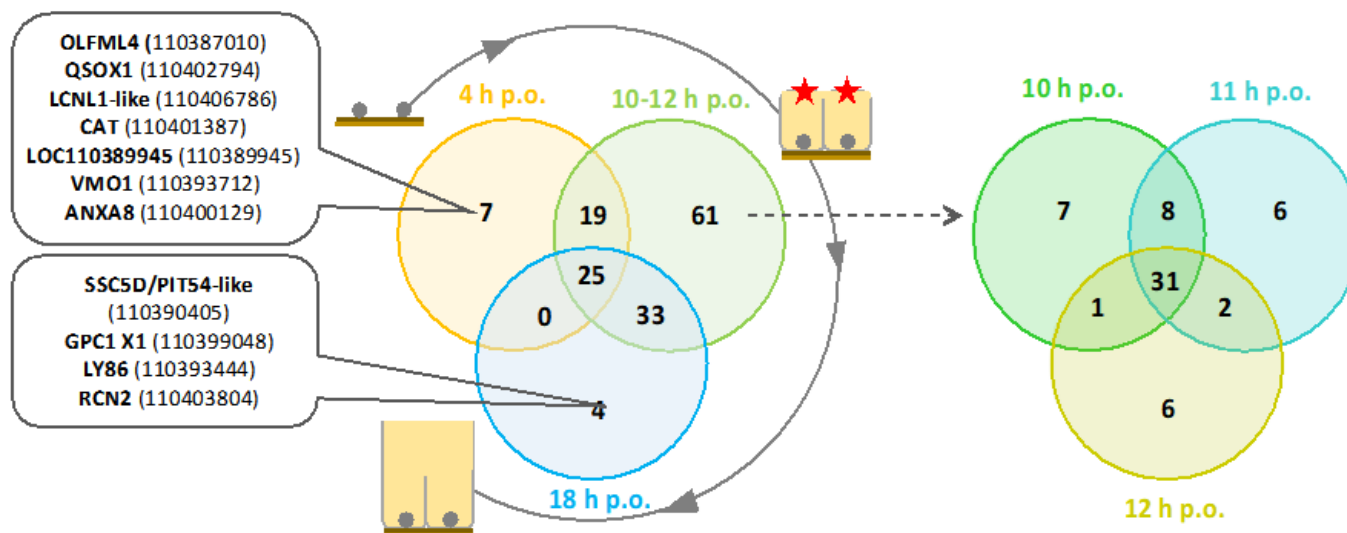




Identified Proteins (9)	Protein short name	Accession N <sup>o</sup> (NCBI)	Gene ID	MW (kDa)	Mean WS				
					4 h	10 h	11 h	12 h	18 h
dromaiocalcin-1-like	<b>DCA-1-like</b>	XP_021238988.1	110391424	21	354.6	2854.7	1656.0	2860.0	3604.5
phosphatidylethanolamine-binding protein 4 isoform X1	<b>PEBP4 X1</b>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

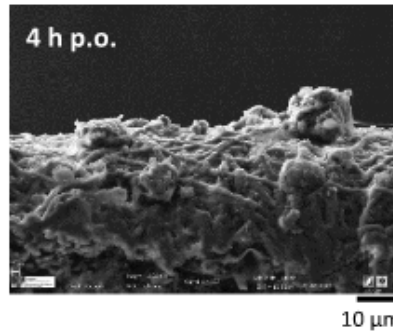


**B**



# The exceptional mechanical properties of Guinea fowl eggshells

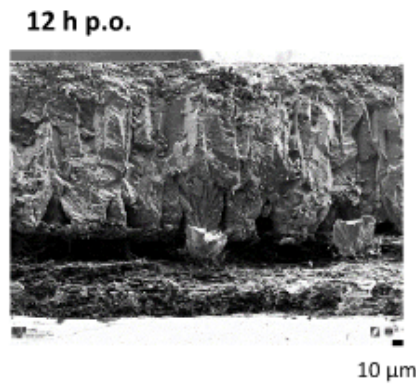
First mineralization events



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 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.

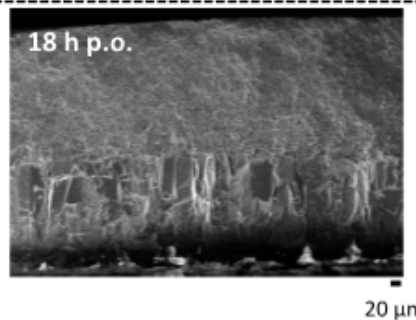
**DCA-1-like**, **OC-17-like**, **OVALX2**, **OC-116-like/MEPE-like**, **OVM**, **ExFAB**, **LYZ C**, **OVOT**, **OCX-32-like/RARRES1-like**, **AVD-like**, **AGP-like**, **EDIL3**, **CLU**, **HPX**, **CST3**



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



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**