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

Joël Gautron

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

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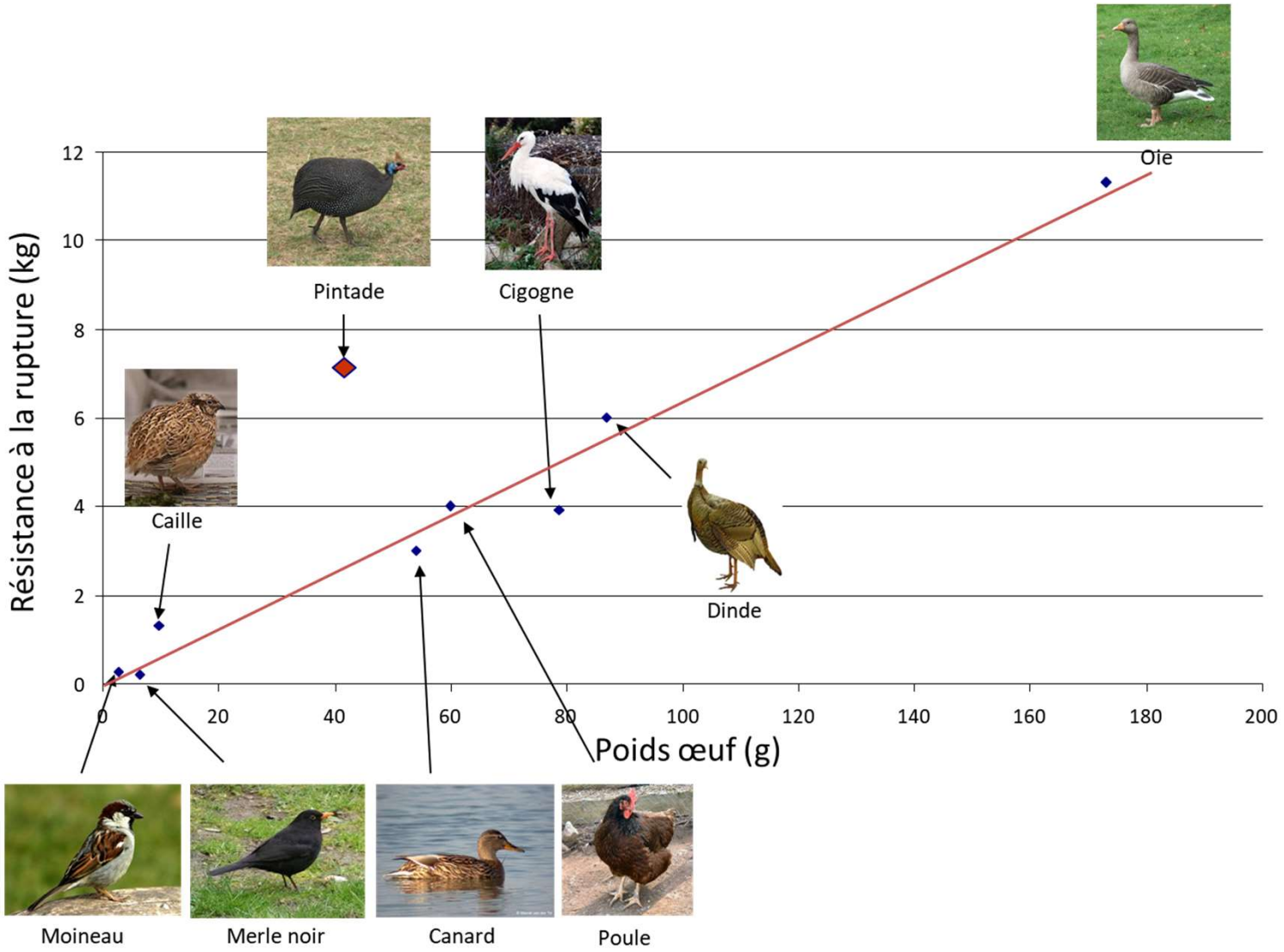
The exceptional mechanical properties of Guinea fowl eggshells



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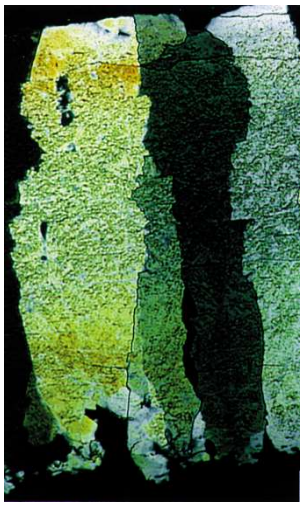


Gallus gallus

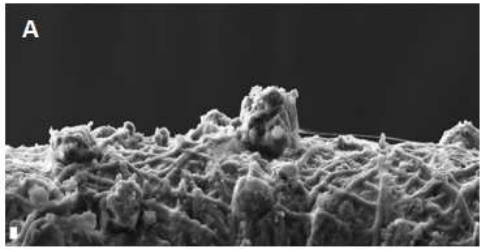
Meleagris gallopavo

Sankofa pyrenaica

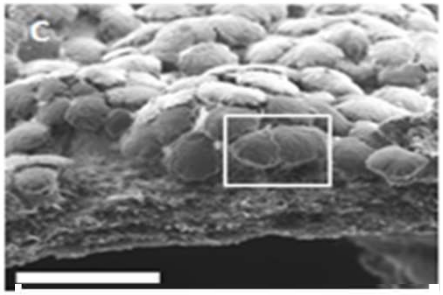
Numida meleagris

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

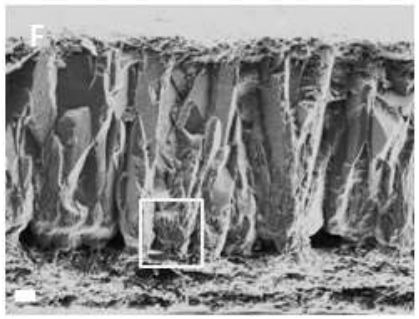
Guinea fowl eggshell biomineralization



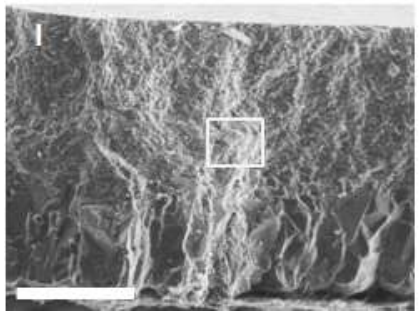
4 hours post ovulation :
Onset of mineralisation with deposition of « seeding sites »



5 Hours P.O.:
One hour later, the eggshell membrane is almost fully covered by semi-spherical aggregates of calcite crystals

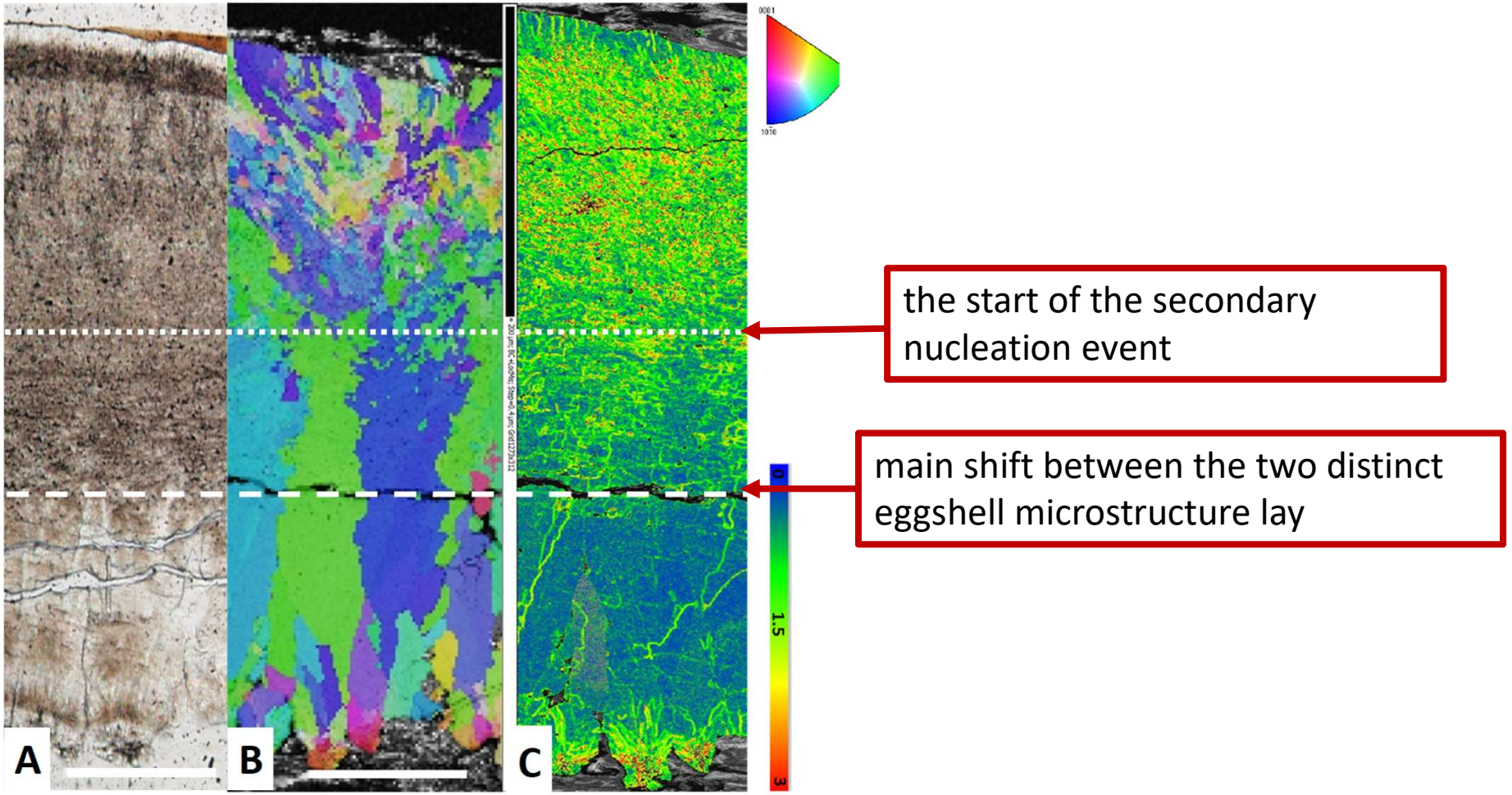


11 hours P.O.:
Cross-section view of an eggshell collected at 11 hrs p.o., showing a sharp microstructure change from large columnar calcite crystal units to calcite microcrystals



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

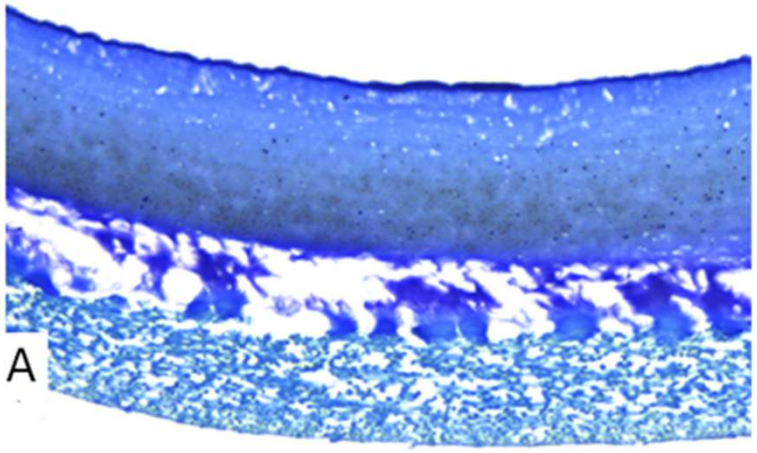


A) Optical microscopy view of the cross-section of a fully formed eggshell showing the non-homogenous distribution of occluded organic matter. B-C) EBSD crystal orientation and local misorientation maps of the eggshell cross-section showing the constituting calcite crystal units

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

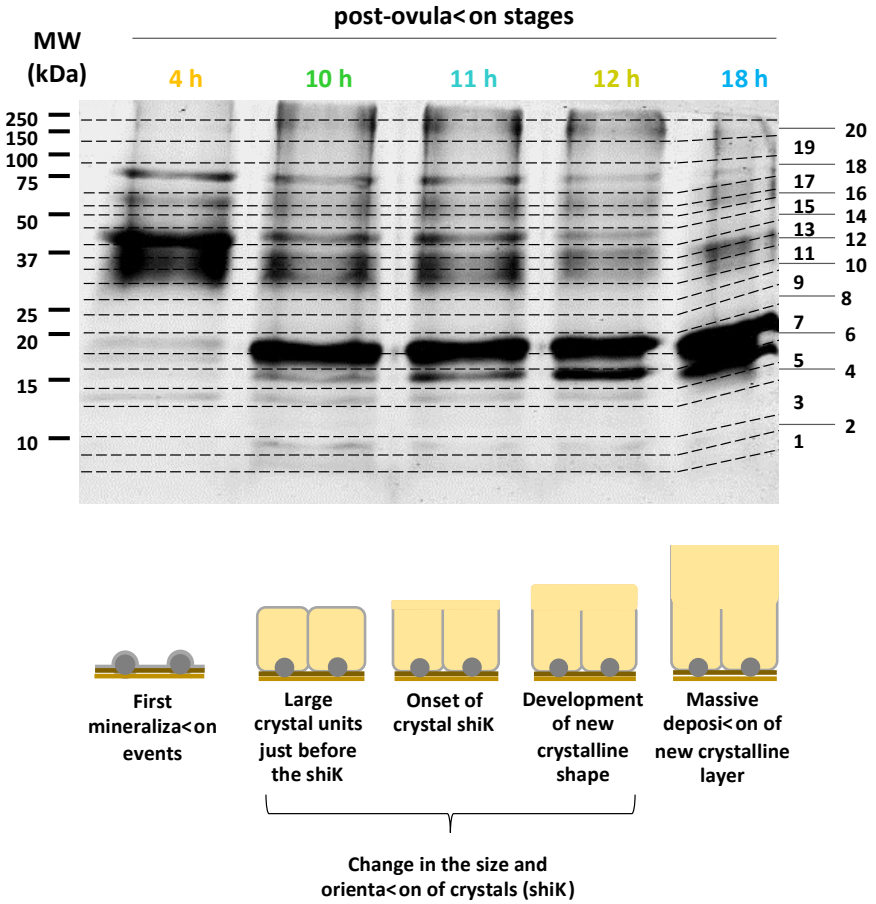
First crystaline layer

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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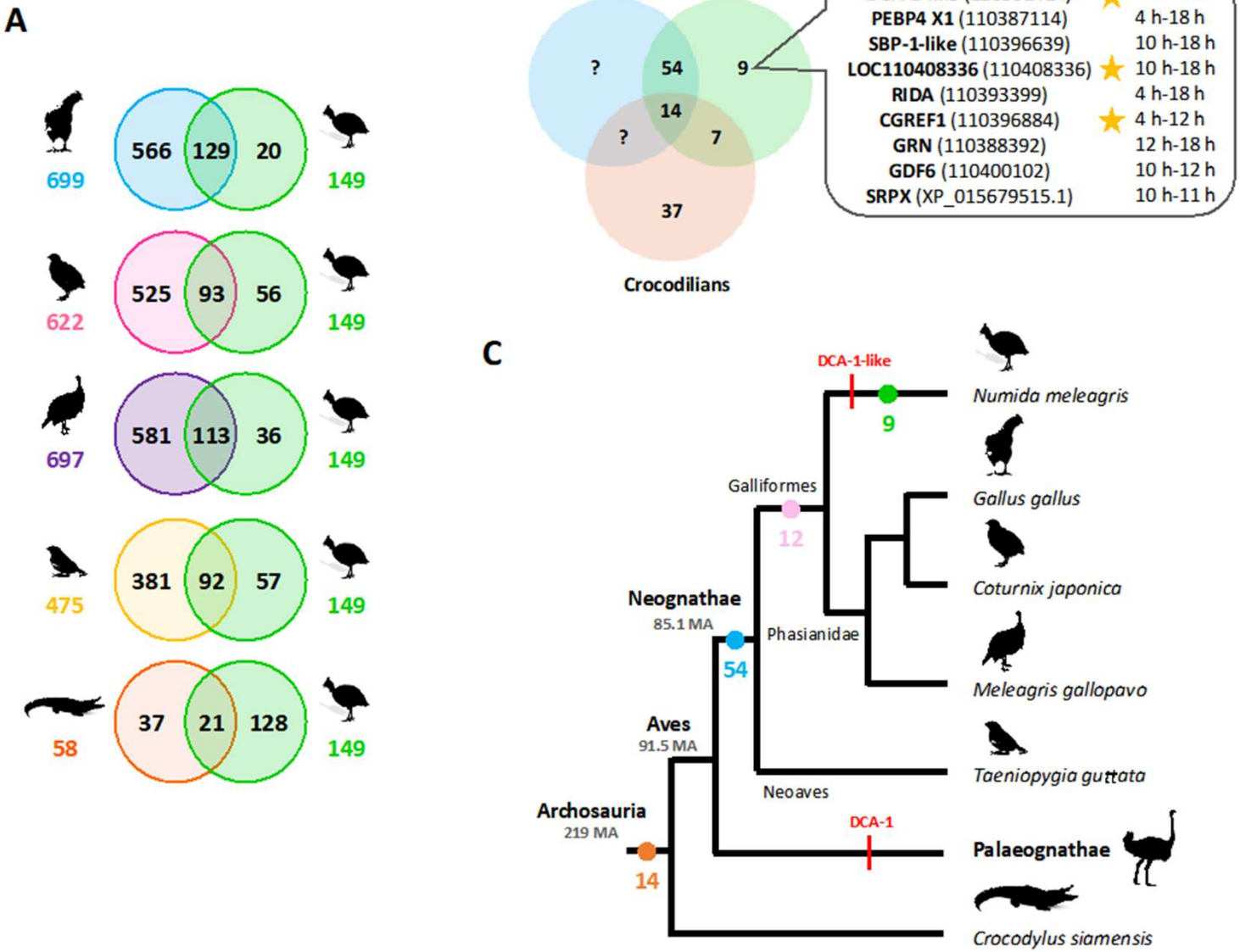

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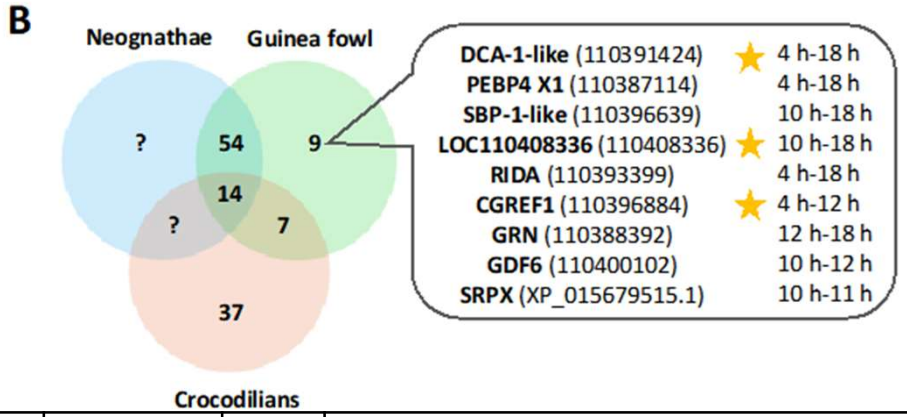

Identified Proteins (9)	Protein short name	Accession N° (NCBI)	Gene ID	MW (kDa)	Mean WS				
					4 h	10 h	11 h	12 h	18 h
dromaiocalcin-1-like	**DCA-1-like**	XP_021238988.1	110391424	21	354.6	2854.7	1656.0	2860.0	3604.5
phosphatidylethanolamine-binding protein 4 isoform X1	**PEBP4 X1**	XP_021230598.1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

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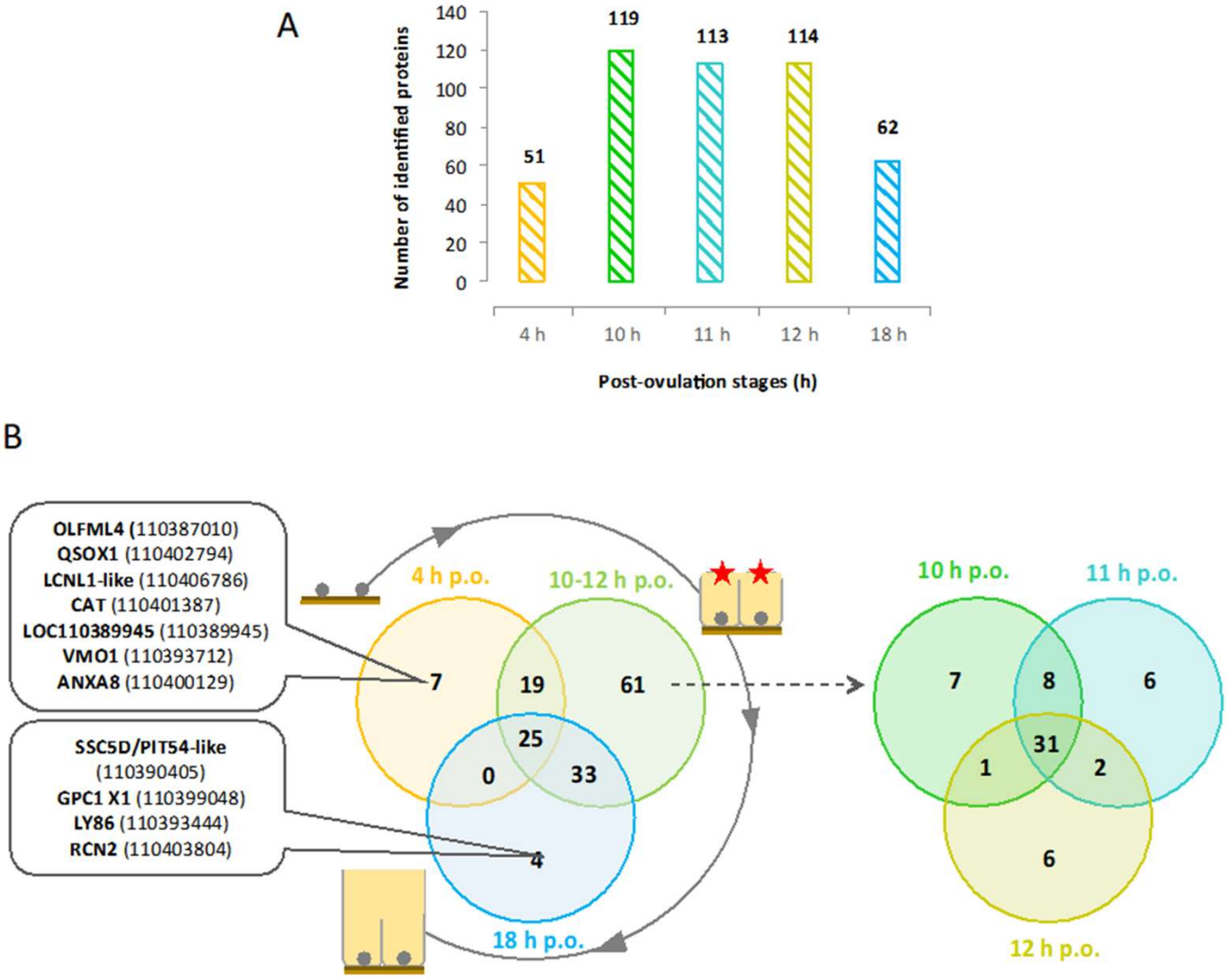

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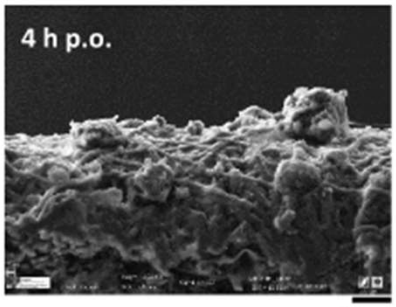
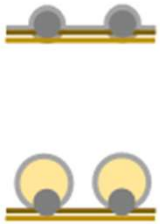
First mineralization events

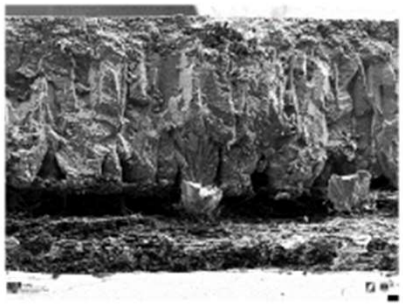




4 h p.o.

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

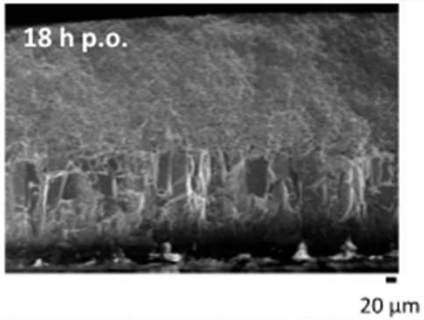
11 h p.o.

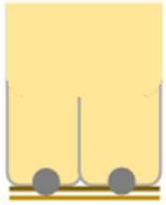
DCA-1-like, OC-17-like, OVALX2, OC-116-like/MEPE-like, OVM, ExFAB, LYZ C, OVOT, OCX-32-like/RARRES1-like, AVD-like, AGP-like, EDIL3, CLU, HPX, CST3

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, Meleagrin-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