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Identification of polymorphisms in Protein Coding Genes which affect eggshell quality traits in layers

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Identification of polymorphisms in Protein Coding Genes which affect eggshell quality traits in layers

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de TOURS





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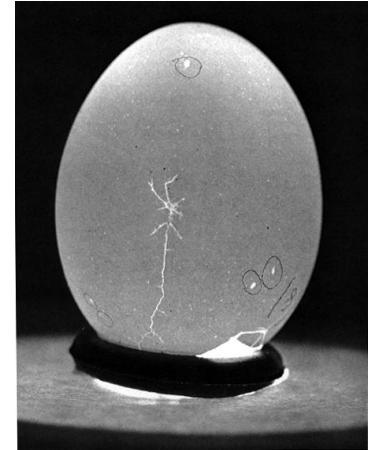
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Background

- Chicken egg is a high quality food, at a low cost
- The eggshell constitutes a natural physical barrier against bacterial penetration
- Microbial spoilage of eggs is promoted by presence of eggshell cracks or micro-cracks



Food safety



**Micro-cracks promote
bacterial penetration**



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Context: set hens free!

- Consumer and big brands demand
 - Cage-free eggs production
 - Sustainability -> Long-life laying hen





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Context: set hens free!

- Consumer and big brands demand
 - Cage-free eggs production
 - Sustainability -> Long-life laying hen

- Increased risk of
 - Microbial contamination
 - Weaker eggshells





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Context: set hens free!

- Consumer and big brands demand
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- Increased risk of
 - Microbial contamination
 - Weaker eggshells

**Importance of
eggshell quality**

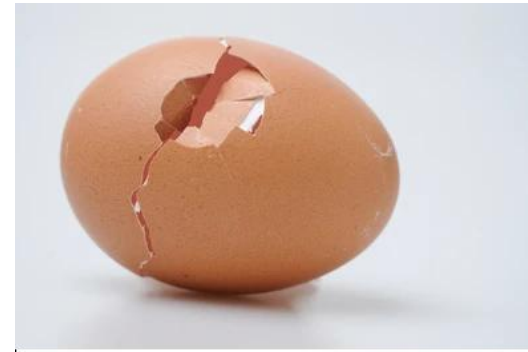


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Factors influencing shell integrity and quality

- Egg manipulation: collecting, grading, packing processes and transportation
- Management / housing systems: nutrition, temperature and lightning conditions
- Eggshell biomineralization process: oviposition time, age of bird
- Genetics : breeds, polymorphisms





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Genetic determinism of eggshell quality

- A large number of QTLs influencing eggshell quality traits (stiffness, strength, shape, diameter)
- Several candidate genes

- Causal polymorphism identification
 - Key step to increase the evaluation accuracy (genomic selection).
 - Link between genes, biological functions and traits.



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Overall approach

- Polymorphisms in genomic sequences from 1000 Gallus genome project (laying hens, broilers, local breeds) Tixier-Boichard et al., 2020 doi:[10.20870/productions-animales.2020.33.3.4564](https://doi.org/10.20870/productions-animales.2020.33.3.4564)
- Functional candidate genes
- QTL affecting eggshell quality (stiffness, strength, shape, diameter)

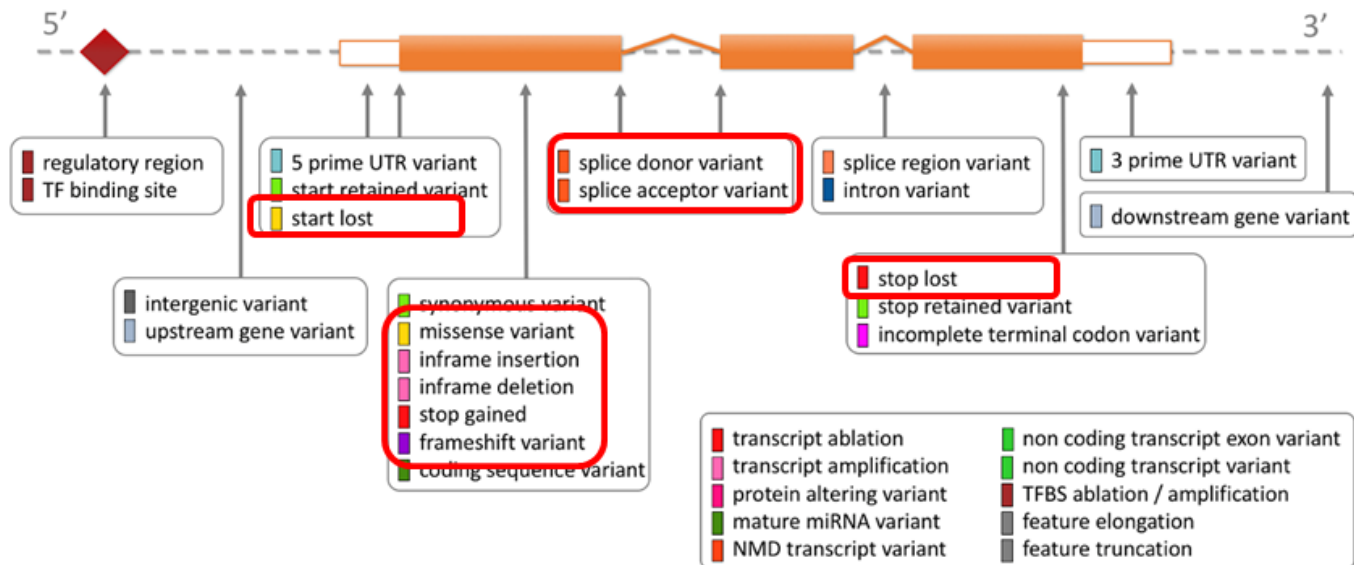


Putative causal mutations, localized in candidate genes for eggshell formation and quality.



Deleterious polymorphisms identification

- May affect protein structure and function





Deleterious polymorphisms identification

- May affect protein structure and function
- Thousand of deleterious variants identified in genomic sequences

	Library	n	Variant	Deleter
Broilers	Chickseq	24	12 898 316	67 986
	Acrigen	16	11 465 902	62 650
	SABRE	6	9 187 271	50 394
Layers	Utopige	90	9 947 217	52 270
	FEEDaGENE	19	7 121 799	40 107
	Epibird	12	7 364 408	38 494
	QTLDJ	4	5 795 085	28 057
	FEEDaGENE_AgENC	4	5 314 924	29 427
Local breeds	Domestic (Diversité)	36	33 410 400	207 596



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Deleterious polymorphism in functional candidate genes

- 465 candidate genes:
 - 333 genes known to be involved in calcification process
 - 225 differentially expressed proteins during biomineralization process



**4849 deleterious loci in
418 candidate genes**



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Deleterious polymorphism in functional candidate genes

- 4 849 deleterious loci in 418 functional candidate genes

Deleterious variant in QTL affecting eggshell quality (stiffness, strength, shape, diameter)

- 5 607 deleterious loci located in 1560 genes
- of which 566 are located in 44 functional candidate genes



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Genotyping

- Consolidated list of 255 deleterious polymorphisms
 - 240 SNP + 15 InDels
 - Corresponding to 46 genes of which 34 are candidate genes
- Amplicon sequencing genotyping on 480 laying hens from 8 different lines (White Leghorn and Rhode Island)
- Identification of 693 polymorphisms in the targeted regions



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Association study strategy

- Within line and overall to support the hypothesis of a direct effect



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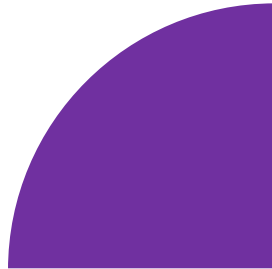
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Association study strategy

- Within line and overall to support the hypothesis of a direct effect

No effect

Never significant effect





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Association study strategy

- Within line and overall to support the hypothesis of a direct effect

No effect

Never significant effect



**Indirect effect
(association)**

Significant effect but
only within line





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Association study strategy

- Within line and overall to support the hypothesis of a direct effect

No effect

Never significant effect



Low effect

Significant effect but
only overall



**Indirect effect
(association)**

Significant effect but
only within line





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Association study strategy

- Within line and overall to support the hypothesis of a direct effect

No effect

Never significant effect



Low effect

Significant effect but only overall



Good candidate

Significant effect overall and within line



Indirect effect (association)

Significant effect but only within line





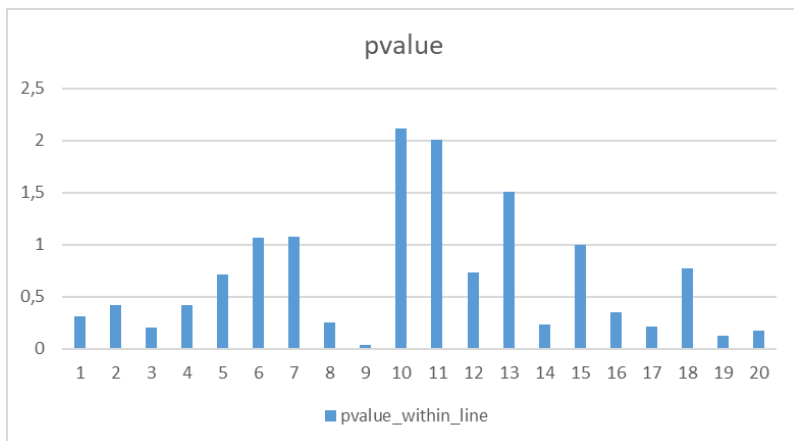
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Example results

- Association study of twenty polymorphisms (MAF>1%) identified in one gene

Within line analysis

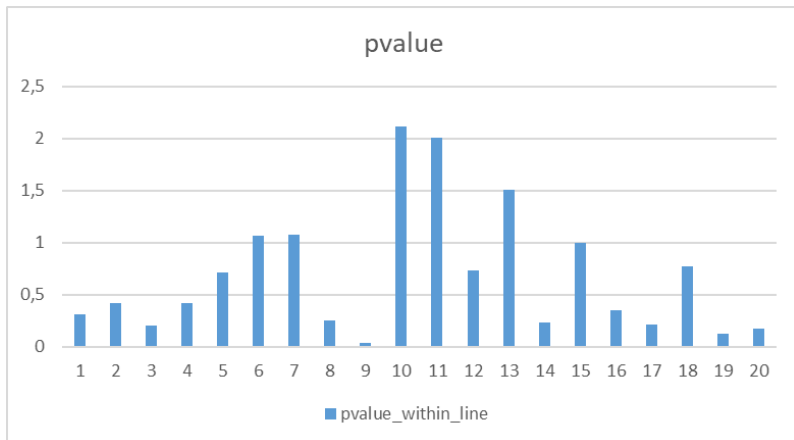




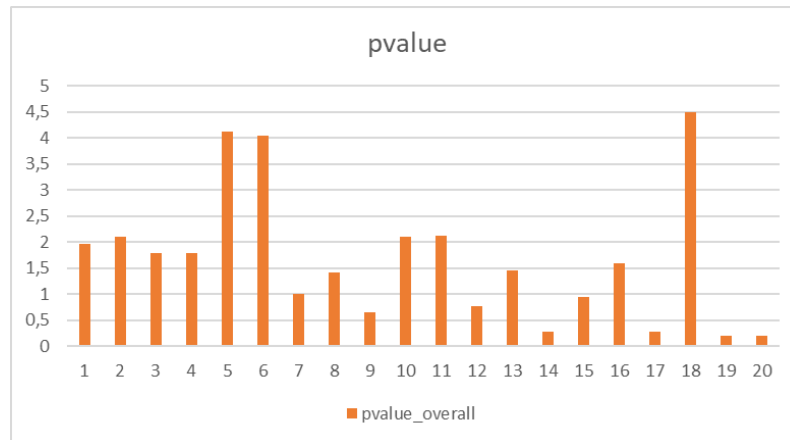
Example results

- Association study of twenty polymorphisms (MAF>1%) identified in one gene

Within line analysis



Overall line analysis

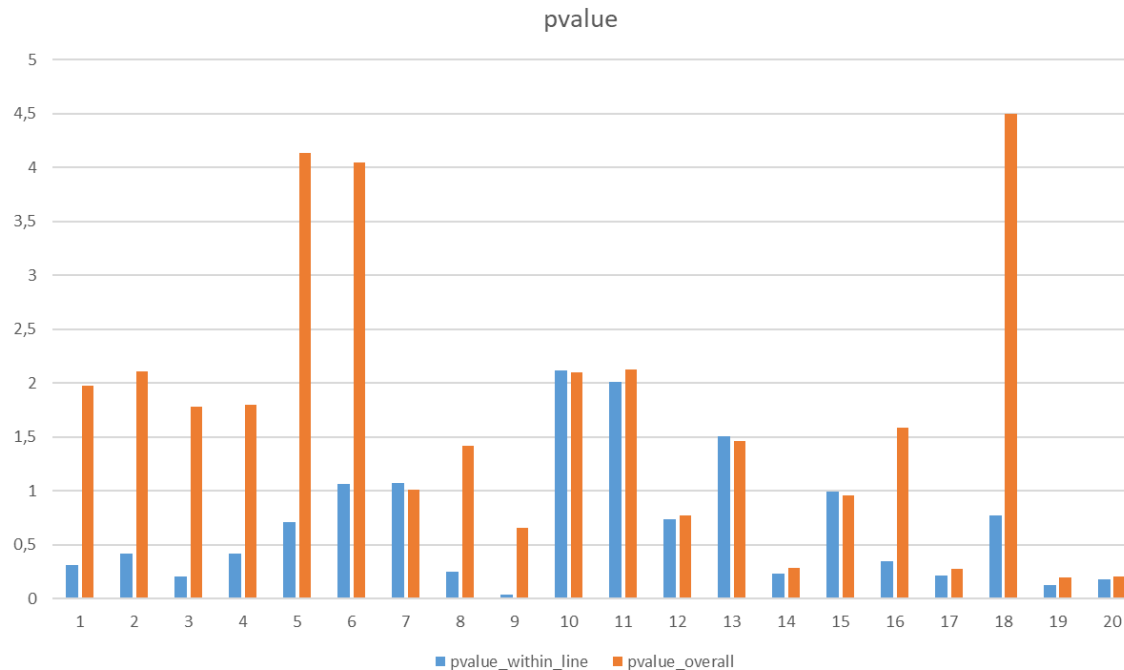




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Example results





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Results

No effect



Low effect



Significant effect but only overall

No Good candidate
Overall and within line effect



**Indirect effect
(association)**





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Results

No effect



Low effect



Significant effect but only overall

No Good candidate
Overall and within line effect



**Indirect effect
(association)**



- Identification of 64 deleterious polymorphisms associated with eggshell strength or eggshell stiffness
- 9 deleterious polymorphisms are predicted to have a high impact of amino acid substitution on protein function



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Next step

- 6 putative causal mutations will be investigated on several laying hen and broiler lines to confirm their causative status
- Functional and structural characterization of protein isoform products from genes carrying these polymorphisms
 - Transcript expression and protein level
 - 3D structure of protein isoforms



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Concluding remarks

- Polymorphisms in coding regions are a small proportion of all polymorphisms
- It's more obvious to draw a link between polymorphism and protein function and trait
- This strategy is generic and can be applied to other species and traits



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