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UNIVERSITÀ DEGLI STUDI DI PADOVA

Exploring the genomic basis of shank and eggshell coloration in Italian native chicken breeds

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Introduction

Eggshell and shank colour are important in poultry selection, breed recognition, and environmental adaptation. This study deepens the genomics of shank and eggshell pigmentation in Italian local chickens to uncover the mechanisms behind these phenotypes.

Materials and Methods

Samples Collection and Genotyping

- 418 animals from 18 local breeds evaluated for shank and eggshell pigmentation.
- Chickens genotyped using the 600K Affymetrix SNP chip array.

Genome-Wide Association Analysis

- Genome-wide association analysis performed to compare the case and control populations for shank and eggshell colour.
- Shanks: (1) case population (201 animals) characterized by a phenotype classified as dark (grey-black and dark green shank); (2) reference population (155 animals) with yellow-shank pigmentation.
- Eggshell: (1) case population (127 animals) with tinted eggshell (different brownness levels); (2) reference population (277 animals) characterized by white eggshell.

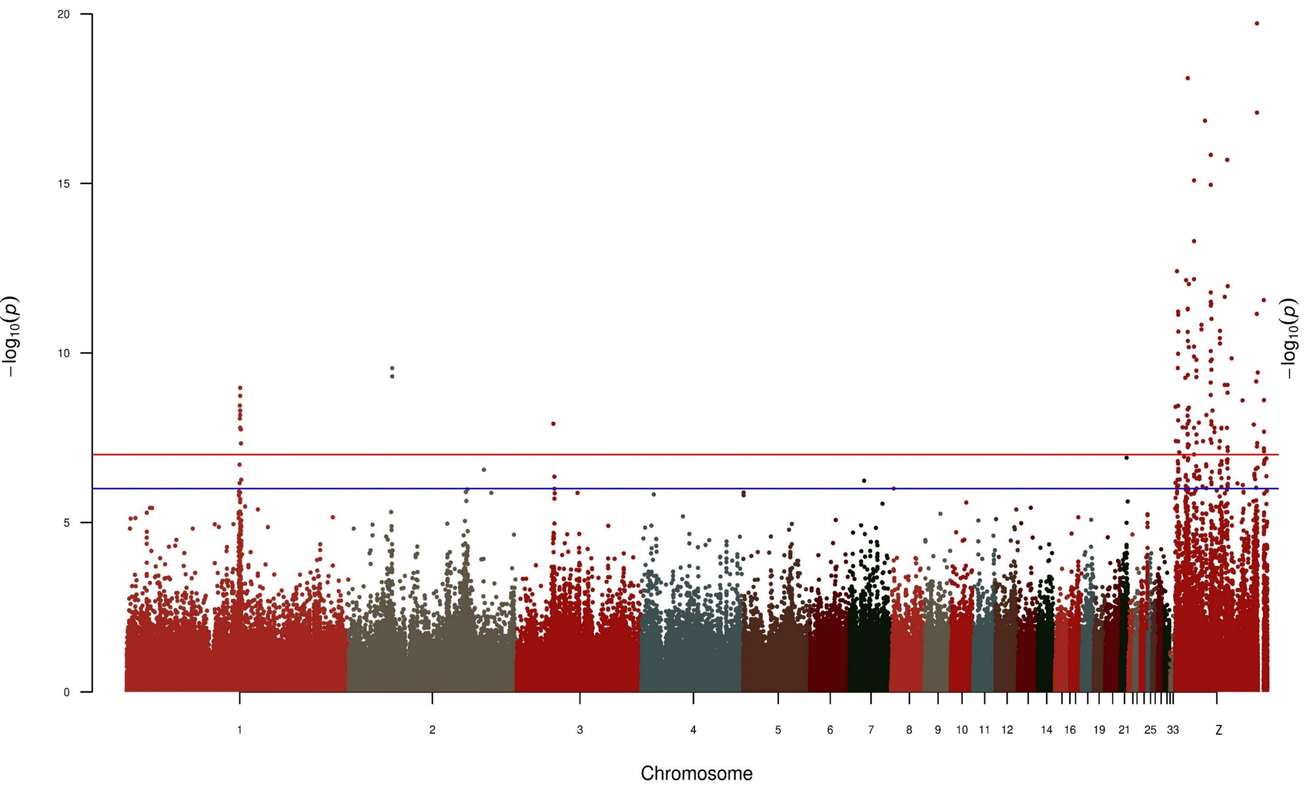
Results and Discussion

- Most interesting locus for shank pigmentation detected on GGAZ, close to the *TYRP1* gene (key role in avian pigmentation).
- Novel loci and genes (e.g., *MTAP*, *CDKN2A*, *CDKN2B*) associated with shank and skin pigmentation, UV protection, and melanocyte regulation were identified.
- A new genomic region for shank pigmentation detected on GGA1, in which *CHODL*, *TMPRSS15*, and *NCAM2* gene were mapped.
- Fewer significant loci identified for eggshell pigmentation, including *SLC7A11* on GGA4 and *MITF* on GGA12 (associated with melanocyte processes and pigment synthesis).

SHANK			
Chromosome and position	**Gene ID**	Description	Reference
1:100867039-101831504	***CHODL, TMPRSS15, NCAM2***	Genomic region related to horn pigmentation in cattle	Zsolnai et al. (2021)
2:38800153-38800475	***EOMES, CMC1, AZI2, RBMS3***	Expression enhanced in embryos of chicken with double comb	Dorshorst et al. (2015)
Z:11112490-12254700	***SLC1A3, RANBP3L, SLC45A2***	SLC family is implicated in pigmentation in white tiger, and SLC1A3 is upregulated in development of stem cells in hair follicles	Xu et al. (2013)
Z: 18925613-18969905	***ERCC8***	Groningen White Headed cattle	Gonzalez-Prendes et al. (2022)
Z: 31545096-32699330	***NFIB, ZDHHC21, CER1, PSIP1, BNC2, TYRP1***	BNC2 strongly associated in human skin pigmentation	Jacobs et al. (2013)
Z: 78827158-79172113	***CDKN2A, CDKN2B***	Play role in the barring phenotype of the chicken by altering the melanocyte cell cycle	Dorshorst and Ashwell (2009)
Z: 78827158-79172113	***MTAP, FEM1C***	Associated with skin and shank pigmentation in chicken	Cha et al. (2023)
Z: 78827158-79213873	***TRIM36, GRAMD3***	Associated with skin and shank pigmentation in chicken	Li et al. (2014)
EGGSHELL			
2:61184687-61271239	***JARID2***	Involved in regulation of gene expression during embryonic development	Whiteley et al. (2021)
4:26751167-30211214	***PCDH18***	PCDH18 very close to SLC7A11 responsible for coloration in mammals (of skin) and in chicken plumage and skin	Chen et al. (2019)
5:15963249-15987149	***PNPLA2, SLC25A22***	Related with production of carotenoids	Ahi et al. (2020)
12:15839118-16014277	***MITF, FAM19A4, ARL6IP5, UBA3***	Linked to pigmentation in cattle and other species, in duck associated with pigmentation of beak	Gonzalez-Prendes et al. (2022)
Z:10028822-10307280	***NPR3, TARS, ADAMTS12, SLC45A2***	SLC45A2 is one of the most important gene in coloration	Dorshorst and Ashwell (2009)

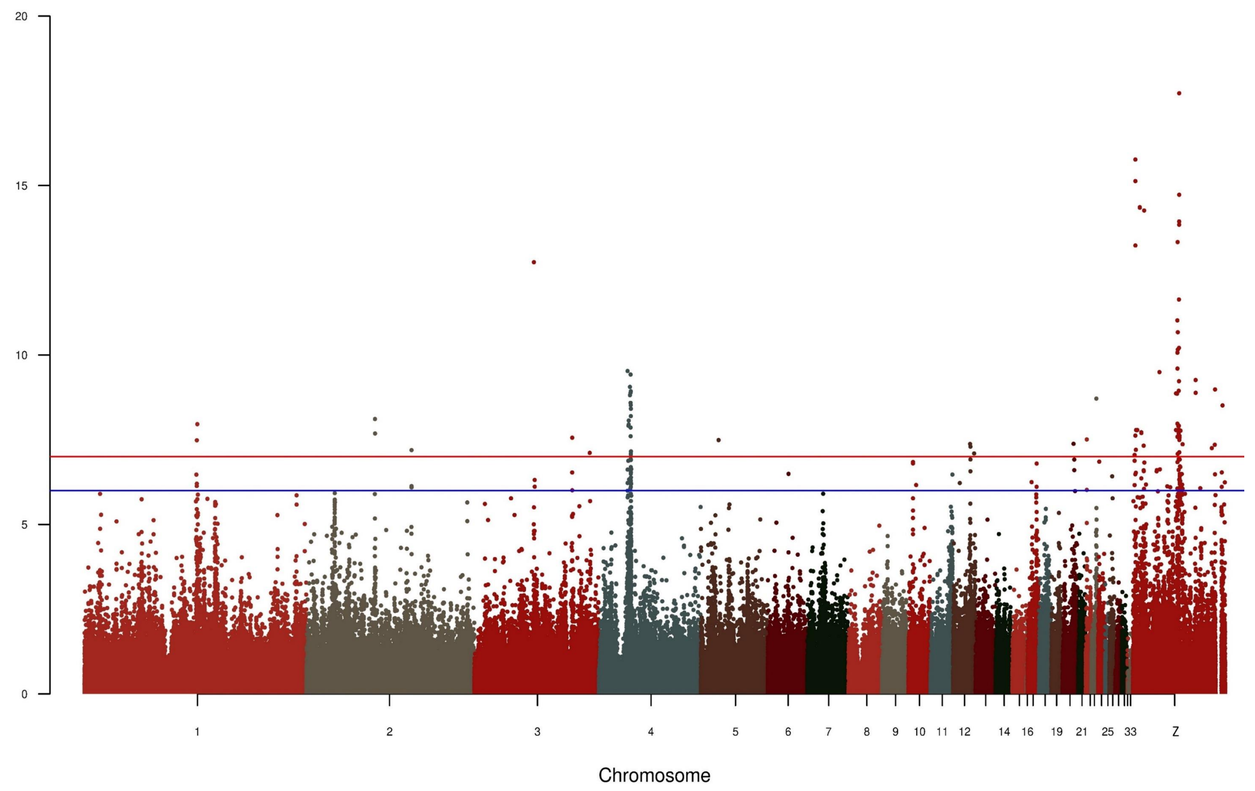
Shank Dark vs Light

Manhattan plot of GWAS comparing case (dark shank) and control population (light shank).



Eggshell Tinted vs White

Manhattan plot of GWAS comparing case (tinted eggshell) and control population (white eggshell).



Conclusions

This study shed light on the genomic architecture underlying shank and eggshell colour in Italian local chicken breeds. These phenotypes play an important role in breed identification and conservation.

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