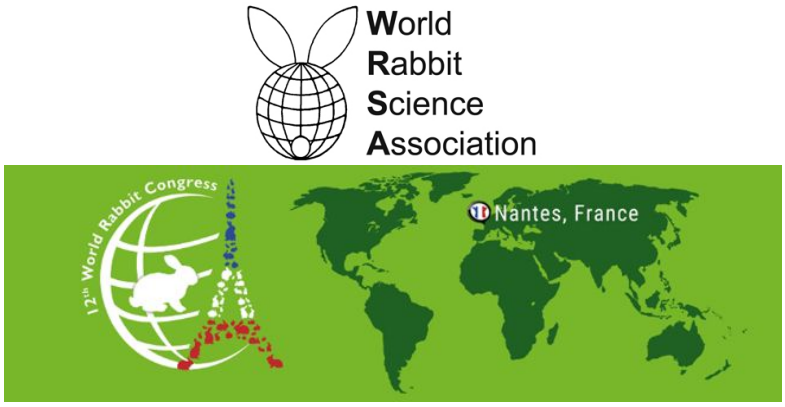

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DECIPHERING THE MOLECULAR ARCHITECTURE OF THE COAT COLOUR VARIABILITY IN A EUROPEAN RABBIT POPULATION

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ABSTRACT

Understanding the molecular mechanism of coloration has been the goal of many genetic and evolutionary studies in a broad number of species. Nevertheless, most of our current knowledge is restricted to colour traits exhibiting relatively simple discrete variation and inheritance patterns. More than a hundred of genes are involved in coloration traits in rodents and many mutations have been identified. In the European rabbit (*Oryctolagus cuniculus*), different coat colours have been selected through domestication and are nowadays fixed in specific breeds. Although numerous mutations affecting coat colour have been discovered in various rabbit breeds, additional variants have still to be identified. Despite the evolution of technologies through the genomics era, understanding the molecular architecture of such complex phenotypes still remains a challenge. Here, we propose a genome-wide investigation of coat colour using rabbit high-density SNP array. We performed genome-wide association studies (GWAS) considering the variation of coat colour as quantitative and binary phenotypes. We identified several significant SNPs marking loci already known to affect coat colour as well as in a few other chromosome regions not yet described to affect this phenotype in rabbits (e.g. a genomic region on chromosome 14). Moreover, we determined the best model of inheritance for each region associated to coat colour. Our results bring new insights into the molecular architecture of the coloration phenotype pinpointing its oligogenic determinism.

Key words: Coat colour, SNP array, GWAS, Dominance/Recessivity.

INTRODUCTION

More than one hundred of genes have been described to affect coloration traits in model species such as drosophila or mice (San-Jose and Roulin 2017). They can be classified according to their phenotypic effects affecting either the development of melanocytes (*KIT* and *MITF* genes) or the melanin synthesis (*TYR*, *TYRP1*, *OCA2*, *MATP* genes) or the switch between eumelanin and pheomelanin production (*MC1R* and *ASIP* genes).

In the European rabbit (*Oryctolagus cuniculus*), different coat colours have been selected through domestication and are nowadays fixed in specific breeds. Therefore, candidate gene approaches have allowed the identification of various mutations responsible of different phenotypes such as the coat colour dilution (Fontanesi et al. 2014; Lehner et al. 2013) and the brown phenotype (Utzeri, Ribani, and Fontanesi 2014). Six main loci (called A for Agouti, B for Brown, C for Colour, D for Dilution, E for Extension and En for English Spotting) are involved in the coloration of the coat. Notably, dominance/recessivity relations do exist between different mutations at one locus that result in distinct phenotypes. For instance, at the C locus, the Cch mutation is responsible of the chinchilla phenotype while Ch and c mutations are associated with Himalayan and albinos coat coloration (Aigner et al. 2000), respectively. Although mutations affecting coat coloration have been discovered in various rabbit breeds, additional variants have still to be identified.

Our objective is a better understanding of the genetic determinism of coat colour variability in the European rabbits using for the first time in this species a genome-wide approach.

MATERIALS AND METHODS

Animals and experimental design

Rabbits are from the French INRA 1001 Californian line (Larzul and De Rochambeau 2005), they are bred in the experimental INRA farm (Toulouse, France) in accordance with the national regulations for animal care and use of animals in agriculture. This line, selected for decreasing residual feed intake, has been originated from Californian rabbits and a coat colour variability is observed within the Himalayan phenotype as described in the following paragraph (Figure 3). The experimental cross between the control line and the tenth generation of selection is described in Garreau et al. (2019), and included 711 rabbits from 20 sires.

We firstly distinguished 5 different rabbit colour groups (from white to chocolate Himalayan) by visual inspection of the whole population: colours were classified as x_1, x_2, x_3, x_4 and x_5. Secondly, we selected few individuals per group (n=15) that were phenotyped for their nose coloration using a colorimeter to validate our subjective classification. A significant correlation was observed between the luminescence (L*) measurement and the note definition (Figure 1A) validating the determined groups. Moreover, an additional group was created since some animals from class x_2 had colored ears but white noses. Altogether, 6 ordered phenotypes were defined, sorted from P1 to P6 (Figure 1B), and 686 rabbits of the experimental design were assigned to one phenotypic group after notation by two independent experimenters.

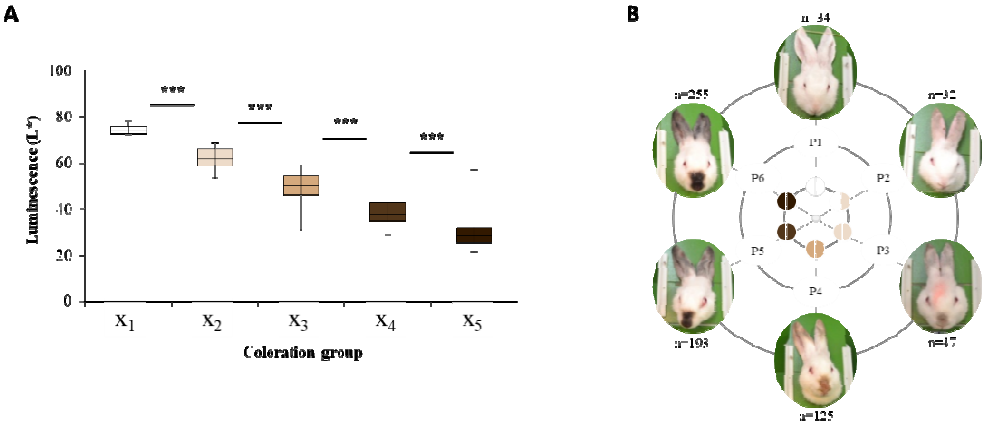

Figure 1: Phenotypic classification of coat colour variability. A- Colorimetric quantification using luminescence (n=15 per group). B- 6 coat colour phenotypes for the experimental design (n=686).

Genotyping data

Ear punch biopsies were collected and genomic DNA was extracted from samples following a salt-based DNA extraction. Rabbits were genotyped at the Centro Nacional de Genotipado (CeGen) platform (Santiago de Compostela, Spain) using the Affymetrix AxiomOrcun SNP Array as recommended by the manufacturer. The SNP-array contains 199,692 molecular markers spanning both chromosomes and scaffolds. The SNP data was filtered using following quality control criteria: call rate ≥ 0.95 and minor allele frequencies ≥ 0.01. After quality control, 686 animals and 162,070 SNPs were used for association analyses.

Statistical Analysis

We used the GEMMA (Genome-wide Efficient Mixed Model Association) software to perform association analyses. Briefly, GEMMA fits both a univariate linear mixed model (LMM) (Zhou and Stephens 2012) and a Bayesian sparse linear mixed model using Markov chain Monte Carlo (BSLMM) (Zhou, Carbonetto, and Stephens 2013); both methods control for population structure. We

also used the SuSiE (Sum of Single Effects) model, a Bayesian analogue of traditional stepwise selection methods, to fine-map the different loci (Wang et al. 2018). To visualize and determine the best classification of individuals given their genotypes, we build a decision tree using the Classification And Regression Trees (CART) algorithm (Breiman et al. 1984) implemented in the rpart R package (Therneau, Atkinson, and Ripley 2015).

RESULTS AND DISCUSSION

To highlight genomic regions associated with coat colour variability in the French INRA 1001 Californian line, a GWAS was conducted (Figure 2). A group of more than one hundred of markers located on Ocu1 (for *Oryctolagus cuniculus* chromosome 1) and close to *TYR* showed significant association results with the best signal for AX-146986391 (P = $2.36*10^{-56}$). Numerous significant signals respectively located on Ocu3 (AX-147059932, P = $9.70*10^{-06}$), Ocu4 close to *ASIP* (AX-147169681, P = $4.84*10^{-06}$), Ocu15 within *KIT* (AX-146983797, P = $6.80*10^{-11}$) were obtained. In addition, groups of variants located on scaffolds GL018754 (AX-147179313, P = $2.83*10^{-06}$) and GL018965 close to *MC1R* (AX-147173908, P = $5.49*10^{-05}$), here regrouped in Ocu23, also showed a trend for association. ***In our European rabbit population, coat color seems under an oligogenic determinism with a few loci contributing significantly to the trait.***

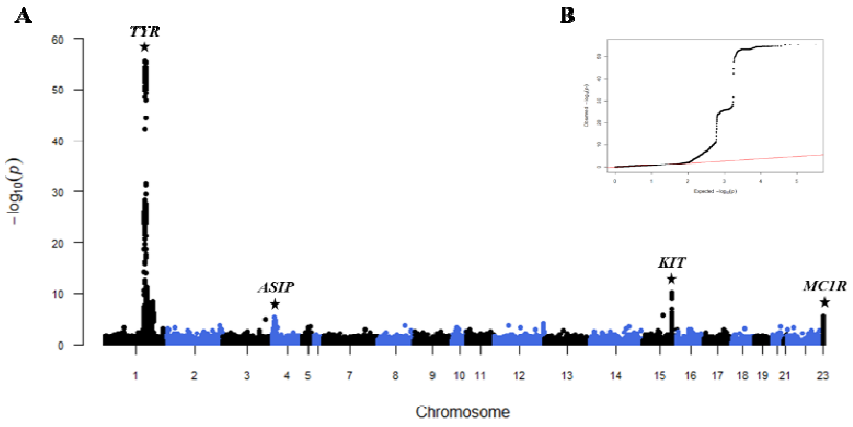

Figure 2: GWAS for coat color in the French INRA 1001 Californian line. A- Manhattan plot shows the significance ($-\log_{10}(P)$) on the y axis and SNPs position sorted by chromosome (OryCun 2.0) on the x axis. B- Quantile-Quantile plot of the $-\log_{10}(P)$.

We then focused on best associated markers to decipher how various regions contribute to the different coat colour phenotypes. The genotypic distribution showed that minor allele for variant AX-146986391 segregated exclusively within the phenotypic class 1 with a recessive inheritance pattern. Moreover, an association analysis testing genomic association with a binary phenotype (0=phenotype P1 *vs*. 1=all other phenotypes) highlighted a unique significant signal on Ocu1 with the best signal under a recessive model. A similar pattern of inheritance was observed for the phenotype P2 that is characteristic of the spotting phenotype (Figure 1B) with an excess of homozygous for the minor allele of AX-146983797. The association signal for the class P2 was explained only by variants of the chromosome 15 with the best p-value under a recessive model. These results suggested that ***two major genes with a recessive determinism, located on Ocu1 and Ocu15, are responsible of white and spotted phenotypes***, respectively.

To better decipher the molecular architecture of other phenotypic groups (Figure 1B), we only considered those individuals in further analyses. We used Bayesian methods, implemented in both GEMMA and SuSiE software, for identifying and fine-mapping quantitative trait loci (QTL) for the remaining coat colour variability. Two loci with large effects, located on Ocu1 and scaffold GL018965, showed high probabilities of being QTLs (70% and 78%, respectively). Two additional QTLs showed intermediate probabilities but also large effects, they are located on Ocu1 (36%) and Ocu4 (55%), and one QTL, located on Ocu14, showed a suggestive probability (20%) of being a QTL. Interestingly, the regions located on Ocu1, Ocu4 and GL018965 and spanning *TYR*, *ASIP* and *MC1R* genes respectively, were already highlighted in the first GWAS analysis (Figure 2) while the second

interval of Ocu1 and Ocu14 were novel loci. In total, ***5 loci accounted for the coat colour variability of phenotypic groups P3 to P6***.

To determine the best classification of individuals for their phenotypic value, we built a decision tree based on their genotypes at the 7 identified loci. The coloration was correctly predicted for 59% of rabbits (Figure 3) and misclassified animals are mainly included in the closest phenotypic group.

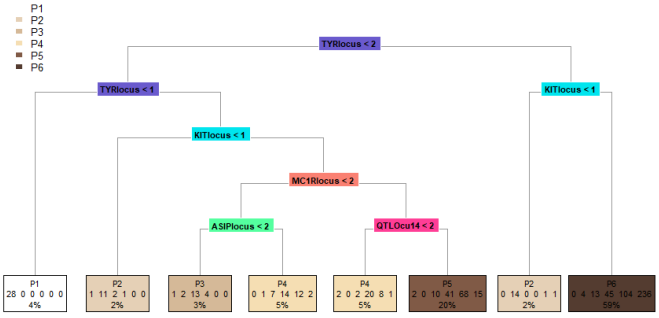

Figure 3: Genotype-phenotype visualization *via* a decision tree. Number of minor alleles at each of the 7 involved loci in the coat colour variability (best associated SNP) was counted (0=homozygous for the minor allele, 1= heterozygous and 2=homozygous for the wt allele). Nodes represent variants of interest, edges discriminate genotypes and leaves are the 6 phenotypic groups (P1 o P6).

CONCLUSION

Altogether, our results suggest that a few regions account for a major component of the phenotypic variance but also additional regions may contribute to the molecular architecture of the coat colour variability.

ACKNOWLEDGEMENTS

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Deciphering the molecular architecture of the coat colour variability in a European rabbit population

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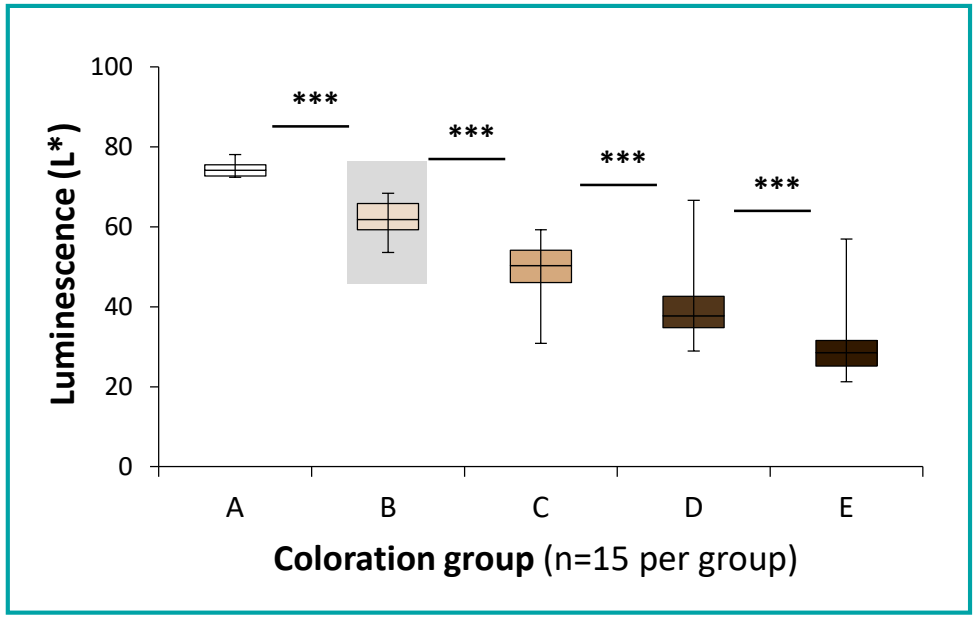
Gradient and pattern of colour within a French Californian population

- Line of rabbits selected on feed efficiency traits *(Drouilhet et al. 2013)*

… but both gradient and pattern of colour segregated within the Himalayan phenotype

- Evaluation of the colour variation of body extremities as a continous trait



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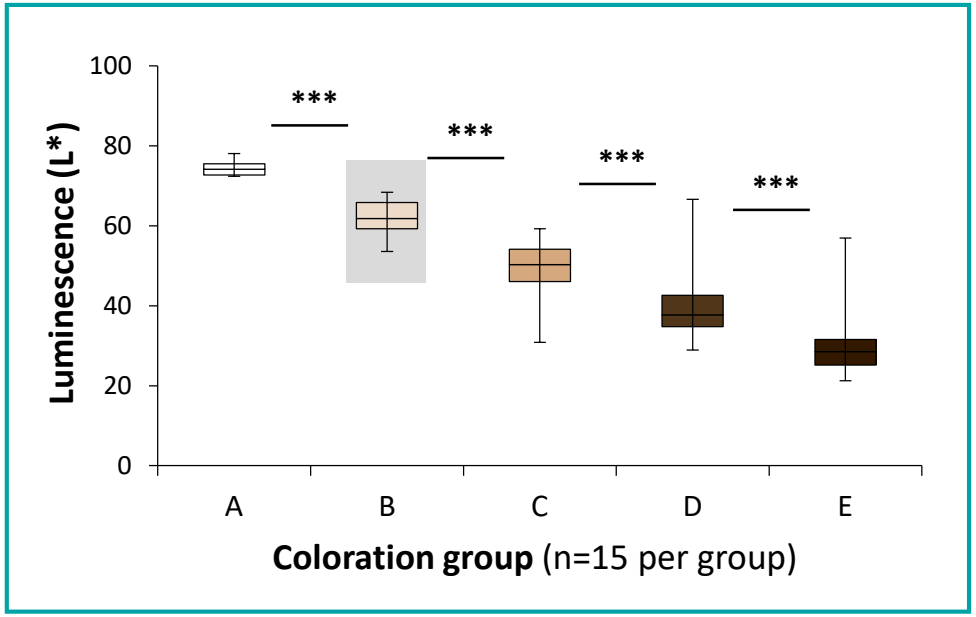
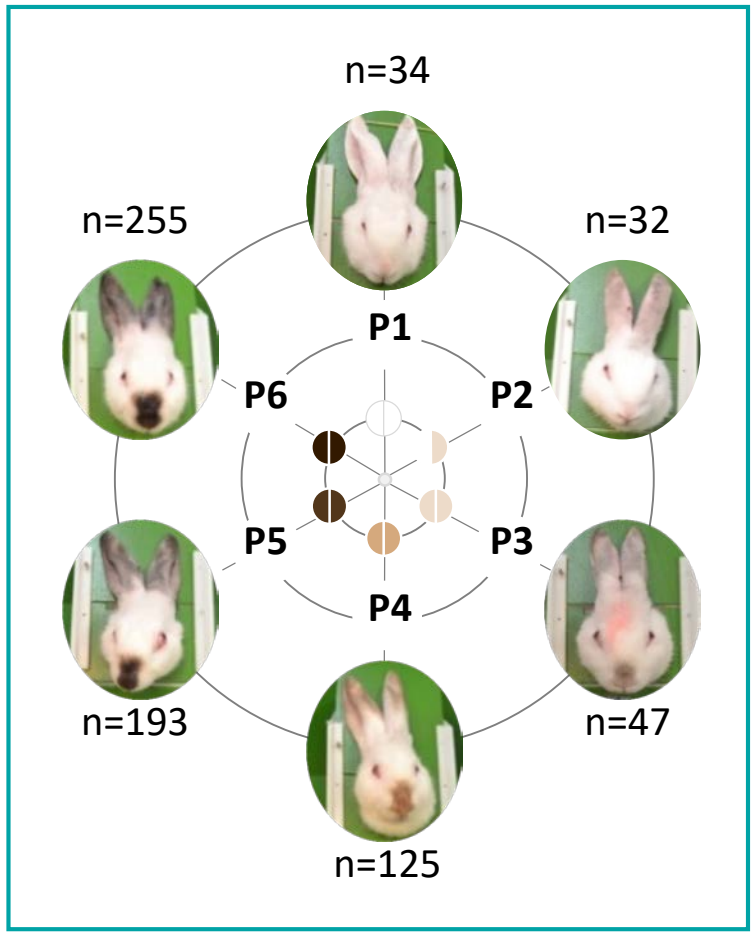


What is the molecular architecture of colour variation of body extremities ?

Description of the familial experimental design *(Garreau et al., 2019)*



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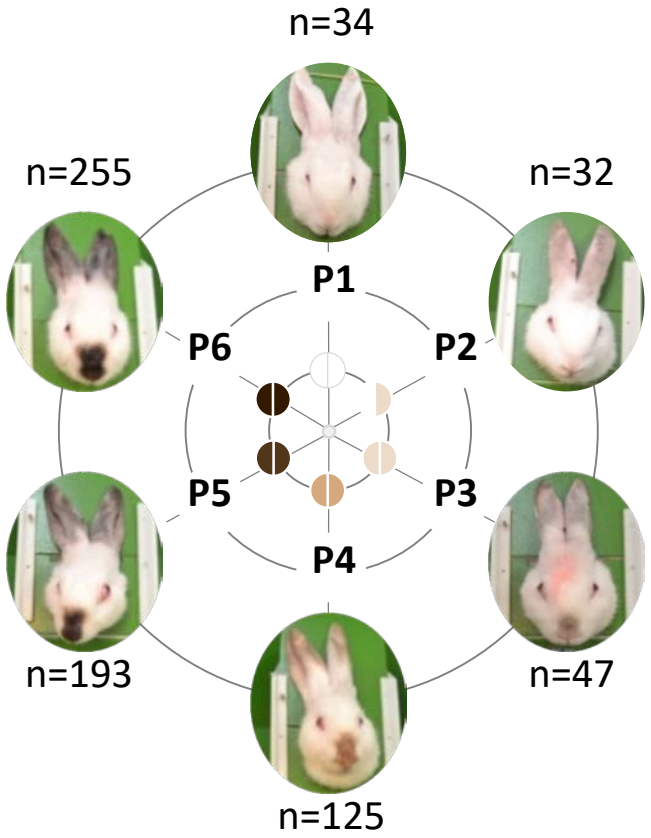
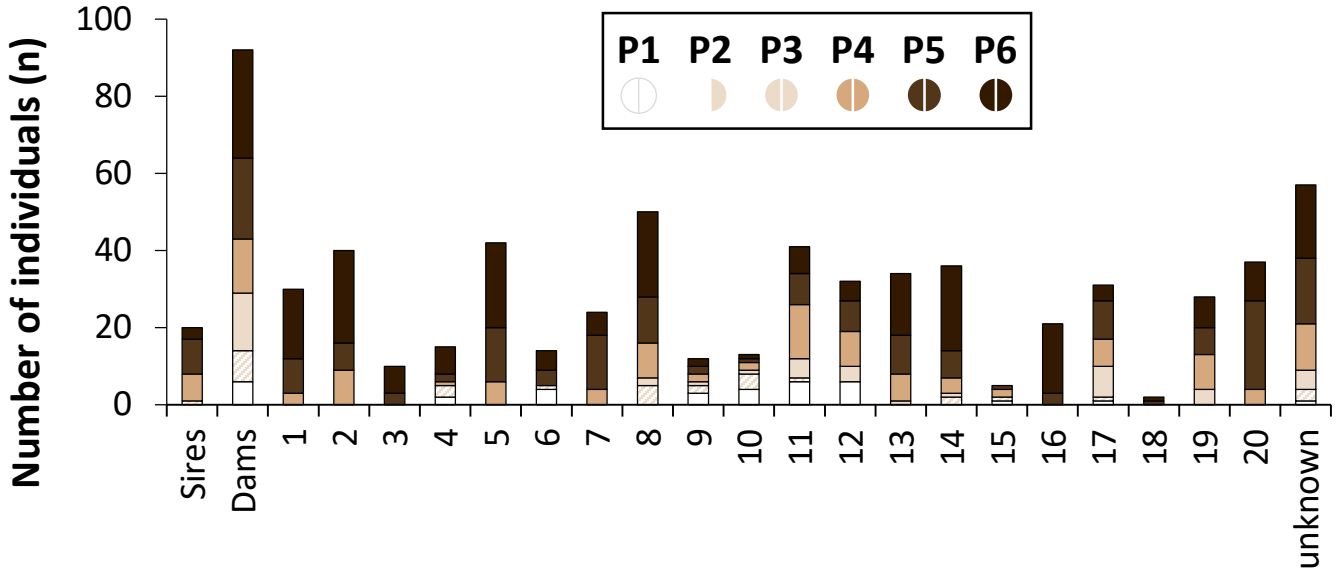




- 20 bucks, 92 does and 574 offspring
- Phenotyping and Genotyping

  6 distinct categories ordered from the lighter to the darker

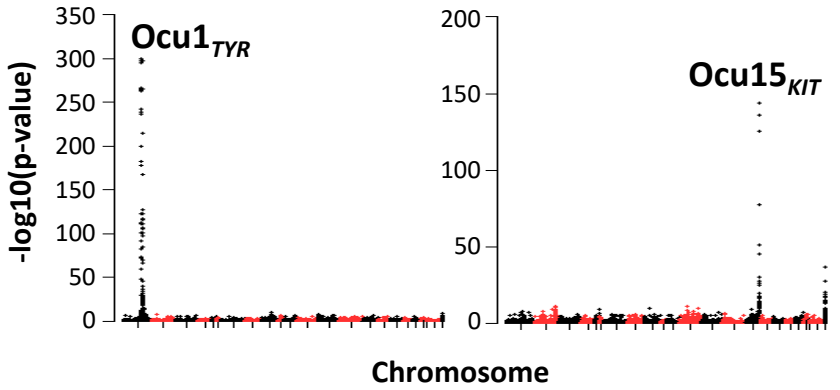
  Affymetrix® AxiomOrcun™ SNP Array (200k)

At least 7 loci associated with coat colour of body extremities

- Two major genes with recessive effects are associated with P1 and P2 phenotypes

  $Ocu1_{TYR}$ locus ≈ P1 white phenotype - $Ocu15_{KIT}$ locus ≈ P2 spotting phenotype

Recessive linear mixed model

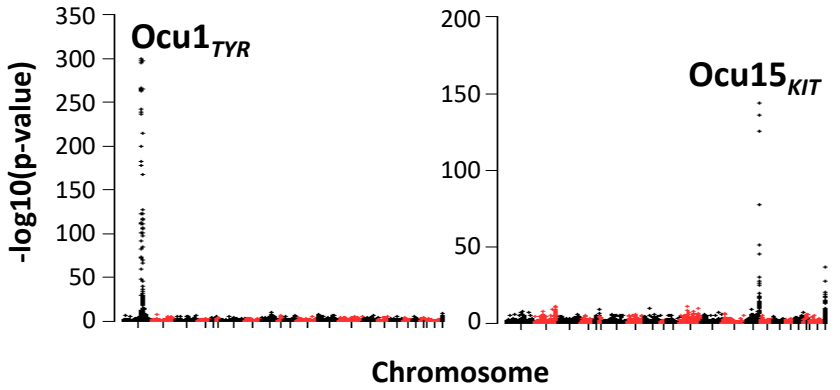


At least 7 loci associated with coat colour of body extremities

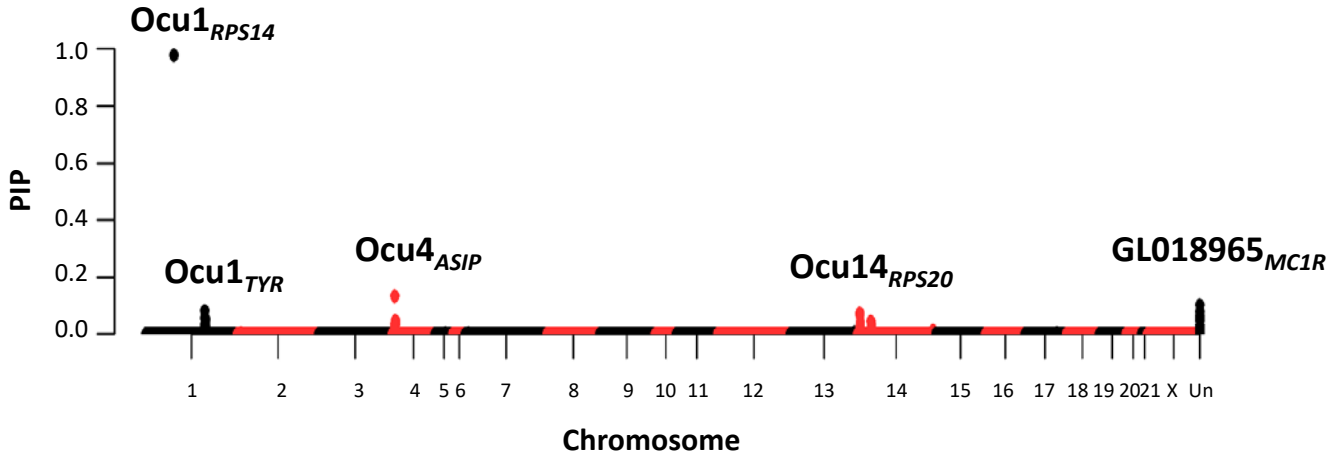
- Two major genes with recessive effects are associated with P1 and P2 phenotypes
  $Ocu1_{TYR}$ locus ≈ P1 white phenotype - $Ocu15_{KIT}$ locus ≈ P2 spotting phenotype

- Five additional loci account for the remaining P3 to P6 coat colour of body extremities

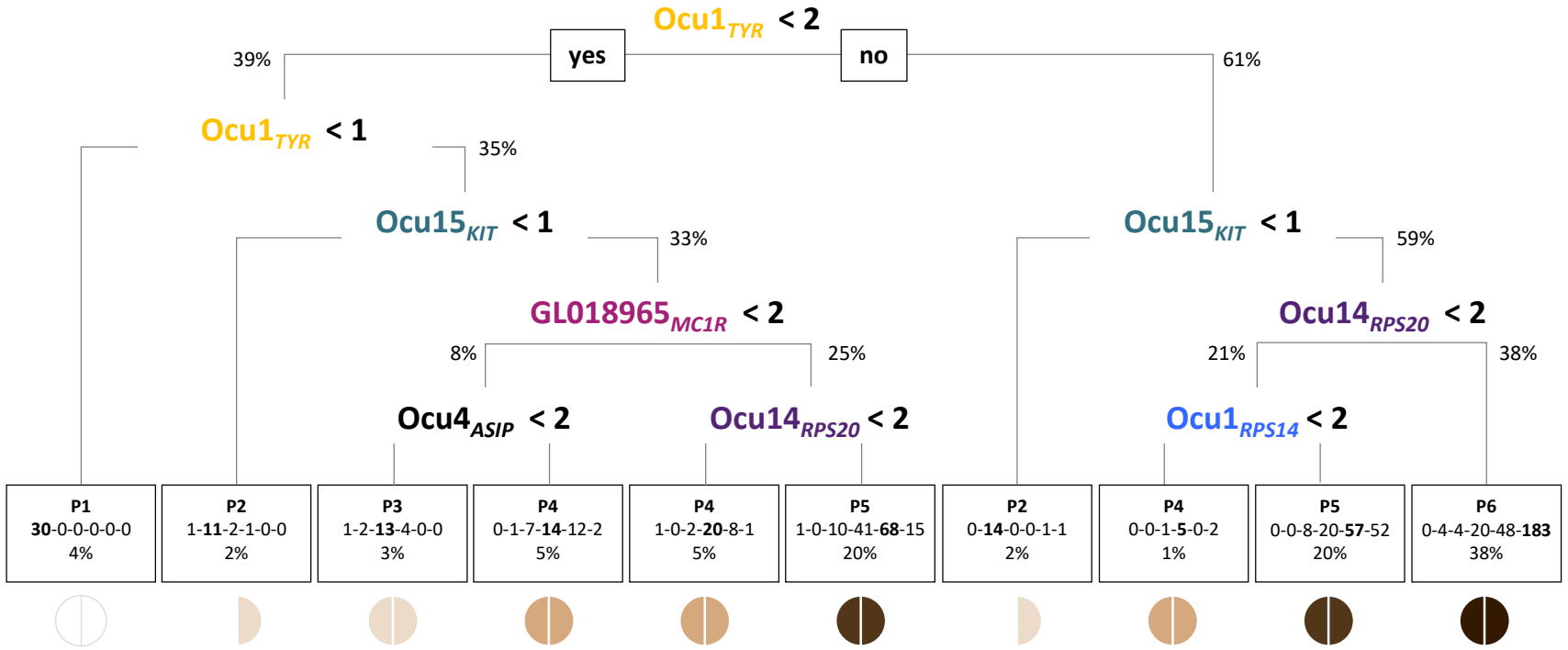
Recessive linear mixed model



Sum of single effects model



Gene-gene interactions contribute to coat colour of body extremities



- ***Ocu4*$_{ASIP}$: *GL018965*$_{MC1R}$ ≈** light P3 and P4 phenotypes
- ***Ocu15*$_{KIT}$: *Ocu*$_{RPS}$ ≈** dark P5 and P6 phenotypes
- ***Ocu1*$_{TYR}$ and *Ocu15*$_{KIT}$** account for P1 and P2 phenotypes, respectively

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Thank you for your attention

Demars J., Labrune Y., Iannuccelli N., Aymard P., Benitez F., Leroux S., Gilbert H. and Riquet J.

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