Sketching Man's Best Friend – Canine DNA Phenotyping for Forensic Purposes

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Introduction

Forensic Short Tandem Repeat (STR) analysis is the gold standard to identify canine individuals, or to link crime scene traces to the donor dog. However, forensic DNA fingerprinting loses its evidential power when no canine suspect DNA is available. To overcome this limitation, we aim to develop a method to predict externally visible traits of dogs based on DNA. Previous studies have already shown that externally visible characteristics of dogs are caused by variations in a surprisingly small number of genes and only a few mutations are probably responsible for the extraordinary diversity of this species [e.g. Boyko et al. 2010]. Due to this special characteristic of dog genetics, developing a suitable marker set for typing external visible traits appears highly promising and could contribute significantly to the toolbox for canine DNA analysis in forensic casework. An outline of this approach and first results are presented here.

Sampling and marker verification

Sampling was performed in Austria, Germany and Switzerland by collecting buccal swabs and took place by direct queries to private dog owners, breeders and by visiting dog shows and obedience schools. The appearance of the sampled dogs were recorded based on a pre-defined list of externally visible characteristics and by photo documentation (Figure 1). Phenotype specific markers that seemed to describe useful visible traits for forensic purposes were taken from literature focusing on the genetic basis of dog phenotypic appearance [e.g. Newton et al. 2000 and Cadieu et al. 2009]. 67 SNP markers were pre-selected for verification testing via Sanger-sequencing. For each marker, ten dogs have been tested, five for each variety of a characteristic.

Figure 1 Example of an obligatory photo taken for a sampled dog.

Table 1 Marker evaluation of the verification tests.

Number of Markers	Description
32	Strong genotype-phenotype association
11	Moderate genotype-phenotype association
11	Uncertain results
10	Not feasible in laboratory work (e.g. primer design failed)
3	Excluded from verification testing due to an unjustifiable effort regarding the potential outcome

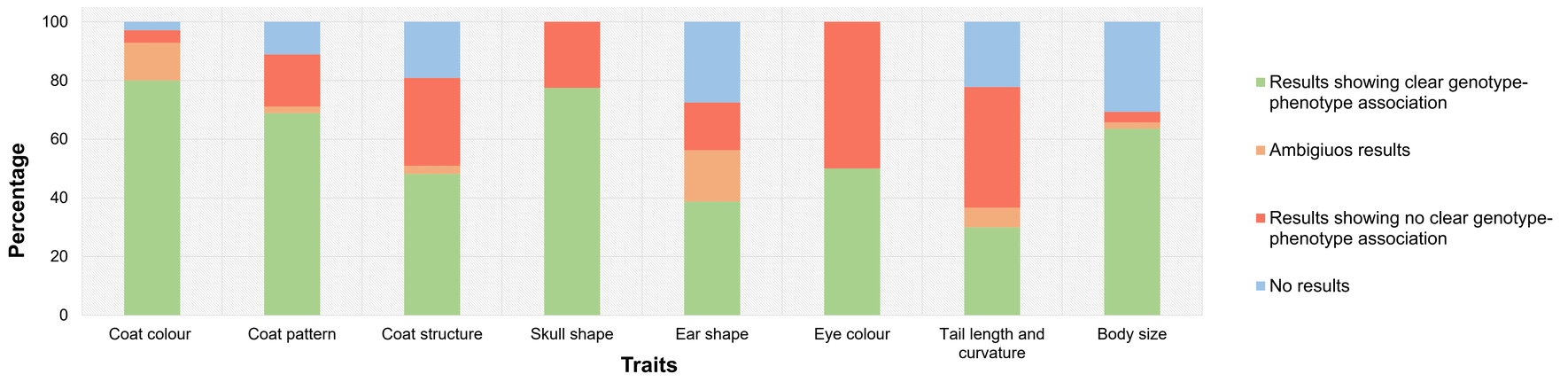

Figure 2 Average marker performance for the relevant canine phenotypic traits in the verification tests.

Results and discussion

Based on the results of the Sanger-sequencing data, the markers have been evaluated (Table 1) and the performance was checked for each trait (Figure 2). It became apparent that roughly two-thirds of the markers show results as anticipated (Table 1), underlining the predictive power of canine phenotype altering variants. Moreover, certain traits such as coat colour appear to be easier to predict than tail related characteristics for instance (Figure 2). Eventually, a set of 43 markers was developed. Three examples for phenotypic SNPs and their effects on the canine outward appearance are shown in Figure 3.

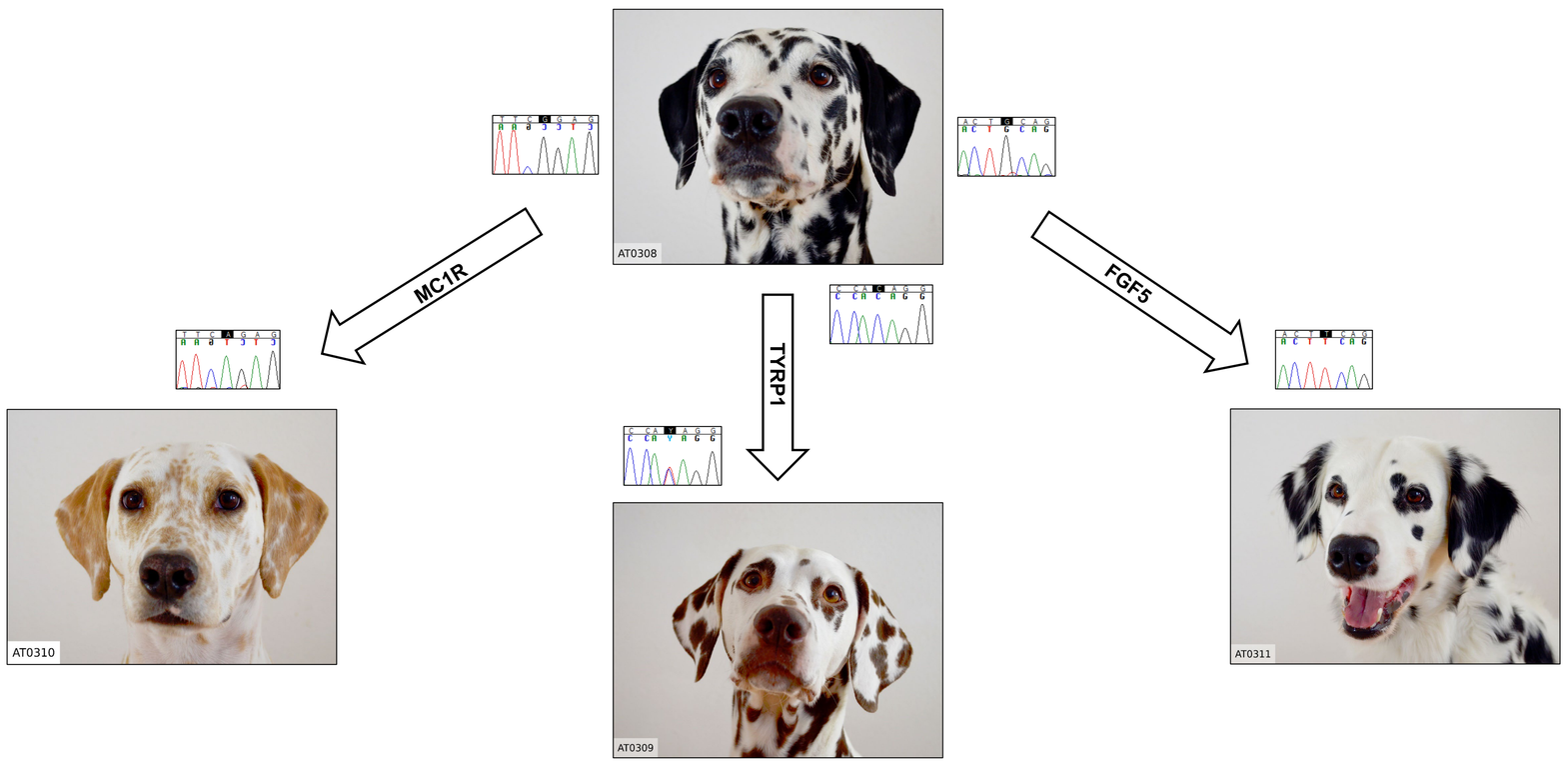

Figure 3 Different phenotypic manifestations in Dalmatian dogs based on SNPs in the *MC1R*, *TYRP1* and *FGF5* genes. Screenshots of the particular sequence variants found in the corresponding dog individuals are shown (indicated in black). All images displayed refer to purebred Dalmatians, having similar characteristics regarding their size, ear and skull shape, tail length and coat pattern. The most noted Dalmatian phenotype, including a short coat and black spots on white background, can be seen in dog AT0308. The SNP in *MC1R* (melanocortin 1 receptor) leads to the yellow coat colour in dog AT0310. The variant in the *TYRP1* (Tyrosinase-related protein 1) gene induces the brown dots in dog AT0309. Besides the coat colour alteration, this SNP leads to a brown coloured nose and amber eyes. A variant changing the hair length of dogs can be detected in *FGF5* (Fibroblast growth factor 5), as seen in the long-haired phenotype in dog AT0311.

Conclusion

The results of the verification testing support the idea of applying the predictive power of canine phenotype markers for forensic purposes. Next step leading to a practicable forensic molecular genetic tool is going to be the transfer to massively parallel sequencing (MPS) and the analyses of a comprehensive sample set.

References

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