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ORIGINAL ARTICLE

Tracing the origin of 'blue Weimaraner' dogs by molecular genetics

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Summary

Weimaraner dogs are defined by light brown coat colour termed grey including several shadings ranging from silver and deer to mouse grey. In contrast, the so-called blue Weimaraners (BW) with lightened black-pigmented coat have been proposed to represent spontaneous revertants in the Weimaraner breed. In order to investigate the genetic determinants of the characteristic grey coat colour versus those of BW, known variation in coat colour genes including *TYRP1* and *MLPH* were analysed in a number of grey and blue dogs. Variations at the *B* locus cause grey coat colour in Weimaraners via two non-functional *TYRP1* copies *(bb)* including the b^s, b^d and b^c alleles. In all BW, at least one functional *TYRP1* allele (*Bb* or *BB* genotype) was identified. Defined microsatellite alleles in *TYRP1* intron 4 are linked to this functional *B* allele in BW. These alleles were also detected in various other dog breeds, but not in grey Weimaraners. The combination of a dominant trait for blue versus grey together with a specific *TYRP1* haplotype in BW suggests that blue coat colour is not the result of spontaneous (back-) mutation in grey Weimaraners. This inference is even emphasized by the presence of a unique Y-chomosomal haplotype in a male offspring of the supposed ancestor of the BW population which – according to pedigree information – carries a copy of the original Y chromosome. Thus, molecular genetic analyses of coat colours combined with Y-chromosomal haplotypes allow tracing the origin of atypical dogs in respective canine populations.

Introduction

The Weimaraner, originally designated as Weimar pointer, represents an old breed of hunting dogs originating in Germany, where the first breed standard was established in 1878 (Schmidt 1989). The German breed comprises two varieties in one population (Schrameyer *et al.*, 2005) distinguished by their coat types, the more common short-hair (SH) and the long-hair variety (LH). Besides these differences in hair length, there are also coat colour variations in the Weimaraner breed including grey shadings ranging from silver and deer to mouse grey (Figure 1; cf. FCI standard Nr. 99/13.02.2002/D). The grey coat colour is the result of brown eumelanin pigmentation together with lightening of colour by the dilute phenotype. This characteristic colour led to the designation 'the greys' in order to denominate the entire breed. In addition, traditional Weimaraners exhibit amber eyes, grey nails and a grey or flesh-coloured nose. In contrast to this colour standard, a darker phenotype resulting from black eumelanin pigmentation combined with the dilute phenotype has been designated as 'blue Weimara-

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Figure 1 Typically grey Weimaraner (left) and blue Weimaraner (BW, right), both of the short-hair variety.

ner' (BW). Amber eye colour and a charcoal or blue nose are further characteristics (see Figure 1). In present-day BW, similarly as in the greys, a range of blue shades is observed from relatively 'dark blue' with little dilution to 'silver blue' with more dilution.

BWs are mostly bred in the US, and the American population of BW can be traced back to a single dog with dark coat colour originating from Germany according to breeding records. This ancestor of the BW population has been photo-documented in 1947 and produced 195 registered offspring. Until now, the derivation of his atypical coat colour and his progeny has not been examined using molecular genetics.

According to the breeding records, the blue colour is inherited in a dominant manner, while grey is inherited recessively. Up to date, BWs have been crossed regularly with their grey counterparts for probably more than 60 years according to breeding records. Thus, the genetic background of the grey Weimaraner and BW may be quite similar. Genes involved in the phenotypic expression of canine black coats represent prime candidates for studying colour differences and the origin of BW. Here, we investigate which gene determines the blue phenotype in these dogs. Because the blue coat colour phenotype in respective dogs has been interpreted as dilute black, genes causing black coat colour in other breeds are strong candidates for differential colouring of grey and BW. For an overview of canine colour genes, see Schmutz & Berryere (2007).

Among the candidate genes decisive for grey versus blue coat colour explored here are the melanocortin receptor 1 (*MC1R, E-locus*) and its ligands encoded by *ASIP* (*A*-locus) and *CBD103* (*K*-locus). These proteins are involved in a process termed pigment-type switching determining yellow/red pheomelanin pigmentation versus black or brown eumelanistic coat colour (Candille *et al.* 2007). Yet, black coat colour in dogs can also be caused by deletion of one codon in the gene *CBD103*, altering the affinity of this ligand to the MC1R receptor resulting in dominantly inherited dark (eumelanistic) coat (Candille *et al.* 2007). On the other hand, black coat colour in the German Shepard dog is caused by a mutation in *ASIP*, also encoding a ligand for the MC1R receptor. In this case, black is inherited in an autosomal recessive manner (Kerns *et al.* 2004) and hence probably less relevant for grey Weimaraner versus BW. Another possibility for the formation of black or brown eumelanin pigment is a functional MC1R signalling pathway together with the *TYRP1* gene, encoding tyrosinase-related protein 1, a gene expressed in the melanosome. In this gene, three common variations are frequently found in different dog breeds: brown coat is recessively inherited to black if one or more of the following alleles are present in each copy of the *TYRP1* gene: b^c, b^s or b^d. In contrast, one functional *TYRP1* copy (*B* allele) without any of these variations determines black coat (Schmutz *et al.* 2002).

In addition to these loci that define the basic coat colour, 'dilute' defines whether the original coat colour is somewhat lightened, meaning whether the present pigment is 'diluted' on the body surface. Dilute phenotypes are associated with the gene encoding melanophilin (*MLPH)* and are inherited autosomal recessively (genotype designated *dd*; Welle *et al.* 2009; Philipp *et al.* 2005). The presumably causative mutation in *MLPH* was recently identified (Drögemüller *et al.* 2007). In the presence of brown eumelanin pigmentation together with dilute the dog appears grey, whereas black eumelanin pigment together with dilute is designated blue. Dilute phenotypes characterize grey and BW as expressed in lightened brown or black coats, the molecular genetic basis of which was still to be established.

In this study, DNA sequence variations were investigated accounting for the difference in coat colour between the traditional light brown-coated greys and light black-coated BW, by characterizing the *CBD103, MC1R*, *MLPH* and *TYRP1* candidate genes.

Finally, this study should reveal whether blue coat colour can be explained by known genetic variations in black coat colour-related genes or whether a single new mutation is responsible for the phenotypic difference between grey Weimaraners and BW. Could this coat colour mutation even be traced back to the first BW recorded in the stud books? To clarify this question, the Y-chromosomal haplotype of an offspring of the first BW was analysed in this study in order to compare it with known Y chromosomes from the greys. Because the Y chromosome is largely excluded from meiotic recombination (Raudsepp & Chowdhary 2008), Y haplotype markers can be used to trace ancestral haplotypes in combination with breeding record information (Parra *et al.* 2008). Coat colour gene and Y-chromosomal variations may thus reveal genetic interrelations of grey Weimaraners and BW.

Material and methods

Samples

Twenty-four registered BW from Canada, USA, France and Germany were investigated as well as one 'grey Weimaraner' descending a 'blue × blue Weimaraner' cross from the US. Most of the studied dogs are closely related belonging to one of two large pedigrees; six additional unrelated BW not comprised in these pedigrees were included in this study. All blue dogs were of the SH variety. In addition, twenty registered unrelated grey Weimaraners were examined, of both the SH and the LH varieties from Germany. Additional dogs from different breeds (four Australian Shepards, a Curly Coated Retriever, two Dachshunds, a Cocker Spaniel, a Springer Spaniel, a Poodle, three German Pinschers, three Large Munsterlanders and three Löwchen) with (partial) black coat colour were selectively characterized. Buccal swabs or blood samples were received as authorized by the owners of the dogs. DNA was isolated according to standard protocols (Miller *et al.* 1988) or the QIAamp DNA Blood MiniKit (Qiagen, Hilden, Germany) following the manufacturer's protocol. After DNA extraction, DNA concentration was measured using a NanoDrop ND-1000 spectrophotometer (Nanodrop Inc., Rockland, DE, USA).

Standard PCR protocol and sequence analysis

For the analysis of candidate genes, we surveyed known variations in four genes: *CBD103, MC1R, MLPH, TYRP1* (for allele and genotype designations see Schmutz *et al.* 2002 and Candille *et al.* 2007). Typing was performed following standard polymerase chain reaction (PCR) protocols. PCRs were run in a thermocycler (Biometra, Göttingen, Germany). Ten μl PCR amplification mixture containing 50 ng DNA in 100 mM Tris (pH 8.3), 1 U Taq Polymerase (Genecraft, Münster, Germany), 0.2 mM of each dNTP, 0.7 mM of each primer (Table S1) and 1.5–2 mM $MgCl_2$ in 500 mM KCl was run in 96-well microtiter plates (Thermowell Costar, Corning, NY, USA). Alternatively, a Mastermix (Hotstar Mastermix; Qiagen) was used, PCRs were performed under standard PCR conditions (Dekomien & Epplen 2002) with annealing temperatures indicated in Table 1A and 30 cycles in a thermocycler. Different PCR-based analysis methods were applied for genotyping the aforementioned genes. PCR products were resolved and visualized on 2–3% agarose gels stained with

Table 1 Observed haplotypes in the *TYRP1* gene of typically grey Weimaraners and BW (A) and haplotype counts (B) in typically grey Weimaraners and BW

A						
Haplotype		1	2	3	4	5
Position	Variation	Grey		Grey and blue		Blue
Exon 2	C41S	*S*	*S*	*S*	C	C
Intron 4	STR	381	385	381	383	377
Exon 5	Q331X	Q	Q	Q	*X*	Q
Exon 5	345delP	P	P	*delP*	P	P
Allele		b^c	b^c	b^{cd}	b^s	*B*
B						
Haplotype		1	2	3	4	5
Grey coat colour (n = 21)		5	1	26	10	0
Blue coat colour (n = 24)		0	0	13	7	28

BW, blue Weimaraners

ethidium bromide. In order to control the genotyping results, several randomly chosen PCR products with evaluated genotypes were exemplarily sequenced. Sequencing reactions were carried out by the dideoxy chain termination method using the Dyenamic ET Terminator Kit (GE Healthcare, Munich, Germany) according to the manufacturer's instructions. Sequences were run on an automated capillary DNA sequencer (MegaBACE 1000, GE Healthcare, Germany). Sequences were edited, assembled and aligned using the program SeqMan (DNAstar, Madison, WI, USA).

CBD103 p.23delG (ΔG23, K^B allele) genotyping

The 'tailed primer PCR' (Jagiello *et al.* 2004) was used for *CBD103* typing in order to resolve the 3 -bp difference between wild type (k^y allele) and ΔG23 (K^B allele) genotype (Candille *et al.* 2007), which cannot be resolved on agarose gels. This method requires three oligonucleotides for amplification: a tailed forward primer (tailed F), a reverse primer and a fluorescence labelled primer (labelled F) corresponding to the 5′-tail sequence of tailed F. PCR conditions were the same as described in the standard PCR protocol except for the primer concentrations which were as follows: 0.2 pmol tailed F; 2.5 pmol labelled F and; 2.5 pmol reverse primer. PCRs for microsatellite amplification were also performed under standard PCR conditions (Dekomien & Epplen 2002) of 57°C annealing temperature and 30 cycles in a thermocycler. PCR products were resolved on a Beckmann CEQ8000 and sized and scored with the respective analysis software.

MC1R p.R306X (*E* allele) genotyping

The *MC1R* p.306X allele (*e*) and wild-type allele (*E*) see Schmutz *et al.* (2002) was typed by PCR-restriction fragment length polymorphism (RFLP) analysis. In addition to the standard PCR protocol, 12% DMSO was included in the PCR mix, and a primer mismatch was introduced as indicated in Table S1 in order to distinguish between wild type and 306X allele. PCR products were digested with the restriction enzyme BglII (New England Biolabs, Ipswich, MA, USA).

MLPH c.106C≥T (*d* allele) genotyping

The c.106C>T variation used for typing the *dd* genotype is not a direct test involving the causal mutation, but a SNP associated with the dilute phenotype in a variety of dog breeds (Philipp *et al.* 2005). The c.106C>T (*d* allele) and wild-type allele (*D*) were analysed by PCR-RFLP. PCR products were digested with the restriction enzyme BsrI (New England Biolabs).

TYRP1 exon 2 p.C41S (b^c allele) genotyping

Denaturing high-performance liquid chromatography (dHPLC) analysis of *TYRP1* exon 2 was performed for p.C41S genotyping. After standard PCR amplification (for conditions see Table 1A) PCR products of the grey Weimaraner and BW DNA samples were used either unmixed or were mixed with a sample of a German Doberman Pinscher in order to reveal homozygous mutation carriers by heteroduplex formation (for methodological details see e.g. Schlang *et al.* 2008). The mixed or unmixed PCR products were denatured at 94°C for 3 min and gradually cooled to 20°C for heteroduplex formation. Thereafter, PCR products were subjected to dHPLC on a WAVE® system (Cheshire, UK) with the analysis software Navigator 2.2. dHPLC analysis has already been described elsewhere (Oefner *et al.*, 1998). The flow speed and the ratio of the buffer system were determined using the WAVE system software. In addition to the C41S mutation, two SNPs were demonstrated in the PCR product resulting in different elution curves. These SNP corresponded to one haplotype associated with the wild-type allele or the mutation as identified by sequence analysis of exemplary canine DNAs according to Schmutz *et al.* (2002).

TYRP1 exon 5 p.Q331X (b^s allele) genotyping

The *TYRP1* allele b^s (Schmutz *et al.* 2002) was analysed by PCR-RFLP. A primer mismatch was introduced as indicated in Table S1 in order to distinguish between wild type and b^s allele. PCR products were digested with the restriction enzyme NdeI (New England Biolabs).

TYRP1 exon 5 p.345delP (b^d allele) genotyping

The *TYRP1* variant 345delP (b^d allele, see Schmutz *et al.* 2002) was analysed by PCR-RFLP. PCR products were digested with the restriction enzyme MnlI (Fermentas GmbH, St. Leon-Rot, Germany).

Microsatellite typing at the *TYRP1* locus

The 'tailed primer PCR' (Jagiello *et al.* 2004) was used for microsatellite typing as described in *CBD103*

ΔG23 (K^B allele) genotyping with standard PCR protocol and primer sequences according to Table S1B for the grey Weimaraner as well as BW DNA samples. In addition, black dogs from different breeds as indicated earlier were typed for the STR 36350491 marker. The *TYRP1* gene is known to determine black coat colour in all of these aforementioned breeds (Schmutz *et al.* 2002).

Y-chromosomal haplotyping

For Y-chromosomal haplotyping, seven male BW as well as one grey Weimaraner (B25) descending from a blue × 'blue Weimaraner' cross as an offspring from the ancestor of the US BW population were investigated. For this, altogether nine markers (five SNP and four microsatellite markers) on the Y chromosome (Bannasch *et al.* 2005; Natanaelsson *et al.* 2006) were investigated according to Kropatsch *et al.* (in press) and compared to Y-chromosomal haplotypes in the German Weimaraner population as specified (Kropatsch *et al.* in press).

Haplotype and statistical analysis

Haplotypes for the *TYRP1* gene were generated using PHASE haplotype software, Version 2.1.1 for Windows (Stephens & Donnelly 2003). In order to evaluate significant associations, chi-square tests were performed using SigmaStat Version 2.03 (Systat Software Inc., Chicago, IL, USA).

Results

Analysing several coat colour genes relevant for the expression of black (blue) coat revealed uniform genotypes in known variations of the *CBD103* and *MC1R* genes in grey Weimaraners and BW (see Table S2). Therefore, the analysed alleles appear irrelevant for the difference in these coat colour phenotypes. Moreover, both, grey and BW exhibit the same variation within the *MLPH* gene in homozygous state, the *dd* genotype and the respective dilute phenotype (Table S2). Based on these results, brown eumelanin pigmentation together with dilute appears grey, whereas black pigment with dilute appears blue. In contrast to the aforementioned genes, *TYRP1* variations determine whether the coat colour in these dogs is grey versus blue. Multiple *TYRP1* alleles (*B*, b^c, b^s or b^d) contribute to the formation of black (*Bb* or *BB* alleles) or brown eumelanin pigment (*bb* alleles). These known variations were used as markers in the *TYRP1* gene in order to define haplotypes in grey and blue Weimaraners (Figure 2). Additionally, two microsatellite markers were used for haplotype reconstruction (Figure 2). *STR 36350491* is located in intron 4 of *TYRP1* and *FH2319* located 1100 kb 3′ of the gene. *FH2319* was not comprised in a distinct *TYRP1* haplotype and, therefore, disregarded for these analyses.

Assuming linkage between the different polymorphic sites in the comparatively small *TYRP1* gene and based on pedigree information (Figure 3), five different haplotypes were constructed in grey Weimaraners and BW (see Table 1; individual typing data are listed in Table S3). Haplotype 5 (C-377-Q-P) occurred exclusively in BW, it was evident in all blue dogs of this study (Table 1). This unique haplotype in BW gives rise to a functional *TYRP1* copy (*B* allele) and is the only haplotype exhibiting a 377 -bp allele of the microsatellite marker *STR 36350491*. Haplotypes 3 and 4 were found in both, grey Weimaraners and BW, whereas haplotypes 1–2 were exclusively found in the greys. Altogether, haplotypes 1–4 contain non-functional copies of the *TYRP1* gene constituting the alleles b^c, b^s or b^d. Table 1B summarizes the haplotype distribution among grey versus blue dogs. Haplotype 5 was never observed in the greys. The chi-square statistics for association testing of these haplotypes with blue coat colour was calculated as 38.634 with four degrees of freedom, rendering a p-value of <0.001 which indicates significant association. This finding of a characteristic haplotype in blue dogs corresponds with the data from two large BW pedigrees (Figure 3) where the presence of a haplotype containing the 377 -bp

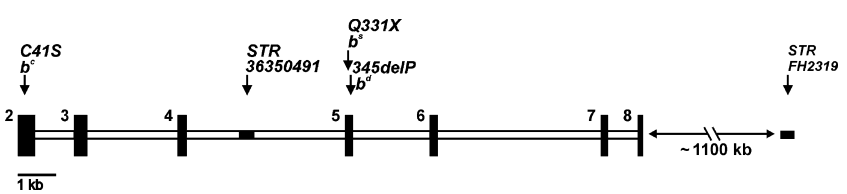

Figure 2 *TYRP1* gene organisation in dogs with coat colour variations and microsatellite markers (STR). Exon/intron organization [UCSC genome browser canFam2 assembly May 2005, chromosome 11, cDNA (AY052751)] with exon numbering according to Schmutz *et al.* (2002).

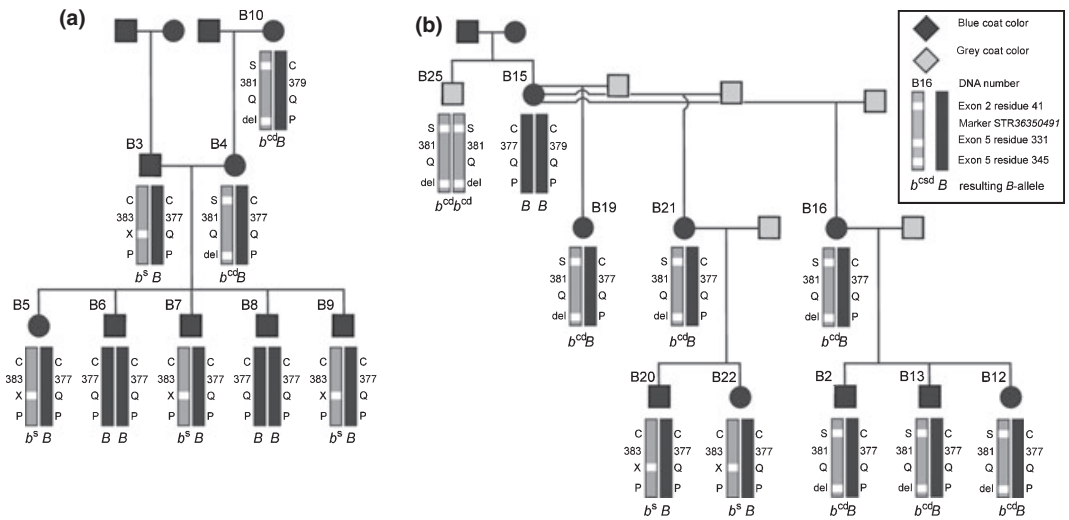

Figure 3 TYRP1 haplotypes in two BW pedigrees (a) and (b). Vertical black bars represent haplotypes of functional *TYRP1* alleles, grey bars represent non-functional *TYRP1* alleles with respective mutation(s) symbolised in white. The alleles represented from top down are: C41S, STR 36350491, Q331X, 345delP.

allele is linked to a functional copy of the *TYRP1* allele. The typical *TYRP1* C-377-Q-P haplotype 5 was apparent in the vast majority of BW, it is shared by all but two of the 'blues'. These latter two BW exhibited a potentially slipped (Levinson & Gutman 1987) 379 -bp allele of *STR 36350491* in the otherwise completely identical *TYRP1* haplotype. In the pedigree data of Figure 3b, one grey dog (B25) descending from a blue × blue Weimaraner breeding exhibits two non-functional copies of the *TYRP1* gene without the typical blue 377 allele underscoring the fact that this gene determines grey versus blue coat colour. Moreover, Figure 3b also indicates that all descents from a grey × blue crossing exhibit the typical 377 allele together with a functional C-377-Q-P *TYRP1* haplotype 5. Although different haplotypes were deduced in the greys of this study (Figure 2), none harboured either a 377- or 379 -bp allele of *STR 36350491* (Table 1). Yet, 377- and 379 -bp alleles can also be found in several other dog breeds with (partial) black eumelanin pigmentation (see Table S4). The latter dogs belong to breeds where *TYRP1* is known to determine the coat colour phenotype.

In addition to the detailed analysis of *TYRP1* variations, the Y-chromosomal haplotype of a male descendant (individual B25, see Table S4) of the ancestor of the American BW population was analysed. B25 represents a male offspring in the 8th generation after the blue Weimaraner ancestor, whereby exclusively male-to-male transmissions were concerned. Via grandfather to father to son to grandson etc., the original Y-chromosomal haplotype should have been preserved. In present-day grey Weimaraners, four different Y-chromosomal haplotypes are present (Table S5). In the BW, three different Y-chromosomal haplotypes of the four haplotypes typical for grey Weimaraners can be found. The newly identified, perhaps ancestral blue Y-chromosomal haplotype of B25 is not represented among the four different Y chromosomes exclusively observed in present-day German Weimaraners (see Table S5; for Y-haplotyping data also see Kropatsch *et al.* in press).

Discussion

Based on the knowledge of colour genetics as established by Little (1957), it was proposed that the difference in coat colours of grey versus BW depends on alleles of the *B* locus, and dilute is associated with the diluted coat colour in both types (Jarmie 1967). This hypothesis was confirmed by the genotyping data in this study. Dilute (*d*; *MLPH* gene) determines whether the original coat colour is changed to grey and blue in grey Weimaraners and BW. The genotyping data confirmed that colour lightening for both, brown or black coat colour as determined by the *B* locus (*TYRP1* gene) is associated with *dilute*. This is also known for the Doberman pincher where the blue Doberman colour is associated with dilute (Philipp *et al.* 2005). In the Doberman, this phenotype is often associated with a disease affecting hair follicles, the colour dilution alopecia (CDA), similar to black hair follicular dysplasia

in other breeds (see e.g. von Bomhard *et al.* 2006). In Weimaraners, less-pronounced CDA disease has been observed in rare cases (Laffort-Dassot *et al.* 2002). In general, the mechanism by which blue is determined in other dog breeds depends on the presence of *dilute* alleles in homozygous state and genetic determinants of black coat.

TYRP1 genotyping confirmed that variations in this gene are crucial for the coat colour difference observed in grey Weimaraners versus BW. All greys showed two mutant *TYRP1* alleles, in various combinations, whereas all BW shared at least one functional *TYRP1* (*B)* allele. The three common *TYRP1* gene variations are frequently found responsible for brown pigment formation in different dog breeds including the greys. Several hunting breeds show similar varieties of brown alleles (Schmutz *et al.* 2002), suggesting that brown alleles existed in hunting dogs before the development of modern-day breeds. Nevertheless, reversion from a 'brown' allele in Weimaraners to a 'black' allele appears highly unlikely regarding average mutation rates of approximately 10^{-8} per nucleotide and approximately 10^{-4} for short tandem repeats. Based on the haplotypes present in the greys, minimally two mutations would have been necessary to occur in order to lead to a reversion from grey to blue coat colour. For example, the back mutation of the amino acid exchange C41S plus a mutation from the 381 short tandem repeat allele into a 377 allele would change the S-381-Q-P haplotype 1 of greys into a blue C-377-Q-P haplotype 5. Probabilities for such scenarios range from 10^{-10} to 10^{-12}. Hence, the coat colour of BW appears more readily explained by cross-breeding of dogs carrying a *B* allele into the population before or after the official establishment of the Weimaraner breed. Theoretically, some BW carrying a *B* allele may have been maintained unofficially from the time before the Weimaraner breed was established, but such hypotheses cannot be verified beyond doubt in retrospect.

In addition to the coat colour data, Y-chromosome haplotyping revealed a unique haplotype in the ancestor of 'grey' Weimaraner B25 that differs significantly from the four haplotypes found exclusively in the greys. This fact indicates that BW originated at least partly outside of the Weimaraner population by introduction of a typical 377 -bp *TYRP1* haplotype potentially along with a unique Y-chromosomal haplotype. On the basis of breeding records concerning the offspring of the anecdotal BW ancestor, a different Y-chromosomal haplotype could also be taken as an argument for cross-breeding, where a dog with diverging *TYRP1* and Y-chromosomal haplotypes was introduced into the Weimaraner population before or after the presence of the anecdotal BW. There was only one descendant from the BW ancestor available for this study. It would be interesting to know the haplotypes of additional descendants for evaluating the meaning of this unique haplotype in Weimaraners.

Overall, the analysis of pedigree information, coat colour typing in combination with Y-chromosomal haplotyping represents a strategy to analyse unusual phenotypes in a canine population. In the context of the BW study, this information provides an argument for a cross-breeding event in combination with the *TYRP1* haplotype data. Yet, BW are repeatedly backcrossed onto the genetic background of the greys for over 60 years. Therefore, the causal difference of both phenotypes may boil down to the single discrepancy in one *TYRP1* allele encoding a functional versus a non-functional copy of the TYRP1 protein.

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Supporting Information

Additional Supporting Information may be found in the online version of this article:

Table S1 (A) PCR primers (5′–3′) for the analyses of coat colour gene variations and (B) microsatellite markers of the *TYRP1* gene

Table S2 Coat colour genotypes for grey Weimaraners and BW

Table S3 TYRP1 haplotypes in 21 grey and 24 BW. Bold type letters indicate the TYRP1 mutations. The 379 bp allele is potentially because of a slippage mutation of the 377 bp allele

Table S4 Genotyping microsatellite marker STR 36350491 in intron 4 of the *TYRP1* gene. Individual dogs were analysed from breeds where the *B* locus is known to contribute to specification of the coat colour

Table S5 Y-chromosomal haplotypes from grey and BW. Individual B25, a 'grey Weimaraner' offspring from a blue × blue breeding and progenitor of an anecdotal BW ancestor, reveals a Y haplotype never observed among typically grey Weimaraners

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