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The Origin and Genetic Architecture of Anomalous Coat Color in the Severely Inbred Scandinavian Wolf Population

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Abstract

Understanding how genetic drift and inbreeding can shape phenotypic traits in small and isolated populations, by facilitating the expression of rare genetic variants, can be important for the conservation and management of such populations by reflecting their demographic history and constraints. After the grey wolf (*Canis lupus*) recolonized the Scandinavian peninsula (Norway and Sweden), the population has remained small and isolated. In consequence, the population is highly inbred and originates from seven individuals, only. With insufficient immigration due to isolation, the loss of genetic variation through genetic drift and inbreeding is severe. Various negative effects of inbreeding have already been documented, such as inbreeding depression and several congenital deformities. Additionally, there have been reports of Scandinavian wolves exhibiting anomalous coat color characterized by hypopigmentation displayed as white tail tips, and for some individuals, also larger white patches. In this study, I utilized long-term monitoring data of the population, dating back to its recolonization, to examine the occurrence of anomalous coat color by investigating its origin and inheritance, genetic mechanism, and effects of inbreeding on its expression.

SNP genotyping revealed a distinct haplotype linked to the MITF gene that co-segregated with anomalous coat color. The only genotyped individual homozygous for this haplotype was anomalously colored, whereas heterozygous carriers had either normal or an unknown phenotype, and the majority of wolves did not carry the haplotype at all. This demonstrates that anomalous coat color can be attributed to genetic variation involving the MITF gene and is likely linked to the disruption of melanocyte development, leading to unpigmented areas and the occurrence of hypopigmentation. The origin of this haplotype could be traced back to the third founder of the population, reproducing for the first time in 1991. All genotyped offspring of this founder were heterozygous carriers, whereas the haplotype was absent in the founding pair and their descendants. The haplotype showed a recessive mode of inheritance, where two copies were required for the expression of anomalous coat color. With evidence of recessive inheritance, inbreeding was identified as an important driver of the expression and occurrence of the trait through increased homozygosity and sharing of identical by descent (IBD) segments.

The occurrence of anomalous coat color in a small and severely inbred population illustrates how reduced genetic variation, resulting from genetic drift and inbreeding, can shape phenotypic traits by exposing rare recessive genetic variants. The anomalous coat color observed in the population can therefore serve as an indicator for underlying genetic

mechanisms driven by demographic history and constraints. Furthermore, the genetic constraints shaped by few founders, small population size, and isolation over several decades point to the importance of maintaining genetic diversity by facilitating gene flow and introduction of new genetic variation, but also how such vital immigration can bring about unforeseen side effects. Finally, these findings highlight the importance of genetic monitoring of small and isolated populations, which can provide a framework for linking demographic history and genetic mechanisms to address long-standing questions relating to the genetics and conservation of populations.

Sammendrag

Kunnskap om hvordan genetisk drift og innavl kan forme fenotypiske egenskaper i små og isolerte populasjoner, ved å øke uttrykkelsen av sjeldne genetiske varianter, kan være av viktighet for bevaring og forvaltning av slike populasjoner ved å reflektere demografisk historie og begrensinger. Etter ulven (*Canis lupus*) rekoloniserte den skandinaviske halvøya (Norge og Sverige) har populasjonen vært liten og isolert. Som følge er populasjonen svært innavlet og stammer kun fra syv individer. Begrenset immigrasjon grunnet isolasjon, har ført til betydelig tap av genetisk variasjon gjennom drift og innavl. Flere negative effekter av innavl er blitt dokumentert, som innavlsdepresjon og flere medfødte misdannelser. I tillegg har det blitt rapportert flere skandinaviske ulver med avvikende fargedrakt karakterisert ved hypopigmentering gjennom hvite haletipper, og for noen individer også større hvite felt. I denne studien benyttet jeg meg av omfattende og langvarig overvåkningsdata av populasjonen som strekker seg helt tilbake til rekoloniseringen, for å kartlegge forekomsten av avvikende fargedrakt ved å undersøke dens opphav og nedarving, genetiske mekanismer, og effekter av innavl.

SNP genotyping viste en distinkt haplotype tilknyttet MITF genet som samsegregerte med avvikende fargedrakt. Det eneste genotypede individet homozygot for denne haplotypen hadde avvikende fenotype, hvorav heterozygote bærere hadde enten normal eller ukjent fenotype, mens flesteparten av genotypede ulver bar ikke haplotypen i det hele tatt. Dette demonstrerer at avvikende fargedrakt kan tilskrives genetisk variasjon som involverer MITF genet, og som sannsynligvis skyldes feil i utviklingen av melanocytter, noe som fører til upigmenterte områder og forekomsten av hypopigmentering. Opphavet til haplotypen kunne spores tilbake til den tredje grunnleggeren, som først reproduserte i 1991. Alle hans genotypede avkom var heterozygote bærere av haplotypen, mens den var ikke til stede i grunnleggerparet eller noen av deres etterkommere. Haplotypen viste recessiv nedarving, hvor to kopier var nødvendig for uttrykkelsen av avvikende fargedrakt. Med bevis for recessiv nedarving ble innavl identifisert som en viktig drivkraft for uttrykkelsen og forekomsten av egenskapen gjennom økt homozygositet og deling av identisk ved arv (IBD) segmenter.

Forekomsten av avvikende fargedrakt i en liten og svært innavlet populasjon illustrerer hvordan redusert genetisk variasjon, gjennom genetisk drift og innavl, kan forme fenotypiske egenskaper gjennom eksponering av sjeldne recessive genetiske varianter. Den avvikende fargedrakten observert i populasjonen kan derfor være en indikator for underliggende genetiske

mekanismer drevet av demografisk historie og begrensninger. Videre så er de genetiske begrensningene, formet av få grunnleggere, liten populasjonsstørrelse og isolasjon over flere tiår, med på å belyse viktigheten av å tilrettelegge genflyt og introduksjon av ny genetisk variasjon, men samtidig også hvordan viktig immigrasjon kan føre med seg uforutsette effekter. Disse funnene demonstrerer også viktigheten av genetisk overvåkning av små og isolerte populasjoner, som kan benyttes som et rammeverk for å koble demografisk historie og genetiske mekanismer til å besvare viktige spørsmål knyttet til genetikk og bevaring av populasjoner.

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1. Introduction

Small and isolated populations face severe challenges from the loss of genetic variation, which compromises their ability to adapt to future environmental change, leaving them vulnerable to extinction (Frankham et al., 2002, pp. 45-47; Keller & Waller, 2002; Lacy, 1997). The loss of genetic variation is due to random genetic drift (Allendorf, 1986; Wright, 1931), where some genetic variants are passed on to viable offspring and others are lost from the population (Frankham et al., 2002, pp. 178-179; Lacy, 1987). Although small populations might restore genetic variation by gene flow, isolation causes the transfer of genetic material between populations to be restricted or more or less absent (Frankham et al., 2002, pp. 167-168 & 314-319; Hedrick & Garcia-Dorado, 2016; Newman & Tallmon, 2001).

Population isolation and loss of genetic variation entail an elevated risk of inbreeding (mating between relatives), which amplifies the effect of genetic drift and leads to further loss of genetic variation (Keller & Waller, 2002). Small populations originate from a restricted number of founders, leading to increased relatedness between individuals (Crow & Kimura, 1970, pp. 61-62; Frankham et al., 2002, p. 187). As a result of relatedness, individuals are more likely to share homologous chromosome segments that are identical by descent (IBD), i.e., they are identical copies derived from a common ancestor (Kardos et al., 2017). Consequently, when relatives mate, their offspring are more likely to inherit identical copies of the same alleles from both parents (Fig. 1; Shafer & Kardos, 2025). The inheritance of such IBD segments leads to reduced genetic variation through increased homozygosity across chromosomes known as runs of homozygosity (ROH; Bosse et al., 2012; Kardos et al., 2017; Shafer & Kardos, 2025).

Increased homozygosity enhances the proportion of recessive alleles masked by the dominant allele in the heterozygous state, to be expressed in the homozygous state (Bosse et al., 2012; Crow & Kimura, 1970, pp. 61-62; Waller & Keller, 2020). Most genetic predispositions are recessive, and the expression of such genetic variants can lead to reduced survival and fertility (fitness) of inbred offspring (i.e., inbreeding depression; Charlesworth & Willis, 2009; Hedrick & Garcia-Dorado, 2016; Lande, 1998), potentially limiting population growth. Because of their negative effects on fitness, such genetic variants are expected to be selected against and removed from the population (Futuyama, 2023, pp. 111-112). However, since genetic drift is a stronger force in small populations, it can counteract selection against these variants, possibly

leading to increased frequencies or even the fixation of them (Charlesworth, 2009; Charlesworth & Willis, 2009; Nei et al., 1975).

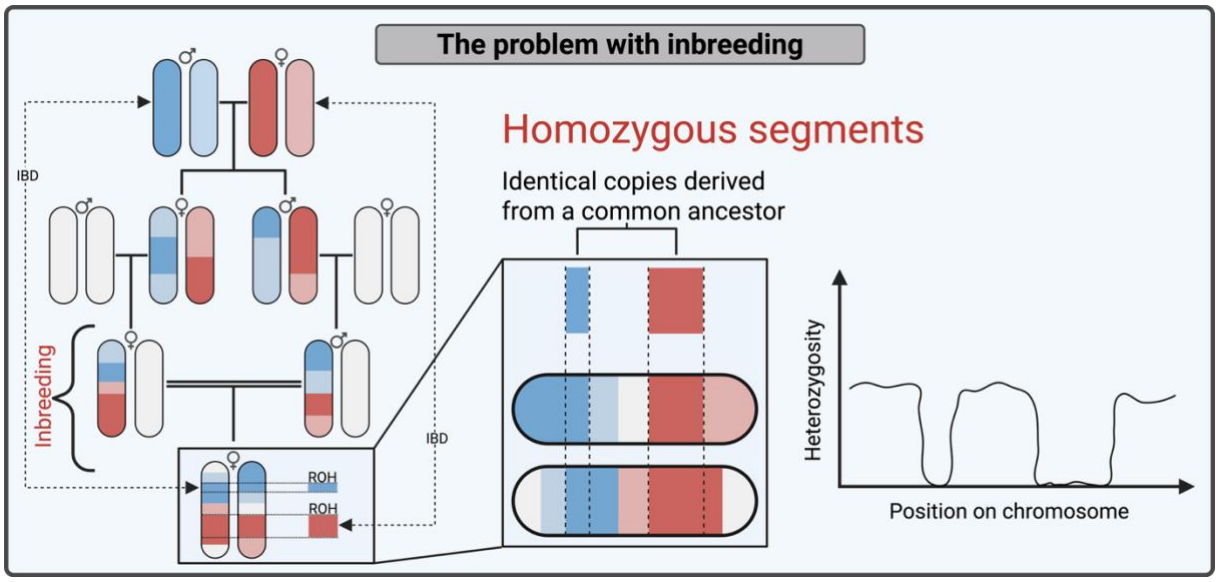


Figure 1. *The pedigree illustrates identical by descent (IBD), where identical segments are shared across chromosomes (ROH). Blue color represents the paternal chromosomes, red represents the maternal chromosomes, whereas white represents chromosomes of unrelated individuals. When relatives mate (i.e., first cousins), it results in the inheritance of identical copies of the same segments (blue and red) derived from common ancestors (IBD).*

A population of particular interest owing to the pronounced effects of genetic drift and inbreeding, is the Scandinavian wolf population. The population has remained small and isolated, facing inbreeding since the recolonization on the Scandinavian peninsula (Fig. 2; Ellegren et al., 1996, 1999; Seddon et al., 2006; Vila et al., 2003). After being declared functionally extinct in the 1960s, a pair of immigrants from the Finnish-Russian population reproduced in 1983 close to the border between Norway and Sweden, founding the current population (Liberg et al., 2005; Vila et al., 2003; Wabakken et al., 2001). After reproducing for three successive years (1983-1985), the female was shot, and the male later disappeared. However, their offspring continued to breed within the same territory through incestuous matings from 1987-1994 (Akesson et al., 2016; Liberg et al., 2004, 2005). During 1991, a second pack was established as a male immigrant reproduced with a female offspring of the founding pair. The immigration of this male initiated reduced inbreeding through increased heterozygosity and population growth, following the introduction of new genetic material (Liberg et al., 2005; Vila et al., 2003).

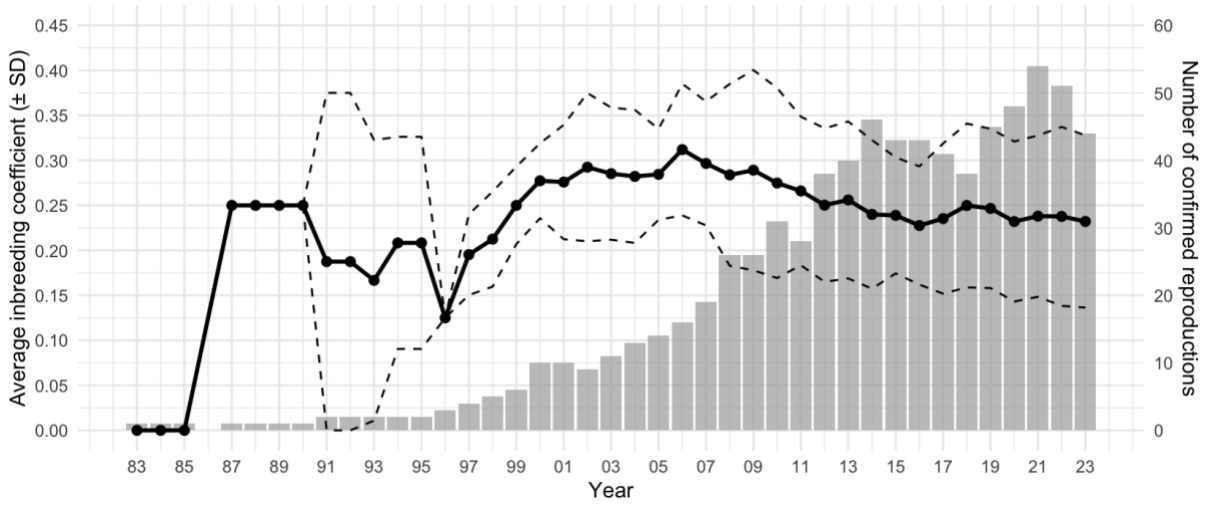


Figure 2. *Annual average inbreeding coefficient (F) for Scandinavian reproductions and number of confirmed reproductions during the period 1983-2023. Solid line represents the average F, and stippled line denotes the standard deviation, providing information on variation in F between packs across years. The bars display annual number of confirmed reproductions. Data on inbreeding was obtained from Naturvårdsverket (2024), while information on confirmed reproductions was gathered from Wabakken et al. (2001) for the period 1983-1998, and from annual reports from Rovdata (https://rovdata.no/Ulv/Rapporter.aspx, last accessed 20.05.2025) for the period 1998-2023. Notice there are two different y-axes.*

Prior to 2008, the population solely originated from these three founders, but over time, there have been additional immigrants from the Finnish-Russian population. Only four of these immigrants (two males in 2008, one female in 2013, and one male in 2021) have managed to establish genetic contact and introduced new genetic material to the population, and can therefore be regarded as additional founders (Liberg et al., 2024, p. 200; Svensson et al., 2024, p. 213; Svensson et al., 2025). Consequently, the current population comprising approximately 400 individuals, only originates from seven individuals (2 females and 5 males), suffering from a small genetic basis and high levels of inbreeding, close to mating between full siblings (F = 0.23 ± 0.09 SD; Akesson et al., 2024; Liberg et al., 2024, pp. 200-201; Svensson et al., 2025). As a result, inbreeding remains a serious concern for the long-term survival and viability of the population.

Few founders followed by prolonged and extensive inbreeding spanning several decades have led to various negative effects of inbreeding. Several individuals have accumulated long ROH, with some being inbred to an extent that almost entire chromosome pairs are IBD (Kardos et al., 2018). Furthermore, inbreeding depression has been documented through lower breeding success and juvenile survival (Liberg et al., 2005), and even sterility among males exhibiting

cryptorchidism (Petersen et al., 2021; Räikkönen et al., 2013). Other negative effects are increasing trends of anomalies, such as congenital defects in the positioning and angles of teeth and skeleton (Räikkönen et al., 2013).

Another potential effect of inbreeding in Scandinavian wolves is the occurrence of anomalous coat color, characterized by hypopigmentation through deficient pigment where normally present (Personal communication with Ø. Flagstad, 28.05.2025; Baxter et al., 2004; Lin & Fisher, 2007). Several studies consider anomalous coat color a potential indicator for population genetics, suggesting that inbreeding, driven by isolation and genetic drift, gives rise to coat color anomalies owing to increased homozygosity leading to the expression of recessive phenotypes (Brito & Valdivieso-Bermeo, 2016; Gong et al., 2021; Hofmeester et al., 2021; Manzo et al., 2022; Tensen & Fischer, 2024). Since the initial appearance in 2004, an increasing proportion of packs where one or several individuals exhibit anomalous coat color has been reported. These individuals (pups, juveniles, and adults) exhibit hypopigmentation through white tail tips, and in some cases, also larger white patches (Fig. 3)

Figure 3. *Examples of normal and anomalous coat color in Scandinavian wolves. There is considerable variation within both phenotypic variants. Photographs: Miljødirektoratet/SNO. Created with BioRender.com.*

Such coloration strongly deviates from the typical wolf characteristics with a uniform black tail tip and lack of significant irregular white patches (Körberg et al., 2014; Miller, 1912, pp. 305-307; Murie, 1944, pp. 9-10; Smith et al., 2020, pp. 108-109). Though wolves have diverse coat coloration with considerable individual variation ranging from yellow to light brown or reddish, accentuated with varying shades of black, grey and off-white (Mech, 1970, pp. 16-17; Miller, 1912, pp 305-307; Paquet & Carbyn, 2003, p. 484), the observed hypopigmentation deviates from what is considered normal variation and is thus considered anomalous.

Cases of hypopigmentation in wild canids are rare considering their lack of adaptive significance (except in Arctic regions) and possible negative pleiotropic effects (e.g., deafness, bone abnormalities; Goding & Arnheiter, 2019; Gong et al., 2021; Körberg et al., 2014; Stritzel et al., 2009), and their genetic mechanism remains poorly researched and understood (Arce et al., 2019; Castelló, 2018, pp. 8-9; Chatellenaz & Zaracho, 2021; de Mello et al., 2020). Coat color is controlled by a complex process involving several genes, signaling pathways, and transcription factors (Charon & Lipka, 2015), and is primarily determined by the presence of pigment producing cells known as melanocytes (Lin & Fisher, 2007). As pigment is synthesized within special organelles (melanosomes) within these cells, mutations in genes necessary for their development, survival and migration can lead to the absence of pigment production, which in turn can result in hypopigmentation through nonpigmented areas of variable size and shape (David et al., 2014; Mikheil et al., 2017). One gene important for melanocyte development is the melanocyte inducing transcription factor (MITF; Brancalion et al., 2022; Wakamatsu & Ito, 2021, p. 50). Genetic variation affecting the MITF gene can impair melanocyte development, ultimately affecting pigmentation (Bismuth et al., 2005), and has been linked to hypopigmentation in several species (Baxter et al., 2004; Charon & Lipka, 2015; Hauswirth et al., 2012; Hofstetter et al., 2019). For instance, hypopigmentation is characteristic for many dog breeds (*C. lupus familiaris*), where several causal variants linked to MITF have been identified (Brancalion et al., 2022; Karlsson et al., 2007; Körberg et al., 2014; Rothschild et al., 2006), and may influence pigmentation in wolves as well, potentially causing cases of hypopigmentation.

In this study, I aimed to address 1) the origin of the anomalous coat color in the Scandinavian wolf population, and determine its mode of inheritance, 2) possible effects of inbreeding on its expression, and 3) identify the underlying genetic mechanism. Based on these objectives, three major hypotheses were formulated. As inbreeding and increased homozygosity lead to

increased expression of recessive alleles, I hypothesize that 1) the anomalous coat color is likely a recessive inherited trait that is expressed through inbreeding due to increased probability of inbred offspring inheriting two identical copies of the recessive allele, allowing the expression of the trait that is otherwise masked in heterozygotes. Furthermore, as the trait was first observed in 2004, after the immigration of the three initial founders and before the immigration of the four successive ones, I hypothesize that 2) the trait was introduced by one of the initial founders. Lastly, considering previous associations between hypopigmentation and genes affecting melanocytes, I hypothesize that 3) the MITF gene is an important genetic determinant for the expression of the anomalous coat color.

2. Material and methodology

2.1. Data material

The Scandinavian wolf population has been monitored since the recolonization on the Scandinavian peninsula (Akesson et al., 2024; Vila et al., 2003; Wabakken et al., 1999, 2001). Information and data collected from the annual monitoring and by the Scandinavian Wolf Research Project (SKANDULV) have been gathered in the Scandinavian large carnivore monitoring database Rovbase 3.0 (https://rovbase30.miljodirektoratet.no/), commissioned by the environmental agencies in Norway and Sweden (Miljødirektoratet and Naturvårdsverket), both of which are responsible for collecting data for large carnivore management. From this database, I will extract phenotypic information (normal and anomalous colored individuals) for all wolves where this is available, as well as information on individual wolves, including the relationship between them compiled in a pedigree. I will also use genotypic data from a sample of normal and anomalous colored wolves, as well as early-generation individuals, to obtain genetic information.

2.1.1. Phenotypic data

Identification of individuals exhibiting normal and anomalous coat color was based on photographs of dead and live Scandinavian wolves between 2002-2024 (Fig. A1; Fig. A2). The majority of photographs were retrieved from Rovbase 3.0, with supplementary photographs provided by the Swedish Veterinary Agency (SVA: https://www.sva.se/). Additional photographs from field observations from SKANDULV and personnel from the Norwegian Nature Inspectorate (SNO) were also included. All photographs related to a given individual

with a unique ID, enabling individual phenotyping by linking photograph and phenotype to a specific individual.

Anomalous coat color is characterized by both white tail tips and larger white patches, however, individuals were phenotyped solely based on the color of their tail tip. I chose to use this selection criterion because white patches complicate the distinction between normal and anomalous coat color, as light to white color is a general feature of the wolf pelage and varies naturally among individuals (Mech, 1970, pp. 16-17). This makes overall body color an unreliable determinant for distinguishing between normal and anomalous coat color and could lead to individuals being incorrectly assigned to a given phenotype. However, the tail tip color of wolves is characteristically black (Ay et al., 2023; Mech, 1970, pp. 16-17; Sand et al., 2007), and provides a more reliable basis for differentiation. Consequently, this study focuses exclusively on tail color as an indicator of normal and anomalous coat color. Tail color was treated as a binary variable, with black color as an indicator for normal phenotype and white color as an indicator for anomalous phenotype.

Figure 4. *Example of photographs that were excluded. Photograph A) was excluded due to poor lighting conditions and angle, B) the tail was outside the picture frame, C) the tail was hidden, and D) as the tail was outside the*

picture frame. Sensitive information (covered in black) excluded for privacy policy reasons. Photographs: Miljødirektoratet/SNO. Created with BioRender.com.

The sampling process for photographs was highly selective when distinguishing between the two phenotypes. Incorrect assignment of individuals to a given phenotype could impact the analyses by disturbing the effects of inbreeding, origin, inheritance, as well as the association between phenotype and genetic variants. To avoid mis-categorizing and enhance the reliability of analyses, photographs were excluded if the tail were in any sort of conditions (e.g., scabies, hidden, bent), outside picture frame, or if there were any uncertainty in the color of the tail tip (e.g., poor angle or lighting conditions), ensuring that only photographs that displayed the tail and could confidently be assigned to a definitive phenotype, were included (Fig. 4).

In total, 476 individuals were successfully phenotyped, of which 433 were assigned to the normal phenotype and 43 assigned to the anomalous phenotype (Table 1; Table A1). After compilation, the data was also quality controlled to ensure that each photograph and phenotype corresponded to the right individual. I also verified that all the excluded photos were indeed not scorable under the criteria given above.

Table 1. Overview of the total number of phenotyped individuals divided by the two phenotypic groups and the source from which the photos were obtained.

Source	**N. individuals**		
	Normal	**Anomalous**	**Total**
Rovbase 3.0	315	29	344
SVA	115	6	121
Observations	3	8	11
Total	433	43	476

2.1.2. Pedigree

Complete pedigrees are difficult to establish in the wild (Bérénos et al., 2014; Pemberton, 2008), but long-term monitoring and continuous sampling of the Scandinavian wolf population have enabled the unique re-construction of a virtually complete pedigree for almost all individual wolves in the population from 1983-2023 (Akesson et al., 2024; Liberg et al., 2005). From this pedigree (n = 3,329), I will use a sample pedigree of 1,060 individuals (Fig. 5), including all reproductive individuals since 1983, as well as all phenotyped individuals.

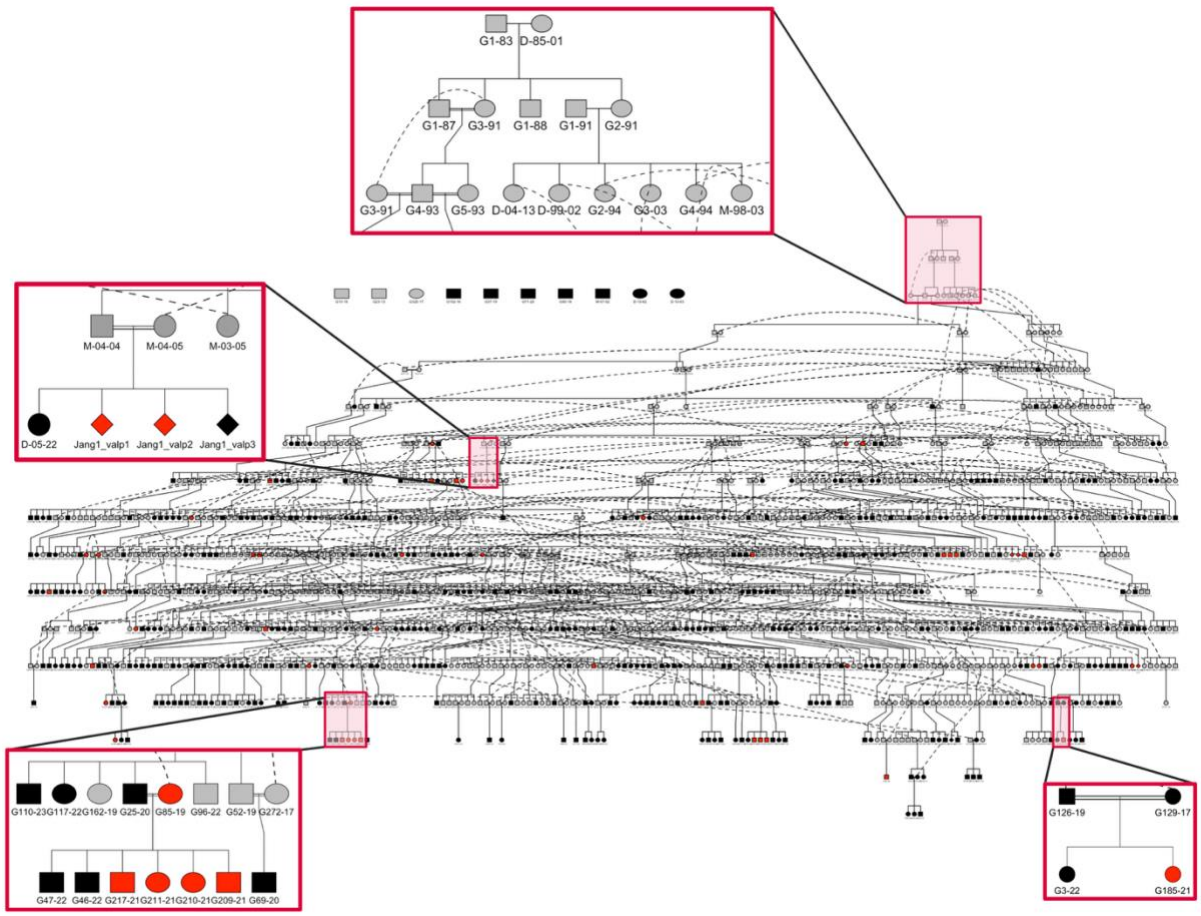


Figure 5. *Sample pedigree from the Scandinavian wolf population from 1983-2023 (n = 1,060). Number underneath symbols represent individual IDs. Squares and circles represent males and females, respectively. Diamonds represent individuals of unknown sex. Symbol color represents phenotypes, where red indicates anomalous phenotype, black indicates normal phenotype, and grey indicates unknown phenotype. Double solid lines represent incestuous matings (i.e., sibling and parent-offspring), and single solid lines represent matings between unrelated individuals. There are several generational overlaps and complex relationships between individuals, with dashed lines representing inbreeding loops. Notice that 10 individuals are not connected to the pedigree. The pedigree was visualized using the R-package "pedtools" and excerpts were created using BioRender.com.*

The pedigree contained individual ID and sex, and the ID of their father and mother. There were in total 17 generations with 289 unique families, including 242 unique sires and 236 unique dams, and 17 individuals with unknown parents. Only seven of the individuals with unknown parents are considered founders. This is because they have introduced new genetic material into the population, as their offspring themselves have successfully reproduced, a prerequisite for individuals to be regarded as a founder (Akesson et al., 2024; Liberg et al., 2024, p. 200; Svensson et al., 2025). The remaining 10 individuals included 2 Scandinavian-born individuals with unknown parents, 5 non-reproducing immigrants, and 3 reproducing immigrants, of which

none of their offspring have yet contributed to reproductions. This pedigree was used for further analyses to gather information on relatedness and inbreeding, as well as origin and inheritance of the anomalous phenotype.

Additional information on individual wolves was obtained from Rovbase 3.0 and included information on year of death and data on first reproductive events for all pairs, to trace the anomalous phenotype over time and across generations.

2.1.3. Genotypic data

CanineHD genotypes

Genetic information was obtained for a total of 77 individuals, extracted from a larger dataset of whole genome sequence data (originally published in Kardos et al., 2018 and Viluma et al., 2022, and jointly genotyped in Smeds & Ellegren, 2023) merged with SNP (single nucleotide polymorphism) data genotyped on an Affymetrix Axiom HD array [filtering in PLINK: --geno 0.02 –mind 0.02 –maf 0.05 –hwe $1e^{-10}$] (Smeds, Ellegren & Åkesson, unpublished manuscript). SNPs can be used as genetic markers in association analyses to investigate genetic differences between individuals where a phenotype is available, to identify specific genomic regions associated with the phenotype under investigation (Bush & Moore, 2012). Furthermore, they can be used to identify haplotypes by assessing co-inherited genetic markers linked to a trait of interest (Yang et al., 2008). The data included 38 phenotyped individuals (1 non-reproducing immigrant and 37 Scandinavian-born wolves; Table A2), of which 37 had normal phenotype and 1 had anomalous phenotype, as well as 39 early-generation individuals (1 founder and 38 Scandinavian-born wolves; Table A3) born prior to and shortly after the trait´s first appearance in 2004.

2.2. Data analyses

To investigate the occurrence of anomalous coat color in the Scandinavian wolf population, including its origin, inheritance, genetic architecture, and effects of inbreeding, I applied a set of different approaches as described below. All statistical analyses were conducted using the open source language and environment for statistical computing and graphics R version 4.4.1 (2024-06-14; R Core Team, 2024), whereas the association and haplotype analysis were performed using the High Performance Cluster Orion.

2.2.1. Phenotype frequencies

The occurrence of anomalous coat color was assessed by calculating the frequencies of the normal ($freq_{normal}$) and anomalous ($freq_{anomalous}$) phenotype relative to the number of phenotyped individuals. Individuals from observations (n = 11, 3 normal and 8 anomalous) were excluded as they were biased towards the anomalous phenotype. The total number of individuals included in the calculation was 465, of which 430 had normal and 35 had anomalous phenotype. The frequencies were calculated using these formulas:

$$freq_{normal} = \frac{N_{normal}}{N_{total}}, \qquad freq_{anomalous} = \frac{N_{anomalous}}{N_{total}}$$

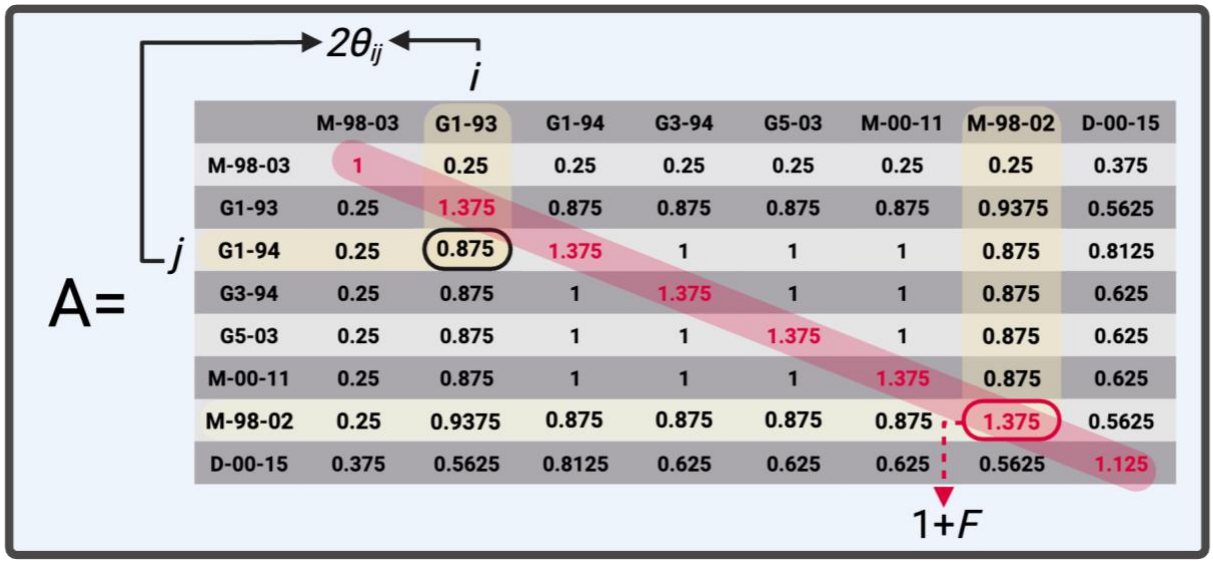
Furthermore, a generalized linear model (GLM) with a binomial distribution, only including individuals with known sex and death year (n = 464), was used to assess if there was a difference in the proportion of males and females exhibiting normal and anomalous phenotype as well as whether the distribution of phenotypes varied across years during the period 2002-2024. The model was fitted with phenotype as the response variable and sex and death year as explanatory variables.

2.2.2. Inbreeding and relatedness

Inbreeding was estimated using the inbreeding coefficient (*F*). *F* represents the probability that two parental alleles at a random locus are IBD (Falconer & Mackay, 1996, p. 58; Knief et al., 2015; Wright, 1922), and therefore measures the amount of identical genes of a common ancestor an individual inherits from its parents (Keller & Waller, 2002). *F* ranges from 0 to 1, with higher values indicating a higher degree of inbreeding and homozygosity due to greater parental relatedness (Frankham et al., 2002, p. 257). *F* was estimated from the pedigree for every individual (n = 1,060), assuming unrelated founders (Falconer & Mackay, 1996, pp. 82-83; Knief et al., 2015), using the R-package "pedigreemm" (Bates et al., 2024).

The pedigree was used to define the additive genetic relationship matrix (**A**), containing all the pairwise values of relatedness between individuals (Fig. 6; Lynch & Walsh, 1998, p. 756; Wilson et al., 2010), using the "getA()" function in the pedigreemm package (Nilforooshan & Saavedra-Jiménez, 2020). Off-diagonal elements of the matrix represent relatedness between individuals, and diagonal elements represent self-relatedness given as 1 + *F*. Here, *F* equals

Wright´s coefficient of inbreeding (Wright, 1922), with values higher than 1 representing the *F* for a given individual (Henderson, 1976; Postma & Charmantier, 2007; Wilson et al., 2010).



	M-98-03	G1-93	G1-94	G3-94	G5-03	M-00-11	M-98-02	D-00-15
M-98-03	1	0.25	0.25	0.25	0.25	0.25	0.25	0.375
G1-93	0.25	1.375	0.875	0.875	0.875	0.875	0.9375	0.5625
G1-94	0.25	0.875	1.375	1	1	1	0.875	0.8125
G3-94	0.25	0.875	1	1.375	1	1	0.875	0.625
G5-03	0.25	0.875	1	1	1.375	1	0.875	0.625
M-00-11	0.25	0.875	1	1	1	1.375	0.875	0.625
M-98-02	0.25	0.9375	0.875	0.875	0.875	0.875	1.375	0.5625
D-00-15	0.375	0.5625	0.8125	0.625	0.625	0.625	0.5625	1.125

Figure 6. *Subsection of the additive genetic relationship matrix (A) representing the pairwise values of relatedness between individuals as two times the kinship coefficient (θ; Lynch & Walsh, 1998, p. 756). The relatedness between two individuals,* ***i*** *and* ***j****, has an expected additive genetic covariance of 2θ****ij****, where θ****ij*** *is the probability that an allele drawn at random from individual* ***i*** *is IBD to one drawn at random from individual* ***j*** *(Wilson et al., 2010). The off-diagonal element between individual G1-93 and G1-94 indicates they are half-siblings and that 25% of their alleles are IBD. Whereas the diagonal element of individual M-98-02 has a relatedness to itself greater than 1, indicating inbreeding. F for this individual is 0.375 (1 – F). Created with BioRender.com.*

F for all individuals was retrieved from the diagonal element of **A**, and quality controlled using the "inbreeding()" function in the pedigreemm package, and also compared to previous pedigree-based *F* estimated for a subset of individuals (n = 50) in Kardos et al. (2018; see Table A4 for comparison).

Additionally, a generalized linear model (GLM) with a binomial distribution was used to assess whether *F* differed between the two phenotypic groups. The model was fitted with phenotype as the response variable and *F* as the explanatory variable.

2.2.3. The animal model

Phenotypic variation arises from a combination of genetic and environmental factors, where related individuals often exhibit similarities due to shared genes and environment (Futuyama, 2023, p. 177; Kruuk & Hadfield, 2007). Using phenotypic resemblance among individuals of known additive relationship (genetic similarity) through the **A** matrix (see 2.2.2. Inbreeding and

relatedness), the animal model aims to partition this variation by including them as random effects to estimate their variance components after adjusting for fixed effects believed to influence the trait (Falconer & Mackay, 1996, p. 125; Kennedy et al., 1988; Wilson et al., 2010). The model also estimates the degree to which phenotypic variance is explained by genetic factors, reflecting the degree of resemblance between related individuals, or stated differently, the degree to which phenotypes correspond to specific genotypes, by estimating narrow-sense heritability (h^2; Wilson et al., 2010).

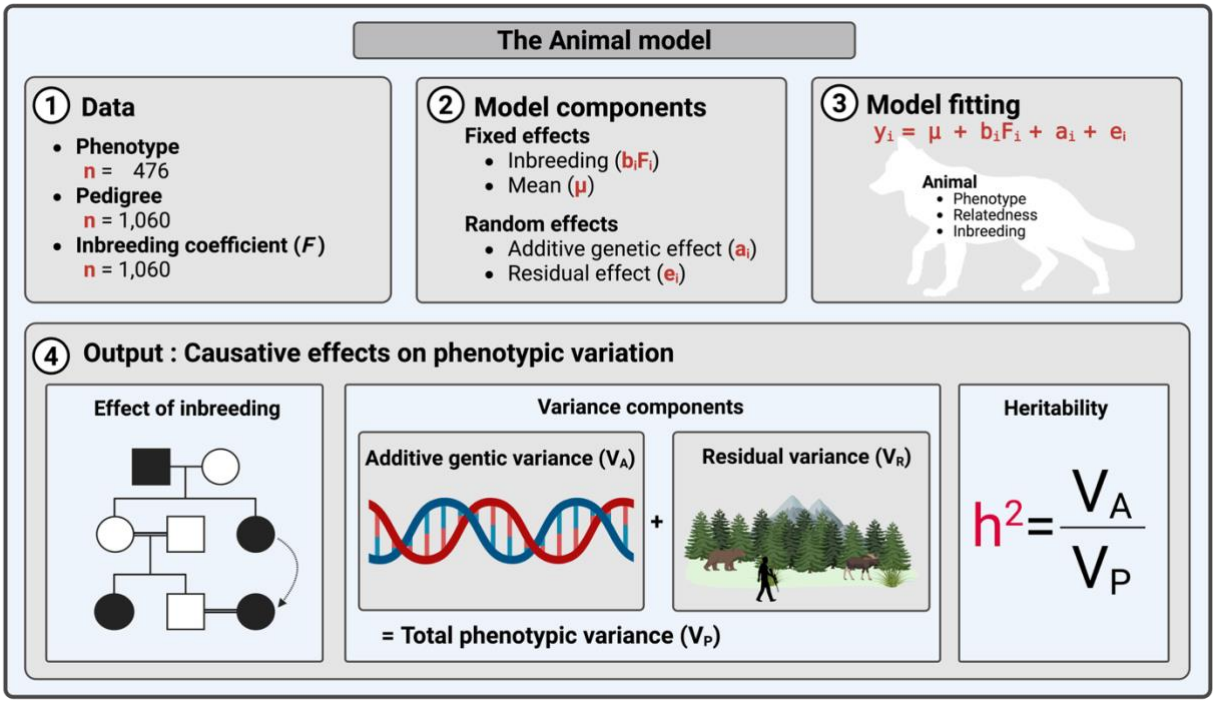


Figure 7. *Conceptual visualization of the animal model approach. The model was used to estimate the effect of inbreeding on phenotypic variation and partition the phenotypic variance (V_P) into the additive genetic variance (V_A) and residual variance (V_R) components adjusted for fixed effects. Variance components were further used to estimate the narrow-sense heritability (h^2). Created with BioRender.com.*

A univariate animal model including both fixed and random effects was fitted with tail phenotype as the response variable to estimate the effect of inbreeding and the narrow-sense heritability (h^2; Fig. 7). Pedigree-based inbreeding coefficients (F) were included as a fixed effect. Variance components were estimated by fitting the additive genetic effect and residual error of individuals as random effects, allowing the partitioning of phenotypic variance into additive genetic variance (V_A), and the residual variance (V_R) accounting for unexplained sources of variance (e.g., non-additive genetic effects and environmental effects). From these variance components, comprising the total phenotypic variance ($V_P = V_A + V_R$), the narrow-sense heritability was estimated ($h^2 = V_A / V_P$) (Kruuk, 2004). The model was fitted as:

$$y_i = \mu + b_i F_i + a_i + e_i$$

where y_i is the tail phenotype, μ is the mean phenotypic value in the population, b_i is the regression coefficient of inbreeding (F_i), a_i is the additive genetic effect, and e_i is the residual error for a given individual (Kruuk, 2004; Lynch & Walsh, 1998, pp. 755-757). The additive genetic effect has a variance equal to the additive genetic variance ($\sigma_a^2 \cdot \mathbf{A}$), **A** being the additive relationship matrix, whereas the residual error has a variance equal to the residual variance ($\sigma_e^2 \cdot \mathbf{I}$), with **I** being an identity matrix of dimensions equal to the number of observations, meaning the error terms were considered independent (Lynch & Walsh, 1998, pp. 755-756).

The model was fitted using the R-package "ASReml-R" version 4.2.0.332 (The VSNi Team, 2023), which is a specialized tool for fitting mixed models using Restricted Maximum Likelihood (REML). REML applies an efficient algorithm known as Average Information (AI), and utilizes sparse matrix methods, enabling the analysis of large and complex datasets (Butler et al., 2017; Johnson & Thompson, 1995; Lynch & Walsh, 1998, pp. 779-781). The model was set with a maxiter of 100 iterations.

The significance of the fixed effect of inbreeding was estimated using Wald analysis of variance, whereas the significance of the random additive genetic effect was estimated using a Likelihood ratio test using the "lrt()" function in "ASReml-R" to compare the fit of the full model with the reduced model excluding the random additive genetic effect (Butler et al., 2017):

$$y_i = \mu + b_i F_i + e_i$$

2.2.4. The genetic mechanism of anomalous coat color

The genetic mechanism of anomalous coat color was investigated using two different approaches. The first method involved an exploratory approach using a genom wide association analysis (GWAS) without prior knowledge of the genetic mechanism involved, to identify specific genomic regions involved in the expression of the trait (Bush & Moore, 2012). The second approach was targeted and hypothesis driven involving a more detailed investigation of the candidate gene MITF using a haplotype analysis.

Genome wide association analysis

GWAS is a tool for investigating associations between a trait and genetic markers (SNPs) at particular locations in the genome (loci) (Uffelmann et al., 2021). By investigating differences

in the frequency of genetic markers between normal and anomalous colored wolves, it is possible to identify markers associated with the anomalous phenotype (Fig. 8; Korte & Farlow, 2013; Santure & Garant, 2018; Uffelmann et al., 2021).

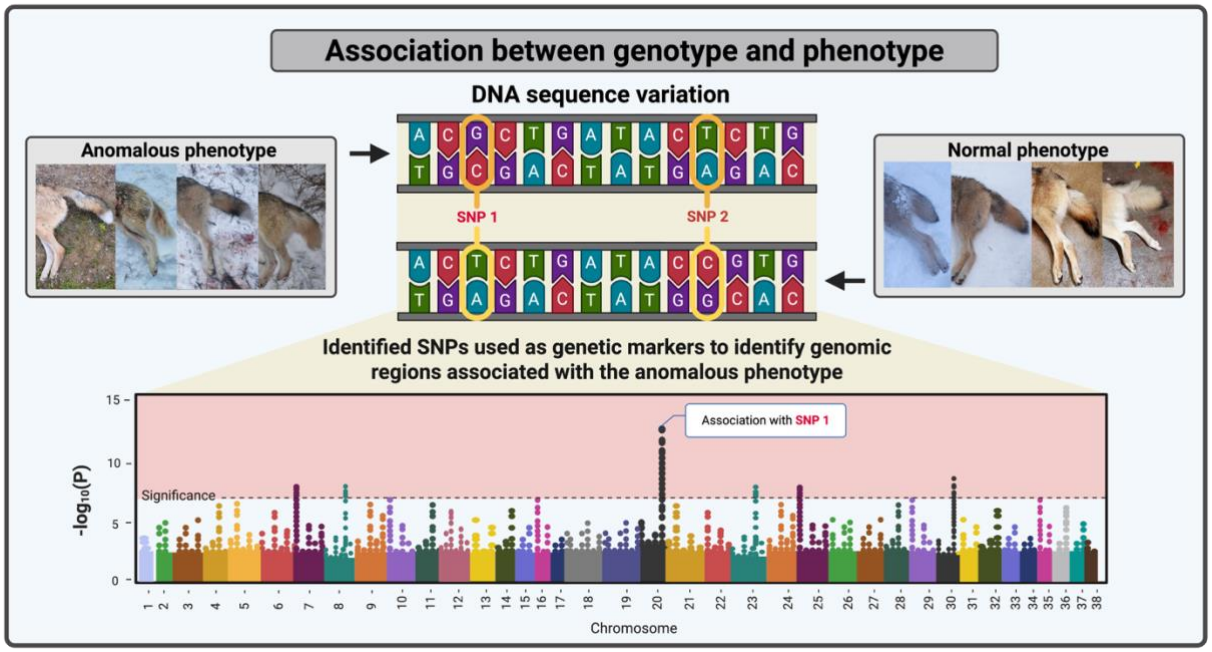


Figure 8. *Conceptual visualization of the GWAS approach. DNA sequence variation (SNPs) can give rise to phenotypic differences. Genotypic information for individuals with normal and anomalous phenotype can be used in association analyses to identify genetic markers associated with the anomalous phenotype. Created with BioRender.com.*

For the GWAS analysis, I used SNP genotype data for 38 individuals with phenotypic information. The data was quality controlled using the software PLINK version 1.9 for cleaning of genetic markers (Purcell et al., 2007; Uffelmann et al., 2021). Quality control involved filtering of SNPs based on sample call rate (mind <0.1), genotyping call rate (geno<0.1), minor allele frequency (maf<0.01), and deviations from Hardy Weinberg equilibrium (hwe<0.001). These filters exclude individual samples and SNPs with more than 10% missing genotype data, which might indicate genotyping errors or low quality (Marees et al., 2018). Furthermore, SNPs with a minor allele frequency of 1% were removed, since they are more prone to errors as a limited number of samples carry the same minor allele (Smith et al., 2019; Turner et al., 2011). Lastly, SNPs significantly deviating from HWE were filtered out as they may indicate genotype errors (Marees et al., 2018). After filtering, the final dataset comprised 237,208 markers with an overall genotyping rate of 99.79%, indicating high quality across samples and SNPs (quality control summary can be found in Fig. A3, Fig. A4 and Table A5).

The GWAS was performed using the software tool Genome Wide Complex Trait Analysis (GCTA version 1.94.4; Yang et al., 2011) and only included autosomal markers (chromosome 1-38). A mixed linear model-based analysis was implemented using the "mlma-loco" module, where the chromosome that the focal SNP is located on is left out of the genomic relationship matrix (GRM), and tested as a fixed effect, whereas the SNPs on the remaining chromosomes are fitted as random effects (Brekke et al., 2023; Yang et al., 2011). The model was fitted as:

$$y = a + bx + g + e$$

where y is the tail phenotype, a is the trait mean, b is the fixed additive effect of the SNP tested for association, and X is the SNP genotypes for the heterozygote and the two homozygotes. g is a vector of random effects ~ N (0, $\mathbf{G}\ \sigma_a^2$), where **G** is the genomic relatedness matrix calculated from the SNPs on all chromosome, except the chromosomes where the SNP tested for association is located, and σ_a^2 is the genetic variance, and e is the residual term ~ N (0, $\mathbf{I}\ \sigma_e^2$), where σ_e^2 is the residual variance and **I** is an identity matrix. The significance threshold for association was determined by using a Bonferroni correction at $\alpha = 0.05$, determined by $p = 0.05/237{,}208 = 2.11 \cdot 10^{-7}$. The CanFam3.1 genome assembly was used to investigate possible candidate genes based on the associated markers identified in the GWAS analysis (National Library of Medicine, 2011).

Haplotype analysis of MITF candidate gene

MITF is an important coat color gene, as it encodes a transcription factor that regulates the development and migration of melanocytes (Körberg et al., 2014; Levy et al., 2006). Genetic variants affecting MITF might therefore affect melanocytes, leading to disrupted pigment production and hypopigmentation, making MITF an obvious candidate gene. Genetic markers within and adjacent to this gene were examined to determine potential association with anomalous coat color by conducting a haplotype analysis, to identify groups of linked and co-inherited genetic variants (alleles) transmitted from parent to offspring, forming a haplotype (Yang et al., 2008). Haplotypes can provide a broader understanding of the genetic mechanism of traits than genome wide association analyses only testing individual SNPs for association, which may lead to lower detection of causal variants (Al Bkhetan et al., 2019; Zakharov et al., 2013).

Genotype data for 25 SNPs spanning a genomic region of 204,296 base pairs, located within (n = 8) and surrounding (n = 17) the MITF gene (Fig. A5), were extracted for 77 individuals (38 phenotyped and 39 early-generation) and quality controlled using PLINK (see Genome wide association analysis for methodology and Table A6 and Fig. A6 for summary). A distinct haplotype identified in an anomalously colored individual, which was homozygous across all 25 markers, indicated a possible association with this MITF-linked haplotype. This haplotype was therefore used as a reference for further analysis to determine whether there was an association with anomalous coat color and to trace its origin and inheritance. Manual haplotype phasing was undertaken for individuals with the normal phenotype and early-generation individuals in order to identify carriers of the same set of SNP alleles, representing the same haplotype, or divergent sets of SNP alleles, representing other haplotypes in the population. Given that the population was initially founded by three individuals along with high levels of inbreeding, haplotype diversity and recombination have been limited (Chapman & Thompson, 2003; Hagenblad et al., 2009; Morrell et al., 2006; Viluma et al., 2022), likely causing conservation of founder haplotypes across generations through strong linkage disequilibrium (LD; Clark, 2004). This made it possible to make inferences about origin and inheritance, and most importantly, the potential involvement of MITF in the expression of anomalous coat color in the population.

3. Results

3.1. Frequency of anomalous coat color

In total, 476 individuals were phenotyped, of which 433 had normal phenotype and 43 had anomalous phenotype. Omitting individuals from observations, the frequency of the anomalous phenotype was 7,53% ($freq_{anomalous}$), including 14 (40%) females and 21 (60%) males. Whereas the frequency of the normal phenotype ($freq_{normal}$) was 92,47%, including 181 (42,09%) females, 248 (57,67%) males, and 1 (0.23%) individual of unknown sex (Fig. 9). There was no significant difference in the proportion of males and females exhibiting normal and anomalous phenotype ($p = 0.792$), and no significant trend in the distribution of normal and anomalous phenotype over time ($p = 0.274$) during the period 2002-2024.

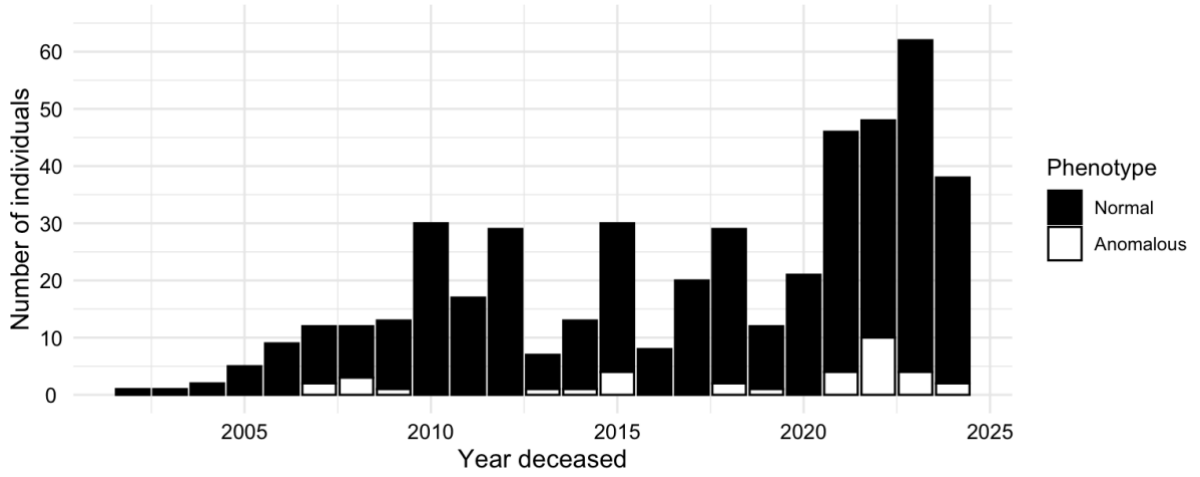


Figure 9. *Number of phenotyped individuals and their respective proportions, excluding observations (n = 11), distributed by the year they were deceased during the period 2002-2024 (n = 465).*

3.2. Degree of inbreeding

The majority of Scandinavian wolves were inbred, of which 93% (991) were inbred ($F > 0$), while only 6,5% (69) were non-inbred ($F = 0$; Fig. 10). During the period 1983-2023, F ranged from 0 to 0.49, with an average F close to mating between full siblings (0.23 ± 0.09 SD; Table 2).

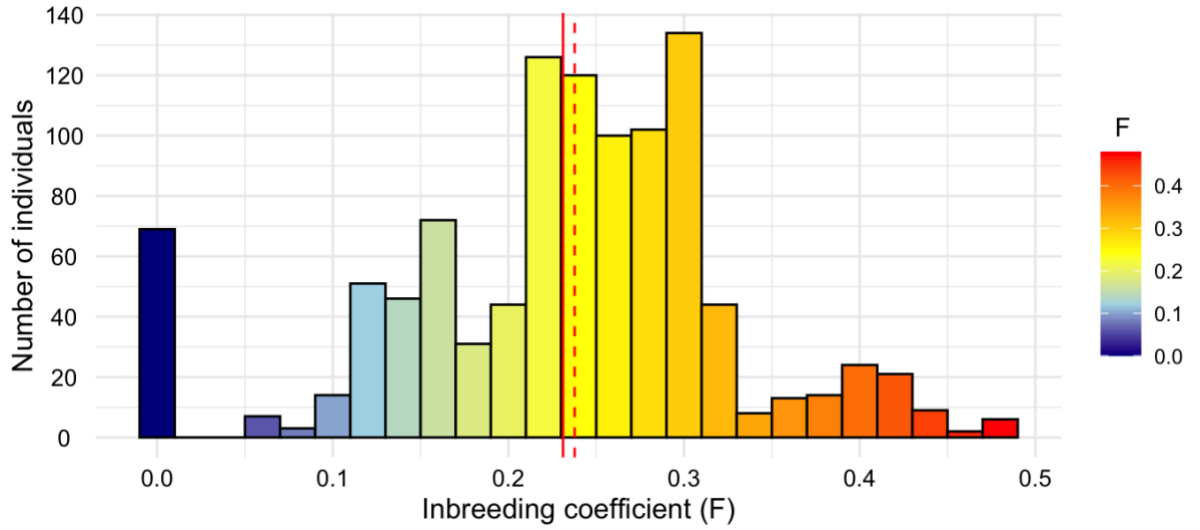


Figure 10. *Distribution of F estimated from the pedigree for all individuals (n = 1,060). F ranged from low (0-0.1), moderate (0.1-0.2), average (0.2-0.3), high (0.3-0.4), to extreme (0.4-0.5) levels. Stippled red line represents the median (0.237), and the solid red line represents the mean (0.231).*

Table 2. Descriptive statistics of *F* estimates in total, for all phenotyped individuals and for each phenotypic group.

	N	Min *F*	Max *F*	Median *F*	Mean F (± SD)
Phenotype	476	0.00	0.4858	0.2485	0.2445 (0.088)
Normal	433	0.00	0.4858	0.2485	0.2426 (0.089)
Anomalous	43	0.1192	0.4027	0.2854	0.2632 (0.076)
Total	1,060	0.00	0.4858	0.2377	0.2311 (0.096)

For individuals with normal phenotype, *F* ranged from 0 to 0.49, with the majority having *F* lower than 0.25. Whereas for individuals with anomalous phenotype, *F* ranged from 0.12 to 0.40, with the majority of individuals having *F* higher than 0.25. There was no significant difference in *F* between the two phenotypic groups (p = 0.145; Fig. 11).

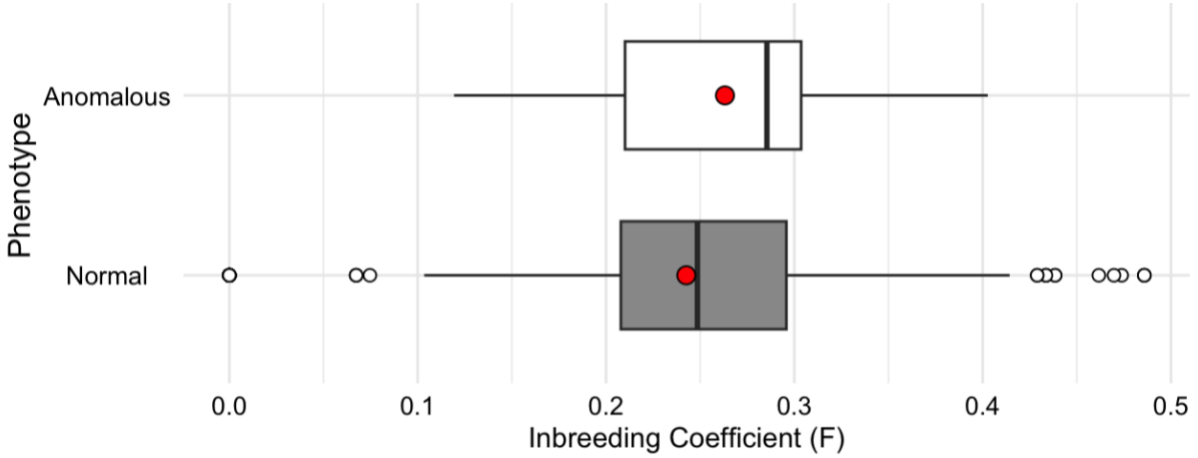


Figure 11. *Boxplot representing the distribution of F within and between the two phenotypic groups. Black horizontal line represents the median and the red dot represents the mean.*

3.3. The animal model

Estimates of the fixed effect of inbreeding

There was no significant effect of inbreeding on phenotypic variation detected based on the animal model (p = 0.78; Table 3; Fig. A7).

Table 3. Output for the fixed effects in the animal model, including estimates, standard error, z. ratio, and the p-value obtained from the Wald analysis of variance.

Fixed effects	Solution	Std. error	z. ratio	p-value
Intercept	0.0284	0.0847	0.3363	0.737
Inbreeding	0.0583	0.2124	0.2747	0.783

Variance components and narrow-sense heritability estimates

The additive genetic variance (V_A) was estimated at 0.051, and the residual variance (V_R) was estimated at 0.042 (Table 4). Together they made up the total phenotypic variance (V_P) of 0.0937, where V_A accounted for 55% and V_R accounted for 44,97%. The contribution of V_A to phenotypic variance was evident, as the inclusion of the additive genetic effect significantly improved the fit of the model (χ^2 (1) = 52.042, $p < 0.0001$; Fig. A8). The narrow-sense heritability (h^2) of tail phenotype was estimated at 0.55, indicating that 55% of the phenotypic variance could be attributed to genetic variation explained by V_A.

Table 4. Estimates for variance components obtained from the animal model, including standard error, z. ratio and their proportion contributed to the phenotypic variance.

	Component	**Std. error**	**z. ratio**	**Proportion of V_P**
V_A	0.0516	0.0129	2.9888	55.03
V_R	0.0421	0.0072	5.8455	44.97

3.4. Genome wide association analysis

There was no significant association detected between genetic markers and the anomalous phenotype (p > Bonferroni correction; Fig. 12; Table 5). The SNPs with the lowest p-values all clustered within chromosome 11, with the majority positioned within a restricted genomic region (22,368,134 – 23,937,628 bp). There were no genes associated with coat color within this region, and the closest coat color gene, being the tyrosinase-related protein 1 (TYRP1), associated with shifts from black to brown pigment production (Van Buren et al., 2021), was located 9.3 megabases (9,300,000 bp) downstream of this region.

Table 5. Summary of the GWAS results. Chr indicates the chromosome the SNP is located on, SNP positions are in base pairs, A1 and A2 are the two alleles observed at the given SNP, frequency and effect of A1, standard error of the effect, and p-value indicating degree of significance (significance threshold 2,11e^{-07}).

Chr	SNP	bp	A1	A2	Freq	b	Std. error	p-value
11	89256	22368134	A	G	0.0263158	0.5	0.114192	1.19444e^{-05}
11	89293	23076669	G	A	0.0263158	0.5	0.114192	1.19444e^{-05}
11	89311	23268094	C	T	0.0263158	0.5	0.114192	1.19444e^{-05}
11	89320	23355706	G	A	0.027027	0.500001	0.114278	1.21257e^{-05}
11	89330	23569124	T	C	0.0263158	0.5	0.114192	1.19444e^{-05}
11	89352	23756076	G	A	0.0263158	0.5	0.114192	1.19444e^{-05}
11	89370	23937628	C	T	0.0263158	0.5	0.114192	1.19444e^{-05}

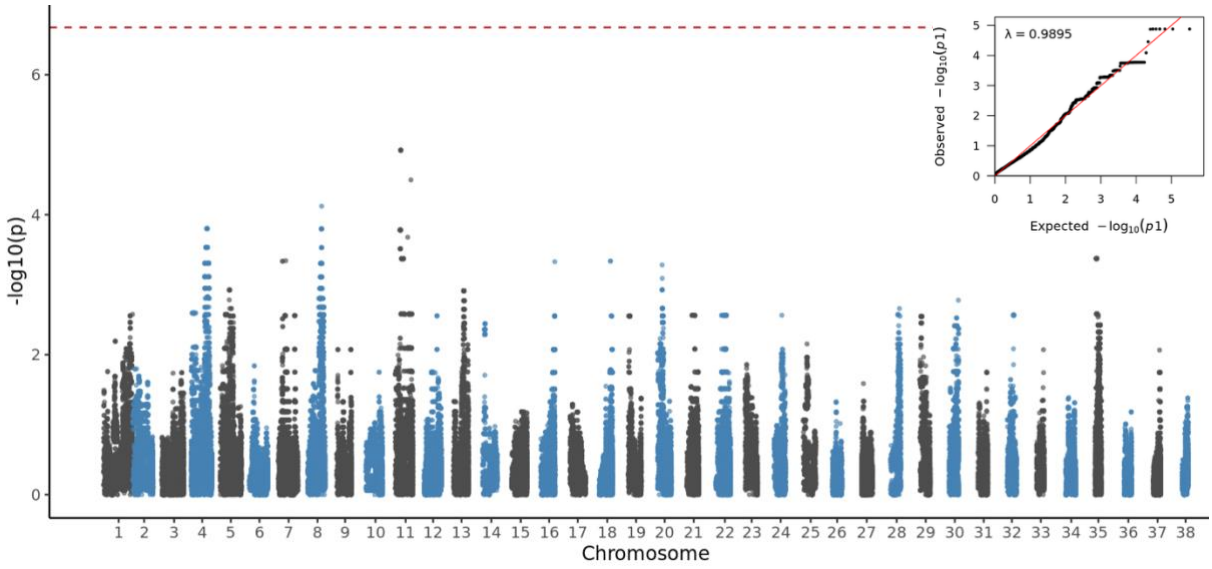


Figure 12. *Manhattan plot visualizing the GWAS results based on the 38 phenotyped individuals (1 anomalous and 37 normal). The plot shows the strength of the association between the anomalous phenotype and each SNP represented as a dot ordered on the x-axis according to their genomic position (chromosome 1-38). The y-axis represents the strength of their association as the -log10 transformed p value (Uffelmann et al., 2021). Red stippled line represents the threshold for significance using the Bonferroni correction. The right corner plot is the quantile-quantile plot showing the observed versus expected p-values under no association, with dots indicating the -log10(p) of the SNPs, and the diagonal line represents the expected values under no association. Observed p-values aligned well with expected values as evident from the genomic inflation factor (λ) close to 1, indicating marginal inflation (e.g., population structure or genotyping error).*

3.5. Haplotype analysis

Association with MITF

The distinct haplotype (H1) located within and adjacent to the MITF gene carried by the anomalously colored individual (D-08-15; Fig. 13) showed that the trait and the haplotype co-segregated, indicating a possible association with MITF. The H1 haplotype was identified in an additional 30 of the 77 analyzed individuals (Fig. 13; genotypes for all individuals can be found in Table A7). The 30 individuals were heterozygous carriers of the SNP specific alleles that coincided with those of the H1 haplotype, and they had either normal (n =14) or an unknown phenotype (n = 16). One of the carriers was an immigrant from the Finnish-Russian population from 2020 (G71-20) (Fig. 13). Another individual (D-10-25) showed almost complete homozygosity for the haplotype, but carried a deviating allele on SNP 20, which may be explained by a genotyping error or a point mutation at that specific position. In total, 46 individuals were noncarriers, of which 23 had normal and 23 had an unknown phenotype.

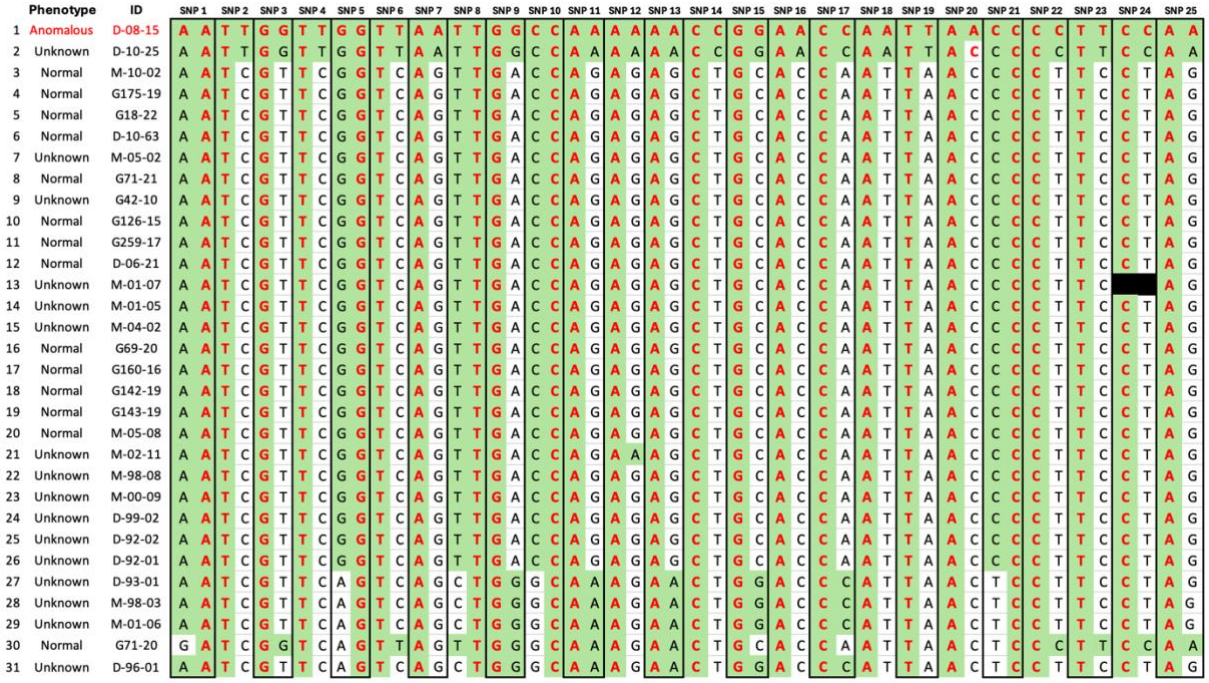


Figure 13. *Genotypes across 25 SNPs within and adjacent to the MITF gene for the 31 carriers of the H1 haplotype. Each row shows an individual´s genotype, whereas the column shows the two alleles at each SNP position. The first individual highlighted in red is the anomalously colored individual homozygous for the H1 haplotype. Green color indicates a match with the reference allele in the H1 haplotype at that given position and is also highlighted in red to show the presence in all individuals. White color represents the presence of alternative alleles and indicates no match with the reference allele. Black background color indicates missing data.*

Origin

The origin of the H1 haplotype identified in the anomalously colored individual was further investigated in 18 early-generation individuals, including the founding female of Nyskoga (D-85-01), 3 of her offspring, and an additional 8 descendants, as well as 6 offspring of the third founder (G1-91) of Gillhov. Investigation of these two groups of wolves, Nyskoga and Gillhov, resulted in the identification of 3 additional haplotypes (H2, H3, and H4) that differed from that of the anomalously colored individual (Fig. 14).

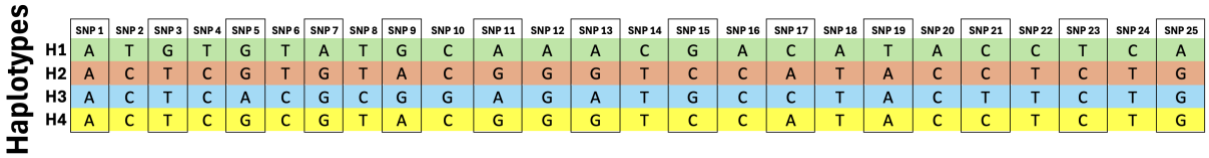


Figure 14. *Overview of the 4 identified haplotypes. Haplotype H1 is the haplotype found in homozygous state in the anomalously colored individual. The remaining haplotypes (H2, H3, and H4) were identified from the Nyskoga lineage.*

Three of the four haplotypes could be traced back to the Nyskoga lineage. From the data, I was able to identify the haplotypes of the female founder of Nyskoga (H3 and H4; Fig. 15), and 10 of her 11 descendants showed almost complete fixation for one of the haplotypes (H4; Fig. 15), demonstrating that the male founder, as well, carried this haplotype. One descendant also carried another haplotype (H2) not compatible with the H4 haplotype due to a single deviating allele on SNP 6. This was considered as the second haplotype of the male founder but might as well be the result of a genotyping error or a point mutation (Fig. 15).

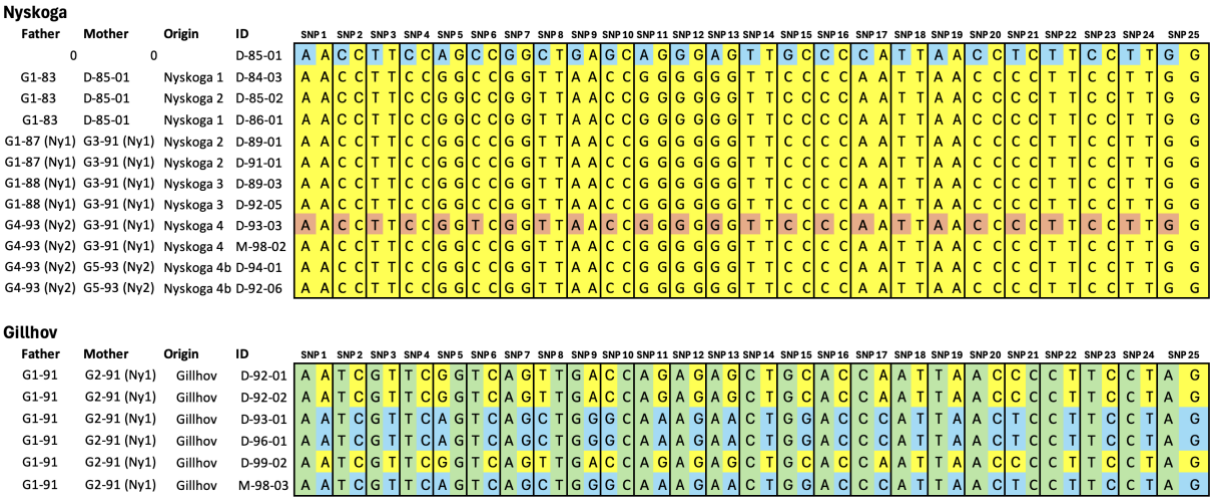


Figure 15. *Genotypes across 25 SNPs within and adjacent to the MITF gene for the 18 early-generation individuals. Nyskoga shows the two haplotypes of the female founder (D-85-01) from the founding pair in 1983, three of her offspring, and 8 descendants. Whereas Gillhov shows the haplotypes for the 6 offspring of the third founder (G1-91).*

For the offspring of the third founder (G1-91, Gillhov), three haplotypes are present. Two of the haplotypes were also found in the Nyskoga lineage (H3 and H4), transmitted by the reproductive female in Gillhov, who was an offspring of the founding pair in Nyskoga. The other haplotype, not identified in any of the Nyskoga wolves, was the same haplotype found in the anomalously colored individual (H1). All genotyped offspring of the third founder were heterozygote carriers of the SNP specific alleles of the H1 haplotype.

Inheritance

From the pedigree, the H1 haplotype could be traced back to the third founder (Fig. 16). The anomalous phenotype first appeared in the Jangen 1 territory, which was the result of mating between two descendants, both of them grandchildren to one of his female offspring; M-98-03, confirmed to carry the H1 haplotype (Fig. 16). The reproductive male of Jangen 1 was the full sibling of the anomalously colored individual confirmed to be homozygous for the H1 haplotype (D-08-15; Fig.16). The haplotype was also found to be carried by three other full siblings; M-01-07, D-10-25 and G42-10 (Fig. 13). The anomalous phenotype was only expressed for individuals homozygous for H1 haplotype, but not for heterozygote carriers, indicating that the allele defined by this haplotype at the MITF gene is recessive (Fig. 16).

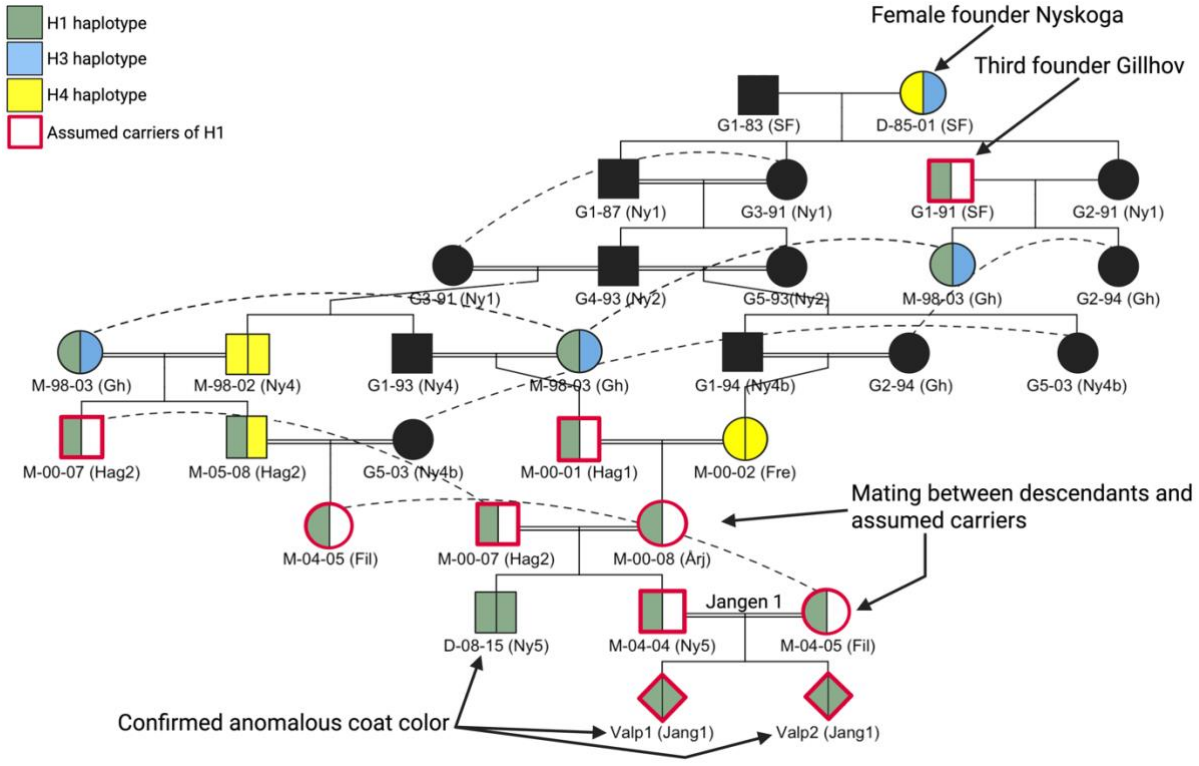


Figure 16. *Visualization of likely inheritance and transmission of the H1 haplotype and the anomalous coat color from the third founder (G1-91), only including reproductive individuals involved in the initial appearance of the trait. Symbols represent the homologous chromosomes of an individual, and the two colors correspond to the*

defined haplotypes in Fig. 14 inherited from their parents. Individuals with two copies of the same color symbolize homozygotes, whereas two different colors symbolize heterozygotes. Individuals with red outline had no genetic information but were assumed to be carriers of the H1 haplotype, as inferred from their own phenotype (anomalous) or the haplotypes of their close relatives. Made with the R-package "pedtools" and with BioRender.com.

4. Discussion

In this study, I investigated the occurrence of anomalous coat color in the Scandinavian wolf population. I found support for all three hypotheses, including recessive inheritance and effects of inbreeding, involvement of the MITF gene, and that the trait was introduced by one of the initial founders of the population. I identified a distinct haplotype spanning across the MITF gene, that was associated with the anomalous coat color. This haplotype was defined as a recessive allele at the MITF locus. Haplotype analysis demonstrated that this haplotype was introduced to the population by the third founder, reproducing for the first time in 1991. Because of subsequent genetic drift, this haplotype increased in frequency in the population and was expressed a few generations later as a result of inbreeding, leading to increased homozygosity and the inheritance of identical-by-descent segments.

4.1. Origin of the MITF haplotype linked to anomalous coat color

The distinct haplotype within a restricted genomic region of chromosome 20, defined by 25 genetic markers spanning across the MITF gene, was present in 31 of 77 analyzed individuals, including one individual with anomalous coat color (D-08-15). Notably, this individual was the only homozygous carrier of the haplotype, whereas heterozygous carriers had either normal or an unknown phenotype, strongly suggesting that the haplotype is associated with anomalous coat color. Investigation of the haplotype in early-generation individuals showed that the appearance of this haplotype coincided with new genetic material introduced by the third founder in 1991. Interestingly, Viluma et al. (2022) found a substantial proportion of new alleles appearing after 1993 to cluster within chromosome 20, where the MITF gene is located, and that these alleles coincided with haplotype blocks of this individual. Three additional haplotypes, which could be traced back to the Nyskoga lineage, were also identified, summing up to a total of 4 haplotypes, which is consistent with Viluma et al. (2022), who found on average 4.2 haplotypes contributed by the initial founders. Notably, all genotyped offspring of the third founder were heterozygote carriers of the haplotype found in the anomalously colored individual. A few generations after the arrival of the third founder, the anomalous phenotype

was expressed in wolves where both the mother and father must have been carriers of the recessive allele defined by the haplotype introduced by the third founder (Fig. 16).

4.2. Recessive inheritance and effects of inbreeding

Recessive traits often appear in a few affected individuals as they are often masked in heterozygotes (Crow & Kimura, 1970, p. 61). This was exactly what I observed, as the majority of individuals carrying the H1 haplotype were heterozygous, which is in agreement with previous findings of an excess of heterozygotes after 1996 (Bensch et al., 2006; Vila et al., 2003). Given the recessive mode of inheritance, inbreeding is an important driver for the expression of anomalous coat color. Indeed, inbreeding increases homozygosity by the inheritance of identical alleles from both parents, owing to parental sharing of IBD segments (Shafer & Kardos, 2025). This is apparent in the Scandinavian wolf population, considering several individuals have accumulated long runs of homozygosity (ROH), with some being inbred to an extent that almost entire chromosome pairs are IBD, lacking any variation (Kardos et al., 2018).

With the arrival of the third founder there was an increase in heterozygosity and evidence of inbreeding avoidance and outbreeding, mediated by his reproductive success and rapid spread of his alleles, with evidence of almost all individuals born after 1993 carrying at least one of his alleles (Bensch et al., 2006; Vila et al., 2003). However, this effect was only temporary, as in 1997 the average heterozygosity decreased (Vila et al., 2003) due to inbreeding, as descendants of the third founder started to mate with each other. This culminated among other less conspicuous effects of inbreeding, to the expression of anomalous coat color in 2004. From the pedigree, it is also evident that there was an increase in inbreeding over time with mating between descendants of the third founder, leading up to the initial occurrence of the anomalous phenotype (Fig. A10).

Although several factors support the significance of inbreeding, the animal model did not detect any significant effect of inbreeding on phenotypic variation in the population ($p = 0.78$). The ability of the model to detect an effect might have been limited considering almost all individuals were inbred (93%), with an average F close to mating between full siblings ($F = 0.23$). Further limitations could be the non-significant difference in average F between the two phenotypic groups ($p = 0.145$). Indeed, all individuals exhibiting anomalous coat color were

inbred, indicating an effect of inbreeding on its expression. However, both phenotypic groups were highly inbred, which might have complicated the isolation of the effect of inbreeding and probably obscured any detectable effect in the animal model. Lastly, one cannot ignore the disparity of the sample size with only 43 individuals with anomalous and 433 with normal phenotype, which might also have constrained statistical significance.

4.3. MITF as a genetic determinant for anomalous coat color

The haplotype associated with anomalous coat color included genetic markers within a restricted region covering the MITF gene, which demonstrates that genetic variants linked to this gene are involved in the expression of the trait. Although GWAS is a widely used tool to identify coat color associated candidate genes and causal variants (Hédan et al., 2019; Tietgen et al., 2021; Zhang et al., 2023), and the use of dense SNP genotype data, likely increasing the probability for detecting associations (Klein, 2007), no significant association with any of the markers within or adjacent to MITF was detected. There may be several reasons for this. Genome wide association analyses are designed to identify associations between traits and a single variant (SNPs), hence, if several variants explain a small portion of the variation within a trait, it may not result in the detection of an association (Al Bkhetan et al., 2019). Moreover, one important and highly limiting factor of the analysis is the profoundly skewed and small sample size. GWAS normally require a large sample size, with similar representation in the affected and unaffected group (Korte & Farlow, 2013; Visscher et al., 2017). The analysis included only 38 individuals, of which only one was affected (anomalously colored), which is likely to have limited the detection ability significantly (Korte & Farlow, 2013; Uffelmann et al., 2021).

Despite the disparity in sample size, I successfully identified an association with the MITF gene by including several genetic markers using a haplotype analysis. The clear association detected from this analysis by a unique pattern for the anomalously colored individual, suggests that the 25 SNPs of the haplotype may have small individual impact on the trait and therefore do not reach the threshold for detection, but that the combined effect of these variants may still be important for the expression of anomalous coat color. This might be supported by Körberg et al. (2014), who investigated canine MITF variants in relation to hypopigmentation and suggested that a combined effect of several, rather than single variants, are responsible for hypopigmentation. However, it might be that these markers are simply associated with the

causal variants and do not have a direct effect themselves. Even so, according to Couzin (2002), haplotypes associated with specific traits indicate that the genetic variants involved in its expression are likely to lie within the haplotype. Therefore, although the definitive causal variants remain unknown, the haplotype analysis supports MITF as a central candidate gene and that variants linked to this gene may give rise to anomalous coat color.

The effect of MITF is reasonable, considering it has previously been linked to hypopigmentation in several species, including dogs (Baxter et al., 2004; Charon & Lipka, 2015; Hauswirth et al., 2012; Hofstetter et al., 2019), and is likely to cause hypopigmentation in wolves as well. Indeed, since the MITF gene is important for melanocyte development, mutations in the MITF locus may impair melanocyte development, leading to unpigmented areas (MITF; Brancalion et al., 2022; Wakamatsu & Ito, 2021, p. 50). In the case of the Scandinavian wolf population, this kind of hypopigmentation is expressed through white tail tips and larger white patches. Admittedly, further research is needed to resolve the exact causal variants, which would require phenotype and genetic information on additional anomalously colored wolves, to increase the sample size and the power and probability of detecting associations (Spencer et al., 2009; Tam et al., 2019). Furthermore, genetic information should preferably be obtained by whole genome sequencing (WGS), as it can identify both known and novel variants, such as mutations and structural variants (Uffelmann et al., 2021; Visscher et al., 2017).

4.4. Natural variation for coat color determining genes

Although genetic variation linked to anomalous coat color originated from the third founder, the origin prior to this individual remains unknown. There have been speculations that this is evidence of hybridization and subsequent introgression of dog-specific mutations (Personal communication with Ø. Flagstad, 28.05.2025). Indeed, hybridization has introduced variants that have given rise to deviating coat color in wolves and coyotes (*C. latrans*) with a melanistic (black) coat color in North America (Anderson et al., 2009). Such melanistic individuals have also been observed after hybridization events in the Scandinavian and Finnish-Russian population (Tirronen & Kuznetsova, 2024; Wabakken et al., 2018), however, none of them have contributed to reproductions in Scandinavia, as evident from the pedigree (Liberg et al., 2005; Liberg et al., 2024, pp. 203-204; Ministry of Agriculture Forestry of Finland, 2019). Although hybrids are strictly prohibited and removed, and detailed genetic monitoring of all reproductive individuals makes it possible to detect hybridization (Bischof et al., 2019; Liberg et al., 2024,

pp. 203-204), one cannot exclude the possibility of hybrid events that may have taken place before recolonization several centuries ago (Caniglia et al., 2013, 2020; Dziech, 2021; McFarlane & Pemberton, 2019; Smeds et al., 2021; Stronen et al., 2022). Nevertheless, various studies regarding the origin of the Scandinavian wolf population strongly support a pure wolf origin (Skage et al., 2017; Smeds et al., 2021; Vila et al., 2003), with no evidence of dog introgression in either population (Fennoscandia; Smeds et al., 2021). It is therefore unlikely that the anomalous coat color could be explained by recent hybridization. Furthermore, although hypopigmentation is a common feature in many dog breeds (Brancalion et al., 2021), it is possible that the genetic variants involved in hypopigmentation originated from ancient wolf populations prior to domestication (Bannasch et al., 2021). This variation was probably captured during the domestication of the wolf and later selected for in several dog breeds.

Anomalous coat color could therefore instead be the result of genetic variation stemming from the wolf gene pool (Apollonio et al., 2004; Khosravi et al., 2015; Ollivier et al., 2013) or from variation due to mutations (Protas & Patel, 2008; Randi & Lucchini, 2002). There is scarce evidence of wolves with anomalous white tail tips, such as those observed in Scandinavia, in North America (Denali National Park, USA, Borg, 2016 & Borg & Klauder, 2019; Vancouver Island, Canada, Burr et al., 2022; Minnesota, USA, Gable et al., 2024; Personal communication M. Gilbert, 24.02.25), and has, only recently, been observed in a single individual in the Finnish-Russian population as well (Personal communication Ø. Flagstad, 20.06.2025). Still, such traits are rarely expressed, due to low levels of inbreeding or that there is selection against such variants due to possible pleiotropic effects and/or reduced camouflage (Gong et al., 2021; Körberg et al., 2014; Stritzel et al., 2009).

One would expect important phenotypic traits such as coat color to be driven by natural selection and be relatively uniform and preserved over time, considering their impact on fitness (i.e., camouflage, thermoregulation, sexual selection; Caro & Mallarino, 2020). However, the ability of selection to counteract drift and remove unfit or even deleterious alleles, as well as select for adaptive ones, depends on the size of the population (Charlesworth, 2009; Lande, 1988; Leonard et al., 2014). For this reason, genetic drift might become the major force driving phenotypic traits in small and isolated populations, and could lead to phenotypic change through increased frequencies of rare color variants by overriding the force of selection (Leonard et al., 2014; Tensen & Fischer, 2024). Anomalous coat color in wolves may therefore derive from the natural combinations of wolf alleles in coat color determining genes (Apollonio et al., 2004),

which generally occur at low frequencies due to selection against hypopigmentation. However, with prolonged and extensive inbreeding spanning several decades, such variants can be expressed more frequently in Scandinavian wolves than in other larger and less inbred populations.

4.5. Implications for conservation management

For small, isolated, and inbred populations, such as the Scandinavian wolf population, immigration is advantageous by increasing genetic variability and counteracting negative effects caused by inbreeding (Akesson et al., 2022). However, immigration might also cause unforeseen side effects, which might affect populations negatively (Bell et al., 2019; Hedrick & Garcia-Dorado, 2016; Ingvarsson, 2002; Robert et al., 2003). The immigration of the third founder led to an immediate rescue of the severely inbred population by increasing heterozygosity and facilitating outbreeding (Vila et al., 2003). However, it is also evident that this founder brought with him genetic variants linked to the anomalous coat color. Additional research and monitoring are needed to investigate possible pleiotropic effects of hypopigmentation. Regardless of this, it is still necessary to facilitate immigration. Indeed, even though some negative variants may be introduced, immigrants generally have positive effects, contributing to ensuring the long-term viability of genetically depleted populations (Akesson et al., 2022; Frankham, 2015; Kardos et al., 2021).

5. Conclusion

This study represents the first attempt to answer long-standing questions regarding the occurrence of anomalous coat color in the Scandinavian wolf population. I have documented its origin and inheritance as well as the underlying genetic mechanism and effects of inbreeding. The results demonstrate that the anomalous coat color is associated with a distinct haplotype involving the MITF gene, indicating that this gene is involved in the expression of the anomalous coat color in the population. The origin of this haplotype coincided with the arrival of the third founder, suggesting that the anomalous coat color was introduced by this individual. Furthermore, the haplotype showed a recessive mode of inheritance, which further demonstrated that inbreeding affects both the occurrence and expression of the anomalous coat color through increased homozygosity and inheritance of IBD segments.

The expression of anomalous coat color in a small and highly inbred population illustrates how genetic drift and inbreeding can become strong forces shaping phenotypic traits in such populations and further demonstrates the influence of inbreeding on the expression of rare genetic variants through increased homozygosity. The anomalous coat color can therefore reveal underlying genetic mechanisms, supporting anomalous coat color as an indicator for population genetics. From a conservation and management perspective, the occurrence of anomalous coat color demonstrates how few founders and small population size highly limit the genetic variability of populations, as well as the potential of inbreeding to expose recessive traits. Furthermore, it highlights the importance of maintaining genetic diversity and facilitating the immigration of unrelated individuals with new genetic variation, but also how vital immigration can result in unforeseen side effects, such as hypopigmentation. Although the specific genetic variants remain unknown, this study forms a framework for future research, which should focus on monitoring and genetic sampling of anomalously colored individuals to confirm the functional role of MITF and its genetic variants, as well as to assess potential pleiotropic effects and fitness consequences linked to hypopigmentation.

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7. Appendices

Appendix 1. Overview of phenotypic data material and the selection process.

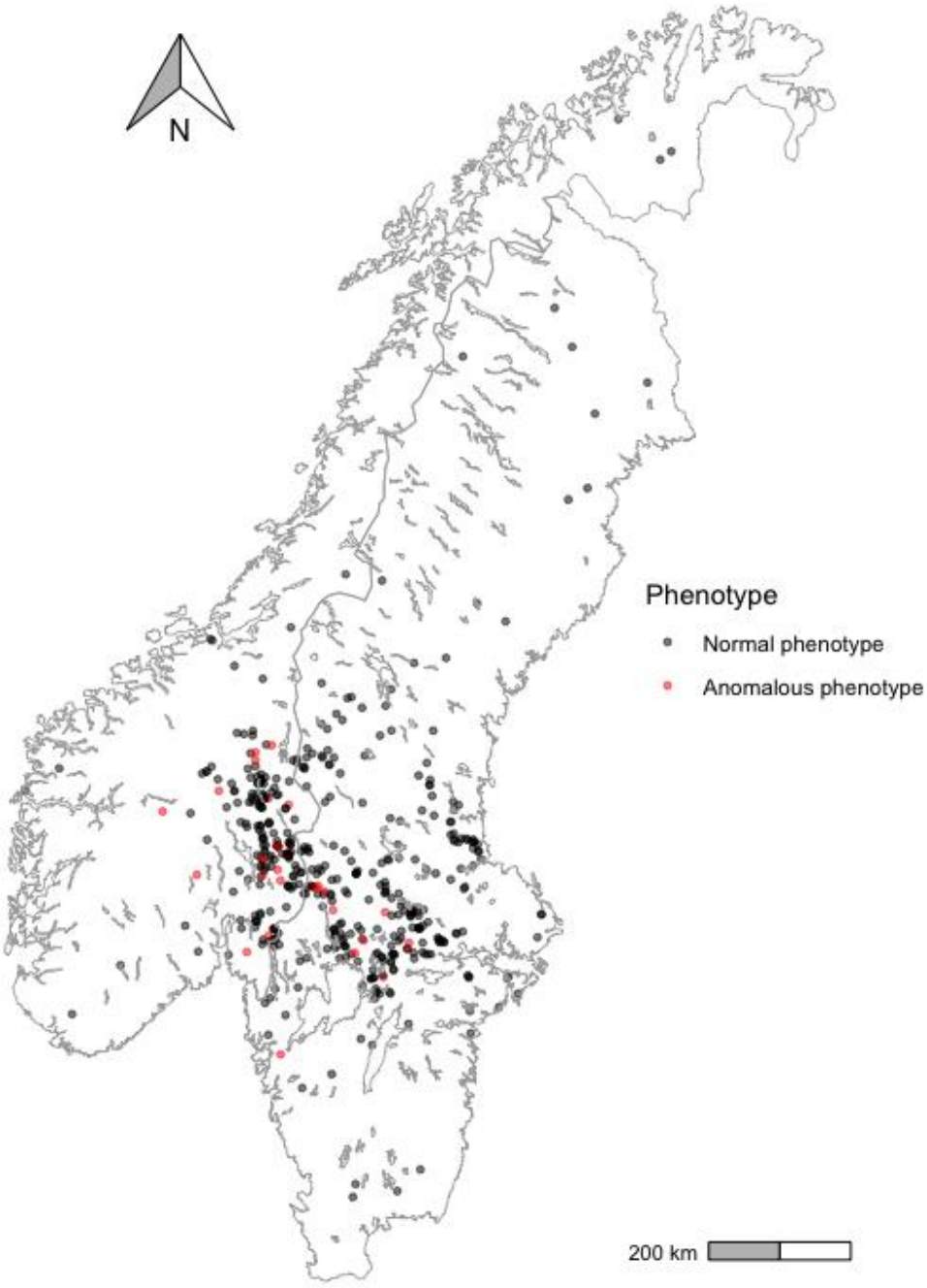

Figure A1. Map of locations where phenotyped individuals collected in Rovbase and SVA (n = 465) were culled or found deceased. Color of symbols indicates phenotypes, where red indicates anomalous phenotype (n = 35), and black indicates normal phenotype (n = 430). The map does not include individuals detected during observations (n = 11) as these had unknown coordinates. In total, 188 were retrieved in Norway (anomalous n = 23, normal n = 165), and 277 individuals were retrieved in Sweden (anomalous n = 12, normal n = 265).

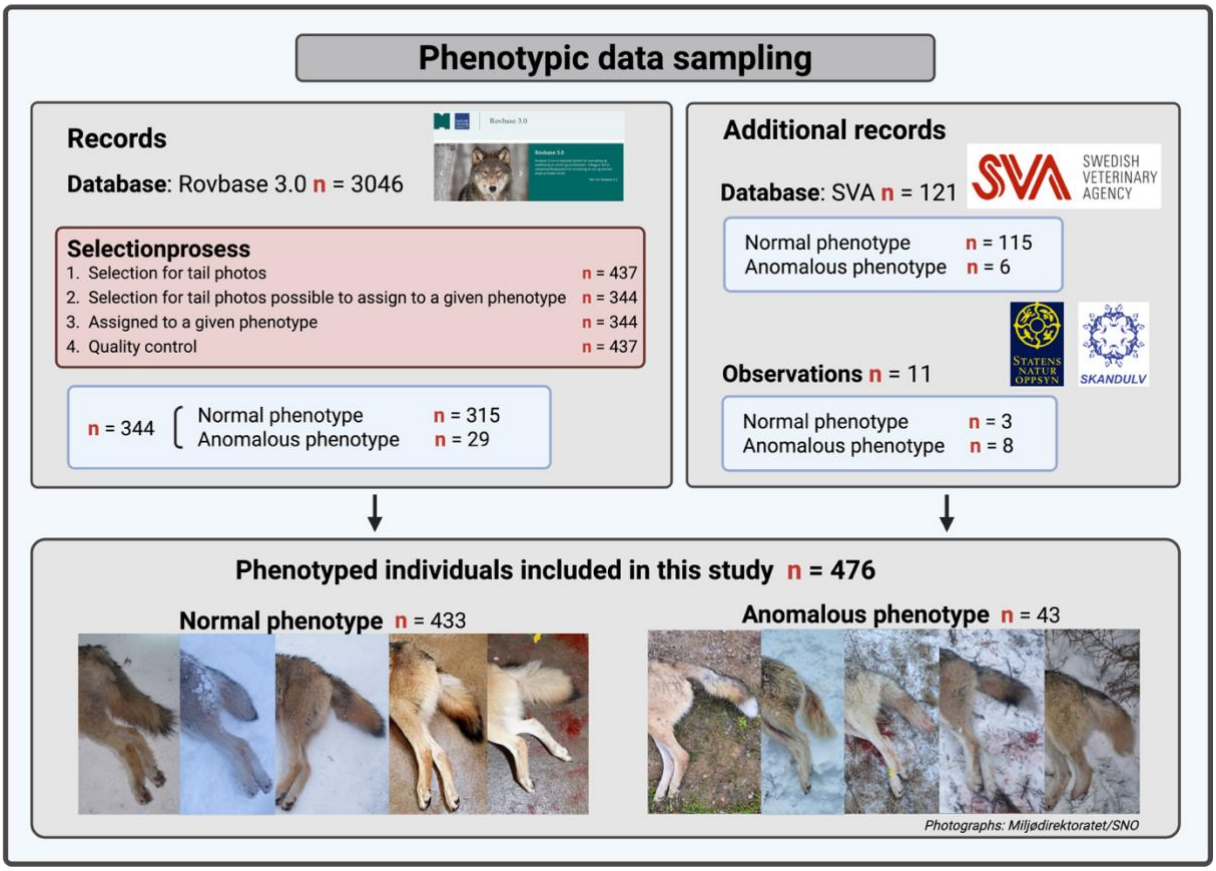

Figure A2. Overview of the sampling process for phenotypic data. Photographs were obtained from Rovbase 3.0 and retrieved from SVA, along with photographs from observations by SKANDULV and SNO. A total of 476 individuals were phenotyped. Created with BioRender.com.

Table A1. Overview of phenotypic data from each source grouped by phenotype and sex.

Phenotype	**Normal**			**Anomalous**			**Total**
Source	**Rovbase**	**SVA**	**Obs**	**Rovbase**	**SVA**	**Obs**	
Unknown sex	1	0	1	0	0	5	7
Females	131	50	2	11	3	1	198
Males	183	65	0	18	3	2	271
Total	315	115	3	29	6	8	476

Appendix 2. Overview of genotypic data.

Table A2. Overview of the 38 genotyped individuals included in the GWAS and haplotype analysis. Information includes individual ID, phenotype, sex, generation, death year, and *F*.

	Individual	Phenotype	Sex	Generation	Death year	*F*
1	D-10-62*	Normal	F	0	2010	0
2	D-10-63*	Normal	F	0	2010	0
3	G71-20*	Normal	M	0	2020	0
4	M-05-08	Normal	M	4	2007	0,125
5	D-08-15	Anomalous	M	6	2008	0,2695
6	D-06-21	Normal	F	7	2006	0,2998
7	M-06-08	Normal	M	7	2008	0,2656
8	D-10-40	Normal	F	7	2010	0,2675
9	M-10-02	Normal	M	7	2012	0,2607
10	G42-11	Normal	F	7	2012	0,227
11	G33-08	Normal	M	8	2011	0
12	G63-12	Normal	M	8	2015	0,2761
13	G122-12	Normal	M	9	2012	0,3519
14	G70-14	Normal	F	9	2015	0,1468
15	G96-12	Normal	M	9	2021	0
16	G11-12	Normal	M	10	2012	0,1266
17	G26-13	Normal	M	10	2012	0,4143
18	G132-12	Normal	M	10	2015	0,1575
19	G98-16	Normal	F	10	2017	0,2932
20	G126-15	Normal	M	10	2018	0,304
21	G175-19	Normal	M	10	2022	0,134
22	G11-15	Normal	M	11	2017	0,0673
23	G103-15	Normal	M	11	2017	0,2223
24	G104-15	Normal	M	11	2021	0,0673
25	G156-14	Normal	M	11	2021	0,2246
26	G88-16	Normal	F	11	2021	0,2149
27	G45-19	Normal	M	11	2021	0,2223
28	G259-17	Normal	F	11	2022	0,3118
29	G160-16	Normal	F	11	2023	0,2338
30	G71-21	Normal	M	12	2021	0,3066
31	G142-19	Normal	M	12	2021	0,3066
32	G143-19	Normal	M	12	2021	0,3066
33	G264-17	Normal	M	12	2022	0,2064
34	G9-18	Normal	F	12	2022	0,2355
35	G18-22	Normal	M	13	2022	0,2342
36	G238-19	Normal	M	13	2022	0,3937

37	G69-20	Normal	M	14	2021	0,2694
38	G73-21	Normal	M	14	2021	0,4056

*D-10-62 and D-10-63 are Scandinavian born with unknown parents, G71-20 non-reproducing immigrant.

Table A3. Overview of the 39 early-generation individuals included in the haplotype analysis. Time period was based on the period their parents reproduced, as exact birth year was unknown, with information gathered from the overview of first reproductive events of pairs, and from Wabakken et al. (2001) and annual reports from Rovdata (https://rovdata.no/Ulv/Rapporter.aspx, last accessed 20.05.2025). Nyskoga had confirmed reproductions from 1987-1994, but some uncertainty remains in how long each pair mated.

	Individual	Time period	Territory
1	D-85-01	-	Founder and reproductive female of Nyskoga 1
2	D-84-03	1983-1985	Nyskoga 1
3	D-85-02	1983-1985	Nyskoga 1
4	D-86-01	1983-1985	Nyskoga 1
5	D-89-01	1987	Nyskoga 2
6	D-91-01	1987	Nyskoga 2
7	D-89-03	1988-1990	Nyskoga 3
8	D-92-05	1988-1990	Nyskoga 3
9	M-98-02	1991	Nyskoga 4
10	D-93-03	1991	Nyskoga 4
11	M-98-03	1991-1993	Gillhov
12	D-99-02	1991-1993	Gillhov
13	D-92-02	1991-1993	Gillhov
14	D-96-01	1991-1993	Gillhov
15	D-92-01	1991-1993	Gillhov
16	D-93-01	1991-1993	Gillhov
17	D-94-01	1992-1994	Nyskoga 4b
18	D-92-06	1992-1994	Nyskoga 4b
19	M-01-04	1993-1994	Hagfors 1
20	M-00-02	1994-1996	Fredriksberg
21	D-00-15	1994-1996	Fredriksberg
22	M-00-09	1994-1996	Fredriksberg
23	M-01-09	1995-1998	Hagfors 2
24	M-01-05	1995-1998	Hagfors 2
25	M-98-08	1995-1998	Hagfors 2
26	M-01-10	1997-2000	Koppang 1
27	M-04-02	1997-2001	Årjäng
28	M-02-09	1997-2001	Årjäng
29	M-02-11	1997-2001	Årjäng
30	D-05-23	1997-2001	Årjäng

31	M-05-02	1998-2001	Filipstad
32	D-00-12	1998-2003	Filipstad
33	M-98-01	1998-2003	Filipstad
34	M-01-06	2000-2002	Hasselfors 1
35	M-05-06	2002-2003	Filipstad 2
36	G42-10	2000-2008	Nyskoga 5
37	M-01-07	2000-2008	Nyskoga 5
38	D-10-30	2000-2008	Nyskoga 5
39	D-10-25	2000-2008	Nyskoga 5

Appendix 3. Quality control of estimated *F* from pedigree data.

Table A4. Overview of *F* for a subset of individuals estimated previously in Kardos et al. (2018, Supplementary information: Table S1) (F_K) and own estimates (F).

	Individual (*founders)	F_K	F
1	D-85-01*	0	0,000
2	G23-13	0	0,000
3	G31-13*	0	0,000
4	M-07-02	0	0,000
5	M-09-03*	0	0,000
6	M-10-10*	0	0,000
7	M-98-02	0,375	0,375
8	D-99-02	0	0,000
9	M-98-03	0	0,000
10	M-98-08	0,125	0,125
11	M-00-09	0,125	0,125
12	D-00-15	0,125	0,125
13	D-05-23	0,234	0,234
14	M-01-10	0,234	0,234
15	M-03-07	0,234	0,234
16	M-03-06	0,188	0,188
17	M-09-17	0,188	0,188
18	G9-05	0,188	0,188
19	D-10-20	0,188	0,188
20	D-07-24	0,215	0,215
21	D-06-14	0,261	0,261
22	M-09-05	0,227	0,220
23	D-10-30	0,27	0,270
24	M-06-03	0,302	0,302
25	D-06-16	0,305	0,305
26	M-06-04	0,302	0,302
27	D-11-17	0,324	0,324
28	M-05-07	0,302	0,302
29	D-08-20	0,324	0,324
30	D-07-09	0,438	0,438
31	D-07-17	0,438	0,438
32	M-07-06	0,437	0,437
33	D-08-10	0,434	0,434
34	G47-11	0	0,000
35	G37-10	0	0,000
36	M-11-02	0	0,000

37	D-10-53	0	0,000
38	G106-13	0,166	0,166
39	D-08-21	0,223	0,227
40	D-11-58	0,257	0,257
41	D-08-19	0,267	0,267
42	D-10-50	0,275	0,275
43	G32-12	0,306	0,306
44	D-10-68	0,311	0,311
45	G32-15	0,361	0,361
46	G126-13	0,307	0,307
47	G97-13	0,306	0,306
48	D-07-16	0,434	0,434
49	G50-12	0,413	0,414
50	D-10-44	0,486	0,486

Appendix 4. Quality control and overview of SNP genotype data for the GWAS analysis and population structure information.

Table A5. Summary of genetic marker quality control for 38 phenotyped individuals using PLINK, showing the initial, retained, excluded, and total number of SNPs remaining after filtering.

Quality control filters	Remaining SNPs	Excluded SNPs	Lost in per cent
Initial dataset	237,670		
Sample < 0.1 call rate	237,670	0	0
SNP < 0.1 call rate	237,670	0	0
SNP maf < 0.01	237,283	387	0.1628
HWE < 0.001	237,208	75	0.0315
Total	237,208	462	0.1943

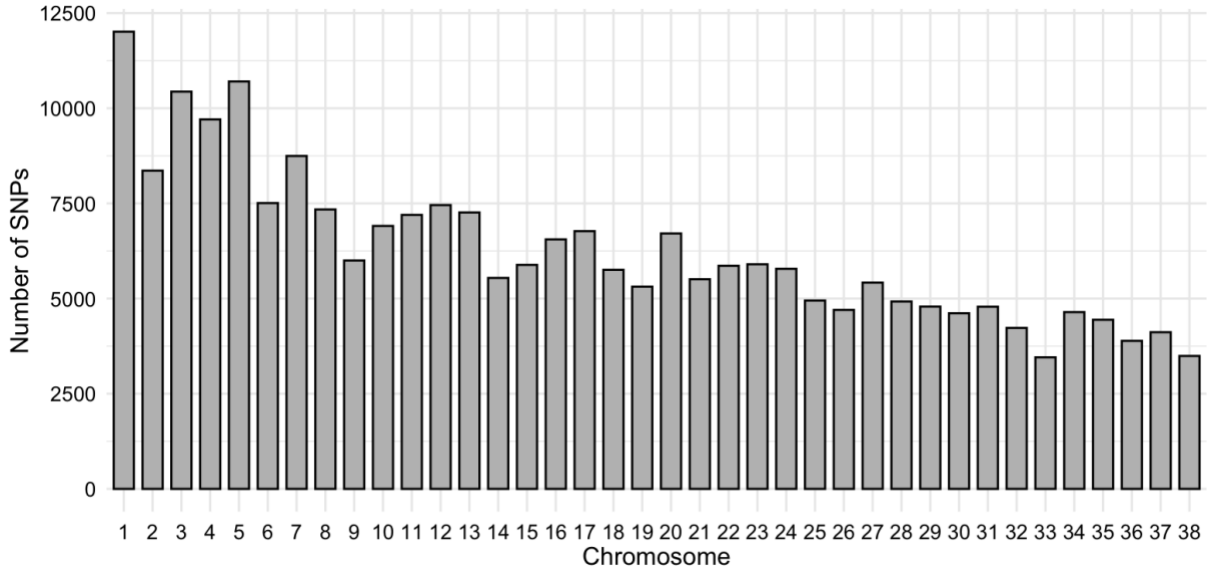

Figure A3. Distribution of the 237,208 SNPs across the 38 autosomes used in the GWAS analysis.

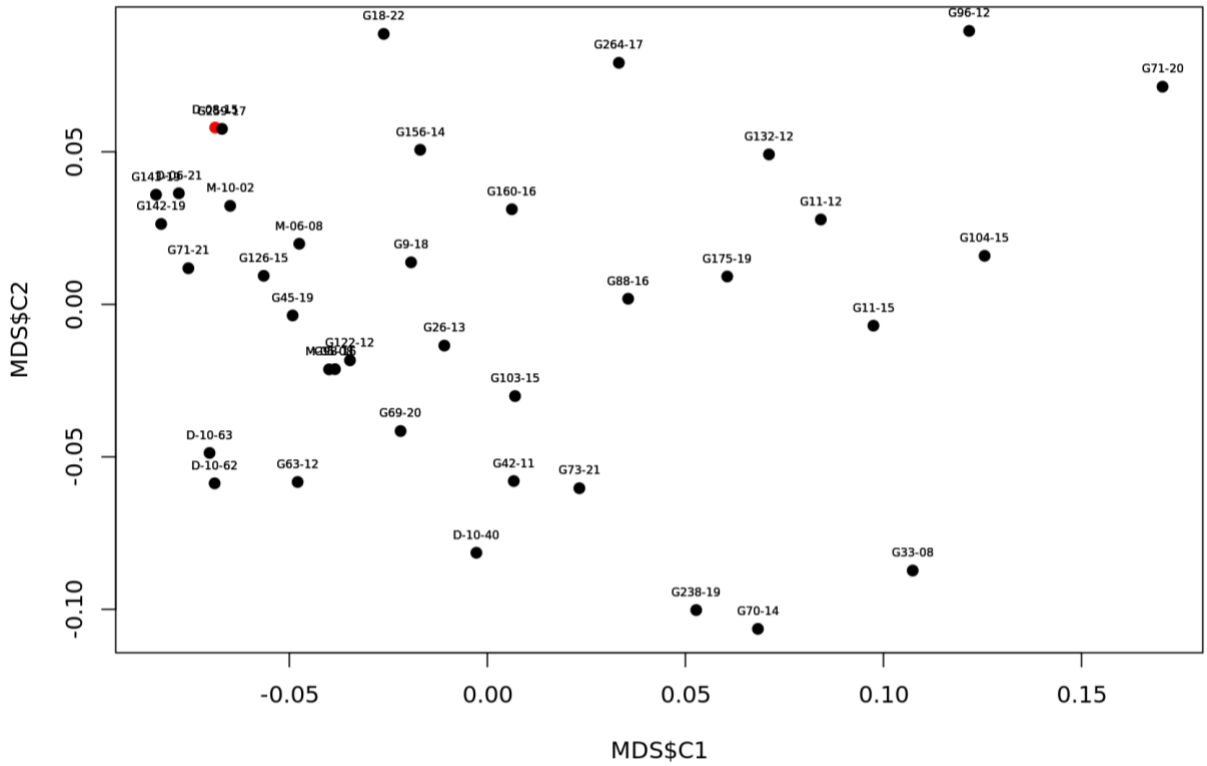

Figure A4. Multidimensional scaling (MDS) plot showing the genetic relatedness for the 38 phenotyped individuals from the 237, 208 SNPs. Each point represents an individual, with black indicating individuals with normal phenotype, and red indicating the single anomalously colored individual (D-08-15). Closer points indicate higher genetic similarity between individuals. Plot made with the "--mds-plot" function in PLINK.

Appendix 5. Quality control of SNP genotype data for the 77 individuals used in the haplotype analysis and information on population structure.

Table A6. Summary of genetic marker quality control for the 77 phenotyped and early-generation individuals included in the haplotype analysis, using PLINK. Overview of the initial, retained, excluded, and total number of SNPs remaining after filtering. 236,037 SNPs and remained after filtering, with a genotyping rate of 99.7%.

Quality control filters	Remaining SNPs	Excluded SNPs	Lost in per cent
Initial dataset	237,670		
Sample < 0.1 call rate	237,670	0	0
SNP < 0.1 call rate	237,475	195	0.082
SNP maf < 0.01	236,681	794	0.334
HWE < 0.001	236,037	644	0.2725
Total	236,037	1,633	0.688

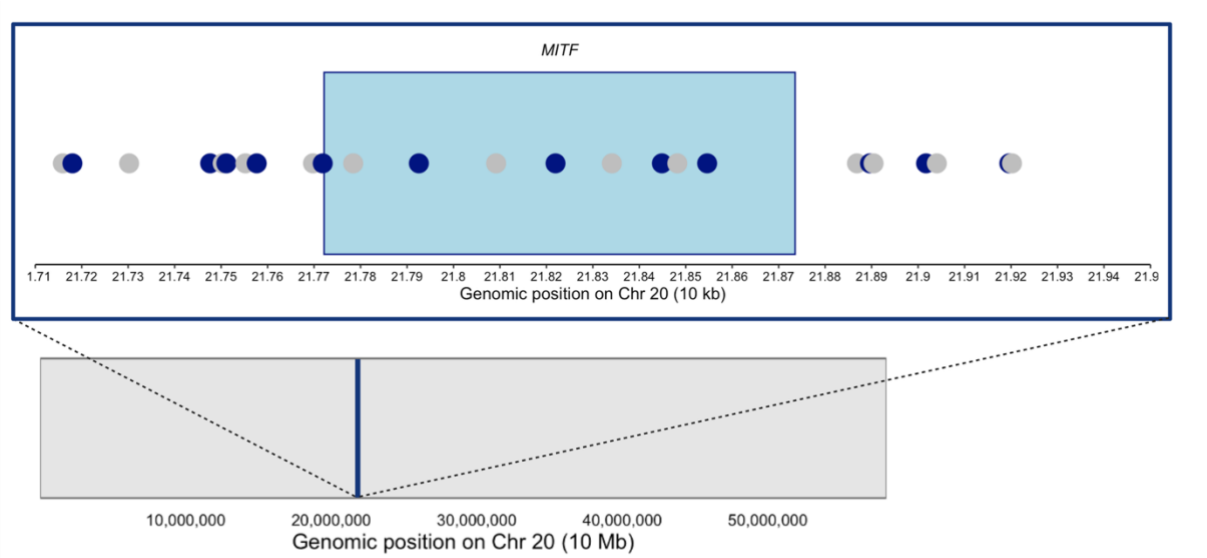

Figure A5. Overview of the 25 SNPs within and adjacent to the MITF gene on chromosome 20. Chromosome 20 contains 58,134,056 bp, with the MITF gene spanning across 21,772,147 to 21,873,545 bp. In total, 8 SNPs were located within MITF. 10 Mb equals 10,000,000 bp and 10 kb equals 10,000 bp. Made with R-package "ggplot" and BioRender.com.

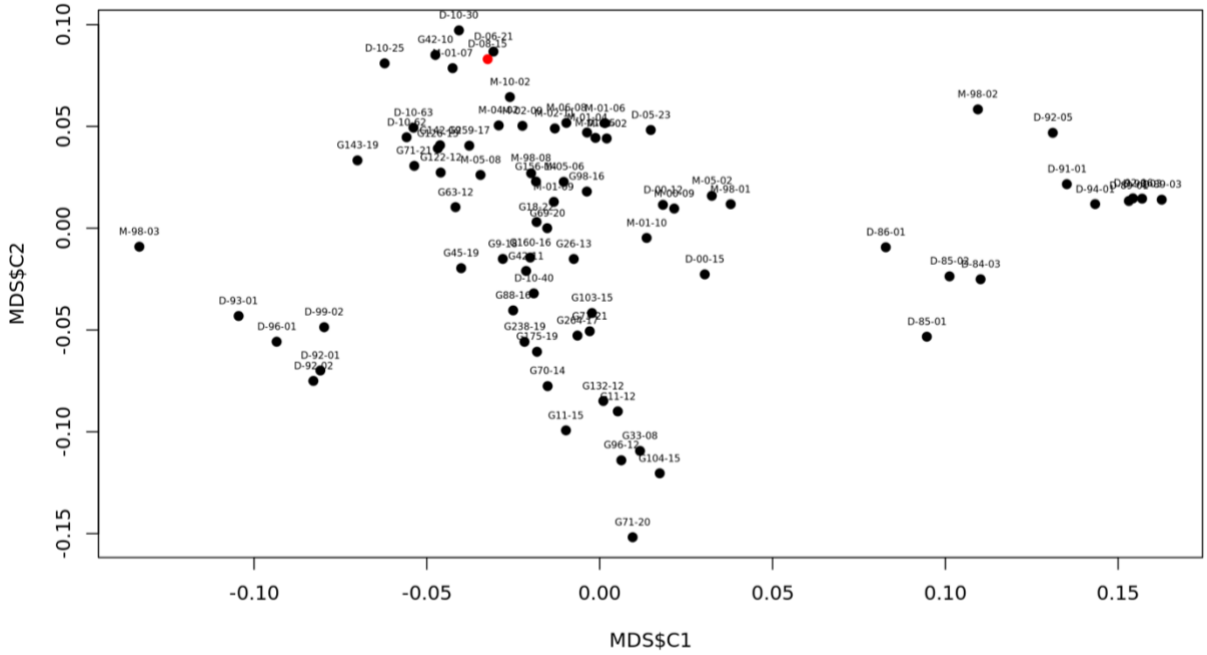

Figure A6. Multidimensional scaling (MDS) plot showing the genetic relatedness for the 77 phenotyped and early-generation individuals estimated from 236,037 SNPs. Each point represents an individual, with red indicating the single anomalously colored individual (D-08-15). Closer points indicate higher genetic similarity between individuals. Plot made with the "--mds-plot" function in PLINK.

Appendix 6. Overview and implementation of the animal model using ASReml-R.

```
library(asreml)

ainv <- ainverse(pedigree)                                    #Inversed A matrix

Animalmodel <-asreml(fixed=Phenotype~inbreeding_coeff.        #Running the animal model
              random= ~ vm(id,ainv),
              residual = ~ idv(units),
              data= data, maxiter = 100,
              na.action = na.method(x="omit",y="omit"))

#ASReml Version 4.2 23/10/2024 12:38:06                       #Model reached convergence after
#Model fitted using the sigma parameterization.                9 iterations
#LogLik        Sigma2     DF     wall
#1     -1435.152           1.0    474   12:38:06
#2     -910.3487           1.0    474   12:38:06
#3     -330.8356           1.0    474   12:38:06
#4      74.69115           1.0    474   12:38:06
#5      297.2904           1.0    474   12:38:06
#6      365.0274           1.0    474   12:38:06
#7      376.9291           1.0    474   12:38:06
#8      377.5800           1.0    474   12:38:06
#9      377.5830           1.0    474   12:38:06


variance_components <- summary(Animalmodel)$varcomp           #Retrieving variance components
#             component   std.error  z.ratio bound %ch
#vm(id, ainv) 0.05160161 0.012936440 3.988857     P   0
#units!units  0.04216648 0.007213489 5.845504     P   0
#units!R      1.00000000          NA       NA     F   0

heritability <- vpredict(Animalmodel,h2.phenotype~V1/(V1+V2)) #Estimating heritability
#             Estimate      SE
#h2.phenotype 0.550311 0.09908604

summary(Animalmodel, coef = TRUE)$coef.fixed                  #Estiamtes of fixed effects
#                 solution  std error   z.ratio
#(Intercept)      0.02849988 0.08472114 0.3363964
#inbreeding_coeff 0.05837090 0.21242488 0.2747837

#Intercept
#Upper 95% CI
0.02849988 + 1.96*(0.08472114) =  0.1945533
#Lower 95% CI
0.02849988 - 1.96*(0.08472114) = -0.1375536

#Inbreeding
#Upper 95% CI
0.05837090 + 1.96*(0.21242488) =  0.4747237
#Lower 95% CI
0.05837090 - 1.96*(0.21242488) = -0.3579819

wald.asreml(Animalmodel,ssType="conditional",denDF="numeric") #Wald variance analysis

#ASReml Version 4.2 23/10/2024 12:40:00
#Model fitted using the sigma parameterization.
#LogLik        Sigma2     DF     wall
#1      377.5830           1.0    474   12:40:01
#2      377.5830           1.0    474   12:40:01
#$Wald

#Wald tests for fixed effects.
#Response: Phenotype

#Df denDF   F.inc   F.con Margin      Pr
#(Intercept)       1 102.0 0.12970 0.11320        0.73725
#inbreeding_coeff  1 324.2 0.07551 0.07551      A 0.78365

#$stratumVariances
#df   Variance vm(id, ainv) units!units
#vm(id, ainv) 405.66403 0.07835563    0.7013171           1
#units!units   68.33597 0.04216628    0.0000000           1
```

Figure A7. Overview of the full model set up, estimation of variance components, heritability, and the fixed effect of inbreeding. Created with BioRender.com.

```
Reduced_model<-asreml(fixed= Phenotype ~ inbreeding_coeff,
                      random= ~ 1,
                      residual = ~ idv(units),
                      data= data, maxiter = 100,
                      na.action = na.method(x="omit", y="omit"))

#ASReml Version 4.2 19/05/2025 19:14:53
#Model fitted using the sigma parameterization.
#LogLik          Sigma2     DF     wall
#1     -3430.847           1.0     474   19:14:53
#2     -2401.331           1.0     474   19:14:53
#3     -1248.506           1.0     474   19:14:53
#4     -409.7959           1.0     474   19:14:53
#5      93.50317           1.0     474   19:14:53
#6      286.6796           1.0     474   19:14:53
#7      342.4455           1.0     474   19:14:53
#8      351.1866           1.0     474   19:14:53
#9      351.5610           1.0     474   19:14:53
#10      351.5620            1.0     474    19:14:53

#Likelihood ratio test for the significance of additive genetic effect the "lrt",
#assuming nested random models
lrt(Animalmodel, Reduced_model, boundary = FALSE)
#df LR-statistic Pr(Chisq)
#model1/model_reduced  1       52.042 5.432e-13 ***
  ---
#  Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
```

Figure A8. Likelihood ratio test of the full and reduced animal model to test for the significance of the additive genetic effect. Created with BioRender.com.

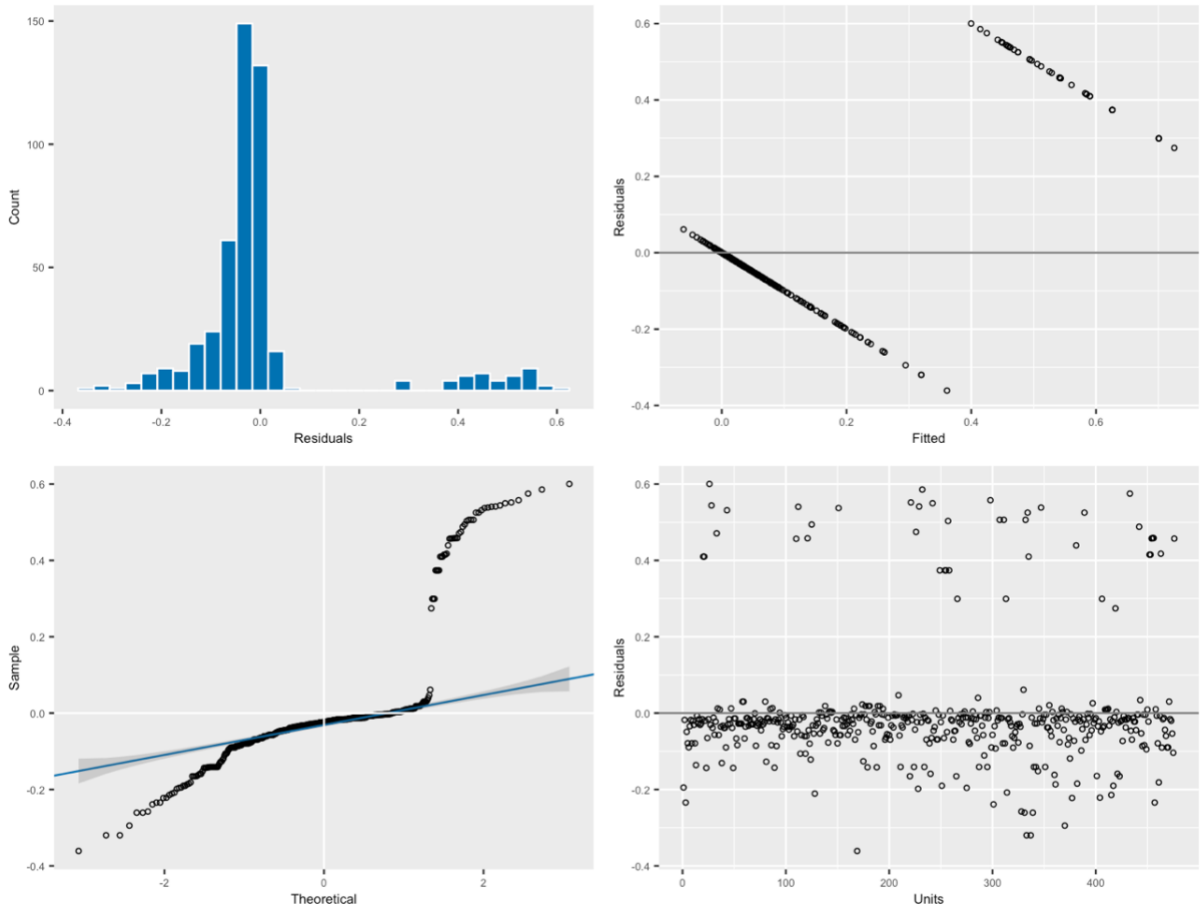

Figure A9. Output from the animal model with phenotype as the response variable using ASReml-R.

Appendix 7. Overview of the result for the haplotype analysis.

Table A7. Overview of the preliminary result of the haplotype analysis, including genotypes across the 25 markers within and adjacent to the MITF gene for all 77 individuals. Individuals in green were carriers of the H1 haplotype and carried at least one SNP specific allele, at each SNP, compatible with the H1 haplotype, whereas red indicates individuals that carried deviating alleles representing other haplotypes in the population.

	Phenotype	ID	SNP1	SNP2	SNP3	SNP4	SNP5	SNP6	SNP7	SNP8	SNP9	SNP10	SNP11	SNP12	SNP13	SNP14	SNP15	SNP16	SNP17	SNP18	SNP19	SNP20	SNP21	SNP22	SNP23	SNP24	SNP25
1	Normal	M-10-02	A A	T C	G T	T C	G G	T C	A G	T T	G A	C C	A G	A G	A G	C T	G C	A C	C A	A T	T A	A C	C C	C T	T C	C T	A G
2	Unknown	M-01-09	A A	C C	T T	C C	A G	C C	G G	C T	G A	G C	A G	G G	A G	T T	G C	C C	C A	T T	A A	C C	T C	T T	C C	T T	G G
3	Normal	G26-13	A A	C C	T T	C C	A A	C C	G G	C C	G G	G G	A A	G G	A A	T T	G G	C C	C T	T A	A A	C C	T T	T T	C C	T T	G G
4	Normal	G175-19	A A	T C	G T	T C	G G	T C	A G	T T	G A	C C	A G	A G	A G	C T	G C	A C	C A	A T	T A	A C	C C	C T	T C	C T	A G
5	Normal	G11-15	G A	C C	G T	C C	A A	T C	G G	C T	G G	G G	A A	G G	A A	T T	G C	C C	C A	T T	A A	C C	T T	C T	T C	C T	A G
6	Normal	G104-15	G A	C C	G T	C C	A A	T C	G G	C T	G G	G G	A A	G G	A A	T T	G C	C C	C A	T T	A A	C C	T T	C T	T C	C T	A G
7	Normal	G18-22	A A	T C	G T	T C	G G	T C	A G	T T	G A	C C	A G	A G	A G	C T	G C	A C	C A	A T	T A	A C	C C	C T	T C	C T	A G
8	Normal	G70-14	A A	C C	T T	C C	A A	C C	G G	C C	G G	G G	A A	G G	A A	T T	G G	C C	C T	T A	A C	C T	T T	T C	C T	T G	G
9	Anomalous	D-08-15	A A	T T	G G	T T	G G	T T	A A	T T	G G	C C	A A	A A	A A	C C	G G	A A	C C	A A	T T	A A	C C	C C	T T	C C	A A
10	Normal	G96-12	G A	C C	G T	C C	A G	T C	G G	T T	G A	G C	A G	G G	A G	T T	C C	C C	A A	T T	A A	C C	T T	C T	T C	C T	A G
11	Normal	D-10-63	A A	T C	G T	T C	G G	T C	A G	T T	G A	C C	A G	A G	A G	C T	G C	A C	C A	A T	T A	A C	C C	C T	T C	C T	A G
12	Normal	D-10-62	A A	C C	T T	C C	G G	C C	G G	T T	A A	C C	G G	G G	G G	T T	C C	C C	A A	T T	A A	C C	C C	T T	C C	T T	G G
13	Normal	G71-20	G A	T C	G G	T C	A G	T T	A G	T T	G G	G C	A A	A G	A A	C T	G C	A C	C A	A T	T A	A C	T C	C C	T T	C C	A A
14	Normal	G33-08	A A	C C	T T	C C	A G	[redacted]	G G	C T	G A	G C	A G	G G	A G	T T	G C	C C	C A	T T	A A	C C	T C	T T	C C	T T	G G
15	Unknown	M-00-02	A A	C C	T T	C C	G G	C C	G G	T T	A A	C C	G G	G G	G G	T T	C C	C C	A A	T T	A A	C C	C C	T T	C C	T T	G G
16	Normal	G63-12	A A	C C	T T	C C	G G	C C	G G	T T	A A	C C	G G	G G	G G	T T	C C	C C	A A	T T	A A	C C	C C	T T	C C	T T	G G
17	Unknown	M-05-02	A A	T C	G T	T C	G G	T C	A G	T T	G A	C C	A G	A G	A G	C T	G C	A C	C A	A T	T A	A C	C C	C T	T C	C T	A G
18	Normal	G71-21	A A	T C	G T	T C	G G	T C	A G	T T	G A	C C	A G	A G	A G	C T	G C	A C	C A	A T	T A	A C	C C	C T	T C	C T	A G
19	Unknown	G42-10	A A	T C	G T	T C	G G	T C	A G	T T	G A	C C	A G	A G	A G	C T	G C	A C	C A	A T	T A	A C	C C	C T	T C	C T	A G
20	Normal	G42-11	A A	C C	T T	C C	A G	C C	G G	C T	G A	G C	A G	G G	A G	T T	G C	C C	C A	T T	A A	C C	T C	T T	C C	T T	G G
21	Normal	G11-12	A A	C C	T T	C C	A G	C C	G G	C T	G A	G C	A G	G G	A G	T T	G C	C C	C A	T T	A A	C C	T C	T T	C C	T T	G G
22	Normal	G126-15	A A	T C	G T	T C	G G	T C	A G	T T	G A	C C	A G	A G	A G	C T	G C	A C	C A	A T	T A	A C	C C	C T	T C	C T	A G
23	Normal	G259-17	A A	T C	G T	T C	G G	T C	A G	T T	G A	C C	A G	A G	A G	C T	G C	A C	C A	A T	T A	A C	C C	C T	T C	C T	A G
24	Normal	G73-21	A A	C C	T T	C C	A G	C C	G G	C T	G A	G C	A G	G G	A G	T T	G C	C C	C A	T T	A A	C C	T C	T T	C C	T T	G G
25	Normal	G132-12	A A	C C	T T	C C	G G	C C	G G	T T	A A	C C	G G	G G	G G	T T	C C	C C	A A	T T	A A	C C	C C	T T	C C	T T	G G
26	Normal	G98-16	A A	C C	T T	C C	A G	C C	G G	C T	G A	G C	A G	G G	A G	T T	G C	C C	C A	T T	A A	C C	T C	T T	C C	T T	G G
27	Normal	G122-12	A A	C C	T T	C C	G G	C C	G G	T T	A A	C C	G G	G G	G G	T T	C C	C C	A A	T T	A A	C C	C C	T T	C C	T T	G G
28	Normal	G88-16	G A	C C	G T	C C	A G	T C	G G	T T	G A	G C	A G	G G	A G	T T	C C	C C	A A	T T	A A	C C	T C	T T	C C	C T	A G
29	Normal	G45-19	A A	C C	T T	C C	G G	T C	G G	T T	A A	C C	G G	G G	G G	T T	C C	C C	A A	T T	A A	C C	C C	T T	C C	T T	G G
30	Normal	D-06-21	A A	T C	G T	T C	G G	T C	A G	T T	G A	C C	A G	A G	A G	C T	G C	A C	C A	A T	T A	A C	C C	C T	T C	C T	A G
31	Normal	G264-17	A A	C C	T T	C C	A G	C C	G G	C T	G A	G C	A G	G G	A G	T T	G C	C C	C A	T T	A A	C C	T C	T T	C C	T T	G G
32	Unknown	M-01-07	A A	T C	G T	T C	G G	T C	A G	T T	G A	C C	A G	A G	A G	C T	G C	A C	C A	A T	T A	A C	C C	C T	T C	[redacted]	A G
33	Unknown	M-01-05	A A	T C	G T	T C	G G	T C	A G	T T	G A	C C	A G	A G	A G	C T	G C	A C	C A	A T	T A	A C	C C	C T	T C	C T	A G
34	Unknown	M-05-06	A A	C C	T T	C C	G G	C C	G G	T T	A A	C C	G G	G G	G G	T T	C C	C C	A A	T T	A A	C C	C C	T T	C C	T T	G G
35	Unknown	M-04-02	A A	T C	G T	T C	G G	T C	A G	T T	G A	C C	A G	A G	A G	C T	G C	A C	C A	A T	T A	A C	C C	C T	T C	C T	A G
36	Normal	M-06-08	A A	C C	T T	C C	G G	C C	G G	T T	A A	C C	G G	G G	G G	T T	C C	C C	A A	T T	A A	C C	C C	T T	C C	T T	G G
37	Normal	G69-20	A A	T C	G T	T C	G G	T C	A G	T T	G A	C C	A G	A G	A G	C T	G C	A C	C A	A T	T A	A C	C C	C T	T C	C T	A G
38	Normal	G160-16	A A	T C	G T	T C	G G	T C	A G	T T	G A	C C	A G	A G	A G	C T	G C	A C	C A	A T	T A	A C	C C	C T	T C	C T	A G
39	Normal	G9-18	A A	C C	T T	C C	G G	C C	G G	T T	A A	C C	G G	G G	G G	T T	C C	C C	A A	T T	A A	C C	C C	T T	C C	T T	G G
40	Normal	G156-14	0 0	C C	T T	C C	G G	C C	G G	T T	A A	C C	G G	G G	G G	T T	C C	C C	A A	T T	A A	C C	C C	T T	C C	T T	G G
41	Normal	G103-15	A A	C C	T T	C C	A G	C C	G G	C T	G A	G C	A G	G G	A G	T T	G C	C C	C A	T T	A A	C C	T C	T T	C C	T T	G G
42	Normal	G142-19	A A	T C	G T	T C	G G	T C	A G	T T	G A	C C	A G	A G	A G	C T	G C	A C	C A	A T	T A	A C	C C	C T	T C	C T	A G
43	Normal	G143-19	A A	T C	G T	T C	G G	T C	A G	T T	G A	C C	A G	A G	A G	C T	G C	A C	C A	A T	T A	A C	C C	C T	T C	C T	A G
44	Normal	M-05-08	A A	T C	G T	T C	G G	T C	A G	T T	G A	C C	A G	A G	A G	C T	G C	A C	C A	A T	T A	A C	C C	C T	T C	C T	A G
45	Unknown	M-02-09	A A	C C	T T	C C	G G	C C	G G	T T	A A	C C	G G	G G	G G	T T	C C	C C	A A	T T	A A	C C	C C	T T	C C	T T	G G
46	Unknown	M-02-11	A A	T C	G T	T C	G G	T C	A G	T T	G A	C C	A G	A A	A G	C T	G C	A C	C A	A T	T A	A C	C C	C T	T C	C T	A G
47	Normal	D-10-40	A A	C C	T T	C C	A G	C C	G G	C T	G A	G C	A G	G G	A G	T T	G C	C C	C A	T T	A A	C C	T C	T T	C C	T T	G G
48	Normal	G238-19	G A	T C	G G	T C	A G	T T	A G	T T	G G	G G	[redacted]	G G	A A	T T	G C	A C	C A	A T	T A	A C	T C	C C	T T	C C	A A
49	Unknown	D-00-15	A A	C C	T T	C C	G G	C C	G G	T T	A A	C C	G G	G G	G G	T T	C C	C C	A A	T T	A A	C C	C C	T T	C C	T T	G G
50	Unknown	D-10-30	A A	T C	G T	T C	G G	T C	A G	T T	G A	C C	A G	A G	A G	C T	G C	C C	C A	A T	T A	A C	C C	C T	T C	C T	A G
51	Unknown	M-98-03	A A	T C	G T	T C	A G	T C	A G	C T	G G	G C	A A	A G	A A	C T	G G	A C	C C	A T	T A	A C	T C	C T	T C	C T	A G
52	Unknown	M-01-06	A A	T C	G T	T C	A G	T C	A G	C T	G G	G C	A A	A G	A A	C T	G G	A C	C C	A T	T A	A C	T C	C T	T C	C T	A G
53	Unknown	M-01-04	A A	C C	T T	C C	A G	C C	G G	C T	G A	G C	A G	G G	A G	T T	G C	C C	C A	T T	A A	C C	T C	T T	C C	T T	G G
54	Unknown	M-98-08	A A	T C	G T	T C	G G	T C	A G	T T	G A	C C	A G	A G	A G	C T	G C	A C	C A	A T	T A	A C	C C	C T	T C	C T	A G
55	Unknown	M-00-09	A A	T C	G T	T C	G G	T C	A G	T T	G A	C C	A G	A G	A G	C T	G C	A C	C A	A T	T A	A C	C C	C T	T C	C T	A G
56	Unknown	M-01-10	A A	C C	T T	C C	A G	C C	G G	C T	G A	G C	A G	G G	A G	T T	G C	C C	C A	T T	A A	C C	T C	T T	C C	T T	G G
57	Unknown	D-89-03	A A	C C	T T	C C	G G	C C	G G	T T	A A	C C	G G	G G	G G	T T	C C	C C	A A	T T	A A	C C	C C	T T	C C	T T	G G
58	Unknown	D-92-05	A A	C C	T T	C C	G G	C C	G G	T T	A A	C C	G G	G G	G G	T T	C C	C C	A A	T T	A A	C C	C C	T T	C C	T T	G G
59	Unknown	D-94-01	A A	C C	T T	C C	G G	C C	G G	T T	A A	C C	G G	G G	G G	T T	C C	C C	A A	T T	A A	C C	C C	T T	C C	T T	G G
60	Unknown	D-99-02	A A	T C	G T	T C	G G	T C	A G	T T	G A	C C	A G	A G	A G	C T	G C	A C	C A	A T	T A	A C	C C	C T	T C	C T	A G
61	Unknown	D-93-03	A A	C C	T T	C C	G G	T C	G G	T T	A A	C C	G G	G G	G G	T T	C C	C C	A A	T T	A A	C C	C C	T T	C C	T T	G G
62	Unknown	D-05-23	A A	C C	T T	C C	G G	C C	G G	T T	A A	C C	G G	G G	G G	T T	C C	C C	A A	T T	A A	C C	C C	T T	C C	T T	G G
63	Unknown	D-89-01	A A	C C	T T	C C	G G	C C	G G	T T	A A	C C	G G	G G	G G	T T	C C	C C	A A	T T	A A	C C	C C	T T	C C	T T	G G
64	Unknown	D-92-02	A A	T C	G T	T C	G G	T C	A G	T T	G A	C C	A G	A G	A G	C T	G C	A C	C A	A T	T A	A C	C C	C T	T C	C T	A G
65	Unknown	D-91-01	A A	C C	T T	C C	G G	C C	G G	T T	A A	C C	G G	G G	G G	T T	C C	C C	A A	T T	A A	C C	C C	T T	C C	T T	G G
66	Unknown	D-92-06	A A	C C	T T	C C	G G	C C	G G	T T	A A	C C	G G	G G	G G	T T	C C	C C	A A	T T	A A	C C	C C	T T	C C	T T	G G
67	Unknown	D-96-01	A A	T C	G T	T C	A G	T C	A G	C T	G G	G C	A A	A G	A A	C T	G G	A C	C C	A T	T A	A C	T C	C T	T C	C T	A G
68	Unknown	M-98-02	A A	C C	T T	C C	G G	C C	G G	T T	A A	C C	G G	G G	G G	T T	C C	C C	A A	T T	A A	C C	C C	T T	C C	T T	G G
69	Unknown	D-92-01	A A	T C	G T	T C	G G	T C	A G	T T	G A	C C	A G	A G	A G	C T	G C	A C	C A	A T	T A	A C	C C	C T	T C	C T	A G
70	Unknown	D-00-12	A A	C C	T T	C C	G G	C C	G G	T T	A A	C C	G G	G G	G G	T T	C C	C C	A A	T T	A A	C C	C C	T T	C C	T T	G G
71	Unknown	D-84-03	A A	C C	T T	C C	G G	C C	G G	T T	A A	C C	G G	G G	G G	T T	C C	C C	A A	T T	A A	C C	C C	T T	C C	T T	G G
72	Unknown	D-85-01	A A	C C	T T	C C	A G	C C	G G	C T	G A	G C	A G	G G	A G	T T	G C	C C	C A	T T	A A	C C	T C	T T	C C	T T	G G
73	Unknown	D-93-01	A A	T C	G T	T C	A G	T C	A G	C T	G G	G C	A A	A G	A A	C T	G G	A C	C C	A T	T A	A C	T C	C T	T C	C T	A G
74	Unknown	M-98-01	A A	C C	T T	C C	G G	C C	G G	T T	A A	C C	G G	G G	G G	T T	C C	C C	A A	T T	A A	C C	C C	T T	C C	T T	G G
75	Unknown	D-85-02	A A	C C	T T	C C	G G	C C	G G	T T	A A	C C	G G	G G	G G	T T	C C	C C	A A	T T	A A	C C	C C	T T	C C	T T	G G
76	Unknown	D-86-01	A A	C C	T T	C C	G G	C C	G G	T T	A A	C C	G G	G G	G G	T T	C C	C C	A A	T T	A A	C C	C C	T T	C C	T T	G G
77	Unknown	D-10-25	A A	T T	G G	T T	G G	T T	A A	T T	G G	C C	A A	A A	A A	C C	G G	A A	C C	A A	T T	A C	C C	C C	T T	C C	A A

Appendix 8. Pedigree overview for the first occurrences of anomalous coat color in the population.

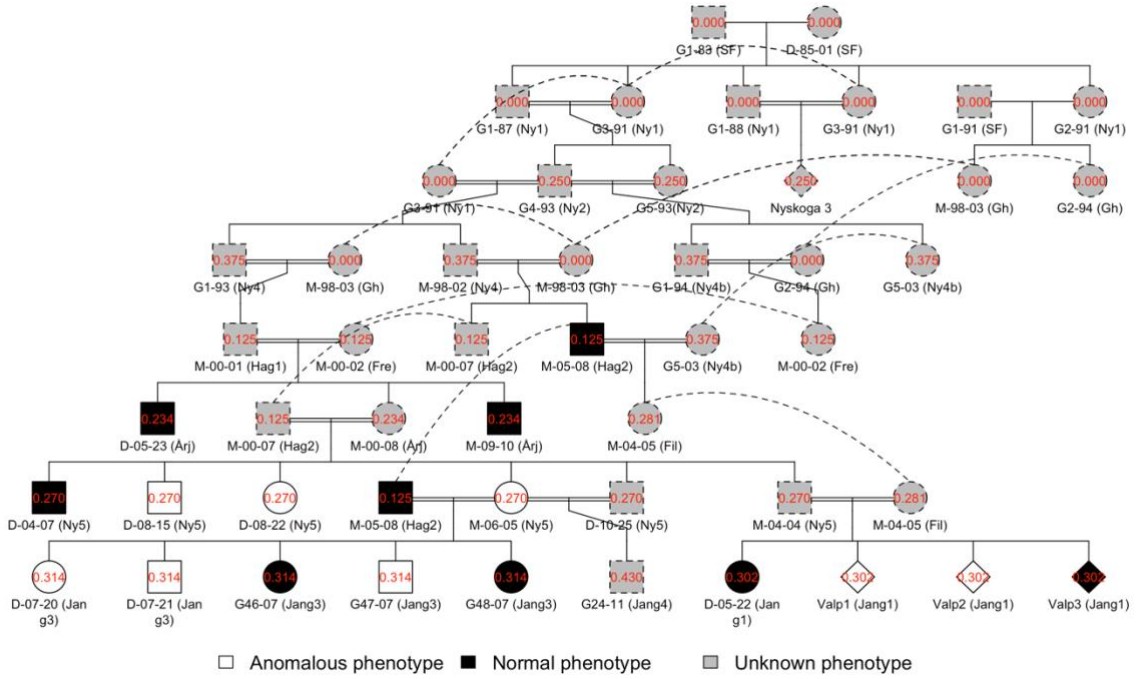

Figure A10. Sample of the pedigree (n = 40) demonstrating possible origin and inheritance of the anomalous phenotype. There are 15 reproductive pairs. Number underneath symbols represent individual IDs and within symbols represent inbreeding coefficients (*F*). Abbreviations after ID-number represent natal territory (e.g., Ny1 represents Nyskoga 1). Squares and circles represent males and females, respectively. Diamonds represent individuals of unknown sex. Color of the symbols represents phenotypes, where white indicates anomalous phenotype, black indicates the normal phenotype, and grey represents unknown phenotype. Double solid lines represent incestuous matings (i.e., sibling and parent-offspring), and single solid lines represent matings between unrelated individuals. Nyskoga 3 represents reproduction and not the number of offspring and was included to show the occurrence of several incestuous matings between descendants of the founding pair (information gathered from overview of first reproductive events of pairs). Notice that all offspring of the third founder are also descendants of the founding pair. The pedigree was visualized using the R-package "pedtools".

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