Effects of inbreeding management on genetic defects in dogs

Paulette Nieuwenhuis

REG.NR.1031810:

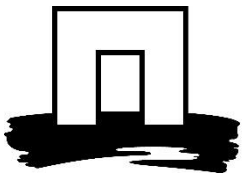
Major thesis Animal Breeding and Genomics (ABG 80436)

October + 2021

Supervisors/examiners:

Jack Windig

Thesis: Animal Breeding and Genomics



WAGENINGEN
UNIVERSITY & RESEARCH

Acknowledgements

I want to thank all the people that helped me during the times of these weird Covid-19 period. I want to thank the people that peer-reviewed parts of my thesis and provided invaluable insights. And finally, I want to thank my supervisor, Jack Windig for all his patience, time, guidance and help to develop this thesis.

Abstract

Between dog breeds there is a lot of genetic diversity, but within breeds it is under pressure. In many breeds there is a lot of inbreeding, what leads to the exposure of deleterious alleles. The effect of deleterious alleles can vary between completely lethal and partly detrimental and the latter lead to inbreeding depression. Deleterious alleles or disorders can be described in a theoretical framework, that includes the strength or effect (s) of the deleterious alleles and the proportion of this deleterious effect that is expressed in the heterozygotes (h). S varies between 0 (neutral) and 1 (completely lethal) and h also varies between 0 (completely recessive) and 1 (completely dominant). Breeders often use different breeding strategies to keep the population healthy. We selected 8 disorders from the 803 known traits and disorders, and their s and h values were estimated based on literature. Four breeds differing in effective population size were used for the simulations, namely Golden Retriever, Friese Stabij, Markiesje, and Saarloos Wolfhond. The framework of s and h was useful in the simulations however, s and h are difficult to estimate. This is due to the lack of information on the size of the effect of the disorders in literature. Higher lethality (s) and dominance (h) of the disorder both cause the disorder to be more easily eliminated from the population. Disorders with a very small effect (s) will have more intermediate allele frequencies and have the risk of getting fixed in the population. The allele frequencies of the disorders that we found during the simulations were higher compared to the allele frequencies found in literature. Large effective population sizes lead to more intermediate allele frequencies and segregating alleles in the population, whereas small populations lead to more elimination and fixation of alleles. A higher age of onset leads also to more segregation, especially in small populations, compared to a lower age of onset. Minimizing kinship leads also to more segregation. So, disorders with a large effect are easy to find and easily eliminated, especially in small populations. However, using inbreeding as a strategy to eliminate these large disorders is not a wise strategy, because the disorders with a small strength have the risk of getting fixated.

Table of content

Introduction

Nowadays, the Federation Cynologique Internationale (FCI) recognizes 353 dog breeds (Fédération Cynologique Internationale, 2018). These comprise a lot of genetic diversity, evident for example, if we compare a Chihuahua with a Danish dog. However, if we look within breeds some breeds have smaller populations, compared to other breeds (Hasselgren, 2013). These breeds often have less genetic diversity, more inbreeding and genetic defects. It is important to consider for all closed populations the effects of inbreeding and inbreeding depression in breeding management.

Inbreeding and inbreeding depression

Inbreeding occurs when two related parents are mated (Swindell & Bouzat, 2006). In closed populations this is, at least in the long run, inevitable and under random mating inbreeding occurs at a higher rate in small populations, due to the smaller number of possible combinations that have low kinships. When inbreeding increases, genetic diversity will decrease (Frankham et al., 2004). A consequence of inbreeding is the loss of heterozygosity, which may lead to the exposure of recessive deleterious alleles. These deleterious alleles can be classified as lethal, near lethal, or detrimental and are often fully recessive, but can be nearly recessive, or partially recessive, (Charlesworth & Charlesworth, 1999; Hedrick & Garcia-Dorado, 2016). With this a theoretical framework was made, that includes the strength or effect (s) of the deleterious alleles and the proportion of this deleterious effect that is expressed in the heterozygotes(h). Whereby both s varies between 0 (neutral) and 1 (completely lethal) and h varies as well between 0 (completely recessive) and 1 (completely dominant, box 1).

When these deleterious alleles lead to a reduction of birth rates and other traits, it is considered inbreeding depression (Hedrick & Garcia-Dorado, 2016; Swindell & Bouzat, 2006). This generally implies a loss in fitness. The age of onset is expected to be important for the fitness. A dog that will be sick before the age of one, is not likely to have offspring. A dog that will be sick only after the age of 5 is more likely to have offspring. Consequently, the disease is more likely to stay in the population when the age of onset is higher. A more lethal disorder is likely to be easier to eliminate from the population, because an affected dog will probably not have offspring, due to natural selection or due to breeders that will not use these dogs for breeding. A more dominant disorder is more easily found and is therefore also expected to be easier to eliminate from the population, especially in small populations. So the level of dominant alleles will be smaller in small populations. However, a recessive disorder with a very small s is expected to stay in the population and multiples of these disorders with a small s will lead to inbreeding depression. Inbreeding depression can be so detrimental that it can lead to the decline or extinction of animal populations (Swindell & Bouzat, 2006).

Box 1: Theoretical framework of strength (s) and dominance (h)

When there is no mutation, migration and selection in a population, and there is random mating, allele and genotype frequencies are in Hardy-Weinberg (HW) equilibrium (Frankham et al., 2004). For a single locus with alleles A and a, frequencies are denoted by p and q respectively:

$$p + q = 1$$

The expected frequencies under Hardy- Weinberg (HW) equilibrium are: AA is p^2, aa is q^2, and Aa is 2pq. Hardy Weinberg equilibrium assumes no mutation, migration, or selection and random mating, HW equilibrium will only be obtained in a large population, with normal Mendelian Segregation of alleles, equal fertility of parent genotypes, equal fertilizing capacity of gametes and equal survival for all genotypes. This means that there is no difference in fitness between these alleles or between homozygous or heterozygous, in other words the alleles are neutral (Frankham et al., 2004).

Although, genetic drift will lead to fluctuation, frequencies will on average remain stable for neutral alleles and it will stay in HW. However, when one of the alleles is not neutral and is deleterious there will be no HW equilibrium. Detrimental mutation produces genetic variation. On the other side purifying, selection and genetic drift lead to less genetic variation(Frankham et al., 2004).

There are two major groups of deleterious alleles, the lethals or near lethals, and the detrimentals. The lethals or near lethals have large effects and tend to be completely recessive, because when lethals or near lethals are dominant, they tend to disappear very quickly from the population (Lobo, 2008). The detrimentals are the ones that have a small or moderate effect and are more often partially recessive (Hedrick & Garcia-Dorado, 2016). Age of onset is also an important factor. A deleterious disorder that starts before the age of 1 will more likely disappear, because these dogs will not have offspring. Dogs with a disorder that starts only at the age of 5 will likely have produced offspring that inherited these deleterious alleles.

The fitness of the different genotypes can be described by

$$AA = 1$$

$$Aa = 1 - hs$$

$$aa = 1 - s$$

The s denotes the selection differential: the reduction in fitness of the homozygous detrimental (aa), and h is the proportion of the selection differential expressed in the heterozygote. S varies between 0 (neutral) and 1 (completely lethal) and h varies as well between 0 (completely recessive) and 1(completely dominant). A more dominant (h) allele leads to a faster decrease in fitness (figure 1, Hedrick & Garcia-Dorado, 2016).

However, up to now this framework is not often used in dog breeding. The more common model used is penetrance. Penetrance is the proportion of individuals having a particular genotype, affected with the disease, in other words in which the detrimental allele is expressed in the phenotype. Penetrance can be used together with the framework. A completely lethal disorder where 80% of the animals with aa die, will lead to a s of 0.8. If the penetrance is 20% for the heterozygous animals (Aa) this results in an h of 25%, because 0.8=1-0.25*0.8.

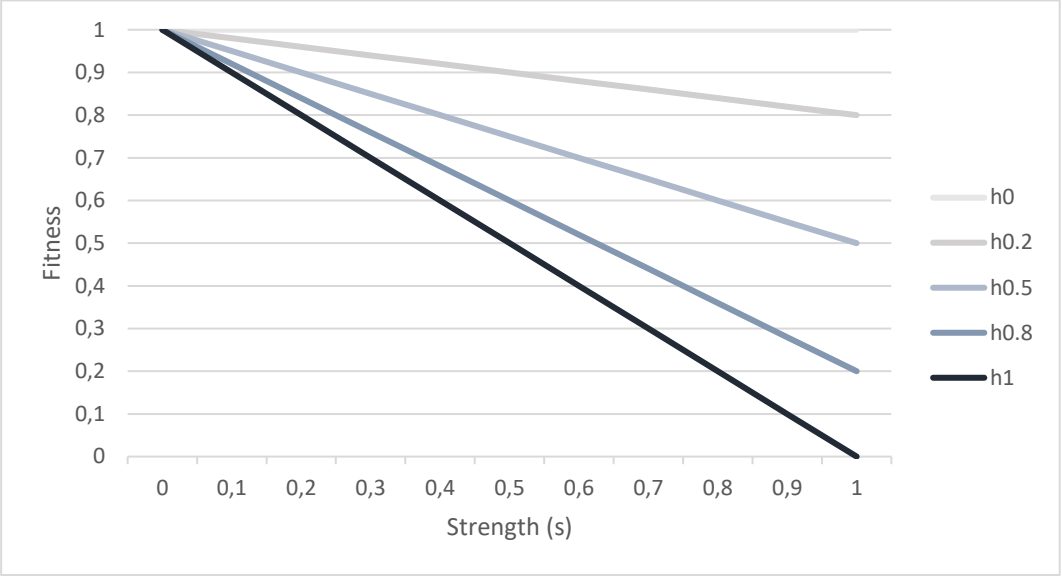


Figure 1: the effects of different strengths (s) and dominance (h) of an disorder on the fitness of the heterozygote

The reduction in mean fitness, when there is no inbreeding and HW proportions, due to the lower fitness of homozygotes and/or heterozygotes for segregating deleterious mutations is called mutation load. The mutation load is as follows (Hedrick & Garcia-Dorado, 2016):

$$L = 2hsq(1-q) + sq^2$$

Where q is the frequency of detrimental allele a and L the mutation load. The 2q(1-q) is the frequency of Aa that is multiplied by the dominance (h) and the strength (s). The sq^2 is the strength (s) multiplied by the frequency of aa. The level of dominance (h) determines the extent that heterozygotes are exposed to selection.

Hedrick & Garcia-Dorado used this framework to make a mathematical model of the mutation/selection, which they used to make predictions (2016). But this is out of the scope of this thesis. In this thesis the framework will be used for simulations and see the effects of genetic management.

Genetic drift and purging

Allele frequencies change, and two processes are important in this respect. Genetic drift are changes in the genetic composition of a population due to random sampling in the population (Frankham et al., 2004). Chance is larger in small populations, compared to large populations, and thus genetic drift higher.

Purging is the elimination of deleterious alleles from populations due to natural selection (Frankham et al., 2004). Animals with genetic defects will have less (strong) offspring, compared to the counterparts that don't have these genetic defects. The random process of genetic drift can override natural selection and purging (Primack, 1995). Both genetic drift and purging lead to the loss of genetic diversity (Primack, 1995). For purging this is partly in the form of the loss of deleterious alleles (Primack, 1995). Purging is especially effective when the effect of deleterious alleles is stronger. The

fluctuation in allele frequencies due to genetic drift is random and may lead to fixation of alleles (frequency is 1) or the loss of alleles (frequency is 0).

Heritable diseases and breeding strategies

There are a lot of different heritable diseases in dogs. According to OMIA (Online Mendelian Inheritance in Animals), an online database with heritable disorders in animals, there are 803 traits and disorders (OMIA, 2020, accessed on 2th of August 2021). Common examples are, forms of cancer, Progressive Retinal Atrophy, Hereditary Epilepsy, Hip Dysplasia, Patella Luxation, Elbow Dysplasia, and von Willebrand's disease. However, a lot of these diseases are polygenic, meaning that not one gene, but multiple genes are responsible for the expression of the disease. Apart from true monogenic diseases, there are diseases where only one or two genes may be responsible for the major part of the effect, and several other genes are only responsible for a small portion of the effect (Orr, 2005). The disorders also differ in severity or strength (s) and in dominance (h), which may lead to the difference in allele frequency in common disorders (Donner et al., 2018).

Donner, et al found the allele frequencies for 30 disorders in mixed breeds and purebreds (2018). According to them, these are the top 30 most frequently observed disease variants in mixed breed dogs. Most of these 30 disorders are monogenic. These allele frequencies are based on DNA tests and tests for monogenic disorders are easier. It is easier to select against monogenic disorders as well. These easier tests and easier selection might cause researchers and breeders to focus more on these monogenic diseases, creating a greater gap of knowledge between monogenic and polygenic disorders.

These heritable diseases in dogs decrease the health of dog breeds. Apart from often used DNA tests, breeders use different breeding strategies to eliminate heritable diseases and to decrease inbreeding, which lead indirectly to a slower increase in deleterious allele frequency. Some of these strategies are, decreasing the number of litters sired by one male, increase the (effective) population, and using animals with a low kinship. There are also some strategies that are less desirable, due to their negative effects. One of these strategies is to increase inbreeding in order to expose the deleterious recessive alleles, and consequently eliminate them. However, if inbreeding is increased, the frequency of other deleterious alleles may increase (Frankham et al., 2004).

The average increase of inbreeding in a population from one generation to the next generation is called the inbreeding rate. It is recommended to have a maximal inbreeding rate between 0.5 and 1.0 to keep a healthy population (FAO, 1998; FAO, 1998). A low inbreeding rate will lead to less deleterious alleles that are exposed within a population.

Kinship is the probability that two alleles, taken from each individual at random, will be identical by descent (Frankham et al., 2004). Some males that do well in shows may for example be used more often by breeders to mate with their females. This will increase the amount of offspring from these males, that will increase the level of kinship. However, to increase the health within the population it is recommended to not let males have to many offspring and use individuals with a low kinship to the rest of the population (Frankham et al., 2004). The use of low kinship or the increase of the effective population size, may help create a healthier population.

Another strategy is to increase the population size. An increase of the population may lead to an increase in genetic diversity. However, an increase in the population size will not lead to an increase

in genetic diversity if these animals only come from a few animals. The use of different breeding strategies will lead to a healthier population and for that a healthier breed.

To evaluate the effect of heritable disorders and defects on dogs, in respect of purging and genetic management, a selection needs to be made of these disorders. Purging is highly effective when it comes to dominant disorders (Hedrick & Garcia-Dorado, 2016). However, with recessive disorders and especially the ones that are low in strength and effect, purging is not really that effective (Hedrick & Garcia-Dorado, 2016). It is very difficult to breed against these recessive heritable disorders, because they are not always known. Although, these recessive disorders may not have a large effect individually together, they may have an effect on the fertility of an animal. So, we don't know what happens with these natural processes, like purging and genetic drift in combination with different breeding strategies and their effects on recessive disorders.

Simulations

Simulations are a useful tool to understand the progression of these types of recessive disorders in populations, and the effect of breeding management in combination with selection (purging) and genetic drift on these recessive disorders. The strength of the effect of a disorder and the dominance (h) are very useful to add to the simulations. The aim of this research is to evaluate known genetic defects in dogs, with respect to genetic management, genetic drift, and purging. In order to fulfill this aim, the following research questions are made

- What known monogenic, heritable disorders of dogs are there?
- Can we relate the common heritable disorders in dogs to the theoretical framework of strength (s) and dominance (h)?
- What is the effect of genetic management and the effective population size on purging and drift of deleterious alleles of different strength and negative effects?

Material and Methods

Monogenic heritable disorders in dogs

To determine which monogenic disorders are known in dogs the Online Mendelian Inheritance in Animals (OMIA) database was used (OMIA, 2020, accessed on 2th of August 2021). This is a database with a catalogue of different heritable disorders, traits and genes in 261 different species. OMIA contains information of these disorders with references and relevant links. There are in total 803 traits and disorders known in dogs, according to OMIA database (Accessed on 2th of August 2021). Of these 803 traits and disorders, about 371 traits and disorders have a known causal variant.

To select disorders for this thesis, different criteria were used. First, we separated disorders from traits. After that we selected those disorders that have a known cause and are monogenic. Next, we selected disorders that are seen in multiple breeds. Finally, we selected disorders that had a difference in mortality and age of onset. The selected disorders are shown in Table 2. Donner, et al (2018) evaluated for 30 common disorders the frequency in mixed breeds and purebreds. Six of the disorders in table 2 had a known frequency in mixed and purebreds found in this study (table 3). Although, there could have been more disorders selected, inclusion of more disorders than those seen in Table 2 and 3 was not necessary for this thesis.

Relating the theoretical framework to common heritable disorders

To evaluate the frequency of the different disorders we used the theoretical framework of the strength (s) and dominance (h). For every disorder the level of s and h was estimated between 0 and 1. The more lethal the disorder is, the higher s will be. However, s may also be increased due the assumption that a breeder will not use a dog with such a disorder. For level h it is the same principal. The more dominant a disorder is, the higher h will be.

We will also evaluate the effects of different levels of s and h on the allele frequency. For this a hypothetical disorder will be simulated with different levels of s (0.01, 0.05, and 0.1 till 1) and different levels of h (0.01, 0.05, and 0.1 till 1) combined with two levels of s (0.2 and 0.8). The specifics of one of the four dog breeds of this thesis, the Saarloos Wolfhond (dog breed) will be used for these simulations.

Effect genetic management on frequency of disorders

To evaluate the effects of genetic management on the frequencies of the disorders simulations were done. For the simulations we have used the program Pointer (former name GenManSim; Windig & Hulsegge, 2021), software developed by the Animal Breeding and Genomics group at the Wageningen University. This program uses different information about population size, biological data, breeding policy, population structure and genome to evaluate inbreeding and genetic management in captive populations.

To determine the average effect and variation of genetic management on the frequencies of the disorder, the number of years simulated was set to 100 and the number of repeats was set to 50. The effect of chance is taken into account by taking the average of 50 repeats and the oldest breeds exist now for about 100 years.

Four breeds with large differences in population size were selected for the simulations, to see effects in inbreeding level and allele frequencies in the disorders (table 1) over the range of effective population sizes present in dog breeds. The Golden Retriever (GR) is one of the most often kept dog

breed in NL very large and popular breed (table 1), whereas the Markiesje and especially the Saarloos Wolfhond (Saarloos) have very small populations (table 1). The Friese Stabij is not as popular as the GR, but also not as small as the Markiesje or as the Saarloos.

To simulate the inbreeding rate and allele frequencies of the four different breeds, parameters were needed, including breeding population, amount of litters, litter size, age of the first litter, and age of the breeding animals (Table 1). These parameters are filled in one of the 6 tabs in Pointer. Whereby, Pointer is keeping reproduction rate, population size, and age structure constant and only the breeding animals are simulated. The assumption is that managers/breeders are keeping the breeding population size constant, versus animals in the wild, where there are fluctuations due to predators, feed and illnesses (Frankham et al., 2004). For each breed there was also their own number of top sires that sired a specific (large) percentage of the population (table 1).

For every breed the simulations were done for every disorder separately. So, per simulation only one disorder was simulated with their specific s and h (Table 3), which are specified in Pointer. These simulations were then repeated with genetic management. For the genetic management, the constraint of kinship with the remainder of the breed (=Mean Kinship = MK) was used. This is the most effective and easiest method to implement. Pointer uses for every year the males and females with a MK that is lower than the average MK of the population. This is done separately for females and males. Next, to determine the effects of different levels of s, simulations were done with the Saarloos and 12 levels of s between 0 and 1. To also know the effects of h, different levels of h with two levels of s (0.2 and 0.8) were simulated in the Saarloos.

Tables and figures are made from the output of Pointer. The allele frequency and inbreeding at year 100, and years till fixation are used for these results.

Table 1: Parameters of the different breeds

Breeds		Golden Retriever		Stabij		Markiesje		Saarloos	
Population size									
Fathers		150		105		33		11	
Mothers		600		222		56		28	
Litters		300		111		28		14	
Total population		750		327		89		39	
Top sires		5		5		2		5	
% sired		25		15		6		15	
Biological data									
Litter size %	1 pup	2		4		7		9	
	2 pup	3		4		8		9	
	3 pup	5		6		16		12	
	4 pup	7		8		24		12	
	5 pup	7		12		26		13	
	6 pup	11		15		14		16	
	7 pup	16		18		4		11	
	8 pup	18		16		1		10	
	9 pup	15		10		-		6	
	10 pup	7		7		-		2	
	11 pup	5		-		-		-	
	12 pup	3		-		-		-	
	13 pup	1		-		-		-	
Age first litter of Dam		18 months						-	
		Sire	Dam	Sire	Dam	Sire	Dam	Sire	Dam
Age parents	1 year	12	9	7	6	25	7	9	0
	2 year	20	23	16	26	33	31	18	22
	3 year	18	21	18	21	21	21	32	28
	4 year	15	17	16	18	10	17	18	21
	5 year	12	13	14	13	5	10	12	14
	6 year	9	10	11	9	3	8	7	9
	7 year	6	5	10	5	2	4	2	5
	8 year	4	2	6	2	1	2	1	1
	9 year	3	0	2	0	-	-	1	0
	10 year	1	0	0	0	-	-	-	-

Results

The heritable monogenic disorders and the corresponding s & h

The disorders that are selected are shown in Table 2 and some of the disorders that are used in the simulations are shown in Table 3. These disorders are further explained below.

Von Willebrands Factor

Von Willebrands factor (vWD) is a heritable bleeding disorder and is a common heritable disease in dogs (Kramer et al., 2004; Venta et al., 2000). There are three clinical types for vWD. Type 1 is the most common and least severe (Venta et al., 2000). Depending on the breed, type 1 will be recessive or dominantly inherited. The version that is dominantly inherited has an incomplete penetrance. The other two types, type 2 and 3, are both autosomal recessive in dogs. Type 3 is the most severe of all three types (Venta et al., 2000). Carriers of type 3 may show phenotypically some of the disorder, however, they are not really affected, because of this (Kramer et al., 2004;Venta et al., 2000). All three types appear in general to be recessive, except for type 1 in some breeds, so h is in general 0. vWD is in general a disorder with large influences on the health of dogs, however, there is clearly a gradation in severeness between the three types. So, s will be lower for type 1 (0.4), compared to type 2 and 3. Type 3 is more severe, than type 2, so s will be 0.65 for type 2 and 0.7 for type 3 (Table 2).

Progressive Retinal Atrophy

Progressive Retinal Atrophy (PRA) is a group of canine retinal dystrophies disorders. It is a very common disorder and is founded in at least 15 breeds (Petersen-Jones, 1998). The retina progressively degenerates, what leads to night blindness and the loss of peripheral and central visual fields (Curtis & Barnett, 1993; Dekomien et al., 2010). In most breeds, PRA is autosomal inherited. However, in some breeds like the Siberian husky it is on the X-chromosome and in the bullmastiff PRA it is autosomal dominant (Llera & Yuill, n.d.). Although, PRA is not a lethal disease, it could be assumed that breeders do not want to breed with carriers and especially not with affected animals. An owner that buys a pup from a breeder would likely not want a dog that goes blind. For this reason, PRA is set to 0.3 for s and because most types are recessive, h is set to 0.

Degenerative Myelopathy

Degenerative Myelopathy (DM) is a progressive neurological disease that affects adult dogs. Dogs with this disorder will suffer from myelin and axon loss (Nikolovski & Atanaskova, 2010). This disorder is found in multiple breeds, but most often found in the German Shephard (Nikolovski & Atanaskova, 2010). DM is an autosomal recessive disorder according to OMIA, however, compound heterozygous animals may suffer from this disease as well, next to animals that are homozygous for this disorder (Pfahler et al., 2014). The age of onset for DM is between 4/5 and 14 years and is not often seen in young dogs (Averill, 1973 cited by Nikolovski & Atanaskova, 2010; Hunter, n.d.-a). The first signs of DM are covert progressive ataxia, which are rare complicated neurological disorders, and paresis of the lower limbs and ultimately leads to the loss of ability to stand (De Silva et al., 2019; Nikolovski & Atanaskova, 2010). There is also no cure for this disorder, only medication and exercise may help delay further development of this disease (Nikolovski & Atanaskova, 2010). Because this disease has an effect on the quality of life, but can be maintained with medicine, s was set to 0.4. h is set to 0, because it is generally autosomal recessive.

Urolithiasis

Urolithiasis are struvite stones in the bladder that consist of magnesium ammonium phosphate hexahydrate (Palma et al., 2013; Seaman & Bartges, 2001). The different components within the struvite stones are always present in urine, however, the formation of these components to stones depends on the local urinary microenvironment, diet, concurrent therapy, and metabolic factors (Palma et al., 2013). Although, there are a lot of external factors that may lead to struvite stones, there is also a heritable factor known. Being homozygous for the SLC2A9 mutation leads to a higher risk that a dog will develop Urolithiasis (Cosgrove et al., 2015). Urolithiasis is easy to treat with antibiotics and a special diet (Palma et al., 2013; Seaman & Bartges, 2001). S is put at 0.2, because Urolithiases is very treatable and dogs that are homozygous for SLC2A9 do not have to develop the disorder. Urolithiases is autosomal recessive, so h is 0.

Ichthyosis

Ichthyosis are skin disorders, with an abnormal epidermal cornification (Tamamoto-Mochizuki et al., 2016). Ichthyosis is classified based on mode of inheritance, phenotypes and causative gene mutations (Oji et al., 2010) and is seen in multiple breeds (Tamamoto-Mochizuki et al., 2016). The variant in the Golden Retriever was used for the simulations. This variant is characterized by epidermal scaling and is seen at a very young age (6 weeks; Tamamoto-Mochizuki et al., 2016). Although, it is not a lethal disorder, it is more likely that a breeder will not use a dog with Ichthyosis in his breeding program. Therefore, s is set to 0.5 and because it is a recessive disorder, is h set to 0.

Centronuclear Myopathy

Centronuclear Myopathy (CNM) is an autosomal recessive disorder that is characterized by muscle weakness (Maurer et al., 2012). The general symptoms of CNM are intermittent weakness and ataxia (Bley et al., 2002), and these are neurological coordination disorders. The age of onset is at a very young age and may already start at 6 or 7 weeks (Bley et al., 2002). They often develop CNM before the age of 1 (Gortel et al., 1996). The symptoms develops further and reach their worst around 1 year old (García-Martinez et al., 2012). There is no treatment for CNM (García-Martinez et al., 2012), that often leads to the euthanasia of these dogs, however they can have a normal life span. Therefore, s is set to 0.6 and h is 0.

Table 2: Information over the selected disorders.

Disorder	Mode of inheritance	Gene	Age when disease strikes/starts	Strength (course of disease)	Variants	References
Van Willebrands factor	Autosomal	VWF	After bleeding episodes, after birth.	Type 3 is deadly with severe bleeding episodes	3 variants	(Kramer et al., 2004; OMIA, 2020)
Wilson disease	Autosomal recessive	COMMD1 and ATP7B	Before and after birth	Hepatitis, progressive cirrhosis of the liver and premature death	2 variants	(Forman et al., 2005; OMIA, 2020)
Haemophilia A	X-linked recessive	F8	Before and after birth	After bleeding episodes	0 variants	(Kehl et al., 2021; OMIA, 2020)
Degenerative myelopathy	Autosomal recessive	sSOD1, SP110	Between 4-14 years	Affects the spinal cord, resulting in slowly progressive hind limb weakness and paralysis. Degeneration of the spinal cord	0 variants	(Hunter, n.d.; OMIA, 2020)
Centronuclear Myopathy	Autosomal recessive	HACD1	6 weeks - 7 months	Muscle weakness	Multiple (different names)	(Gentilini et al., 2011; OMIA, 2020)
Progressive Retinal Atrophy	Autosomal and x linked	Multiple genes	Depending on the variant (2-3 months or 3-9 years)	Affect photoreceptor cell and lead to blindness	15 variants	(Llera & Yuill, n.d.; OMIA, 2020)
Congenital Stationary or Night blindness	Autosomal recessive	LRIT3	After 6 months	Vision loss and night blindness	0 variant	(OMIA, 2020; Wag, n.d.-a)
Narcolepsy	Autosomal recessive	HCRTR2	Affects young dogs	Sudden collapse and loss of movement	0 variants	(Hunter, n.d.-b ;OMIA, 2020)
Dwarfism	Autosomal recessive	LHX3, (s)POU1F1, GH1	Before and after birth and diagnosed after 2 months of age	Lack of Growth hormone leads to slower growth rate, lack of primary guard hairs gradually, and retention of puppy coat. Leads also to a shorter lifespan	5 variants	(Greco, 2019; OMIA, 2020)
Ichthyosis	Autosomal recessive	NIPAL4, PNPLA1, ASPRV1, SLC27A4, TGM1	Deteriorates with age	Skin condition whereby the epidermal cells terminal differentiation undergo from basal keratinocytes to the highly specialized corneocyte.	5 variants	(Downing, n.d.;OMIA, 2020)
Neuropathy, sensory	Autosomal recessive	FAM134B	Signs between 2-7 months of age	Hyperextension of the limbs, progressive proprioceptive ataxia with intermittent knuckling of the paws, and self-mutilation wounds in the distal part of the limbs	0 variants	(LABOKLIN, n.d.; OMIA, 2020)
Muscular dystrophy	Autosomal recessive and x linked recessive	Multiple genes	Diagnosed in early life	Affects the muscles	5 variants	(Barnette, n.d.; OMIA, 2020)
Epidermolysis bullosa	Autosomal recessive	Multiple genes	Diagnosed in early life (at birth or few weeks after birth)	Raw skin and fluid filled blisters. Often results in death after 3 months	4 variants	(OMIA, 2020 ;Wag, n.d.-a)
Nephritis	Autosomal dominant and recessive and x linked	COL4A5 (x-linked)	Early age	Kidney disease characterized by inflammation of the glomeruli. Often results in death after 15 months	3 variants	(OMIA, 2020; Wag, n.d.-b)
Urolithiasis	Autosomal recessive	SLC2A9	Worsens with age	Urinary stones	0 variants	(ACVS, n.d.; OMIA, 2020)

Table 3: Frequencies of the selected disorders and variations in mixed breeds and purebred dogs.

Disorder	Variant[1]	penetrance	Strength (s)[2]	Proportion effect(h)[3]	Start (T)[4]	Frequency (P) in %		References
						Mixed breed	Purebred	
Van Willebrand's factor	Type 1	Incomplete penetrance	0.4	0	After birth	0.768	1.460	(Donner et al., 2018; Gentilini et al., 2011; Kramer et al., 2004; OMIA, 2020; Venta et al., 2000)
	Type 2		0.65	0		0.595	0.708	
	Type 3	Full penetrance	0.7	0		Not known	Not known	
Progressive Retinal Atrophy	In Golden retriever (TTC8)	Full penetrance	0.3	0	Diagnoses at 5 years	0.136	0.026	(Donner et al., 2018; Downs et al., 2014; Llera & Yuill, n.d.; OMIA, 2020)
Degenerative Myelopathy	sSOD1, SP110	Incomplete penetrance	0.4	0	Between 4 and 14 years	7.771	5.414	(Awano et al., 2009; Bryant & Meffert, 1993; Donner et al., 2018; Hunter, n.d.; OMIA, 2020)
Urolithiasis	SLC2A9	Incomplete penetrance	0.2	0	Between 3 and 6 years	2.155	1.319	(ACVS, n.d.; Bannasch et al., 2008; Donner et al., 2018; OMIA, 2020)
Ichthyosis	Golden retriever (PNPLA1)	Almost full penetrance	0.5	0	Worsens with age	0.710	0.699	(Donner et al., 2018; Guaguere et al., 2013; Downing, n.d.; OMIA, 2020)
Centronuclear Myopathy	Golden retriever(HACD1)	Full penetrance mutation with variable expressivity	0.6	0	6 weeks - 7 months	0.121	0.117	(Donner et al., 2018; Gentilini et al., 2011; OMIA, 2020; Maurer et al., 2012)

[1]*Different mutations/variations of the disease.*

[2]*Strength of the effect of the disorder*

[3]*Proportion of the effect of the disorder*

[4]*Age of onset of the disease*

Effect of different effective population size on inbreeding and allele frequency

Inbreeding (Delta F) was the lowest for Stabij (ΔF 0.45) compared to the other 3 breeds (ΔF 0.54, 1.05, and 2.08, Table 4). The generation interval was for all 4 breeds between 2.9 and 3.9 years, with the Markiesje the lowest generation interval and Stabij the highest. The simulations were also done with and without genetic management, which led to a decrease in inbreeding in all 4 breeds (-0.102, -0.066, -0140, and -0.182). With the decrease of inbreeding, the generation interval increased (+0.14, +0.29, +0.34, and +0.24). Also, with genetic management the inbreeding (Delta F) was lowest for the Golden Retriever (ΔF 0.033), compared to the other 3 breeds (ΔF 0.043, 0.164, and 0.292).

Overall the allele frequencies of the detrimental alleles after 100 years were higher in the numerically breeds with a larger population, when compared to the smaller breeds (table 5 to 8). The differences were smaller between the bigger breeds compared to the smaller breeds. Some allele frequencies were higher in the Golden Retriever and some in the Stabij. The same is true for the Saarloos and the Markies. Genetic management led to a higher allele frequency and year till fixation in all disorders in all four breeds. Except for PRA in the Markies and Saarloos where it led to a decrease (table 7, 0.31 to 0.27 and table 8, 0.369 to 0.337).

The disorders were more often (more repeats) eliminated in the smaller breeds compared to the bigger breeds, where the allele frequency more often remained segregating. Progressive Retinal Atrophy was even fixed in the Saarloos. No fixation in the other breeds was seen (figure 2 and 3). The higher allele frequency, due to genetic management, is also shown in the disorders that were eliminated. For the Markies and Saarloos the number of repeats in which the disorders were eliminated was decreased and the number of repeats in which the allele frequency of the disorders was segregating was increased (figure 2 and 3).

Table 4: The effects of different population effective population size and genetic management on the inbreeding and generation interval. Results of the simulations over 100 years with the average[2] and range[3] of 50 repeats.

	Genetic management[1]	Golden Retriever	Friese Stabij	Markiesje	Saarloos Wolfhond
		Average and Range			
Inbreeding pups	-	0.135[2]	0.109	0.304	0.474
		(0.107 – 0.176)[3]	(0.089 -0.141)	(0.271 – 0.376)	(0.404 – 0.548)
	+	0.033	0.043	0.164	0.292
		(0.029 – 0.038)	(0.037 -0.056)	(0.146 – 0.193)	(0.264 – 0.349)
Generation interval (year)	-	3.73	3.92	2.91	3.25
	+	3.87	4.21	3.25	3.49
Delta F (%)	-	0.54	0.45	1.05	2.08
		(0.52 - 0.57)	(0.44 – 0.47)	(1.02 – 1.09)	(2.01 -2.16)
	+	0.13	0.18	0.58	1.20
		(0.13 -0.13)	(0.18 – 0.19)	(0.57 – 0.60)	(1.17 – 1.23)

[1] without (-) and with (+) genetic management

Effects of the differences in strength and dominance on the frequencies

For the first three disorders (vWb type 1, 2 and 3) dominance (h) was set to 0 and the age of onset was 0 years. An increase in strength of the disorder led to a decrease in the allele frequency in the two bigger breeds Table 5 and 6). However, in the Saarloos the increase in strength of the vWb2 led to an increase in the allele frequency instead of a decrease (table 7).

A higher age of onset led to an increase in the allele frequency. PRA, DM and URO had a higher age of onset (5, 4, and 3 years) and all three disorders had higher frequencies in all four breeds, whereby the frequency for PRA was the highest (Table 5,6,7,8). A higher age of onset led also to a higher year till fixation. The allele frequencies of the three disorders PRA, DM and URO were still segregating at 100 years (Table 5, 6, 7, 8). The disorders with an age of onset of zero were more often eliminated compared to the disorders that have a later age of onset. For example, PRA, DM and URO have a higher number of repeats in which alleles were segregating, compared to the other disorders (figure 2 and 3).

Table 5: The effects of the different disorders with different s and age of onset in the golden retriever. Results of the simulations after 100 years with the average[3] and range[4] of 50 repeats.

	Allel frequency		**Year till fixation/elimination**		**Number of repeats in which alleles were segregating**	
Genetic management[2]	-	+	-	+	-	+
vWb1[1]	0.0326[3]	0.0434	98.56	99.96	45	50
	(0 – 0.1128)[4]	(0.0005 – 0.0991)	(81 – 100)	(98 – 100)		
vWb2[1]	0.0222	0.0431	97.84	100	42	50
	(0.0000 - 0.0828)	(0.0126 – 0.1081)	(74 -100)	(100 – 100)		
vWb3[1]	0.0178	0.0461	97.54	100	9	50
	(0.0000 - 0.0791)	(0.0086 – 0.1263)	(68 -100)	(100 – 100)		
PRA[1]	0.1766	0.2009	100	100	50	50
	(0.0207 -0.4319)	(0.0876 – 0.3352)	(100 - 100)	(100 – 100)		
DM[1]	0.0840	0.1160	100	100	50	50
	(0.0051 - 0.2557)	(0.0344 – 0.2190)	(100 – 100)	(100 – 100)		
URO[1]	0.1124	0.1361	100	100	50	50
	(0.0042 - 0.2692)	(0.0374 - 0.2387)	(100 – 100)	(100 – 100)		
ICH[1]	0.0370	0.0438	97.94	100	43	50
	(0.0000 - 0.1297)	(0.0035 – 0.1383)	(81 – 100)	(100 – 100)		
CNM[1]	0.0240	0.0374	97.78	100	42	50
	(0.0000 - 0.1294)	(0.0026 – 0.0863)	(72 – 100)	(100 – 100)		

[1]vWb: van Willebrand's factor type 1,2 or 3, PRA: Progressive Retinal Atrophy, DM: Degenerative Myelopathy, URO: Urolithiasis, ICH: Ichthyosis, CNM: Centronuclear Myopathy
[2] without (-) and with (+) genetic management

Table 6: The effects of the different disorders with different s and age of onset in the Stabij. . Results of the simulations after 100 years with the average[3] and range[4] of 50 repeats.

	Allel frequency		Year till fixation/elimination		Number of repeats in which alleles were segregating	
Genetic management[2]	-	+	-	+	-	+
vWb1[1]	0.0315[3]	0.0485	97.16	99.90	41	49
	(0 – 0.1385)[4]	(0 – 0.1410)	(66 – 100)	(96 – 100)		
vWb2[1]	0.0296	0.0507	98.40	100	45	50
	(0.0000 – 0.0944)	(0.0036 – 0.1308)	(66 – 100)	(100 – 100)		
vWb3[1]	0.0275	0.0439	96.96	99.62	11	49
	(0.0000 – 0.11421)	(0.0000 – 0.1337)	(57 – 100)	(85 – 100)		
PRA[1]	0.1770	0.2090	100	100	50	50
	(0.0159 – 0.4092)	(0.0720 – 0.3545)	(100 – 100)	(100 – 100)		
DM[1]	0.1048	0.1109	100	100	50	50
	(0.0144 – 0.1628)	(0.0360 – 0.2391)	(100 – 100)	(100 – 100)		
URO[1]	0.1091	0.1290	100	100	50	50
	(0.0079 – 0.3040)	(0.0526 – 0.2271)	(100 – 100)	(100 – 100)		
ICH[1]	0.0263	0.0445	96.44	99.56	50	47
	(0.0000 – 0.1239)	(0.0000 – 0.1068)	(72 – 100)	(88 – 100)		
CNM[1]	0.0373	0.0478	97.98	99.96	43	49
	(0.0000 – 0.1131)	(0.0000 – 0.1146)	(65 – 100)	(98 – 100)		

[1]vWb: van Willebrand's factor type 1,2 or 3, PRA: Progressive Retinal Atrophy, DM: Degenerative Myelopathy, URO: Urolithiasis, ICH: Ichthyosis, CNM: Centronuclear Myopathy

[2] without (-) and with (+) genetic management

Table 7: The effects of the different disorders with different s and age of onset in the Markies. Results of the simulations after 100 years with the average[3] and range[4] of 50 repeats.

	Allele frequency		**Year till fixation/elimination**		**Number of repeats in which alleles were segregating**	
Genetic management[2]	-	+	-	+	-	+
vWb1[1]	0.0128[3]	0.0355	66.94	85.64	11	29
	(0 – 0.1810)[4]	(0 – 0.2037)	(30 – 100)	(40 – 100)		
vWb2[1]	0.0199	0.0291	71.04	86.56	16	25
	(0.0000 – 0.1379)	(0.0000 – 0.1557)	(28 – 100)	(35 – 100)		
vWb3[1]	0.0103	0.0193	65.14	82.60	9	19
	(0.0000 – 0.0905)	(0.0000 – 0.1696)	(19 – 100)	(43 – 100)		
PRA[1]	0.3111	0.2715	100	99	48	48
	(0.0000 – 0.9698)	(0.0000 – 0.6207)	(92 – 100)	(69 – 100)		
DM[1]	0.1034	0.1513	96	99	41	45
	(0.0000 – 0.3448)	(0.0000 – 0.4254)	(46 – 100)	(80 – 100)		
URO[1]	0.1333	0.1879	94	99	36	49
	(0.0000 – 0.4655)	(0.0000 – 0.4811)	(48 – 100)	(76 – 100)		
ICH[1]	0.0199	0.0260	69.72	85.18	14	26
	(0.0000 – 0.1810)	(0.0000 – 0.1207)	(28 – 100)	(40 – 100)		
CNM[1]	0.0152	0.0271	75.24	80.80	19	22
	(0.0000 – 0.1478)	(0.0000 – 0.1404)	(33 – 100)	(29 – 100)		

[1]vWb: van Willebrand's factor type 1,2 or 3, PRA: Progressive Retinal Atrophy, DM: Degenerative Myelopathy, URO: Urolithiasis, ICH: Ichthyosis, CNM: Centronuclear Myopathy

[2] without (-) and with (+) genetic management

Table 8: The effects of the different disorders with different s and age of onset in the Saarloos. Results of the simulations after 100 years with the average[3] and range[4] of 50 repeats.

	Allel frequency		Year till fixation/elimination		Number of repeats in which alleles were segregating	
Genetic management[2]	-	+	-	+	-	+
vWb1[1]	0.0074[3]	0.0320	56.20	72.60	5	18
	(0 – 0.1071)[4]	(0 – 0.2029)	(19 – 100)	(32 – 100)		
vWb2[1]	0.0087	0.0181	53.84	66.96	4	13
	(0.0000 – 0.1857)	(0.0000 – 0.1500)	(24 – 100)	(24 – 100)		
vWb3[1]	0.0126	0.0257	59.68	72.60	9	15
	(0.0000 – 0.1429)	(0.0000 – 0.2328)	(20 – 100)	(33 – 100)		
PRA[1]	0.3697	0.3373	97	98	43	46
	(0.0000 – 1.0000)	(0.0000 – 0.8714)	(16 – 100)	(38 – 100)		
DM[1]	0.1441	0.1481	88	94	30	39
	(0.0000 – 0.6071)	(0.0000 – 0.6509)	(39 – 100)	(54 – 100)		
URO[1]	0.1423	0.1862	90	95	35	39
	(0.0000 - 0.5714)	(0.0000 – 0.6143)	(20 – 100)	(55 – 100)		
ICH[1]	0.0114	0.0149	57.74	68.14	7	12
	(0.0000 – 0.1571)	(0.0000 – 0.1667)	(20 – 100)	(22 – 100)		
CNM[1]	0.0186	0.0253	55.96	70.76	8	12
	(0.0000 – 0.2286)	(0.0000 – 0.1929)	(21 – 100)	(28 – 100)		

[1]vWb: van Willebrand's factor type 1,2 or 3, PRA: Progressive Retinal Atrophy, DM: Degenerative Myelopathy, URO: Urolithiasis, ICH: Ichthyosis, CNM: Centronuclear Myopathy

[2] without (-) and with (+) genetic management

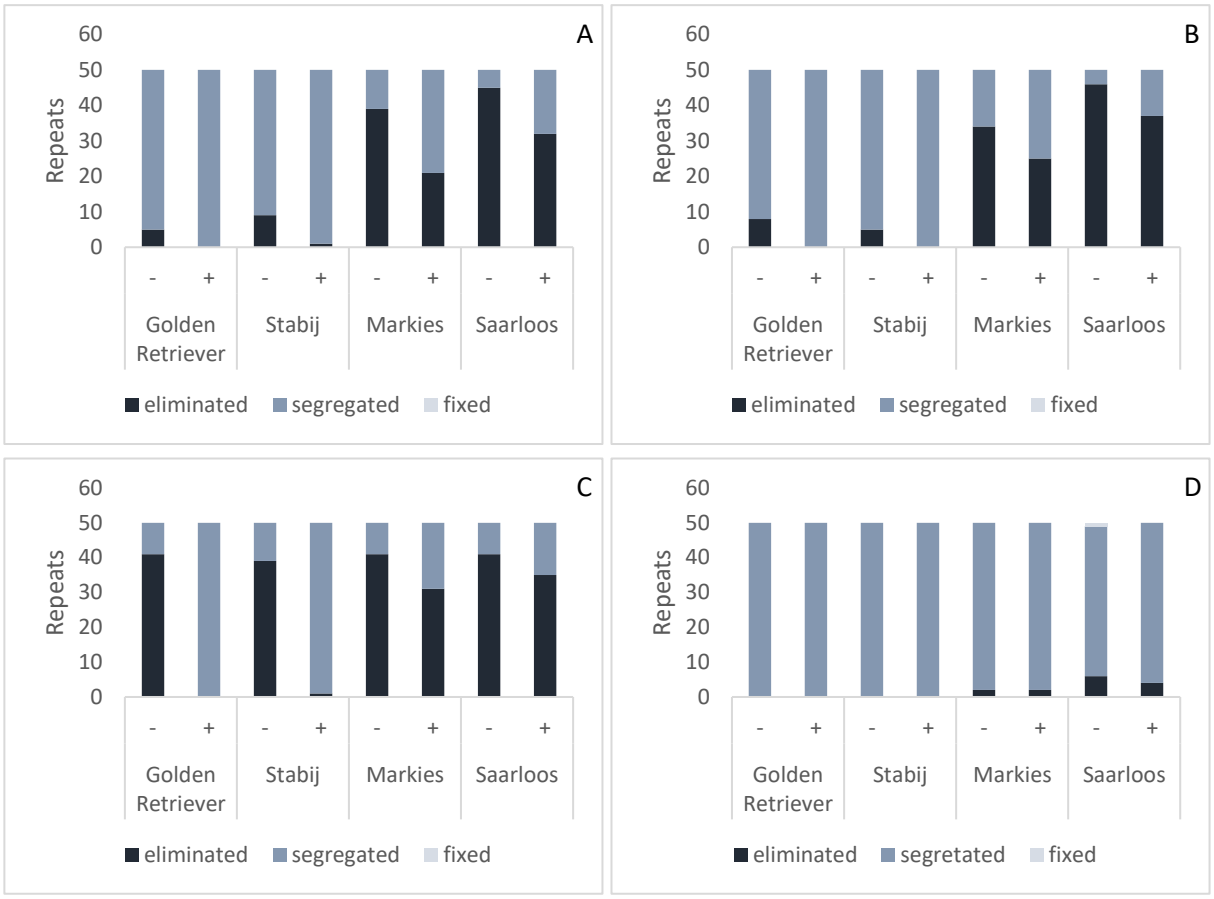


Figure 2: An overview of the distribution (eliminated, segregating or fixed) of the 50 repeats of 4 different disorders in the Golden Retriever, Stabij, Markies and Saarloos after 100 years without (-) and with genetic management (+). A: Van Willebrand's factor type 1. B: Van Willebrand's factor type 2. C: Van Willebrand's factor type 3. D: Progressive Retinal Atrophy.

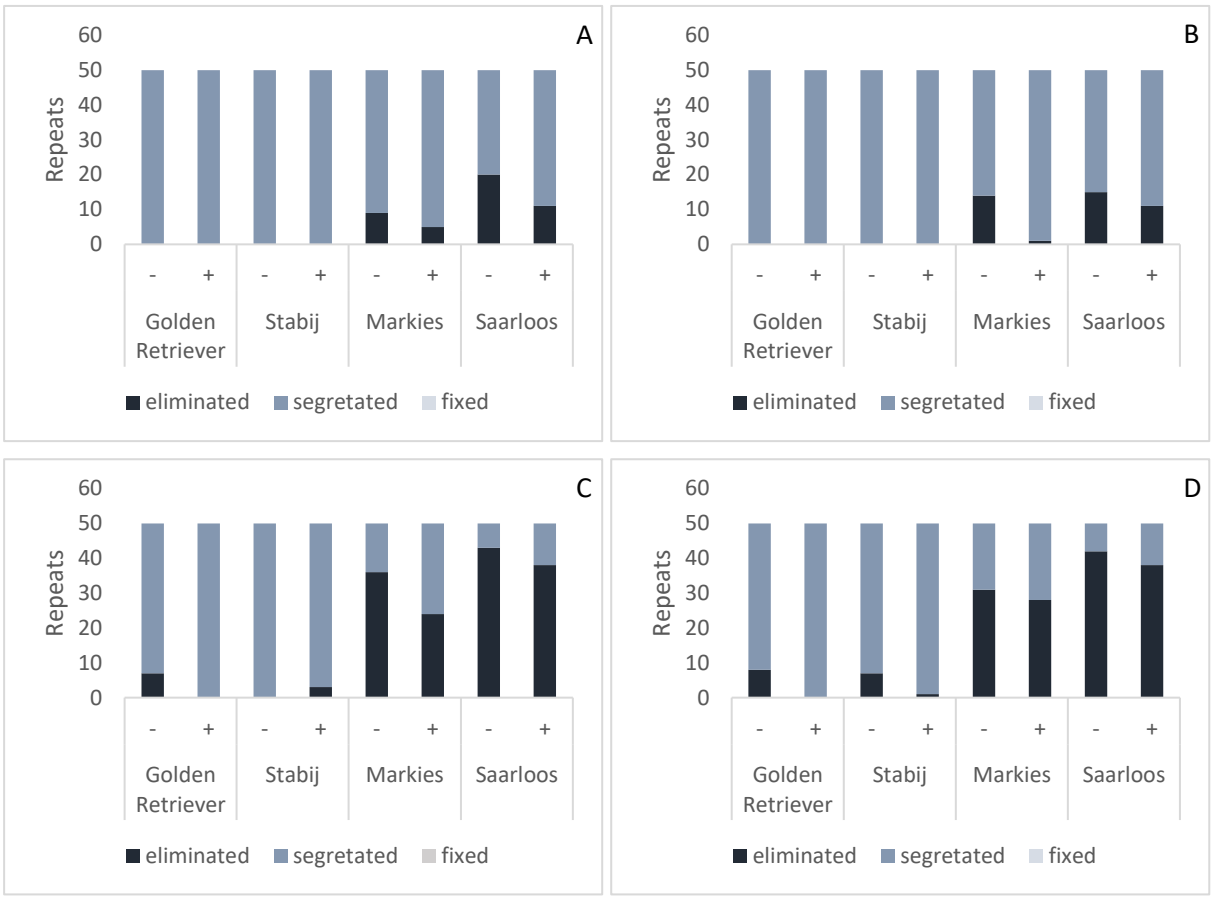


Figure 3: An overview of the distribution (eliminated, segregating or fixed) of the 50 repeats of 4 different disorders in the Golden Retriever, Stabij, Markies and Saarloos after 100 years without (-) and with genetic management (+). A: Degenerative myelopathy. B: Urolithiasis. C: Ichthyosis D: Centronuclear Myopathy.

Allele frequencies compared with literature

The average allele frequencies after 100 years that we found during the simulations were compared with the allele frequencies we found in literature (mixed breeds and purebreds) as shown in Figure 4. It shows that the allele frequencies in literature are lower (vWb2 0.071 & 0.0060) than those found in the simulations (0.022; 0.0296; 0.0199; 0.0087). The allele frequencies that are found without genetic management are also more in line with the ones in literature, compared to the allele frequencies with genetic management. But even without genetic management the differences are very large, with PRA being the largest (0.0003 and 0.0014 vs 0.018, 0.018, 0.031, and 0.037). Only Saarloos is very close for vWb1 (0.0074) and vWb2 (0.0087) to the frequencies found in literature for the mixed breeds (0.0077 and 0.0071).

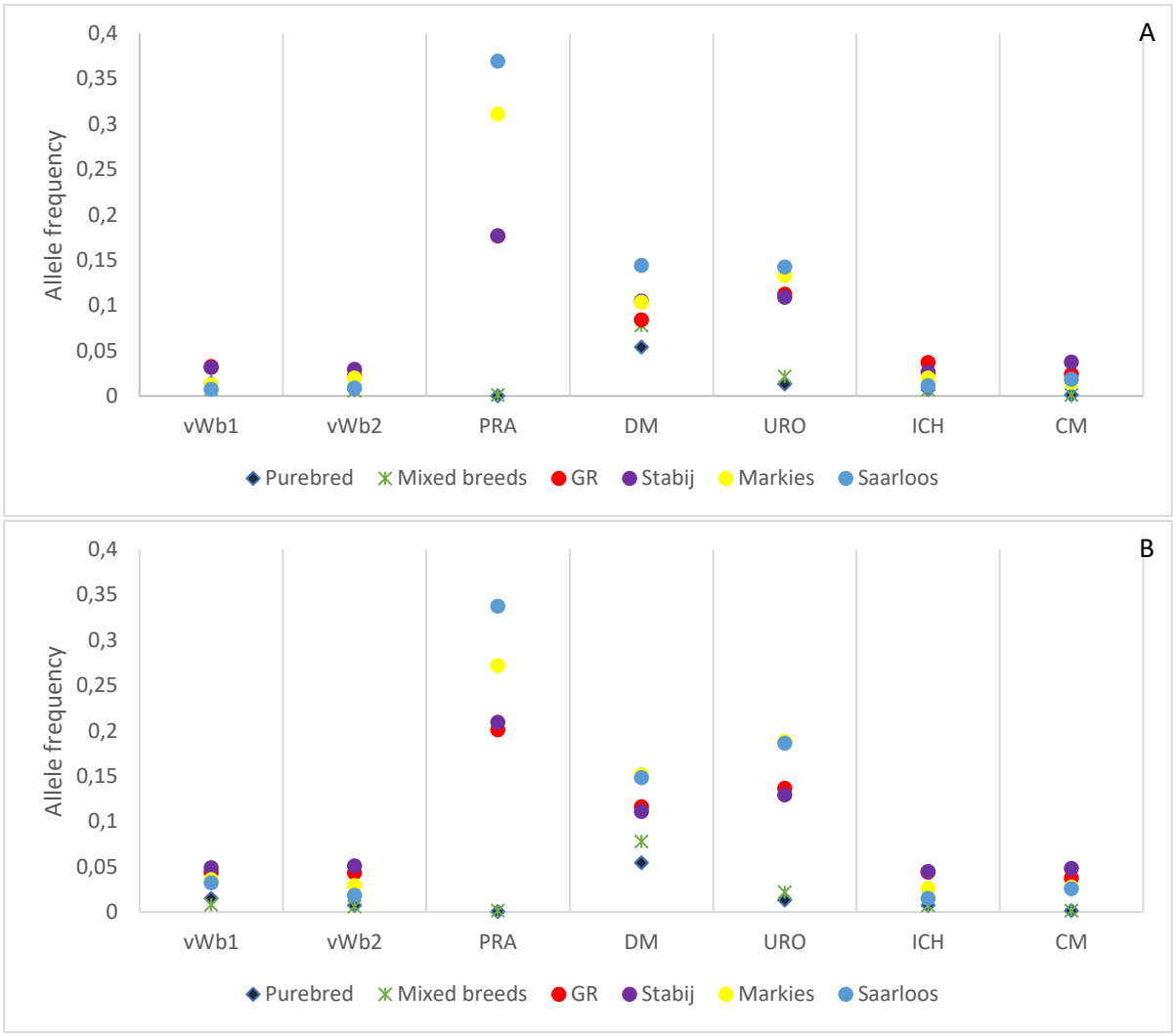


Figure 4: The allele frequency of purebreds and mixed breeds found in literature vs the allele frequencies found in the simulations after 100 years for the golden retriever, Stabij, Markies, Saarloos without (A) and with genetic management (B).

Effect of strength and dominance

The more lethal or stronger the disorder, the more repeats of the disorder in which it is eliminated (figure 2 A, B, C and figure 5, 6 and 7). This is also shown in figure 6, whereby a higher strength leads to more elimination and a very low strength leads to fixation and segregation.

A dominant disorder is even more easily eliminated (figure 7) and this is increased when the strength of the disorder is also higher. For example with a dominance of 0.01, the number of repeats are higher for a higher strength (0.8, 46 repeats eliminated) compared to a lower strength (0.2, 36 repeats eliminated). Although, with dominance 0.3 till 0.6 all the repeats are eliminated, with 0.7 and 0.8 there are some that are segregating.

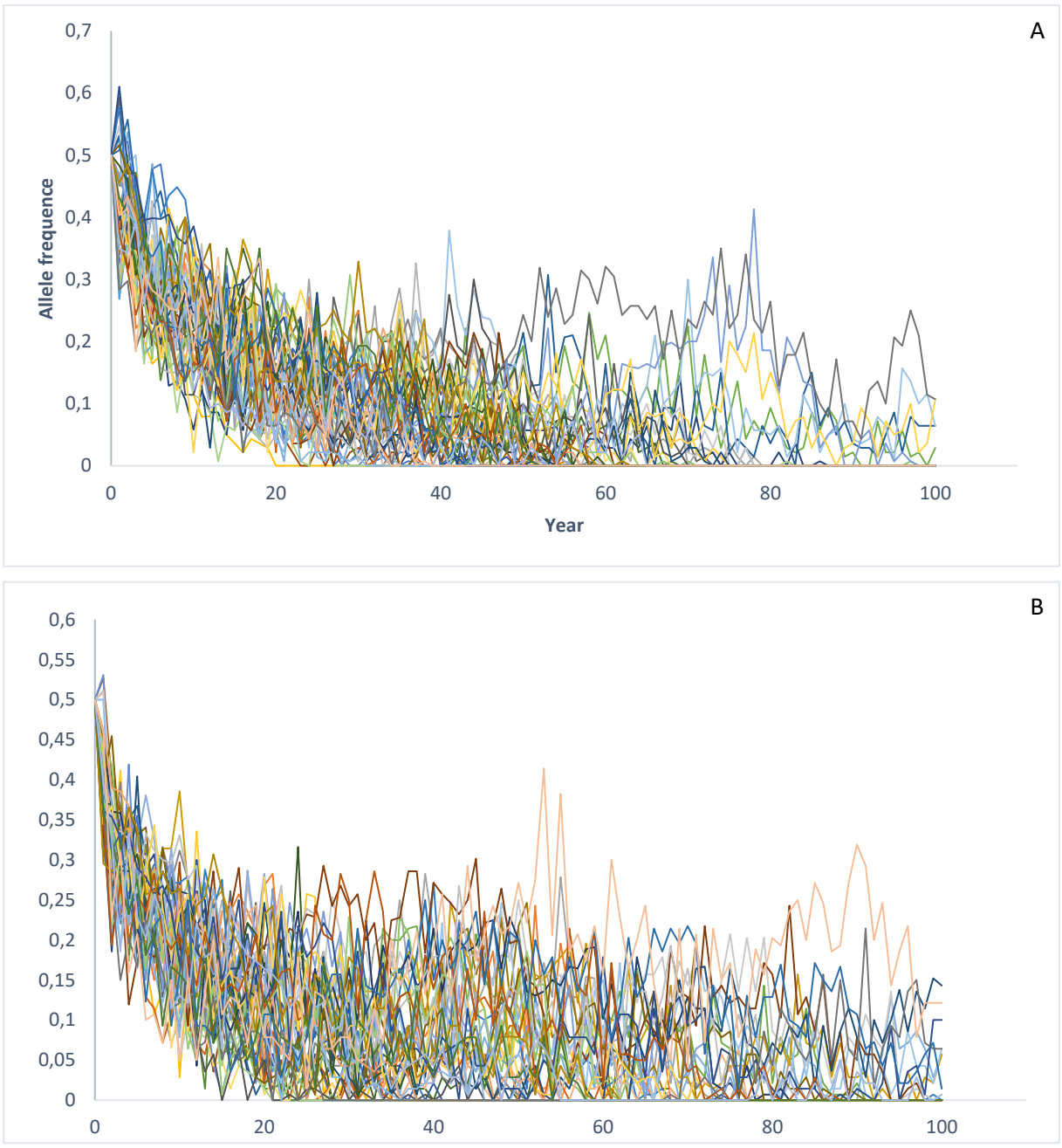


Figure 5: Allele frequency of the van Willebrand's factor type 1 (A) and type 3 (B) over 100 years in the Saarloos

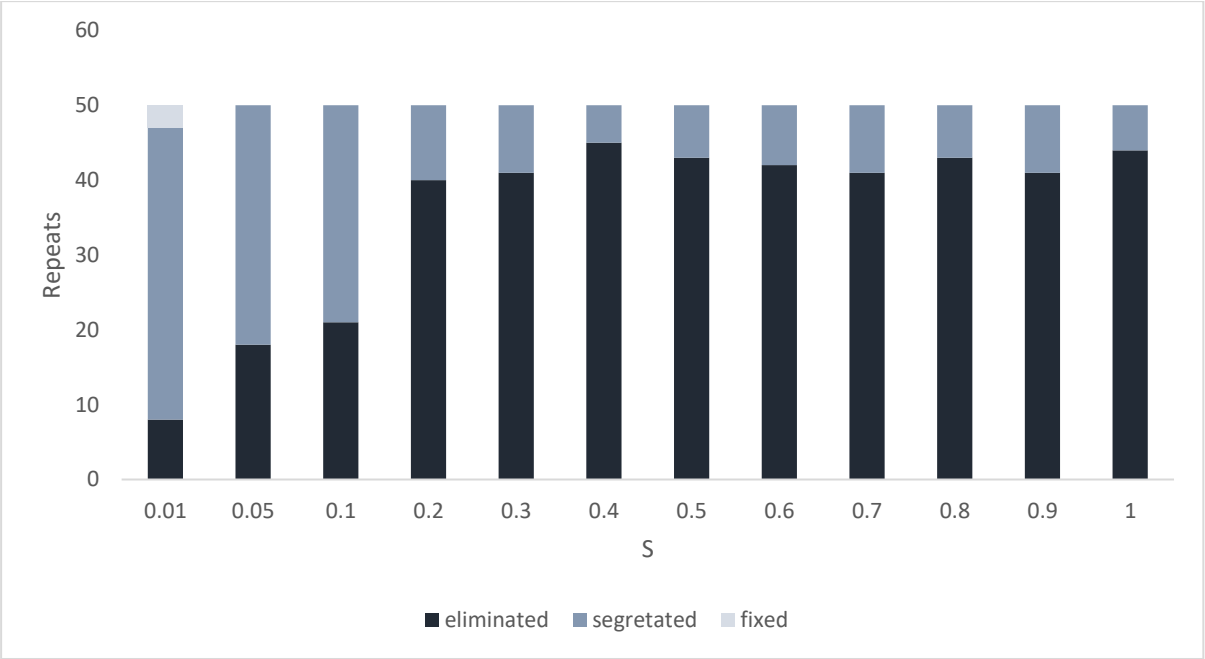


Figure 6: The effect on allele frequencies of different strength (s). Results of the simulations in the Saarloos with a hypothetical disorder with different strengths.

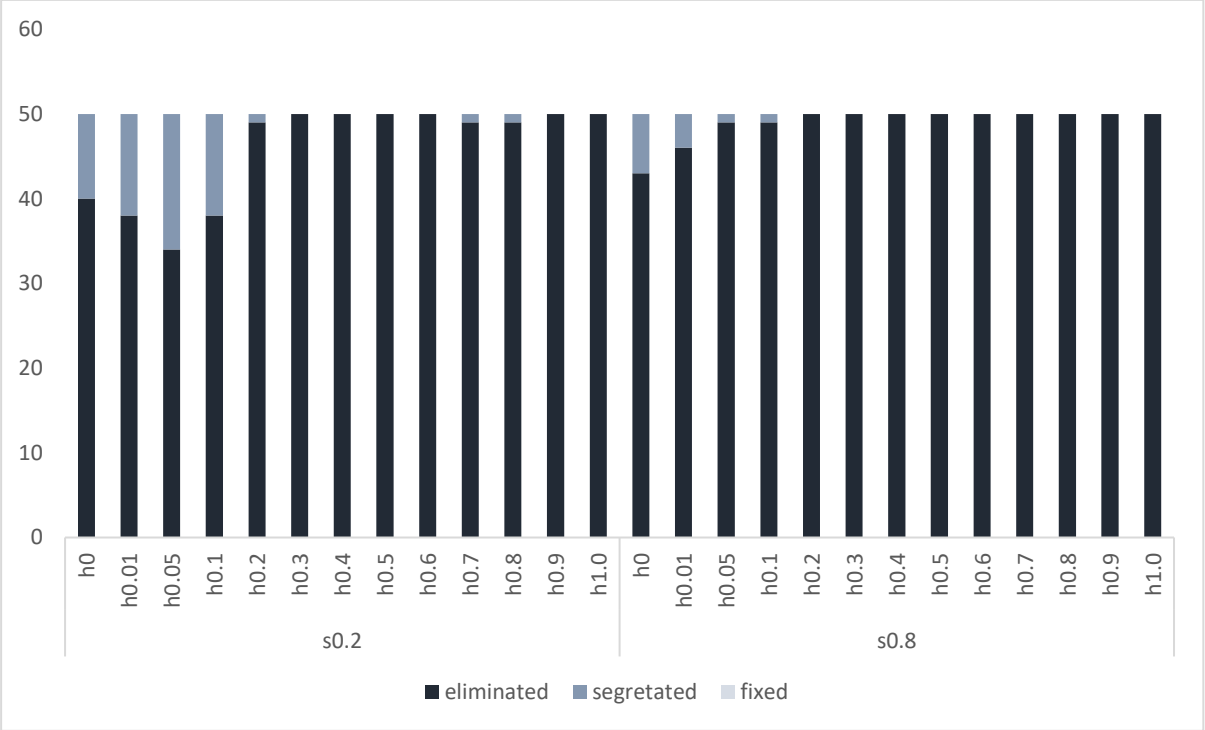


Figure 7: The effect on allele frequencies of different strength (s) and dominance (h). Results of the simulations in the Saarloos with a hypothetical disorder with different strengths.

Discussion

The aim of this study was to look at the effects of strength and dominance on allele frequencies of common genetic disorders in dogs, as well as at the ability to estimate parameters with the theoretical framework. The theoretical framework had not been used for breeding in practical situations. It was only described in wildlife populations (Hedrick & Garcia-Dorado, 2016). Therefore, this study investigated if the theoretical framework is also useful for known heritable disorders and the effect of effective population size on the allele frequencies of these disorders. This study found that the theoretical framework is a useful tool to show the effect of different strength (s) and dominance (h) on common disorder and breeding practices. Large effective population sizes lead to more intermediate allele frequencies and segregation, whereas small effective population sizes lead to more elimination and fixation of allele frequencies.

Known heritable disorders

There are a multitude of disorders (371, OMIA accessed on 2th of august 2021) known in dogs with a causal variant (OMIA, 2020). However, only a few (~10 disorders) were needed for the simulations. There were multiple disorders suitable for this thesis, however, most of the disorders are lacking information, especially about the allele frequency. For 30 disorders in dogs the allele frequency was described (Donner et al., 2018). Which lead to the decision to use these 30 disorders. It was also very important that the disorders had a difference in strength, were recessive, were monogenic, and were frequently observed in dogs. Polygenic disorders would be too complicated to use in the simulations and for now to relate to the framework. Although there were only 8 disorders used for the simulations, the results clearly showed that disorders with a high strength (≥0.7) will be easier eliminated (purged) from the populations, especially out of a small population. The allele frequency of the disorders that have a similar low strength will likely keep on segregating in large populations and may get fixed in small populations. Purging will be less effective with disorders with a low strength. Disorders with other s or h in different populations could show different results in allele frequencies. Also, we only evaluated the effect of different levels of s and h in a breed with a very small effective population size (Saarloos, Figure 6 & 7). Thus, it would be valuable to repeat these simulations in combination with a breed that has an intermediate population size and one with a large population size.

Some of the disorders have multiple variants, where one variant would be dominant and the other recessive. An example is vWb type 1, where there are cases from a variant with dominant inheritance and incomplete penetrance in some breeds (Venta et al., 2000). However, we only used the recessive variants in this thesis. It could be interesting to see how the same disorder develops within a population with a difference in dominance. However, it is likely that the disorders with a higher dominance will be eliminated more easily from the population, especially if s is also higher (figure 7). We only used one disorder per simulation. Realistically, an animal would carry multiple disorders that often have a small effect. Some of these disorders may get fixed in a small population. So, it could be interesting to simulate the effect of not one, but multiple disorders per simulation within a breed and to see the effect on fertility and inbreeding. It is likely that some of the disorders will get fixed, where others will not. The question only lies in how large the chance is that multiple disorders get fixed at the same time in a small population and in a large population. However, the chance of fixation is higher for a small population, compared to a large population, due to inbreeding, purging and genetic drift.

Theoretical framework is useful for simulations

The theoretical framework of strength (s) and dominance (h) of a disorder was not yet described for dog breeding. It was only used for wild populations (Donner et al., 2018). We have estimated the values of the theoretical framework to common disorders in dogs and used this for simulations. However, there are a lot of components that could be improved. We estimated the values for s and h based on the knowledge about the severity of the disorder (lethality) and the level of dominance, but there could be a large human error in these estimations. For example, we choose for vWb1 0.4, for vWb2 0.65, and for vWb3 0.7, but vWb1 could also have been 0.2/0.3, vWb2 could have been 0.5/0.6, and vWb3 could have been 0.8/0.9. Although, the outcome would not have been very differently, because the three types are still differing in lethality. There are a lot of assumptions that can lead to different values. The estimation was based on how lethal a disorder is or if breeders would likely not breed with a dog carrying the disorder. But s is the reduction in the number of offspring in the next generation. The estimation of s and h of a disorder may be very different if another person would estimate it. Also, a dog doesn't have to die immediately from a disorder. It may still reproduce, but with less offspring or it may receive medicines to survive. However, would a breeder use a dog knowing it would have a lethal disorder, that means that this dog will not have any offspring. S will then be increased. So, it would be good to have a standardization for these values for multiple disorders.

The level of penetrance may help for the standardization of h and s. For example, a lethal disorder with 80% (penetrance) of the animals with the deleterious allele that die from this disorder, leads to a s of 0.8 and an h of 0.25. Except, the penetrance and the death rate is not known for many disorders. vWb1 illustrates this, where the mutation penetrance increases as the von Willebrand's Factor plasma level decreases (Berber, 2012). This makes it very difficult to estimate s of a disorder. Some of the disorders had an incomplete penetrance (Table 3), what means that not every affected dog will develop the disorder. This makes it even more difficult to estimate s, because the affected dogs that are not developing the disorders are likely overlooked. In a study on boxers none of the animals that tested homozygous for the disorder were definitely diagnosed for DM. Therefore, an estimation for the prevalence could not be estimated (Zeiler et al., 2013). Disorders can originate from different mutations that can also be specific to a breed. All these different mutations can react differently and lead to a different s and h. For example, PRA has a mutation within the Golden Retriever, but there are other mutations that are found in other breeds (Downs et al., 2014), or there are like vWb factor where it is found in multiple breeds (Kramer et al., 2004; Oost et al., 2004). CM has full penetrance in a population but varies in the severity of the symptoms in individual dogs (Maurer et al., 2012). This will lead to a different s for every dog, because some dogs could have mild symptoms and may have offspring. In the previous examples it is still clear that there is a visible effect of the disorder. There are also effects that are not easily quantifiable or easily linked to the disorder. There could, for example be a reduction in the litter size, which leads to a higher s. However, this should be monitored at a population level for multiple years in order to be useful for the estimation for s. So, it is very difficult to make a standardization for s and h and make a good estimation.

There is a database for vets (PETscan) about heritable disorders, which could give useful information for the estimation of s and h. Vets update how often a disorder occurs and about new upcoming disorders (LICG, 2019). There are 2 main causes why this is not a complete database: first, not every animal that dies gets an autopsy and not every autopsied animal has a conclusive cause of death. Secondly, not every vet is connected and they should also keep records of the dogs that died or are euthanized due to a disorder. Thus, a database with mortality, age of diagnosis, development of the

disorder, and number of offspring should be included. In this way s and h could be more standardized and used similarly in simulations, which may lead to better assessments of breeding strategies. However, the database should be open for multiple people (universities, companies, breedclubs) that want to study them.

The more lethal the disorder and the earlier the age of onset is, the easier a disorder is eliminated from a population (Figure 6 and 7). These disorders are also easily found and are therefore easier to eliminate with natural selection and human selection. However, the disorders that are less lethal and have thus a small s will not be as easily eliminated. They are difficult to find and are not noticed on an animal level, but may have a large effect on a population level (Hedrick & Garcia-Dorado, 2016). A disorder with a very small effect has the risk of getting fixed (s0.01, figure 6). So, this could also happen with multiple small disorders. Multiple disorders with a small effect may each not have a large effect on its own, but could have a strong effect together, which could lead to a decrease in fertility (Hedrick & Garcia-Dorado, 2016). Eliminating large disorders by inbreeding would therefore not be a wise strategy, because you may fixate multiple disorders with a small effect. Inbreeding also leads to more disorders that come to the surface and may lead to detrimental effects. The good effects of purging require a very slow inbreeding rate and are often taking effect after an early fitness decline (Hedrick & Garcia-Dorado, 2016). This may give potential dangers, but it is not known how large these dangers are. However, a breeder would likely not notice these early fitness declines within the population.

The age of onset has a large influence as well. A higher age of onset leads to more segregation of a disorder. So, it would be important that a disorder is recognized early to eliminate it from the population. DNA testing is a good strategy to use for an early diagnosis, so that there is a choice to not use these dogs in the breeding program.

The allele frequencies found during the simulations were larger, compared to the allele frequency found by Donner et al. (2018). It was very difficult to estimate s and h, due to the lack of information about the disorders. It could be that s was wrongly estimated. It is not known how many dogs exactly die due to the disorders, if they can survive with medication, and how many there are still used for breeding. This can all change the estimation of s and h.

Breeders also often use DNA tests to select against disorders, which may have led to the lower allele frequencies found in literature. Or breeders will use as few relatives as possible of dogs that have a known disorder. Breeders are also often importing animals from other countries, which may have led to the difference in allele frequencies. Also, the allele frequencies in literature were based on purebreds and mixed breeds, whereas we took the average from only four pure breeds separately.

A larger effective population size leads to intermediate allele frequencies and segregation

A large effective population size leads to more intermediate allele frequencies and segregation, while small effective population sizes lead to elimination or fixation of a disorder. Disorders with a small effect will sometimes be fixed and disorders with a large effect will be eliminated (Figure 6 and 7). However, at equilibrium, small populations are predicted to have a lower inbreeding load, whereby larger populations are expected to have more severe decrease in fitness under inbreeding (Hedrick & Garcia-Dorado, 2016). So, detrimentals should not be included in the inbreeding load, because these disorders that sometimes are fixed may not lead to a decrease in fitness when there is inbreeding. If there is inbreeding in large populations, the disorders with segregating allele frequencies may come

more to the surface and may become fixed. This may be another reason that large populations have a larger decrease in fitness, compared to smaller populations.

Genetic management leads to more segregation of the disorders in all four breeds. Minimizing kinship leads to a decrease in inbreeding, but it also means that every animal will be used regardless of their fitness (Hedrick & Garcia-Dorado, 2016). So, it could be that a dog is used that has a low kinship but is also heterozygous for some disorders. Using low kinship lessens the effect of natural selection and could neutralize it in the case of fecundity traits (Hedrick & Garcia-Dorado, 2016). Therefore, it may be important to combine multiple strategies for breeding, like founder flush cycles. With founder flush cycle a population thrives and increases in genetic diversity after a bottleneck (Bryant & Meffert, 1993; Meffert, 1999). However, it is not likely that breeders would want to take the risk of letting their dog breed undergo a bottleneck, because the risk is that the breed could go extinct. If available, a DNA test would then be a better strategy for dog breeders to use in combination with low kinship.

Conclusion

There are a lot of disorders known in dogs. Some have been investigated more and have more information, than other disorders, which leads to more availability of data for the simulations. Monogenic, recessive disorders are able to be related to the theoretical framework. However, it was very difficult to give a good estimation of the level of s and h. Using the level of penetrance and a good database from vets will lead to more information about the disorders and this help with a better estimation and a standard way to estimate the level s and h. A standardization for the level of s and h would give more accuracy between different studies done by different people.

A larger effective population size leads to intermediate allele frequencies and segregation. Disorders with a large effect will disappear more easily from the populations, especially from small populations. However, the disorders with a small effect will stay within the population, and have the risk of getting fixed in small populations. In large populations they were more likely to segregate.

Recommendations

To improve the reliability of the simulations, it is advised to standardize the level of strength and dominance. The level of penetrance and more information about the disorders may help with this. It is important that vets keep a database with records about the disorders. In this case the framework could be more available in practice for the simulations and therefore in dog breeding.

Breeders should not try to eliminate disorders with inbreeding, because they will have the risk to fixate the disorders with a small strength. The best way to eliminate disorders is to use DNA tests and use the free animals in combination with the carriers. If the population is too small, they should only use the animals that are free from a disorder. However, the disorders that have no DNA test (yet) can be controlled by using genetic management strategies, like low kinship, keeping the effective population high, less litters per sire, etc. The allele frequencies of the disorders will then be segregating but will not get fixated.

References

ACVS. (n.d.). *Urinary Stones*. American College of Veterinary Surgeons. Retrieved April 14, 2021, from https://www.acvs.org/small-animal/urinary-stones

Averill, D. R. (1973). Degenerative myelopathy in the aging German shepherd dog: clinical and pathologic findings. *Journal of the American Veterinary Medical Association*, *162*(12), 1045–1051.

Awano, T., Johnson, G. S., Wade, C. M., Katz, M. L., Johnson, G. C., Taylor, J. F., Perloski, M., Biagi, T., Baranowska, I., Long, S., March, P. A., Olby, N. J., Shelton, G. D., Khan, S., O'Brien, D. P., Lindblad-Toh, K., & Coates, J. R. (2009). Genome-wide association analysis reveals a SOD1 mutation in canine degenerative myelopathy that resemblesnamyotrophic lateral sclerosis. *Proceedings of the National Academy of Sciences of the United States of America*, *106*(8), 2794–2799. https://doi.org/10.1073/pnas.0812297106

Bannasch, D., Safra, N., Young, A., Karmi, N., Schaible, R. S., & Ling, G. V. (2008). Mutations in the SLC2A9 gene cause hyperuricosuria and hyperuricemia in the dog. *PLoS Genetics*, *4*(11), 1000246. https://doi.org/10.1371/journal.pgen.1000246

Barnette, C. (n.d.). Muscular dystrophy. In *VCA Animal Hospital*. Retrieved April 14, 2021, from https://vcahospitals.com/know-your-pet/muscular-dystrophy

Berber, E. (2012). The molecular genetics of von Willebrand disease. In *Turkish Journal of Hematology* (Vol. 29, Issue 4, pp. 313–324). Galenos Yayinevi. https://doi.org/10.5505/tjh.2012.39205

Bley, T., Gaillard, C., Bilzer, T., Braund, K. G., Faissler, D., Steffen, F., Cizinauskas, S., Neumann, J., Vögtli, T., Equey, R., & Jaggy, A. (2002). Genetic aspects of labrador retriever myopathy. In *Research in Veterinary Science* (Vol. 73, Issue 3, pp. 231–236). W.B. Saunders. https://doi.org/10.1016/S0034-5288(02)00034-6

Bryant, E. H., & Meffert, L. M. (1993). The effect of serial founder-flush cycles on quantitative genetic variation in the housefly. *Heredity*, *70*(2), 122–129. https://doi.org/10.1038/hdy.1993.20

Charlesworth, B., & Charlesworth, D. (1999). The genetic basis of inbreeding depression. *Genetical Research*, *74*(3), 329–340. https://doi.org/10.1017/S0016672399004152

Cosgrove, L., Hammond, G., & McLauchlan, G. (2015). Primary portal vein hypoplasia and SLC2A9 mutation associated with urate urolithiasis in a Spanish water dog. *Canadian Veterinary Journal*, *56*(11), 1153–1157. /pmc/articles/PMC4608468/

Curtis, R., & Barnett, K. C. (1993). Progressive retinal atrophy in miniature longhaired dachshund dogs. *British Veterinary Journal*, *149*(1), 71–85. https://doi.org/10.1016/S0007-1935(05)80211-8

De Silva, R., Greenfield, J., Cook, A., Bonney, H., Vallortigara, J., Hunt, B., & Giunti, P. (2019). Guidelines on the diagnosis and management of the progressive ataxias. In *Orphanet Journal of Rare Diseases* (Vol. 14, Issue 1, pp. 1–10). BioMed Central. https://doi.org/10.1186/s13023-019-1013-9

Dekomien, G., Vollrath, C., Petrasch-Parwez, E., Boevé, M. H., Akkad, D. A., Gerding, W. M., & Epplen, J. T. (2010). Progressive retinal atrophy in Schapendoes dogs: mutation of the newly identified CCDC66 gene. *Neurogenetics*, *11*(2), 163–174. https://doi.org/10.1007/s10048-009-0223-z

Donner, J., Anderson, H., Davison, S., Hughes, A. M., Bouirmane, J., Lindqvist, J., Lytle, K. M., Ganesan, B., Ottka, C., Ruotanen, P., Kaukonen, M., Forman, O. P., Fretwell, N., Cole, C. A., &

Lohi, H. (2018). Frequency and distribution of 152 genetic disease variants in over 100,000 mixed breed and purebred dogs. *PLoS Genetics*, *14*(4), e1007361. https://doi.org/10.1371/journal.pgen.1007361

Downing, R. (n.d.). *Ichthyosis in Dogs*. VCA. Retrieved April 14, 2021, from https://vcahospitals.com/know-your-pet/ichthyosis-in-dogs

Downs, L. M., Wallin-Håkansson, B., Bergström, T., & Mellersh, C. S. (2014). A novel mutation in TTC8 is associated with progressive retinal atrophy in the golden retriever. *Canine Genetics and Epidemiology*, *1*(1), 1–12. https://doi.org/10.1186/2052-6687-1-4

FAO. (1998). Secondary guidelines for development of national farm animal genetic resources management plans: management of small populations at risk. *Food and Agricultural Organization*, 215. http://www.fao.org/3/a-w9361e.pdf

Fédération Cynologique Internationale. (2018). *Presentation of our organisation*. FCI. http://www.fci.be/en/Presentation-of-our-organisation-4.html

Forman, O. P., Boursnell, M. E. G., Dunmore, B. J., Stendall, N., Van De Sluis, B., Fretwell, N., Jones, C., Wijmenga, C., Rothuizen, J., Van Oost, B. A., Holmes, N. G., Binns, M. M., & Jones, P. (2005). Characterization of the COMMD1 (MURR1) mutation causing copper toxicosis in Bedlington terriers. *Animal Genetics*, *36*(6), 497–501. https://doi.org/10.1111/j.1365-2052.2005.01360.x

Frankham, R., Ballou, J., & Briscoe, D. (2004). *A primer of conservation genetics*. https://books.google.com/books?hl=nl&lr=&id=vvAfAwAAQBAJ&oi=fnd&pg=PT10&dq=a+primer+of+conservation+genetics&ots=LFsLJTdJo9&sig=EVfyooh48Va4Wtf2Bz4QMntfkf8

García-Martinez, J. D., Rivero, M. A., López-Albors, O., Arencibia, A., Vázquez, J. M., Ayala, I., & Gil, F. (2012). Late onset centronuclear myopathy with severe atrophy in an adult Labrador retriever. In *Journal of Applied Animal Research* (Vol. 40, Issue 1, pp. 69–72). https://doi.org/10.1080/09712119.2011.628237

Gentilini, F., Zambon, E., Gandini, G., Rosati, M., Spadari, A., Romagnoli, N., Turba, M. E., & Gernone, F. (2011). Frequency of the allelic variant of the ptpla gene responsible for centronuclear myopathy in Labrador retriever dogs as assessed in Italy. *Journal of Veterinary Diagnostic Investigation*, *23*(1), 124–126. https://doi.org/10.1177/104063871102300122

Gortel, K., Houston, D. M., Kuiken, T., Fries, C. L., & Boisvert, B. (1996). Inherited myopathy in a litter of Labrador retrievers. *Canadian Veterinary Journal*, *37*(2), 108–110. https://www.ncbi.nlm.nih.gov/pmc/articles/PMC1576622/

Greco, D. S. (2019). *Juvenile-onset Panhypopituitarism in Dogs - Endocrine System - Veterinary Manual*. https://www.msdvetmanual.com/endocrine-system/the-pituitary-gland/juvenile-onset-panhypopituitarism-in-dogs

Guaguere, E., Thomas, A., Grall, A., Bourrat, E., Lagoutte, L., Degorce-Rubiales, F., Hitte, C., Bensignor, E., Fontaine, J., Pin, D., Queney, G., & Andre, C. (2013). Autosomal Recessive Ichthyosis in Golden Retriever Dogs: Distribution and Frequency of the Pnpla1 Mutant Allele in Different Populations. In *Advances in Veterinary Dermatology* (pp. 82–84). https://doi.org/10.1002/9781118644317.ch11

Hasselgren, C. (2013). *Registration figures worldwide – from top thirty to endangered breeds*. FCI International. https://dogwellnet.com/content/population-statistics/general-breeds-specific-disease-inform/information-on-breeding-animals/registries-health-information/registration-figures-worldwide-–-from-top-thirty-to-endangered-breeds-r292/

Hedrick, P. W., & Garcia-Dorado, A. (2016). Understanding Inbreeding Depression, Purging, and

Genetic Rescue. In *Trends in Ecology and Evolution* (Vol. 31, Issue 12, pp. 940–952). Elsevier Ltd. https://doi.org/10.1016/j.tree.2016.09.005

Hunter, T. (n.d.-a). *Degenerative Myelopathy in Dogs*. VCA Animal Hospital. Retrieved April 13, 2021, from https://vcahospitals.com/know-your-pet/degenerative-myelopathy-in-dogs

Hunter, T. (n.d.-b). *Narcolepsy in Dogs*. VCA Animal Hospital. Retrieved April 14, 2021, from https://vcahospitals.com/know-your-pet/narcolepsy-in-dogs

Kehl, A., Haaland, A. H., Langbein-Detsch, I., & Mueller, E. (2021). A sine insertion in F8 gene leads to severe form of hemophilia a in a family of rhodesian ridgebacks. *Genes*, *12*(2), 1–8. https://doi.org/10.3390/genes12020134

Kramer, J. W., Venta, P. J., Klein, S. R., Cao, Y., Schall, W. D., & Yuzbasiyan-Gurkan, V. (2004). A von willebrand's factor genomic nucleotide variant and polymerase chain reaction diagnostic test associated with inheritable type-2 von willebrand's disease in a line of german shorthaired pointer dogs. *Veterinary Pathology*, *41*(3), 221–228. https://doi.org/10.1354/vp.41-3-221

LABOKLIN. (n.d.). *Sensory Neuropathy (SN)*. LABOKLIN (UK)| Genetic Diseases | Dogs|. Retrieved April 14, 2021, from https://www.laboklin.co.uk/laboklin/showGeneticTest.jsp?testID=8596&testID=8596

LICG. (2019). *PETscan maakt gerichte aanpak van erfelijke ziektes bij hond en kat mogelijk*. LICG. https://www.licg.nl/nieuws/petscan-maakt-gerichte-aanpak-van-erfelijke-ziektes-bij-hond-en-kat-mogelijk/

Llera, R., & Yuill, C. (n.d.). *Progressive Retinal Atrophy in Dogs | VCA Animal Hospital*. Medical Conditions, Pet Services. Retrieved April 14, 2021, from https://vcahospitals.com/know-your-pet/progressive-retinal-atrophy-in-dogs

Lobo, I. (2008). Mendelian ratios and lethal genes. *Nature Education*, *8*(6), 437–449. https://www.nature.com/scitable/topicpage/mendelian-ratios-and-lethal-genes-557/

Maurer, M., Mary, J., Guillaud, L., Fender, M., Pelé, M., Bilzer, T., Olby, N., Penderis, J., Shelton, G. D., Panthier, J. J., Thibaud, J. L., Barthélémy, I., Aubin-Houzelstein, G., Blot, S., Hitte, C., & Tiret, L. (2012). Centronuclear Myopathy in Labrador Retrievers: A Recent Founder Mutation in the PTPLA Gene Has Rapidly Disseminated Worldwide. *PLoS ONE*, *7*(10). https://doi.org/10.1371/journal.pone.0046408

Meffert, L. M. (1999). How speciation experiments relate to conservation biology. *BioScience*, *49*(9), 701–711. https://doi.org/10.2307/1313594

Nikolovski, G., & Atanaskova, E. (2010). Degenerative Myelopathy in Dogs. *Macedonian Veterinary Review*, *33*(1), 33–37.

Oji, V., Tadini, G., Akiyama, M., Blanchet Bardon, C., Bodemer, C., Bourrat, E., Coudiere, P., Digiovanna, J. J., Elias, P., Fischer, J., Fleckman, P., Gina, M., Harper, J., Hashimoto, T., Hausser, I., Hennies, H. C., Hohl, D., Hovnanian, A., Ishida-Yamamoto, A., … Traupe, H. (2010). Revised nomenclature and classification of inherited ichthyoses: Results of the First Ichthyosis Consensus Conference in Sorze 2009. *Journal of the American Academy of Dermatology*, *63*(4), 607–641. https://doi.org/10.1016/j.jaad.2009.11.020

OMIA. (2020). Omia: Online Mendelian Inheritance in Animals. In *The University of Sydney*. https://www.omia.org/home/

Oost, B. A., Versteeg, S. A., & Slappendel, R. J. (2004). DNA Testing for Type III von Willebrand Disease in Dutch Kooiker Dogs. *Journal of Veterinary Internal Medicine*, *18*(3), 282–288.

https://doi.org/10.1111/j.1939-1676.2004.tb02546.x

Orr, H. A. (2005). The genetic theory of adaptation: A brief history. In *Nature Reviews Genetics* (Vol. 6, Issue 2, pp. 119–127). Nature Publishing Group. https://doi.org/10.1038/nrg1523

Palma, D., Langston, C., Gisselman, K., & McCue, J. (2013). Canine struvite urolithiasis. *Compendium: Continuing Education For Veterinarians*, *35*(8). http://vetfolio-vetstreet.s3.amazonaws.com/7d/07b8d0bd6911e28e71005056ad4736/file/PV2013_Palma_CE.pdf

Petersen-Jones, S. M. (1998). A review of research to elucidate the causes of the generalized progressive retinal atrophies. *Veterinary Journal*, *155*(1), 5–18. https://doi.org/10.1016/S1090-0233(98)80028-2

Pfahler, S., Bachmann, N., Fechler, C., Lempp, C., Baumgärtner, W., & Distl, O. (2014). Degenerative myelopathy in a SOD1 compound heterozygous Bernese mountain dog. In *Animal Genetics* (Vol. 45, Issue 2, pp. 309–310). https://doi.org/10.1111/age.12118

Primack, R. B. (1995). A primer of conservation biology. In *Cambridge University Press*. https://doi.org/10.2307/2404967

Seaman, R., & Bartges, J. W. (2001). Canine Struvite Urolithiasis. *Compendium on Continuing Education for the Practicing Veterinarian*, *23*(5), 407–420. http://assets.prod.vetlearn.com.s3.amazonaws.com/mmah/8d/0f26d123d84ac4af5deccff59f23cc/filePV_23_05_407.pdf

Swindell, W. R., & Bouzat, J. L. (2006). Selection and inbreeding depression: effects of inbreeding rate and inbreeding environment. *Evolution*, *60*(5), 1014. https://doi.org/10.1554/05-493.1

Tamamoto-Mochizuki, C., Banovic, F., Bizikova, P., Laprais, A., Linder, K. E., & Olivry, T. (2016). Autosomal recessive congenital ichthyosis due to PNPLA1 mutation in a golden retriever–poodle cross-bred dog and the effect of topical therapy. *Veterinary Dermatology*, *27*(4), 306-e75. https://doi.org/10.1111/vde.12323

Venta, P. J., Li, J., Yuzbasiyan-Gurkan, V., Brewer, G. J., & Schall, W. D. (2000). Mutation Causing von Willebrand's Disease in Scottish Terriers. *Journal of Chemical Information and Modeling*, *14*(1), 10–19.

Wag. (n.d.-a). *Congenital Stationary Night Blindness in Dogs*. Retrieved April 14, 2021, from https://wagwalking.com/condition/congenital-stationary-night-blindness

Wag. (n.d.-b). *Epidermolysis Bullosa in Dogs*. Retrieved April 14, 2021, from https://wagwalking.com/condition/epidermolysis-bullosa

Wag. (n.d.-c). *Hereditary Nephritis (Samoyed) in Dogs*. Retrieved April 14, 2021, from https://wagwalking.com/condition/hereditary-nephritis-samoyed

Windig, J. J., & Hulsegge, I. (2021). Retriever and pointer: Software to evaluate inbreeding and genetic management in captive populations. *Animals*, *11*(5), 1–21. https://doi.org/10.3390/ani11051332

Zeiler, G. E., Van der Zwan, H., & Oosthuizen, M. C. (2013). Genetic testing of canine degenerative myelopathy in the south african boxer dog population. *Journal of the South African Veterinary Association*, *84*(1), 5. https://doi.org/10.4102/jsava.v84i1.1005

Appendix

Annex to MSc thesis – Data management plan

Data management plan belonging to the MSc thesis performed at the Animal Breeding and Genomics Group by Paulette Nieuwenhuis, completed in October 2021.

Agreements

1. The data used in this thesis project have been described in this document and have been stored in a systematic manner (at least in separate folders for all sections as described below). Data includes all data as mentioned in the results section of your report.
2. The data management plan has been discussed with the MSc thesis supervisor and he/she has agreed on the location for data storage.
3. In case of confidentiality, contact details of the responsible person from the company/institution that has ownership of the data are mentioned in this document.
4. **The data can be found in/on/through Jack Windig.**

Section A - Raw data

File names	Received from	On date
Practical wildlife conservation genetics	Jack Windig	2021

Comments:

This was a file with input data about the four breeds.

Section B – Data analysis (e.g. script files)

Mention here the (script) files you used for the analysis of your data

File names	Created in (month, year)	Remarks
Markiesje	2021	There are two files with kinship and without kinship
Golden Retriever	2021	There are two files with kinship and without kinship
Saarloos	2021	There are two files with kinship and without kinship
Friese stabij	2021	There are two files with kinship and without kinship

Section C – Final data

All data files that were used for the Results section of your report.

File names	Created in (month, year)	Remarks
Resultaten eind	2021	