Pedigree analysis and optimisation of the breeding programme of the Markiesje and the Stabyhoun

Aiming to improve health and welfare and maintain genetic diversity

Harmen Doekes

REG.NR.: 920809186050

Major thesis Animal Breeding and Genetics (ABG-80436)

January, 2016

Supervisors/examiners:

Piter Bijma - Animal Breeding and Genomics Centre, Wageningen University
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Thesis: Animal Breeding and Genomics Centre

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Commissioned by:

Nederlandse Markiesjes Vereniging
Nederlandse Vereniging voor Stabij- en Wetterhounen

Preface

This major thesis is submitted in partial fulfilment of the requirements for the degree of Master of Animal Sciences of Wageningen University, the Netherlands. It comprises an unpublished study on the genetic status of two Dutch dog breeds, the Markiesje and the Stabyhoun. that was commissioned by the Breed Clubs of the breeds, the 'Nederlandse Markiesjes Vereniging' and the 'Nederlandse Vereniging voor Stabij- en Wetterhounen'. It was written for readers with limited pre-knowledge. Although the thesis focusses on two breeds, it addresses issues that are found in many dog breeds.

The study was conducted in 2015 at the chair group Animal Breeding & Genetics of Wageningen University, under supervision of Kor Oldenbroek and Jack Windig. I would like to thank my supervisors for their useful feedback, technical support and communication with the Breed Clubs and other relevant contacts. I am furthermore grateful to the various people of the Breed Clubs who provided me with the breeds' studbooks and adequately answered my questions.

Throughout the execution of the research project I have developed my general research skills. An example of such a skill is independent decision making. Although my supervisors steered the direction of the project, I was largely free to decide on what to focus (which was both a challenge and a useful experience). In addition to developing my general research skills, I have enlarged my knowledge of population genetics and have gained insight into the world of pedigree dogs.

I sincerely hope that the results of this study can support the Breed Clubs in optimising their breeding programmes and thereby maintain genetic diversity and improve the animal health and welfare in both breeds.

Wageningen, January 2016
Harmen Doekes

Contents

Summary

The strong artificial selection pressure and the management in (small) closed populations has led to a considerable amount of inbreeding and loss of genetic diversity in many dog breeds, which has resulted in the manifestation of inherited disorders. In this study, two of the nine original Dutch dog breeds, the Markiesje (studbook since 1970s) and the Stabyhoun (studbook since 1940s), were extensively analysed with the aim to formulate breeding recommendations that can support the Breed Clubs in maintaining genetic diversity and improving health and welfare in their breeds. First, a pedigree analysis was performed in which general population parameters and parameters related to genetic diversity were calculated. Second, the prevalence of inherited disorders found in the breeds was estimated and potential risk factors for these disorders were identified. Last, the effectiveness of various breeding strategies on reducing the inbreeding rate was evaluated using computer simulations.

The Markiesje is a small breed with 120 pups born per year. In the Markiesje population, 26.2% of the males and 36.7% of the females were selected for breeding. The average litter size was 4.2 pups and the generation interval 3.4 years. The Stabyhoun is a larger breed with 700 pups born per year. In the Stabyhoun population, 7.8% of the males and 17.8% of the females were selected for breeding. The average litter size was 6.3 pups and the generation interval 4.4 years.

Respectively 13% and 33% of the genetic diversity originally present in the 54 founders of the population Markiesjes and the 30 founders of the population Stabyhouns has been lost. In general, both breeds have had an unacceptably high risk on accumulating inherited disorders, with inbreeding rates per generation exceeding 1% (effective population size < 50). The inbreeding and coancestry rates were, however, found to be decreasing in both breeds. The effective number of founders and the founder genome equivalent of respectively 14.6 and 4.1 for the Markiesje and 6.3 and 1.5 for the Stabyhoun, also indicated a large loss of genetic diversity in both breeds through an unequal contribution of founders and genetic bottlenecks. In the Markiesje, two influential founders were identified whose genes were together responsible for 31% of the gene pool, 46% of the average inbreeding coefficient and 44% of the average coancestry of Markiesjes born in 2010-2015. In the Stabyhoun, five influential founders were identified whose genes were together responsible for 86% of the gene pool, 95% of the average inbreeding coefficient and 95% of the average coancestry of Stabyhouns born in 2010-2015. Three inherited disorders were identified in the Markiesje: patellar luxation (PL; estimated prevalence of 21.7%), progressive rod-cone degeneration (PRCD; 1.4%), and a neuropathology (0.8%). PL is polygenic and PRCD is monogenic autosomal recessive. The neuropathology is assumed to be monogenic autosomal recessive. Six inherited disorders were identified in the Stabyhoun: hip dysplasia (HD; 20.8%), elbow dysplasia (ED; 18.8%), epilepsy (0.9%), persistent ductus arteriosus (PDA; 0.8%), cerebral dysfunction (CD; 0.6%), and von Willebrand Disease type-I (vWD-I; 69.8%). HD, ED, epilepsy and PDA are polygenic disorders, whereas CD and vWD-I are monogenic autosomal recessive. Simulations showed that the sire breeding restrictions applied by the Breed Clubs are effective in limiting the inbreeding rate, especially in the Stabyhoun (for which a popular sire effect was present). Enlarging the number of breeding individuals was in absolute numbers mostly effective in the small population of Markiesjes. Steering on relatedness, e.g. by minimising population coancestry, was shown to be very effective in reducing the inbreeding rate in both breeds.

The current population status of and genetic diversity in both breeds is far from ideal. In the case of the Stabyhoun, it is recommended to either steer systematically on relatedness or outcross with other breeds in order to reduce the (increase in) relatedness. In the case of the Markiesje, outcrossing with look-alikes is recommended to enlarge the population without causing a major increase in relatedness. Other breed-specific recommendations can be found in the conclusion of this report.

Samenvatting (Dutch summary)

De sterke artificiële selectiedruk en het fokken in (kleine) gesloten populaties heeft in veel hondenrassen geleid tot een hoog inteeltniveau, een lage genetische diversiteit en verschillende erfelijke aandoeningen. In deze studie zijn twee Nederlandse hondenrassen, het Markiesje (stamboek sinds de jaren zeventig) en de Stabij (stamboek sinds de jaren veertig), geanalyseerd met als doel tot aanbevelingen te komen die de rasverenigingen kunnen bijstaan in het behouden van de genetische diversiteit en het verbeteren van het dierenwelzijn in de rassen. Eerst is een stamboekanalyse uitgevoerd, waarbij algemene kengetallen en parameters met betrekking tot genetische diversiteit zijn berekend. Daarna is de prevalentie van erfelijke aandoeningen in de rassen geschat. Als laatste is het effect van diverse fokstrategieën op de inteelttoename in kaart gebracht met computersimulaties.

Het Markiesje is een klein ras met jaarlijks ongeveer 120 geboren pups. Meer dan een kwart (26,2%) van de mannetjes en meer dan een derde (36,7%) van de vrouwtjes werd tot nu toe geselecteerd voor de fok. De gemiddelde worpgrootte was 4,2 pups en het generatie-interval 3,4 jaar. De Stabij is een groter ras met ongeveer 700 pups per jaar. Rond een dertiende (7,8%) van de mannetjes en een zesde (17,8%) van de vrouwtjes werd tot nu toe geselecteerd voor de fok. De gemiddelde worpgrootte was 6,3 pups en het generatie-interval 4,4 jaar. Van de genetische diversiteit die aanwezig was in de 54 basisdieren in het Markiesje en de 30 basisdieren in de Stabij is respectievelijk 13% en 33% verloren gegaan. Gedurende het overgrote deel van de tijd dat de rassen bestaan is de inteelttoename per generatie in beide rassen ver boven de 1% geweest (effectieve populatiegrootte < 50), een waarde die als ondergrens wordt gebruikt om aan te geven dat een ras een onaanvaardbaar hoog risico loopt op de accumulatie van erfelijke aandoeningen. De inteelttoename en verwantschapstoename nemen echter wel af in de rassen. Het effectieve aantal basisdieren en het genoom equivalent waren respectievelijk 14,6 en 4,1 voor het Markiesje en 6,3 en 1,5 voor de Stabij, wat aangeeft dat een groot deel van de genetische diversiteit verloren is gegaan door een onevenredige bijdrage van de basisdieren en door genetische flessenhalzen. In het Markiesje zijn twee basisdieren gevonden die samen verantwoordelijk zijn voor 31% van de genenpoel, 46% van de gemiddelde inteeltcoëfficiënt en 44% van de gemiddelde verwantschap van de Markiesjes geboren in 2010-2015. In de Stabij zijn vijf basisdieren gevonden die samen verantwoordelijk zijn voor 86% van de genenpoel, 95% van de gemiddelde inteeltcoëfficiënt en 95% van de gemiddelde verwantschap van de Stabijs uit 2010-2015. Drie erfelijke aandoeningen zijn geanalyseerd in het Markiesje: patella luxatie (PL; geschatte prevalentie van 21,7%), progressieve staafjes-kegeltjes degeneratie (PRCD; 1,4%) en een neuropathologie (0,8%). PL is polygeen en PRCD is monogeen autosomaal recessief. De oorzaak van de neuropathologie is onbekend maar het wordt verondersteld dat deze aandoening ook monogeen autosomaal recessief is. In de Stabij zijn zes erfelijke aandoeningen geanalyseerd: heupdysplasie (HD; 20.8%), elleboogdysplasie (ED; 18.8%), epilepsie (0.9%), persisterende ductus arteriosus (PDA; 0.8%), cerebrale dysfunctie (CD; 0.6%) en de von Willebrand ziekte type-I (vWD-I; 69.8%). HD, ED, epilepsie en PDA zijn polygeen en CD en vWD-I zijn monogeen autosomaal recessief. Met behulp van computersimulaties is laten zien dat de huidige fokrestricties van de rasverenigingen effectief zijn in het beperken van de inteelttoename, met name in de Stabij (waarbinnen geregeld populaire reuen overmatig zijn gebruikt). Het vergroten van de fokpopulatie was in absolute aantallen vooral effectief in de kleinere populatie van Markiesjes. Sturen op verwantschap was zeer effectief in het beperken van de inteelttoename in beide rassen.

Concluderend is de huidige populatiestatus van en de genetische diversiteit in beide rassen verre van optimaal. Aan de rasvereniging van de Stabij wordt aangeraden om ofwel consequent op verwantschap te sturen ofwel systematisch uit te kruisen met andere rassen. Dit om de (toename) in de verwantschap te verminderen. Aan de rasvereniging van het Markiesje wordt aangeraden om door te gaan met het inkruisen van look-alikes en de populatie zo te laten groeien, zonder dat hierbij de verwantschap sterk toeneemt. Overige aanbevelingen zijn te vinden in de conclusie van dit rapport.

1. Introduction

The Markiesje and the Stabyhoun are two of the nine existing original Dutch dog breeds. Although both breeds are thought to descend from the Spioen - a small spaniel type hunting dog that was already present in West-Europe in the Middle Ages - they were not purebred until the 20th century. Since the foundation of the Stabyhoun Breed Club ('Nederlandse Vereniging voor Stabij- en Wetterhounen') in 1947 and the Markiesjes Breed Club ('Nederlandse Markiesjes Vereniging') in 1979, both rare breeds have developed as (semi-) closed populations and approximately 2,500 Markiesjes and 16,700 Stabyhouns have been registered in the Breed Clubs' studbooks.

During the last decades the interest in purebred dogs and the knowledge and awareness of their genetic health problems has increased tremendously. This has led to various breeding-initiatives, both on the (inter)national level and within breed clubs. As a result of the interest of the Markiesjes and the Stabyhoun breed clubs in the genetic health of their breeds, it was decided to perform a comprehensive pedigree analysis and evaluate breeding strategies for both populations. The results of these analyses are presented in the current report.

In the introduction an extensive theoretical framework is provided. In the first paragraphs background information is given on the historical processes of dog domestication, breed development and the emergence and prevention of genetic disorders. Subsequently, the Markiesje and the Stabyhoun are introduced. The chapter concludes with the aim and research questions of the study.

1.1. Dog domestication and the development of breeds

The grey wolf (*Canis lupus*) is known to be the closest modern relative of the domestic dog (*Canis familiaris*), with less than 0.2% difference in their mtDNA-sequence and the ability to interbreed (Wayne, 1993). Although it is widely supported that dogs descend from the wolf lineage, the origin of dog domestication is still a large matter of debate. Genomic and archaeological studies estimate the divergence time between dogs and wolfs variably from 9,000 to over 33,000 years ago and mention China, Israel and Croatia as likely domestication centres (Pollinger et al., 2010; Thalmann et al., 2013). However, recent sequencing of wolf genomes does not support any of the wolf lineages in these three hypothesized regions as being the source lineage (Freedman et al., 2014). Instead, this finding suggests that either the diversification in the modern grey wolf populations took place after dogs were domesticated or that the wild ancestors of dogs have gone extinct. The latter theory is supported by Skoglund et al., who sequenced the genome of a 35,000-year-old Taimyr wolf and found that this individual diverged from the modern wolf around the same time as the wolf and dog lineages split up (Skoglund et al., 2015). Skoglund et al. also suggest that the modern dog is derived from multiple wolf populations, so via multiple domestication events. This suggestion is based on evidence of introgression of Taimyr wolves with modern arctic breeds, as is graphically depicted in figure 1. The likely extinction of the dog's wild ancestors, the admixture between the wolf and dog lineages and the occurrence of population bottlenecks in both lineages make it very difficult to determine the exact time and origin of domestication (Freedman et al., 2014).

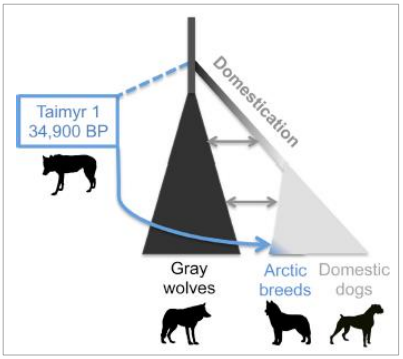

Figure 1. *Trifurcation of canine lineages (Skoglund et al., 2015)*

Irrespective of the exact time and location of domestication, there is no other animal species that has lived in such a close association with humans over so many millennia as the dog (Parker et al., 2004). During this time, dogs have adopted and fulfilled numerous roles in society, varying from hunting and herding sheep to providing companionship. At the same time, the dog has become the most

morphologically diverse mammalian species, with enormous variation in e.g. size, coat colour and coat length (Boyko et al., 2010). Especially since the 19th century many breeds, i.e. uniform groups with common ancestors and distinguishable and strictly defined physical and behavioural characteristics, have been developed through artificial selection. At the same time, breed clubs were formed and breed standards, describing a breed's ideal individual, were set up (Parker, 2012). Although most purebred dogs are nowadays kept as companion animals, many breed standards still contain elements of the original working purpose of the breed. An example is the Kooiker dog that was originally used for luring and driving ducks into special cages and for which energetic, endurance, fast and fierce are still keywords in the behavioural description of its breed standard (VHNK, 2015).

By the beginning of the 20th century, national canine organisations like the UK Kennel Club, the American Kennel Club and the Dutch Kennel Club ('Raad van Beheer op Kynologisch gebied Nederland') were formed. These organisations are generally responsible for the national breeding policy and registration of purebred dogs. In 1911 an international federation of kennel clubs, the 'Fédération Cynologique Internationale' (FCI), was founded. The FCI currently includes 91 member states and recognizes 343 distinct breeds, which are classified in 10 groups based on similarities in appearance, personality and their role in society (Oldenbroek & Windig, 2012; FCI, 2015). Over a 1,000 distinct breeds can be distinguished if unrecognized breeds are included (Morris, 2002).

1.2. The emergence of inherited disorders

During the process of artificial selection, that created the possibility for an owner to choose his or her ideal dog type, two major (genetic) health issues have emerged. These issues manifest themselves in the form of inherited disorders and have gained more and more attention in the (Dutch) media and society, especially since documentaries by the BBC, Zembla and Radar (BBC One, 2008; Zembla, 2010; Radar, 2011). The social relevance of the topic is furthermore indicated by the large number of dogs and dog owners. In the Netherlands there are 1.5 million dogs, of which a third is purebred, and one out of the five households owns one or more dogs (Raad van Beheer, 2014). About 40% of the Dutch purebred dogs is thought to suffer from a genetic disorder during the first half of their life (Gubbels, 2012). The Dutch opinion towards pedigree dogs is ambivalent, not only between but also within individuals. About 95% of the Dutch owners of animals with a risk of breed-related welfare problems will purchase the same breed or species again and 75% will recommend it to others (Pompe et al., 2013). Despite the public wish for animal welfare to be the top priority, there is a demand for dogs with a recognizable and predictable appearance and behaviour (Raad van Beheer, 2014).

The two types of inherited disorders that can be distinguished are those related to the breed standard (conformation related) and those not directly related to the breed standard (non-conformation related). In 2009-2010 a total of 84 conformation related and 312 non-conformation related inherited disorders were identified in the top 50 UK Kennel Club registered breeds (Asher et al., 2009; Summers et al., 2010). Currently, in 2016, a total of 661 inherited disorders - both conformation related as unrelated - have been described in the dog-section of the Online Mendelian Inheritance in Animals database. Of these disorders roughly 40% is reported to follow the Mendelian inheritance pattern (OMIA, 2016).

Type I: conformation related inherited disorders

Conformation related inherited disorders are the result of an extreme selection on appearance-based criteria. The related characteristics are called harmful breed characteristics. Breed characteristics are considered harmful when individuals of the breed are brought to a veterinarian, behaviour therapist or other specialist in order to treat the characteristic and/or associated problems (Raad van Beheer, 2014). An example of a harmful breed characteristic is an extremely shortened skull, which leads to brachycephalic airway obstructive syndrome in large proportions of some breeds (Fasanella et al., 2010). Another - less obvious - example is canine hip dysplasia (HD), i.e. the malformation of the hip

joint. This disorder is mostly found in large- and giant-breed dogs and the prevalence of HD positively correlates with the average weight and body-mass index (BMI) of breeds (Comhaire & Snaps, 2008; Witsberger et al., 2008). A high weight or BMI could therefore be seen as a harmful breed characteristic for HD.

Type II: non-conformation related inherited disorders

The manifestation of non-conformation related disorders is the result of evitable and inevitable inbreeding and the associated loss of genetic diversity (see paragraph 1.3). Every animal carries multiple harmful recessive alleles that are partly or completely masked by the present dominant alleles. As inbreeding enhances the probability to be homozygous for an allele (table 1), including the harmful allele, it leads to a higher proportion of affected individuals in the population (Charlesworth & Charlesworth, 2010). Examples of non-conformation related inherited disorders are progressive retinal atrophy (PRA) and von Willebrand's disease (vWD) (Summers et al., 2010).

Table 1. *Change in Hardy-Weinberg genotype frequencies due to inbreeding (Charlesworth & Charlesworth, 2010)*

	Frequency*	
Genotype	Outbred population	Inbred population
Homozygous normal (AA)	p^2	$p^2 + Fpq$
Heterozygous (Aa)	2pq	2pq (1-F)
Homozygous mutant allele (aa)	q^2	$q^2 + Fpq$

* *p* is the normal (dominant) allele frequency, *q* the mutant (recessive) allele frequency and *F* the average inbreeding coefficient (see paragraph 1.3).

1.3. The dog genome, genetic diversity, inbreeding and coancestry

With approximately 2.4 billion base pairs and 20,000 genes the size of the dog genome is similar to that of humans and other mammals. The number of chromosomes, 38 autosomal chromosome pairs and one pair of sex chromosomes, is higher than in humans (Ensembl, 2015).

Genetic diversity is the variation in genomes of individuals in a population. A commonly used measure for genetic diversity is the gene diversity (GD), which reflects the number of alleles that are present at a locus and the evenness with which these alleles occur in the population. It can be calculated for a single locus as (Lacy, 1995):

$$GD = 1 - \sum({p_i}^2)$$

where p_i is the frequency of allele *i* and the summation is over all alleles present in the population. To get a genome-wide measure of GD it can be averaged over all loci. In large populations with a low level of relatedness the GD approaches 1.

A different approach is to look at the variation on the DNA-sequence level, using the nucleotide diversity. A population's nucleotide diversity is the average probability that two randomly picked nucleotides at a site differ in state (Charlesworth & Charlesworth, 2010). Parker et al. showed that the total human and dog populations share a similar nucleotide diversity of 8×10^{-4}. They also estimated that more than 27% of the total genetic variation within dogs can be accounted for by variation among breeds, compared with 5-10% among human populations (Parker et al., 2004). This gives an indication of the substantial loss of genetic diversity within dog breeds.

There are four evolutionary forces that are well known to influence genetic diversity:

1. *Mutation.* Mutations in germ cells increase genetic diversity. Although the mutation rate in dogs is an order of magnitude higher than in humans, with reported mutation frequencies for

microsatellites ranging from 1.1 x 10^{-2} to 3.9 x 10^{-3} per locus per generation (Parra et al., 2010), this evolutionary force is relatively weak. Harmful mutations are generally lost via negative selection and new beneficial or neutral mutations are often lost via drift before they get the chance to increase in frequency (Charlesworth & Charlesworth, 2010).

2. *Migration*. Gene flow, i.e. the transfer of alleles through migration, can either increase genetic diversity via immigration or decrease it via emigration. Migration between different dog breeds is referred to as outcrossing. For migration to occur within a breed there has to be population stratification, either in the form of breeding sublines or of geographically separated subpopulations. Subpopulations typically have their own allele frequencies and the level and direction of migration between the subpopulations can strongly influence the genetic diversity in the metapopulation (Charlesworth & Charlesworth, 2010; Oldenbroek & Windig, 2012).
3. *Selection*. The biased contribution of parental genotypes to the offspring's gene pool, as a direct result of selection, causes a loss of genetic diversity. Selection is primarily artificial in dog breeds, rather than natural. The first major step of artificial selection occurs at the establishment of a new breed, when founders are selected. The number of founders used is often small, which causes a low initial genetic diversity. This phenomenon is referred to as the founder effect. Diversity is further lost through selective breeding during the development of the breed; only those individuals that conform well to the breed standard are used for breeding. Selective breeding not only involves enhancing the frequency of desirable alleles, but also reducing the frequency of undesirable traits, such as inherited disorders. A commonly seen phenomenon in selective breeding is the popular sire effect, in which a few popular sires are excessively used for breeding (Leroy, 2011; Oldenbroek & Windig, 2012). Another aspect that influences genetic diversity through selection, but is less often mentioned, is the (un)willingness of owners to breed with their dog.
4. *Random drift*. Random drift refers to the decrease of genetic diversity through the process of Mendelian sampling (Charlesworth & Charlesworth, 2010). As dog breeds often have a small census size and sometimes undergo genetic bottlenecks, they are strongly subjected to drift (Oldenbroek & Windig, 2012).

Inbreeding, i.e. the mating between relatives, is strongly related to genetic diversity. As illustrated in table 1, inbreeding causes a change in a population's genotype frequencies but does not influence the population's allele frequencies. In combination with selection and drift, however, it can change allele frequencies and result in loss of alleles (Charlesworth & Charlesworth, 2010).

A certain extent of inbreeding is inevitable in small closed populations like dog breeds. This inevitable inbreeding, together with the deliberate inbreeding that is used to breed individuals that conform to the breed standard, causes the manifestation of inherited disorders. As many reproductive- and health-related traits are optimal in individuals that are heterozygous for the genes underlying these traits, inbreeding also leads to a reduced fitness: a phenomenon known as inbreeding depression (Charlesworth & Charlesworth, 2010; Oldenbroek & Windig, 2012).

Inbreeding is quantified with the inbreeding coefficient (F_i), which is the probability that two alleles present at an arbitrary locus in individual *i* are derived from the same ancestral allele, i.e. that they are identical by descent (IBD). Inbreeding is the direct result of relatedness between parents, which is quantified with the coefficient of coancestry (f_{ij}), also known as the coefficient of kinship. This coefficient is defined as the probability that an allele selected randomly from an autosomal locus in individual *i* and an allele selected randomly from the same autosomal locus of individual *j* are IBD. The f_{ij} equals the F of a hypothetical offspring of individual *i* and *j*. In a population under constant selection and with random mating, the average inbreeding of one generation therefore equals the

average coancestry of the previous generation ($\bar{F}_t = \bar{f}_{t-1}$). The change in both parameters is also equal in this situation ($\Delta\bar{F} = \Delta\bar{f}$). A faster increase in $\bar{F}$ than in $\bar{f}$ might indicate subpopulation stratification; within the subpopulations the $\bar{F}$ and $\bar{f}$ would increase collectively, but on the whole population level the $\bar{f}$ would stay constant. A faster increase in $\bar{f}$ than in $\bar{F}$ can occur when the least-related individuals are mated or for example only one sire is used for the whole new generation (Caballero & Toro, 2000; Oldenbroek & Windig, 2012).

Maintaining genetic diversity, combined with restricting inbreeding, is an important aspect in the management of captive and domesticated populations, as it is related to a population's adaptive potential and restricts inbreeding depression and the manifestation of inherited disorders (Ivy & Lacy, 2012; Oldenbroek & van der Waaij, 2015).

1.4. Prevention and restriction of inherited disorders

Regarding the prevention and restriction of conformation related disorders the focus should lie on a responsible breeding goal. Such a breeding goal should have health and life expectancy as leading criteria, followed by the breed's behavioural and appearance-based criteria. It should not focus on a single or just a few traits, but on the whole individual (Oldenbroek & Windig, 2012). In addition to a responsible breeding goal, monitoring and steering of genetic diversity is thought to be essential to restrict the manifestation of non-conformation related disorders (Lewis et al., 2010; Leroy & Rognon, 2012; Oldenbroek & Windig, 2012).

Monitoring of genetic diversity

Genetic diversity can be monitored on the basis of genealogical data. Figures commonly used for monitoring are the number of pups per generation, the number of sires and dams per generation, the distribution of pups over the parents/ancestors and the $\Delta\bar{f}$ and $\Delta\bar{F}$. A commonly used measure is the effective population size (N_e), which is defined as the number of reproducing individuals in an idealized population that leads to the same genetic diversity as in the population under study. The idealized population has various assumptions. First, there is an equal number of males and females. Second, all individuals have an equal probability to produce offspring and the number of offspring per individual only varies due to chance. Third, mating is random, so there is no selection. Last, the size of the breeding population is constant over time (Charlesworth & Charlesworth, 2010). The effective population size is classically based on the increase in homozygosity over time, using the following formula (Falconer et al., 1996):

$$N_e{}^{IBD} = \frac{1}{2\Delta IBD}$$

where the ΔIBD is traditionally the average rate of inbreeding $\Delta\bar{F}$, but can also be the average rate of coancestry $\Delta\bar{f}$ or the individual inbreeding or coancestry rate. Other estimators of N_e are for example based on the sex ratio or the variance in family size (Gutiérrez et al., 2008; Leroy et al., 2013). The classic formula for the estimation of N_e using the sex ratio is (Falconer et al., 1996):

$$Ne^S = \frac{4N_mN_f}{N_m + N_f}$$

where N_m and N_f are the number of reproducing males and females, respectively.

A different approach of measuring (the loss of) genetic diversity in a population is by looking at the probability of gene origin. This involves the calculation of founder equivalents and the computation of genetic contributions of ancestors/founders to the current gene pool, average inbreeding coefficient and average coancestry (Lacy, 1989; Caballero & Toro, 2000).

Steering of genetic diversity: the use of breeding strategies

Steering of genetic diversity in captive/domesticated populations is often performed on the basis of the $\Delta\bar{f}$, $\Delta\bar{F}$ and the associated effective population size (Ivy & Lacy, 2012). Effective population size thresholds of 50 and 500 have been proposed with regard to the risk on extinction of a population on respectively the short and long run (Leroy et al., 2013). According to the risk classification system of the Food and Agriculture Organisation (FAO), a livestock breed is critically endangered when the total number of breeding females is lower than 100 or the total number of breeding males is equal to or lower than 5, corresponding to an Ne^S of approximately 20. Such a breed is categorized as endangered when the total number of breeding females is lower than 1000 or the total number of breeding males is below or equals 20, corresponding to an Ne^S of approximately 80 (FAO, 2007).

In table 2 risk categories are distinguished for the occurrence of inherited disorders in dog breeds, which are based on the inbreeding rate and the associated inbreeding population size (Oldenbroek & Windig, 2012). For example, a ${N_e}^F$ of 100 ($\Delta\bar{F}$ of < 0.5%) is here considered as the minimum to prevent a high frequency of inherited disorders on the long term.

Table 2. *Risks of different inbreeding rates (directly translated from (Oldenbroek & Windig, 2012))*

Inbreeding rate ($\Delta\bar{F}$)	Risk on problems	Minimum no. of stud dogs*	Long-term expectancy
> 1%	Unacceptably high	< 25	Extinction of breed because of accumulation inherited disorders
0.5-1%	High	25-50	High frequency of inherited disorders
0.25-0.5%	Moderate	50-100	Inherited disorders do occur
< 0.25%	Small	> 100	Inherited disorders occur only occasionally

* Assuming that the number of breeding females is at least equal to the number of stud dogs and that the number of progeny that is selected for breeding is approximately equal for every stud dog.

Various breeding strategies have been described to restrict the inbreeding rate and limit the loss of genetic diversity. These strategies, which vary in effect, can be roughly divided in the following categories:

1. *Enlarging the effective population size.* This can be realized by using a larger proportion of the breed for breeding, using a more equal sex ratio, importing individuals from the same breed from abroad and by outcrossing (Oldenbroek & Windig, 2012). One should try to prevent bottlenecks, i.e. temporary small populations, as they can cause a severe loss in genetic diversity (Charlesworth & Charlesworth, 2010; Leroy & Baumung, 2011). The variation in the number of progeny among parents, which also reduces the N_e, can be managed for a large part using breeding restrictions.
2. *Setting breeding restrictions on individuals*. This includes restricting the number of litters per parent and restricting the number of offspring of a parent that may be used for breeding (Leroy & Baumung, 2011; Windig et al., 2014). Both methods prevent the excessive use of popular individuals for breeding, but the latter method is stricter as it takes the variation in the number of progeny per litter into account.
3. *Applying mating programs*. The optimum contribution method, first described by Meuwissen (Meuwissen, 1997), maximises genetic diversity in closed populations. It is based on estimated breeding values (EBV) of individuals adjusted for their average relationships. A second and more commonly used method is minimum coancestry mating, in which every dam is mated to the least related sire. Line breeding, which can be considered as a third mating program, is a type of deliberate inbreeding in which relatives (often cousins) are mated to fix certain characteristics. When enough lines are used the genetic diversity on the population

level can be maintained (Oldenbroek & Windig, 2012). A last mating program mentioned here is minimising population coancestry by excluding individuals from the breeding program that have a higher than average mean kinship with the rest of the breed (Windig et al., 2014).

Selecting and applying the best breeding strategy is limited by several factors. First, there are many independent decision makers, i.e. individual breeders, involved in dog breeding. This makes a fully controlled mating program like the optimal contribution method impracticable. Enlargement of the effective population size can also be limited by the existence of many independent owners (that might not want to breed with their dog) and could theoretically also be limited by a small demand for dogs of the breed. Last, the population parameters that are used to determine a breeding strategy fluctuate both between breeds and within a breed over time (Lewis et al., 2014). Therefore, the most appropriate strategy should be chosen based on the situation in a specific breed (Windig et al., 2014).

In addition to the mentioned strategies, health breeding programmes can be implemented to directly select against inherited disorders. Collins et al. introduced the Breed-Disorder Welfare Impact Scores (BDWIS), which is based on the prevalence and severity of a disorder and the average proportion of its life that an individual is afflicted by the disorder (Collins et al., 2011). The BDWIS can be used for setting the breeding priorities within the breeding goal of a breed.

Registration

Comprehensive registration of the ancestry of individuals is essential for monitoring and steering of genetic diversity. The total Dutch purebred dog population is registered in the national studbook called 'het Nederlands Honden Stamboek' (NHSB), which is managed by the Dutch Kennel Club and is internationally recognized. Within the NHSB three types of pedigree certificates are distinguished: those in the main studbook, those in the appendices of the main studbook and those in the provisional studbook. The provisional studbook is meant for growing (Dutch) breeds that are not yet recognized by the FCI (Raad van Beheer, 2015). For every registered individual the NHSB contains its NHSB-registration number, name, date of birth, its parents' registration numbers, colour, chip/tattoo number and - if provided – information about the breeder and owner. In addition to the NHSB, breed clubs generally have their own monitoring databases, in which they can include additional data from e.g. health screenings or club matches.

1.5. National initiatives to improve purebred dog health- and welfare

In recent years several national projects were started/executed with the aim to improve the health and welfare of Dutch purebred dogs.

In 2011-2013 the project *Relatedness and inbreeding in purebred dogs*, funded by the Dutch Kennel Club and the Ministry of Economic Affairs, was executed. As part of this project Wageningen University developed software that can be used to monitor and predict inbreeding and coancestry in breeds. This software is now used by the Dutch Kennel Club and will be made available to Breed Clubs to monitor their breeds and improve breeding programmes. The software is also used in the present research project. In addition to the developed software, a book on the breeding of purebred dogs with regard to inbreeding and coancestry was published in 2012 (Oldenbroek & Windig, 2012).

In 2013 a project commissioned by the Ministry of Economic Affairs started, with the aim to determine the incidence of conformation and non-conformation related inherited disorders in the dog and cat populations in the Netherlands. A pilot study with a few popular breeds formed the basis for the development of a database that will include health data of all the treated dogs in the Netherlands. Based on the results of analyses with this database, priorities can be set and diagnostic tests will be developed. The Faculty of Veterinary Medicine, Utrecht University, is currently developing this database (Expertisecentrum Genetica Gezelschapsdieren, 2014).

In 2014, the Dutch Kennel Club introduced the project plan *Fair-breed* ('Fairfok') with the aim to improve the health, welfare and behaviour of Dutch purebred dogs and thereby improve the public opinion on purebred dogs. One of the ambitions of this project is that the current pedigree certificate will become a quality certificate of the breeding process. In this way it will give insight in the breeder's effort to breed healthy individuals. Another ambition is to reduce the proportion of Dutch purebred dogs with a genetic disorder, from the current 40% to a maximum of 25% in 2019 and a maximum of 10% in 2024. Within the project plan concrete measures are described in order to realize the ambitions. One of these measures is the creation of a DNA-database. Since the summer of 2014 a DNA-sample is taken from all new-born purebred dogs to confirm the pups' ancestry. The same DNA can be used for large-scale studies on genetic disorders, hopefully leading to new diagnostic tests that can be used for selecting against these disorders (Raad van Beheer, 2014).

1.6. The Markiesje

The Markiesje, also known as the Dutch Tulip Hound, is the youngest recognized Dutch dog breed. Although their origin lies in the 17th and 18th century in France, Germany and the Netherlands (as is based on paintings; figure 2), Markiesjes were never purebred until the 1970s. In this decade a group of Dutch people started searching for dogs with a high resemblance to the Markiesje in the paintings. After some (unsuccesfull) experimental crossing with the Phalène, Papillon and Cavalier King Charles Spaniel the first purebred Markiesjes were born. Pom, a female look-alike imported from France, gave birth to three nests and became the ancestress of the breed. In 1979 the Markiesjes Breed club - Nederlandse Markiesjesvereniging in Dutch - was formed (Romijn & Dirkse, 2014). Since then, using outcrossing with an semi-open stud book, approximately 2,500 Markiesjes have been registered.

Figure 2. *'Marquise' de Pompadour with a Markiesje (Drouais, 1763)*

In 1999 the provisional studbook of the NHSB was opened for the Markiesje. The breed clubs aims for international recognition by the FCI in the future (Romijn & Dirkse, 2014).

Brief breed description

The Markiesje is a slender build Spioen with a height of 33-38 cm and a body length to height ratio of approximately 10 to 9. A typical body weight is in the range of 5-9 kg. According to the breed standard, the Markiesje is elegant and alert and should never be dwarfish. Its coat is semi-long, glossy and black and it has well-feathered ears, legs and tails. Up to 40% of its coat is allowed to be white, but only when the whiteness is on its chest, head (except the cheeks), neck, legs or tip of the tail. A Markiesje should be friendly, calm and intelligent and should not bark excessively nor show any signs of anxiousness or aggressiveness. Although not yet recognized, the breed would be classified in group 9 of the FCI, which contains companion and toy dogs (Morris, 2002; Romijn & Dirkse, 2014).

General breeding rules and the Breed Advisory Committee

In order to manage the breeding direction and maintain a healthy population there are the Breed Club's breeding rules, which are largely in line with - and sometimes slightly stricter than - the national breeding rules. The most important rules with regard to the present study, are (Nederlandse Markiesjesvereniging, 2015):

- A bitch may not be mated to her grandfather, father, son, grandson, brother nor half-brother.
- A mating between a dog and bitch may not be repeated, unless the number of progeny eligible for breeding is less than 3 or when the mating is desirable for health research.

- The minimal breeding age of dogs and bitches is 18 months. A bitch has a maximum mating age of 72 months if she did not gave birth to a litter before and of 96 months if she did gave birth before. A stud dog has no maximum mating age.
- A stud dog may be used unlimited for mating during a year, but with the restriction of maximal 5 successful litters in his entire life (successful means at least 1 progeny registered in the NHSB). A bitch may give birth to maximal 5 litters during her life. The restriction of 5 litters per parent was set around 2008 (van Ederen, 2015).

In addition to the breeding rules, the Breed Advisory Committee of the Breed Club gives breeding advice and checks upon new-born litters. When pups are one and a half years old, they are called to a young dog-examination, where it is decided if they are eligible for breeding (Romijn & Dirkse, 2014).

Inherited disorders in the Markiesje

A few inherited disorders have been identified in the Markiesje: patellar luxation (PL), the progressive rod-cone degeneration of progressive retinal atrophy (PRA-PRCD) and a neuropathological disorder.

Patellar luxation (PL)

PL was first observed in the breed in 2007. PL is a condition in which the kneecap dislocates from its normal position, leading to lameness. The disorder is multifactorial and has a moderate heritability: approximately 0.17 in Dutch Flat-Coated Retrievers and 0.27 in Kooiker dogs (Lavrijsen et al., 2013; Wangdee et al., 2014). A few loci have been associated with the disease (Lavrijsen et al., 2013; Pradit & Nganvongpanit, 2014).

PL is diagnosed via physical examination by a veterinarian. Four grades of PL are distinguished, as shown in the table below (Alam et al., 2007).

Table 3. *Grades and assessment criteria for patellar luxation (Alam et al., 2007)*

Grade	Assessment criteria
I	The patella can be manually dislocated but returns to its normal position when released.
II	The patella spontaneously luxates during standing/walking and remains luxated until manually replaced to the normal position.
III	The patella is permanently luxated but can be repositioned manually.
IV	The patella is permanently luxated and cannot be repositioned manually.

PL can be lateral and/or medial and can occur in one or both knee joints (Alam et al., 2007).

Progressive rod-cone degeneration (PRCD)

This form of progressive retinal atrophy (PRA) was first observed in the breed in 2010. In PRCD the light receptors in the retina degenerate, leading to a reduced vision and eventually to complete blindness. The disorder is monogenic, autosomal recessive and is caused by a homozygous mutation in the second codon of a protein-coding gene on dog chromosome 9. The dog PRCD gene is orthologous to the human PRCD gene on chromosome 17, for which various sequence variations are known to be associated to retinal degeneration in humans (Zangerl et al., 2006). PRA was found in 38 of the 50 most popular dog breeds in the UK and was thereby the third most common disorder across these breeds (Summers et al., 2010). There is no known treatment for PRCD (Romijn & Dirkse, 2014).

Neuropathological disorder

In 2003 a unknown neuropathology was observed in the breed. Affected pups develop a spastic paresis, have difficulty with walking and have to be euthanized. Utrecht University is currently investigating the genetic cause of the disease. The disorder is thought to be monogenic autosomal recessive (Mandigers, 2015).

Management directed against inherited disorders

To limit the propagation of the abovementioned inherited disorders, the Markiesjes Breed Club has implemented health screenings and disorder-specific breeding rules (table 4).

Table 4. *Overview of inherited disorders and related health screenings/breeding rules in the Markiesje*

Disorder	Putative mode of inheritance	Health screenings	Breeding rules
Patellar luxation (PL)	Multifactorial	PL-examination	Only "free x free" and "free x grade1" are allowed
Progressive rod-cone degeneration (PRCD)	Monogenic, autosomal recessive	PRCD-DNA test or ECVO examination	Only "free x free", "free x carrier" and as exception "free x affected" are allowed
Neuropathological disorder	Monogenic, autosomal recessive	-	Affected individuals, their parents and their litter mates are excluded

Since 2007 potential breeding individuals have to undergo a PL-examination. The PL-examination is conducted once-off, when the dog is at least 12 months old, by a veterinarian according to the Meutstege protocol. Since 2011 potential breeding individuals also have to undergo an ECVO (European College of Veterinary Ophthalmologists) eye-examination or the PRCD-DNA test. The ECVO eye examination can be conducted when the dog is at least 18 months old and is only valid for one year, so has to be repeated. The PRCD-DNA test is strongly recommended by the Breed Club, as it also identifies carriers of the disease (van Ederen, 2015).

Regarding PL, only breeding with the combinations "PL-free x PL-free" and "PL-free x PL-grade1" is allowed. Regarding PRCD, the combinations "PRCD-free x PRCD-free", "PRCD-free x PRCD-carrier" and in exceptional cases "PRCD-free x PRCD-affected" are allowed. Regarding the neuropathological disorder, all affected individuals, their parents and their litter mates are excluded from the breeding program (Nederlandse Markiesjesvereniging, 2015).

1.7. The Stabyhoun

The Stabyhoun, colloquially known as the Stabij, Bijke or Frisian Pointer is thought to descend from Spaniel-type dogs that came to the Netherlands in the Middle-Ages (figure 3). Stabyhouns have been documented in historical documents and paintings dating back to 1800. The breed has its true origin on the countryside of Friesland, where Stabyhouns were used for among others hunting, guarding the farm and pest control. In the early 1900s the Stabyhoun was extensively used for hunting moles and polecats. To improve their hunting skills, Stabyhouns were crossed with Wetterhouns, another Frisian hunting breed. Since 1938, however, the concern regarding the preservation of these two breeds grew and during World War II the organised breeding of the separate breeds started. In 1942 both breeds were recognized by the Dutch Kennel Club and the FCI. The breed club 'Nederlandse Vereniging voor Stabij- en Wetterhounen' was formed in 1947 (Dooper, 2004). Since 1952 approximately 16,700 Stabyhouns have been registered in the NHSB.

Figure 3. *Spaniel-type dog (Jan Steen, 1625-1679)*

Internationally the breed has increased in popularity since 2000 and the breed club has currently sister associations in Sweden, Finland, Denmark, Norway, the UK and the USA. Nowadays most Stabyhouns are kept as companion animals, but some are still used for e.g. pest control as well (NVSW, 2015b).

Brief breed description

The Stabyhoun is a powerfully-built Pointer which is higher than long and neither very robust nor fragile. It is devoted, obediently, watchful and calm and is suited to be a household pet. Its coat is long and straight and white with black or brown patches and/or speckles. The ideal height for males is 53 cm and for females 49 cm. The body weight is in the range of 20-25 kg. The Stabyhoun is classified in group 7 of the FCI, which includes the Pointing Hunting dogs (NVSW, 2015e).

Breeding rules and the Breed Advisory Committee

The most important breeding rules of the Breed Club regarding the present study are (NVSW, 2015e):

- A bitch may not be mated to her grandfather, father, son, grandson nor brother.
- Combinations that would give birth to a litter with a COI of > 10%, as calculated over three ancestral generations, are strongly discouraged.
- A mating between a dog and bitch may not be repeated, except in special circumstances.
- The minimum breeding age of dogs and bitches is 18 months. A bitch has a maximum mating age of 72 months if she did not gave birth to a litter before and of 96 months if she did gave birth before. A stud dog has no maximum mating age.
- A stud dog may be used twice for mating during a year and is restricted to maximal 10 successful litters in his entire life (successful means at least 1 progeny registered in the NHSB), of which maximal 8 in the Netherlands. A bitch has a maximum of 5 successful matings and cannot be mated within 24 months of the previous mating.

The Breed Advisory Committee of the Breed Club assesses mating requests for compliance of the breeding policy, gives breeding advice and checks upon new-born litters. When pups are one and a half years old, they are called to the young dog-examination (NVSW, 2015a).

Inherited disorders in the Stabyhoun

The following inherited disorders have been identified in the Stabyhoun population: hip dysplasia (HD), elbow dysplasia (ED), epilepsy, patent ductus arteriosus (PDA), cerebral dysfunction and von Willebrands disease, type I (vWD-type I).

Hip dysplasia (HD)

In 1972 the first screening of a Stabyhoun for HD was performed. HD is a developmental orthopedic disorder which involves the malformation of the hip joint. The disorder can cause pain and discomfort while exercising and can lead to lameness. Although HD is observed in dogs of all sizes, it is more common in large and giant dogs, with a reported prevalence of up to 70% in among others Golden Retrievers, Rottweilers and German Shepherds (Paster et al., 2005; Lavrijsen et al., 2014; Sanchez-Molano, 2014). The disorder is multifactorial and has a moderate heritability, estimated from 0.20 to 0.43 across various UK dog breeds (Lewis et al., 2013) and from 0.0 to 0.37 for separate HD-related traits in Dutch breeds (Lavrijsen et al., 2014). Genome wide analyses have identified candidate genes for HD and breed-specific genetic prognostic models have been developed (for example (Bartolomé et al., 2015)). These models could be used to replace the current phenotypic selection against HD by a more accurate genomic selection. Genomic selection, however, seems infeasible for most dog breeds as it requires a large amount of SNP data and an extensive registration (Oldenbroek & Windig, 2012).

The assessment of hip dysplasia varies across countries, but is generally based on X-rays. In 1999, the FCI adopted a classification system that uses radiologically ascertainable features. In this system, the HD-result depends on the shape and depth of the acetabulum, the occurrence of subluxation, the Norberg angle (NA) and on the presence of osteoarthrosis (OA). The NA is a measure for the connection between the femoral head and acetabulum and is approximately 105° in normal hip joints (Flückiger, 2007). In table 5 the FCI classification system is summarized.

Table 5. *Grades and assessment criteria of canine hip-dysplasia (Flückiger, 2007)*

Grade	Degree of HD	Assessment criteria
HD-A	No signs of HD	Congruent femoral head and acetabulum *and* NA ≈ 105°
HD-B	Nearly normal	Slightly incongruent femoral head and acetabulum *or* NA < 105°
HD-C	Mild HD	Incongruent femoral head and acetabulum *and* NA ≈ 100° *and/or* flattening of craniolateral rim of acetabulum *and* no more than slight signs of OA
HD-D	Moderate HD	Obvious incongruity with subluxation *and* NA > 90° *and* flattening of craniolateral rim *and/or* signs of OA are present
HD-E	Severe HD	Luxation or subluxation *and* NA < 90° *and* obvious flattening of craniolateral rim *and* signs of OA are present

In some countries, e.g. in the USA, the OFA (Orthopaedic Foundation for Animals) score is used rather than the FCI system. The OFA-system distinguishes 7 grades (Flückiger, 2007).

Elbow dysplasia (ED)

ED was first registered for a Stabyhoun in 1989. ED is used collectively for four developmental abnormalities in the elbow joint that can all cause osteoarthrosis (OA) and lameness (IEWG, 2015):

- Ununited anconeal process (UAP): the anconeal process, a small piece of bone that is essential for joint stability, fails to fuse with the olecranon of the ulna.
- Fragmented medial coronoid process (FCP): the medial coronoid process fails to unite with the ulna. The loose piece of cartilage in dogs with UAP and FCP causes irritation, infection and damage to the joint.
- Osteochondrosis (OC) or osteochondritis dissecans (OCD): local failure in ossification, leading to a thickened cartilage layer (OC) that may develop in a dislodged single or fragmented cartilage flap (OCD).
- Incongruity (INC): a not proper positioning of the joint surfaces, generally caused by an unequal growth of the radius and/or ulna or by a malformed trochlear notch (the socket in the ulna that connects with the head of the humerus). Incongruity leads to an abnormal distribution of the pressure on the joint.

ED is like HD especially frequent in large and giant breeds and has reported prevalence-values of over 60% for some breeds. A heritability ranging from 0.0 to 0.39 is reported for ED related traits in Dutch breeds (Lavrijsen et al., 2014). A substantial higher heritability has been reported for types of ED in other countries, for example 0.57 for FCP in German Shepherd Dogs in Germany (Lewis et al., 2013).

The first clinical signs of ED can generally be seen at an age of 4-9 months. For the assessment of ED a classification system is developed by the International Elbow Working Group (IEWG). This system uses four grades with signs of OA, osteosclerosis and primary lesions as assessment criteria (table 6).

Table 6. *Grades and assessment criteria of canine elbow dysplasia (IEWG, 2015)*

Grade	Degree of ED	Assessment criteria
0	No ED	No evidence of OA, no osteosclerosis *nor* any primary cause
1	Mild ED	Signs of mild OA (osteophytes of < 2 mm) *or* minor osteosclerosis
2	Moderate ED	Signs of moderate OA (osteophytes of 2-5 mm) *or* obvious osteosclerosis *or* suspect of primary lesion*.
3	Severe ED	Signs of severe OA (osteophytes of ≥ 5 mm) *or* obvious primary lesion*.

*Primary lesions include an ununited anconeal process (UAP), fragmented medial coronoid process (FCP), osteochondritis dissecans (OCD) and incongruity (INC).

Mostly positive genetic correlations have been reported between HD and ED (Lewis et al., 2013).

Epilepsy

Epilepsy is the most common neurological disorder in dogs, with an overall prevalence of idiopathic epilepsy of 0.5 to 5%. Epilepsy involves the occurrence of recurrent and unprovoked epileptic seizures, during which there is an excessive or synchronous neuronal activity in the brain. Symptoms of seizures include collapsing, muscle twitching, loss of consciousness and foaming at the mouth. The symptoms generally start to occur when a dog is 3-6 months. Besides idiopathic epilepsy, in which the seizures are the direct result from one or more genetic defect(s), there is also epilepsy caused by environmental factors such as metabolic disorders (Ekenstedt et al., 2012).

The much higher prevalence of idiopathic epilepsy of up to 9% in some breeds, and of up to 33% in some families, indicates a strong genetic contribution (Ekenstedt et al., 2012). The disorder is generally considered to be polygenic autosomal, although some epilepsies might be caused by single-locus mutations. To date only a few risk genes have been identified, with *ADAM23* as the most promising locus. The genetic heterogeneity of the disease, the non-genetic causes and the limited amount of data slow down the further identification of risk genes (Koskinen et al., 2015).

Patent ductus arteriosus (PDA)

PDA is the first or second most common canine congenital heart disorder in which the Ductus Arteriosus - the blood vessel that connects the pulmonary artery and aorta in a foetus - does not close within the first week(s) after birth. Affected individuals have an abundant recirculation of blood through the heart, which can eventually lead to terminal (left) heart failure (Oyama et al., 2010).

An overall prevalence of congenital heart disorders of 0.50-0.68% has been reported in hospital surveys. A quarter to a third of these cases involved PDA, leading to an estimated prevalence of 0.1-0.2%. Dogs like the Chihuahua, Collie and the Maltese, and in some regions also larger dogs like the German Shepherd and the Newfoundlander, are most frequently affected by PDA. Females are reported to have a 1.7 to 3.0 times higher predisposition to PDA than males (Oyama et al., 2010; Oliveira et al., 2011). The higher prevalence in some breeds suggests a genetic contribution. The disorder is thought to be a polygenic threshold trait with a high heritability (Patterson et al., 1971).

Cerebral dysfunction (neuro)

Cerebral dysfunction is a neurological inherited disorder found in a few Stabyhoun litters. Affected pups start to display compulsive behaviour, do not eat independently, emaciate, are thought to have a lot of pain, and have to be euthanized when they are a few months old. In 2015, an autosomal recessive mutation was identified by the Faculty of Veterinary Medicine of University Utrecht as the genetic cause of the disease (NVSW, 2015d).

Von Willebrand disease, type I (vWD-I)

VWD-I was first observed in the breed in 2007. Von Willebrand Disease is the most common inherited bleeding disorder in dogs and is characterized by deficiencies in the von Willebrand Factor (vWF), an important protein for blood coagulation. Type I is the most common and mildest of the three distinguished types of vWD. It is caused by a mutation (G > A) in the *vWF* gene and characterized by a lowered concentration but normal functioning of vWF. There is incomplete dominance: heterozygous carriers of the type I mutation generally have less than half of the normal plasma vWF and homozygous carriers have a small amount to almost no vWF. The main clinical sign is a longer coagulation time (Barr & McMichael, 2012; Shaffer et al., 2015). A DNA-test is available for the disorder (NVSW, 2015c).

Management directed against inherited disorders

To limit the propagation of the abovementioned disorders, the Stabyhoun Breed Club has implemented (mandatory) health screenings and disorder-specific breeding rules (table 7).

Table 7. *Overview of inherited disorders and related health screenings/breeding rules in the Stabyhoun*

Disorder	Putative mode of inheritance	Health screenings	Breeding rules
Hip dysplasia (HD)	Multifactorial	X-rays[†]	• HD-D or –E are excluded • Combinations between HD-A or –B and HD-A, -B or –C are allowed
Cerebral dysfunction (neuro; CD)	Monogenic, autosomal recessive	DNA-test[‡]	• Affected individuals are excluded • Combination of two carriers is not allowed
Elbow dysplasia (ED)	Multifactorial	X-rays	• Individuals with grade 3 or that had to undergo surgery are excluded • Proven carriers excluded*
Epilepsy (idiopathic)	Polygenic	-	• Affected individuals are excluded • Proven carriers are excluded*
Patent ductus arteriosus (PDA)	Polygenic	-	• Affected individuals are excluded • Proven carriers are excluded*
Von Willebrands disease, type I (vWD-I)	Monogenic, autosomal, incomplete dominance	DNA-test	-

[†] Screening is mandatory for potential breeding individuals.
[‡] DNA-test was developed and made mandatory for breeding individuals in 2015.
*Parents of 2 affected litters are considered to be proven carriers.

The screening of potential breeding individuals for HD is mandatory since at least 1985. Individuals with moderate or severe HD (D or E) are excluded from breeding and individuals with mild HD (C) may only be mated with individuals with normal or nearly normal hip joints (A or B).
Since 2015 the DNA-test for the mutation underlying cerebral dysfunction is mandatory for potential breeding individuals. Individuals of which both parents are known to be homozygous normal do not have to undergo the DNA-test (NVSW, 2015e).

Screening for elbow dysplasia is recommended, but not mandatory for breeding individuals in Netherlands (it is mandatory in some of the Scandinavian countries). Individuals that have had to undergo surgery or are diagnosed with ED grade 3, however, are excluded from breeding.

Regarding epilepsy and PDA no health screenings are available yet (NVSW, 2015e). In 2013 blood samples of Stabyhouns were taken by the Faculty of Veterinary Medicine of University Utrecht to study the genetics underlying PDA. This might result in some sort of health screening in the future.
Regarding vWD-I there is a passive breeding policy. A DNA-test is available but screening of breeding individuals is not mandatory. The combinations 'homozygous mutant x homozygous mutant' and 'homozygous mutant x heterozygous carrier' are, however, not recommended by the Breed Club (NVSW, 2015c).

1.8. Aim and research questions

The aim of the present study was to formulate sound breeding recommendations that can support the Breed Clubs in maintaining the genetic diversity and improving the health and welfare in their breeds. This required insight in the current 'population status' (population size, percentage of individuals selected for breeding, generation interval, etc.) of and the genetic diversity in the breeds. Information on the inherited disorders in the breeds and the effectiveness of various breeding strategies was furthermore required, leading to the following research questions:

1. What is the current population status of and the genetic diversity in the Markiesje and the Stabyhoun and how have these developed since the foundation of the breeds?

2. What is the prevalence of the inherited disorders found in the Markiesje and the Stabyhoun and which risk factors for these disorders can be identified?
3. What is the effect of various breeding strategies on the inbreeding rate and generation interval in the Markiesje and the Stabyhoun?
4. What is the effect of excluding individuals affected by and/or carriers of genetic disorders from breeding on the inbreeding rate in the Markiesje and the Stabyhoun?
5. How can, given the population status and genetic diversity, the breeding of the Markiesje and the Stabyhoun be optimised?

In order to answer the above questions, various analyses were conducted. How these analyses were conducted is described in the next chapter.

2. Materials and methods

Data of all registered Markiesjes and Stabyhouns was extracted from ZooEasy, the monitoring software used by both breed clubs. The initial dataset contained 2,473 Markiesjes and 16,688 Stabyhouns. The 66 Markiesjes and 9 Stabyhouns that had neither registered parents nor registered progeny were excluded from the analyses. Of the remaining 2,407 Markiesjes and 16,679 Stabyhouns the unique registration number, sex and name were known. With the exception of three Markiesjes, also the date of birth was known. The three Markiesjes without known date of birth were excluded from year based analyses. Varying per individual the date of decease, the parents' registration numbers and the results of health screenings were known.

Subsets of the original dataset were used for the three analyses: the pedigree analysis (research question 1), the analysis of inherited disorders (research question 2) and the simulation of breeding strategies (research questions 3 & 4).

2.1. Pedigree analysis

For both populations a simplified file was constructed containing the ID, the sire's ID, the dam's ID, the sex, the date of birth and the date of decease of each individual. These files were scanned for possible duplicates, bisexual individuals (used as both sire and dam) and progeny born before their parents. Further analysis was performed using Microsoft Excel 2010, the Inbreeding monitor and the Coancestry, Inbreeding and Contribution (CFC)-software.

General population parameters

The following general population parameters were calculated and visualized using Microsoft Excel:

- *Life span*: the average difference between the date of decease and the date of birth. This parameter was calculated using all individuals with a known date of birth and known date of decease and were born before a certain date (the date on which the data was obtained minus the maximum life span). All animals born after this date were excluded from the calculation in order to prevent bias. Markiesjes with a decease date of 01/01/2001 were excluded from the calculation, as this particular decease date was later added by the Breed Club for those individuals that were assumed to be dead.
- *Litter size*: the number of pups per unique litter. Unique litters were defined as unique combinations of dam, sire and date of birth.
- *The mean age of the sires/dams at the birth of their (selected) progeny*: the average difference between the date of birth of the parent and the date of birth of the (selected) progeny. This parameter was calculated both per litter and per progeny. The mean age of the parents at the birth of their selected progeny, known as the generation interval, was similarly calculated with the extra criterion that the offspring's ID had to occur at least once as a parent.
- *The number of (selected) progeny per sire/dam*: the number of times an individual's ID occurred as a sire/dam. The number of selected progeny was similarly calculated, again with the extra criterion that the offspring's ID occurred at least once as a parent.

The mean values of the parameters above were compared between males and females with independent samples t-tests. When equal variances could not be assumed, the Satterthwaite-approximation was used. Large sample sizes ensured the validity of the t-tests, despite the non-normal distribution of many of the parameters.

The current population size was estimated via a few steps. First, individuals with a known decease date and individuals that would have been older than the maximum life span were assumed to be dead. Second, a probability to be alive was assigned to each remaining individual, which was based on the

age-distribution of individuals with known dates of birth and decease. All these 'fractions' of individuals were then summed to get the estimate of the number of living individuals.

Pedigree completeness

Pedigree completeness was determined, because it strongly influences the outcome of the coefficients of inbreeding (COI) and coancestry (MacCluer et al., 1983). Three measures for pedigree completeness were used:

1) *Completely known ancestral generations (up to >5 generations):* calculated by tracing back ancestors per generation until at least one ancestor is unknown.
2) *Average equivalent complete generations (EcG):* the sum over all known ancestors, where every ancestor is weighted by $1/2^n$, with n being the number of generations separating the ancestor from the individual.
3) *Longest ancestral path (LAP):* the number of generations that separates an individual from its furthest ancestor. This number is equivalent to the pseudo-generations number (ψ) and is computed using the fact that the ψ of an individual always equals the maximum ψ of the parents plus one (Sargolzaei et al., 2006).

The first two parameters were computed per year of birth with the inbreeding monitor. The distribution of LAPs for the whole population was computed with CFC. The interpretation of the three parameters is illustrated in figure 4. In this example, the number of completely known ancestral generations for individual *f* is 1 (as two grandparents are missing) and the LAP is 3. The EcG equals 1.75, which is the sum of the weighted values for all the known ancestors, i.e. two parents (2 * 1/2), two grandparents (2 * $1/2^2$) and one great grandparent ($1/2^3$).

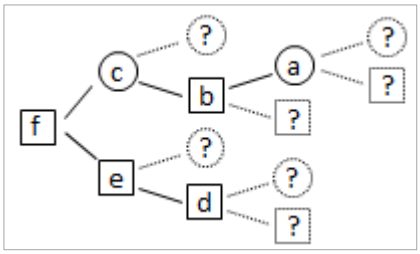

Figure 4. *Example of a pedigree for calculating completeness*

Coefficients of inbreeding and coancestry

The inbreeding coefficient of individuals (F_i) and the coancestry between individuals (f_{ij}) were computed with the Inbreeding monitor and with CFC, which both use an indirect algorithm that is based on the multiplication of the numerator relationship matrix. This algorithm was developed by Colleau (Colleau, 2002) and modified by Sargolzaei et al. (Sargolzaei et al., 2005). Individuals were grouped by year of birth to calculate the average COI ($\bar{F}$) and average coancestry ($\bar{f}$) per year.

Genetic diversity I: inbreeding rate and effective population size

The change of genetic diversity throughout the development of the breeds was evaluated in two ways: with the rate of inbreeding/coancestry and associated effective population sizes and with parameters based on the probability of gene origin. The rate of inbreeding for two consecutive years was first calculated using (Oldenbroek & Windig, 2012):

$$\Delta\bar{F}_y = \frac{(\bar{F}_x - \bar{F}_{x-1})}{(1 - \bar{F}_{x-1})}$$

where $\Delta\bar{F}_y$ is the inbreeding rate per year, $\bar{F}_x$ is the average COI of individuals born in year *x* and $\bar{F}_{x-1}$ is the average COI in the previous year. The $\Delta\bar{F}_y$ is multiplicative, rather than additive. As the heterozygote genotype frequency in an inbred population equals to 2pq(1-F), as shown in table 1, $\Delta\bar{F}$ actually measures the fraction of heterozygosity that disappears over time. A natural log transformation was used to approximate a linear relationship:

$$\ln(1 - \bar{F}_x) \approx -\Delta\bar{F}_y\, x + \ln(1 - \bar{F}_0)$$

where *x* is the number of years in the period, $\Delta\bar{F}_y$ is the average rate of inbreeding per year in the period and $\bar{F}_0$ and $\bar{F}_x$ are the average COI at year 0 and year *x*, respectively. To obtain the inbreeding rate per generation, the $\Delta\bar{F}_y$ was multiplied with the generation interval.
An equivalent approach was used for the calculation of the coancestry rate ($\Delta\bar{f}$).

The inbreeding and coancestry effective population sizes were then calculated for various periods with the classical formulae (Falconer et al., 1996):

$$N_e{}^F = \frac{1}{2\Delta\bar{F}_{gen}} \qquad N_e{}^f = \frac{1}{2\Delta\bar{f}_{gen}}$$

where $\Delta\bar{F}_{gen}$ and $\Delta\bar{f}_{gen}$ are the rate of inbreeding and the rate of coancestry per generation, respectively.

Genetic diversity II: probabilities of gene origin
Parameters related to the probability of gene origin - founder equivalent, founder genome equivalent and effective number of non-founders - were computed with CFC to further describe the genetic diversity. Founders were defined as individuals with no known parents and were assumed to have no genetic relationship with any other animal in the pedigree than with their own descendants. Individuals with one missing parent were defined as semi-founders. The true- and semi-founders were responsible for the initial genetic diversity present in the analysed populations.

The founder equivalent (f_e), or effective number of founders, is defined as the number of equally contributing founders that is expected to produce the same genetic diversity as in the population under study. A difference between the total number of founders and the f_e indicates an unequal contribution of founders, so selection. For a reference group consisting of all individuals born in 2010-2015, the f_e was computed with CFC. The software implements the following formula of Lacy (Lacy, 1989; Sargolzaei et al., 2006):

$$f_e = \frac{1}{\sum_{j\in FOUN}\left[\frac{\sum_{i\in G}(t_{ij})}{n_g}\right]^2}$$

where FOUN is the set of founders, G is the set of individuals in the reference group, t_{ij} is the fraction of genes that individual *i* derived from founder *j* (as an element of the numerator relationship matrix) and n_g is the number of individuals in the group.

The founder genome equivalent (f_{ge}), which is similar to the f_e but additionally corrects for the proportion of the founder genomes that is lost by drift, was computed with the formula of Caballero and Toro (Caballero & Toro, 2000; Sargolzaei et al., 2006):

$$f_{ge} = \frac{1}{2\bar{f}}$$

where $\bar{f}$ is the average coancestry of the group under consideration. The difference between f_e and f_{ge} is an indication of the amount of genetic drift during non-founder generations. This difference is quantified by the effective number of non-founders (f_{ne}), which was computed with CFC as (Caballero & Toro, 2000; Sargolzaei et al., 2006):

$$f_{ne} = \left[\frac{1}{f_{ge}} - \frac{1}{f_e}\right]^{-1}$$

The genetic diversity in the reference population relative to the diversity in the founders was calculated with (Lacy, 1995):

$$GD_t = 1 - \frac{1}{2f_{ge}} \qquad GD_t{}^* = 1 - \frac{1}{2f_e}$$

where the formula on the left accounts for both the unequal founder contribution and genetic drift and the formula on the right only accounts for the unequal founder contribution. The loss of GD due to selection and drift was subsequently determined with $1 - GD_t$ and the loss of GD due to only selection was determined with $1 - GD_t{}^*$, assuming that the initial GD was approximately 1. The loss due to drift accumulated over non-founder generations was calculated as (Caballero & Toro, 2000)

$$GD_t{}^* - GD_t = \frac{1}{2f_{ne}}$$

Contributions of founder genes to the gene pool (GP), average inbreeding coefficient ($\bar{F}$) and average coancestry ($\bar{f}$) of the reference group were also computed with CFC. This computation applied the simple rule of thumb that an autosomal allele randomly picked of an individual has a probability of 1/2 to originate from any parent, 1/4 from any grandparent and $1/2^x$ to an ancestor in the x^{th} ancestral generation. The contributions to $\bar{F}$ and $\bar{f}$ components were computed by a method of Sargolzaei and Colleau, which involves the decomposition of $\bar{F}$ and $\bar{f}$ in contributions of Mendelian sampling variances of ancestors and then links these to the contributions of genes of nodal common ancestors (Sargolzaei & Colleau, 2006; Sargolzaei et al., 2006).

2.2. Analysis of inherited disorders

The pedigree data files were extended with the available information on the genetic disorders of interest. For the Markiesjes this concerned the PL status as the result of the examination by a veterinarian using the Meutstege protocol, the PRCD status as the result of the ECVO eye-examination by a veterinarian and the results of the PRCD DNA-test. Regarding the neuropathology only the reported affected individuals were known. For the Stabyhoun it concerned the HD and ED status as obtained from X-rays and the CD and vWD-I status as a result of performed DNA-tests. Regarding epilepsy, PDA, and CD only the reported affected individuals were known.

A disorder's prevalence was generally estimated as the number of reported affected individuals (n_a) in a period divided by the total number of individuals born in the same period (n_{tot}), which also includes the unaffected individuals (n_u). Whenever the results of health screenings and DNA tests were available, these were used to get a more accurate estimate of the prevalence. In these situations the prevalence in the screened individuals, the 'maximal prevalence' , was compared to the prevalence in all individuals, the 'minimal prevalence'. When sufficient data was available, the prevalence was estimated for a recent period (e.g. 2010-2014) to get insight in the current incidence of the disorder.

Sex predispositions were investigated with the relative risk (RR) and odds ratio (OR). Both were calculated from the male and female prevalence:

$$RR = \frac{p_1}{p_2} \quad OR = \frac{p_1 \,/\, (1 - p_1)}{p_2 \,/\, (1 - p_2)}$$

where p_1 is the estimated prevalence for group 1 (n_{a1}/n_{tot1}) and p_2 is the estimated prevalence for group 2 (n_{a2}/n_{tot2}). For low prevalence values the RR and OR give very similar results. For the computation of the 95%-confidence interval (CI_{95}) the natural logarithm of the RR and OR was taken, as the sampling distribution of ln(RR or OR) is approximately normal for large sample sizes. Then the

confidence interval bounds on the logarithmic scale were calculated according to the formulae (Ott & Longnecker, 2010):

$$\ln(RR) \pm z_{0.05/2} * \sqrt{\frac{n_{u1}/n_{a1}}{n_{tot1}} + \frac{n_{u2}/n_{a2}}{n_{tot2}}} \qquad \ln(OR) \pm z_{0.05/2} * \sqrt{\frac{1}{p_1} + \frac{1}{1-p_1} + \frac{1}{p_2} + \frac{1}{1-p_2}}$$

in which $z_{0.05/2}$ equals 1.96. The approximate bounds of the CI of the OR were obtained via exponentiation of the interval bounds on the ln-scale.

For monogenic disorders the mutant allele frequency (q) was estimated, using the proportion of affected individuals in the population (q^2). It was checked whether these traits were in Hardy-Weinberg Equilibrium (HWE) or not.

Other disorder-specific analyses, e.g. with regard to the association between HD and ED, are explained in the relevant paragraphs in the results chapter.

2.3. Simulation of breeding strategies

The simulation program Dog Breed Management was used to evaluate the effect of different breeding strategies on the inbreeding rate, the generation interval and the frequency of inherited disorders. This program was developed by Wageningen University as part of the project *Relatedness and inbreeding in purebred dogs* (paragraph 1.5).

Dog Breed Managament starts with the setup of the population. The program assumes that at the start of the simulation all individuals are unrelated and non-inbred. The simulation itself consists of four steps that are repeated for each simulated year (figure 5). First, progeny is generated based on the initialized mating parameters. Second, the inbreeding coefficients of the progeny and the age and number of living animals are evaluated. Third, animals are culled based on the age distribution of the breeding population and the applied mating restrictions. Last, breeding individuals are selected from the refreshed breeding population (Windig et al., 2014).

The populations of Markiesjes and Stabyhouns were set up using general population parameters such as the number of (fe)males available for breeding, the age distribution of the breeding individuals, the number of litters per year and the distribution of the litter size. These parameters were derived from the results of the pedigree analysis. The number of available breeding individuals and litters were estimated based on the average number of breeding individuals in the period 2010-2014. If a popular sire effect was present in the breed, i.e. if one or a few dogs sired a large proportion of the litters in 2010-2014, the number and contribution of the top sires was also entered in the simulation. Mating restrictions were used to simulate the current breeding policy in both breeds. For example, the maximum number of litters per sire per life was set to 5 for the Markiesjes and 10 for the Stabyhouns. The frequencies of monogenic inherited disorders, as obtained from the analysis of inherited disorders, were also entered in the baseline simulations. Mating restrictions regarding these disorders were simulated by selecting against affected individuals and/or against carriers. Selection against affected individuals was implemented using a ‘lethal age’, i.e. the age up to which affected individuals were allowed to breed. This age was set to 0 when affected individuals were not allowed to breed and the disease status was known prior to the minimum breeding age. Selection against carriers was simulated using the complete exclusion of male carriers and/or female carriers from breeding. Selection against polygenic disorders could not be simulated with the Dog Breed Management software.

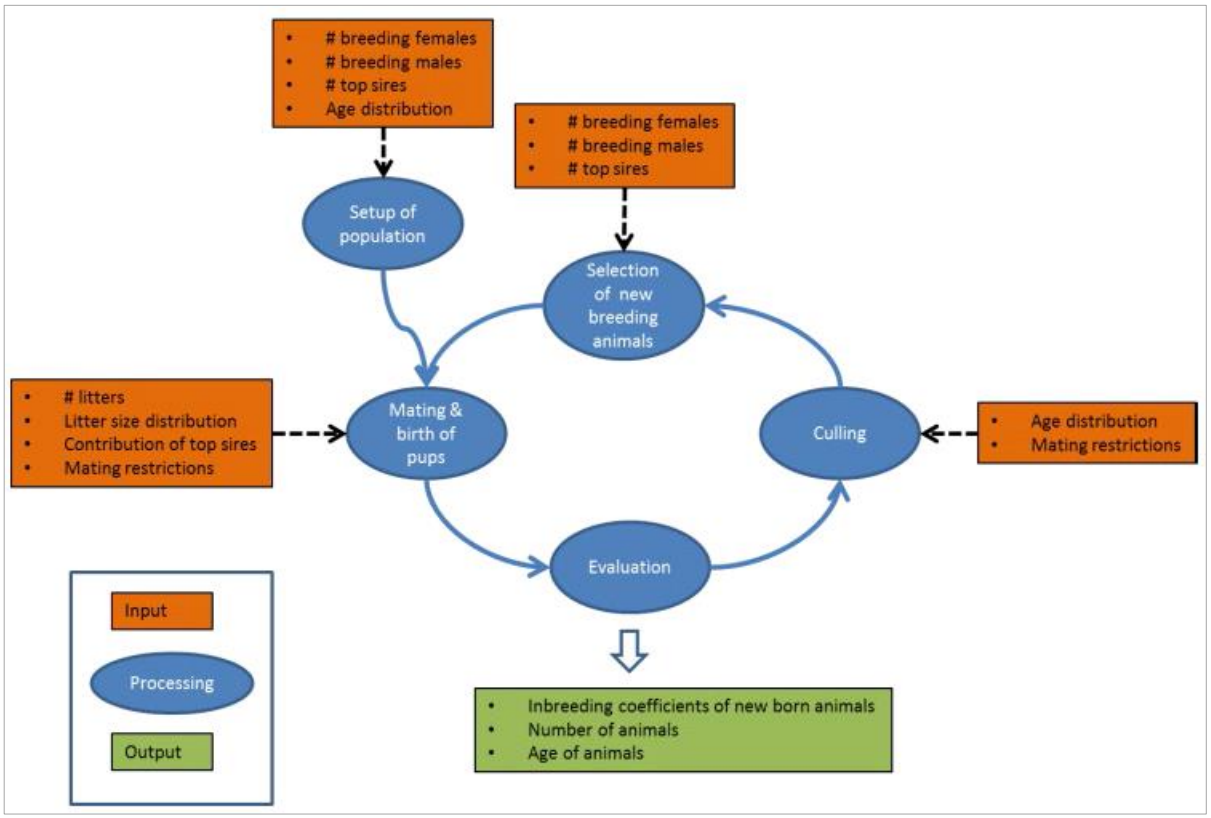

Figure 5. *Schematic overview of the simulation program Dog Breed Management (Windig et al., 2014)*

Simulations were run with 25 replicates and over 50 years per replicate (unless otherwise specified in the results).

3. Results Markiesje

In this chapter the results of the pedigree analysis, analysis of inherited disorders and simulation of breeding strategies for the Markiesje are presented.

3.1. Pedigree analysis

In the dataset with 2,407 Markiesjes there were no duplicate entries, bisexual individuals nor individuals that were born before their parents.

General population parameters

In table 8 general population parameters, averaged over all Markiesjes in the dataset, are summarized. Standard deviations and the range are included to show the (large) spread in and the bounds of the parameter values. The median is included to indicate the skewness of the parameter's distributions. Graphs of the distributions are included in appendix I.

Table 8. *General population parameters Markiesje*

Parameter	Mean ± std.	Median	Range (min. - max.)	n
Life span (in years)	12.5 ± 4.50	13.91	0.25 – 18.69	356*
Litter size	4.18 ± 1.54	4	1 – 8	553□
Age (in years) of:				
- sires at birth progeny	3.10 ± 1.54	2.72	0.90 – 11.44	2318†
- dams at birth progeny	3.92 ± 1.66	3.53	0.51 – 9.25	2318†
- sires at birth selected progeny	2.92 ± 1.44	2.57	0.90 – 10.00	549‡
- dams at birth selected progeny	3.92 ± 1.78	3.51	0.51 – 9.25	556‡
Number of:				
- progeny per sire	8.67 ± 5.02	8	1 – 24	205•
- progeny per dam	7.04 ± 5.06	5	1 – 27	267•
- selected progeny per sire	2.48 ± 2.34	2	0 – 14	133○
- selected progeny per dam	1.88 ± 2.26	1	0 – 12	176○

*Individuals born in 1977-1997 with a known date of birth and known date of decease.

□ Unique litters with known date of birth and known sire and dam.

† Progeny with known date of birth and known date of birth of sire/dam.

‡ Selected progeny with known date of birth and known date of birth of sire/dam.

• Sires/dams born before 2008 (ensuring that on average a parent has had >95% of its progeny).

○ Sires/dams born before 2002 (ensuring that on average the parents' progeny has reached the age on which >95% of the selected progeny has become parent).

There was no significant sex difference in mean life span (P= 0.275). Sires were on average younger at the birth of their progeny than dams (P= 0.000 for all progeny and for selected progeny). Males were less frequently selected for breeding than females: 26.2% of the males and 36.7% of the females born before 2008 were selected (see figure 6 as well). Sires had on average slightly more progeny than dams (P= 0.001 for all progeny, and P= 0.047 for selected progeny).

The number of newly registered individuals per year, comprising new-born pups and founders, increased in the first four decades of the breed and now fluctuates between the 90 and 140 (figure 6). Despite the high proportion of Markiesjes that was selected, the number of pups selected for breeding did generally not exceed 30 per year. The small observed census size, in combination with a few temporary setbacks (e.g. in 2007), will have caused substantial random drift.

Based on the known decease dates and the life span distribution the number of currently living individuals (in June 2015) was estimated to be 1219.

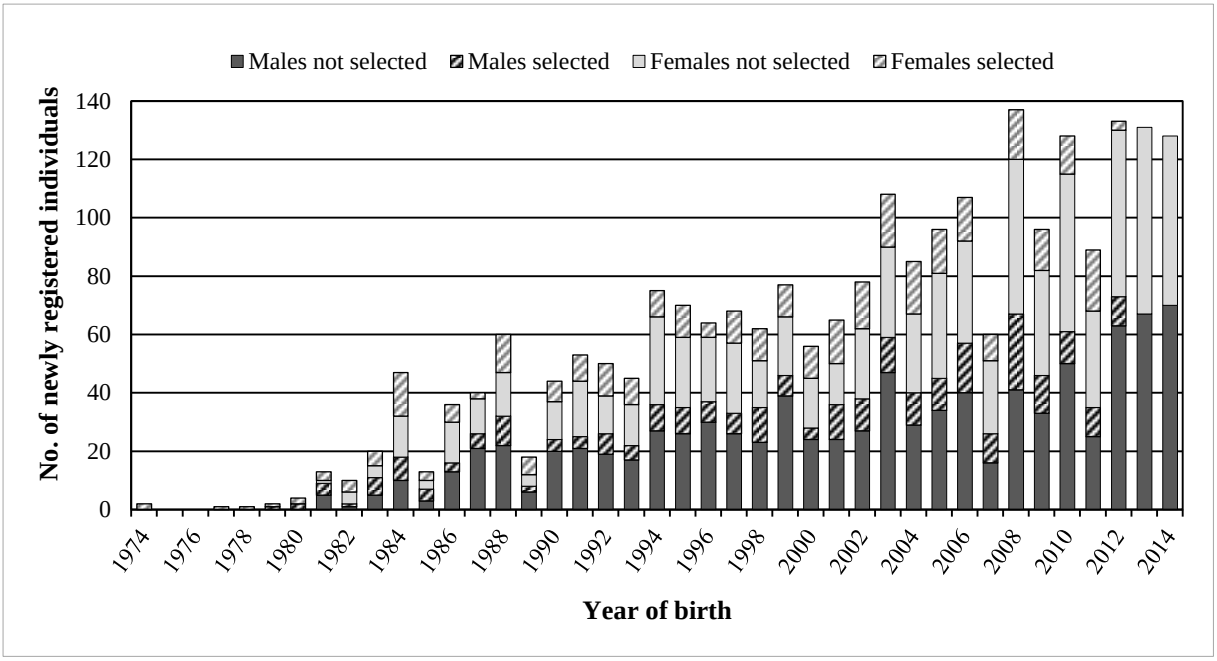

Figure 6. *Number of newly registered Markiesjes per year of birth, comprising new-born pups and founders, that were either selected for breeding or not (yet)*

The mean age of the parents at the birth of their progeny increased over time, from 3.02 y in 1983-1992 to 3.75 y in 2010-2014. The generation interval increased from 3.27 y in 1983-1992 to 3.72 y in 2008-2012. A mean generation interval of 3.42 was found for all parents born before 2002.

The number of (selected) progeny per parent stayed more or less constant over the years, both for dams as for sires (figure 7). The fluctuations over time were largely caused by the natural variation in litter size and the number of litters per parent that varied between 1 and 4 litters.

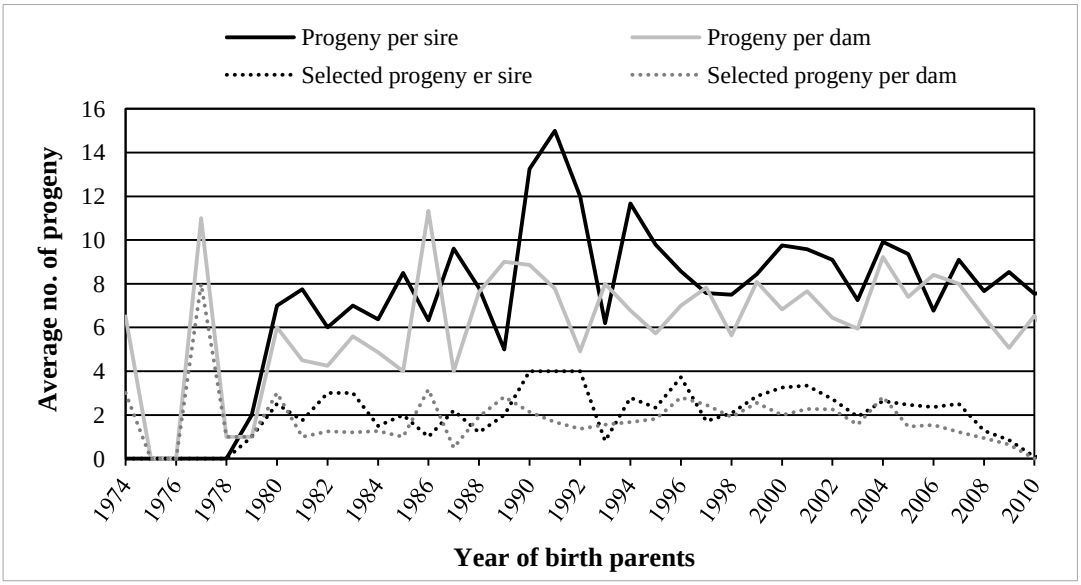

Figure 7. *Average number of (selected) progeny per Markiesje-parent from 1974 to 2010*

Litter size and life span showed no trends over time aside from minor fluctuations around the mean.

Pedigree completeness

During the development of the breed there was a steady increase in the pedigree completeness, as is shown by figure 8.

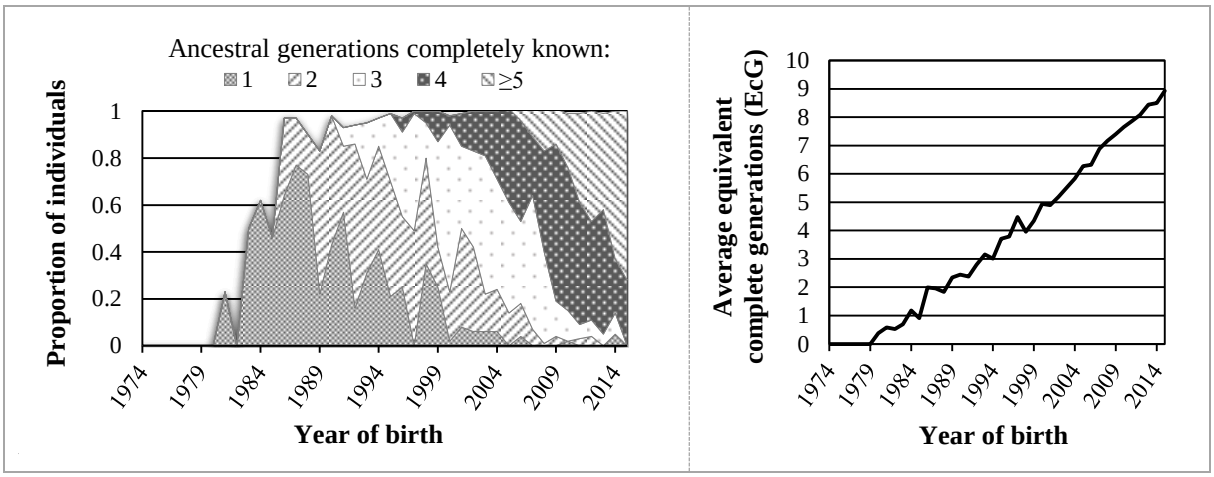

Figure 8. *Pedigree completeness: proportion of Markiesjes with x ancestral generations completely known (left) and average equivalent complete generations (right) from 1974 to 2015*

Of more than 90% of the Markiesjes born after 2012 at least 4 ascending generations were completely known and of more than 70% of the Markiesjes born in 2015 at least 5 ancestral generations were completely known. The average equivalent complete generations (EcG) increased from 0 at the formation of the breed to almost 9 in 2015.

The maximum longest ancestral path (LAP) in the dataset was 18 and over 60% of the Markiesjes had a LAP of >10. The distribution of LAPs is included in appendix I.

Inbreeding and coancestry

A total of 1952 inbred individuals were identified. The increase in the average COI ($\bar{F}$) and the average coancestry ($\bar{f}$, including self-relationships) over time is shown in figure 9.

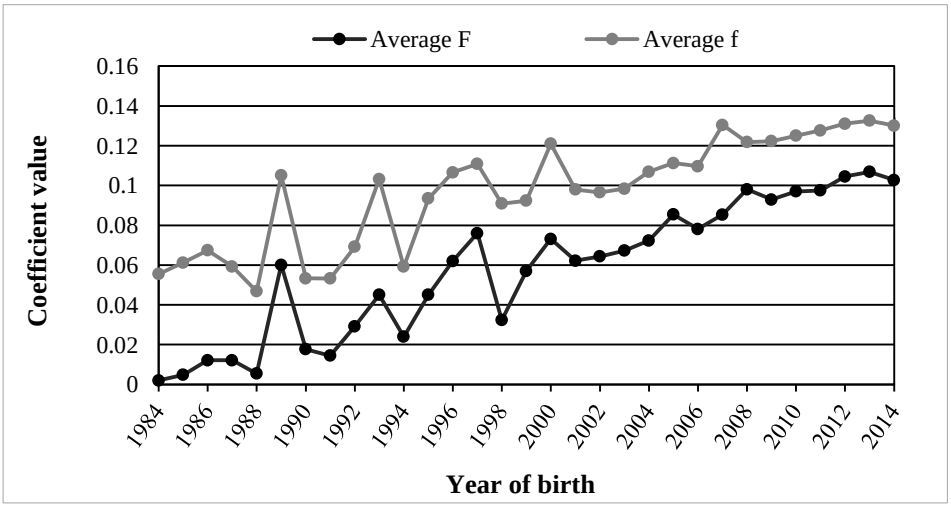

Figure 9. *Average inbreeding ($\bar{F}$) and average coancestry ($\bar{f}$, including self-coancestry) in the population of Markiesjes from 1984 to 2014*

When fluctuations were ignored $\bar{F}$ followed $\bar{f}$ at an interval of approximately 8 years. This interval is larger than the generation interval (of 3.42), indicating a relative suppression of the inbreeding. The inbreeding and coancestry rates, however, were approximately equal since 1988. The peak in $\bar{F}$ in 1989 can be explained by the birth of two relatively highly inbred litters in that year (with a COI of

0.08 and 0.125). The low $\bar{F}$ in 1998 was largely due to three litters that had a founder as parent and therefore a COI of 0. These three litters comprised approximately a third of the pups, i.e. 20 of the 63 pups, born in this year.

Genetic diversity 1: inbreeding/coancestry rate and effective population size

The current gene diversity (GD) was calculated to be 87% of the initial genetic diversity. An inbreeding rate per generation ($\Delta\bar{F}_{gen}$) of 1.27% was found for 1984-2015, corresponding to an effective population size based on inbreeding rate (Ne^F) of 39.22. These values are based on the slope of the logarithmic regression in figure 10.

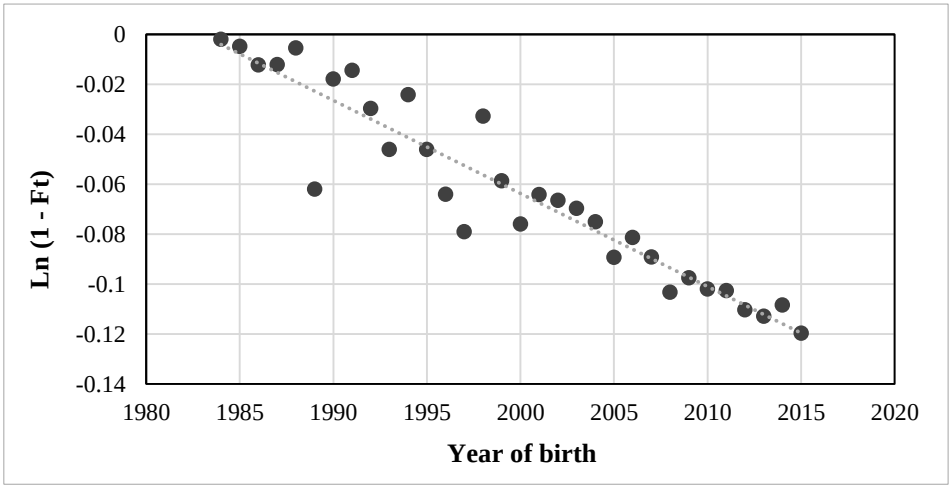

Figure 10. *Logarithmic regression of 1- $\bar{F}_x$ against the year of birth for the period 1984-2015. The slope represents the increase in the average COI per year ($\Delta\bar{F}$)*

The increase in COI per year and the associated Ne^F was quite constant throughout the existence of the breed. To get insight in trends over time, the $\Delta\bar{F}_{gen}$ and Ne^F were calculated and visualized for periods of five years (figure 11). The large observed Ne^F for the period 1996 to 2000 can be explained by the relatively high $\bar{F}$ in 1996 and 1997 and the outlier in 1998. Despite the decrease in $\Delta\bar{F}_{gen}$ since 2001, the breed still falls within the highest risk category of table 2 and is therefore considered to have an unacceptably high risk on the accumulation of inherited disorders and perhaps even extinction of the breed.

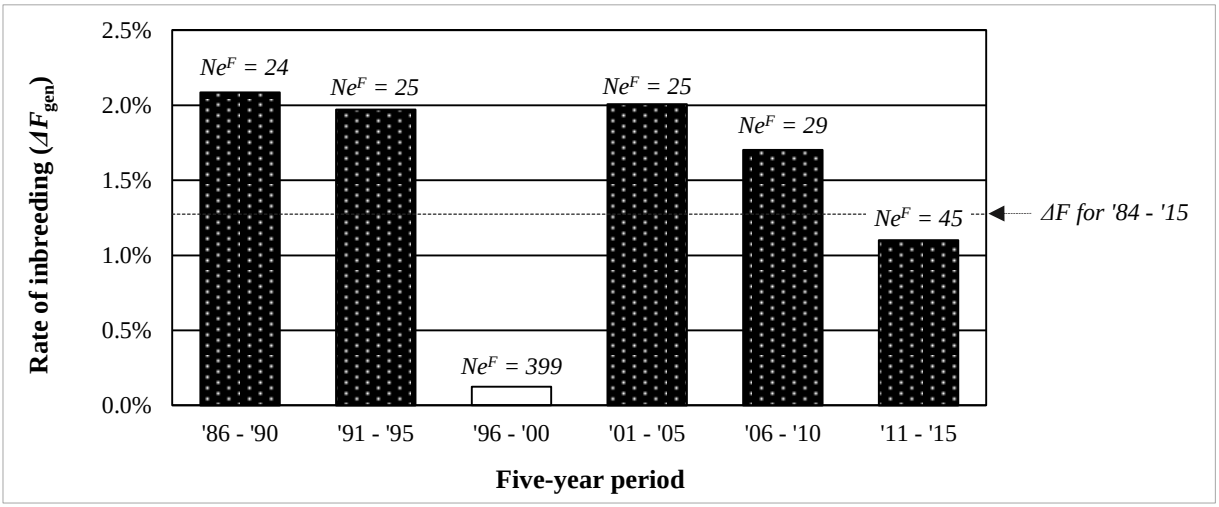

Figure 11. *Rate of inbreeding ($\Delta\bar{F}_{gen}$) and inbreeding effective population size (Ne^F) in the Markiesje per five-year period from 1986 to 2015. Bars are filled according to the risk categories in table 2: black bars with white dots indicate an (unacceptably) high risk, with $\Delta\bar{F}_{gen}$ > 1.0%, and empty white bars correspond to a (very) small risk, with $\Delta\bar{F}_{gen}$ < 0.25% .*

The coancestry effective population size (Ne^f) from 1984 to 2015 was 47.61 and thereby slightly higher than the Ne^F. The difference between the Ne^f and Ne^F was mainly due to the high base level of $\bar{f}$, caused by the inclusion of self-coancestry, and the not-increasing $\bar{f}$ between 1984 and 1990. The Ne^f fluctuated more over time than the Ne^F, but stayed - with the exception of the first period and the period between 1996 and 2000 - below 58.

Genetic diversity 2: probability of gene origin

In total 54 founders were identified in the dataset (f_{tot}). Of these founders 70.4% was born before 1990 and 92.6% before 1997. In addition to these founders, there were 31 Markiesjes with only a registered dam and 13 Markiesjes with only a registered sire; these 44 semi-founders were all born before 1997.

For the reference group, consisting of all individuals born in 2010-2015, the probability of gene origin was computed. The founder equivalent (f_e) was 14.63, the founder genome equivalent (f_{ge}) was 4.08 and the effective number of non-founders (f_{ne}) was 5.65. The ratio f_e/f_{tot} of 0.27 indicates a small to moderate deviation from an equal contribution of founders. This deviation is further illustrated in table 9, in which the ten founders with the highest contribution to the gene pool (GP) of the reference group are listed.

The proportion of the initial genetic diversity that was lost due to an unequal contribution of founders ($1 - GD^*$) was 3.4%. The total proportion lost due to both selection and drift ($1 - GD$) was 12.2%. This led to a loss in genetic diversity due to drift accumulated over non-founder generations of 8.8%. A loss of 0.88% per generation was estimated based on ~10 generations that have passed since the founders lived.

Table 9. *The 10 founders with the highest contribution to the gene pool (GP) of Markiesjes born in 2010-2015 (n = 641)*

				Contribution to		
Registration number	Name	Sex	Year of birth	GP*	$\bar{F}^\dagger$	$\bar{f}^\dagger$
100 GO GEEN VR	Pom	F	1977	0.170427	0.030980	0.034289
242 GO GEEN VR	Rasta	M	1985	0.138879	0.016425	0.019375
106 GO GEEN VR	Sonja	F	1974	0.062671	0.008773	0.010034
448 GO GEEN VR	Lotje	F	1988	0.055617	0.004189	0.005422
1145 G0 VR 2458830	Tosca	F	2000	0.048728	0.001208	0.002159
168 GO GEEN VR	Trixie	F	unknown	0.046561	0.006977	0.007920
343 GO GEEN VR	Wobbel	F	1984	0.046257	0.003510	0.004506
608 G0 GEEN VR	Hummeltje	F	1991	0.041034	0.002047	0.002958
371 G0 GEEN VR	Jody	F	1988	0.033971	0.001538	0.002089
796 GO GEEN VR	Jori	F	1994	0.025418	0.000696	0.001245

* The probability of gene origin, i.e. the fraction of genes in the group passed on by the founder.

† Contribution of genes of founders to the average COI and the average coancestry of the reference group, with $\bar{F}$ = 0.102558 and $\bar{f}$ = 0.122608 for the Markiesjes born in 2010-2015.

From table 9 it can be derived that the probability that a randomly picked gene from a randomly picked individual born after 2009 originally comes from Pom or Rasta is more than 30%. The genes of these two founders together contributed 0.047 (46%) to the average COI and 0.054 (44%) to the average coancestry. The genes of the top 5 founders contributed 47.6%, 60.0% and 58.1% to the GP, average COI and average coancestry, respectively. There were 21 founders (39%) without any contribution to the current GP. The 44 semi-founders contributed 14.2% to the current GP.

3.2. Analysis of inherited disorders

The results for patellar luxation, progressive rod-cone degeneration and the neuropathology are presented below. Other inherited disorders that were found in the breed such as epilepsy and cataract were not analysed, as only a few Markiesjes were affected by these disorders.

Patellar luxation (PL)

A total of 644 Markiesjes were examined for the presence of PL by veterinarians following the Meutstege protocol. All these individuals were born after 1984 and 67.4% was born after 2006, when the health screening of potential breeding individuals on PL was made mandatory. The total prevalence of PL, of grade I up to IV combined, was 21.74% in the screened individuals (table 10). No clear selection effect was visible; the prevalence in all screened individuals born before 2006 was 23.0%, the prevalence in Markiesjes born in 2007-2009 was 16.4% and in Markiesjes born after 2009 it was 24.0%. The prevalence of grade II up to IV, which only includes the Markiesjes with spontaneous luxation, was 3.73%.

Females were slightly more often screened than males, i.e. 29.9% versus 25.7% of total. The prevalence for PL was higher for females than for males; a female:male RR of 1.365 (CI_{95}: 1.009 – 1.845) for grade I to IV was found.

Table 10. *PL status of 644 examined Markiesjes born between 1984-2014*

	Males		Females		Total	
PL status	n	%	n	%	n	%
Grade I	48	15.89	68	19.88	116	18.01
Grade II	6	1.99	15	4.39	21	3.26
Grade III	1	0.33	1	0.29	2	0.31
Grade IV	0	0	1	0.29	1	0.16
Unaffected	247	81.79	257	75.15	504	78.26
Total	*302*	*100*	*342*	*100*	*644*	*100*

For 57 of the affected individuals it could be derived whether one or both knee joints were affected. Of these animals 42 (74%) had PL in both the knees. Of 39 knee joints for which the luxation direction could be determined 11 (28%) were medial, 16 (41%) lateral and 12 (31%) bidirectional.

Progressive rod-cone degeneration - Progressive Retinal Atrophy (PRCD-PRA)

There were 303 Markiesjes with a registered PRCD status from both the DNA-test and the ECVO-eye examination, 208 individuals with only the DNA-test result and 64 with only the ECVO result. The results of both tests are shown in table 11 and 12.

Table 11. *Observed genotype of Markiesjes with PRCD DNA-test and expected genotypes under HWE*

PRCD genotype	$n_{observed}$	$\%_{observed}$	$n_{expected}$
Homozygous normal	399	78.08	399
Heterozygous/carrier	105	20.55	105
Homozygous mutant	7	1.37	7
Total	*511*	*100.00*	*511*

Table 12. *PRCD status of Markiesjes with ECVO eye-examination*

PRCD status	n	%
Affected	8	2.13
Provisionally not clear	2	0.53
Clear	366	97.34
Total	*376*	*100.00*

In the group that has undergone the DNA-test the prevalence was 1.37%, the frequency of the normal allele (*p*) equalled 0.88 and the frequency of the mutant allele (*q*) equalled 0.12. These allele frequencies led to an expected number of individuals per genotype under Hardy-Weinberg equilibrium

(HWE) that was rounded exactly equal to the observed number of individuals (table 11). As the goodness-of-fit test statistic χ^2 and the p-value approached 0 and 1, respectively, the group that has undergone the DNA-test was in HWE, suggesting no selection. However, when looking at the mutant allele frequency over time, the selection applied by the Breed Club was effective: q decreased from 0.17 in 2006-2009 (n = 103) to 0.10 in 2010-2014 (n = 364).

The prevalence in the Markiesjes that underwent the ECVO-eye examination was 2.13% and thereby slightly higher than in the DNA-test group. No sex predisposition was found for PRCD.

Neuropathology

A total of 11 Markiesjes were registered as affected by the neuropathology. These individuals were distributed over 6 litters and were all born in 2003-2013. An overview of the 6 affected litters is given in the table below.

***Table 13**. Overview of litters with Markiesjes affected by neuropathology, showing the number of (affected) pups, the litter's F compared to the $\bar{F}$ of the year of birth and the number of (nodal common) ancestors*

				No. pups				No. ancestors	
Litter	Date of birth	Sire ID	Dam ID	Affected	Total	F_{litter}	$\bar{F}_{\text{year of birth}}$	NCA*	Total
A	09-01-2003	1119 G3	1085 G2	3	6	0.0805	0.0673	29	62
B	07-03-2006	1228 G3	1120 G3	3	5	0.0827	0.0781	42	78
C	04-10-2008	1628 G4	1480 G3	1	5	0.0786	0.0981	46	102
D	29-10-2010	1762 G5	1490 G4	1	4	0.1082	0.0970	61	121
E	07-02-2012	1923 G3	1701 G4	1	5	0.1288	0.1045	89	130
F	12-11-2013	1902 G4	1738 G5	2	6	0.1158	0.1068	78	160

***** Nodal common ancestors are ancestors that occur both in the paternal and maternal line of an individual's pedigree. They are the ancestors on which the individual/litter is inbred.

The COI of the affected litters was higher than the average COI of all Markiesjes born in the same year, with the exception of litter C. This indicates that the manifestation of the disorder might be the result of inbreeding. However, this observation could be easily due to chance as well. Approximately a third (11/31) of the pups in the 6 affected litters were affected, which is higher than the expected proportion of 0.25 for a monogenic autosomal recessive disorder. The observed proportion, however, did not significantly differ from 0.25 (P = 0.128; 1-sided binomial test).

Under the assumption that the neuropathology is a monogenic autosomal recessive disorder, the mutant allele frequency for all animals born after 2003 was estimated to be 9% (q = 0.090). Under HWE this would give an heterozygote frequency ($2pq$) of 0.17, corresponding to a total of 218 carriers born after 2002. As affected individuals and their siblings and parents were excluded from breeding, the actual number of carriers is expected to be lower than 218.

Partial inbreeding coefficients of the NCAs were calculated to get insight in potential carriers and the possible origin of the mutant allele (still assuming a monogenic autosomal recessive disorder). The NCAs with a relatively high contribution to the COI of the affected individuals are shown in table 14. The contribution of NCAs in this table is averaged over the 6 affected litters. In appendix II a similar table (table II) is included, in which the influential NCAs are shown per affected litter. Assuming that the mode of inheritance is autosomal recessive, the NCAs with a high ratio between their contribution to the COI of the affected litter ($F_{ij.litter}$) and their contribution to the average COI of the reference of the reference population ($\bar{F}_{ij.ref}$) are potential carriers of the mutant alleles. Examples of these NCAs are 713 G3 for litter B, 1198 G3 for litter E and 1088 G3 for litter F.

Table 14. *Nodal common ancestors (NCAs) of affected litters with a difference between the litters' average partial inbreeding coefficient ($\bar{F}_{ij.lit}$) and the average partial inbreeding coefficient of the reference population ($\bar{F}_{ij.ref}$) of more than 0.001 and/or a $\bar{F}_{ij.litters}$ that is more than 2 times the $\bar{F}_{ij.ref}$. The j in F_{ij} represents the NCA and the i the inbred individual/litter. The reference population consists of all unaffected individuals born since 2003, excluding litter mates of affected individuals (n =1299).*

NCA ID	$\bar{F}_{ij.lit}$	$\bar{F}_{ij.ref}$	$\bar{F}_{ij.lit} - \bar{F}_{ij.ref}$	$\bar{F}_{ij.lit} / \bar{F}_{ij.ref}$
713 G3	0.004261	0.000246	0.004016	17.35
1088 G3	0.002841	0	0.002841	x
348 G1	0.006471	0.004555	0.001916	1.42
242 GO	0.008329	0.006493	0.001836	1.28
415 G2	0.002806	0.001309	0.001498	2.14
1198 G3	0.001420	0.000232	0.001189	6.13
108 G0	5.93E-05	1.49E-06	5.78E-05	39.81
730 G3	0.000355	7.74E-05	0.000278	4.59
701 G2	0.000355	7.83E-05	0.000277	4.54
594 G2	0.000355	8.85E-05	0.000267	4.01
390 G2	0.001347	0.000577	0.000770	2.33

The contribution of genes of founders to the gene pool, the average COI and average coancestry of the affected litters was also compared with the contribution of the same founders to the reference group. The result of this comparison is given in table III in appendix II. Again, if the mode of inheritance is indeed autosomal recessive, one of the founders in this table (e.g. 796 GO, 168 GO or 106 GO) might have introduced the mutant allele in the population.

3.3. Simulation of breeding strategies

The results obtained in the pedigree analysis were used as input for the simulation program. The input used for the baseline simulation is included in appendix IV.

Below the results of simulating different breeding strategies – setting sire breeding restrictions, steering on relatedness with mating programs and enlarging the breeding population size – are presented. The influence of selection against monogenic disorders on the inbreeding rate is also evaluated.

Sire breeding restrictions

Various combinations of life-based and year-based sire restrictions were simulated. The effect of these restrictions on the inbreeding rate per generation and on the generation interval (as the mean of 25 simulation runs) is shown in table 15A and 15B, respectively.

Table 15A. *Effect of restricting the use of sires* per year and life on the mean $\Delta\bar{F}_{gen}$ (in %) of 25 simulated populations of Markiesjes*

Max no. of litters / year	Max no. of litters / life:				
	No	20	10	5	2
No	0.96	-	-	-	-
20	0.96	0.96	-	-	-
10	0.96	0.96	0.94	-	-
5	0.96	0.96	0.94	0.91	-
2	0.93	0.93	0.93	0.89	0.74

*Dams were restricted to maximal 5 litters per life

Table 15B. *Effect of restricting the use of sires* per year and life on the mean generation interval (in years) of 25 simulated populations of Markiesjes*

Max no. of litters / year	Max no. of litters / life:				
	No	20	10	5	2
No	2.94	-	-	-	-
20	2.94	2.94	-	-	-
10	2.94	2.94	2.93	-	-
5	2.94	2.94	2.93	2.90	-
2	2.95	2.95	2.94	2.92	2.80

*Dams were restricted to maximal 5 litters per life

The inbreeding rate in the simulation was lower than, but close to, the observed inbreeding rate in the breed (which was 1.1% for the last five years). The inbreeding rate stayed fairly similar over the different breeding restrictions and showed only a slight decrease when applying very strict breeding restrictions. The limited effectiveness of the breeding restriction can be explained by the absence of a popular sire effect in the simulation/breed. The current breeding restriction applied by the Markiesjes Breed Club, of maximal 5 litters per sire per life, leads to one of the lowest inbreeding rates in table 15A. Strict life-based restrictions, of ≤ 10 litters per sire per life, decreased the generation interval decreased, as shown in table 15B.

Steering on relatedness with mating programs

Three mating programs that are based on relatedness were simulated: minimum coancestry mating, minimising population relatedness by excluding those individuals from breeding that have a higher than average mean kinship and the use of optimal contributions. The effectiveness of these methods on reducing the inbreeding rate were compared, both with and without the breeding restriction of 5 litters per sire per life in the simulation (table 16).

Table 16. *Effect of various mating programs on the mean $\Delta\bar{F}_{gen}$ (in %) and mean generation interval in the 25 simulated populations of Markiesjes (100 years each), both with and without breeding restriction*

Breeding restriction	Mating program	$\Delta\bar{F}_{gen}$			Generation interval
		Overall	Year 0-20	Year 20-100	
None	None	0.95	0.97	0.95	2.96
	Min. coancestry mating	1.16	0.36	1.23	3.53
	Min. population coancestry	0.58	0.60	0.57	3.31
	Optimal contributions	1.12	0.87	1.20	3.22
Max. 5 litters per sire per life	None	0.91	0.88	0.91	2.89
	Min. coancestry mating	0.75	0.38	0.77	3.24
	Min. population coancestry	0.56	0.59	0.56	3.27
	Optimal contributions	0.77	0.66	0.78	3.61

Minimum coancestry mating was found to be mainly effective on the short run. Combining this method with a breeding restriction increased its effectiveness on the longer term. The overall effect was, however, still substantially smaller than for minimising population coancestry, which was the most effective simulated strategy. Applying the latter mating program reduced the inbreeding rate per generation over the 100 simulated years by more than 0.35%, both with and without breeding restriction. The effect of using optimal contributions was unexpectedly low. Without a breeding restriction method this mating program resulted in a inbreeding rate that was even higher than the inbreeding rate that was found without using a mating program.

Enlarging the breeding population size

The effect of increasing the breeding population size on the inbreeding rate in the Markiesje is shown in table 17. Increasing the number of breeding individuals was found to substantially decrease the inbreeding rate. Enlarging the breeding population size by 1.25 times (i.e. 8 more males and 14 more females per year) for example decreased the inbreeding rate in the simulated populations by 0.19%. Enlarging the population size 1.5 times led to a 0.32% decrease in $\Delta\bar{F}_{gen}$ and was thereby almost as effective as minimising population coancestry. The decrease in $\Delta\bar{F}_{gen}$ per extra breeding individual was larger when enlarging the breeding population size with a relatively small factor, as illustrated by the decrease of 0.19%, 0.32% and 0.46% for respectively 1.25, 1.5 or 2 time enlargement (adding 22,

55 or 89 individuals) of the population. In absolute numbers the addition of breeding females was more effective than the addition of breeding males; 28 extra females (+50%) resulted in a decrease of 0.20% whereas adding 33 males (+100%) decreased the inbreeding rate by 0.26%.

***Table 17**. Effect of increasing the number of available breeding males and females per year on the mean ΔF_{gen} (in %) of 25 simulated populations of Markiesjes*

Males available for breeding	Females available for breeding						
	n = 56 (baseline*)	n = 59 (+5%)	n = 62 (+10%)	n = 70 (+25%)	n = 84 (+50%)	n = 98 (+75%)	n = 112 (+100%)
n = 33 (baseline*)	0.91	0.89	0.84	0.78	0.71	0.62	0.55
n = 35 (+5%)	0.88	0.87	0.80	0.75	0.67	0.61	0.55
n = 36 (+10%)	0.89	0.88	0.81	0.76	0.64	0.59	0.54
n = 41 (+25%)	0.85	0.85	0.79	0.72	0.65	0.58	0.52
n = 50 (+50%)	0.80	0.79	0.78	0.70	0.59	0.54	0.51
n = 58 (+75%)	0.78	0.76	0.72	0.66	0.52	0.52	0.46
n = 66 (+100%)	0.75	0.72	0.68	0.62	0.55	0.51	0.45

* The estimated number of yearly available breeding individuals for the period 2010-2014

No clear effect of enlarging the breeding population on the generation interval was found.

Selecting against monogenic inherited disorders

The effect of different types of selection against PRCD is shown in table 18. Selection against this disorder hardly affected the inbreeding rate and average COI at 50 years. The inbreeding rate in the first (five) years increased slightly with stricter selection. This difference was, however, neglectable compared to the difference in the $\bar{F}$ at 50 years.

***Table 18.** Effect of different types of selection against PRCD on the mean $\Delta\bar{F}_{gen}$ and the mean mutant allele frequency (with an initial q of 0.116) in 25 simulated populations of Markiesjes*

Selection against	$\bar{F}$ at 50y (in %)	$\Delta\bar{F}_{gen}$ (in %)		Mean fixation y in fixed runs (n)	Mean q at 50y in non-fixed runs (n)
		0-5y	6-50y		
none of the individuals	14.30	0.79	0.94	27 (7)	0.154 (18)
homozygotes	14.27	0.62	0.90	29 (9)	0.070 (16)
homozygotes and ♂ heterozygotes	14.09	0.82	0.91	14 (50)	NA (0)
homozygotes and ♀ heterozygotes	13.85	0.95	0.91	8 (50)	NA (0)
homozygotes and all heterozygotes	13.98	0.89	0.92	1 (50)	NA (0)

Complete selection against both homozygotes and heterozygotes resulted logically in fixation of all the runs in the first year. Selection against female heterozygotes, in addition to homozygotes, seemed to be more effective than selection against male heterozygotes, as the average number of years to fixation was lower.

The simulated selection against the neuropathology (initial q of 0.090 and assuming a monogenic disorder) showed no effect on the inbreeding rate at all. The average number of years to fixation was, like with selecting against PRCD, higher when selecting against male heterozygotes (12 years) than when selecting against female heterozygotes (8 years).

4. Results Stabyhoun

In this chapter the results of the pedigree analysis, analysis of inherited disorders and simulation of breeding strategies for the Stabyhoun are presented.

4.1. Pedigree analysis

One dam was born later than her offspring. This dam was excluded from year based analyses. There were no duplicate entries nor any bisexual individuals.

General population parameters

In table 19 general population parameters for the Stabyhouns in the dataset are summarized. Standard deviations and the range are included to show the (large) spread in and the bounds of the parameter values. The median is included to indicate the skewness of the parameter's distributions. Graphs of the distributions are included in appendix III.

Table 19. *General population parameters Stabyhoun*

Parameter	Mean ± std.	Median	Range (min. - max.)	n
Life span (in years)	12.17 ± 3.16	12.84	0.34 – 18.68	346*
Litter size	6.32 ± 2.40	7	1 – 13	2634□
Age (in years) of:				
- sires at birth progeny	4.91 ± 2.34	4.45	0.81 – 14.84	16649†
- dams at birth progeny	4.14 ± 1.71	3.85	0.81 – 14.59	16643†
- sires at birth selected progeny	4.77 ± 2.36	4.29	0.82 – 12.05	1819‡
- dams at birth selected progeny	4.06 ± 1.76	3.73	0.81 – 10.85	1816‡
Number of:				
- progeny per sire	32.32 ± 40.30	17	1 – 392	432•
- progeny per dam	13.80 ± 9.76	11	1 – 78	1001•
- selected progeny per sire	4.34 ± 6.97	2	0 – 58	331○
- selected progeny per dam	1.71 ± 1.97	1	0 – 10	795○

*Individuals born in 1960-1997 with a known date of birth and known date of decease.
□ Unique litters with known date of birth and known sire and dam.
† Progeny with known date of birth and known date of birth of sire/dam.
‡ Selected progeny with known date of birth and known date of birth of sire/dam.
• Sires/dams born before 2007 (ensuring that on average a parent has had >95% of its progeny).
○ Sires/dams born before 2002 (ensuring that on average an individuals' progeny has reached the age on which >95% of selected progeny has become parent).

There was no significant sex difference in mean life span (P= 0.156). Sires were on average older at the birth of their progeny than dams (P= 0.000). Males were less frequently selected for breeding than females: 7.8% of the males and 17.6% of the females born before 2007 was selected (see figure 12 as well). This large sex difference was, as expected, also present for the number of progeny: sires had on average 32.32 progeny in total and 4.34 selected progeny, whereas dams had on average 13.80 progeny in total and 1.71 selected progeny (P= 0.000 for both).

The number of newly registered Stabyhouns per year, i.e. new-born pups and founders, increased from about 50 in 1960 to 300 in 1977 (figure 12). Before 1960 less than 10 Stabyhouns per year were registered. In 1978 the number dropped and fluctuated around 200 for two decades, whereafter it further increased. Since 2010 the number of new-born pups fluctuates around 700.

Based on the known dates of decease and the life span distribution the number of currently living individuals (in August 2015) was estimated to be 6959.

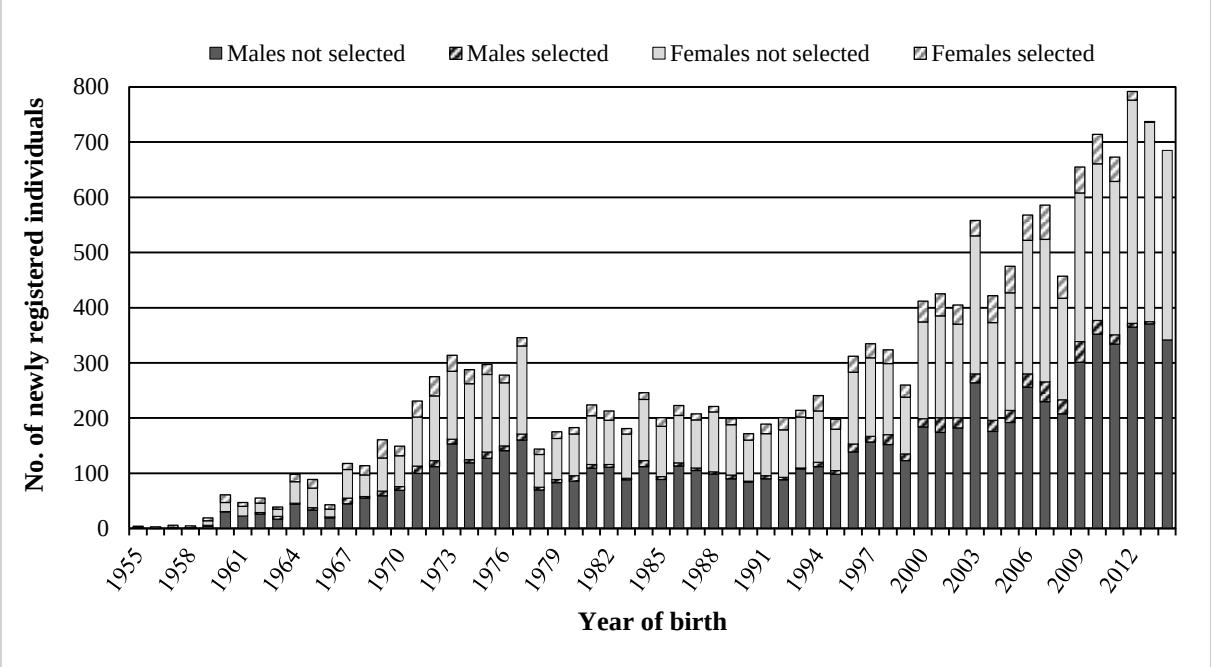

Figure 12. *Number of newly registered Stabyhouns per year of birth, comprising new-born pups and founders, that were either selected for breeding or not (yet)*

The mean age of the parents at the birth of their progeny increased over time, from 3.77 years in 1965-1974 to 4.52 years in 2010-2014. The generation interval increased from 3.78 in 1965-1974 to 4.53 in 2005-2009. A mean generation interval of 4.42 was found for all parents born before 2002.

The number of (selected) progeny per dam stayed more or less constant over the years, whereas the number of (selected) progeny per sire showed major fluctuations (figure 13). The high peaks in the graph are due to the use of a limited number of sires and an excessive use of a few popular sires. The peaks in 1954 and 1958 were due to the only two sires born in those years, which had respectively 58 and 123 progeny. The major peaks in 1963, 1965, 1971 and 1991 were based on respectively 5, 5, 13 and 6 sires. Within these years there were many popular sires with more than 100 progeny and more than 25 selected progeny. One sire, named Kast Fen ‘t Houneheim and born in 1971, was extremely

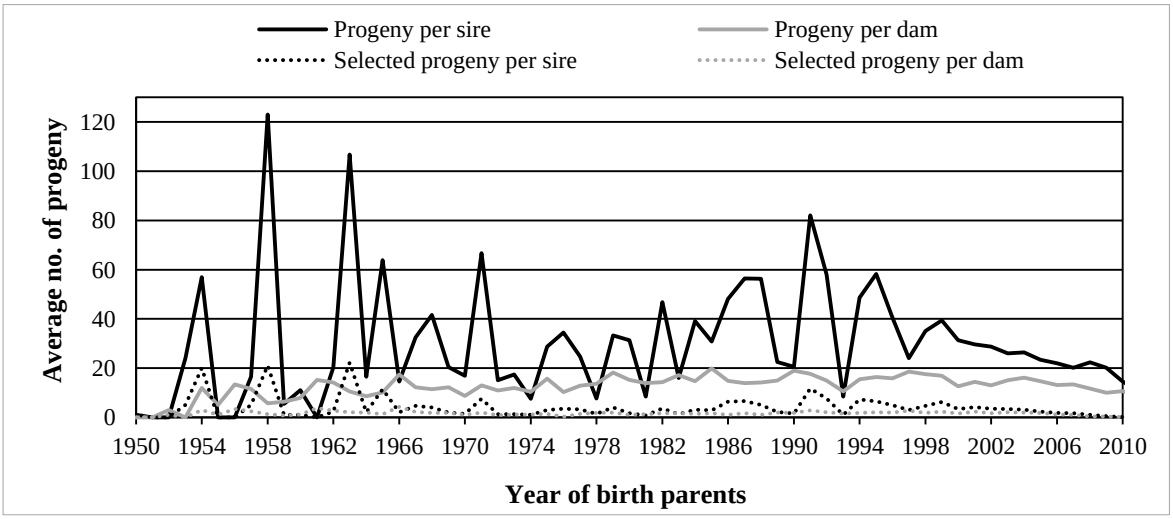

Figure 13. *Average number of (selected) progeny per Stabyhoun-parent from 1950 to 2010*

popular with a total of 392 progeny and 58 selected progeny. The observed popular sire effect was still present in the last decades, although less extreme. In 2010-2014 there were on average 73 distinct sires per year while the 5 most popular sires were responsible for 11% of all the pups in this period.

Litter size and life span did not show a trend over time aside from minor fluctuations around the mean.

Pedigree completeness

During the first two decades only founders and their progeny were registered, explaining the first peaks in figure 14 (left). Since 1958 the pedigree completeness has increased along with the growth of the breed and since 1994 all registered Stabyhouns have had ≥ 5 ancestral generations completely known. The EcG fluctuated during the first decades and then steadily increased to almost 15 in 2015.

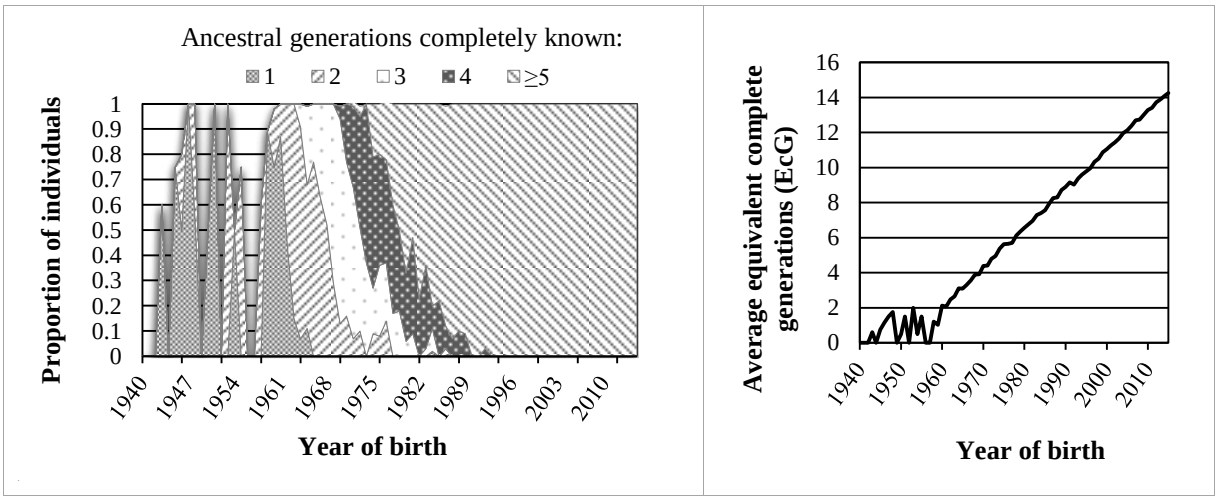

Figure 14. *Pedigree completeness: proportion of Stabyhouns with x ancestral generations completely known (left) and average equivalent complete generations (right) from 1940 to 2015*

The maximum longest ancestral path (LAP) was 24 and over 76% of the Stabyhouns had a LAP of >10. The distribution of LAPs is included in appendix III.

Inbreeding and coancestry

A total of 16404 inbred Stabyhouns were identified in the studbook. The increase in the average COI ($\bar{F}$) and the average coancestry ($\bar{f}$, including self-relationships) over time is shown in figure 15.

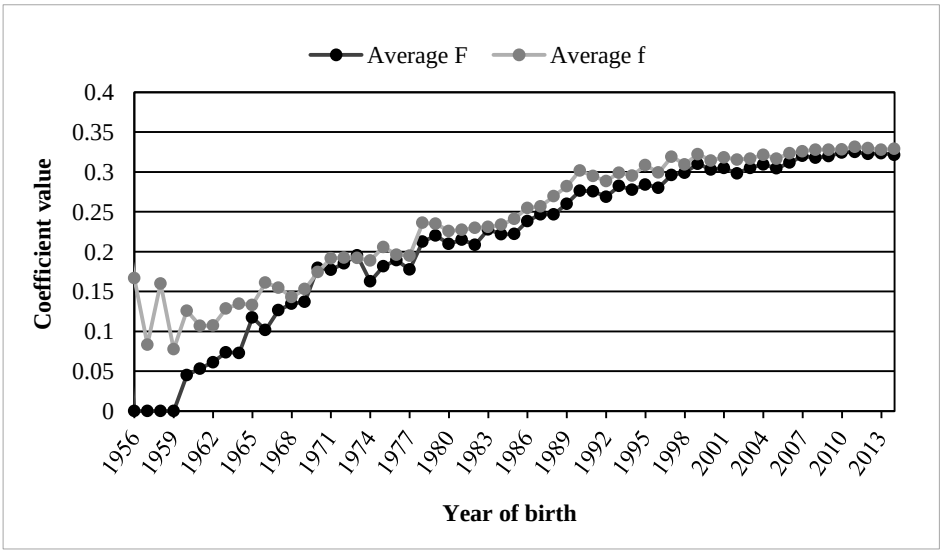

Figure 15. *Average inbreeding ($\bar{F}$) and average coancestry ($\bar{f}$, including self-relationships) in the Stabyhoun population from 1956 to 2014*

The size of the interval between $\bar{F}$ and $\bar{f}$ decreased during the first decades of the breed. Since 1968, this interval has been 2-3 years, which is smaller than the average generation interval of 4.42 years. To explore the influence of international population stratification on the relatively low relatedness, the coancestry per country was compared to the overall coancestry (table 20).

Table 20. *Average inbreeding ($\bar{F}$) and average coancestry ($\bar{f}$) in the largest subpopulations of Stabyhouns in 2005-2015*

Country	No. individuals	$\bar{F}$	$\bar{f}$	Year of birth first registered individual
The Netherlands	4579	0.3203	0.3259	1940
Sweden	724	0.3189	0.3369	1991
Finland	322	0.3233	0.3424	1990
Denmark	547	0.3183	0.3394	1994
Norway	65	0.2955	0.3454	1998
United States of America	220	0.3231	0.3408	1994
Overall	*6505*	*0.3199*	*0.3253*	-

Within most international subpopulations the $\bar{f}$ was 1-2% higher than the overall $\bar{f}$, indicating only a small effect of population stratification.

Genetic diversity 1: inbreeding rate and effective population size

The current gene diversity (GD) was estimated to 67% of the initial GD. An average inbreeding rate per generation ($\Delta\bar{F}_{gen}$) of 2.96% was found for the period of 1956 to 2015, corresponding to an effective population size based on this rate (Ne^F) of 16.91 (figure 16).

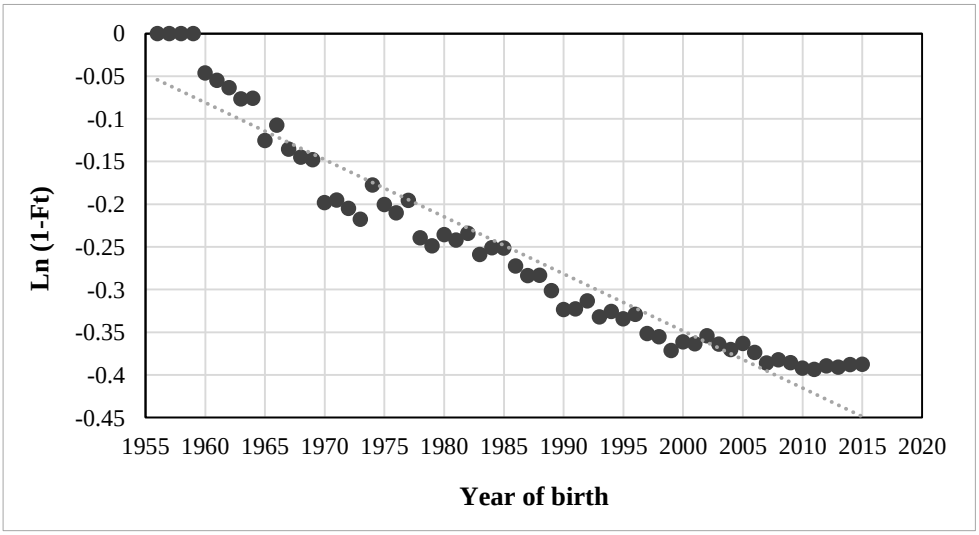

Figure 16. *Logarithmic regression of 1- $\bar{F}_x$ against the year of birth of Stabyhouns for the period 1956-2015. The slope represents the increase in the average COI per year* ($\Delta\bar{F}$)

The seemingly curved, rather than linear, relationship in figure 16 indicates a decrease of $\Delta\bar{F}$ over time. This decrease in $\Delta\bar{F}$ over time is further visualized per period of 10 years in figure 17. Especially during the first decades of the breed the $\Delta\bar{F}_{gen}$ was very large, with values of over 4%. Despite the steady decrease in $\Delta\bar{F}_{gen}$, the population has stayed in the highest risk category of table 2 for over half a century. Around 2000, the $\Delta\bar{F}_{gen}$ came below the 1% threshold. In the period 2010-2015 the $\Delta\bar{F}_{gen}$ was even negative and the Ne^F for this period could therefore not be calculated.

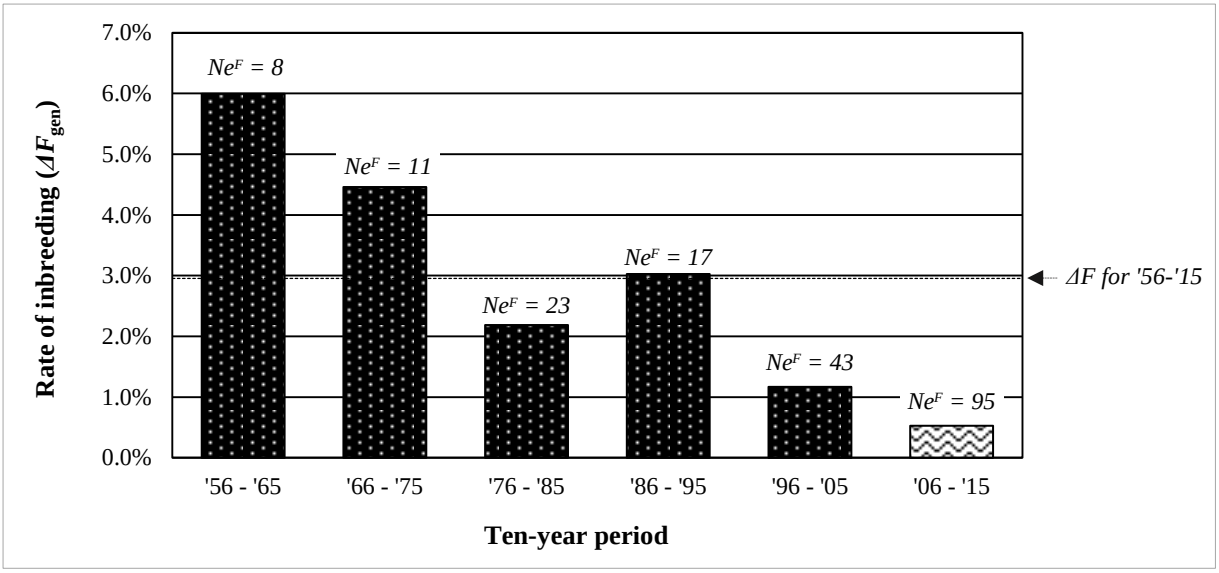

Figure 17. *Rate of inbreeding ($\Delta\bar{F}_{gen}$) and inbreeding effective population size (Ne^F) in the Stabyhoun per ten-year period from 1956 to 2015. Bars are filled according to the risk categories in table 2: black bars with white dots indicate an (unacceptably) high risk with $\Delta\bar{F}_{gen}$> 1.0%, and the zigzag indicates a high risk with a $\Delta\bar{F}_{gen}$ between 0.5% and 1.0%.*

The coancestry effective population size (Ne^f) from 1956 to 2015 was 20.25 and thereby slightly higher than the Ne^F.

Genetic diversity 2: probability of gene origin

A total of 30 founders were identified (f_{tot}) in the studbook. Of these founders 11 were born in 1940-1950, 18 in 1950-1960 and 1 in 1982. There were no semi-founders present.

Table 21. *The 10 founders with the highest contribution to the gene pool (GP) of Stabyhouns born in 2010-2015 (n = 3764)*

Registration number	Name	Sex	Year of birth	Contribution to GP*	Contribution to $\bar{F}^{\dagger}$	Contribution to $\bar{f}^{\dagger}$
NIET GEREG. 2	Staby	F	1944	0.207360	0.076155	0.077042
NIET GEREG. 1	Bruno	M	1950	0.207360	0.076155	0.077042
NHSB G0 253630	Autgertsje	F	1954	0.195788	0.063645	0.064540
VR 27	Albert	M	1940	0.122866	0.042787	0.043331
VR 14	Aukje	F	1940	0.122866	0.042787	0.043331
NHSB G0 250780	Adelheit	F	1958	0.045278	0.010575	0.010814
NHSB G0 227627	Aagje	F	1957	0.034630	0.003364	0.003570
NHSB G0 250784	Aisje	F	1956	0.017613	0.001684	0.001788
NHSB G0 227624	Hertha	F	1956	0.017194	0.002717	0.002812
VR 50	Durk	M	1944	0.012087	0.001584	0.001652

* The probability of gene origin, i.e. the fraction of genes in the group passed on by the founder.
† Contribution of genes of founders to the average inbreeding coefficient and the average coancestry of the group, with $\bar{F}$ = 0.323316 and $\bar{f}$ = 0.327879 for the Stabyhouns born in 2010-2015.

The founder equivalent (f_e) for the 2010-2015 reference group was 6.30, the founder genome equivalent (f_{ge}) 1.52 and the effective number of non-founders 2.01. The f_e / f_t ratio of 0.21 implies a considerable amount of selection during the existence of the breed. The unequal contribution of

founders is further illustrated in table 21, in which the ten founders with the highest contribution to the gene pool (GP) of the reference group are listed. These ten founders are together responsible for over 98% of the current GP. Over 85% is contributed by the five most influential founders: Staby, Bruno, Autgertsje, Albert and Aukje. The genes of these five founders together contributed 0.302 to the average inbreeding coefficient (93%) and 0.305 the average coancestry (also 93%). There were 16 founders (53%) without any contribution to the current GP.

The proportion of genetic diversity that was lost due to an unequal contribution of founders ($1 - GD^*$) was 8.1%. The total proportion of genetic diversity lost due to selection and drift ($1 - GD$) was 32.8%. The total loss due to random drift in the non-founder generations was therefore 24.7%, which resulted in an average loss of 2.03% per generation based on the ~12 generations that have passed since the founders lived.

4.2. Inherited disorders

In this paragraph the results per inherited disorder are presented. At the end of the paragraph, in table 26, an overview of all the analysed disorders is given.

Hip dysplasia (HD)

A total of 2621 Stabyhouns, all born between 1964 and 2014, had a registered HD status. Of these individuals 55.9% was diagnosed with at least HD-B and 32.7% with at least HD-C. The individuals with at least HD-C were considered as affected.

The prevalence of HD was further evaluated for Stabyhouns born in the period 2007-2012 and registered in the Netherlands, Sweden, Finland, Denmark, Norway and the United States of America (table 22A). The overall prevalence of HD in this period and these countries was between 5.4% and 20.8%. A *minimal prevalence* of 5.4% was found under the assumption that all affected individuals in the population were also screened. If for example only half of the affected individuals in the population was screened, the actual prevalence would be 10.8%. A *maximal prevalence* of 20.8% was found in the screened or 'hospital' population.

The minimal and maximal prevalence had a range of respectively 1.7-24.8% and 4.5-46.4% in the different countries. Both minimal and maximal prevalence were highest in Sweden and Finland. The Netherlands showed the lowest minimal prevalence. These findings coincide with a relatively high proportion of Stabyhouns that is screened in Sweden and Finland (> 60%) and a relatively small proportion that is screened in the Netherlands (17.4%).

When affected individuals were considered as individuals with at least HD-B, an overall minimal and maximal prevalence of respectively 10.3% and 39.4% were found.

Females initially seemed to have a higher minimal prevalence than males, with their odds of being diagnosed with at least HD-C being 1.413 times the odds for males (CI_{95}: 1.229 - 1.625). This sex predisposition, however, was not present for the maximal prevalence (for which a not significant OR of 1.136 was found). The observed sex difference on the population level can be (largely) explained by the confounding fact that females were more often screened than males; 60.6% of the screened population was female whereas 50.5% of the whole population was female.

A positive relationship between body weight and HD-severeness was found in Stabyhouns of which the HD status, body weight and shoulder height were known (table 23). Fisher's protected LSD confirmed that the mean weight in groups HD-C, -D and -E was higher than the mean weight in groups HD-A and –B at the 0.05 significance level.

The relationship between BMI, calculated as an individuals' weight divided by its squared shoulder height, and HD-score was less clear. Because of the unequal variance for BMI over the HD-scores (table 23), the non-parametric Kruskal-Wallis test was performed. This test showed that the mean BMI

was not equal for all groups, but was not able to identify any significant pairwise differences. However, a positive relationship between BMI and HD-status seems to be present in table 23.

Table 23. *Mean ± standard deviation of weight and BMI per HD-status in the Stabyhoun*

	HD-status				
	A (n = 653)	B (n = 253)	C (n = 231)	D (n =130)	E (n = 7)
Weight (kg)	20.62 ± 2.91	20.42 ± 2.77	21.31 ± 2.95	21.52 ± 2.84	22.86 ± 4.45
BMI (kg/m^2)	81.81 ± 9.36	81.59 ± 9.66	85.91 ± 29.21	88.75 ± 55.31	85.50 ± 15.11

A positive relationship was also found between the proportion of Stabyhouns affected by HD and their age at screening. This relationship was thought to be independent of the relationship between HD and body weight, as the correlation between age and body weight in the considered Stabyhouns was very poor ($R^2 = 0.02$). The proportion of screened Stabyhouns with HD-C, HD-D or HD-E increased with a higher age (figure 18); the total proportion of affected individuals was for example 0.48 in screened Stabyhouns of >3 years old and 0.22 in screened Stabyhouns between 1 and 1.5 years old.

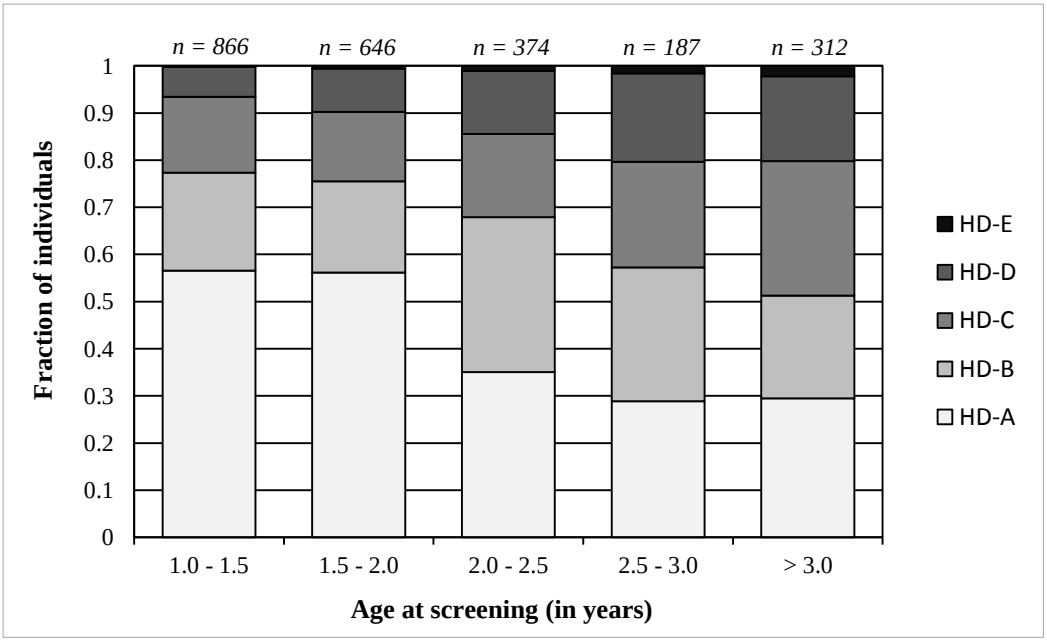

Figure 18. *Fraction of Stabyhouns per HD-score per age-class for Stabyhouns with a known age at HD-screening*

Table 22A. *ED and HD status of screened Stabyhouns born in 2007 - 2012 in the Netherlands (NL), Sweden (SE), Finland (FI), Denmark (DK), Norway (NO) and the United States of America (USA)*

		NL		SE		FIN		DK		NO		USA		Total	
	Status	n	%	n	%	n	%	n	%	n	%	n	%	n	%
HD	HD - A	397	83.2	93	35.2	23	20.9	41	47.7	26	83.9	33	75.0	613	60.6
	HD - B	15	3.1	106	40.2	36	32.7	21	24.4	2	6.5	9	20.5	189	18.7
	HD - C	49	10.3	53	20.1	39	35.5	15	17.4	0	0	2	4.5	158	15.6
	HD - D	16	3.4	12	4.5	10	9.1	8	9.3	3	9.7	0	0	49	4.8
	HD - E	0	0	0	0	2	1.8	1	1.2	0	0	0	0	3	0.3
	Total	*477*	*100*	*264*	*100*	*110*	*100*	*86*	*100*	*31*	*100*	*44*	*100*	*1012*	*100*
ED	Grade 0	26	52.0	206	86.2	80	78.4	61	83.6	25	83.3	35	89.7	433	81.2
	Grade 1	1	2.0	30	12.6	17	16.7	9	12.3	3	10.0	2	5.1	62	11.6
	Grade 2	0	0	2	0.8	4	3.9	1	1.4	0	0	2	5.1	9	1.7
	Grade 3	0	0	0	0	1	1.0	2	2.7	1	3.3	0	0	4	0.8
	Affected*	23	46.0	1	0.4	0	0	0	0	1	3.3	0	0	25	4.7
	Total	*50*	*100*	*239*	*100*	*102*	*100*	*73*	*100*	*30*	*100*	*39*	*100*	*533*	*100*

* Individuals registered as affected, but without known grade.

Table 22B. *Minimal and maximal prevalence of ED and HD in Stabyhouns born in 2007 – 2012 per county of registration*

		NL	SE	FIN	DK	NO	USA	Total
Total number of born individuals		*2743*	*441*	*206*	*326*	*33*	*115*	*3864*
HD	% of total screened	17.4	59.9	53.4	26.4	93.9	38.3	26.2
	Minimal prevalence*	2.4	14.7	24.8	7.4	9.1	1.7	5.4
	Maximal prevalence*	13.6	24.6	46.4	27.9	9.7	4.5	20.8
ED	% of total screened	1.8	54.2	49.5	22.4	90.9	33.9	13.8
	Minimal prevalence*	0.9	7.5	10.7	3.7	15.2	3.5	2.6
	Maximal prevalence*	48.0	13.8	21.6	16.4	16.7	10.3	18.8

* Minimal prevalence = $n_{affected} / n_{total}$ x 100; Maximal prevalence = $n_{affected} / n_{screened}$ x 100. Affected individuals have at least HD-C or ED-grade 1

Elbow dysplasia (ED)

A total of 942 Stabyhouns, all born between 1989 and 2014, had a registered ED status. The prevalence of ED in these individuals equalled 19.3%, considering dogs with grade 1 or higher dysplastic. In the more recent period 2007-2012 the maximal prevalence was slightly lower, namely 18.8% (table 22B). A minimal prevalence of 2.6% was found for this period. The minimal and maximal prevalence showed a range of respectively 0.9-15.2% and 10.3-48.0% in the different countries. The Netherlands had both the lowest minimal prevalence and the highest maximal prevalence, which can be (partly) subscribed to the small proportion that is screened for ED (1.8%) and the 23 Stabyhouns that were registered as 'affected' without a known grade.

For all the screened individuals a male:female OR of 1.524 (CI_{95}: 1.100 – 2.110) was found. This higher risk for males, however, was not significant for the group 2007-2012 (OR CI_{95}: 0.891 – 2.137).

The relationship between HD and elbow dysplasia (ED) was investigated using the 850 Stabyhouns that were examined for both disorders. The odds to be affected by ED was 1.96 times higher for HD-affected Stabyhouns than for HD-unaffected Stabyhouns (CI_{95}: 1.259 – 3.040). In table 24 it is shown that this association was consistent over the different grades of the disorders. A chi-square test for independence could not be performed on the clusters in this table, because of the limited number of severely affected individuals. Clustering HD-B and HD-C together (instead of A and B), however, solved this issue and showed a very similar and significant pattern (χ^2 = 13.62 and P = 0.009).

Table 24. *ED-status percentages of Stabyhouns per HD status*

		% per ED-status		
HD-status	n	Grade 0	Grade 1	Grade 2 / 3
A / B	639	90.30	8.45	1.25
C	153	84.31	11.11	4.58
D / E	60	78.33	15.00	6.67

To explore the relationship between body weight and ED all affected individuals (grade 1 up to 3 and the separate 'affected' group) were clustered together. The mean weight of these affected individuals was 22.08 kg, which tended to be higher than the mean weight of 20.88 kg of unaffected individuals (two-sided t-test: P = 0.087). Regarding BMI no significant difference was found, although the mean BMI was higher for affected individuals than for unaffected individuals (85.02 versus 81.91).

Epilepsy

A total of 132 Stabyhouns with registered epilepsy were identified. These individuals were all born between 1977 and 2013. In the period 2008-2012 an average of 5.6 pups with epilepsy were reported and registered per year, resulting in an estimated prevalence of 0.85%.

Males were significantly more often reported as affected than females, with an OR of 1.86 (CI_{95}: 1.28 – 2.68). This sex difference was less pronounced and not significant in the more recent period 2000 – 2013, for which an OR of 1.40 (CI_{95}: 0.88 – 2.23) was found. The predisposition was completely absent in the period 2008-2013.

Patent ductus arteriosus (PDA)

There were 62 Stabyhouns registered as affected by a heart disorder, of which 48 were indisputably affected by PDA. Most of the other 15 individuals were either registered as affected by a different heart disorder or as healthy after some initial signs of dysfunction (like heart murmur). A few individuals lacked registration on the type of dysfunction.

The first individual registered with PDA was born in 1999. A prevalence of 0.48% was found for the period 1999-2014. The prevalence was higher in recent years, namely 0.75% for the period 2009-2014. No sex difference was found in the prevalence of PDA (CI_{95} of male-female OR: 0.45 – 1.41).

Cerebral dysfunction (CD)

A total of 16 pups were registered as affected by CD. These pups were born between 2009 and 2012 in 5 distinct litters. Based on the number of affected pups and the total number of pups in these years, a prevalence and mutant allele frequency (*q*) of respectively 0.58% and 0.076 were estimated. No significant sex predisposition was found.

In 2004 another litter with three pups affected by CD was born. These pups, however, were not included here as they were not registered.

The DNA-test performed on unaffected Stabyhouns in 2014 had 170 homozygous normal individuals and 26 carriers as outcome. Using the HWE equation this gives a mutant allele frequency (*q*) that is 13.08 times smaller than the frequency of the normal allele (*p*). As this ratio is in line with the estimated *q* of 0.076 there is no indication of a deviation from HWE.

Von Willebrand Disease, type-I (vWD-I)

There were 53 individuals with a registered DNA-test result. These Stabyhouns were all born in 2000-2013. The distribution of their genotypes is presented in table 25.

Table 25. *Observed genotype of Stabyhouns with registered vWD-I screening result and expected genotypes under HWE (n = 53)*

VWD-I genotype	$n_{observed}$	$\%_{observed}$	$n_{expected}$
Homozygous normal	16	30.19	18.72
Heterozygous	31	58.49	25.56
Homozygous mutant	6	11.32	8.72
Total	*53*	*100*	*53*

In the group with a registered screening result the *p* and *q* equalled respectively 0.594 and 0.406. The best estimate for the overall prevalence of vWD-I in the population, combining the prevalence of both homozygous mutants and the less severely affected heterozygotes, was 69.8%. There were more heterozygotes than expected under HWE, as shown in the table. This difference, however, was not significant (chi-square goodness of fit: $\chi^2 = 2.40$ and P = 0.301), which is in line with the passive breeding policy regarding vWD-I.

Overview disorders

An overview of the analysis of inherited disorders for the Stabyhoun population is given in table 25.

Table 26. *Overview of main results per inherited disorder in the Stabyhoun*

Disorder	Prevalence (%)	Allele freq. (q)	Remarks
HD	5.4 - 20.8	NA	No sex predisposition, females more often screened than males, sign. relationship with body weight and age and association with ED
ED	2.6 - 18.8	NA	Males seem to be more often affected than females, tendency to relationship with body weight, association with H
Epilepsy	0.85	NA	Males seem to be more often affected than females
PDA	0.75	NA	No sex predisposition
CD	0.58	0.076	No sex predisposition, no significant deviation from HWE
vWD-I	69.8	0.406	No sex difference, no significant deviation from HWE

4.3. Simulation of breeding strategies

The results obtained in the pedigree analysis were used as input for the simulation program. The input used for the baseline simulation is included in appendix IV. The results of simulating different breeding strategies are presented below.

Sire breeding restrictions

Sire restrictions were generally very effective in reducing the inbreeding rate in the simulated Stabyhoun populations, as shown in table 27A. The high effectiveness can be explained by the presence of a popular sire effect in the (simulated) breed. Very strict breeding restrictions per life, however, were found to be less effective than moderate breeding restrictions per life.

When simulating stricter breeding restrictions per life the generation interval shortened, irrespective of the simulated breeding restriction per year (table 27B).

Table 27A. *Effect of restricting the use of sires* per year and life on mean $\Delta\bar{F}_{gen}$ (in %) of the 25 simulated Stabyhoun populations*

Max no. of litters / year	Max no. of litters / life:				
	No†	20	10	5	2
No	4.37	-	-	-	-
20	2.89	0.98	-	-	-
10	1.04	0.66	0.73	-	-
5	0.48	0.42	0.44	0.63	-
2	0.31	0.32	0.31	0.26	0.30

*Dams were restricted to maximal 5 litters per life

† The 5 most popular sires sired 11% of offspring

Table 27B. *Effect of restricting the use of sires* per year and life on the mean generation interval (in years) of the 25 simulated Stabyhoun populations*

Max no. of litters / year	Max no. of litters / life:				
	No†	20	10	5	2
No	4.04	-	-	-	-
20	4.08	3.30	-	-	-
10	4.04	3.67	3.65	-	-
5	4.01	3.87	3.80	3.63	-
2	3.98	3.99	3.92	3.66	2.85

*Dams were restricted to maximal 5 litters per life

† The 5 most popular sires sired 11% of offspring

The breeding restrictions that are currently implemented by the breed club - a maximum of 2 litters per sire per year and 10 per life (of which 8 in the same country) - resulted in one of the lowest inbreeding rates in table 27A. The decrease in generation interval was relatively small and the average COI after 50 years was the lowest for all the simulated combinations of breeding restrictions.

Steering on relatedness with mating programs

Steering on relatedness was shown to be very effective when breeding restrictions were absent and slightly to moderately effective in the presence of sire breeding restrictions (table 28).

Table 28. *Effect of various mating programs on the mean $\Delta\bar{F}_{gen}$ (in %) and mean generation interval (in years) of 25 simulated Stabyhoun populations (100 years each), both with and without breeding restrictions*

Sire breeding restrictions	Mating program	$\Delta\bar{F}_{gen}$			Generation interval
		Overall	Year 0-20	Year 20-100	
None	None	4.40	4.36	4.43	3.99
	Min. coancestry mating	4.17	3.29	4.21	4.27
	Min. population coancestry	0.42	0.39	0.42	4.00
	Optimal contributions	0.27	0.23	0.27	4.53
Max. 2 litters per sire per year and 10 litters per sire per life	None	0.30	0.32	0.30	3.95
	Min. coancestry mating	0.24	0.03	0.26	4.40
	Min. population coancestry	0.17	0.18	0.17	4.27
	Optimal contributions	0.18	0.15	0.18	4.64

Miminum coancestry mating was mainly effective on the short term. On the long run, minimising population coancestry and the use of optimal contributions both led to a reduction in $\Delta\bar{F}_{gen}$ of 0.12-0.13% on top of the reduction caused by the applied breeding restrictions. These methods also seemed to increase the generation interval by approximately a half year.

Enlarging the breeding population size

The effect of enlarging the breeding population size on the inbreeding rate is shown in table 29A. Only the addition of many extra Stabyhouns to the breeding population led to a substantial decrease in $\Delta\bar{F}_{gen}$. The breeding population size had to be enlarged 1.75 to 2 times (i.e. more than 250 extra breeding individuals) to get to a decrease in $\Delta\bar{F}_{gen}$ that was similar to the decrease realized with the use of minimal population coancestry or optimal contributions. In absolute numbers it was more effective to add extra breeding males than females, which can be explained by the male:female ratio in the baseline scenario that was lower than 1:2.

***Table 29A**. Effect of increasing the number of available breeding males and females per year on the mean $\Delta\bar{F}_{gen}$ (in %) of the 25 simulated Stabyhoun populations*

Males available for breeding†	Females available for breeding						
	n = 222 (baseline*)	n = 233 (+5%)	n = 244 (+10%)	n = 278 (+25%)	n = 333 (+50%)	n = 389 (+75%)	n = 444 (+100%)
n = 105 (baseline*)	0.31	0.30	0.29	0.28	0.25	0.24	0.22
n = 110 (+5%)	0.30	0.30	0.29	0.27	0.25	0.23	0.22
n = 116 (+10%)	0.29	0.29	0.29	0.26	0.25	0.23	0.21
n = 131 (+25%)	0.28	0.28	0.28	0.26	0.23	0.21	0.20
n = 158 (+50%)	0.26	0.25	0.25	0.23	0.21	0.19	0.19
n = 184 (+75%)	0.25	0.24	0.24	0.22	0.20	0.20	0.17
n = 210 (+100%)	0.24	0.22	0.21	0.20	0.18	0.17	0.16

* The estimated number of yearly available breeding individuals for the period 2010-2014
† Sires were restricted to maximal 2 litters per year and maximal 10 litters per life

***Table 29B**. Effect of increasing the number of available breeding males and females per year on the generation interval (in years) of the 25 simulated Stabyhoun populations*

Males available for breeding†	Females available for breeding						
	n = 222 (baseline*)	n = 233 (+5%)	n = 244 (+10%)	n = 278 (+25%)	n = 333 (+50%)	n = 389 (+75%)	n = 444 (+100%)
n = 105 (baseline*)	3.92	3.96	3.92	3.94	3.88	3.76	3.68
n = 110 (+5%)	3.93	3.98	3.93	3.95	3.89	3.79	3.66
n = 116 (+10%)	3.94	4.01	3.98	3.94	3.92	3.85	3.72
n = 131 (+25%)	3.94	3.94	3.95	3.94	3.92	3.92	3.84
n = 158 (+50%)	3.96	3.97	3.96	3.96	3.97	3.96	3.95
n = 184 (+75%)	3.95	3.98	3.96	4.01	4.01	4.01	3.96
n = 210 (+100%)	4.02	3.93	3.95	4.03	3.97	3.98	4.01

* The estimated number of yearly available breeding individuals for the period 2010-2014
† Sires were restricted to maximal 2 litters per year and maximal 10 litters per life

For scenarios with a small number of available breeding males and a large number of available breeding females the generation interval was relatively short (table 29B). Although this finding was unexpected, it can be explained by the low male:female ratio as well. When there were relatively few sires available, they were more often used at a low age in the simulation in order to keep the number of litters per year equal to half the number of available females. The average age at which sires reached

their breeding restriction limit of 10 litters per life would therefore go down, resulting in a lower generation interval in these scenarios.

Selecting against monogenic inherited disorders

The effect of direct selection against monogenic disorders in the Stabyhoun is shown in table 29 (for selection against vWD-I) and table 30 (for selection against CD).

Table 30. *Effect of different types of selection against vWD-I on the $\Delta\bar{F}_{gen}$ and the mutant allele frequency (with an initial q of 0.406) in 25 simulation runs of 50 years each.*

Selection against	$\bar{F}$ at 50y (in %)	$\Delta\bar{F}_{gen}$ (in %)		Mean fixation y in fixed runs (n)	Mean *q* at 50y in non-fixed runs (n)
		0-5y	6-50y		
none of the individuals	5.39	0.44	0.42	NA (0)	0.373 (25)
homozygotes	5.56	0.41	0.44	NA (0)	0.051 (25)
homozygotes and ♂ heterozygotes	5.75	0.81	0.44	23 (25)	0.001 (1)
homozygotes and ♀ heterozygotes	5.97	0.59	0.44	28 (24)	NA (0)
homozygotes and all heterozygotes	6.13	1.27	0.43	1 (25)	NA (0)

Table 31. *Effect of different types of selection against CD on the $\Delta\bar{F}_{gen}$ and the mutant allele frequency (with an initial q of 0.076) in 25 simulation runs of 50 years each.*

Selection against	$\bar{F}$ at 50y (in %)	$\Delta\bar{F}_{gen}$ (in %)		Mean fixation y in fixed runs (n)	Mean *q* at 50y in non-fixed runs (n)
		0-5y	6-50y		
none of the individuals	5.39	0.44	0.42	41 (1)	0.068 (24)
homozygotes	5.40	0.51	0.43	34 (4)	0.036 (21)
homozygotes and ♂ heterozygotes	5.70	0.51	0.45	15 (25)	NA (0)
homozygotes and ♀ heterozygotes	5.54	0.44	0.45	18 (25)	NA (0)
homozygotes and all heterozygotes	5.53	0.52	0.44	1 (25)	NA (0)

In the tables above it is underlined that selection against a monogenic disorder with a high prevalence can increase the $\Delta\bar{F}_{gen}$ in primarily the first years of selection. Selection against a rare disorder, like CD, hardly affects the $\Delta\bar{F}_{gen}$ and consistent selection against carriers of such a disorder results in a fast fixation of the normal allele.

For both vWD-I and CD selection against male heterozygotes led to a lower mean fixation year than selection against female heterozygotes. However, the $\Delta\bar{F}_{gen}$ for the first years was lower in the latter scenario.

5. Discussion

The enormous variety between dog breeds in population status and genetic diversity is underlined by this study. In addition to the difference in population size, with the Markiesje as one of the smallest and the Stabyhoun as one of the largest original Dutch dog breeds (Hoving & Cnossen, 2009), the analysed breeds differ in many population parameters (table 32).

Table 32. *Comparison of population parameters of the Markiesje and the Stabyhoun*

Category	Parameter	Markiesje	Stabyhoun
General	Year of breed club foundation	1979	1942
	Living population size	1200	7000
	Number of pups born per year (for recent years)	120	700
	Percentage of males selected for breeding (%)	26.2	7.8
	Percentage of females selected for breeding (%)	36.7	17.6
	Litter size	4.2	6.3
	Generation interval (years)	3.42	4.42
Genetic diversity I	Current GD as proportion of founder GD	0.87	0.67
	Average COI in 2015 (%)	11.3	33.4
	Inbreeding rate for whole existence of breed (%)	1.27	2.96
	Inbreeding rate for period 2005-2014 (%)	1.04	0.53
	Interval between inbreeding and coancestry (years)	8.0	2.5
Genetic diversity II	Total number of founders	54	30
	Founder equivalent	14.6	6.3
	Founder genome equivalent	4.1	1.5
	Effective number of non-founders	5.7	2.0
Inherited disorders	Prevalence* of:		
	- PL, polygenic (%)	21.74	NA
	- PRCD, monogenic (%)	1.37	NA
	- Neuropathology, monogenic (%)	0.8	NA
	- HD, polygenic (%)	NA	20.8
	- ED, polygenic (%)	NA	18.8
	- Epilepsy, polygenic (%)	NA	0.85
	- PDA, polygenic (%)	NA	0.75
	- CD, monogenic (%)	NA	0.58
	- vWD-I, monogenic (%)	NA	69.8
Effectiveness of breeding strategies on decreasing inbreeding rate	Sire breeding restrictions	Low	Very high
	Applying minimum coancestry mating	High	High
	Enlarging population size	Very high	Low

* For PL, PRCD, HD and vWD-I the maximal prevalence, i.e. the prevalence within screened individuals, is given. For the other disorders the minimal prevalence, i.e. the percentage of all individuals that is registered as affected. For vWD-I it is the percentage of screened individuals that is heterozygous or homozygous for the mutant allele (both are affected).

The current genetic diversity in the Markiesje (87% of the GD that was present in 54 founders and 44 semi-founders) is considered to be larger than in the Stabyhoun (67% of GD present in 30 founders). The current GD in both breeds is likely overestimated, because the founders that are assumed to be unrelated could be related in reality. This overestimation latter is especially an issue for breeds with a long history prior to registration (Mäki, 2010). As the Stabyhoun was already bred in Friesland for over a century prior to the set-up of the studbook, the mean coancestry in this breed will be a strong underestimation. The violation of the founder assumption is expected to be less severe in the Markiesje, as the founders of this breed came from geographically distinct locations.

The Markiesje

Despite the decreasing inbreeding rate since 2001, the Markiesje is still considered to have an unacceptably high risk on the accumulation of inherited disorders. The overall inbreeding effective population size of 39 falls into the lower part of the range of effective population sizes for breeds studied in France (Leroy et al., 2013) and Australia (Shariflou et al., 2011) that showed ranges in Ne^F of 33-257 and 26-1090, respectively. As the average coancestry determines what the average COI can be on the long term (Oldenbroek & Windig, 2012), and the COI has followed the coancestry at an interval of more than twice the generation interval up till now, it is expected that the average COI will further increase in the (near) future if no additional measures are taken.

The small census size of the population is considered to be the main reason for the fast increase in inbreeding. Despite the remarkably high percentage of Markiesjes that is used for breeding compared to the Stabyhoun and to other breeds (Hoving & Cnossen, 2009; Voges & Distl, 2009; Leroy & Baumung, 2011), the absolute number of breeding Markiesjes is low, causing inevitable inbreeding. The simulations results indeed showed that enlarging the effective population size was highly effective in reducing the inbreeding rate. Adding females to the breeding population appeared to be more effective than adding males. Although this finding was unexpected, it can be explained by the relative excess of males in the baseline scenario. As every female is only allowed to give birth once every two years and males can mate multiple times a year and are young when they sire, it is more useful to invest in extra females than in extra males.

Enlarging the population size in a responsible way is, however, not a simple task. A breed-specific factor that limits breed growth is litter size. Although the observed litter size is exactly equal to the mean litter size of 4.2 for small dogs of 5-10 kg (Borge et al., 2011), it is low compared to the mean litter size of 5.4 for breeds of all sizes reported by Borge et al. and very low compared to the litter size of the Stabyhoun. In addition, the small number of Markiesjes available for breeding limits the possibility of enlarging the population without causing a major increase in relatedness. A good option would therefore be to make use of the studbook's semi-openness and enlarge the size of the breed via outcrossing with look-alikes (i.e. dogs that resemble the Markiesje but have an unknown ancestry). The effectiveness of outcrossing on improving the genetic health of the breed will logically depend on the number of 'outsiders' used and on the relatedness among these outsiders and between the outsiders and the registered Markiesjes. Because most Markiesje-look-alikes come from geographically distinct locations, it is thought that they have a relatively low relatedness to the current studbook. However, it is recommended to test look-alikes on relatedness before admitting them to the studbook. If the number of available look-alikes is limited, or the look-alikes have a high relatedness to the studbook, outcrossing with other breeds could be considered for further breed growth. Such outcrossing should always be performed systematically (see e.g. (Oldenbroek & Windig, 2012) for an outcrossing scheme) and it should be prevented that a large part of the population is crossed with a small group of strongly related individuals. Because one of the requirements for international recognition is a sufficiently large - and generally closed - population (FCI, 2003), it is advisable to enlarge the breed via outcrossing prior to FCI recognition.

Another factor that accelerates the loss of genetic diversity in the Markiesje is the generation interval, which is short compared to the generation interval of 4 to 5 years that is observed for the Stabyhoun and most other breeds (Mäki, 2010; Shariflou et al., 2011). The short generation interval is primarily caused by the use of young sires. Although a short generation interval might enable fast artificial selection, it also causes a faster increase in inbreeding over time. In addition to reducing this increase, a longer generation interval enables to intervene in the breeding process more easily (Oldenbroek & Windig, 2012). The observed increase in generation interval during the existence of the breed is therefore thought to be a very positive development.

A third factor to consider when aiming to maintain genetic diversity involves the founders and their contributions to the current gene pool. The number of observed founders (54) was moderate compared to other breeds (Oliehoek et al., 2009; Mäki, 2010; Shariflou et al., 2011) and created a reasonably broad initial genetic diversity together with the 44 semi-founders. The ratio of f_e/f_{tot} of 0.27 indicates an unequal contribution of the founders and associated loss of genetic diversity that is average compared to other breeds for which this ratio ranges from 0.12 to 0.42 (Voges & Distl, 2009; Mäki, 2010; Shariflou et al., 2011). The effective number of non-founders was high, indicating a relatively low amount of genetic diversity lost due to genetic drift relative to selection, compared to the Stabyhoun and other large breeds. This finding was unexpected because of the small size of the breed. Two over-represented founders, Pom and Rasta, were shown to be responsible for 30% of the current gene pool. To maintain the alleles of other founders as well, it is advisable to breed with Markiesjes that are less related to Pom and Rasta in future.

Other general breeding strategies that were analysed are breeding restrictions and mating programs. The sire breeding restrictions applied by the breed club were found to be effective in restricting the inbreeding rate. However, in view of the recommendation to enlarge the population size, it could be considered to loosen the restriction of maximal 5 litters per sire per life to 10 litters per life, as this hardly seemed to influence the inbreeding rate in the simulated populations (as long as no top sires will rise). Minimising population coancestry was also effective in reducing the inbreeding rate and might be an option for the breed, in addition to enlarging the population size.

The current number of inherited disorders found in the Markiesje (3) is low compared to other breeds. In a study of 50 breeds in the UK, the total number of disorders per breed ranged from 4 to 77 (Summers et al., 2010). The low number of disorders is no guarantee for the future, however, as the inbreeding rate remains unacceptably high.

The prevalence of PL in the screened Markiesjes was similar to the reported prevalence in other dog breeds in the Netherlands, such as 23.6% in Dutch Flat-Coated Retrievers (Lavrijsen et al., 2013) and 24% in Kooiker dogs (Wangdee et al., 2014). The higher risk for females is in agreement with findings in many breeds with small dogs (Priester, 1972; Alam et al., 2007; Lavrijsen et al., 2013; Soontornvipart et al., 2013). This sex predisposition might be related to hormonal differences or to X-linked factors that affect the expression of PL. Another hypothesized explanation is the influence of a temporarily increased body weight during gestation. This hypothesis could not be investigated for the Markiesje, as the body weight of the screened individuals was not known. In contrast to the female sex predisposition in small dogs, large dogs were reported to have a male predisposition for PL (Gibbons et al., 2006). The number of knee joints for which the luxation direction was known in the current study was too small for reliable inference. The most common luxation direction differs strongly between breeds (Alam et al., 2007; Lavrijsen et al., 2013; Wangdee et al., 2014). Selection against PL, as applied by the breed club, did not seem to be very effective. In future, techniques like genomic selection could help to combat polygenic disorders like PL.

Although over 29 breeds are known to be affected by PRCD (Downs et al., 2014), no prevalence estimates could be found for this disorder. The selection measures applied by the breed club were found to be effective, as the prevalence of PRCD decreased over time.

The neuropathology was and still is assumed to be monogenic recessive, as the disorder skips generations and is found in multiple individuals in a few affected litters. Because males and females seem to be equally often affected, the disorder is assumed to be autosomal. The proportion of affected individuals in the affected litters was, however, slightly (but not significantly) higher than expected for a monogenic autosomal recessive disorder. A possible explanation for this finding is the presence of epistasis, in which the effect of the neuropathology allele depends on the presence of an allele at another locus. In the Dalmatian, it is for example known that the occurrence of canine congenital

sensorineural deafness (CSSD) is associated with QTLs that influence pigment formation (Kluth & Distl, 2013). The GWAS that is currently being performed by Utrecht University will hopefully identify the genetic cause of the neuropathology so that a DNA-test can be developed and complete selection becomes feasible. Complete selection against the neuropathology and PRCD did not substantially increase the inbreeding rate in the simulated populations, because of the low allele frequencies. However, when excluding affected individuals and carriers from breeding it should be realised that all of their genes are lost if they are not passed on via other individuals.

The Stabyhoun

The situation in the Stabyhoun is clearly different from that in the Markiesje. The current breeding population size is considered to be large enough for maintaining a healthy population, but due to the history of the breed the situation is rather problematic. For almost half a century the breed has undergone a very high inbreeding rate and a very low Ne^F of less than 20. During this time, a third of the genetic diversity in the founders has been lost and inherited disorders have accumulated. The inbreeding rate has decreased steadily over time, which is thought to be the result of the growing population size. This decrease was especially apparent in the last one to two decades, which is thought to be due to the implementation of stricter breeding rules (e.g. sire breeding restrictions and a maximum inbreeding coefficient over three ancestral generations).

The low number of (possibly related) founders and the absence of semi-founders together caused a narrow initial genetic diversity in the breed. Both selection and genetic drift have been shown to reduce the initial diversity substantially over time. Selection during the development of the breed was with an f_e/f_{tot} ratio of 0.21 a bit stronger than in the Markiesje and led to an remarkable unequal contribution of founders; the five most influential founders contributed over 85% to the current gene pool. The presence of extremely over-represented founders is sometimes observed in other breeds as well, e.g. in Icelandic Sheepdogs (Oliehoek et al., 2009). Part of the selection in the Stabyhoun took place via the excessive use of sires. The loss of diversity because of genetic bottlenecks was unexpectedly high in the Stabyhoun compared to the amount of selection and the genetic bottleneck effect in the Markiesje. This is thought to be due to the low percentage of selected individuals.
Breeding restrictions were shown to be very effective in reducing the inbreeding rate in the simulated Stabyhoun populations (as a popular sire effect was present). The breeding restriction that is currently applied by the breeding restriction, however, is already one of the most effective restrictions. Minimising population coancestry was also very effective, whereas enlarging the population size was less effective.

The number of inherited disorders was twice as high in the Stabyhoun (6) as in the Markiesje but was still low compared to the 50 most popular breeds in the UK (Summers et al., 2010).
In the current study a minimal and maximal prevalence were used for HD and ED. These were defined as the proportion of individuals registered as affected in the screened population and in the total population, respectively. The minimal prevalence is thought to be a severe underestimation of the real prevalence, as many affected individuals are not screened. The maximal prevalence, on the other hand, is considered a slight overestimation of the true prevalence, as dogs suspected of having HD/ED are most likely more often screened than non-suspects. Because of the compulsory screening of HD prior to breeding, however, also non-suspects are screened for this disorder and the true prevalence will approach the maximal prevalence. For ED the difference between the true prevalence and the maximal prevalence will likely be higher than for HD. As other studies always reason from the maximal prevalence, this parameter is used for the comparison with other breeds.
The prevalence of HD in screened Stabyhouns of 20.8% was quite high compared to other breeds (Witsberger et al., 2008; Comhaire, 2014). It was substantially higher than the average prevalence of

13.3% that was reported for spaniel type pointing dogs, i.e. breeds classified in FCI group 7 section 1.2, in the Netherlands (Lavrijsen et al., 2014). The female sex predisposition that is observed in other breeds (Malm et al., 2007; Lavrijsen et al., 2014) is in line with the significant OR for the minimal prevalence. This predisposition could possibly be explained by differences in growth rate or sex hormones or other characteristics that are linked to the sex-chromosomes. The not-significant OR for the maximal prevalence, however, indicates the importance of distinguishing both types of prevalence.
The positive relationships between HD and body weight and between HD and age at screening have been reported in many other studies (e.g. (Malm et al., 2007; Comhaire & Snaps, 2008; Witsberger et al., 2008)). The relationship with age is not surprising, as osteoarthritis - one of the main assessment criteria of HD - is known to get worse with aging. One should, however, be careful when interpreting the relationship with age, as young individuals are often examined when they might be used for breeding and while they are not suspected to be affected by HD. In contrast, when older individuals are screened they are often already suspected to be affected. The relationship between age and HD might therefore be less clear than presented.
The prevalence of ED was also high compared to the prevalence in other dog breeds in the Netherlands (Lavrijsen et al., 2014). The higher risk for males was reported for some other breeds as well, like for Dutch Labrador Retrievers (Lavrijsen et al., 2014) and German Rottweilers (Beuing et al., 2000). The tendancy to a relationship with age is also observed in previous studies (Mäki et al., 2000; Sturaro et al., 2005), as well as the association between HD and ED (Mäki et al., 2000; Lavrijsen et al., 2014).
The observed prevalence of epilepsy falls in the lower region of the reported range for the general canine prevalence of idiopathic epilepsy of 0.5 – 5% (Ekenstedt et al., 2012; Kearsley-Fleet et al., 2013; Koskinen et al., 2015). The sex predisposition found for males is in agreement with findings in other breeds (Kearsley-Fleet et al., 2013).
PDA is observed relatively often in the Stabyhoun compared to other breeds in which this congenital heart disease is found (Oyama et al., 2010; Oliveira et al., 2011). The higher risk for females, with a female:male OR of 2.7 - reported for Italian dogs of various breeds (Oliveira et al., 2011), was not found to be present in the Stabyhoun.
Of the Stabyhouns that were screened for vWD-I, 11% was affected by the disorder as homozygote and 58% was less severely affected as heterozygote. The prevalence of the disorder is therefore moderate to high compared to other breeds screened by the company vetGen (vetGen, 2005).
With simulations it was shown that the monogenic disorder CD can be selected out of the breed over a short period of time, without any substantial increase of the inbreeding rate (in contrast to the vWD-I with the higher allele frequency).
Selection against polygenic disorders could not be simulated with the Dog Breed Management program. This, however would be of large value for breeds with multiple polygenic disorders.

6. Conclusion and recommendations

The population status of and genetic diversity in the Markiesje and the Stabyhoun are far from ideal. Through selection and genetic bottlenecks the genetic diversity has decreased substantially since the foundation of the breeds. For many decades both populations have had an inbreeding rate that corresponds to an unacceptably high risk on the accumulation of inherited disorders. Various inherited disorders are present in the dogs that are nowadays born. Despite the decrease in inbreeding rate in both populations, the breeds are still considered to be under risk.

The main cause for the unacceptably high inbreeding rate in the Markiesje was shown to be the small breeding population size. Therefore, it is highly recommended to enlarge the number of breeding individuals. Using the semi-openness of the studbook and admitting more look-alikes is considered to be the best option for enlarging the breed without an enormous increase in relatedness. It is therefore advisable to wait with international recognition, which would imply stricter regulations, until the breed is sufficiently large. It is recommended to test look-alikes on their relatedness with the breed prior to admitting them to the studbook. If the number of available look-alikes is limited, or the look-alikes have a high relatedness to the studbook, it is recommended to systematically outcross with other breeds to increase the population size.
While enlarging the population size it is wise to focus on lengthening the generation interval (especially for sires) as well, because a longer interval results in a slower increase in inbreeding over time and enables to intervene in the breeding process more easily.

The main issue in the Stabyhoun was shown to be the strong selection that especially occurred during the earlier decades of the breed (among others via the excessive use of popular sires) and has resulted in an incredibly high inbreeding rate, a very skewed founder contribution, various inherited disorders and a high relatedness in the current population. Systematically outcrossing with other breeds, or steering on relatedness, is recommended to reduce the (increase in) relatedness. In addition, it is recommended to maintain the general way of breeding of the last 10 years, as it seems to be effective in restricting the inbreeding rate. Also, exchange between Stabyhouns from different countries should be stimulated, especially while the subpopulations are small.
In addition to the breed-specific recommendations, it is recommended for both breeds to:

- Keep the current sire breeding restrictions. In the Stabyhoun these were shown to be very effective. In the Markiesje they were also shown to be effective. In view of enlarging the population size, the restriction per life could be loosened from 5 to 10 litters in the Markiesje.
- Minimise population coancestry by selecting breeding individuals that are less than average related to the rest of the breed. Although a complete mating program is hard to implement, the use of individuals that are strongly related to the most influential founders (Pom and Rasta in the Markiesje; Staby, Bruno, Autgertsje, Albert, Aukje in the Stabyhoun) could be limited.
- Continue with selecting against inherited disorders. Selecting against monogenic disorders was shown to hardly increase the inbreeding rate (less influence with lower allele frequency). Strict selection against severe disorders, such as the neuropathology in the Markiesje and CD in the Stabyhoun, can therefore be justified. For less severe disorders it is, however, recommended to exclude carriers bit by bit in order to prevent losing diversity. Selection against polygenic disorders in the breeds was not proven to be effective and could neither be simulated. In future, techniques like genomic selection might help to combat these disorders.

A combination of the abovementioned recommendations can strongly improve the situation in both breeds; genetic diversity can be largely maintained and the dogs' health and welfare can improve by preventing severe inbreeding depression and limiting the occurrence of inherited disorders.

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Appendices

Appendix I. Markiesje: distributions of general population parameters

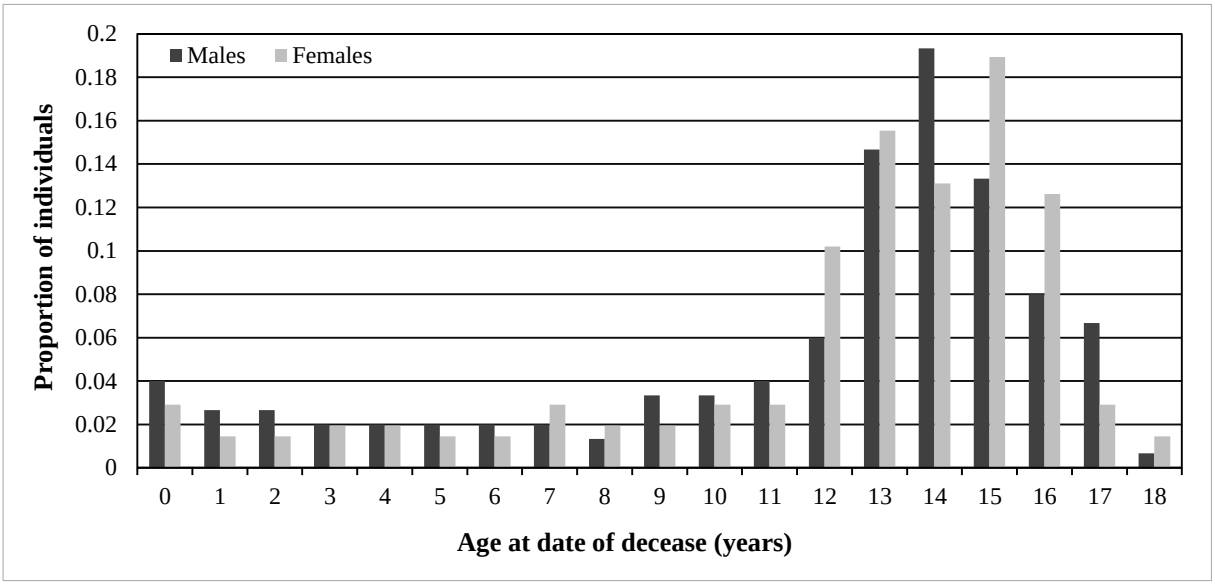

Figure I. *Age of male (n = 150) and female (n = 206) Markiesjes born before 1998 at their date of decease*

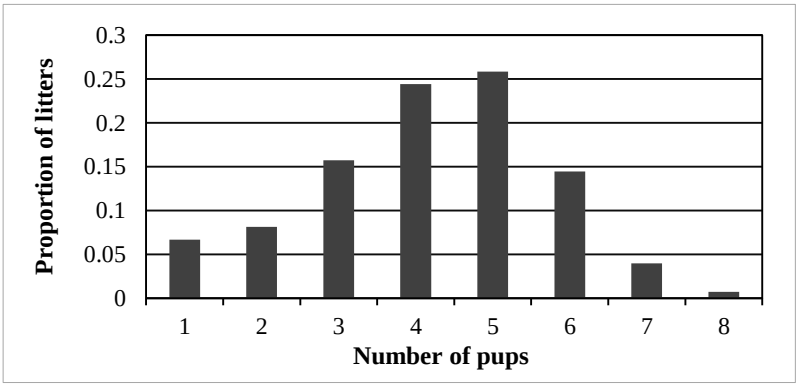

Figure II. *Number of pups per litter (n = 553) for the Markiesje*

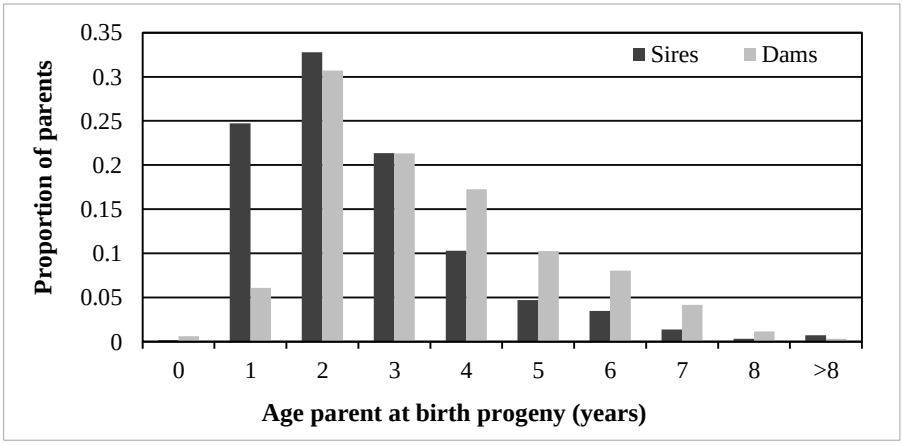

Figure III. *Age of Markiesje sires (n = 2318) and dams (n = 2318) at the birth of their progeny*

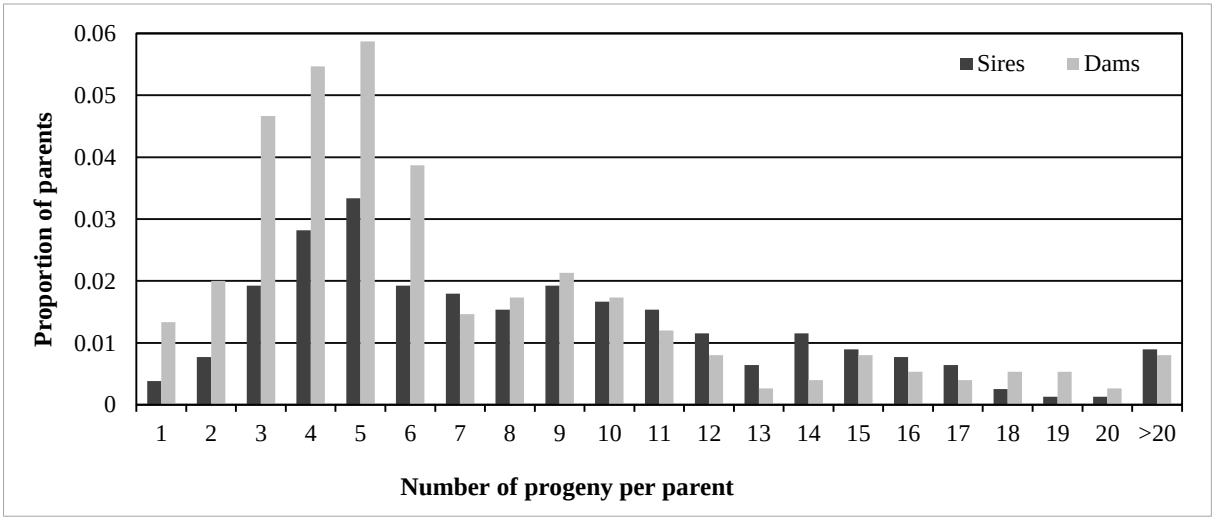

Figure IV. *Number of progeny per sire (n = 750) and dam (n = 780) born before 2008. The proportion of Markiesjes without progeny was 0.74 for males and 0.63 for females (not shown).*

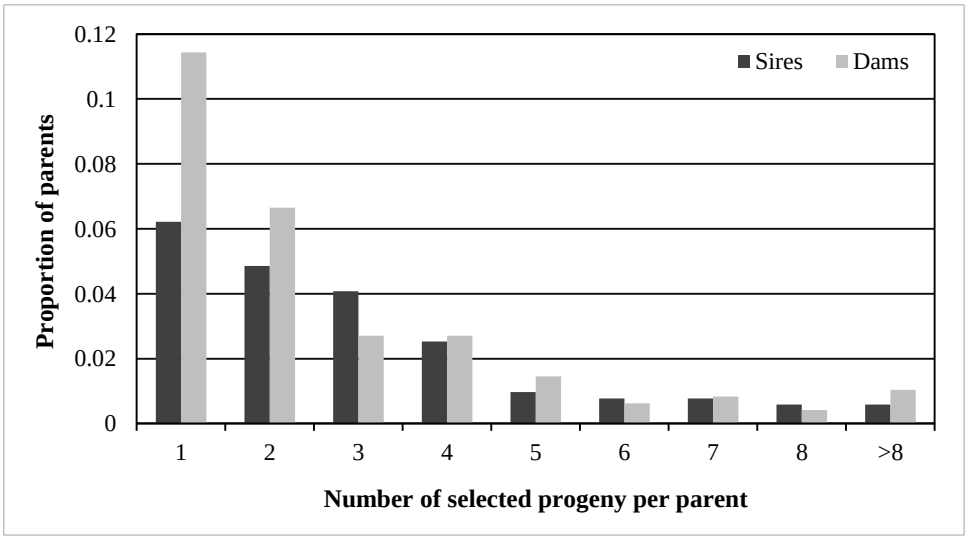

Figure V. *Number of selected progeny per sire (n = 515) and dam (n = 481) born before 2002 for the Markiesje. The proportion of parents without selected progeny was 0.79 for sires and 0.72 for dams (not shown).*

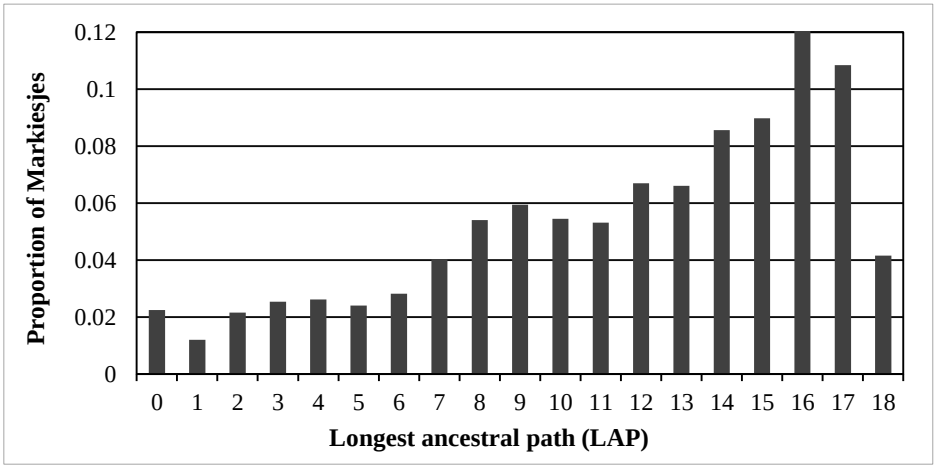

Figure VI. *Longest ancestral paths (LAP) in the Markiesje*

Appendix II. Markiesjes affected by neuropathology: decomposition of COI and founder contributions

Table IIA. *Nodal common ancestors (NCAs) of affected litters A up to D with a difference between the litter's partial inbreeding coefficient ($F_{ij.litter}$) and the average partial inbreeding coefficient of the reference population ($\bar{F}_{ij.ref}$) of more than 0.001 and/or a $F_{ij.litter}$ that is more than 2 times the $\bar{F}_{ij.ref}$. The j in F_{ij} represents the NCA and the i the inbred individual. The reference population consists of all unaffected individuals born since 2003, excluding the litter mates of affected individuals (n =1299).*

Litter	NCA ID	$F_{ij.litter}$	$\bar{F}_{ij.ref}$	$F_{ij.litter} - \bar{F}_{ij.ref}$	$F_{ij.litter} / \bar{F}_{ij.ref}$
A	348 G1	0.015625	0.004555	0.011070	3.43
	415 G2	0.007813	0.001309	0.006504	5.97
	242 GO	0.010986	0.006493	0.004493	1.69
	390 G2	0.003906	0.000577	0.003329	6.77
	477 G1	0.003906	0.002070	0.001836	1.89
	123 G0	0.003235	0.001806	0.001429	1.79
	108 G0	0.000137	1.49E-06	0.000136	92.19
	168 GO	0.000313	0.000149	0.000164	2.10
B	713 G3	0.015625	0.000246	0.015379	63.61
	557 G2	0.015625	0.004515	0.011110	3.46
	284 G1	0.012207	0.009304	0.002903	1.31
	400 G1	0.001953	0.000537	0.001416	3.64
	448 GO	0.001953	0.000537	0.001416	3.64
	459 G1	0.003906	0.002890	0.001016	1.35
	168 GO	0.000420	0.000149	0.000271	2.82
	295 GO	0.000244	0.000114	0.000130	2.14
	108 G0	8.01E-05	1.49E-06	7.86E-05	53.78
C	242 GO	0.009979	0.006493	0.003486	1.54
	740 G2	0.003906	0.000713	0.003193	5.45
	459 G1	0.005859	0.002890	0.002969	2.03
	546 G3	0.003906	0.002569	0.001338	1.52
	211 G1	0.020233	0.018956	0.001277	1.07
	514 G2	0.001465	0.000728	0.000737	2.01
	371 G0	0.001160	0.000427	0.000733	2.72
	338 G1	0.000366	0.000107	0.000259	3.43
	456 G1	0.000244	3.31E-05	0.000211	7.38
	295 GO	0.000244	0.000114	0.000130	2.14
D	459 G1	0.010254	0.002890	0.007363	3.55
	211 G1	0.025112	0.018956	0.006156	1.32
	730 G3	0.003906	7.74E-05	0.003829	50.45
	701 G2	0.003906	7.83E-05	0.003828	49.90
	594 G2	0.003906	8.85E-05	0.003818	44.13
	665 G2	0.003906	0.000398	0.003509	9.82
	100 GO	0.009162	0.006450	0.002713	1.42
	284 G1	0.0116810	0.009304	0.002376	1.26
	242 GO	0.008633	0.006493	0.002139	1.33
	556 G2	0.000488	0.000167	0.000322	2.93
	111 GO	9.08E-05	2.79E-05	6.3E-05	3.26
	124 G0	9.08E-05	2.79E-05	6.3E-05	3.26

Table IIB. *Continuation of table II: affected litters E and F*

Litter	NCA ID	$F_{ij.litter}$	$\bar{F}_{ij.ref}$	$F_{ij.litter} - \bar{F}_{ij.ref}$	$F_{ij.litter} / \bar{F}_{ij.ref}$
E	1198 G3	0.015625	0.000232	0.015393	67.48
	880 G3	0.015625	0.000779	0.014846	20.06
	991 G4	0.007813	0.000457	0.007355	17.092
	950 G2	0.007813	0.000467	0.007346	16.73
	770 G2	0.007813	0.001482	0.006331	5.27
	741 G2	0.002930	0.000155	0.002775	18.94
	211 G1	0.021086	0.018956	0.00213	1.11
	415 G2	0.003204	0.001309	0.001896	2.45
	325 G1	0.000916	0.000202	0.000714	4.54
	547 G3	0.000732	0.000182	0.000550	4.02
	504 G3	0.000427	6.2E-05	0.000365	6.89
	572 G1	0.000244	1.12E-05	0.000233	21.88
	NIET GE	0.000231	0.000115	0.000116	2.00
	111 GO	7.89E-05	2.79E-05	5.1E-05	2.83
	124 G0	7.89E-05	2.79E-05	5.1E-05	2.83
F	1088 G3	0.015625	0	0.015625	x
	284 G1	0.013744	0.009304	0.004439	1.48
	978 G1	0.003906	0.000576	0.003330	6.78
	949 G2	0.003906	0.000674	0.003232	5.80
	1104 G2	0.003906	0.000893	0.003014	4.38
	726 G2	0.000977	0.000102	0.000875	9.58
	702 G2	0.000977	0.000260	0.000717	3.76
	775 G3	0.000977	0.000333	0.000644	2.94
	400 G1	0.001091	0.000537	0.000554	2.03
	448 GO	0.001091	0.000537	0.000554	2.03
	547 G3	0.000488	0.000182	0.000306	2.68
	666 G2	0.000244	6.96E-05	0.000175	3.51

Table III. *Contributions of genes of founders to the gene pool (GP), average inbreeding coefficient ($\bar{F}$) and average coancestry ($\bar{f}$) of Markiesjed affected by the neuropathology and a reference group of Markiesjes born since 2003, excluding the litter mates of affected individuals. Only the founders with a higher contribution to the affected individuals than to the reference group are shown.*

	Affected individuals (n = 11): Contribution in % to			Reference group (n = 1299): contribution in % to			Difference			Factor different		
ID founder	GP_a	$\bar{F}_a$	$\bar{f}_a$	GP_r	$\bar{F}_r$	$\bar{f}_r$	$GP_a - GP_r$	$\bar{F}_a - \bar{F}_r$	$\bar{f}_a - \bar{f}_r$	GP_a / GP_r	$\bar{F}_a / \bar{F}_r$	$\bar{f}_a / \bar{f}_r$
445 GO	1.705	0	0.155	0	0	0	1.705	0	0.155	-	-	-
207 GO	1.705	0	0.213	0.042	0	1.3E-4	1.663	0	0.213	40.78	-	1698.94
341 G0	3.409	0	0.426	0.084	0	2.5E-4	3.325	0	0.426	40.78	-	1698.94
605 GO	2.699	0	0.249	0.838	6.2E-3	0.013	1.861	-6.2E-3	0.236	3.22	0	19.41
220 GO	1.332	0.058	0.186	0.490	5.7E-3	0.013	0.842	0.052	0.173	2.72	1.11	13.89
796 GO	3.480	0.053	0.347	2.546	0.051	0.110	0.934	1.9 E-3	0.237	1.37	28.72	3.16
196 GO	2.945	0.177	0.405	2.416	0.142	0.190	0.530	0.035	0.215	1.22	5.00	2.13
242 GO	15.383	1.806	2.907	13.607	1.487	1.742	1.776	0.319	1.165	1.13	5.66	1.67
168 GO	5.017	0.664	1.024	4.545	0.634	0.717	0.472	0.030	0.306	1.10	22.46	1.43
106 GO	6.875	0.880	1.381	6.268	0.824	0.941	0.607	0.056	0.440	1.10	15.79	1.47
295 GO	2.406	0.110	0.284	2.254	0.069	0.116	0.152	0.041	0.169	1.07	2.67	2.46
448 GO	5.899	0.370	0.850	5.566	0.371	0.493	0.333	-3.3E-4	0.357	1.06	-1139.18	1.73

Appendix III. Stabyhoun: distributions of general population parameters

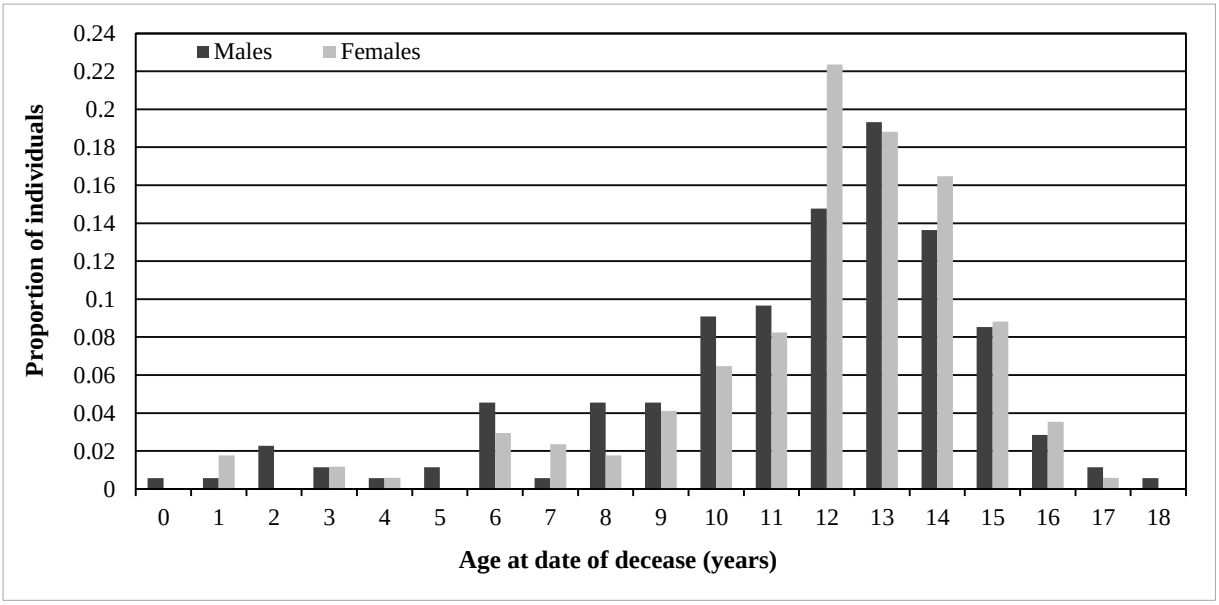

Figure VII. *Age of male (n = 176) and female (n = 170) Stabyhouns born before 1998 at their date of decease*

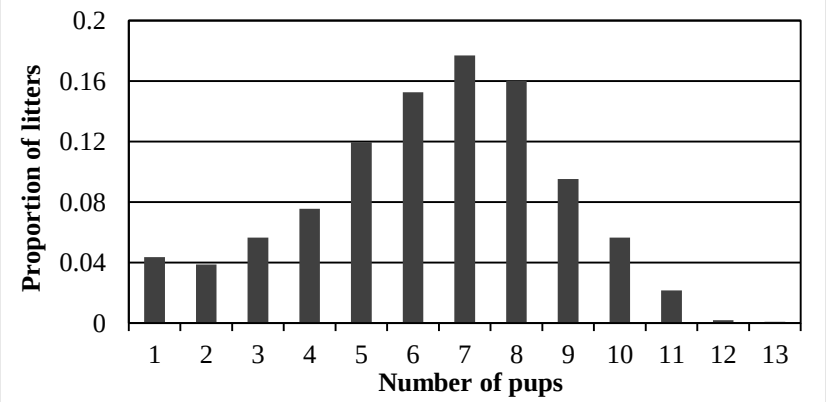

Figure VIII. *Number of pups per litter (n = 2634) for the Stabyhoun*

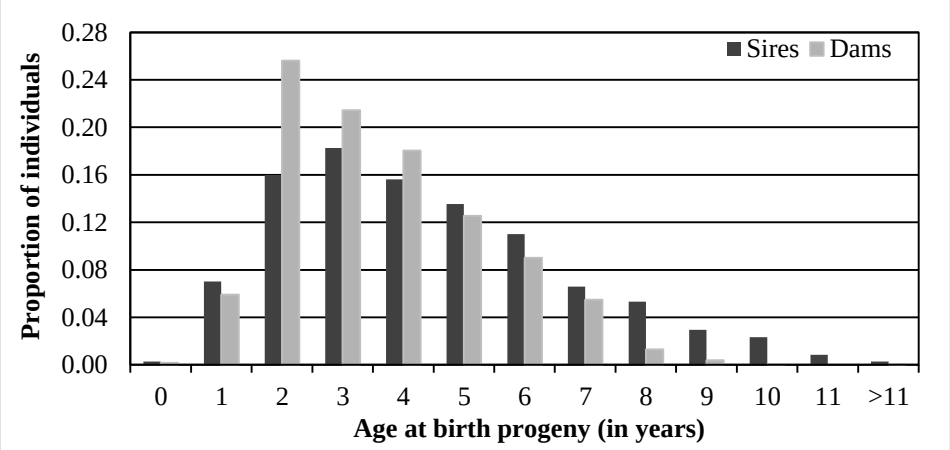

Figure IX. *Age of Stabyhoun sires (n = 16649) and dams (n = 16643) at the birth of their progeny*

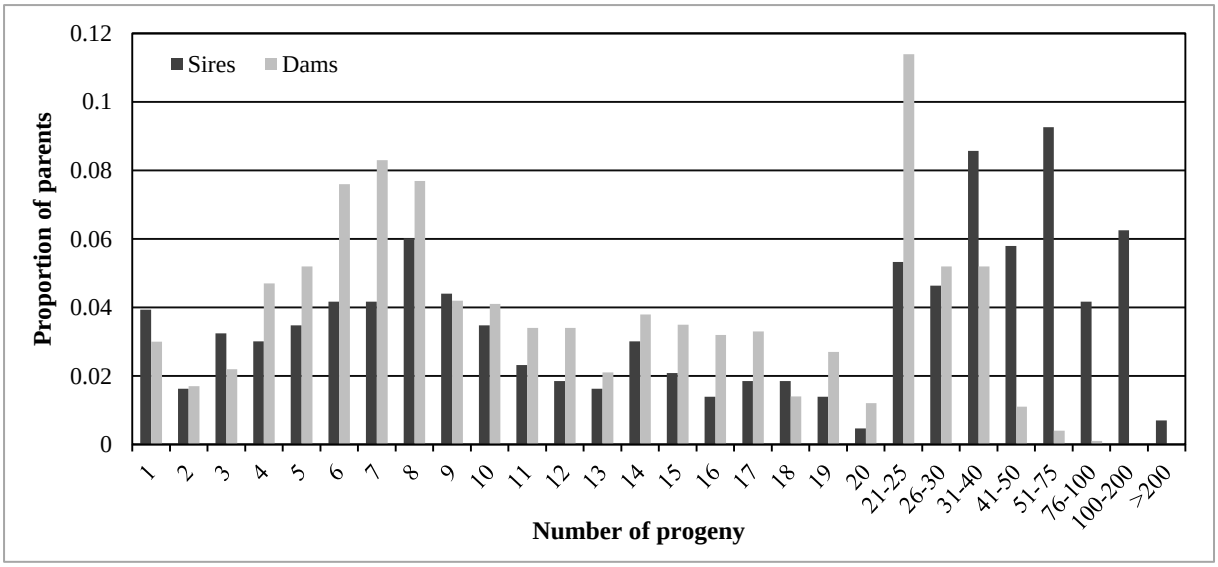

Figure X. *Number of progeny per sire (n = 432) and dam (n = 1001) born before 2007. The proportion of Stabyhouns without progeny was 0.92 for males and 0.82 for females (not shown).*

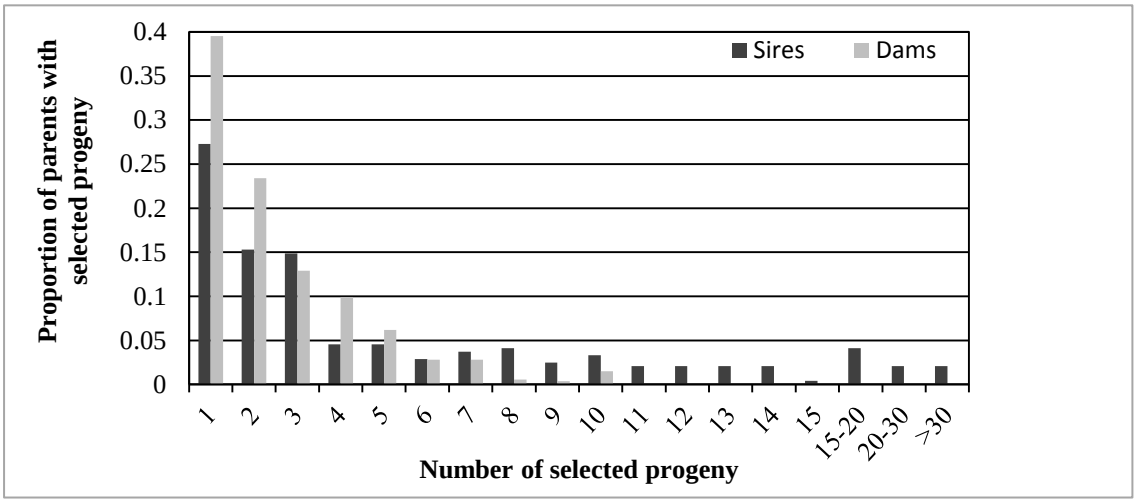

Figure XI. *Number of selected progeny per sire (n = 331) and dam (n = 795) born before 2002 for the Stabyhoun. The proportion of parents without selected progeny was 0.27 for sires and 0.33 for dams (not shown).*

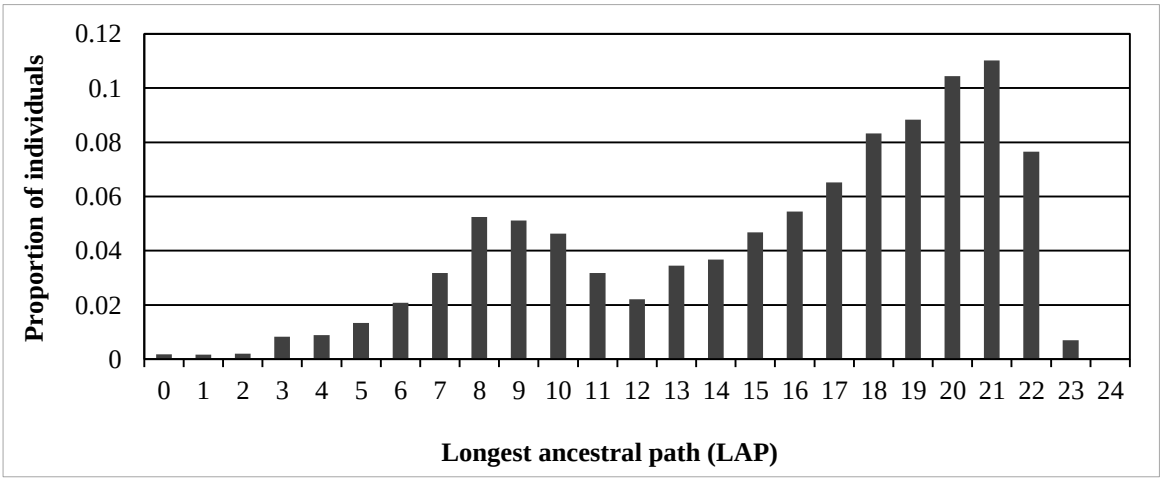

Figure XII. *Longest ancestral paths (LAP) in the Stabyhoun (n = 16679)*

Appendix IV. Dog Breed Management: input baseline simulations

Table IV. *Input values baseline simulation (no breeding restrictions, no population stratification and no simulated inherited disorders). Values were based on the*

Parameter		Markiesje		Stabyhoun	
No. of simulated years		50		50	
No. of simulation runs		25		25	
No. of sires available / year		33		105	
No. of dams available / year		56		222	
No. of litters / dam / year		0.5		0.5	
Max litter size		8		11	
Fraction of litters per litter size					
	1	0.07		0.04	
	2	0.08		0.04	
	3	0.16		0.06	
	4	0.24		0.07	
	5	0.26		0.12	
	6	0.14		0.15	
	7	0.04		0.18	
	8	0.01		0.16	
	9	-		0.10	
	10	-		0.06	
	11	-		0.02	
Max. parental age		9		11	
Fraction of parents per age (y)		Sires	Dams	Sires	Dams
	1	0.25	0.07	0.07	0.06
	2	0.33	0.31	0.16	0.26
	3	0.21	0.21	0.18	0.21
	4	0.10	0.17	0.16	0.18
	5	0.05	0.10	0.14	0.13
	6	0.03	0.08	0.11	0.09
	7	0.01	0.04	0.07	0.05
	8	0.01	0.02	0.05	0.02
	9	0.01	0	0.03	0
	10	-	-	0.02	0
	11	-	-	0.01	0
Min. age dam (months)		18		18	
No. of popular sires		0		5	
% of offspring of these sires		0		11.0	
No. of breeding groups		1		1	
Sires per breeding group		33		105	
Dams per breeding group		56		222	
No. of (monogenic) disorders		0		0	
Max. no. of litters per dam / life		5		5	
Max. no. of litters per sire / y		500		500	
Max. no. of litters per sire / life		500		500	
Max. no. of sons per sire		500		500	
Max. relatedness parents		1.0		1.0	
Minimise relatedness parents		no		no	
Minimise relatedness parents below the average relatedness		no		no	
Max. COI parents		1.0		1.0	
Use of optimal contributions		no		no	
Use of initial A-matrix		no		no	
No. of group mating schemes		1		1	
Group name and fraction sires from own and other groups		first	1.0	first	1.0