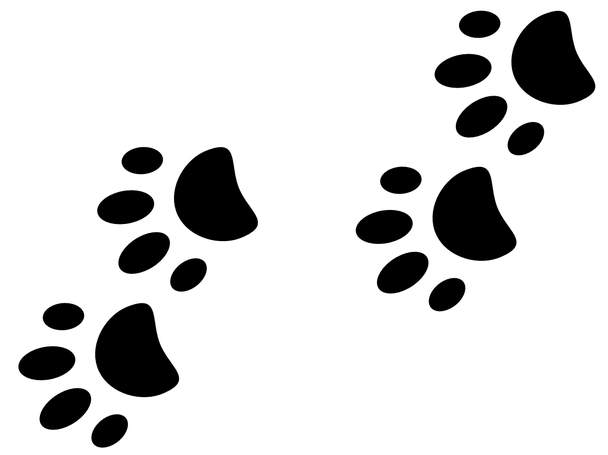
Breeding Better Buddies

Helping purebred dogs
get back on their paws

ACT 3.079
Cato Berkelaar
Karel de Bruijn
Swen Diepstraten
Ellen van der Heide
Max van Kommer
Talita Kuipers
Mia Smetsers

TABLE OF CONTENTS



01. PREFACE

Health is always of elevated importance in the purebred dog breeding world. There is a lot of media highlighting the negative aspects of purebred dogs, and claims that dog breeders do not consider the health of dogs they are breeding. However, this is usually far from the truth. Breeders are often the ones wanting the best for their breed, and regularly become involved in the running and managing of the breeding associations – in their free time as volunteers. Despite their commitment and effort, there are still many issues from past decisions in the purebred dog world. This is the result of years of selective breeding of individuals with desirable traits a disproportionately high number of times. The small number of founding ancestors at the origins of dog breeds amplifies this.

These past breeding practices have resulted in the multitude of different dog breeds which we see today, but are also the stem of the major health challenges today. The past use of popular sires has resulted in high levels of inbreeding, which in turn is one of the causes for the high number of hereditary diseases found in purebred dogs – issues which are not mutually exclusive, and both aggravated by small population sizes. Today, most breed associations have regulations to prevent the same mistakes that were made in the past, but the damage has already been done. The association for the Bouvier des Flandres (Boe4), General Association for Saarloos Wolfdog Enthusiasts (AVLS), and Dutch Association for Stabyhoun and Wetterhoun (NVSW) are amongst the breed associations of the Netherlands that are concerned with combatting the challenges faced when breeding for health. The aforementioned associations have asked for advice on interventions they can incorporate into their breeding program that stem from a scientific background. This work is the accumulation of that.

In this guide, we will first introduce the general profiles of the four breeds under investigation: the Bouvier des Flandres, the Saarloos Wolfdog, the Stabyhoun and the Wetterhoun. Following then, we will go on to discuss the genetic background of the issues surrounding dog breeding and consider some solutions. Having gone over this, we will review the information from the breeders' perspective, and examine some of the challenges faced by breeders and the viewpoints of dog owners in the breed associations. Finally, we will raise some ethical considerations that encourage the reader to think critically about the topics we have discussed, and hopefully re-examine their point of view, followed by a summary of the guide.

02. GLOSSARY

Allele: A variant of a gene.

Artificial selection: Selection of traits based on human choices.

Bottlenecks: Randomly determined events that limit genetic variation in a population(1).

Breed barrier: A fictional barrier that prevents dogs from the same breed in a closed population to breed together. For example, the separation between pedigree and non-pedigree dogs.

Carrier: An individual who possesses an allele for a trait but does not express it, thereby passing it on to its offspring.

Cataracts: An eye condition, in which there is a clouding on the lens of the eye.

Cerebral dysfunction: A deterioration in the cerebral processes.

Chromosome: A strand of DNA that is present in the cell nucleus that contains part of the hereditable information. The number of chromosomes vary for each species(2).

Cynology: Scientific study of dogs.

Dam: The mother of the offspring.

Deleterious alleles: A version of a gene that if expressed has a negative effect on the health of the individual.

Dilated cardiomyopathy: A heart muscle disease.

DNA: Deoxyribonucleic acid; a macro molecule that is present in the cell nucleus that contains the genetic information of the individual(2).

Dominant: The trait is expressed even if only one allele is present(2).

Drip method: A method that is used for an outcross, where another breed is introduced slowly into the original breed multiple times.

Effective population: The number of individuals in a population that take part in reproduction, derived from the inbreeding rate. It is the size of an imaginary population with the same inbreeding rate, but the parents of the animals are chosen randomly from the population, and they are also mated randomly. In such population each animal has an equal chance of having offspring and are assumed to be not related to each other(2).

Epilepsy: A neurological disorder which causes seizures.

Estimated Breeding Values (EBV): Estimation of the genetic merit for a particular trait.

Elbow dysplasia: Deformity of the elbow.

Expressed: Characteristics or genes that appear in the phenotype.

Fertility: The ability to produce offspring.

Founder effect: A reduction in genomic variation that happens when a small group of individuals are isolated from a larger population(3).

Founders: Individuals with unknown parents in the pedigree, usually founders were used to establish the pedigree**.**

Gene therapy: A technique that is used to prevent or treat disease.

Gene: Part of the DNA on a chromosome that is transferred as a unit to the offspring. The gene determines some traits in the offspring(2).

Generation interval: The time that it takes in order to replace the group of adult individuals by the next generation. It can be described as the average age that the individuals produce offspring(2).

Genetic diversity: A measure that quantifies the proportion of genetic variety within a population(4).

Genetic merit: Overall genetic improvement brought by selection.

Genetic pool: All genes that are present in a population(5).

Genetic rescue: A tool to conserve the breed, and increase the genetic diversity and minimize extinction risk, usually in small, isolated populations.

Genome: The complete set of DNA in a cell(6).

Genomic estimated breeding values (gEBV): Breeding values derived from information of an animal's DNA(7).

Genotype: The pair of alleles that occurs on a gene. This can be used for the set of genotypes that determine a specific trait(2).

Glaucoma: Eye disease characterized by blindness and elevated pressure of the eye(8).

Hereditary disease: A disease that is caused by gene transfer from the parents to the offspring.

Heterozygous: An individual that has two different alleles for a gene(2).

Hip dysplasia: Deformity of the hip. It causes degenerative changes in the hip joint(9).

Homozygous: An individual that has two copies of the same allele in the gene(2).

Hypothyroidism: A deficiency of the thyroid gland and hormones. This results in symptoms as a decreased metabolic rate and dermatological problems(10).

Inbreeding: Breeding of related individuals; homozygosity of alleles due to a shared ancestor in the pedigree of the father and mother(2).

Inbreeding depression: The deterioration in vitality of the animal that is due to the high degree of inbreeding of the animals. Health and fertility traits are most sensitive to inbreeding(2).

Inbreeding rate: The change of the inbreeding level over time.

Look-alikes: Dogs that in almost all aspects conform to the breed standard, but that are not affiliated with the pedigree and so do not have the official papers to be considered a purebred.

Monogenetic: Relating to one gene.

Mutation: A spontaneous change in the order of the DNA on a chromosome which can be passed on to offspring when it happens in the sex chromosomes. A mutation becomes noticeable when the change in the DNA also reflects a change in a noticeable certain trait (for example a disease or change in hair colour)(2).

Natural selection: The process through which populations adapt and change according to changes in their environment.

Nucleotides: These form the base of the structure of nucleic acids as DNA(11).

Optimal contribution in breeding: Each animal has an optimal number of offspring that will determine if it will be used for breeding. To optimally contribute to the population.

Outcross: Breeding with other breeds or individuals from a different population to increase the genetic diversity.

Patent ductus arteriosus: The most common inheritable heart disease in dogs. It causes heart failure and could lead to death(12).

Phenotype: The value of a characteristic that you measure or perceive. It is the result of all genetic and environmental factors affecting that characteristic(2).

Polygenic: Relating to multiple genes.

Progeny: Another term for offspring.

Progressive retinal atrophy: A group of inherited retinal disorders which can lead to blindness(13).

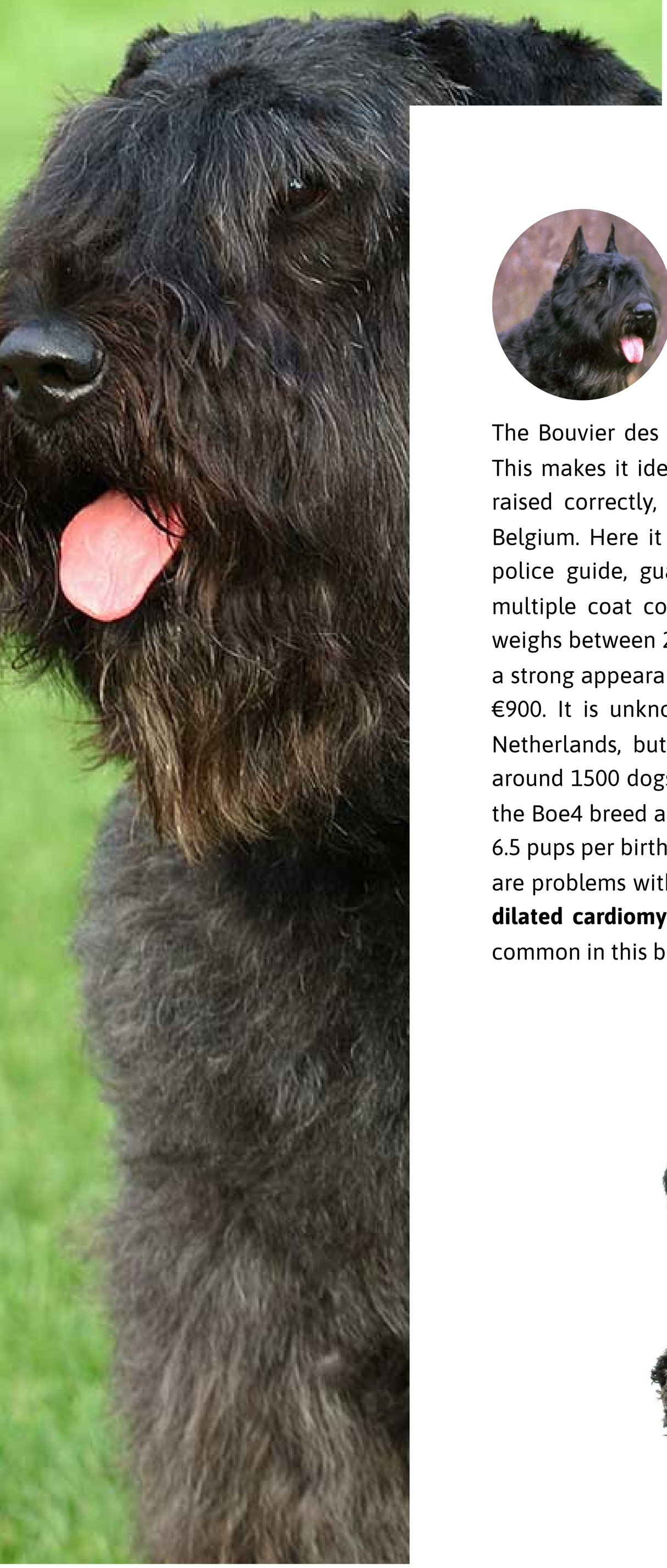
Recessive: One allele does not contribute to the trait when in the presence of a dominant allele, but only when two recessive alleles are present(2).

Sire: The father of the offspring.

Severe Combined Immunodeficiency Disease (SCID): A group of genetic disorders caused by mutations in genes which are involved in the development and functioning of immune cells, essential for infection fighting(14).

Von Willebrand's disease (type I): A disease in which blood does not clot properly, causing increased bleeding time(15).

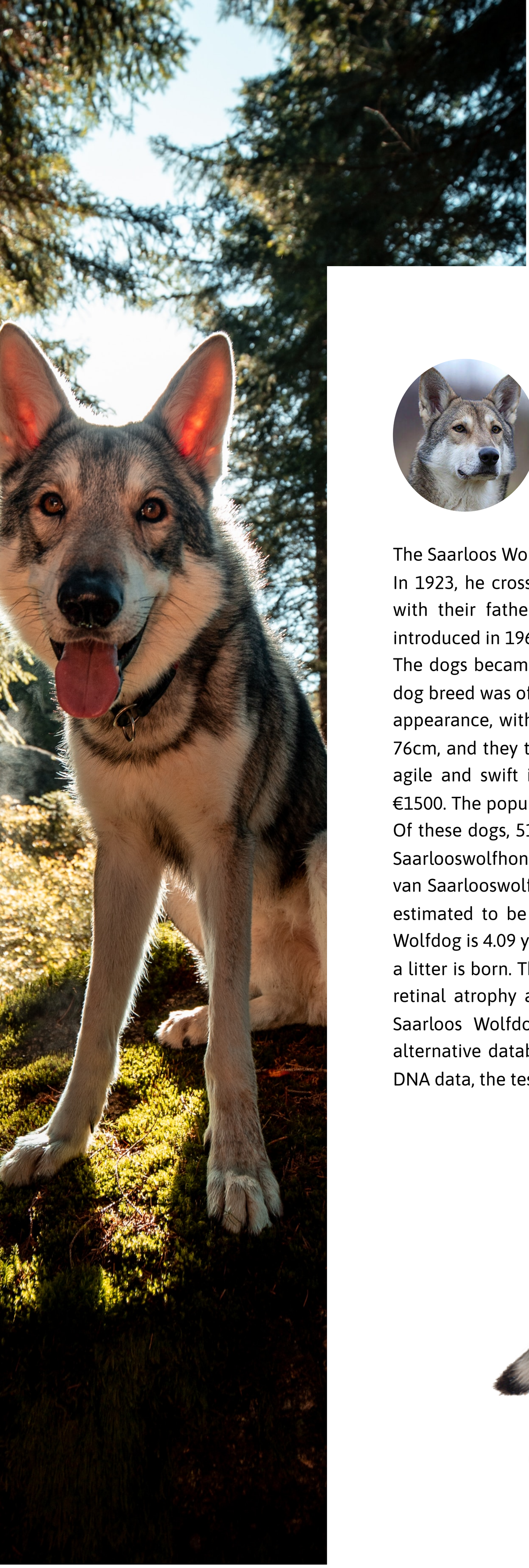
03. PROFILES



BOUVIER DES FLANDRES

The Bouvier des Flandres is described as a guarding, loyal, smart and kind breed. This makes it ideal as a family dog. The Bouvier is however a dog that should be raised correctly, as it has a very strong personality. It originates from Flandres, Belgium. Here it was used as an all-round farm dog. Today the dog is used as a police guide, guard and search and rescue dog. The Bouvier des Flandres has multiple coat colours and is usually between 59 and 68cm high (Figure 3.1). It weighs between 27 and 40kgs. The body of the dog is short and compact, and it has a strong appearance. The Bouvier des Flandres costs on average between €600 and €900. It is unknown how many Bouvier des Flandres there currently are in the Netherlands, but estimates based on the pedigree data indicate that there are around 1500 dogs. It is however unknown how many of these dogs are members of the Boe4 breed association. On average the Bouvier des Flandres has a litter size of 6.5 pups per birth. The largest issue regarding the health of the Bouvier des Flandres are problems with the eyes in the form of **progressive retinal atrophy**. Furthermore, **dilated cardiomyopathy**, a disease related to the heart of the dog, is also fairly common in this breed.

Figure 3.1 Bouvier des Flandres conformation.



SAARLOOS WOLFDOG

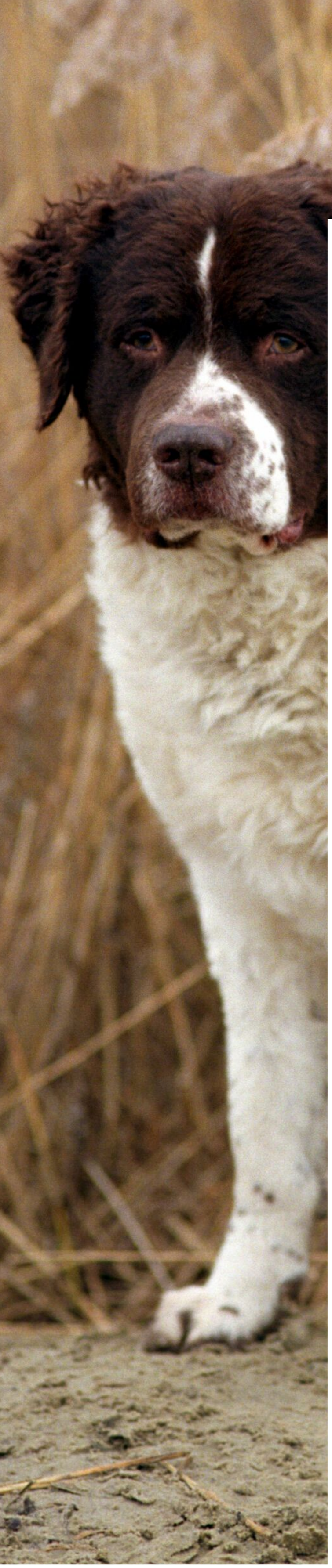
The Saarloos Wolfdog is a breed of dog named after the founder, Leendert Saarloos. In 1923, he crossed a German Shepherd with a wolf. These pups were bred again with their father. These dogs formed the basis of the breed. A new wolf was introduced in 1963, which resulted in the characteristics that the breed is known for. The dogs became more wolf-like, and they were found to be very shy. In 1975 the dog breed was officially recognised as a breed. The Saarloos Wolfdog has a wolflike appearance, with a hint of German Shepherd (Figure 3.2). They can measure up to 76cm, and they typically weigh around 36 to 45kg. The dogs are muscular, but still agile and swift in their movements. The average Saarloos Wolfdog puppy costs €1500. The population size of the Saarloos Wolfdog in The Netherlands is 719 dogs. Of these dogs, 514 are members of the Algemene Vereniging voor Liefhebbers van Saarlooswolfhonden (AVLS), and 61 are members of the Nederlandse Vereniging van Saarlooswolfhonden (NVSWH). The amount of Saarloos Wolfdogs that breed is estimated to be around 100 individuals. The **generation interval** of the Saarloos Wolfdog is 4.09 years, which means that the parents are on average 4.09 years when a litter is born. The most common diseases of the Saarloos wolfdog are progressive retinal atrophy as well as **hip- and elbow dysplasia**. The inbreeding level of the Saarloos Wolfdog differs; based on Zooeasy the inbreeding was 55%, in the alternative database, created by the AVLS, the inbreeding was 46% and based on DNA data, the tested dogs had an average of 36% inbreeding.

Figure 3.2 Saarloos Wolfdog conformation.

STABYHOUN

The Stabyhoun (Dutch: Stabijhoun) name is derived from the words “sta mij bij” (English: stand by me). It originates from the Friesland area of the Netherlands, and they have been documented in historical artworks since 1800. They were used for hunting, pest control and animal guarding. In the past the Stabyhoun was crossed with the Wetterhoun to improve their hunting skills, but after WWII the breeds were separated. In 1942, the Stabyhoun was recognised by the Dutch Kennel Club (Raad van Beheer; RvB) and the breed club “Nederlandse Vereniging voor Stabij- en Wetterhounen” was formed 5 years later (16). The Stabyhoun’s general appearance is described as ‘functional and powerful’ yet neither too robust nor too fine. Their body is slightly longer than their height at the withers (ideal height at withers males: 50–53cm; females 48–50cm). It is a pied dog in black/ brown with white markings (Figure 3.3). There is feathering on the chest, collar, forelegs, trousers and tail. The face is of gentle and friendly expression. The behavioural temperament is described as independent but affectionate and with strangers the Stabyhoun may be reserved at first but not afraid (17). An average Stabyhoun puppy from the association (NVSW) costs €1250. The NVSW reports a population size of 4569 males and 4605 females, totalling 9174 individuals. Of these 9174 Stabyhouns, only (20.4 %) are used in the breeding program, 12.6% of the females, and 7.8% of the males. Once females are a minimum of 4.5 years of age, they can have their first litter - of which 6.33 puppies is the average litter size. The Stabyhoun experiences increased risk of the following inherited disorders: hip and elbow dysplasia, **epilepsy**, **patent ductus arteriosus**, **Von Willebrand’s disease (type I)** and **cerebral dysfunction**. The inbreeding level presented by the NVSW is 32%.

Figure 3.3 Stabyhoun conformation.



WETTERHOUN

The Wetterhoun is native to Friesland, a province in the North of the Netherlands. Originally, it was bred as a hunting dog, particularly over wet and marshy terrain (*wetterhûn* = water dog in Frisian). Today, the Wetterhoun is still used as a hunting dog. They are diverse and adaptable and thus are also reported as excellent guard dogs since they are reserved around strangers. Also, they are described as a good family dog (18). Individuals of this breed are medium sized, powerful dogs, with a distinctive curly, waterproof coat of white and black or white and liver colour combinations (Figure 3.4). The ideal male size is 59 cm, and that of females is 55 cm, and weigh 25-30 kg. The puppies cost €1250, a price which is fixed by the NVSW association (H. van den Hoek, personal communication, 17 April 2023). The NVSW reports the population size of the Wetterhoun as a total of 1238 individuals in the association, with 629 females and 609 males. It is unclear how many Wetterhouns there are in total in the Netherlands. Of these 1238 registered Wetterhouns, only 170 (13.7%) are used in the breeding, 16.2% of the females, and 11.2% of the males. Regulations from the NNSW state that females have to be a minimum of 4 years old before having their first litter, and have an average litter size of 6.15 puppies. Commonly reported diseases in the breed include hip dysplasia, numerous eye problems (**cataracts**, progressive retinal atrophy and **glaucoma**), ear infections, **hypothyroidism,** Von Willebrand's Disease, and to a lesser degree, epilepsy and **Severe Combined Immunodeficiency Disease (SCID)**. The inbreeding rate in the NVSW is reported to be 35%.

Figure 3.4 Wetterhoun conformation.

04. GENETICS

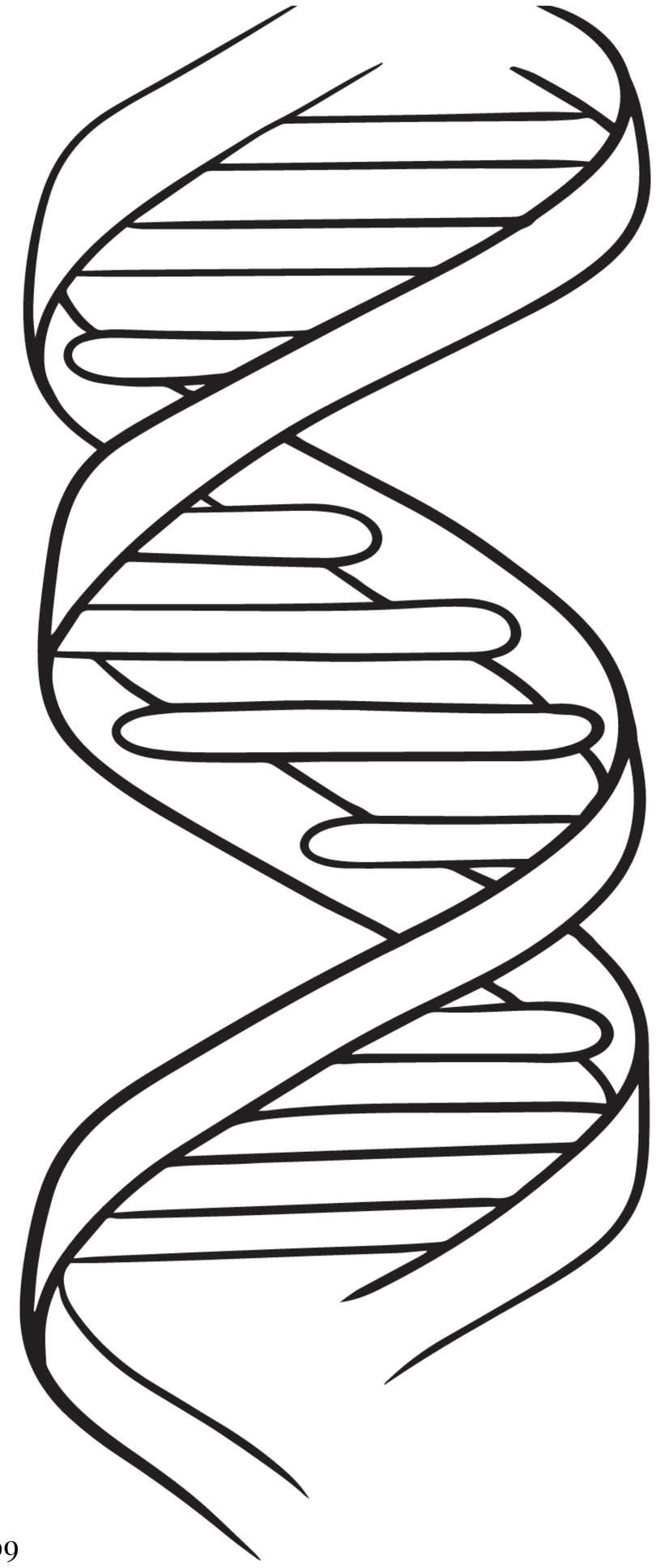
INTRODUCTION

Dog breeding is inseparable from genetics. Human selection of desired traits has created all of the breeds as they are known today. By careful breeding, the preferred traits can be amplified, and the negative traits can be minimized. Inbreeding is becoming an ever-growing problem, which results from the low genetic diversity that is present within the dog breeds. Breeding associations and purebred dog owners have a lot of questions regarding these problems, and how to tackle them. Furthermore, there is a lot of misinformation within associations and online, and this chapter will focus on correcting this misinformation and providing a scientific background on genetic management techniques.

There is already a lot of information known regarding genetic techniques, but these concepts are often difficult to understand, and spread over various sources. This chapter aims to summarize these concepts, and to provide multiple ways to manage the genetics, and the problems that arise within dog breeding. The focus of this chapter will be regarding techniques and solutions implementing DNA-data, as it is thought from within the team that this is where the breeding associations can benefit the most from. Furthermore, this chapter focusses on how these techniques can be implemented, and what the challenges can be with implementing these techniques.

GENETIC DIVERSITY

WHAT IS GENETIC DIVERSITY?
Genetic diversity is a concept that underlies the evolution and maintenance of all species, including dog breeds. It can be defined as the combined differences in the DNA of all individuals in a population. Large amounts of differences in DNA means high genetic diversity (19).



WHY IS GENETIC DIVERSITY IMPORTANT?
High variety of individual's **genes** in a population allows them to successfully handle with changes in their environment. In nature, if an individual in the population possesses a trait that keeps them alive, they have the advantage over others that do not possess this trait – we say this trait is selected for by **natural selection**. In dog breeding, selection by people is used to create breeds with emphasis on conformational, behavioural and health characteristics. This **artificial selection** is towards traits favoured by humans for aesthetics or function, such as short legs in dachshunds bred to hunt burrowing animals. By doing so, one can indirectly select for genes and traits that are harmful to health and fitness of a breed. In the Dachshund, breeding for short legs and a long back has led to a high incidence of intervertebral disc disease, a disease that affects a large proportion of the breed (20).

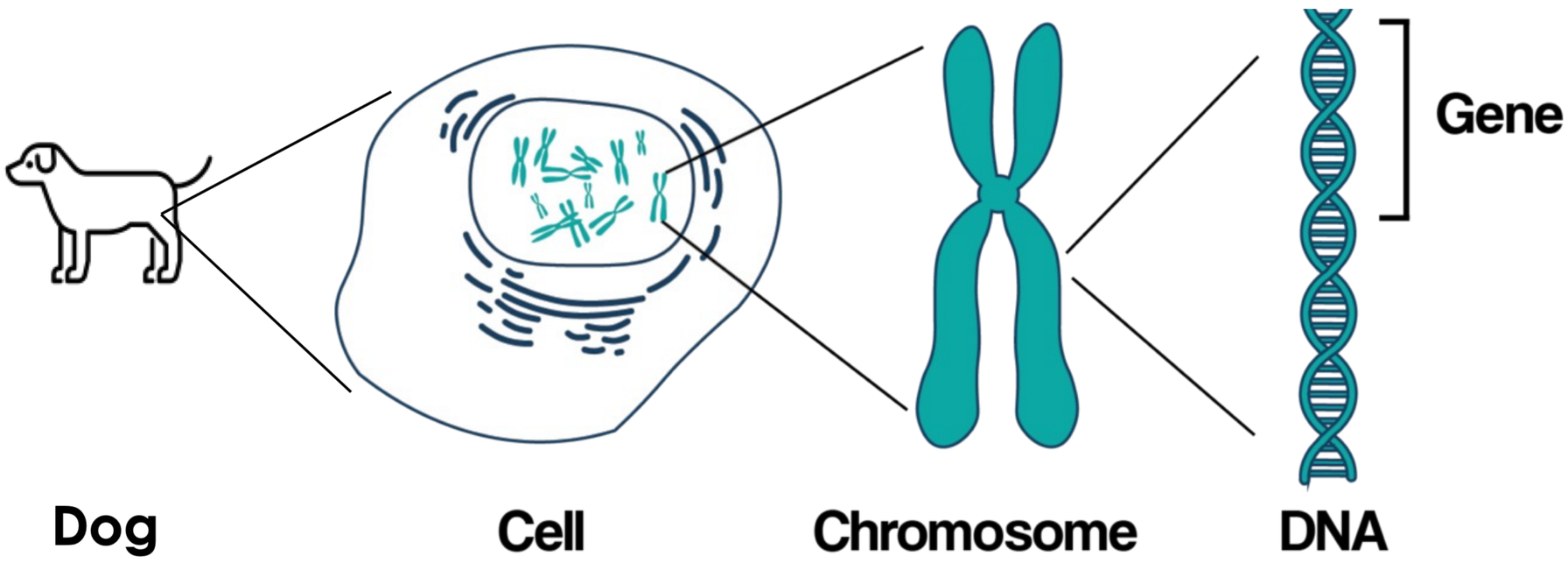
Importantly, having too little differences between the individuals in the breed (low genetic diversity) is harmful because it limits the **genetic pool** and overtime this variation may decrease further. In a small population, individuals have to reproduce with closely related individuals in order to survive and in the pedigree dog world, this effect is much greater. If individuals have the same genes (because they are relatives), no variation is brought into the population and genetic diversity decreases even further. This is known as **inbreeding**(21).

INBREEDING

WHAT IS INBREEDING? WHY IS INBREEDING IMPORTANT TO MANAGE?
Inbreeding occurs when closely related individuals are bred together which is often the case when populations are small and closed off from other populations. Pedigree dog breeds are by definition small, closed populations, often with an even smaller number of breeding individuals. Inbred individuals share ancestors, and they are therefore more likely to have identical copies of genes. This is a problem because these genes can contain disease-causing **mutations** which are officially termed **deleterious alleles**. When they are **expressed,** they can decrease the health of inbred individuals. For that reason, inbred individuals are usually less healthy than noninbred individuals (21).

Additionally, there are **recessive** and **dominant** alleles of genes. Since all cells carry two copies of each **chromosome**, they have two versions of each gene (Figure 4.1). These different versions of a gene are called **alleles**. Dominant alleles show their effect even if the individual only has one copy of the allele (also known as being **heterozygous**). For example, if the allele for brown fur is dominant, you only need one copy of the brown fur allele to have brown fur (although, with two copies you will still have brown fur). Recessive alleles only show their effect if the individual has two copies of the allele (also known as

being **heterozygous**). For example, if the allele for brown fur is dominant, you only need one copy of the brown fur allele to have brown fur (although, with two copies you will still have brown fur). Recessive alleles only show their effect if the individual has two copies of the allele (also known as being **homozygous**). For example, if the allele for blond fur is recessive, to have blond fur you need to have two copies of the blond fur allele.



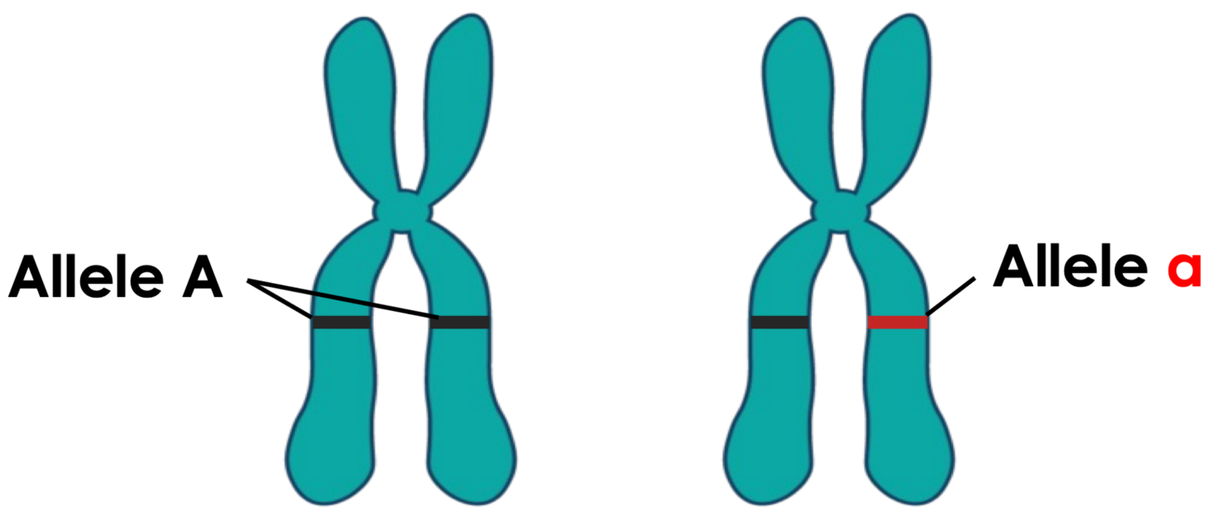


Figure 4.1 Figure demonstrating the homozygous and heterozygous chromosomes and their alleles.

An individual carrying a single deleterious allele can still be healthy and might be able to pass the deleterious allele to the next population; this is called a **carrier** (individuals with **Aa** in Figure 4.2). In a large population, this is not a problem because the chance that recessive deleterious alleles are expressed is very low. However, when the population becomes small, and close relatives end up mating with one another, the **progeny** will have a high chance of carrying two recessive deleterious alleles and hence can express the recessive trait, making it unhealthy (22).

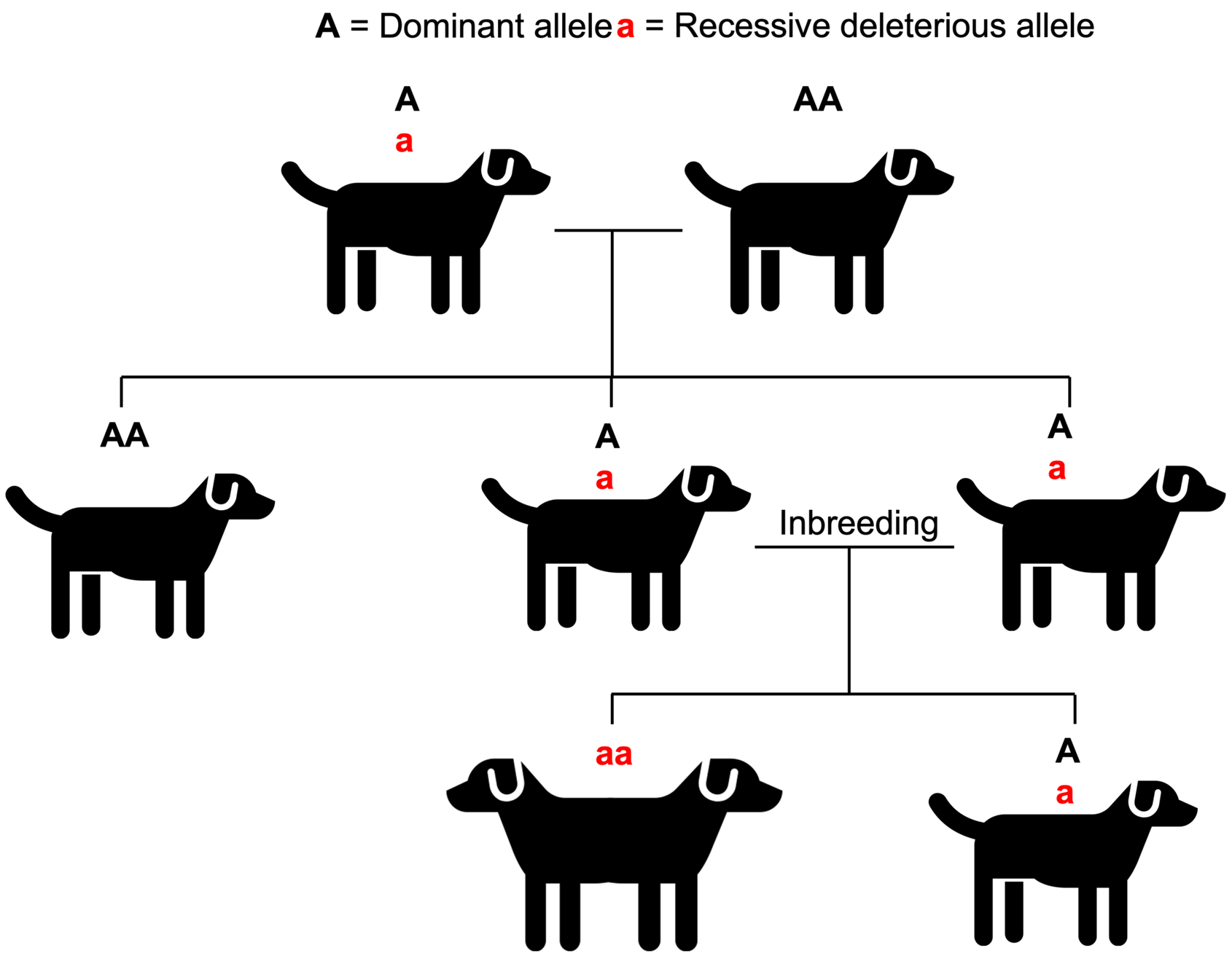


Figure 4.2 Pedigree diagram representing the effect of inbreeding on homozygosity.

In addition, the small number of **founders**, and the overuse of particular individuals when breeding, underlies the presence of inbreeding (23). The small number of dogs that lie at the origin of a new breed results in future generations being a genetic reflection of these initial dogs, this is termed the **founder effect** (24). The overuse of **sires** with highly desirable traits, for example the short legs in the dachshund, has resulted in a more widespread of recessive deleterious alleles, reducing the genetic diversity (25).

The degree of which an individual is inbred, is calculated as an inbreeding coefficient (COI). This is a percentage that represents the degree of inbreeding with 0% in individuals who had completely unrelated parents. If an individual is mated with its parent, the offspring will have an inbreeding coefficient of 25%. All of the dog breeds involved in this research exhibit higher COIs (Saarloos: 36-55%, Wetterhoun: 35% and Stabyhoun: 32%, Bouvier: currently unknown). This proves the urgency of intervention.

THREATS OF INBREEDING DEPRESSION

Years of inbreeding has been shown to manifest traits related to reproduction and disease which can lead to **inbreeding depression;** where a high level of homozygosity (**AA** or **aa**) is seen in the population, leading to a decrease in dog survival and fertility. The rationale is as follows: as related dogs are bred together, more recessive deleterious (bad) alleles (**aa**) are expressed in the population. Being homozygote for recessive deleterious alleles, increases a dog's risk of disease and thus reduces the dog's vitality (state of being strong and active). Decreased vitality is a well-known characteristic of inbreeding depression (26). The negative effect of inbreeding on a dog's vitality and reproductive ability has been shown in multiple dog breeds. Ubbink et al. (1992) investigated the link between homozygosity (a proxy for inbreeding) and risk for specific diseases in Dutch Bouvier des Flandres dogs, such as osteochondrosis, food allergy, autoimmune disease and hypoplastic trachea. They showed that in this population, inbreeding increased the risk for the aforementioned diseases (26). Further studies on Labrador retrievers, German shepherds and Icelandic sheepdog confirm a negative link between level of inbreeding and occurrence of disease (high level of inbreeding leads to a decrease in dog vitality) (27, 28).

Additionally, research on the effect of inbreeding on **fertility** was investigated in the Irish Wolfhound in which they presented that there was a highly significant influence of maternal inbreeding on litter size (a proxy for fertility) (29). This has been supported by research on the Bernese Mountain dog, Basset hound, Cairn terrier, Epagneul Breton, German shepherd dog, Leonberger and West Highland white terrier, German spitz dogs and even in the common ancestor of the domestic dog, the Red Wolf (30, 31, 32). In all these breeds, litter size was much lower for litters produced by **dams** with larger inbreeding coefficients - more inbred individuals (30). A dog's reproductive performance is crucial to any animal breeding enterprise and thus should be of major interest to dog breeders. Fertility not only reflects the genetic health of a population, but also influences selection potential and other important parameters directly connected to the success of the breeding program (33).

HERESITARY DISEASES

WHAT ARE HEREDITARY DISEASES?

Hereditary diseases are diseases that can be passed from parent to child through their DNA. These hereditary diseases can be passed from either one or both parents, and therefore many close family members may have the same diseases. There are many different types of hereditary diseases, ranging from allergies to having a higher chance of developing cancer (34). Diseases are not always expressed, and individuals may "carry" it down the pedigree (Figure 4.2.); this makes it difficult to eradicate these diseases from the population. Diseases that are dependent on one gene are called **monogenic** diseases, and when the disease is dependent on multiple genes, they are called **polygenic**. Monogenic diseases are most often simpler to manage, as they are caused by only a single gene. Monogenic diseases have only 2 **phenotypes**, which when the disease is dominant makes it easier to detect them without tests. Polygenic diseases on the other hand are caused by a multitude of genes (35). Different methods of control are needed for these different types of diseases. Next to being polygenic or monogenic the disease can also be related to a gene that lies on the X or Y chromosome of the dog, or "sex- linked" whereby specifically a male or a female animal has a higher disposition for a disease (36). Overall, hereditary diseases need to be avoided as much as possible, but completely avoiding them is usually not possible, especially in populations with low genetic diversity. Therefore, finding ways to manage them is a necessity in dogs.

WHY ARE HEREDITARY DISEASES IMPORTANT TO MANAGE?

Due to their hereditary nature, it is impossible to separate heritable diseases from DNA. By breeding, the genetic code of both parents recombines into the new animal. When a parent has a dominant disease, or both parents are a carrier for a recessive disease, the offspring has a probability of receiving this disease. Managing hereditary diseases also becomes more difficult if it becomes more prevalent in a population (37). Mitigating the rate of spread of a hereditary disease should be the main objective in most breeding practices; as having a low amount of hereditary diseases is an important objective of sustaining a viable and healthy population (38).

Having many hereditary diseases also contributes to a further lowering of the genetic diversity, as it can force breeders to exclude individuals from the breeding population. According to most breeding regulations, individuals with a disease will not be allowed to breed (39); however, this is impossible since all animals are carrier of several diseases. By removing the individuals who express a disease and the carriers, it is possible to lower the incidence or even remove a disease completely from the population (40). Unfortunately, this is not a simple solution since removing carriers from the breeding proportion of a small, closed population comes at the cost of having a lower genetic diversity. Breeding with a lower population causes the **inbreeding rate** to increase, and by having a higher inbreeding rate, new genetic defects and diseases can arise (41). Overall, removing individuals that are carriers of a hereditary disease can actually have the opposite of the wanted effect. This further confirms the need for a balance within this problem.

Due to artificial selection within pedigree breeding programs, some sires/dams are favoured over others for specific traits they obtain. This leads to this sire being overrepresented within the population (42). If a popular sire is a carrier of a hereditary disease that greatly affects life quality, then this can greatly affect the entire population. With the implementation of DNA-tests, carriers could also be identified within the population based on their genotype, and based on that, selections could be made (43). Artificial selection can also be used for improvement within the population. Using DNA-testing, it is possible to determine which two animals could be mated in order for the offspring to have the lowest probability of having a hereditary disease.

MONOGENIC
Monogenic diseases are based on a mutation of a single gene. They usually arise due to the small number of founders within a breed, followed by a strict selection for the desired traits, and which also includes **bottlenecks** (44). A mutation can also happen within a single gene, resulting in a different phenotype (35). This mutation is then passed on to the next generation. In all dog breeds, approximately 700 monogenetic diseases have been identified and described, of which 230 have known causative mutations (45). There are multiple ways that monogenetic diseases can be combatted, and a multitude of studies have been dedicated towards future treatment with **gene therapy**. Monogenic diseases show two distinct phenotypic categories, a sick phenotype and a healthy phenotype. With high enough severity of the disease, a single individual sick dog will be excluded from the breeding pool by the breeders. For carriers this is more complex. When the sick phenotype is also not visible, this can also cause problems. Furthermore, if many dogs within a population are carriers of a monogenic disease, you cannot exclude all of them. To check the carrier status of a dog, genetic tests can be developed. This has already been done for several diseases, for example **cerebral dysfunction** within the Stabyhoun (46), as well as many more.

POLYGENIC
Polygenic diseases are based on multiple genes and they are generally more difficult to control. Most polygenic disorders have no tests for carriers, but there are phenotypic tests that can identify affected dogs (47). We cannot speak of “healthy” and “sick” phenotypes like we can for monogenic diseases. When an animal has a polygenic disease, there are varying degrees of how affected the animal can be. With polygenic diseases, a multitude of genes combine to cross a threshold and produce a sick dog. These genes are known as liability genes, a form of quantitative genetics related to disease, and in identifying the liability of a dog carrying this gene, how much information per generation is known within the pedigree is more important than the depth of the pedigree (47). The information in the pedigree allows us to assess the quantity of liability genes. A litter with a high prevalence of a polygenic disease is expected to carry more liability genes than a litter with a low instance of this disease. Screening for polygenic diseases is therefore very important, as this gives information on the potentially present liability genes.

When it is yet unknown whether a disease is polygenic or monogenic, the disease should be managed in the same way as if it were a polygenic disease. If in multiple generations normalcy is found within a litter, there is a high chance that the animals do not have a lot of liability genes. But when a dog is diagnosed with a genetic disorder, it should be considered to exclude it from the breeding population, depending on the population size. If this is the case the breeder should take into consideration how severe the disease is, and what the drawbacks are, as well as the rules from associations (44).

DNA DATA

Humans have 23 pairs of chromosomes, while dogs (*Canis lupus familiaris)* have 39 pairs of chromosomes. **Chromosomes** are DNA molecules which make up the genetic material of all living things (Figure 4.1). An organism’s entire set of chromosomes is called the **genome**. The dog genome is made up of approximately 2.4 billion DNA base pairs, or **nucleotides** (48). Nucleotides form the building blocks of DNA, and variation in these building blocks is what makes up the phenotypic variation we see around us. Some locations on the chromosome can have multiple variants. A variant at one specific location is called a ***S***ingle ***N***ucleotide ***P***olymorphism, SNP for short (pronounced “snip”; Figure 4.3). Described simply: at one location where you would expect the nucleotide ‘A’, you may instead find the nucleotide ‘G’. This mutation could have no effect on the health or appearance of the dog, or it could have a large effect on the dog's health/appearance.

If you can quantify and analyse enough SNPs of an individual and compare it to others, you can find out quite a lot about the reasons that we see differences within a population, as well as disease susceptibility.

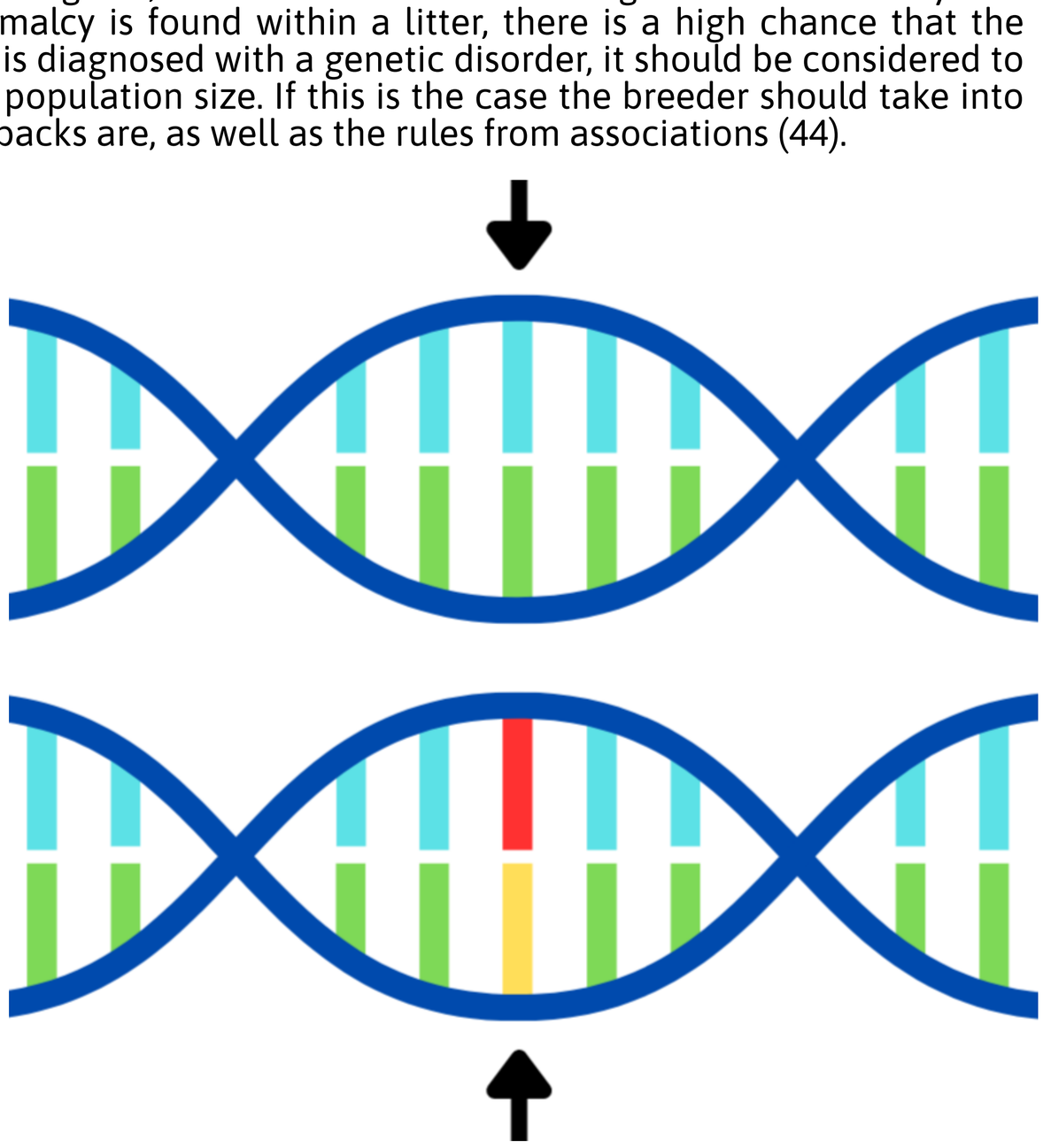


Figure 4.3 Visualisation of a SNP, where the nucleotide at the location of the arrow is different between the two DNA strands.

HOW DOES A SNP CHIP WORK?
A SNP can work as a marker once it is linked to a certain trait or disease. This allows you to investigate the effect and frequency of the trait/disease in the population. The specifics of how a SNP array works can be quite technical, but it is basically a small plastic slide with thousands of small wells that bind DNA to detect genetic variations (49). The four main steps involved in doing a SNP assay are summarised here and in Figure 4.4 on the following page:

1) Making the chip by immobilisation of DNA probes
- The chip is a small slide with thousands of tiny wells in it, with sequence-specific DNA probes that only bind to certain DNA sequences attached to the wells

2) Fragmenting, amplifying and labelling the nucleotides
- DNA is extracted and isolated, and then amplified until there are enough DNA fragments
- These fragments are then labelled with a fluorescent dye

3) Hybridisation
- The labelled DNA fragments are now washed over the chip with probes, so that the fragments can ‘attach’ to the chip

4) Scanning
- When a DNA fragment hybridises (attaches) to the chip, the fluorescent label releases a tiny bit of fluorescence
- This light is detected and recorded, and the results are analysed by a computer

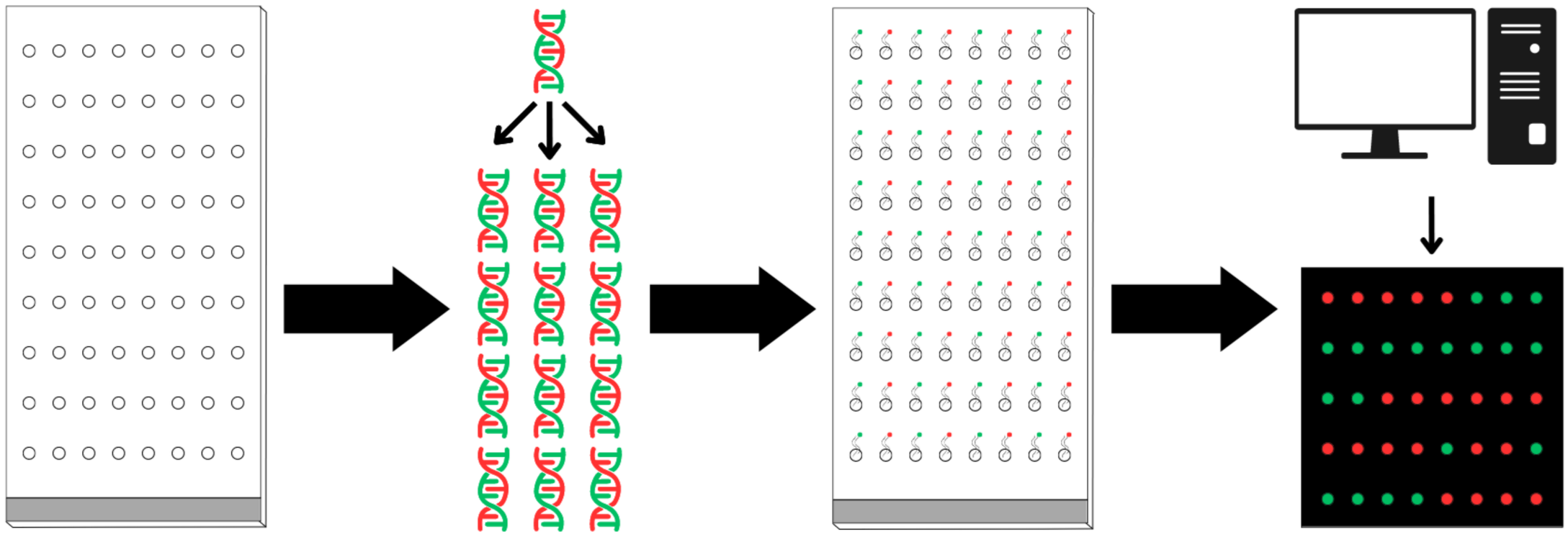


Figure 4.4 Process of doing an SNP array analysis.

In the Netherlands, the RvB has worked together with VHLGenetics to produce a 50K SNP chip, so a slide with 50,000 wells testing 50,000 different SNPs (L. Roest, personal communication, 17 April 2023). The SNPs selected are markers to identify certain traits, multiple diseases, and can be used to determine parentage and heterozygosity of the dog.

WHY IS DNA COLLECTION BENEFICIAL?
Using DNA, you can uncover traits of your dogs that may be invisible to the naked eye, such as Von Willebrand's Disease (VWD), or spot genetic diseases before the individuals are used for breeding, as well as keeping an eye on a breed's levels of inbreeding. For example, a study in the Leonberger dog identified a mutation of high frequency associated with ichthyosis, a severe skin disease. By identifying this mutation, dogs can be screened and breeding with dogs carrying it can be reduced, lowering the incidence of this disease in the population (50). SNP chips can help us pinpoint the specific genetic variants that are associated with various traits and diseases if they occur in large frequencies and the effects are large, which has also been done for a genetic mutation causing blindness in Irish Setters (51).

Multiple studies have already identified that purebred dogs have low genetic diversity, also when compared to mixed-breed dogs (42, 50, 52). Therefore, it is very important to monitor their genetic diversity and intervene before the inbreeding level gets too high. SNP chips can be used to test the genetic diversity within and between breeds. Knowing the relatedness, genetic diversity and disease status of as many individuals in a population as possible can lead to more informed breeding choices (53).

At the end of the day, the more dogs that are genotyped, the more comprehensive the database is, and the better breeding decisions can be made.

LIMITATIONS OF SNP CHIP
Although genotyping may seem like a quick fix in the dog breeding world, it also has its drawbacks. A SNP is only for one specific location in the genome, and a lot of diseases are very complex involving multiple SNPs. Identifying all of these locations can be very difficult for genes of small effect (23). Data quality and analysis quality also have to be reviewed critically, as errors in genotyping or imputation can lead to different results, and sample sizes have a large impact on data quality (54). Further, some traits and diseases are breed specific, thus there is not a SNP chip available which can test all of the relevant SNPs across many dog breeds. Additionally, breed-specific data is often limited. Outside of the more scientific considerations, one should also take into account the ethical implications of genotyping dogs. One factor to consider then is who has access to this data? Who owns this data? What are the consequences for the dogs revealed to have 'poor' genotypes, like those in higher disease risk categories? More on this will follow in the chapter on ethics. Then there is also of course the cost of DNA testing. Many breeders already complain of the high costs of getting a whole litter of puppies tested (A. v.d. Berg, personal communication, 20 April 2023), and one of the reasons that genotyping in the livestock industry hasn't been taken up globally is due to the high costs (55). For genotyping to be readily adopted, the benefits have to outweigh the costs, and the benefits may not be very high if there is no clear breeding goal, or with small population sizes, which may be an issue in the purebred dog world.

SOLUTIONS

Looking to the future, the breeding associations might be wondering how they can incorporate the use of DNA in their breeding programs to better improve their breeds health. It is important to keep in mind the level of genetic diversity in your dog population and therefore, we will discuss the science behind outcrossing and incorporating look-alikes into the breeding population. We suggest the use of SNP chips for uncovering breed purity of look-alikes. Additionally, SNP chips could be incorporated when developing genomic estimated breeding values; values that combine the genotypic, phenotypic and pedigree information per individual. Lastly, we explain and demonstrate how estimated breeding values and an individual's average relatedness (mean kinship) can be computed from DNA data and combined to evaluate an individual's significance in the breeding population, a method termed "optimal contribution".

OUTCROSSING

In highly inbred populations, where the genetic diversity is extremely low and genetic disorders are common, one of the last possibilities for a dog breed to survive is outcrossing with individuals from different populations. **Outcrossing** is therefore also referred to as **genetic rescue**. When a (negative) trait becomes fixed within the population, the only way to correct it is by outcrossing with another population, which brings new genes to the table (56). Outcrossing is defined as

breeding an animal with an animal that is not closely related (57). In the case of purebred dogs this means mating an individual of one breed with that of a different breed. In the purebred dog community, there is a so-called **breed barrier**, in which only dogs of which both parents are registered within the pedigree can be determined as a purebred dog (58). By outcrossing a breed, the inbreeding in their offspring is lowered, and the genetic diversity is improved. This is because the individuals that are mating will not be related, or at least less related than the breed itself (57). By maintaining the genetic diversity within the breed, the breed stays healthier, and happier (42). Outcrossing is usually only necessary in small populations or populations that went through a bottleneck, so keeping populations large and genetically diverse should be a key point for breeders to take into consideration (59). Some breeders are weary of outcrossing as new genes will be introduced into the population, which could bring about phenotypic change and potentially other diseases, as all purebred dogs have problems with genetic defects (A., v.d. Berg, personal communication, 20 April 2023).

HOW TO SELECT A FITTING BREED TO OUTCROSS

The selection of individuals - or even breeds - for an outcross is a difficult task that needs to be balanced well. On one hand, genetic variation can be introduced quickly by outcrossing with a very different breed; on the other hand, the original morphology and genetic variability also need to be considered (59). Outcrossing can not only change the morphology of the breed but also the behavioural and breed characteristics (59). All of these things should be considered during the selection process, as the breeders will want to keep the breed as similar as possible. For outcross selection, usually breeds are first chosen based on their phenotype, behaviour and characteristics. Afterwards, there is the possibility for genetic analyses of the selected breeds. It is strongly advised by Stronen et al. (2017), to genotype the animals that are used for outcrossing. Based on this genotyping, the selection of animals can be validated to have the highest genetic gain per breeding as possible and that the amount of matings are as low as possible.

The amount of outcrossing should be as low as possible, as outcrossing does carry the possibility that the benefits of the greater variability in the gene pool are lessened by the introduction of a new deleterious allele (60). The question then becomes: Do the benefits outweigh the costs? As hereditary diseases are rare and often recessive, with careful planning the benefits can be maximised while the losses are minimized. The considered breeds need to be related to the breed for which the outcross is performed, as breeding dogs that are too unrelated can result in maladaptive traits in the offspring (59). This does come with the benefit of the dogs not losing the trait the breeder wants to keep. An example of this is shown in the outcross of the Griffon Bruxelloi; for this breed the Australian terrier was chosen for an outcross, as they already shared similar characteristics. After the outcross, the prevalence of deleterious diseases was greatly reduced (61).

BACKCROSSING

After an outcross is performed, the litter of this outcross will have an inbreeding level of 0% (57). By crossing these individuals with purebred individuals again, the genetic diversity is increased within the population. In most outcrosses this process is repeated a few times. Every time a backcross is performed, the effects of the outcross are lessened, as the offspring is inheriting more genes of the original breed, and less of the outcrossed breed. This makes it so that an outcross is only a temporary solution, and that it is advised that an outcross alongside backcrossing should be incorporated into breeding programs every couple of years. This is called the **drip method** (Dutch: Druppelmethode) (Figure 4.**5**). Without proper planning of the outcross, and the necessary successive backcrosses the inbreeding of the population could even increase (57). This is also where the benefits of genotyping the individuals comes into play. If a breeder has an overview of which of the possible outcrosses will have the best results, and which backcrosses will have the greatest effect for the genetic gain of the total population, they are able to get the most out of their outcrossing (57, 59). This will limit the number of outcrosses that have to be performed. This would be beneficial for keeping the breed as “pure” as possible, which is a concern of many breeders (for further information, see section Breeders perspective).

Because of years of inbreeding, most purebred dogs have a high degree of homozygosity, which means that they have the same alleles for a specific trait. By outcrossing this degree of homozygosity gets immediately lowered, as with the outcross a different allele than the deleterious allele is passed to the offspring (59). Outcrossing can also be used to perform research on disease. Based on the outcross it can be determined whether a disease is dominant or recessive (62). If a homozygous individual with a disorder is outcrossed with a homozygous individual without a disorder, based on the offspring it can be determined whether the disease is a dominant disease, or a recessive disease.

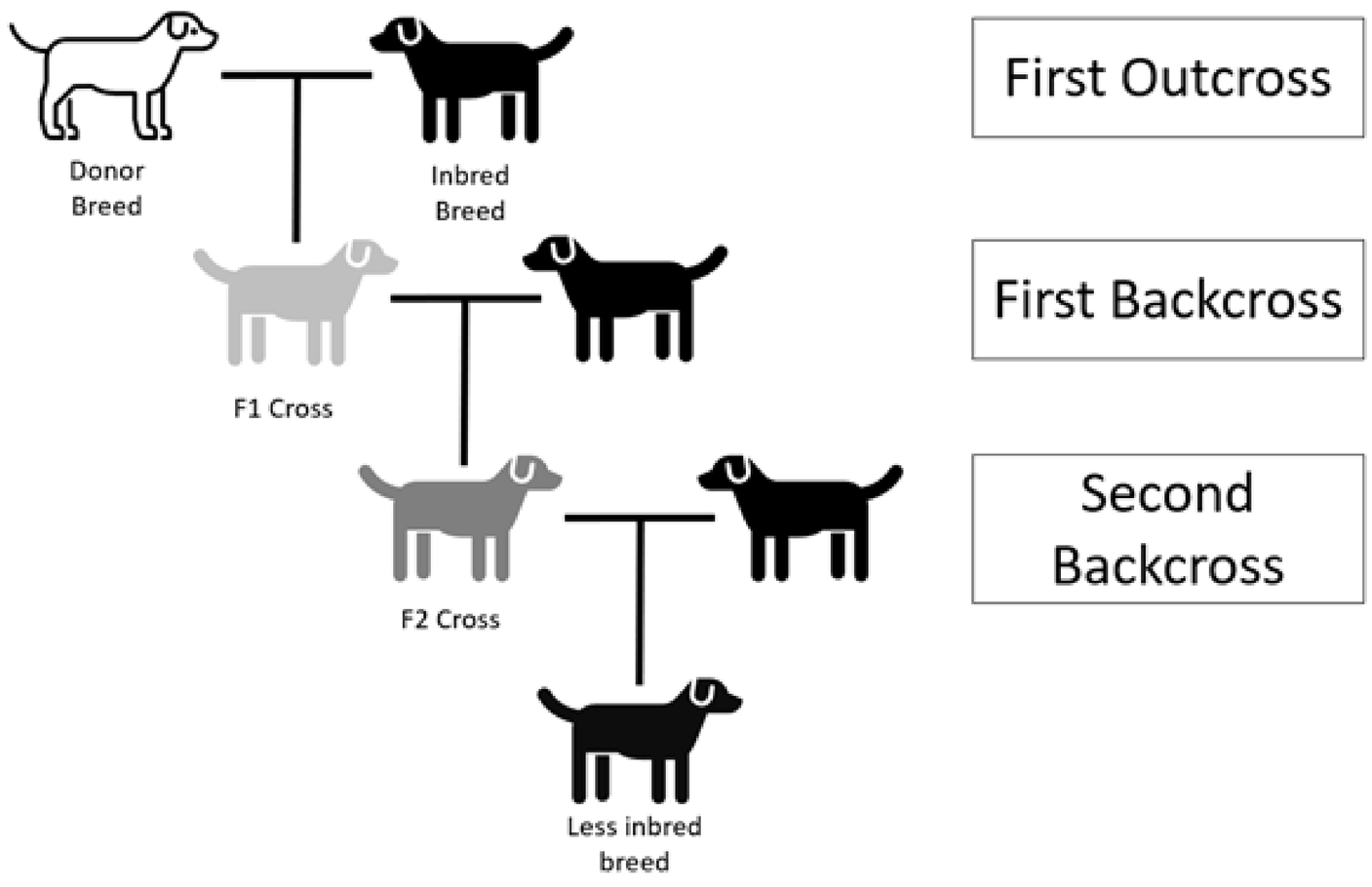


Figure 4.5 Depiction of an outcross, with 2 levels of backcross.

The steps that need to be taken within an outcross therefore are as follows: First a breed needs to be selected. The breed can either be closely related to the outcrossed breed, or distantly related. This consideration should be made based on the degree to which the breeder wants to increase genetic diversity or maintain the characteristics of the breed. Based on the phenotype of the dog, potential partners should be selected and genotyped. Furthermore, based on genotypic information the best combination of potential partners can be selected. These animals could then be mated. The offspring of this mating is selected carefully, and the best offspring are then backcrossed within the closed population. These backcrosses are then continued for multiple generations, where the offspring have a lower amount of foreign DNA every generation. This outcross should be repeated every few generations, as they will have less effect with every breeding (Figure 4.5), as for every breeding, the amount of foreign DNA will half again.

LOOK-ALIKES

As described above, some of the main threats and obstacles around health of pedigree dogs are the small population sizes, low genetic diversity, and high levels of inbreeding (23, 50, 52, 63). It logically follows then that an effective way to combat these problems is to increase the population size. More exchange between populations in different countries may be an option, but then costs and regulations from other countries have to be considered too. Using artificial insemination can overcome some of the costs, as frozen dog sperm can be easily transported to the Netherlands to be used to inseminate dams in the Netherlands. This still, however, poses some logistical challenges to select the sire to be used, which may have an unknown pedigree and so unknown relatedness to the potential dam in question, and of course also entails some costs in the transport of sperm.

Rather than looking for external sources to boost the Dutch population, then, maybe internal sources need to be considered. There is a vast population of **look-alikes**; dogs that in almost all aspects conform to the breed standard, but that are not affiliated with the pedigree and so do not have the official papers to be considered a purebred. There may be a lot of resistance to this approach; enthusiasts of a certain breed may argue that they are not 'pure' in their bloodline and that by including these in the pedigree, you do not know which other genetic defects you are introducing into the current population. Using genotyping, one can overcome these issues. Using SNP chips, you can see if the look-alike is in fact the dog breed in question, and depending on the selected SNPs, you can also have an overview of the disease risk the dog has, allowing you to utilise this reserve of animals in the population.

Using SNP chips to determine breed composition and/or breed purity has already been done in many livestock species (64, 65, 66). (67) were able to assign cattle with incomplete or missing pedigree data of four different breeds with an accuracy of 88.9% (104 out of 117) using only 133 selected SNPs. Considering that the RvB has a mandatory test of 50K SNPs, determining the breed composition should be readily (L. Roest, personal communication, 17 April 2023). As well as having relevant SNPs to use as breed markers, there will also have to be a reference population (66). In the case of the Saarloos Wolfdog, Stabyhoun and Wetterhoun, this should not pose a problem, as there is already a database with the DNA SNP chips which have been collected, as well as updated pedigrees to confirm the DNA data. In the Bouvier des Flandres, this may be more challenging as the database is less up to date.

Determining breed composition and/or breed purity can be done to a high degree of accuracy and is less time consuming than traditional methods like pedigree analyses, and can overcome problems with incomplete pedigrees (64, 66, 68, 69, 70). Further, if useful and informative SNPs are selected, then you will not need a high-density SNP chip to attain a high level of accuracy in breed purity (66, 68, 69, 71).

To determine breed purity, there are several steps involved. This is a generalised overview:

1) Selecting relevant and informative SNPs.
There are several ways to go about this. One method could be to use a high-density SNP chip, which include thousands of SNPs, providing a lot of genetic data (69, 71). Another way could be to select SNPs depending on their frequency and diversity within the breed. SNPs common in a breed and rare in other breeds can be used to distinguish between purebred and mixed individuals (68, 72, 73). Some SNPs are related to breed-specific traits, such as coat colour or ear shape. These can be used as markers (65, 69). This means per breed you would have to select which SNPs to look at for which breed-specific trait. (74) also used machine learning algorithms to identify the SNPs that are strongly associated with certain breeds of a certain ancestry.

Finding the best way to go about it for all breeds may be difficult, as SNPs informative for one breed may not say much about another breed. In an ideal world, every breed would have its own tailor-made SNP chip, but as this is currently not possible due to the cost involved, time and logistics, the same SNP chip is used for all of the breeds. (75) were able to distinguish cattle breeds using 133 selected SNPs; a 50K SNP chip should exceed the minimum requirement to distinguish between dog breeds.

2) DNA collection from a reference population i.e., the breed under study.
If you want to determine the breed purity of, for example, a Stabyhoun look-alike, you would need to collect DNA from the reference population - which in this case would be confirmed purebred Stabyhouns in the pedigree. If possible, it would also be informative to collect DNA from other closely related breeds, such as the Wetterhoun, French Spaniel or other breeds with similar aesthetics, ancestry or breeding purpose. This will make fine-mapping the differences between similar breeds more accurate. As DNA collection is already a mandatory process for the pedigree dogs from the RvB, there is already a good reference population to move ahead with. If breeds are closely related, which is the case with a purebred and a look-alike, then you will probably need 400-500 individuals in the reference population to be absolutely certain (76).

3) Genotyping the DNA samples with a SNP assay.
This process is described earlier in the chapter (see: What is a SNP chip?).

4) Analysing genetic data to estimate individual breed composition of look-alike
This can be done with several different methods. In livestock, one of the main methods used is a Principal Component Analysis (PCA), a statistical procedure to analyse large data sets with multiple dimensions to allow for easier visualisation (66).

5) Comparing breed composition of look-alike to breed composition of the purebred population to determine breed purity.
For this step, a threshold value for breed purity needs to be decided on, above which the look-alike will be accepted into the pedigree (66). In cattle, animals registered with 87.5% pedigree purity are considered purebred (66). One method to do this could be to set the threshold value so that the false positives (mixed-breed dog assigned as purebred) and false negatives (purebred dog assigned as mixed-breed) are balanced out. This allows look-alike purebred dogs to be correctly assigned but look-alike mixed-breed dogs are excluded, as best as possible (66). An acceptable value for dogs may be different to that of cattle, so this is something that needs to be looked at in detail, and may also vary from breed to breed.

6) Validating the results through testing and verification.
Before this process is widely used to test more look-alikes to include in the breeding population, it should be tested using confirmed purebred dogs in the pedigree to see if they were correctly assigned to the breed, as well as mixed or purebreds from another breed are correctly rejected. If the test's accuracy is satisfactory, and dogs are assigned and rejected correctly, then this method can be applied to look-alikes, as well as any other dog (66).

Tapping into this extra reservoir of dogs to include in the breeding population has the potential to significantly boost population numbers and get some more genetic diversity into the pedigree. By having these look-alikes genotyped, you can also have an indication of diseases and other detrimental genes they may have, allowing you to kill two birds with one stone. Overcoming the emotional resistance to this idea may be difficult for many breeders and 'pure' bred enthusiasts. However, if the main goal is to maximise the health of the breeds, look-alikes are interesting to consider.

ESTIMATED BREEDING VALUES

Most of the past breeding choices have been done using phenotypic selection in order to 'fix' a certain trait into the population. Using this process to produce genetic change is challenging because the phenotype is often a poor predictor of the individual's true **genotype**. Thanks to advances in data handling, industrial breeding programs often use a more data-driven method; **Estimated Breeding Values** (EBVs). The values reflect not only each dog's phenotype, but also its genotype (77).

EBVs rank dogs in a population, from the best to worst, for their likelihood of passing desirable/ undesirable genes to their offspring compared to the data of their relatives (78, 79). EBVs identify which young dogs have the highest estimates of **genetic merit** and are thus the ones who should be added to the breeding scheme. It is usually a number from X to -X (where 0 is the average, see Figure 4.6); however, in Sweden the mean is set to 100 (80). Since 2012, EBVs for many breeds have gradually been introduced into the Swedish dog breeding programmes (81). These EBVs are calculated by linking pedigree information with data from, for instance, the registrations of hip dysplasia status. By doing so, every individual's genetic risk could be calculated from the pedigree.

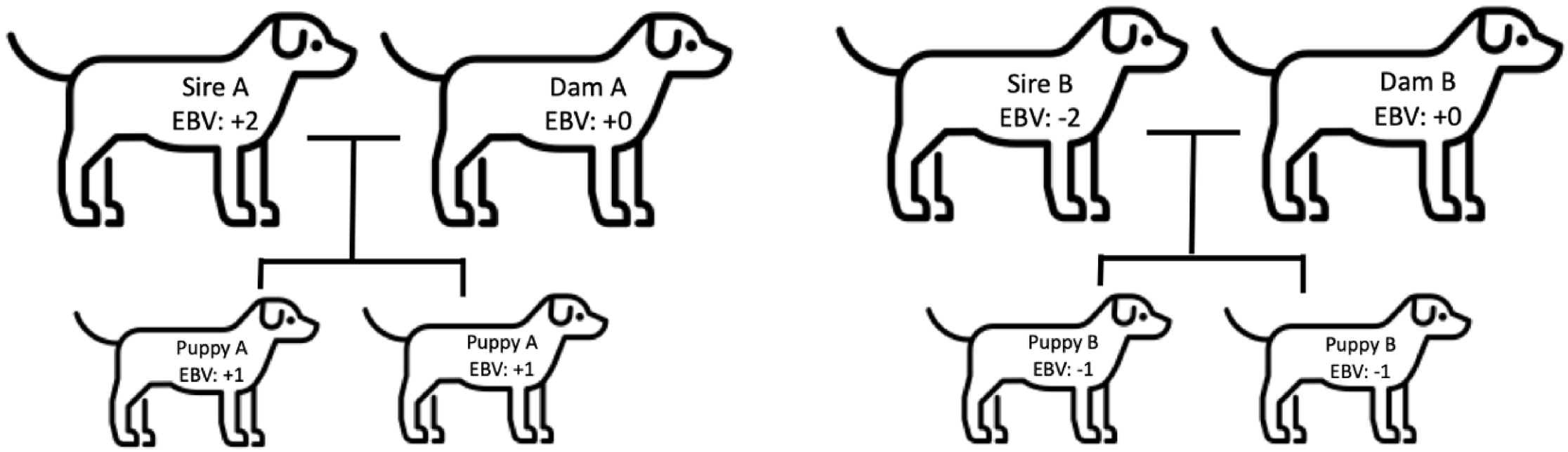


Figure 4.6 Explanation pedigree demonstrating Estimated Breeding Values (EBVs).

It is important to keep in mind that EBVs change over a dog's lifetime and the EBV of a puppy at birth will be the average of its parents, but it will change as the population develops. For the Swedish kennel club, the EBVS of 44 breeds are computerized and are updated weekly in order to provide the breeders the most accurate EBVs (last updates 2023-04-24; https://hundar.skk.se/avelsdata/Initial.aspx).

To establish an effective EBV evaluation system, three requirements must be satisfied (82):

1) The score should be biologically related to the disease phenotype. For example, dogs that receive a low score – depicting a low risk for a disease – should not develop that disease in later life.

2) The phenotype used to define the scores must be heritable.

3) The score should depict the amount by which the average value of the parents differs from that of the population, i.e., a score is given based on the reference population. For example, if all individuals in the population present the same phenotype, there is no genetic difference from parents to the reference population because they are all identical.

Using this method, two hip quality phenotypes were analysed to calculate EBVs for selecting breeders for the German Shepard, Labrador Retrievers and the Golden Retriever. They concluded that genetic selection improved hip quality when using the EBVs developed in 1980. Among first generation puppies, 34% of the German Shepherd Dogs, 55% of the Labrador Retrievers, and 43% of the Golden Retrievers had an excellent hip dysplasia score. Strikingly, after 8 generations of selection based on EBVs, over 93% of the German Shepherd Dogs, 94% of the Labrador Retrievers, and 87% of the Golden Retrievers received an excellent hip dysplasia score proving the efficacy of this method (82).

The accuracy of the EBV increases with numbers of offspring and this may take some time to achieve (83). Since current pedigree dog breeding programs are often too small to use EBV selection, it would be useful to pool pedigree and phenotype data across countries enabling a more accurate calculation of the breeding values. The Fédération Cynologique Internationale and the British Veterinary Association and Kennel Club came together and compared their estimations of genetic merit (EBVs) for hip dysplasia (80). They concluded that both scoring systems capture the same genetic trait and therefore, pooling of this data over multiple countries is useful and can increase the accuracy of EBVs for hip dysplasia. As the Netherlands has no EBV evaluation system in place, it might be beneficial to import dogs with high genetic merit from countries with EBV systems while developing their own EBV evaluation system. It is advised to look to the Swedish and British kennel club for their expertise in incorporating EBVs into the breeding programme.

Given the advancements in DNA marking and SNP chip availability, there has been word of **genomic breeding values (gEBVs)**. These will combine genotype, phenotype and pedigree information in an optimal value to produce a more accurate ranking for each animal in the breeding population, including those that have not been assessed for the trait of interest (84). Genotypic selection has been proven highly successful in dairy cattle (85) and has been suggested for improvement of pedigree dog breeding programmes (86). A study performed in 2011 demonstrated that genotyping a dog's genome (using SNP chips) can be used as an effective risk management strategy for the prevention of canine hip dysplasia (83). Another study confirmed that the prediction accuracy of genomic selection for hip dysplasia scores were generally better than pedigree-based prediction in fewer than 1200 Labrador retrievers (87).

Utilising gEBVs could revolutionise the pedigree dog breeding industry. It will allow small breeding programs, that lack extended pedigree data, to construct a genomic selection program based on the current population's genetic information. gEBVs show also practical use as they could be applied directly at birth – prior to purchase – once DNA of the pup has been collected. By including EBVs generated later in a dog’s life, gEBVs can be used to score complex diseases that result from multiple genes and environmental factors (88). It is suggested that the Dutch pedigree dog breeding programs invest in genotyping their dogs as soon as possible to get an accurate depiction of the genetic information per breed.

GENETIC MANAGEMENT

Genetic management is an important part of the management of any closed population, regardless of its use (e.g. research, companionship, etc.) (89). Dog breeds lose a lot of genetic diversity because of the high selection pressure of breeders. With the loss of genetic diversity, the inbreeding goes up. The increase of inbreeding leads to inbreeding depression and high incidences of heritable, and often recessive diseases (90). Therefore, purebred dog breeds generally have a higher rate of homozygosity than other species (91). Genetic management techniques are used to maintain the overall long-term viability of a breed for continued production of healthy animals and can therefore make sure that in the small population the best animals will be selected to breed (89). In managed populations, genetic diversity can be maximised by selection from the optimal contributions method; which shows how much an animal should contribute in each generation (90). Furthermore, one could also incorporate kinships in this calculation. With kinship, animals are removed from breeding that have a higher-than-average kinship with other breeding candidates (57). All of these methods work with DNA or pedigree data in some way, which can be acquired by dog owners. DNA data is preferred here, as it is more precise. These different methods will be discussed in more detail below.

MEAN KINSHIP

Selection based on mean kinship is a measure of the relatedness between an animal with the population of that animal (90). Animals with a low mean kinship value are on average less related to the population and thus more valuable regarding their genetic diversity. The mean kinship is dependent on the population of the breed, which means that the mean kinship of a dog will change over time (92). Mean kinship is based on an individuals kinship values with every individual in the population. The kinship between two animals is calculated with the relatedness coefficient (*r*), which indicates the relatedness between two animals. When counting paths in a pedigree, the relatedness coefficient can be calculated as follows:

$$Relatedness\ coefficient\ \left(r\right) = \sum_{i=1}^{m} 0.5^{n}\ (1+F)$$

The number of possible pathways that are in a pedigree between the two animals is indicated by *m*, *n* indicates the steps of the pathway that connect the two animals. The inbreeding coefficient (*F*) is the inbreeding coefficient of a common ancestor in the specific pathway. The kinship can be calculated according to the following formula (2):

$$f\ = 0.5 * r$$

The mean kinship is calculated by taking the mean of all kinships of an animal, including the kinship with itself. This will show which animals are most genetically different from the rest of the population. By selecting individuals with the lowest mean kinship for breeding, the population will increase in genetic variation. This does however also come with drawbacks. You cannot simply mate the dog with the lowest mean kinship with all dogs in the population, as this results in the mean kinship becoming higher. This also means that the mean kinship should be updated after each puppy is born. However, as the genes of that dog become common and intermixed, this implies that they cannot be used strategically anymore (2). This is what is observed with the popular sire effect.

By using large numbers of marker loci, DNA data can be used instead of pedigree data to calculate the kinship (93). This gives more accurate calculations, which can be useful for the selection of which individuals should mate, or which animals should at least be a contributor when the genetic diversity within the breed becomes too low (93). Pedigrees of purebred

dogs are not fully complete, and therefore the use of DNA data could become necessary to give an accurate estimation of the kinship between animals. When pedigrees are not correct, or not complete, mistakes in calculations of kinship add up (94). These mistakes will then be used to calculate the mean kinship and could give a different outcome as to which animal is the most genetically diverse. By making use of DNA data, far more data can be extracted (94).

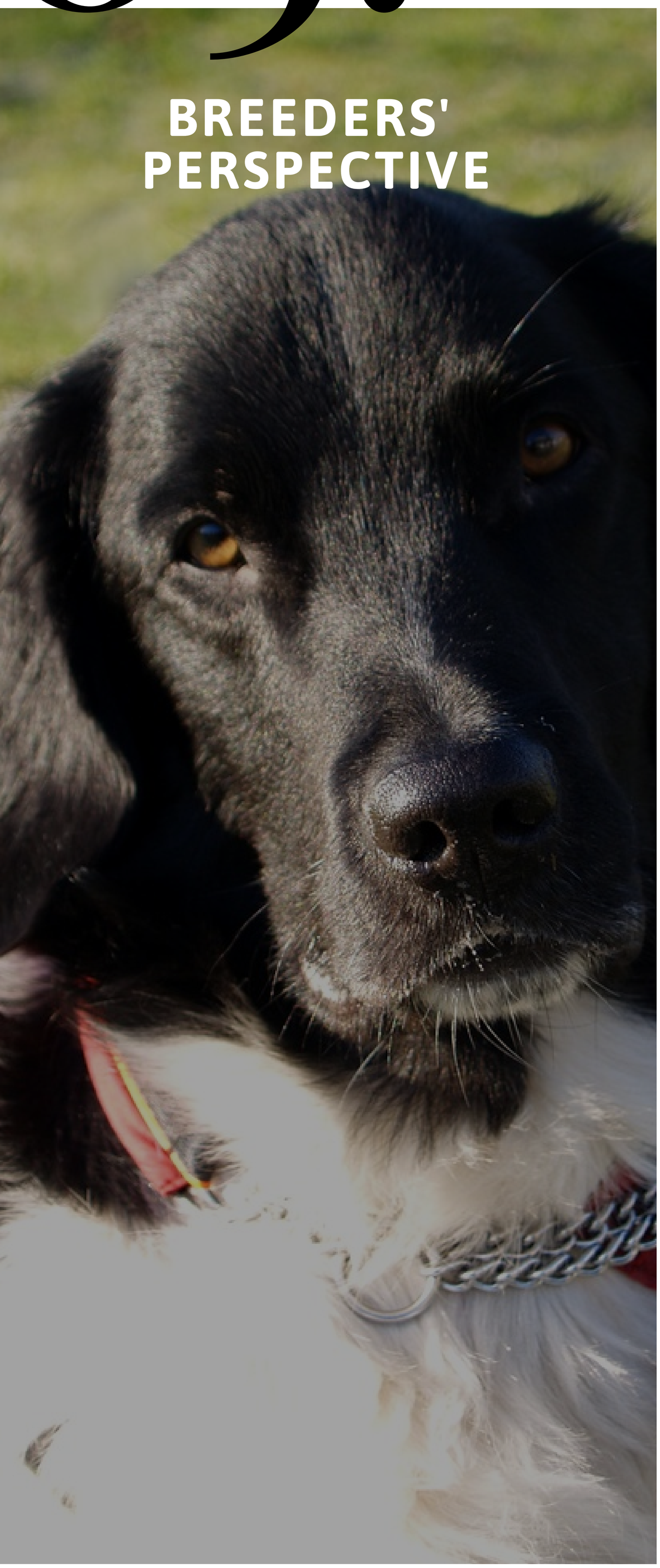
OPTIMAL CONTRIBUTION

Optimal contribution selection is based on the principle that the rate of genetic gain should be maximised, while the rate of inbreeding should be minimised (95). The optimal contribution selection allows you to maximise the EBV, while restricting the mean kinship (95). The principles of this strategy have shown to be effective at maintaining the genetic gain and lower inbreeding rates, and that they can therefore be maintained long term. The accuracy of these methods can be increased by using genomic information (96). Unlike the relationships derived from pedigree information, genomic relationships will vary for a given type of relative, which means that a group of siblings could have different mean kinship values, while having the same amount of relatedness according to their pedigree (97). Genomic data can furthermore help increase the genetic gain in a family, while keeping the inbreeding the same. With this genomic data, the optimal contribution can attain even higher accuracies than previously thought.

For implementation of optimum contribution selection, there are available software packages such as EVolutionary Algorithms (EVA) and R packages (Optisel) which aid in the calculations of optimum contribution selection (98). The EVA software, alongside other software predicts the optimal genetic contributions based on a pedigree (Berg et al., 2006). This program will give breeding recommendations according to the strategy for the optimal contribution selection. In order to use optimum contribution software, a lot needs to be available, such as reliable pedigree data, genetic markers (to determine the kinship), which animals can be used as a candidate, the breeding value or inbreeding rate of the animal and optionally the effective size of the population (98). Those who do not have this information, may see this as a drawback, but should strive to achieve this in order to maintain a healthy breed. Another drawback is that this strategy does not work if all breeders are working independently. Unfortunately, this is often the case for dog breeders, as they do not necessarily follow the advice that breeding associations put out for them (personal communications). If all of the data can be collected and breeders can be convinced to follow the directions given, the use of optimum contribution selection can be a great addition towards the growth of the genetic welfare of purebred dog breeds. Implementation of Optimal contribution selection can be difficult, as the outcome is a percentage of how much each animal should contribute to the next generation. This requires breeders to work together, for which thorough communication is necessary, and could become a problem. However even coming close to optimal contribution can improve the health of the breed.

05.

BREEDERS' PERSPECTIVE



INTRODUCTION

Selective breeding is essential to preserve a breed and can be a useful method to maintain genetic diversity. Breeders play a crucial role in breed population growth, being the main point of reproduction in pedigree dog breeds. If breeders face too many challenges they might quit, which in turn might lower the breeding population. A factor which might reduce the genetic variation even further which may result in accumulation of disease. In the previous chapter, the techniques of implementing DNA-data were explained. The goal of this chapter is to illustrate the challenges that come with implementing DNA usage in breeding programmes, as well as to give recommendations on how to overcome these.

First, we will discuss regulations for breeding both from the Raad van Beheer and the breeding associations. Strict regulations are necessary for breeding healthy dogs, but regulations that are too strict can deter breeders from following the rules. Secondly, we will discuss the financial side of breeding as it has several expenses for the associations, breeders, and owners. As aforementioned, DNA testing of the dogs can provide the breeding association with important information; however, these are also costly, a potential challenge the associations may face when implementing DNA. Lastly, we will discuss the perspective of the breeders, owners, and board on the breeding regulations, willingness to use your dog for breeding, willingness to do a DNA test, willingness to do an outcross, and to know the breeding value of your own dog.

REGULATIONS

RAAD VAN BEHEER (RVB)
The RvB is an overarching body in the field of **cynology** in the netherlands, counting more than 300 member associations. Their general task is to manage the Dutch studbook and set up rules and regulations, such as the mandatory DNA parentage test, which are adjusted approximately every six months(99). Additionl to this, the breeding associations set up their own breeding regulations but these are not allowed to contradict the cynological regulations(99). The RvB is a democratic organisation and thus if change has to be made, all their member associations are able to vote, giving their insights during general meetings (L. Roest, personal communication, 17 April 2023).

The RvB does not only make regulations, but they also monitor the breeding associations and their plans. For example, when a breeding association considers outcrossing their breed, they are obliged to present a plan of approach to the RvB for assessment (L. Roest, personal communication, 17 April 2023). In the case of multiple breeding associations per breed, they have to submit the plan of approach together (99). After approval, the RvB informs the FCI and foreign kennel clubs, and adjusts their pedigree administration. There are no international regulations regarding outcrossing, but outcrossing can happen on an international scale (L. Roest, personal communication, 17 April 2023). For example, the Stabyhoun association (NVSW) has mentioned that they are considering an outcross in collaboration with other countries (L. Roest, personal communication, 17 April 2023). In this case, there is a common understanding between the kennel clubs that they have to accept each other's pedigrees (L. Roest, personal communication, 17 April 2023).

BREEDING ASSOCIATIONS
In this subchapter, the name of the breed is used as synonym for the breeding association for simplification. The breeding associations are as follows:

- Bouvier des Flanders = Boe4
- Saarloos wolfdog = AVLS
- Stabyhoun and Wetterhoun = NVSW

BREEDING REGULATIONS
As aforementioned, the breeding associations are able to develop their own breeding regulations of which all members are obliged to obey (Table 5.1). For example, the breeding regulations of the associations state that for the Bouvier des Flandres a mating can be repeated four times (100). For the Saarloos Wolfdog a mating can be repeated twice under specific circumstances, only if the first nest consists of one or two pups (101). On the other hand, for the Stabyhoun and Wetterhoun a mating can only be repeated once (102, 103). Therefore, the Bouvier des Flandres regulations appear to be less stern considering repeating a mating compared to the other two breeding associations as well as in the number of sire matings; the sire can mate 10 times each calendar year, and a maximum of 50 times in their lifetime. Overall, the Saarloos Wolfdog has the strictest rules for the number of mating in sires: the sire can mate once each calendar year and 3 times during their lifetime. Furthermore, the Stabyhoun sire can mate maximum of 3 times in two years, and a maximum of 10 times in his lifetime, of which only eight can be done in the Netherlands. Compared to the Stabyhoun, the Wetterhoun is stricter with a maximum of 2 times matings in each calendar year, and with a maximum of 4 times in their whole life. So, there is a lot of variation between the associations.

Table 5.1 Overview of some differences between breeding regulations.

	Repeat mating	Number of matings per sire
Bouvier des Flandres	4x	10x each year, 50x in their whole life
Saarloos Wolfdog	2x, under specific circumstances	1x each year, 3x in their whole life
Stabyhoun	1x	3x each two years, 10x in their whole life
Wetterhoun	1x	2x each year, 4x in their whole life

HEALTH RULES
Similar to the breeding regulations, the breeding associations are able to develop their own health regulations of which all members are obliged to obey. As an example, the Bouvier des Flandres state that all individuals have to be screened for hip dysplasia, eye defects, and elbow dysplasia(100) while the Saarloos Wolfdog state that they have to be screened on all health risks specific to the breed: hip dysplasia, hereditary eye defects, pituitary dwarfism, degenerative myelopathy, and elbow dysplasia(101). Furthermore, the Stabyhoun has to be screened on hip dysplasia, and the cerebral dysfunction gene(102), while the Wetterhoun has to be screened on hip dysplasia, and the SCID-gene(103). In order to prevent matings of individuals suffering from heritable diseases, it could help to register the diseases in the system to inform the breeders (H. van den Hoek, personal communication, 17 April 2023). Subsequently, honesty is required from the breeders when registering diseases, this might pose a challenge (H. van den Hoek, personal communication, 17 April 2023).

Dogs presenting high risk for heritable diseases are excluded from the breeding population; however, this threshold varies for each breed. Each breed also states several disqualifying flaws that exclude an animal from breeding. These are stricter for the Stabyhoun and Wetterhoun as they will exclude animals with an under- or over-bite, or an animal that is deaf or blind. The Bouvier des Flanders and Saarloos Wolfdog have fewer disqualifying mistakes: they will exclude an animal that has extreme fears or extremely aggressive.

MONITORING AND SANCTIONS
Naturally, the breeding associations would like their breeders to obey the procedures and rules; if this is not the case, sanctions are put in place for the breeders. These vary in each association, and also vary for the offence. For example, the breeders of the Bouvier des Flandres are lenient in controlling their breeders; however, they are required to notify a mating to the association (A. v.d. Berg, personal communication, 20 April 2023). The Saarloos Wolfdog provides their breeders an official warning after their offence (M. Eggink, personal communication, 17 April 2023). On the other hand, the Stabyhoun and Wetterhoun first issue a warning, after the second offence they are reprimanded. If the behaviour is repeated a third time, they are exiled from the association. According to a representative of the Stabyhoun association, the effectiveness of this method is debated within the association, and it was suggested that the first warning should be published on their website for all members to see (H. van den Hoek, personal communication, 17 April 2023). Below is a visual overview of this entire section on regulations (Figure 5.1).

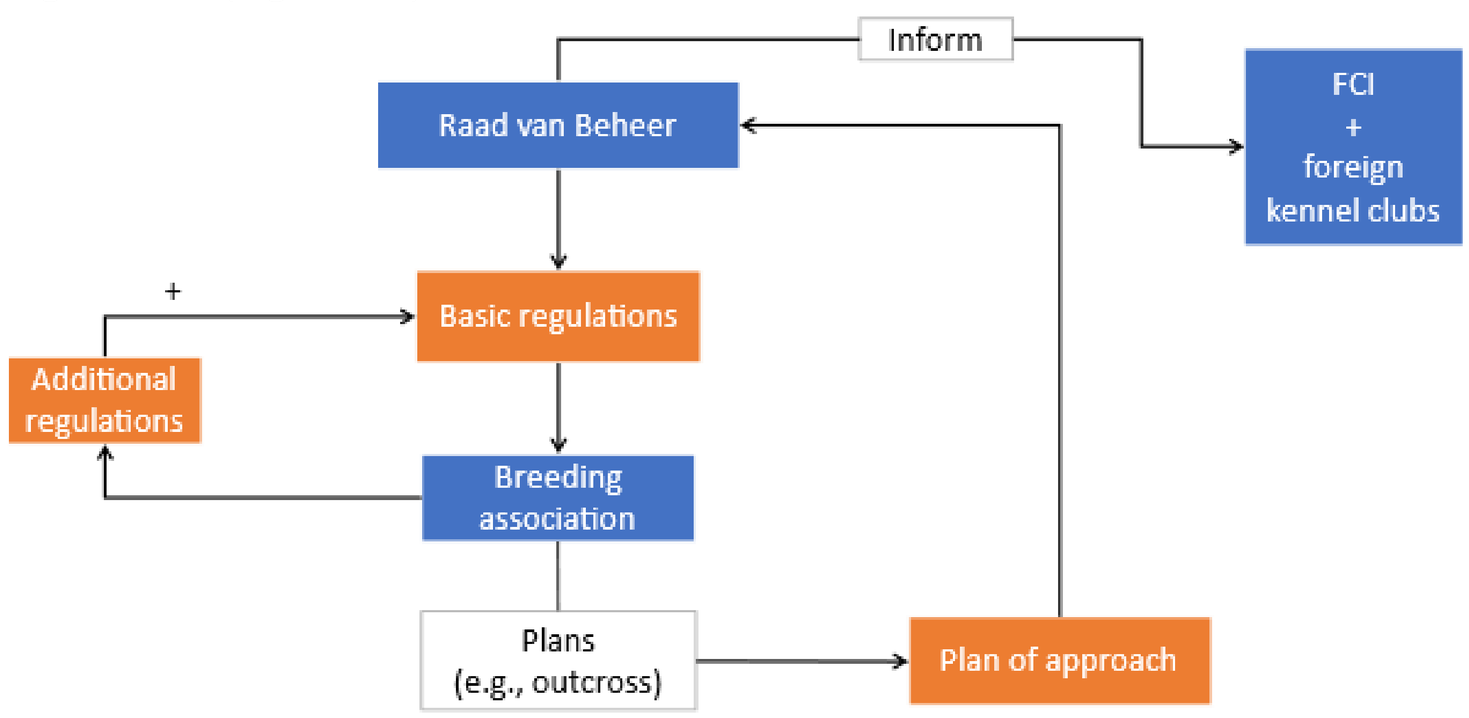


Figure 5.1 Overview of the regulations (orange), involved parties (blue), and actions (white).

FINANCIAL SIDE OF BREEDING

Breeders are confronted with a plethora of costs when they breed in line with the regulations of their association and the RvB. Every breeder has to request a UBN-number (unique company number) from the Rijksdienst voor Ondernemend Nederland (RVO), which costs €19(104). There are also registration fees, which are €99,00 per dog (including litter check and chip).

In order to start a **kennel**, breeders encounter costs to register for a name (€317.60). Furthermore, the name of the kennel has to be checked which will cost €48.20. Afterwards, a breeder can start the breeding process, in which first a combination of two dogs is chosen. The mating of the two dogs will have to be reported to the RvB, as well as the birth declaration. Subsequently, if either of these are late, a fine of €25.20 is issued.

Additional costs are applied when giving a pedigree declared by the RvB and if the pedigree has to be corrected it costs €38.60 - but if there is a logbook: €67.30. The logbook consists of data such as, sport results, championship results and titles (99). If one would ask for a duplicate of the pedigree, it will cost €44.90 without a check and €89.20 with a check. Once an owner has bought the puppy, transferring a dog from the breeder to the owner costs €23.80. (99)

There are many obligatory health-related tests and screenings such as those for hip dysplasia (€ 62,30), elbow dysplasia (€ 74,70), and DNA-profile tests (€ 43,30). Some of which have to be done regularly, such as eye examinations in the Bouvier des Flandres (A. v.d. Berg, personal communication, 20 April 2023). An external company (such as Embark) can perform a DNA test to analyse whether a dog is a purebred or to check their health; however, it costs $199 (at Embark). If these tests are indeed mandatory, one can understand that some may be deterred from applying for such tests but also then obeying the breeding associations(105). A representative of Boe4 expressed his concern that the high costs and strict regulations could cause some breeders to breed from outside the association's supervision (A. v.d. Berg, personal communication, 20 April 2023). A similar concern was expressed by a representative of the Raad van Beheer, as well as a representative of the AVLS (L. Roest, personal communication, 17 April 2023; M. Eggink, personal communication, 17 April 2023).

Reputable breeders will carry out all these tests and screenings, which will probably increase their puppy prices. Since puppies from a reputable breeder are more expensive and consumers can risk being rejected for not being a good fit for the puppy, consumers might be more inclined to purchase one from a commercial breeder(106) or a breeder who plays a little fast and loose with the regulations. The representative of the AVLS confirmed that screening all puppies in an outcross project would lead to major expenses (M. Eggink, personal communication, 17 April 2023). People finding puppies elsewhere could be avoided if the RvB agreed to have the puppies screened only by a certified vet and not include an extra screening by the RvB.

There are also optional costs for the owners of the dogs, such as competing in sports or championships. The championships have entrance costs for each competing dog, as well as permit costs for the association to host the championship (99). Not to forget, the championship medal as well as grooming costs for competing dog owners. Additionally, the NVSW lists several sports and activities (agility, frisbee, fetch and canicross to name a few(107)) that the dog owners could join for which there are additional costs; for example, for training days and competitions.

Overall, dog breeding is expensive. The mandatory costs for breeder are already high and by implementing extra costs for DNA tests or extras screenings the associations may discourage breeders from obeying their rules. We recommend looking into subsidies and suggest that it might be possible to collaborate with research institutes in order to qualify for a subsidy. It could also help to discuss with the RvB what they can do to help the breeders and associations with these costs, since the DNA tests and screenings are beneficial to the breeds, and this indirectly also benefits the RvB. Below is a visual overview of the key players that influence the costs of breeding (Figure 5.2).

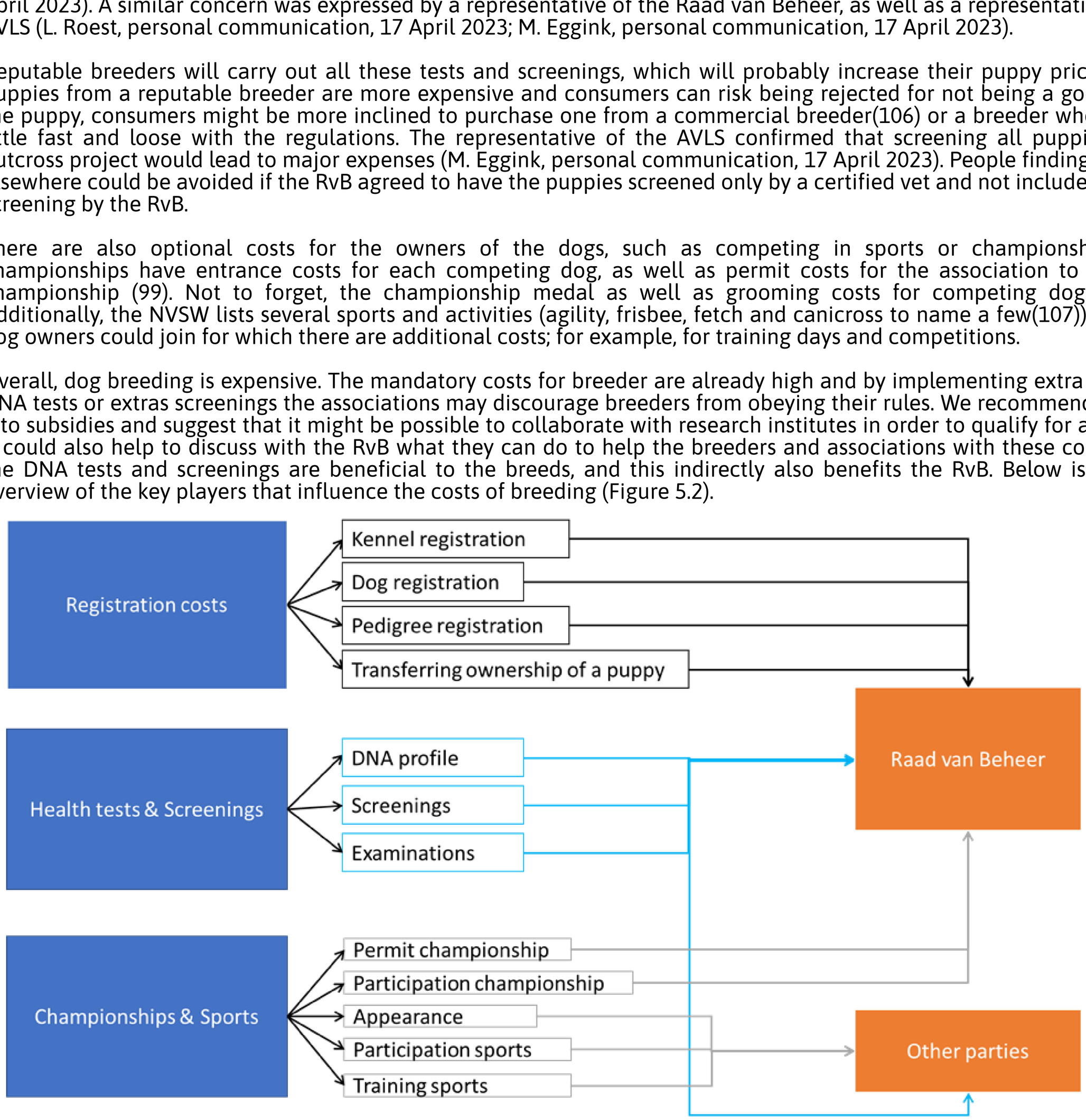


Figure 5.2 Overview of the financial costs for breeding, the other parties include breeding associations, veterinarians, companies such as Embark, etc. The arrow colours for each of the three types of costs are different to give a clearer overview of the origins and destinations of the arrows.

BREEDERS, OWNERS, & THE BOARD

A questionnaire was sent out to get more insight into the owners of the purebred dogs. The owners were asked on the following topics: health problems, breeding with their own dogs, willingness to collect DNA, willingness to do an outcross, and willingness to know the breeding value of their dog. This questionnaire was sent out to the members of the NVSW, AVLS, and Boe4 and to members in various Facebook groups. In total there were 246 responses, indicating the enthusiasm of the association members. There were 62 responses of people who owned a Bouvier des Flanders, 77 responses from Saarloos Wolfdog owners, 92 responses for the Stabyhoun, and only 15 responses for the Wetterhoun. All age categories were represented for each breed (Figure 5.3). Most of the owners that filled out the questionnaire have purebred dogs that have a pedigree, for the Saarloos Wolfdog all dogs had a pedigree (Figure 5.3).

The owners of the Bouvier des Flandres believe that the following characteristics of this breed should be preserved: its strong character, loyalty, alertness and child friendliness. However, this breed is not for every owner, you need to have the time, space, and give enough attention to fulfil the needs of this breed. Additionally, this breed is not recommended for beginners as they can be very stubborn and are slow learners although once they have learned something they will not forget it.

According to the owners of the Saarloos Wolfdog, the breed has a very specific character and is therefore not for everyone. The breed is very loyal to what they consider their pack members but reluctant to other people which will result in a very special owner-dog bond. For this breed you need to be consistent and read into the specifics of the breed before buying a puppy. The dogs are also described as intelligent and gentle.

The Stabyhoun has been described as eager to learn, enthusiastic, energetic, playful, and child friendly. An active owner is selected since these dogs are quite energetic. The dog can be stubborn, and therefore the owner has to be consistent in its orders. Furthermore, the Stabyhoun is stress sensitive as well as insecure and sensitive to sounds and other overwhelming stimuli. This breed is also not for everyone, but they are often described as sweet and gentle dogs.

The Wetterhoun is a unique hunting dog with many qualities, according to the owners. The breed is also of Dutch heritage and therefore is important to preserve. The owners described them as being loyal, social, reliable, and having a will of their own. This dog is not recommended as a first-time breed, because they can be stubborn and are hard to raise; however, if an owner has enough time and patience the breed is cuddly and loyal to their owner.

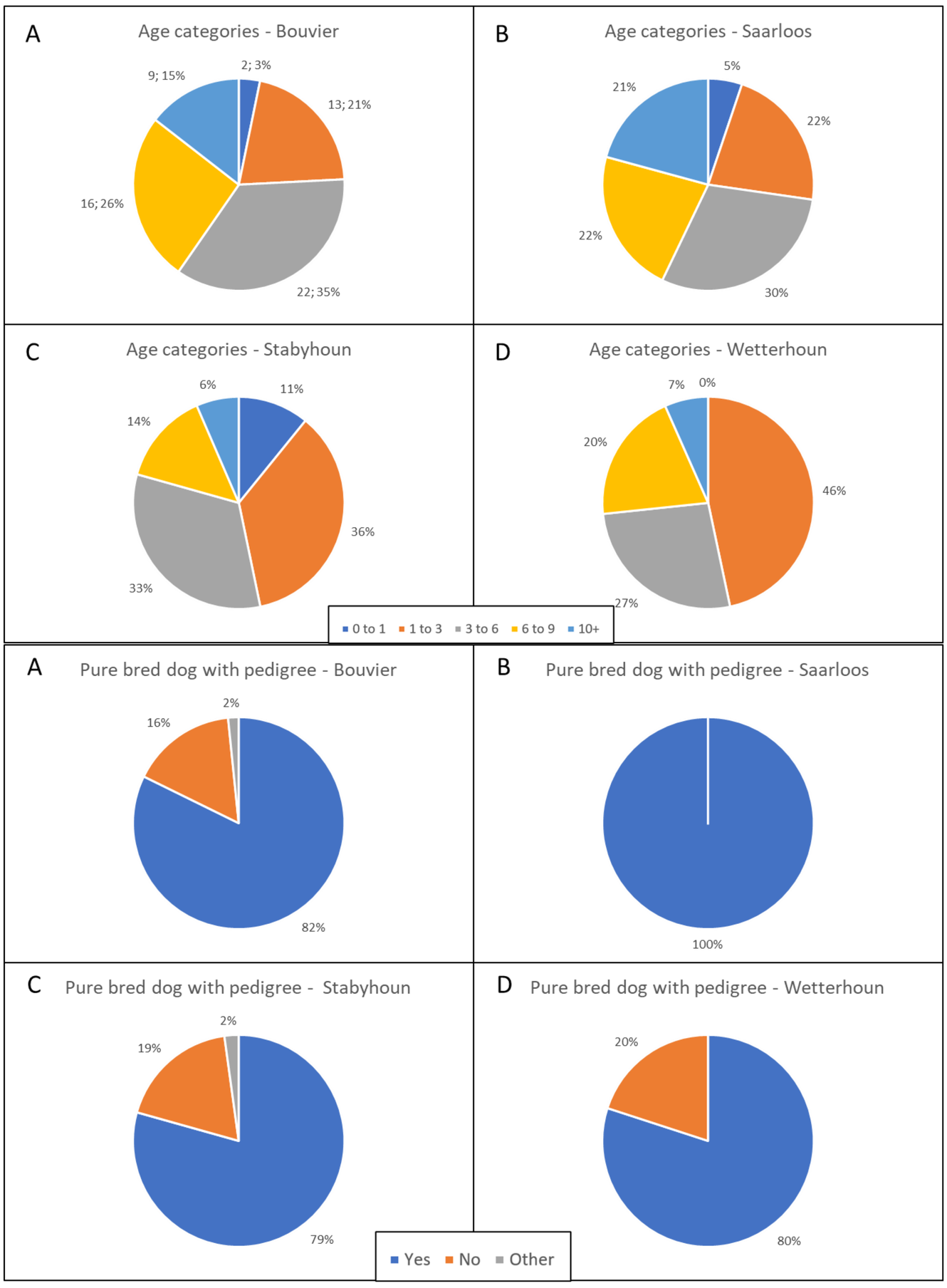


Figure 5.3 The various age categories that were represented for each breed (above) and summary of purebred dogs with or without a pedigree (below). A) Bouvier des Flandres, B)Saarloos Wolfdog, C) Stabyhoun, and D) Wetterhoun.

HEALTH PROBLEMS IN THE DOGS

Fortunately, most of the respondents reported that their dog did not have any health problems (Figure 5.4, on the next page). However, there were some respondents that did report that their dog having a health problem. The dogs all had various health problems; some were breed-specific while others were more general. The health problems that were found in these four breeds were hip dysplasia, elbow dysplasia, several allergies, and epilepsy, confirmed by the profile chapter.

Breed specific health problems of concern were also added by the owners; Bouvier des Flandres mentioned that their dogs suffered from several eye issues, an auto-immune disease, intestinal problems, juvenile cellulite, spondylosis, dementia, deafness, and Radius Curvus Syndrome. Saarloos owners reported arthrosis, heart issues, skin problems, hypothyroidism, mental health problems, and only one descended testicle. Owners of a Stabyhoun mentioned ear issues, back problems, and sensitive stomach or intestines. Wetterhoun owners reported irritated paws and diabetes.

OPINION ON THE BREEDING REGULATIONS

The respondents were asked to share their opinion on the breeding regulations of their respective associations. Breeding with your dog is essential to keep the breed healthy; however, not all matings result in a healthy dog. Breeding regulations are there to guide the breeders with their decisions; however, these regulations need to have a realistic balance between what is feasible and what is ideal. For example, ideally one would like a mating that results in an inbreeding coefficient of maximum 10%; however, if the average inbreeding coefficient of the whole population is above 35% thus, this is not feasible. Therefore, it is clear that the breeding regulations should adjust to the current state. The answers of the respondents were divided into positive, negative, or neutral attitudes

toward the regulations (Figure 5.5). Neutral responses ranged from “no opinion” to responses with equal negative and positive opinions.

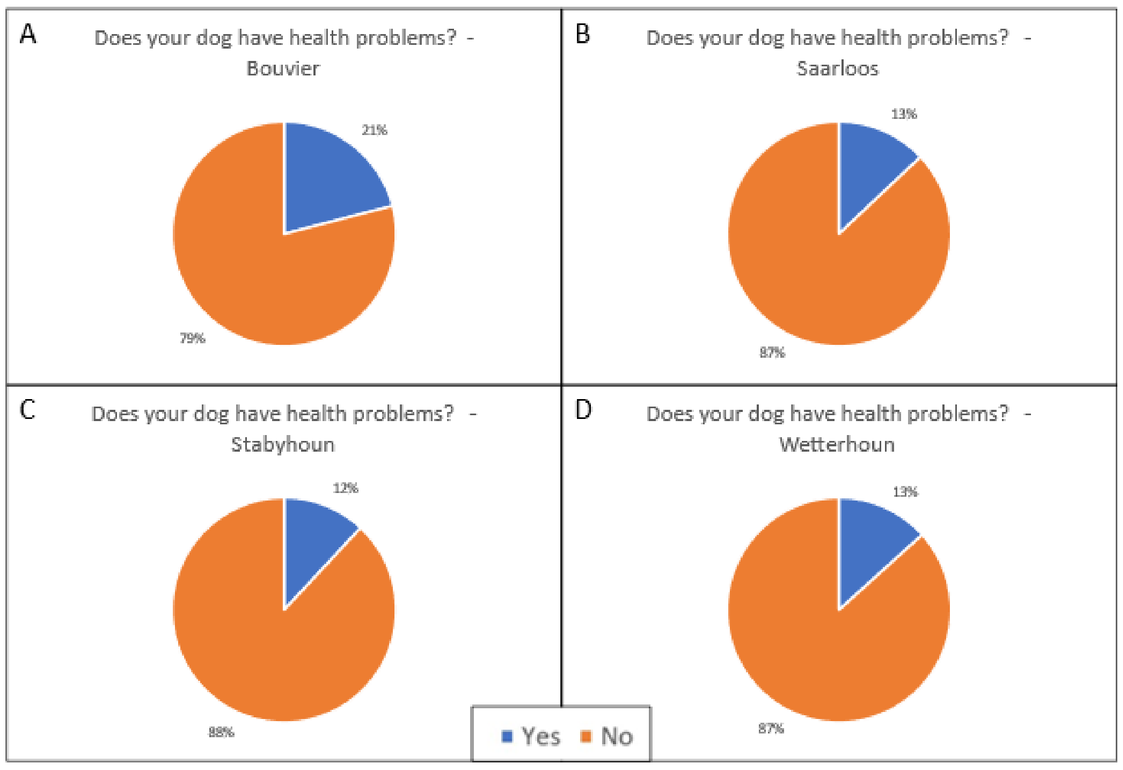


Figure 5.4 Responses to the question: "Does your dog have health problems?" A) Bouvier des Flandres, B) Saarloos Wolfdog, C) Stabyhoun, and D) Wetterhoun.

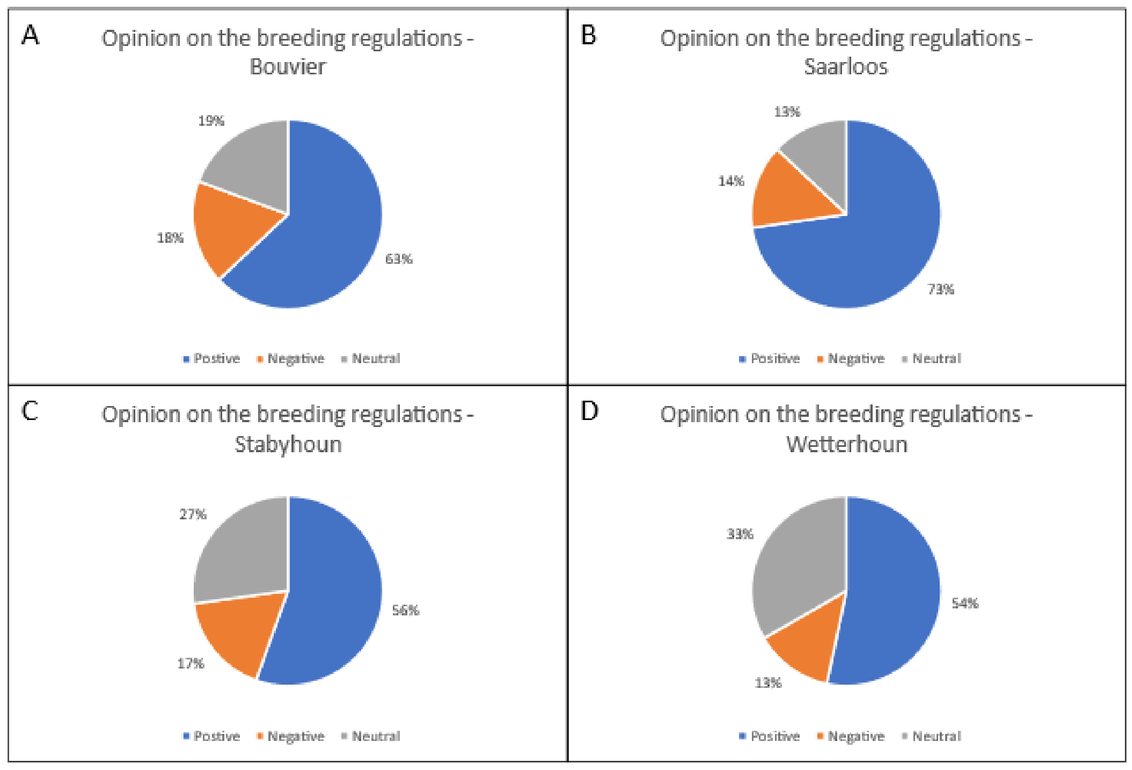


Figure 5.5 Ratios of positive, negative and neutral opinions on the breeding regulations. A) Bouvier des Flandres, B) Saarloos Wolfdog, C) Stabyhoun, and D) Wetterhoun.

First, owners of the Bouvier de Flandres were generally positive about the breeding regulations, stating that they were strict but necessary. Some even mentioned that the regulations should be even stricter. Most respondents with a negative opinion called the regulations vague and thus, causes confusion. Some of the negative responses were about specific parts of the regulations that the respondents disagreed with. For example, one respondent expressed dissatisfaction that blond fur is not allowed; having a Bouvier des Flandres with blond fur is not according to the breed standards; however, including them could increase the breeding population. Many respondents also compared the two different associations. Two people stated that they think the regulations of Boe4 are more focused on the health of the dogs, while the regulations of the Nederlandse Bouvierclub focus more on the breeders’ interests. The respondents typically favoured Boe4, but this result could be due to possible bias. It is interesting to note that several people mentioned that they want the regulations for both associations to be the same which could help to increase the breeding population, as well as communication between the two associations. Together they should reach out to more breeders and owner to keep the breed healthy.

Likewise, the Saarloos owners were also mostly positive to the current regulations. Several respondents stated that they think the regulations are effective in promoting health in the breed, which is important. Otherwise, the practice of the outcross was also a source of positive opinions. On the other hand, negative views were expressed by individuals that think the regulations are too strict, or they were not a fan of regulations in general. We identified an information mismatch between the board and breeders, which was clear from both the questionnaire and the interview: the breeders are not always aware of the difference between deciding on a mating based on DNA and based on pedigree (M. Eggink, personal communication, 17 April 2023). We suggest more elaboration on decisions in order to decrease the knowledge gap between board and breeders. Breeders often complain that the choices made cannot be explained properly on pedigree level which is the root of a lot of debate. For example, when two dogs are selected to mate together, they are from the same line in the pedigree; however, on DNA level they are very dissimilar. Hence, they are a good match even though the pedigree data would suggest otherwise. Similarly, the Saarloos Wolfdog is also comprised of two separate associations where some respondents prefer AVLS, some prefer NVSWH, and some dislike both.

In comparison to the Bouvier des Flandres and Saarloos Wolfdog, the owners of the Stabyhoun were less positive. The respondents described that the regulations are too strict about looks. For example, a Stabyhoun that has the wrong eye colour is excluded from breeding. These strict regulations on looks might raise some ethical questions which will be elaborated on in the ethics chapter. Some respondents also stated what the association could improve on: the maximum number of litters per dog should be lowered or have a mandatory screening for elbow dysplasia. Importantly, not all respondents were negative about the regulations with some respondents stating that they thought that the breeding regulations were acceptable and precise.

Overall, the owners of the Wetterhoun were not elaborate with their explanations. They did mention an outcross, but the responses were conflicting. They commended the associations efforts; however, the breeders would like to receive more help from the association. It was also mentioned that they think that the regulations should focus more on tackling the inbreeding effects.

USING THEIR OWN DOG FOR BREEDING

Not every purebred dog owner wanted to use their own dog for breeding (Figure 5.6). Having a large breeding population is key in maintaining genetic diversity. This is easier said than done, as a lot of factors are involved in breeding such as, costs, space, and emotional attachment to the puppies. It was interesting to see that less than half of the owners have bred with their dog and most of these puppies received a pedigree. The explanations as to why owners do not wish to partake in breeding were similar across all the breeds; either they do not have enough time or space to facilitate the puppies for eight weeks. Another reason was that the dog has health issues or was rejected from the breeding population (some Stabyhoun dogs were rejected based on the appearance). Some owners wanted to use their own dog for breeding, but they were not successful. One should also keep in mind the potential emotional attachment to the puppies: some owners indicated that is an obstacle to find good owners for the puppies and keeping all puppies is usually not an option. Fortunately, there are also people that do breed with their dogs who reason that they want to preserve the breed and keeping the breed healthy. Most of the puppies that were bred from the purebred dogs received a pedigree.

It is noticeable that the portion of the Bouvier des Flandres owners that have used their own dog for breeding is smaller compared to the other breeds. This could be explained by the fact that more than half of their dogs were sterilized. There were also several health problems in the Bouvier des Flanders which excluded them from breeding. Overall, the breeding population of the Bouvier des Flandres decreased and will continue to decrease, unless more owners are stimulated to use their dog for breeding.

It is, therefore, extremely important to stimulate owners to breed their dogs. During the interviews, the associations AVLS, NVSW, and Boe4 were asked how they would stimulate more owners to use their own dogs for breeding. The AVLS indicated that personal contact works best, for example the “AVLS” day, breeders' days, and with webinars on breeding and hope that by doing so they encourage owners to use their own dogs for breeding. The NVSW also hosts a “breeders’ day” where people can check if their dog is suitable for breeding by judges (H. van den Hoek, personal communication, 17 April 2023). If the dog is considered suitable for breeding, they will contact the owners to ask if they can use the dog for breeding. Similar to AVLS, the NVSW also try to stimulate the owners through personal contact. On the other hand, the Boe4 has indicated that there is no clear approach on how to increase the breeding population and stimulate the owners (A. v.d. Berg, personal communication, 20 April 2023). Nowadays, breeders can just choose themselves which dogs they want to pair up. However, they did indicate that there is word of mouth to stimulate owners to breed.

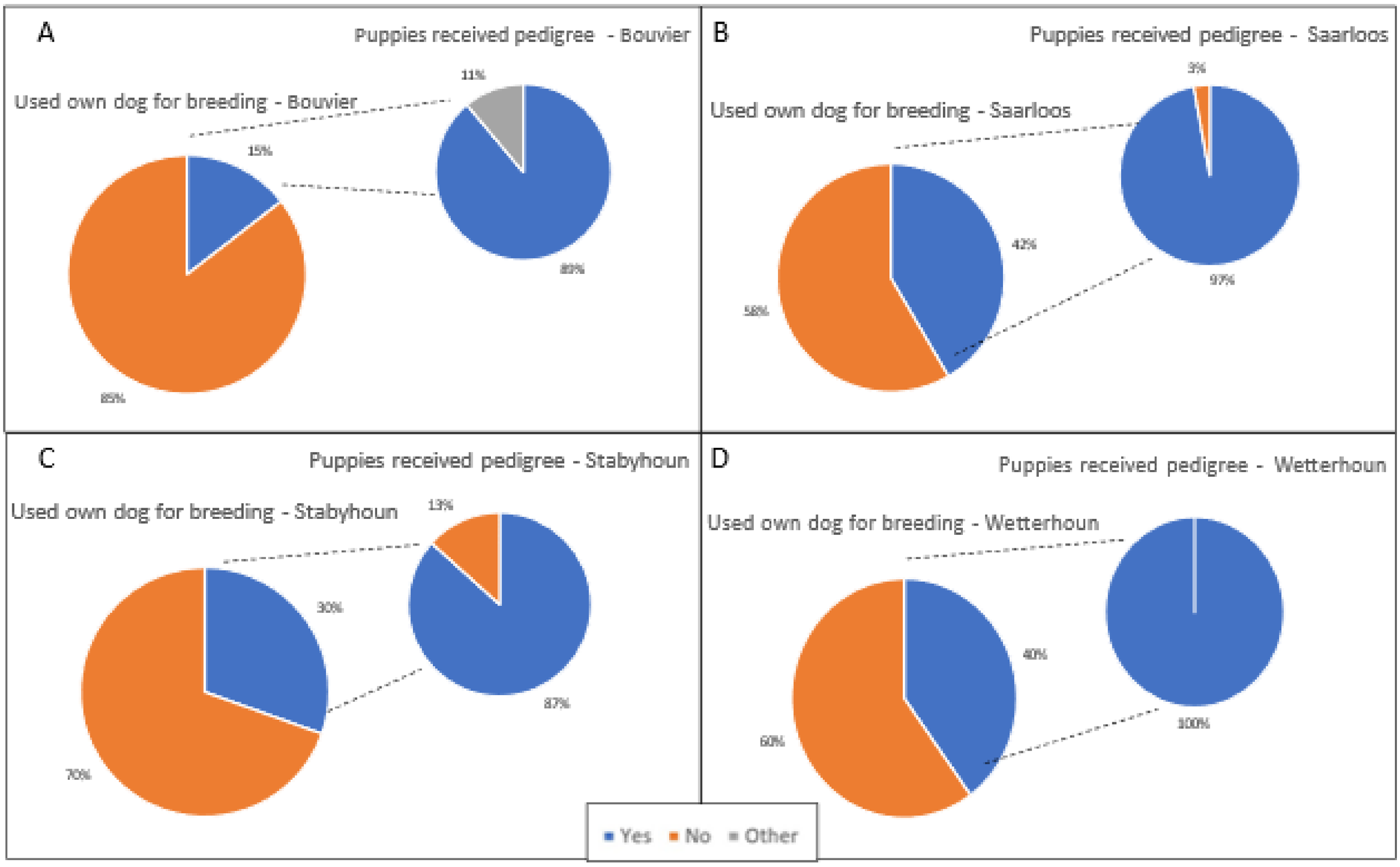


Figure 5.6 Summary of results from the questions: "Have you used your own dog for breeding," and if their puppies received a pedigree. A) Bouvier des Flandres, B) Saarloos Wolfdog, C) Stabyhoun, and D) Wetterhoun.

WILLINGNESS TO COLLECT DNA, AND KNOW THE RESULTS

Collecting the DNA of dogs can give more information on diseases and inbreeding and could potentially provide more information to choose the best dog for mating, in regard to the health of the breed. Luckily, most owners are willing to collect the DNA of their dog (Figure 5.7, on the next page). Their reasoning to collect DNA from their dog varied, such as that it was already done when the puppies received their chip, or to help researchers. Unfortunately, not all owners of purebred dogs are. From the responses of the questionnaire, it became clear that there was a mismatch between the information that is available and what is understood by the owners. Overall, the main reasons to not collect DNA were similar for all breeds. The reasons include that their dogs will not be used for breeding, or it depended on what the aim is for collecting the DNA.

The answers that were indicated by the category “other” were either owners that did not know yet or did not research it yet. Furthermore, owners indicated that it depends on how it is collected and what the costs are. Overall, more information is needed for the purebred dog owners on the aim of DNA collection and how it is collected. This could increase the willingness to collect DNA. Therefore, it is important that the information is easily accessible for the breeders.

The DNA could indicate that a dog has a high risk to develop health issues. Fortunately, most owners are willing to learn this as they want to know if measures can be taken beforehand. Others suggested that the dog could then be excluded/included from breeding or to improve the partner selection. Owners that disagreed indicated that dogs are “living animals”, and an emotional bond has already been formed describing that they do not feel the need to know the results since they do not see the dog as a product. For example, you can get rid of a car when it is not working properly, but you would not get rid of a dog when there is a risk of getting health problems. Responses that were categorized as “other”, were mainly responses that were both negative and positive or if they are not sure if they want to know.

WILLINGNESS TO DO AN OUTCROSS, AND TO USE THEIR FEMALE DOG FOR AN OUTCROSS

An outcross is beneficial for increasing the genetic diversity in a breed. It could decrease the number of heritable diseases and decrease the inbreeding coefficient. Nevertheless, outcrosses are regularly debated within an association. The respondents were asked to share their willingness to do an outcross and if they were willing to use their (hypothetical) female dog for an outcross project (Figure 5.8). The responses were categorized as positive, negative, or other. Owners were classified as “other” when they expressed no opinion, when they did not understand the concept, or when they expressed both positive and negative opinions equally.

The most interesting result seems to be the fact that Bouvier des Flandres owners are not as positive about outcrossing compared to the other associations. In fact, the opinions are extremely divided within the group. Most of the respondents mentioned concerns about preserving the typical characteristics. The respondents with a positive attitude toward

outcrossing mentioned they would do an outcross if it benefited the breed, but only after careful consideration of what other breed to use. On the other hand, the owners with a negative attitude towards an outcross stated that they are afraid to lose the breed specific traits that they hold dear. Another setback is the miscommunication on the definition of an outcross. Outcross is in this project defined as “mating an individual from one breed with that of a different breed; however, many respondents seemed to think this meant crossing two different breeds to create a new breed, for example crossing a Labrador Retriever with a Poodle that resulted in the Labradoodle. We think that there might not be a clear understanding on the process of an outcross with most owners and breeders and we suggest more information should be provided from the associations and researchers to the breeders and owners. It was interesting that when owners were asked if they were willing to use their (hypothetical) female dog for an outcross project, the response was overwhelmingly “no”. Unfortunately, most of the respondents did not provide an explanation. The explanations that we did receive, were similar to the reasons from the owners that were willing to do an outcross. Such as, promoting health, not wanting the breed to change, and for some owners it would depend on the other breed.

In contrary to the Bouvier des Flandres owners, the Saarloos Wolfdog owners were mostly positive about outcrossing. They mentioned that it was necessary to do an outcross, and that it is essential for a breed health. They do express concern about the choices that are being made, such as, preserving the specific traits of the breed, as well as the mating and breeds that were chosen for the outcross. Since, the outcross pups could be dissimilar to the Saarloos Wolfdog, some owners were concerned if it would then be possible to find new owners for the pups. When asked if they were willing to use their (hypothetical) female dog for an outcross project, most responded with a “yes”, as long as they agree with the donor breed.

Overall, the Stabyhoun owners were also mostly positive about outcrossing. They mostly expressed similar opinions to the breeds above. The owners indicated that the outcross should not be necessary if the associations help the breeders with their decisions and mating. Despite the positivity about outcrossing, the opinions were split on whether the owners would use their (hypothetical) female dog for an outcross project. Which is problematic, since female dogs are crucial in breeding – in outcrossing and in general. Unfortunately, most respondents did not answer the question, or they were not sure. Many respondents answered that they did not have a female dog, which probably means that the question was not formulated clearly. The owners that answered “yes”, typically mentioned that they would do it as long as there were suitable buyers for the puppies.

Wetterhoun owners were all either positive or neutral about outcrossing even though there were only fifteen respondents. While they were positive, they do have requirements such as, keeping the breed specific traits. When asked if they were willing to use their (hypothetical) female dog for an outcross project, the opinions were split. There were a few respondents that said they would be willing to use their female dog, but they were not able to do so.

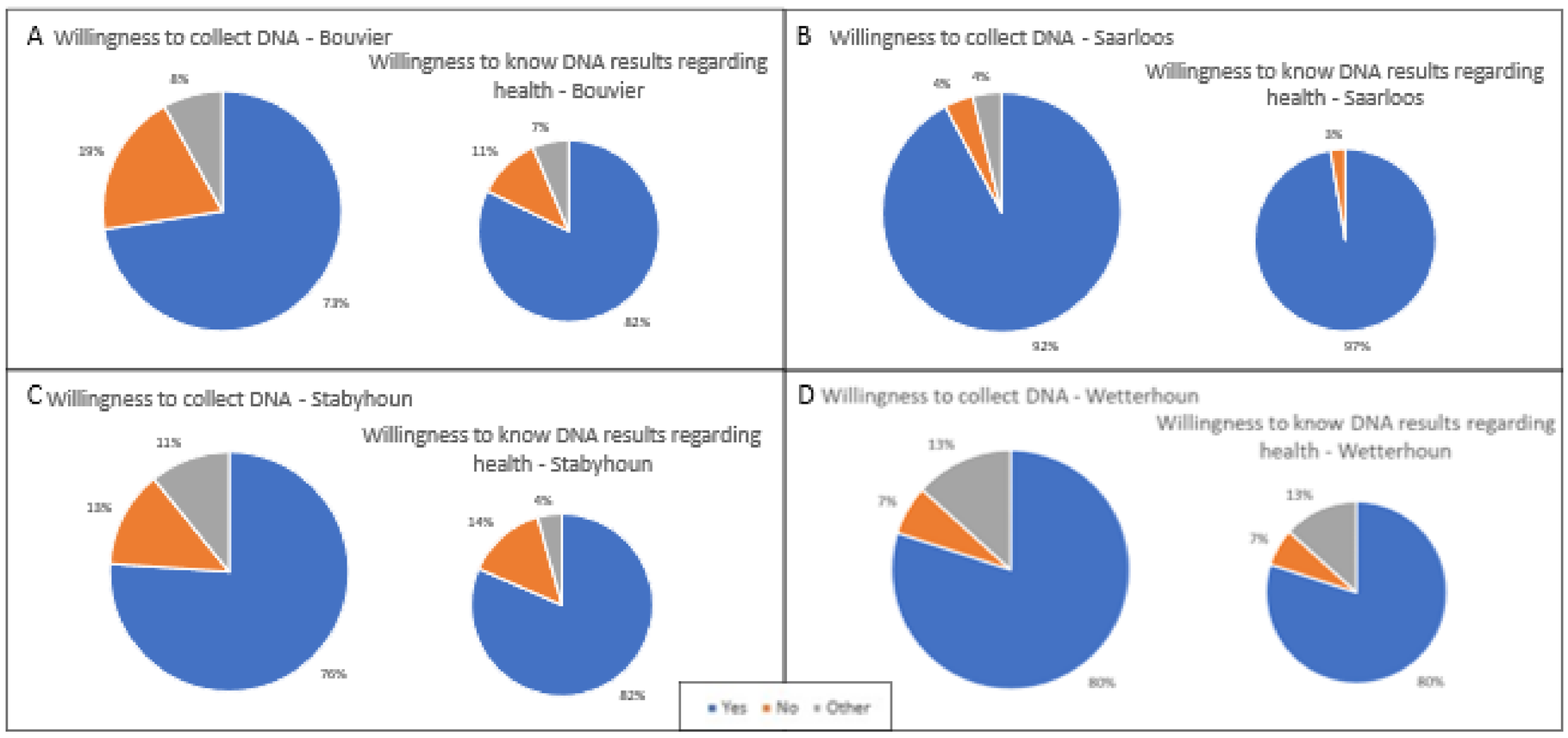


Figure 5.7 The willingness to collect DNA and the willingness to know DNA results regarding health. A) Bouvier des Flandres, B) Saarloos Wolfdog, C) Stabyhoun, and D) Wetterhoun.

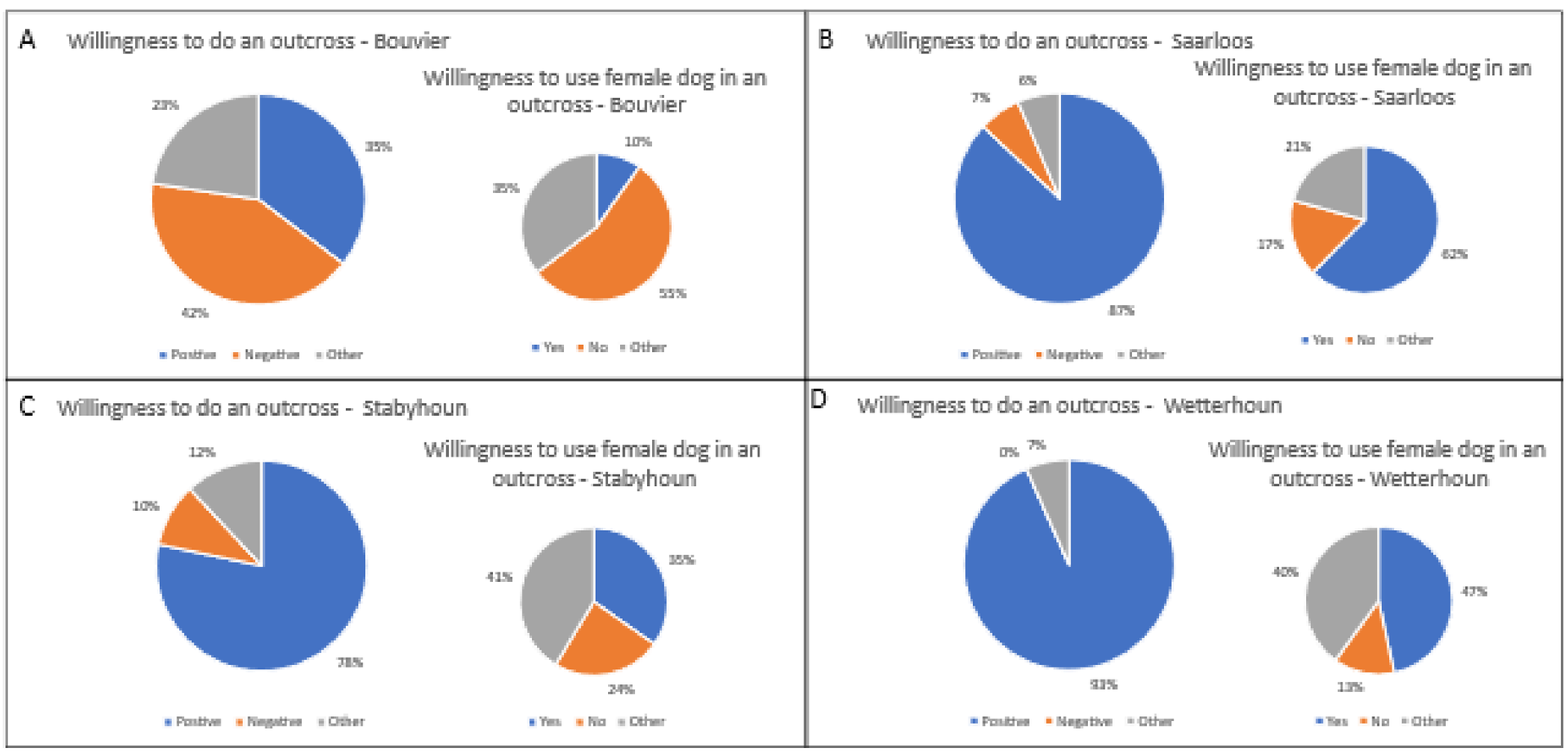


Figure 5.8 Willingness to do an outcross and willingness to use a female dog in an outcross. A) Bouvier des Flandres, B) Saarloos Wolfdog, C) Stabyhoun, and D) Wetterhoun.

WILLINGNESS TO KNOW THE BREEDING VALUE OF YOUR DOG
Breeding values indicate the genetic merit for a particular trait which could help when choosing matings. In Sweden and the UK breeding values are already calculated for the dogs, but this is not yet done in the Netherlands. There was some variety in the willingness to know the breeding value of a trait of their dog (Figure 5.9). For all breeds, the responses that were indicated by “other” ranged from does not apply to the owner having no opinion on it.

The owners of the Bouvier des Flandres are unfortunately mostly unwilling. Their main reasons were that it has to be accepted that it is nature or that it is not needed to calculate this per animal. In the responses it was also stated that a breeding value is too ‘materialistic’. This indicated that the definition and reasoning of the breeding value is unclear to the breeders and owners.

The owners of the Saarloos Wolfdog are mostly willing to know the breeding value of a trait for their dog as their main goal was to breed healthier dogs. However, they also indicated that the breeding values should help to breed healthier dogs, and not to overuse the “better” dogs (those with higher breeding values) more often compared to others. Rightfully so, as this can increase the inbreeding and kinship in the breed. The owners of the Saarloos Wolfdogs were not willing and indicated that one should still think logically when selecting dogs for breeding. Furthermore, the breeding value should not be used for excluding dogs from the breeding programme, but they also mention that the breeding value sounds too ‘scientific’ and ‘capitalistic’. Scientists are often considered untrustworthy, because they are seen as cold and unfeeling, and they often contradict each other(108). As a result, the owners should be weary of the term and concept of a breeding value. The response of breeding value being too capitalistic, indicated once more that the meaning of the word “value” is translated directly, which could make it sound capitalistic. Which again shows the mismatch between the researchers and breeders. We recommend having an open discussion between the breeders and researchers which could create a better connection and could result in more confidence towards each other.

The responses for the Stabyhoun and Wetterhoun were similar to those for the Saarloos Wolfdog. On the one hand, the owners that were willing to know these breeding values indicated that it can be useful for breeding. On the other hand, the owners that indicated that they are not willing needed more information or it was not relevant to them.

Overall, there is still more information needed on the definition and added value of the breeding value. It was interesting to note that some owners indicated that they already knew the breeding value of their dog, but is not yet used in the Netherlands and is, therefore, not possible. It is clear that there is a mismatch between the information on breeding value and translation to the owners. If this mismatch is addressed and solved, it will be beneficial to the future of the breed. We recommend arranging a “breeders day” containing a presentation from a researcher that explains the concept of the breeding value, and how it could be used. This could facilitate credible information transfer from scientist to breeder and possibly then to the owner. We also recommend recording the presentation for later publication, so it is available for all members.

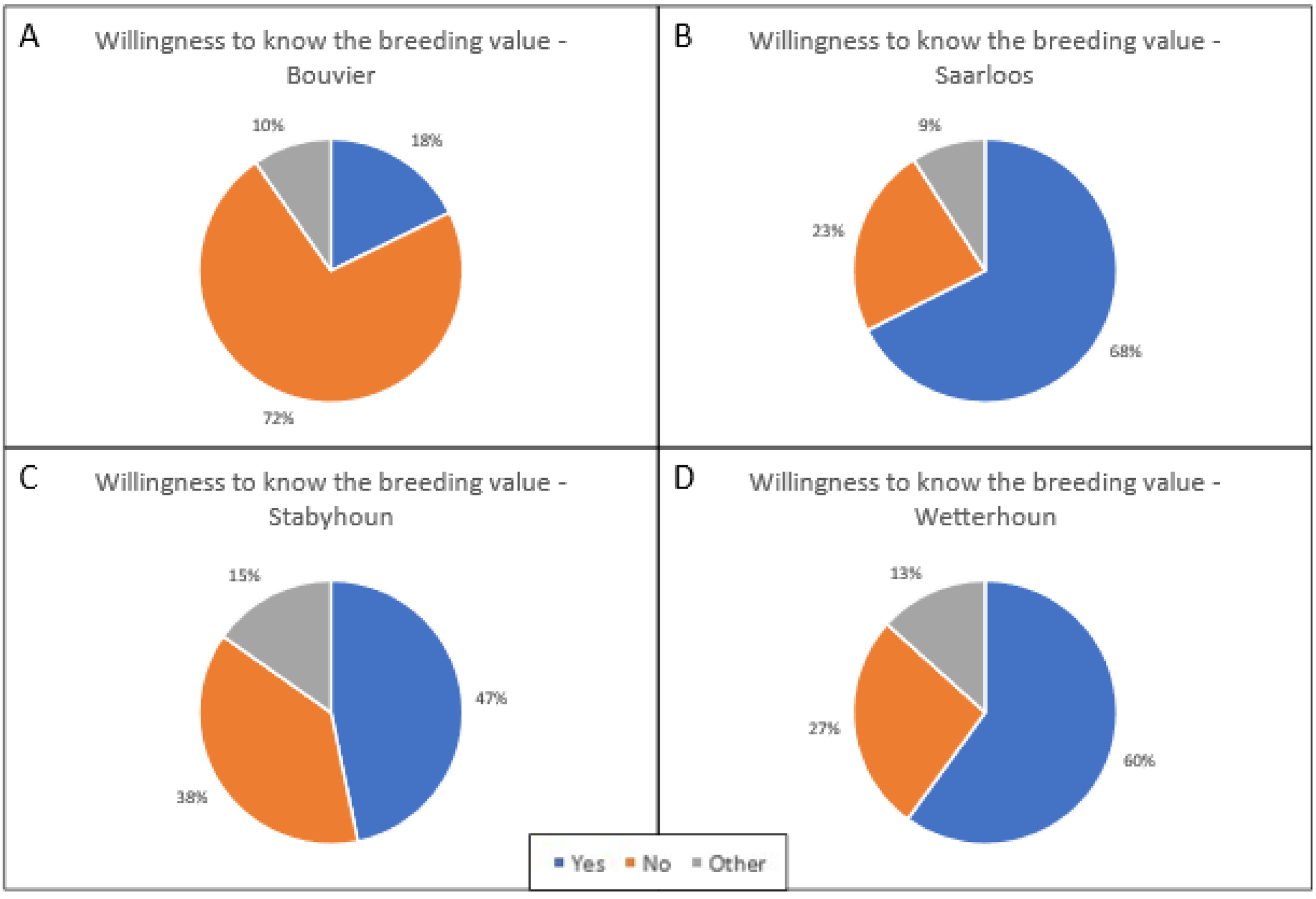


Figure 5.9 The willingness to know the breeding value. A) Bouvier des Flandres, B) Saarloos Wolfdog, C) Stabyhoun, and D) Wetterhoun.

PROS AND CONS OF AN ASSOCIATION FOR THE BREEDER
Discussion on the breeding regulations and decisions of the board
The RvB set several regulations for the breeding associations onto which the breeding associations can add on to. There can be a lot of discussion about the breeding regulations within an association. For example, within the Boe4 there is a lot of discussion with the breeders, mostly because of their expenses (A. v.d. Berg, personal communication, 20 April 2023). The members indicated that breeders who breed outside of the association are able to earn more money because of the fewer mandatory examinations. The RvB will also give a pedigree registration to dogs that are purebred and not bred within the associations. Which can be both positive and negative, since the breeding population increases by giving a pedigree to purebred dogs that were bred outside of the associations. However, this is also negative since it will not stimulate breeders to follow the breeding regulations, which could decrease the overall health and increase the inbreeding levels.

Unlike the Boe4, the AVLS states that there is not much of a discussion between the breeders (M. Eggink, personal communication, 17 April 2023). Nevertheless, the respondents do question the decisions and regulations made by the association. Here, there were multiple responses that indicated that the information given by the board is not fully clear to the members and therefore, the decisions that were made – for example in the outcross – were not received well. During the interview, it became clear that the board gathers information from multiple sources which has educated them well on the procedure, but members of the association do not have access to the same information. We recommend that all gathered information should be made available and readable for the members. It is important that the members are able to effortlessly read the articles, and there should be an open discussion where they have opportunity to ask questions. For example, have an online discussion board on the website of the association. This will prevent that these discussions only take place in Facebook groups, which could provide the members with inconsistent information.

The NVSW stated that the breeders can be stubborn in the choice of mating. For example, the board wants to increase the breeding population; however, many breeders are inflexible in selecting the dogs to mate as are set in having a dog that fits the breeding standards. The board could give advice on the health risks of a mate combination which can result in a pairing that is line with the regulations, but also considers the health risks.

Pros of joining a breeding association
By joining a breeding association one can also reap many benefits. By doing so, you will help to preserve the breed for the future. You are also able to get in contact with other owners and enthusiasts of the breed at various activities organised by the association such as, examinations and championships (M. Eggink, personal communication, 17 April 2023; H. van den Hoek, personal communication, 17 April 2023; A. v.d. Berg, personal communication, 20 April 2023). The three associations also have their own club magazine with stories and research in it. The breeding associations are also actively trying to improve the overall health of the breed and set regulations for this. The breeders that are not a member can breed purebred dogs; however, if they do not follow the regulations the inbreeding and kinship could increase. That is why having an overview of the current ongoings in the breed could help to preserve the breed and keep it healthy.
Overall, the breeding associations have their advantages and disadvantages. However, everyone agrees that the breeds should be preserved for the future, and the dogs have to be healthy.

Recommendations
In this chapter, we have discussed a few challenges and mismatches that come with breeding purebred dogs. To overcome these, we have several recommendations for the breeding associations. First, the breeding associations should maintain social cohesion and involve members in making and deciding on regulations, more than they do now. For example, good ideas can come from the members such as having a maximum number of litters per breeder not only per dog. Second, clear communication between breeding associations; both from the same breed and other breeds. For example, the “Vereniging de Nederlands Schapendoes” publishes the mean kinship on their site so the breeders can easily access it and base their decisions on this. We suggest that associations adopt these ideas from other breed associations. The RvB could help by minimize the costs for breeders and by providing open access to data. This gives all breeding associations access to each other’s data, which can increase the breeding population.

It was interesting to see the clear mismatch between researchers, the board, breeders, and owners. Especially, choices that affect the health and looks of the breed cause a lot of disputes. These could be solved by having all information accessible and easy to read for the members. Also, it could help to arrange days and webinars that are recorded for all members.

06. ETHICS

INTRODUCTION

Some of the earliest evidence of domesticated dogs are from 23.000 years ago in Scandinavia (109). Since then, they have been bred to fill specific purposes giving us all the breeds we know today. They are used for a variety of purposes such as working dogs (sniffer dogs), police and military dogs, hunting, human assistance and much more. However, most dogs are kept as companion animals (110). The main goal of this ethics chapter is to raise questions that we think are important to think about in the future with breeding dogs. The ethical issues are important for the purebred dog owners/buyers, the breeders, the associations and other interested parties.

In this chapter, ethics will be defined as a set of moral principles that governs a person's behaviour or the conducting of activities regarding dog breeding (111). Most people have strong empathy for animals, especially when they have a strong emotional attachment to them (111). Within the purebred dogs' community, there is a great emotional connection to the breeds (especially from the breeders). This relates to treating the dogs you own and breed well. This could be for several reasons; the first is to treat the dog you own well, so it can serve you as a human better. A second reason to treat the dog you own or breed well is that you have a responsibility towards them. Third, you could treat the dogs well because you feel that the animals have a value of their own. In dog breeding, one of the central reasons for treating the dogs well is that they will provide an income. Breeders breed dogs for two main reasons: looks and behaviour (111). With this, it is important to keep in mind that dogs must be bred and treated in a way that their health and well-being are not compromised during their lifespan (111). Within the breeders' world, there is a shift nowadays towards more health-orientated breeding. Animal welfare is defined as "how an animal is coping with the conditions in which it lives" by the World Organisation for Animal Health (OIE)(111). Another important concept is ethical breeding, which means breeding animals befitting their natural looks and behaviour in a sustainable fashion (24).

Firstly, this chapter will go into the ethical challenges of DNA ownership. Secondly, will be about DNA profiling and questions that arise with this as: What to do when you know a dog has a high chance of disease? And Should breeders mention dogs have a high chance of certain diseases? Thirdly, the paragraph will go into aesthetics versus health and fourthly about dog health trade-offs. Lastly, this chapter will end with a closing message to think about.

DNA OWNERSHIP

For the DNA test to be useful, it is important that they are accessible for the breeding associations and if possible, also researchers. The DNA testing company Embark, which is currently being used by AVLS, mentions on their website that dog owners should be able to decide what happens with their dog's DNA data. In this way, dog owners are also able to access and download their dog's raw DNA data. Embark also mentions that they are open to working together with researchers. However, they ask for the permission of the owners if their dog's data can be used in research as they value both scientific openness and privacy (112). Another DNA testing company called Wisdom Panel mentions on their website that it may share anonymous data with researchers and third parties. However, it remains unclear what research is performed with the data. If personal information or information about a specific pet is required Wisdom Panel will explicitly ask for approval (113). So, although they do not explicitly mention that they are the owners of the DNA data, it seems that companies like Embark and Wisdom Panel are in fact the owners of the DNA, and dog owners should keep this in mind. Moreover, it should be noted that these are private companies, with their business model being making a profit out of gathering this data. RvB also has a 50K SNP chip that is required if the breeders want to receive a pedigree. This data belongs to the RvB but breeding associations can periodically request the data after they signed an agreement that the data is only used with the goal of improving the health of purebred dogs (L. Roest, personal communication, (02 May 2023). For human DNA testing companies, it was also concluded that there is a large difference between companies, and many do not meet the international guidelines for privacy, confidentiality and secondary data use (114).

DNA PROFILING

DNA profiling techniques as mentioned before in this report can provide genetic insights into purebred dogs. Each dog could be screened for a great number of genetic diseases in order to make a DNA profile (115). For example, this information can be used to determine if the dog has a heightened risk of developing a specific disease. In this way, genetic testing is a health quality control (116). It can be very beneficial for dog breeders to have this information at an early life stage, to prevent breeding with dogs who carry certain diseases. Moreover, if dog breeders or owners have knowledge of what the dog is susceptible to, they can look out for certain symptoms to be able to act on them at an early stage, which could possibly lead to a better cure or health plan together with a veterinarian. Because of this, for purebred dogs, it is in many cases no longer ethical to breed dogs without testing them on genetic disorders through DNA profiling. The choices the breeders make on what dogs will breed affect the whole gene pool, and therefore it is very important for them to have this knowledge before they choose breeding couples (116). Breeders should use this to go for breeding combinations that improve the health of the dogs, as well as maintain genetic diversity.

Although DNA profiling is very helpful for the breeding of purebred dogs, it could also entail difficulties. For example, when a breeder has a litter, and finds out a puppy has a high risk of a disease developing later in life. As a result, the breeder will most likely not use this dog for further breeding. However, it is not always the case that when a gene for a certain disease is found in the DNA test that the dog will develop it. Dogs with a higher risk of developing a disease be named 'At Risk' instead of 'Affected' (116). Also, one must keep in mind that all dogs are carriers of some hereditary diseases (117). Overall, it is important that the results of genetic tests are well explained and communicated to the breeders and owners of the dogs. With this, it is essential to also consider the emotions that can come with certain test results. The breeder receiving a result about a dog being a carrier for a certain disease can be really overwhelming for the breeder or dog owner. For example, it has to be well communicated to the breeders and owners that there is a chance of a false positive or false negative test result due to crossovers (116). With this, it is important to note that not every DNA test that is commercially available is fully accurate - sometimes upon validation, even opposite associations between genotypes and phenotypes have been observed (24). Therefore, it is vital that official and validated genetic tests are used and trusted. Test results should contain information about the inheritance, penetrance and expressivity of the disease. Additionally, the sensitivity and specificity of the genetic test should be given as well as the percentage of genetic variation of disease represented by the tested loci (116). In this way, genetic testing enhances dog health and breeding options instead of excluding individuals and limiting dog breeding choices.

Overall, it is crucial to realise and understand that with maintaining purebred lines, where a degree of inbreeding is

necessary in order to maintain the breed, the emergence of inherited disorders is expected (115). So, a balance must be found between the purity of a breed, and the health of the individual dogs.

WHAT TO DO WHEN YOU KNOW A DOG HAS A HIGH CHANCE OF DISEASE?
Not all decisions informed by the results of genetic testing will be simple. In some circumstances, for example, a dog that exemplifies the breed standards well could be found to be a carrier for a highly undesirable trait. In situations like these, owners and breeders will be forced to make difficult choices about what to do with these individuals. Breeders will likely turn to veterinarians and their colleagues for advice on how to proceed. Owners and breeders will expect accurate risk assessments for their dogs and will want guidance on how best to proceed (118). Knowing the symptoms to look out for and having the opportunity to develop a care plan with a veterinarian can help maximize the number of healthy years for the dog.

SHOULD BREEDERS MENTION DOGS HAVE A HIGH CHANCE OF CERTAIN DISEASES?
DNA tests make it easier for breeders to identify the susceptibility their dogs have to getting certain diseases. This raises the question; What information should the breeders disclose to potential buyers? One can imagine that as a result a lot of ‘leftover puppies’ could arise which have a higher risk to develop a disease in their life. However, it is impossible to know if a puppy will actually develop the disease later in life when it tests at risk in a DNA test.

Most breeding associations already disclose potential diseases to potential buyers, and it is also disclosed in the puppy contract in some associations, like the AVLS. This helps buyers make informed decisions on whether or not they are able to take care of an unhealthy dog. However, most buyers would likely assume that their dog will not develop diseases but knowing the genetic predisposition of the puppies changes things. Puppies might not get adopted because of their genetics, as they are at risk of developing a disease, as people wouldn't want to care for a dog if they know it has a high chance of developing complications.

At the same time, withholding information from potential buyers is not legal, and a breeder is liable if a dog turns out to have a hereditary disease (119). Breeders should minimize the chance of a hereditary disease by only breeding with healthy individuals. It is impossible to prevent everything, and even when a DNA test is performed it is impossible to say for sure if a dog will develop a disease. Breeders could disclose this information to potential buyers to prevent being held liable for withholding information in a sale. However, disclosing if a dog has a predisposition for a certain disease might prevent that dog from being sold and finding a home.

From the questionnaire, it was revealed from the question: “If a DNA test would show your dog had a predisposition for a certain disease, would you want to know?”. Most dog owners would want to know this. The main reason they wanted to know was so they would be able to organise the dog’s life accordingly/take this into account and know not to breed with the dog. However, this is probably not the case if they have not bought the dog and an emotional connection was not formed yet.

One option might be to include the price for DNA testing in the price of the pup, which is already done by the breeders to get a pedigree from RvB. However, we think this data is underused. Another choice is to let the DNA test be done by a private company like Embark, which is more expensive but also has a larger size SNP chip. The choice can then be made to give all this information only to the breeding associations. This way, owners can request the DNA data of their dog from the association if they want to anticipate possible problems in the future. In addition to this, the breeding associations will have a better picture of which dogs are a good breeding match. This is only a suggestion, and legal advice should be sought before conclusions can be drawn. Another problem might be that the people from the breed associations are all volunteers and thus there can be notable differences in skill and knowledge levels. The question then is; Who is accountable if things go wrong? Another question might be; Who will get access to that information? The dog owners for their own dog, but what about breeders and researchers? In addition, often there are multiple breed associations per breed, it would be best if they could work together in some capacity. However, this means that they would also have to come to an agreement. This would need serious consideration from the associations if they would like to use DNA data to improve the health of their breed.

AESTHETICS VERSUS HEALTH

One of the bigger issues in the purebred dog world is how much value is placed on the dogs' appearance versus their health. In recent years, a large shift towards the health of dogs has taken place. Most breeding associations mainly focus on their breed's health and genetic diversity. However, it can be seen that a lot of people like buyers and some breeders still place too much value on certain aesthetic aspects of the dogs (120). For example, breeding for longer bodies in certain breeds is linked to an increased chance of hip dysplasia in those dog breeds (121). Sometimes this is also directly linked to the breed standards, as can be seen in the Rhodesian ridgeback where the gene that causes the iconic ridge is also linked to the Dermoid Sinus condition (122). This is not necessarily the problem for all dog breeds; for example, the AVLS mentions that the philosophy from their founder is: “Breed for health as they will be beautiful anyway.” (M. Eggink, personal communications, 17 April 2023). Some people might not see breed-related welfare issues as demotivators of buying that specific breed. One possible reason for this might be that the phenotypic traits of a breed, like the Chihuahuas, are positive motivators and do not decrease the popularity of the breed. It is therefore important to not only motivate the breeders but also the public to support choices that reduce inbreeding and breed-related welfare issues (120). Breeding dogs for purposes that were not the original intention gives rise to extremes such as breeds that can only give birth through c-sections such as the pug and English bulldog. These dogs are bred for their big heads and small bodies, which serve no function. Extremes like this are even more ethically and morally questionable (123). Although none of the breeds included in our project present such extremes, the breeding associations should keep in mind when they are breeding for welfare, and when it is for aesthetics.

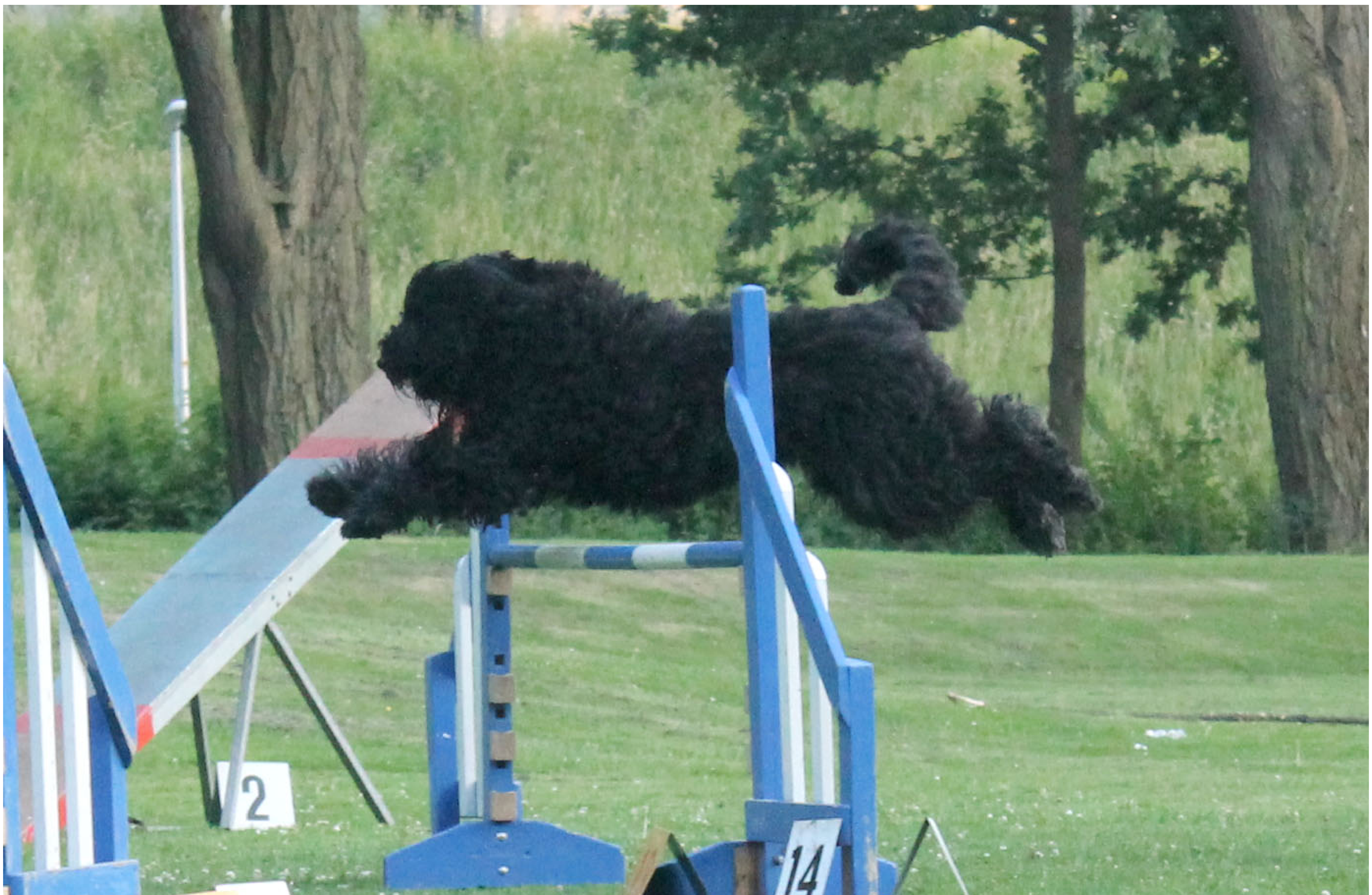
DOG HEALTH TRADE-OFFS

Animal welfare can be considered as three distinct but interconnected aspects as suggested by Fraser et al. (1997). The first aspect is that animals should physically be well, which means they should be free from injury and disease. The second aspect is that an animal should mentally be well. The third aspect is that they should exhibit natural behaviours and live in a way that enriches their social and environmental needs. It is also important to mention that although you should try to minimize hereditable diseases, it is impossible to eliminate them from a population altogether. The RvB thinks the focus should be more on making sure every dog breeds at least once rather than focusing so much on testing every dog for hip dysplasia as it is not a common disease anymore (L. Roest, Personal communications, 23 April 2023).

Another question raised is; Should one focus on a major disease that only affects a small part of the population, or a minor disease that affects a large part of the population (24)? An example might be epilepsy in the Stabyhoun which is a rare but well-known disease in the Stabyhoun population according to H. v.d. Hoek (personal communications, 17 April 2023). This is a very impactful disease for the dog as well as the owners, but the question is if this should be prioritised or for example Von Willebrand's disease type I which affects 69.8% of the population (124). However, most owners never even know their dog has this coagulation disease, as it is the mildest form of Von Willebrand's disease. It should also be checked how feasible it is to try and eliminate a disease from a population. It will be easier to reduce the prevalence of monogenic diseases than polygenic diseases, but one should keep in mind the occurrence of the disease in the population. For example, Von Willebrand's disease in the Stabyhoun, this disease is monogenic, with incomplete penetrance, which means that it is only on one allele and not all dogs that have the right genes suffer from the disease. The question then is: should this disease be prioritised over another disease like epilepsy because more dogs suffer from it, or should epilepsy be prioritised as the suffering is much greater? One possible solution for this might be for the associations to make an index of the diseases and give them weight according to their frequency and impact. This way the associations can select both aspects in a balanced way.

FOOD FOR THOUGHT

Overall, one must keep in mind the trade-off between the wishes of dog owners, breeders and the wellbeing of the individual dogs. Until which point is it ethical to push the limits of a population in terms of their genetic diversity and composition/health? In a way you could say that lovers of these breeds care so much about them, that they also should realize at what point the animals are better off not being bred. One should critically look at their breed and ask themselves: will we really be able to rescue this breed? Or do we have to accept that it is lost and let them go in order to prevent the health issues of the breed, despite all the emotions of the breeders and owners that will come with losing the breed? Because what is really important: the health and wellbeing of the dogs or our preference as humans to have a dog that looks and behaves in a way we want them to? Or could these be united by defining breeding standards focussed primarily on health? The following quote by Croney from 2019 indicates something suggested to think about as owners and breeders of purebred dogs: "It is ironic that the human-dog bond is so highly valued in western developed nations that people will (knowingly and unknowingly) tolerate various types and degrees of harm to dogs in order to perpetuate their existence". We would like to emphasize that we have seen first-hand all the efforts the associations and owners are putting in to improve the health of their breeds and that they have the best intention for their dogs. However, we would like to encourage everyone to always stay critical about what is feasible and when you are impeding the health of dogs to sustain a breed.



07.

CLOSING REMARKS

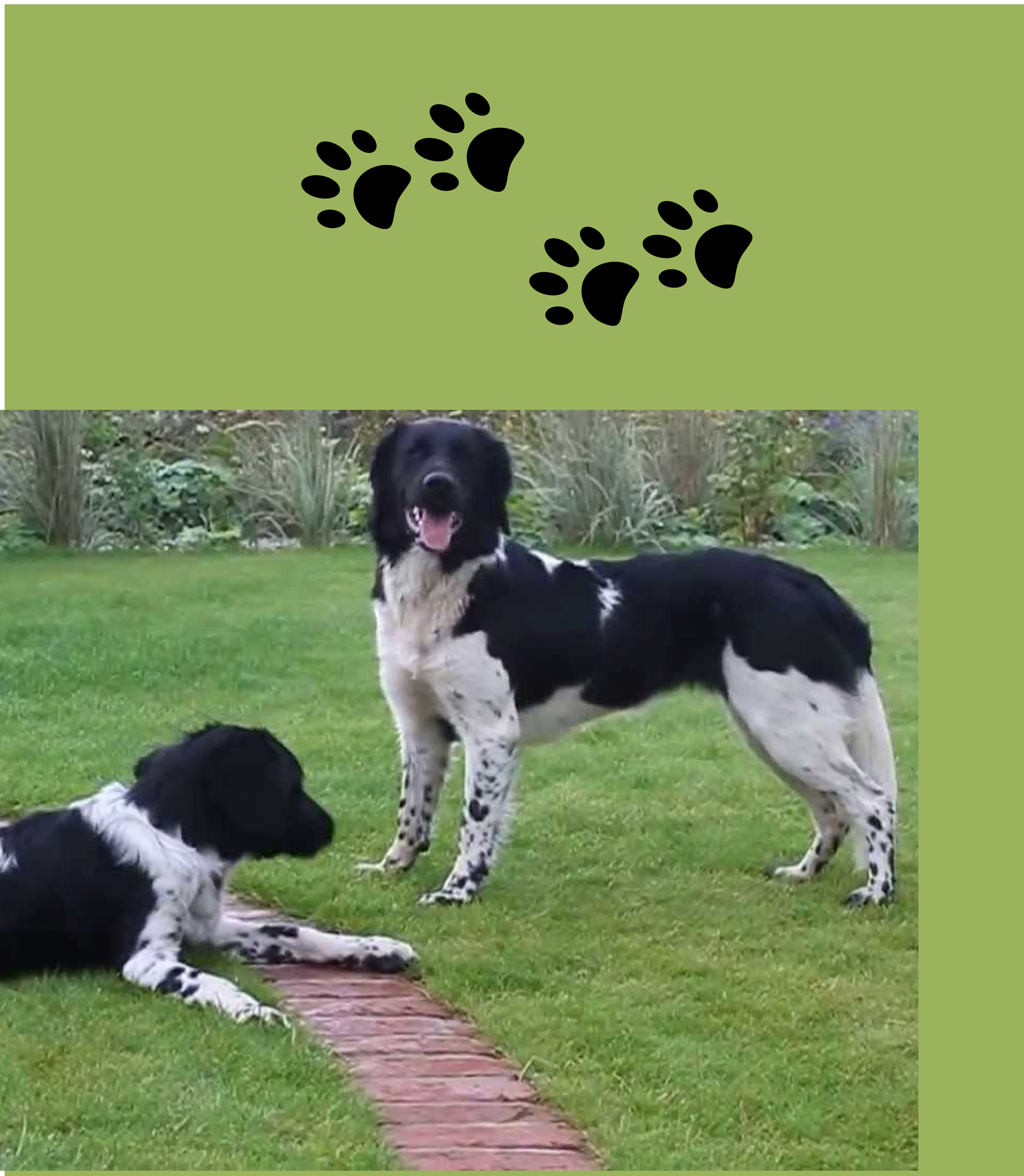
To conclude the guide, we will provide a summary of all the chapters with our recommendations. The importance of maintaining genetic diversity within a population has been discussed and the relationship between genetic diversity and inbreeding is explained. We discuss how years of inbreeding lead to inbreeding depression and the consequences of this. Inbreeding depressions shows itself through hereditary diseases within the population, of which the different types are discussed. Later, we describe how DNA-data can be used to identify genes related to these diseases, within purebred dog populations. We suggest the associations look into using SNP-Chips for this purpose. Although heavily debated, we discuss the benefits of outcrossing and multiple approaches for this. The associations should consider using more related or less related donor breeds to introduce less or more genetic diversity in the population, respectively. We suggest that the potential loss of favoured traits can be combatted by the use of multiple backcrosses. On the other hand, by choosing a more closely related breed, you might not introduce enough genetic variation in the population. Another way of introducing genetic variation is to include look-alikes (dogs that in all aspect conform to the breeds, but do not have the official papers to be considered purebred) into the population. For these dogs, we suggest using SNP- to determine the breed purity. By having these dogs genotyped, you can have an indication of detrimental genes in the population. If the main goal is to maximise the health of the breeds, look-alikes are interesting to consider. A third intervention would be to use EBVs: values of genetic merit per individual that grade the likelihood of passing genes from parent to pup. As the Netherlands currently has no EBV evaluation system in place, and it might be beneficial to import dogs with high genetic merit (or their DNA) from countries with EBV evaluation systems. Furthermore, SNP-chips can also be used to determine gEBVs that combine genotype, phenotype and pedigree information into a single value. Lastly, mean kinship is a value which describes how related each animal is to all other animals within the population and by combining EBVs and mean kinship one can evaluate an individual's significance in the breeding population; a method termed optimal contribution.

In the following chapter, we explain the relationship between the Raad van Beheer (RvB), breeding associations and the breeders. In summary, the RvB monitors the breeding associations and sets up basic rules and regulations the breeding associations should follow. Breeding associations themselves also have sanctions in place for members (breeders) who do not follow these rules. The cost of dog breeding is discussed and therefore, the extra costs for DNA tests or screening could be a challenge the associations may face when implementing this. We recommend that the breeding associations look into subsidies for these. From the widespread questionnaire sent, it was found that most owners are willing to collect the DNA of their dog. The respondents argue that it can be beneficial for research within breeding, but it is unclear what the aim and methods are. Most respondents are willing to learn the results of the DNA test in regard to health as they want to adapt the life of the dog to it, or treat the symptoms of early, but “quality testing” the dog is ethically questionable. The respondents had mixed responses regarding the outcross as there was a lot of misunderstanding of the meaning and decisions of the outcross. They are concerned that even though dogs might remain healthy, you could lose typical characteristics preferred by the breed owners. The respondents had mixed responses regarding breeding values, as the definition of breeding values was unclear. We recommend that the breeding associations maintain social cohesion and involve members in making and deciding on regulations, more than they do now. Furthermore, we also recommend having an open discussion between the breeders and researchers, which could create a better connection and might result in more confidence towards each other. In addition to this, we suggest further communication within and across breeding

associations. Additionally, we think the RvB should help by minimising the costs for breeders and by providing open access to data, allowing breeding associations of the same breed access to each other's data. This is important because it can increase the breeding population. The mismatch of knowledge could be solved by having all information accessible and easy to read for the members. Also, it could help to arrange days and webinars that are recorded and available for all association members.

Finally, the chapter on ethics was intended to make the reader think critically about a few important subjects related to DNA testing in dogs, and purebred dogs in general. We suggest that people think critically about who owns and can access the DNA data after they submit it for testing. Furthermore, not only the advantages and the challenges of DNA profiling need to be taken into account. Results from DNA tests should contain elaboration if a dog might be at risk of a disease. One should keep in mind if it is ethical to discriminate between dogs that are at risk of a disease or not? Additionally, it is important to think about the impact the breed standard can have on the health of purebred dogs; for example, breeding for longer bodies in certain breeds is linked to an increased chance of hip dysplasia in those dog breeds. We also discussed trade-offs regarding purebred dog health such as, if one should focus on a disease that is rare but incredibly severe or a common disease and is not severe. Finally, we stimulate the reader to think critically about purebred dog breeding in general and reflect on how much value they place on a certain trait.

Lastly, we would like to recommend making this guide available to everyone (breeders, owners, and the public). As we think it is important for all people to be more informed about breeding purebred dogs so everyone can play their part in breeding better buddies.



08.

ACKNOWLEDGEMENTS

For the duration of our project, we had some people helping behind the scenes. We would like to thank our coach, Willeke, for her guidance and her feedback throughout the project. Our academic advisor, Mario, also provided key insights and ensured that the final product was relevant and accurate. Further, we would like to express our gratitude to Jack and Rita, our commissioners, for always making time for us and having such immense subject knowledge and input across the entire process. Also, it is important to acknowledge Lèneke and the WUR Science Shop for their efforts in making this project possible. During this project several interviews were conducted with stakeholders of this project, and we would like to thank Anton, Hans, Laura and Marianne for making the time to speak with us, and members of the associations for filling in out questionnaire. Lastly, but certainly not least, we would like to give a big shout out to dogs for being the best buddies.

09. REFERENCES

1. Li H, Roossinck MJ. Genetic bottlenecks reduce population variation in an experimental RNA virus population. Journal of virology. 2004;78(19):10582-7.
2. Oldenbroek J, Windig JJ. Het fokken van rashonden: omgaan met verwantschap en inteelt: Raad van Beheer op Kynologische Gebied in Nederland; 2012.
3. Institute NHGR. Founder effect 2023 [Available from: https://www.genome.gov/genetics-glossary/Founder-Effect#:~:text=A%20founder%20effect%2C%20as%20related,separated%20from%20a%20larger%20population.
4. Hughes AR, Inouye BD, Johnson MT, Underwood N, Vellend M. Ecological consequences of genetic diversity. Ecology letters. 2008;11(6):609-23.
5. Institute NHGR. Gene pool 2023 [Available from: https://www.genome.gov/genetics-glossary/Gene-Pool#:~:text=A%20gene%20pool%20refers%20to,able%20to%20withstand%20environmental%20challenges.
6. Institute NHGR. Genome 2023 [Available from: https://www.genome.gov/genetics-glossary/Genome
7. Calus MP. Genomic breeding value prediction: methods and procedures. animal. 2010;4(2):157-64.
8. Brooks DE. Glaucoma in the dog and cat. Veterinary Clinics of North America: Small Animal Practice. 1990;20(3):775-97.
9. Wilkin GP, Ibrahim MM, Smit KM, Beaulé PE. A Contemporary Definition of Hip Dysplasia and Structural Instability: Toward a Comprehensive Classification for Acetabular Dysplasia. The Journal of Arthroplasty. 2017;32(9, Supplement):S20-S7.
10. Scott-Moncrieff JC. Clinical signs and concurrent diseases of hypothyroidism in dogs and cats. Veterinary Clinics of North America: Small Animal Practice. 2007;37(4):709-22.
11. Cosgrove M. Nucleotides. Nutrition. 1998;14(10):748-51.
12. Buchanan JW, Patterson DF. Etiology of patent ductus arteriosus in dogs. Journal of veterinary internal medicine. 2003;17(2):167-71.
13. Bunel M, Chaudieu G, Hamel C, Lagoutte L, Manes G, Botherel N, et al. Natural models for retinitis pigmentosa: progressive retinal atrophy in dog breeds. Human Genetics. 2019;138:441-53.
14. Fischer A. Severe combined immunodeficiencies (SCID). Clinical & Experimental Immunology. 2000;122(2):143-9.
15. Eikenboom JC, Reitsma PH, Briet E, Peerlinck K. Recessive inheritance of von Willebrand's disease type I. The Lancet. 1993;341(8851):982-6.
16. Doekes H. Pedigree analysis and optimisation of the breeding programme of the Markiesje and the Stabyhoun [Unpublished thesis]: Wageningen Univeristy & Research; 2016.
17. Nederlandse Vereniging voor Stabij en Wetterhounen (NVSW). Breedstandard Stabijhoun. 2023.
18. Fédération Cynologique Internationale (FCI). FCI n.d. [Available from: https://fci.be/en/.
19. Jerold Bell. Genetic Diversity. Cummings School of Veterinary Medicine at Tufts University, Sciences DoC; n.d.
20. Hermansen J, Kuricová M, Lipták T. Intervertebral Disc Disease in Dogs – The Relationship Between Recovery and Timing of Surgery. Folia Veterinaria. 2022;66(3):54-9.
21. Minter M, Nielsen E, Blyth C, Bertola L, Kantar M, Morales H, et al. What Is Genetic Diversity and Why Does it Matter? Frontiers for Young Minds. 2021;9:656168.
22. Museum of Paleontology UC. Understanding Evolution University of California2023 [Available from: https://evolution.berkeley.edu/the-relevance-of-evolution/conservation/inbreeding-depression/.
23. Lewis T, Windig JJ. Dog breeds: towards genomic management of populations with a high incidence of genetic defects. Genomic management of animal genetic diversity Wageningen Academic Publishers. 2018:179-205.
24. Broeckx BJ. The dog 2.0: Lessons learned from the past. Theriogenology. 2020;150:20-6.
25. Leroy G, Baumung R. Mating practices and the dissemination of genetic disorders in domestic animals, based on the example of dog breeding. Animal Genetics. 2011;42(1):66-74.
26. Ubbink G, Knol B, Bouw J. The relationship between homozygosity and the occurrence of specific diseases in Bouvier Belge des Flandres dogs in the Netherlands: Inbreeding and disease in the bouvier dog. Veterinary Quarterly. 1992;14(4):137-40.
27. Mäki K, Groen AF, Liinamo AE, Ojala M. Population structure, inbreeding trend and their association with hip and elbow dysplasia in dogs. Animal Science. 2001;73(2):217-28.
28. Ólafsdóttir GÁ, Kristjánsson T. Correlated pedigree and molecular estimates of inbreeding and their ability to detect inbreeding depression in the Icelandic sheepdog, a recently bottlenecked population of domestic dogs. Conservation Genetics. 2008;9(6):1639-41.
29. Urfer SR. Inbreeding and fertility in Irish Wolfhounds in Sweden: 1976 to 2007. Acta Veterinaria Scandinavica. 2009;51(1).
30. Leroy G, Phocas F, Hedan B, Verrier E, Rognon X. Inbreeding impact on litter size and survival in selected canine breeds. The Veterinary Journal. 2015;203(1):74-8.
31. Andrade FMd, Silva MMd, Krebs G, Feltes GL, Cobuci JA. Inbreeding on litter size of German Spitz dogs. Revista Brasileira de Zootecnia. 2021;50.
32. Rabon Jr D, Waddell W. Effects of Inbreeding on Reproductive Success, Performance, Litter Size, and Survival in Captive Red Wolves (Canis rufus). Zoo biology. 2009;29:36-49.
33. Schrack J, Dolf G, Reichler IM, Schelling C. Factors influencing litter size and puppy losses in the Entlebucher Mountain dog. Theriogenology. 2017;95:163-70.
34. Association. AVM. Making sense of genetic disease in dogs and cats. 2020 [Available from: https://www.avma.org/javma-news/2020-11-01/making-sense-genetic-disease-dogs-and-cats#:~:text=The%20most%20common%20hereditary%20diseases,inflammatory%20bowel%20disease%3B%20diabetes%20mellitus%3B.
35. Génin E, Feingold J, Clerget-Darpoux F. Identifying modifier genes of monogenic disease: strategies and difficulties. Human Genetics. 2008;124(4):357-68.
36. Del Vecchio C, Verrilli F, Glielmo L. When sex matters: a complete model of X-linked diseases. International Journal of General Systems. 2018;47(6):549-68.

37. Norman AJ, Putnam AS, Ivy JA. Use of molecular data in zoo and aquarium collection management: Benefits, challenges, and best practices. Zoo Biology. 2019;38(1):106-18.
38. Laikre L. Hereditary defects and conservation genetic management of captive populations. Zoo Biology. 1999;18(2):81-99.
39. Sharma RK, Sc LM, Ehinger B. Management of Hereditary Retinal Degenerations: Present Status and Future Directions. Survey of Ophthalmology. 1999;43(5):427-44.
40. Laikre L, Ryman N, Thompson EA. Hereditary Blindness in a Captive Wolf (Canis lupus) Population: Frequency Reduction of a Deleterious Allele in Relation to Gene Conservation. Conservation Biology. 1993;7(3):592-601.
41. Frankham R, Ralls K. Inbreeding leads to extinction. Nature. 1998;392(6675):441-2.
42. Leroy G. Genetic diversity, inbreeding and breeding practices in dogs: results from pedigree analyses. The Veterinary Journal. 2011;189(2):177-82.
43. Shuster DE, Kehrli ME, Ackermann MR, Gilbert RO. Identification and prevalence of a genetic defect that causes leukocyte adhesion deficiency in Holstein cattle. Proceedings of the National Academy of Sciences. 1992;89(19):9225-9.
44. Switonski M. Impact of gene therapy for canine monogenic diseases on the progress of preclinical studies. Journal of Applied Genetics. 2020;61(2):179-86.
45. Leeb T, Bannasch D, Schoenebeck JJ. Identification of Genetic Risk Factors for Monogenic and Complex Canine Diseases. Annual Review of Animal Biosciences. 2023;11(1):183-205.
46. Cerebral dysfunction (CDFS): Labogen; [Available from: https://shop.labogen.com/en/genetic-test-order/dog/stabijhoun/1905/cerebral-dysfunction-cdfs.
47. Jerold S. Bell D. Breeding Strategies for the Management of Genetic Disorders Tufts' Canine and Feline Breeding and Genetics Conference2003 [Available from: https://www.vin.com/apputil/content/defaultadv1.aspx?id=3848654&pid=11165.
48. Yates AD, Allen J, Amode RM, Azov AG, Barba M, Becerra A, et al. Ensembl Genomes 2022: an expanding genome resource for non-vertebrates. Nucleic acids research. 2022;50(D1):D996-D1003.
49. Xiang R, MacLeod IM, Daetwyler HD, de Jong G, O'Connor E, Schrooten C, et al. Genome-wide fine-mapping identifies pleiotropic and functional variants that predict many traits across global cattle populations. Nature Communications. 2021;12(1).
50. Letko A, Minor KM, Jagannathan V, Seefried FR, Mickelson JR, Oliehoek P, et al. Genomic diversity and population structure of the Leonberger dog breed. Genetics Selection Evolution [Internet]. 2020; 52(1).
51. Sutter NB, Ostrander EA. Dog star rising: the canine genetic system. Nature reviews Genetics. 2004;5(12):900-10.
52. Parker HG, Kim LV, Sutter NB, Carlson S, Lorentzen TD, Malek TB, et al. Genetic Structure of the Purebred Domestic Dog. Science. 2004;304(5674):1160-4.
53. Meurs KM, Friedenberg SG, Kolb J, Saripalli C, Tonino P, Woodruff K, et al. A missense variant in the titin gene in Doberman pinscher dogs with familial dilated cardiomyopathy and sudden cardiac death. Human Genetics. 2019;138(5):515-24.
54. Van Eenennaam AL, Weigel KA, Young AE, Cleveland MA, Dekkers JCM. Applied animal genomics: results from the field. Annual review of animal biosciences. 2014;2:105-39.
55. Burrow HM, Mrode R, Mwai AO, Coffey MP, Hayes BJ. Challenges and Opportunities in Applying Genomic Selection to Ruminants Owned by Smallholder Farmers. Agriculture. 2021;11(11):1172.
56. Baker BE. Current Dog Breeding Practices Impacts on Health and Preservation of Purebred Dogs. 2020.
57. Windig JJ, Doekes HP. Limits to genetic rescue by outcross in pedigree dogs. Journal of Animal Breeding and Genetics. 2018;135(3):238-48.
58. Farrell LL, Schoenebeck JJ, Wiener P, Clements DN, Summers KM. The challenges of pedigree dog health: approaches to combating inherited disease. Canine Genetics and Epidemiology. 2015;2(1):3.
59. Stronen AV, Salmela E, Baldursdottir BK, Berg P, Espelien IS, Järvi K, et al. Genetic rescue of an endangered domestic animal through outcrossing with closely related breeds: A case study of the Norwegian Lundehund. PLoS One. 2017;12(6):e0177429.
60. Bateson P. Independent inquiry into dog breeding: Bateson; 2010.
61. Knowler SP, v/d Berg H, McFadyen A, La Ragione RM, Rusbridge C. Inheritance of Chiari-like malformation: can a mixed breeding reduce the risk of Syringomyelia? PloS one. 2016;11(3):e0151280.
62. Kijas JW, Miller BJ, Pearce-Kelling SE, Aguirre GD, Acland GM. Canine Models of Ocular Disease: Outcross Breedings Define a Dominant Disorder Present in the English Mastiff and Bull Mastiff Dog Breeds. Journal of Heredity. 2003;94(1):27-30.
63. Leroy G. Genetic diversity, inbreeding and breeding practices in dogs: results from pedigree analyses. Veterinary journal. 2011;189(2):177-82.
64. Frkonja A, Gredler B, Schnyder U, Curik I, Sölkner J. Prediction of breed composition in an admixed cattle population. Animal Genetics. 2012;43(6):696-703.
65. Funkhouser SA, Bates RO, Ernst CW, Newcom D, Steibel JP. Estimation of genome-wide and locus-specific breed composition in pigs. Translational Animal Science [Internet]. 2017; 1(1):[36-44 pp.].
66. Hulsegge I, Schoon M, Windig J, Neuteboom M, Hiemstra SJ, Schurink A. Development of a genetic tool for determining breed purity of cattle. Livestock Science. 2019;223:60-7.
67. Hulsegge et al. (2019)
68. Lewis J, Abas Z, Dadousis C, Lykidis D, Paschou P, Drineas P. Tracing cattle breeds with principal components analysis ancestry informative SNPs. PloS one. 2011;6(4):e18007.
69. Hulsegge B, Calus MPL, Windig JJ, Hoving-Bolink AH, Maurice-van Eijndhoven MHT, Hiemstra SJ. Selection of SNP from 50K and 777K arrays to predict breed of origin in cattle. Journal of animal science. 2013;91(11):5128-34.
70. Kuehn LA, Keele JW, Bennett GL, McDaneld TG, Smith TPL, Snelling WM, et al. Predicting breed composition using breed frequencies of 50,000 markers from the US Meat Animal Research Center 2,000 Bull Project. Journal of animal science. 2011;89(6):1742-50.
71. Matukumalli LK, Lawley CT, Schnabel RD, Taylor JF, Allan MF, Heaton MP, et al. Development and Characterization of a High Density SNP Genotyping Assay for Cattle. PLoS ONE [Internet]. 2009; 4(4).
72. Merdan S, El-Zarei M, Ghazy A, Ayoub M, Al-Shawa Z, Mokhtar S. Genetic Diversity Analysis of Five Egyptian Buffalo Populations Using Microsatellite Markers. Journal of Agricultural Science. 2020;12(4).
73. Ding L, Wiener H, Abebe T, Altaye M, Go R, Kercsmar C, et al. Comparison of measures of marker informativeness for ancestry and admixture mapping. BMC Genomics. 2011;12(1):1-18.
74. Ding et al. (2011) and Kuehn et al. (2011)
75. Hulsegge et al. (2019)
76. Connolly S, Fortes M, Piper E, Seddon J, Kelly M. Determining the number of animals required to accurately determine breed composition using genomic data Proceedings. 2014.
77. Institute of Canine Biology (ICB). Using EBVs to breed better dogs 2017 [Available from: https://www.instituteofcaninebiology.org/using-ebvs-to-breed-better-dogs.html.
78. International Working Dog Registry (IWDR). What are EBVs and how do they help? 2023 [Available from: https://www.iwdr.org/master-knowledge-base/what-are-ebvs-and-how-do-they-help/.
79. The Kennel Club (UK). What are estimated breeding values (EBVs)? 2023 [Available from: https://www.thekennelclub.org.uk/health-and-dog-care/health/getting-started-with-health-testing-and-screening/estimated-breeding-values/.

80. Fikse WF, Malm S, Lewis TW. Opportunities for international collaboration in dog breeding from the sharing of pedigree and health data. The Veterinary Journal. 2013;197(3):873-5.
81. Hedhammar Å. Swedish Experiences From 60 Years of Screening and Breeding Programs for Hip Dysplasia—Research, Success, and Challenges. Frontiers in Veterinary Science. 2020;7.
82. Leighton EA, Holle D, Biery DN, Gregor TP, McDonald-Lynch MB, Wallace ML, et al. Genetic improvement of hip-extended scores in 3 breeds of guide dogs using estimated breeding values: Notable progress but more improvement is needed. PLoS One. 2019;14(2):e0212544.
83. Guo G, Zhou Z, Wang Y, Zhao K, Zhu L, Lust G, et al. Canine hip dysplasia is predictable by genotyping. Osteoarthritis and Cartilage. 2011;19(4):420-9.
84. Thomson PC, Wilson BJ, Wade CM, Shariflou MR, James JW, Tammen I, et al. The utility of estimated breeding values for inherited disorders of dogs. The Veterinary Journal. 2010;183(3):243-4.
85. Liu Z, Seefried F, Reinhardt F, Thaller G, Reents R. Dairy cattle genetic evaluation enhanced with genomic information. Proceedings of the 9th WCGALP, Leipzig, Germany. 2010.
86. Malm S, Fikse WF, Danell B, Strandberg E. Genetic variation and genetic trends in hip and elbow dysplasia in Swedish Rottweiler and Bernese Mountain Dog. Journal of Animal Breeding and Genetics. 2008;125(6):403-12.
87. Sánchez-Molano E, Pong-Wong R, Clements DN, Blott SC, Wiener P, Woolliams JA. Genomic prediction of traits related to canine hip dysplasia. Frontiers in Genetics. 2015;6.
88. Woolliams J, Lewis T, Blott S. Canine hip and elbow dysplasia in UK Labrador retrievers. The Veterinary Journal. 2011;189(2):169-76.
89. Williams-Blangero S, VandeBerg JL, editors. Genetic Considerations in the Management of Captive Nonhuman Primates. International Perspectives: The Future of Nonhuman Primate Resources: Proceedings of the Workshop Held April 17-19, 2002; 2003: National Academies Press.
90. Oliehoek PA, Bijma P, van der Meijden A. History and structure of the closed pedigreed population of Icelandic Sheepdogs. Genetics Selection Evolution. 2009;41(1):39.
91. Kraus C, Snyder-Mackler N, Promislow DEL. How size and genetic diversity shape lifespan across breeds of purebred dogs. GeroScience. 2023;45(2):627-43.
92. Frankham R, Ballou SEJD, Briscoe DA, Ballou JD. Introduction to conservation genetics: Cambridge university press; 2002.
93. Eding H, Meuwissen THE. Marker-based estimates of between and within population kinships for the conservation of genetic diversity. Journal of Animal Breeding and Genetics. 2001;118(3):141-59.
94. Blouin MS. DNA-based methods for pedigree reconstruction and kinship analysis in natural populations. Trends in Ecology & Evolution. 2003;18(10):503-11.
95. Meuwissen THE. Maximizing the response of selection with a predefined rate of inbreeding. Journal of Animal Science. 1997;75(4):934-40.
96. Clark SA, Kinghorn BP, Hickey JM, van der Werf JHJ. The effect of genomic information on optimal contribution selection in livestock breeding programs. Genetics Selection Evolution. 2013;45(1):44.
97. Hill WG, Weir BS. Variation in actual relationship as a consequence of Mendelian sampling and linkage. Genetics Research. 2011;93(1):47-64.
98. Wellmann R. Optimum Contribution Selection 2023 [Available from: https://cran.r-project.org/web/packages/optiSel/vignettes/ocs-vignette.html#example-data-set.
99. RvB. [Available from: https://www.houdenvanhonden.nl/?gclid=CjwKCAjwjMiiBhA4EiwAZe6jQ_B_RuMyyFgapc3kbraqKtTVlSaTMzKO9-PaC9Y3euVl3njMqM_yxBoCVF0QAvD_BwE.
100. Boe4. Verenigingsregelement voor het ras: Bouvier des Flandres Sint Hubert2018 [Available from: https://www.boe4.nl/phocadownloadpap/Gezondheid/Verenigings_Fokreglement.pdf.
101. AVLS. Fokreglement VFR 2013 [Available from: https://avls.nl/fokreglement/.
102. NVSW. Verenigingsfokreglement, Stabijhoun 2019 [Available from: https://www.nvsw.nl/uploads/VFR%20Stabijhoun%202022%20%20versie%20%20AV%202019.pdf.
103. NVSW. Verenigingsfokreglement, Wetterhoun 2016 [Available from: https://www.nvsw.nl/uploads/VFR%20Wetterhoun%202022%20versie%20AV%202018.pdf.
104. RVO. Dierlocatie UBN registreren en wijzigen 2020 [Available from: https://www.rvo.nl/onderwerpen/identificatie-en-registratie-dieren/dierlocatie-ubn.
105. Embark. Embark dog DNA test kits [Available from: https://shop.embarkvet.com/products/embark-dog-dna-test-kit?variant=40103683719228&_ga=2.199896749.2005020937.1683030257-121049627.1683030257
106. Towsey M. Something stinks: The need for environmental regulation of puppy mills. Villanova Environmental Law Journal. 2010;21(1):159.
107. NVSW. Actief met uw hond [Available from: https://www.nvsw.nl/diverse/actief-met-uw-hond.
108. Fiske ST, Dupree C. Gaining trust as well as respect in communicating to motivated audiences about science topics. Proceedings of the National Academy of Sciences. 2014;111(supplement_4):13593-7.
109. Perri AR, Feuerborn TR, Frantz LAF, Larson G, Malhi RS, Meltzer DJ, et al. Dog domestication and the dual dispersal of people and dogs into the Americas. Proceedings of the National Academy of Sciences. 2021;118(6):e2010083118.
110. Farstad W. Ethics in animal breeding. Reproduction in Domestic Animals. 2018;53:4-13.
111. Hens K. Ethical Responsibilities Towards Dogs: An Inquiry into the Dog–Human Relationship. Journal of Agricultural and Environmental Ethics. 2009;22(1):3-14.
112. EmbarkVet. Frequently Asked Questions answered by the Embark team [Available from: https://embarkvet.com/frequently-asked-questions/.
113. Wisdom Panel. Do you share the results with anyone else? 2022 [Available from: https://kinship-wisdom.kustomer.help/en_us/do-you-share-the-results-with-anyone-else-ryxMKxpfo.
114. Laestadius LI, Rich JR, Auer PL. All your data (effectively) belong to us: data practices among direct-to-consumer genetic testing firms. Genetics in Medicine. 2017;19(5):513-20.
115. Meyers-Wallen V. Ethics and Genetic Selection in Purebred Dogs. Reproduction in Domestic Animals. 2003;38(1):73-6.
116. Bell JS. Researcher responsibilities and genetic counseling for pure-bred dog populations. The Veterinary Journal. 2011;189(2):234-5.
117. Rokhsar JL, Canino J, Raj K, Yuhnke S, Slutsky J, Giger U. Web resource on available DNA variant tests for hereditary diseases and genetic predispositions in dogs and cats: An Update. Human Genetics. 2021;140(11):1505-15.
118. Ackerman LJ. The Genetic Connection: A Guide to Health Problems in Purebred Dogs. 2nd ed: Lakewood, Colo. : AAHA Press; 2011. 329 p.
119. ECLI:NL:RBBRE:2012:BY7900. Rechtbank Breda; 2013.
120. Sandøe P, Kondrup SV, Bennett PC, Forkman B, Meyer I, Proschowsky HF, et al. Why do people buy dogs with potential welfare problems related to extreme conformation and inherited disease? A representative study of Danish owners of four small dog breeds. PLOS ONE. 2017;12(2):e0172091.

121. T. Roberts PDM. Selection for breed-specific long-bodied phenotypes is associated with increased expression of canine hip dysplasia. The Veterinary Journal. 2010;183(3):266-72.
122. Salmon Hillbertz NHC, Isaksson M, Karlsson EK, Hellmén E, Pielberg GR, Savolainen P, et al. Duplication of FGF3, FGF4, FGF19 and ORAOV1 causes hair ridge and predisposition to dermoid sinus in Ridgeback dogs. Nature Genetics. 2007;39(11):1318-20.
123. Croney CC. Turning up the Volume on Man's Best Friend: Ethical Issues Associated with Commercial Dog Breeding. Journal of Applied Animal Ethics Research. 2019;1(2):230-52.
124. H. Doekes KO, J. Windig. Genetische analyse van de Stabij. 2016.
125. Fraser D, Weary DM, Pajor EA, Milligan BN. A Scientific Conception of Animal Welfare that Reflects Ethical Concerns. Animal Welfare. 1997;6(3):187-205.