Wageningen University & Research

Animal Breeding and Genomics Group

Breed analysis of the Dutch Bouvier des Flandres population

Possible guidelines to minimise inbreeding levels and reduce occurrence of inherited disease.

By Talita Kuijpers, 1050962

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Supervised by Jack Windig

Abstract

Genetic diversity plays a pivotal role in the evolution and sustainability of species, including pedigree dog breeds. Inbreeding and low genetic diversity can lead to detrimental health effects in dog populations. This study focuses on the Bouvier des Flandres population in the Netherlands as a case study, aiming to formulate comprehensive mating guidelines to maintain genetic diversity and mitigate inbreeding and the prevalence of inherited disorders. The research utilizes a comprehensive pedigree analysis of the Dutch Bouvier des Flandres population to assess its current structure. It examines factors such as population size, the percentage of breeding individuals, generation interval, and inbreeding levels. Simulation modelling is employed to project future inbreeding rates, considering the current breeding regulations. Additional breeding regulations are introduced in the simulation to evaluate their effect on the inbreeding rates. The study also investigates common inherited disorders in the Bouvier population, noting their severity and frequency. The study's findings and proposed mating guidelines have broader implications for preserving genetic diversity and promoting ethical breeding practices in various pedigree dog populations.

Introduction

Genetic diversity is a fundamental concept for the evolution and sustainability of all species, including dog breeds. It encompasses the collective variations in DNA among individuals within a population, indicating the level of genetic diversity (Jerold Bell, n.d.); a higher degree of diversity in DNA reflects a greater genetic diversity within the population. High amounts of variation enable populations to adapt effectively to environmental changes. In natural environments, individuals with favourable traits have a survival advantage and are 'favoured' through the process of natural selection, allowing them to reproduce and disseminate their genes. However, in pedigree dog breeding, human-driven selection focuses on specific physical, behavioural, and health characteristics desired for aesthetic or functional purposes, such as short legs in Dachshunds bred to hunt burrowing animals. Unfortunately, selective breeding can inadvertently lead to the propagation of genes and traits that are detrimental to the health and overall fitness of the breed. An example of this is the high prevalence of intervertebral disc disease in Dachshunds, resulting from breeding for short legs and a long back (Hermansen et al., 2022).

Low genetic diversity, characterized by minimal differences among individuals within a breed, is detrimental as it limits the genetic pool leading to further reduction in the genetic variation over time. In small and closed populations - like pedigree dog breeds - individuals often must reproduce with closely related individuals to ensure survival. In exacerbated situations, this can increase inbreeding dramatically where numerous individuals share identical genes. This can result in a lack of new variation being introduced to the population and a further decline in genetic diversity (Minter et al., 2021). Moreover, the occurrence of inbreeding in pedigree individuals can be attributed to the limited number of founders and the excessive reliance on specific individuals during the initial breeding process (Lewis & Windig, 2018). When a new breed originates from a small group of dogs, subsequent generations become a genetic reflection of these initial dogs, termed the "Founder effect" (Broeckx, 2020). Furthermore, the overuse of popular sires results in a more widespread dissemination of deleterious mutant recessive alleles, increasing the levels of homozygosity while also increasing inbreeding levels further (Lewis & Windig, 2018).

Prolonged inbreeding has been found to manifest traits associated with reproduction and disease, resulting in a phenomenon known as "Inbreeding depression". The rationale is as follows: when closely related dogs are bred together, an increased expression of recessive deleterious alleles occurs within the population. Homozygosity for these harmful alleles elevates the risk of disease, ultimately compromising the vitality of dogs. The manifestation of reduced vitality is a well-documented characteristic of inbreeding depression (Ubbink et al., 1992). Multiple studies conducted on various dog breeds, including Bouvier des Flandres, Labrador Retrievers, German Shepherds, and Icelandic Sheepdogs, have consistently demonstrated a positive correlation between an increasing level of

inbreeding and the occurrence of diseases, highlighting the detrimental impact of high inbreeding levels on dog vitality (Mäki et al., 2001; Ólafsdóttir & Kristjánsson, 2008).

Moreover, the impact of inbreeding on fertility has been extensively examined across various dog breeds. A study on the Irish Wolfhound revealed a significant correlation between maternal inbreeding and litter size, serving as an indicator of fertility. They found that with increased levels of inbreeding one may witness a decrease in litter sizes (Urfer, 2009). This finding has been further supported by research conducted on breeds such as the Bernese Mountain Dog, Basset Hound, Cairn Terrier, Epagneul Breton, German Shepherd Dog, Leonberger, West Highland White Terrier and German Spitz Dogs (Andrade et al., 2021; Leroy et al., 2015). In all these breeds, litters produced by dams with higher inbreeding coefficients (indicating more inbred individuals) exhibited significantly lower litter sizes (Leroy et al., 2015). Reproductive performance is of utmost importance to dog breeders, as it not only reflects the genetic health of a population but also influences selection potential and other crucial parameters linked to the success of a breeding program.

In the past, inbreeding was considered favourable ensuring conformity of the breed. This resulted in a significant increase in inbreeding levels during the 1980s and 1990s across all breeds (Lewis et al., 2015). Unfortunately, this practice has led to the emergence of diseases in various dog breeds. To address this issue, breeding associations have made efforts to reduce the spread of harmful alleles by restricting dogs that express extreme negative traits, such as high hip and elbow dysplasia scores. Moreover, the Dutch government prohibits breeding of animals that have serious defects, diseases, or behaviour problems (Rijksoverheid, n.d.). Additionally, thanks to advancements in DNA profiling, genetic tests have been developed to identify hidden recessive deleterious alleles. However, it is imperative to exercise caution when excluding all these individuals, as excessive removal of individuals can further restrict the gene pool, limit genetic diversity, and promote increased inbreeding. To prevent falling into this cycle, it is necessary to rigorously monitor genetic diversity/inbreeding when mating individuals while also keeping an eye on occurrence of disease.

A plethora of breeding strategies are reported in literature that aim to reduce the inbreeding rate and limit the loss of genetic diversity. One approach is to increase the breeding population size as it has been found that large populations experience a lower decrease in fitness and a lower inbreeding rate than small populations (Denis & Konstantinos, 2006). Increasing the breeding population can be achieved by incorporating non-breeding individuals into the breeding pool such as foreign pedigree animals or ‘look-a-likes (Wang et al., 2017). Some breed organisations have even considered outcrossing – like the Dutch Saarloos Wolfdog (Windig & Doekes, 2018). However, when increasing the breeding population one may encounter resistance from dog owners due to the numerous

responsibilities associated with breeding alone, which could discourage them from participating in the process.

On the other hand, by incorporating breeding restrictions, one can limit inbreeding without increasing the breeding population and the effectiveness can be conveniently tested on various simulation programmes (Calboli et al., 2008; Windig & Hulsegge, 2021). The following interventions have been suggested: limiting parent kinship, monitoring, and restricting individuals with high mean kinship, or restricting breeding by an individual's inherent inbreeding level (Lewis & Windig, 2018; Windig & Oldenbroek, 2015). Moreover, efforts can be made to lessen the popular sire effect by imposing restrictions on the number of matings a sire can have (Lewis & Windig, 2018).

A means of limiting the dissemination of harmful alleles involved in polygenic traits, thereby mitigating spread of inherited disease, is by the use of estimated breeding values (EBVs). They quantify the likelihood of an animal passing on a particular value for a trait to its offspring. EBVs are determined by considering the animal's phenotype, when observable, as well as the phenotypes of its relatives, in combination with their pedigree relationships (Farrell et al., 2015). This is particularly useful for selection on complex/ polygenic traits (i.e., those influenced by multiple genes and environmental factors). Using this method, two hip quality phenotypes were analysed to calculate EBVs for selecting breeders for the German Shepherd, Labrador Retrievers, and the Golden Retriever. 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 (Leighton et al., 2019). Moreover, as genotyping technologies become more affordable, there is a growing potential to employ genomic selection strategies for managing inherited disorders in susceptible breeds on a larger scale. These strategies involve calculating genomic EBVs (gEBVs) using relationships established through genome-wide markers, instead of relying solely on pedigree-based information. Sánchez-Molano et al. (2015) have confirmed that prediction accuracy of genomic selection for hip dysplasia scores were generally better than pedigree-based prediction in fewer than 1200 Labrador retrievers. This opens a new door into the combined use of pedigree and genomic technologies to have a hands-on approach to rising inbreeding levels.

Breed characteristics

The Bouvier des Flandres (henceforth named Bouvier) is a sturdy, full-bodied sheepdog native to north-western France, Belgium, and south-western Netherlands. The name roughly translates to 'cow dog of Flanders' as moving cattle was the Bouvier's primary occupation. Due to its robust build, it was mainly used as a draft or cart dog on farms, driving cows to market or the slaughterhouse (American Kennel Club, 2022). During WWI, the Bouvier breed was nearly eradicated. Surviving Bouviers were put into service pulling ambulance stretchers or cannons, serving as messengers, or searching battlefields for wounded soldiers (National Purebred Dog Day, 2021). Breeding continued after the war and a male Bouvier, *Ch. Nic de Sottegem*, trained as a trench dog, took precedence at contests due to his "ideal stature and courage" and is now considered the founder of the breed (National Purebred Dog Day, 2021). It was only in 1965 that the breed standard was adopted by the Federation Cynologique Internationale (FCI) (Fédération Cynologique Internationale (FCI), n.d.), and was officially recognised as a breed. Later on, some Bouviers served as guard and defence dogs, but as of the 1960s, they were mainly used as a companion and family dog.

The Bouvier is characterised by their shaggy, double coat which is often faded grey or black (**Figure 1**). They are squarely built and exude strength. They have a flat skull and a broad muzzle. Their back is broad and runs horizontally. Their tail is in line with the back and carried high. The ideal height for males is 62–68 cm and for females 59–65 cm. The body weight is in the range of 30–40 kg and can live an average of 11 years. They belong in FCI group 1: Sheepdogs and drovers (Fédération Cynologique Internationale (FCI), n.d.).

Figure 1. *Breed standard of the Bouvier des Flandres. Image obtained from RaadvanBeheer.nl.*

Inherited disorders in the Bouvier

Hip dysplasia (HD)

Medical assessment of the hip, elbow and eye are legally required in the pedigree Bouvier population (Vereniging Boe4, n.d.). Hip dysplasia (HD) is a heritable developmental disorder of the hip joints and is examined by means of x-ray photography. As per the Boe4 breeding regulations, Bouvier puppies have to be a minimum of 12 months to have their HD photos taken. The grading of HD is shown in **Table 1**. Grading of HD and ED is according to the FCI. Dogs that express HD have multiple breeding restrictions: "a dog presenting the result HD-C may not be used for breeding, unless combined with a partner who has the result HD-A. Moreover, dogs presenting an HD result D or E are prohibited from breeding" (Vereniging Boe4, n.d.).

Elbow dysplasia (ED)

Similarly elbow dysplasia (ED) is a heritable developmental disorder of the elbow joint and is also examined by means of x-ray photography. Bouvier puppies have to be a minimum of 12 months to have their ED photos taken. The grading of ED is shown in **Table 1**. These photos are then assessed by three members of the Raad van Beheer panel and results are given to the owners and published online (Raad van Beheer, n.d.; Vereniging Boe4, n.d.). Dogs that express ED have multiple breeding restrictions: "dogs presenting ED result grade 1 may not be used for breeding, unless combined with a partner who has the result ED-free and dogs presenting ED result Grades 2 and 3 are prohibited from breeding" (Vereniging Boe4, n.d.).

***Table 1**. Grading of HD and ED according to the FCI.*

Disease	Degree	Description
Hip dysplasia (HD)	HD A	No signs of HD
	HD B	Near normal hip joints
	HD C	Mild HD
	HD D	Moderate HD
	HD E	Severe HD
Elbow dysplasia (ED)	Free	No signs of ED
	Borderline	Near normal elbow joints
	Grade 1	Mild ED
	Grade 2	Moderate ED
	Grade 3	Severe ED

Eye disorders

Lastly, breeding Bouvier individuals are required to have their eyes tested by the ECVO (European College of Veterinarian Ophthalmologists). During this procedure, a specialised individual performs an eye examination to rule out heritable eye disorders such as cataract, entropion, glaucoma, and others (Vereniging Boe4, n.d.). Moreover, parents animals must have been examined by means of gonioscopy for iridio-corneal angle abnormality (ICAA) before they are used for breeding. The survey should be repeated every 3 years and is valid for 3 years in accordance with ECVO recommendations (ECVO

(European College of Veterinary Ophthalmologists), n.d.). Blind dogs are prohibited from breeding as well as dogs presenting disease/ abnormality that is considered chronic and/or hereditary according to current veterinary standards (Vereniging Boe4, n.d.). Below are the diseases most frequently observed in Bouvier dogs, along with concise descriptions and the breeding regulations endorsed by the Boe4 breeding association, following the ECVO recommended guidelines:

1. Persistent pupillary membranes (PPM)

PPM is characterised by persistent blood vessel remnants in the anterior chamber of the eye which fail to regress by 6 weeks after birth (Hendrix, 2013). These remnants can take the form of strands that may run from iris to iris, iris to cornea or iris to lens. In some cases, sheets of tissue may form in the anterior chamber. This can pose a great threat to vision and when severe, vision impairment or blindness may occur. PPM has been described or suspected to be either autosomal recessive (Basenji and Welsh corgi) or autosomal dominant with incomplete penetration (mastiff) (Chaudieu, 2022). Although in the wirehaired dachshund, it is proposed by deduction that the mode of inheritance is polygenic (Chaudieu, 2022).

In accordance with the ECVO recommendations, the Boe4 breeding association restricts matings of dogs presenting PPM. If the remnant strands are from iris to iris, after consultation with the association, the breeder may decide whether to mate the individual; however, only with an unaffected individual. If remnant strands are from iris to cornea or iris to lens, breeding the affected animal is prohibited.

2. Persistent hyperplastic tunica vasculosa lentis/ Persistent hyperplastic primary vitreous (PHTVL/PHPV)

PHTVL/PHPV is characterised by failure of regression of the primary vitreous (a foetal eye vessel) (Davis, 2017). This disease has proved hereditary in two breeds of dogs, the Doberman Pinscher, and the Staffordshire Bull Terrier, but the specific genetic basis has not yet been outlined (H. Colitz et al., 2000). In the Doberman Pinscher and the Staffordshire Bull Terrier, PHTVL/PHPV occurs as a bilateral condition without other malformations and may be due to inbreeding (H. Colitz et al., 2000). Another study of the Dutch Doberman population presumed that the mode of inheritance is autosomal incomplete dominant (Stades et al., 1991).

Individuals with Grade I PHTVL/PHPV (retrolental pigmented fibrovascular dots are present but do not interfere with vision) may be bred with unaffected individuals once the breeder has conversed with the breeding association. No individual presenting Grade II-VI (see Stades et al. (1991) for grade descriptions) may be bred with any individual.

3. Hereditary cataracts

A cataract refers to opacity of light scattering in the lens of the eye leading to visual impairment or blindness. It is one of the most frequent ocular diseases and represents a leading cause for blindness (Ofri, 2017). Hereditary canine cataracts are often referred to as "primary cataracts". Secondary cataracts develop as a consequence of other eye diseases like progressive retinal atrophy, retinal dysplasia, and glaucoma (Mellersh et al., 2007). It can develop any time between a few weeks from birth to months (Mellersh et al., 2006). To date, a mutation in only one gene (heat-shock transcription factor 4, HSF4) has been identified as a causative agent for cataract over different breeds (Rudd Garces et al., 2022). This disease has been extensively studied in multiple breeds with some suggesting it is autosomal recessive (Gelatt et al., 2003; Mellersh et al., 2007; Mellersh et al., 2006; Rudd Garces et al., 2022). However, a study performed by Mellersh et al. (2009) found that a different mutation to the HSF4 gene in Australian Shepherds has a dominant mode of inheritance, in contrast to the aforementioned HSF4 insertion which is recessive.

The breeding recommendation from ECVO differentiates between clinically important and low priority forms of cataracts. These specifications indicate that no individuals presenting cortical, nucleus or posterior pole cataract are allowed to breed. However, individuals with low priority lens opacities are allowed to breed, only with unaffected individuals.

4. Retinal dysplasia (RD)

RD is characterised by the presence of neuroepithelial folds and rosettes in the sensory retina. The condition in dogs is of clinical importance because of its association with retinal detachment, focal retinal atrophy, cataract and persistence of hyaloid vessels (Toole et al., 1983). In the dog, RD is characterised by detachment from the retinal pigment epithelium of the abnormally developed retina at birth, or during the first 6 weeks of life, the time period when the retina undergoes the major period of postnatal maturation (Iwabe et al., 2020). The age of onset is between 2 – 3 months of age (Ryan Liera, 2023; Toole et al., 1983). The autosomal recessive inheritance of RD is well supported for some though not in all reported breeds, for example, miniature Schnauzer, Sealyham, Yorkshire, American Cocker Spaniel, and Bedlington terriers (Iwabe et al., 2020) and as an autosomal dominant condition with incomplete penetrance in the Labrador Retriever (Appleyard et al., 2006). Moreover, a recent study by Joyce et al. (2021) reported a X-linked mode of inheritance for RD in the English cocker spaniel.

Individuals presenting focal/multifocal (characterised by retinal folds that appear as streaks, dots or vermiform lesions present singly or in multiples (Iwabe et al., 2020)) or geographic (characterised by thickened circular plaque or retinal tissue (Iwabe et al., 2020)) form of RD may be bred by decision of the breeder to an unaffected individual. Dogs presenting total RD are prohibited from breeding, as well as their parents and their offspring.

5. Distichiasis

Distichiasis is a condition characterised by the presence of lashes on the internal edge of the eyelids such that they are in contact with the cornea (Petersen et al., 2015). This congenital hereditary disorder affects various dog breeds, especially purebred breeds that may manifest from 0.3 - 8.9 years of age (Jondeau et al., 2023). While there have been suggestions that distichiasis is inherited as an autosomal dominant trait, it has not yet been formally proven (Petersen et al., 2015). In humans, congenital distichiasis follows an autosomal-dominant pattern with a significant level of penetrance, but the precise mechanism responsible for its occurrence remains to be elucidated (Raymond-Letron et al., 2012).

Dogs presenting distichiasis are allowed to breed with unaffected individuals by decision of the breeder (with consultation from the breeding association). In severe cases, breeding with the affected individual is prohibited.

6. Corneal endothelial dystrophy (CED)

The cornea is an optically clear tissue permitting the transmission of light to the inner structures of the eye. Abnormalities of the corneal structure can result in ocular discomfort, reduced light refraction and blindness. CED is the prominent cause of corneal degeneration in canines. This condition is characterised by a reduction in the clarity of both the eyes, accompanied by a decrease in endothelial cell density and irregular morphology. CED is a hereditary progressive disorder that can result in potential secondary infections and/or blindness (Armour et al., 2019). The mode of inheritance is possibly sex-linked recessive as proposed by Crispin and Barnett (1983) after they investigated 144 cases of corneal dystrophy and found twice as many occurred in the female than in the male. Similarly, the sex distribution of a group of Huskies presenting corneal dystrophy was 58 females to 20 males (MacMillan et al., 1979). According to ECVO, dogs presenting endothelial dystrophy are prohibited from breeding.

7. Progressive retinal atrophy (PRA)

Generalised PRA in domestic dogs is characterised by progressive degeneration of the retina leading to night blindness, followed by loss of peripheral and central visual fields analogous to retinitis pigmentosa, the most prevalent group of inherited retinopathies in man (Dekomien et al., 2010). In most canine breeds, the disease is inherited in an autosomal recessive manner (Dekomien et al., 2010; Hitti-Malin, 2020; Petersen–Jones et al., 1999). Early onset PRA is visible from 2 – 6 weeks of age while late onset PRA manifests at 2 – 5 years of age (Mellersh, 2014). No breeding is permitted with the affected animal, its parents or its offspring according to the ECVO recommendations. The Boe4 breeding association abides by the ECVO recommendations.

8. Entropion

Entropion is a malposition of the eyelid, in which it is folded inward, resulting in contact between the eyelashes and cornea (Maman & Taub, 2011). Symptoms primarily arise from irritation of the ocular surface. Entropion is categorised into two main groups: primary and secondary entropion. Primary entropion is classified as congenital or developmental, which appears between 4 – 7 months of life or following the development of the adult features (Stades & Gelatt, 2008). Pure breeds are more commonly treated for primary entropion, suggesting a hereditary basis and a polygenic inheritance pattern has been proposed (Carrozza et al., 2022). Secondary or acquired entropion develops after an ocular problem such as eyelid trauma, microphthalmos and loss of eyelid support. Breeders may decide whether to breed the individual; however, in severe cases breeding of the affected individual is prohibited.

9. Microphthalmia

Microphthalmia is a developmental disorder of the eye in which one (or both) eyes are abnormally small. According to Kaukonen et al. (2018), it is the result of an amino acid deletion in the retinol-binding protein (RBP4). RBP4 carries Vitamin A from the maternal hepatic stores to the foetus where it helps develop retinoic acid, a potent paracrine-signalling molecule needed for proper development of the vertebrate eye. Apart from genetic mutations, multiple risk factors have been reported for this disease, including a Vitamin A (retinol) deficiency. The disease follows an autosomal recessive inheritance pattern, wherein its expression is contingent upon the maternal genotypes penetrance. The disease manifests only when both offspring and mother carry deletion homozygotes. The maternal penetrance effect arises from a disruption in the transfer of retinol across the placenta (Kaukonen et al., 2018). The disease is congenital and thus is present from birth. Individuals presenting microphthalmos are prohibited from breeding.

10. Glaucoma

Glaucoma is a group of progressive disorders characterised by optic nerve head, retinal ganglion cell death which leads to irreversible vision loss. Primary glaucoma is mainly due to increased ocular pressure (IOP) which causes the membranes of the eye to expand in the socket and damage the retinal ganglion cells; this is without another incident of intraocular disease. Increasing ocular pressure will cause increasing blindness from the peripheral vision and, if left untreated, can exacerbate central vision loss leading to total irreversible blindness (Pizzirani, 2015). Primary angle-closure glaucoma is the most common form of canine primary glaucoma, of which the age of onset is around 7 years (Komáromy & Petersen-Jones, 2015; Strom et al., 2011). In this case, the irido-corneal angle (ICA) is congenitally narrowed (also known as irido-corneal angle abnormality (ICAA)), preventing ocular fluid drainage, ultimately increasing IOP and may be associated with dysplasia of the pectinate ligament (PLD) (Van der Linde-Sipman, 1987). The occurrence of glaucoma in the Bouvier is related to the most severe cases

of PLD; a disease especially common in this breed (Miller et al., 2013; Van der Linde-Sipman, 1987). Glaucoma is a multifactorial disease; therefore, genetics are not the sole determining factor for the presence of the disease. Nevertheless, it is undeniable that a familial history of glaucoma increases an individual's risk of developing the disease (Pizzirani, 2015).

ECVO recognises ICAA as a predisposing factor and hence tests of ICAA as a predictor of glaucoma. Dogs presenting mild to moderate ICAA are only allowed to be bred with unaffected individuals if determined able to breed by the breeder. Dogs presenting severe ICAA are prohibited from breeding. However, the irido-corneal angle may change with age and thus gonioscopy should be performed before breeding and repeated every 3 years. To those individuals presenting primary glaucoma, breeding is prohibited.

A summary of the inherited diseases, their most likely mode of inheritance and the respective breeding rules can be found in the appendix (**Table 2**).

Research problem

Pedigree dog breed associations often face challenges in maintaining genetic diversity within their populations, which can lead to detrimental effects on the health and well-being of the dog population. The research problem lies in the absence of comprehensive mating guidelines specifically tailored to address these issues, resulting in inadequate measures to mitigate the increasing rates of inbreeding and the prevalence of inherited disorders. Thus, there is a need to formulate effective guidelines that consider the population structure, breeding policies, inherited disorders, and potential interventions to preserve genetic diversity in pedigree dog populations, using the Bouvier des Flandres population in the Netherlands as a case study.

Research significance

With the growing concern over genetic diversity and the overall companion animal genetic health, there is an increasing demand for pedigree dog mating guidelines that address the challenges associated with inbreeding and the prevalence of inherited disorders. By incorporating evidence-based research and analysis, these guidelines can provide practical interventions and strategies to promote genetic diversity, improve the well-being of pedigree dog populations, and ensure their long-term viability. Moreover, the generalizability of the guidelines to other pedigree dog populations expands the study's impact, promoting ethical breeding practices and responsible genetic diversity preservation across various breeds and regions.

Aim & Research questions

The main objective of this research is to formulate comprehensive mating guidelines for a pedigree dog breed association. These guidelines will allow them to maintain genetic diversity, thereby improving the health of the dog population. In order to complete the objective, the Bouvier des Flandres population of the Netherlands will be used as a template. Insight into the population parameters (population size, percentage of breeding individuals, generation interval, inbreeding level, etc.) of the Bouvier is thus required. Subsequently, the current Bouvier population and their breeding regulations will be simulated to project the future inbreeding rates. Furthermore, additional breeding regulations will be added to the simulation in order to lessen projected inbreeding rates of the same Bouvier population. An analysis of common inherited disorders in the Bouvier will be performed. From these findings, guidelines will be constructed, instructing pedigree breeders on how to tackle rising inbreeding rates. The following research questions provided the foundation for this research:

1. What is the current population structure of the Dutch Bouvier des Flandres population based on a comprehensive pedigree analysis? How does the current breeding policies affect the inbreeding rate? How will other explored breeding strategies affect the inbreeding rate?
2. What are the common inherited disorders observed in the Bouvier population, and what is the severity and frequency of each?

Materials and methods

Data of all registered Bouvier individuals was received from the Dutch Kennel Club. In this data set, the unique registration number, sex, and name of each registered dog was recorded from 1979 up to and including 2020. The data set had been pre-processed with Retriever computer software, and the output file was provided. The data set contained 48345 dogs of which 3166 did not have a birth year. Within the output file, general population parameters relevant for the determination and management of inbreeding were presented. Such parameters include population size, the number and variation of offspring per male dog per year, and the average of and variation in litter size, generation interval, pedigree completeness, inbreeding (*F*) and the kinship coefficient (*f*). Additionally, inbreeding and kinship rates were estimated and provided in the output document.

Pedigree analysis:

The following general population parameters from the output file were considered during the pedigree analysis:

- *Number of new registrations per year*: provides insight into the breed's popularity throughout the period spanning from 1979 to 2020.
- *Litter size per year*: the count of puppies per distinct litter referring to unique combinations of a mother, father, and birth date.
- *Total number of litters per year*: the cumulative count of unique combinations of mother, father, and birth date within a specific year.
- *Offspring per parent per birth year of parent*: the average number of offspring per parent in its future lifetime.
- *Age of dam/sire at birth of progeny*: the difference between the date of birth of the parent and the date of birth of the progeny.
- *Generation interval*: the mean difference between the date of birth of the parent and the date of birth of the progeny.
- *Top sires and their contribution*: the contribution of top sires to total number of offspring per year.

The inbreeding (*F*) and kinship (*f*) coefficients per year were calculated by the Retriever program using the methods of Meuwissen and Zuo (1992) and Sargolzaei et al. (2005), respectively. Additionally, the Retriever program provides the inbreeding (ΔF) and kinship (Δf) rates calculated over the whole period and for each generation. Generation-based inbreeding rates were computed for each interval of n years using the following equation.

$$\Delta F_{1-n} = \left(\frac{1-F_n}{1-F_1}\right)^{\frac{L}{n}},$$

where F_1 is the average level of inbreeding among pups born during the initial year of the given period, F_n in the last year. L represents the generation interval. For year-based inbreeding rates, the variable L was substituted with a value of 1 and are presented as 'ΔF absolute'. Inbreeding rates were also calculated by regressing $LN(1 - F_y)$ on year and equating the slope to $-\Delta F$ (Pérez-Enciso, 1995*)*. Kinship rates (Δf) calculations were performed using identical procedures, but with the substitution of kinship (f) instead of inbreeding coefficients (F). Effective population sizes (Ne) were also provided and calculated using $Ne = \frac{1}{2\Delta F}$ and $Ne = \frac{1}{2\Delta f}$.

Completeness of the pedigree was determined by analysis of the average equivalent complete generations (EcG) which is a sum of all known ancestors where parents are 0.5, grandparents are 0.25, great-grandparents are 0.125 and great-great-grandparents: 0.0625 etc. Additionally, the number of ancestral generations completely known were visualised by tracing back ancestors per generation until at least one ancestor was unknown.

Policy simulation

The simulation program Pointer (GenManSim) was used to evaluate the effect of different breeding strategies on the inbreeding rate of the Bouvier population. The input data was based on the output of the Retriever program regarding the Bouvier population. Pointer predicts inbreeding rates for populations of different sizes, with different population structures and genetic management techniques (Jack J Windig & Ina Hulsegge, 2021). The summary of input parameters that were used to simulate the Bouvier population can be found below (**Table 3**). An extensive explanation can be found in the Appendix (**Table 5**).

Table 3. *Summary of Retriever output data used as input for Pointer software.*

Parameters	**Value**
Pedigree years from which data is based on	2014 – 2019
Number of litters per year	70
Number of females available for breeding per year	56[a]
Number of males available for breeding per year	30[b]
Contribution of the 4 most popular males to total number of young	31%
Age distribution	See **Figure 2**
Litter sizes	See **Figure 3**

[a] Based on 2014 – 2017. 12 females born per year who will later become mothers. 18.5% of breeding females are replaced each year. Total of 67 breeding females including animals who are still too young to breed now but will become mothers later. 56 is excluding non-breeding animals.
[b] Based on 2014 – 2017. 5 males born per year who will later father. 18.5% of breeding males are replaced each year. Total of 35 male breeding animals including animals who are still too young to breed now but will become fathers later. 30 is excluding non-breeding animals.

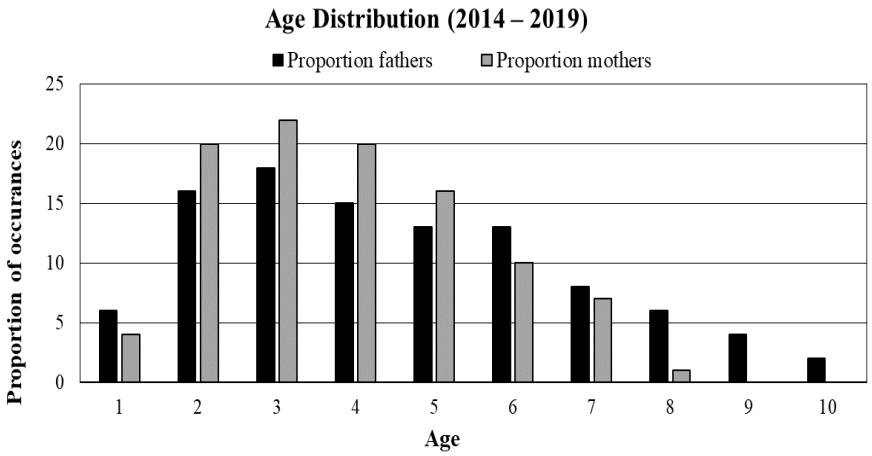

Figure 2. *Age distribution of the Bouvier population from years 2014 – 2019. Based on Retriever output data.*

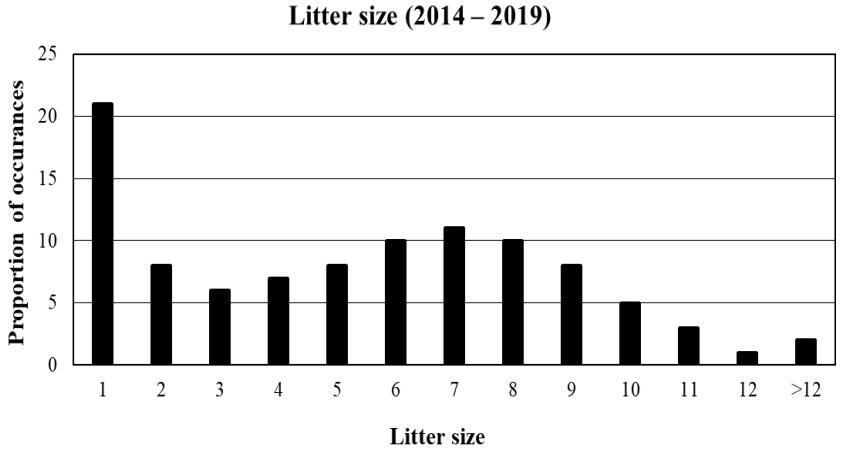

Figure 3. *Popular litter sizes of the Bouvier from 2014 – 2019. Based on Retriever output data.*

Several genetic management techniques can be incorporated into the Pointer simulation, offering a range of options. In order to observe the trajectory of the inbreeding level in the absence of any intervention, a simulation was conducted without implementing a breeding policy. Subsequently, a second simulation was performed incorporating the current breeding policy, to assess its impact on the future inbreeding rates. The current breeding policy is as follows: Bouvier males are currently limited to 5 successful matings per year, with a maximum of 25 matings per lifetime; Bouvier females are limited to 5 litters per individual; a male must be a minimum of 1.5 years (18 months) to sire their first litter and a female must be a minimum of 2 years (24 months) to carry their first litter.

Various alternative policies were simulated with the objective of identifying the optimal approach of genetic management, such as:

- *Enlarging breeding population size*: increasing the number of breeding animals and by allowing additional males to breed, while keeping contribution of top sires constant.

- *Breeding restrictions*: restricting the number of litters sired per animal per year/ over its lifetime. This was done by restricting the number of litters per sire/ dam and the number of sons per male.
- *Restrictions by coefficients*:
    - *Kinship coefficients per parent pairing*: only parents with the lowest kinship were mated.
    - *Mean kinship of breeding individuals*: only individuals with the lowest mean kinship were mated.
    - *Combination of mean kinship and kinship of parents*: individuals with the lowest mean kinship were mated with the lowest kinship counterpart.

Analysis of inherited disorders:

Disease occurrence was extrapolated from the number of examinations performed per year per disease and the results were divided into 3 categories: "Free" (no presence of disease), "Not free" (presence of disease) or "Undetermined". HD, ED and ECVO results were provided by the Boe4 association. The proportion of affected individuals from the total number of tested individuals served as a proxy for disease prevalence, with the assumption that the tested population represents the total population.

Pointer software was used to simulate the effect different diseases had on the inbreeding, allele frequency and genotype frequency of the respective disease. The baseline input parameters were used to set up the simulated population, with the current breeding restrictions. These values can be found in **Table 4**. A literature review was performed which identified a disease's most likely mode of inheritance and age of onset given from studies performed on other breeds. These are summarised in **Table 2**. The diseases included in the simulation were modelled by their prevalence and age of onset by altering their starting frequencies (denoted as '*p*' in Pointer) and their year of effect, respectively. The square root of the percentage of "Not free" individuals was used as the starting frequency in the Pointer software.

Within the simulation, the number of diseases and their selection rates were altered to investigate the effect on the inbreeding levels and disease allele frequencies. The selection rates (denoted by '*s*' in Pointer) demonstrate the severity of the disease such that when $s = 1$ the allele is lethal, $0 < s < 1$ the allele is detrimental and when $s = 0$ the allele is neutral. The chance of survival for a homozygous individual is therefore $1 - s$.

Results

Pedigree analysis results

The general population parameters of the Bouvier are summarised below (**Table 4**). The average and the standard deviation are included to show the spread of the data around the mean. The median indicates the skewness of the distributions.

Table 4. *General population parameters of the Bouvier based on the years between 1989 – 2020.*

Parameter	Mean ± stdev	Median	Range (min – max)	Total
Number of registered individuals				48345
Pups not selected for breeding	1256.38 ± 1003.93	812	273 – 3540	40495
Pups selected for breeding	95.19 ± 83.37	62.5	0 – 277	4684
Number of top sires[a]				7
Number of progeny per sire	12.28 ± 2.12	11.83	8.27 – 15.82	
Litter size	6.07 ± 0.39	6.17	4.84 – 6.72	
Number of offspring per sire[b]	30.26 ± 12.44	30.21	0 – 55.33	
Number of offspring per dam[b]	12.29 ± 3.87	13.49	0 – 16.45	
Generation interval of				
Sires	4.62 ± 0.35	4.55	4.09 – 5.6	
Dams	4.20 ± 0.24	4.18	3.69 – 4.65	
Average age of				
Sires at birth of progeny	3.77 ± 1.96		0 – 8	
Dams at birth of progeny	3.53 ± 1.71		0 – 8	

[a] Number of sires occurring more than once as top sires.
[b] Offspring produced over the whole lifetime of the individual.

The number of newly registered Bouviers per year can be seen in **Figure 4**. The large increase from 1988 to 1989 can be attributed to the change from paper to computerised data collection. It is for this reason that further analysis will comprise of computerised data from 1989 to 2020 (see **Figure 5**). The number of newly registered Bouviers per year has decreased from 3700 individuals to a mere 400 individuals from 1990 to 2020. The peaks in the early 1990s reflects the popularity of the Bouvier in the late 1980s and early 1990s in the Netherlands. However, since then there has been a steady decrease in its popularity over time.

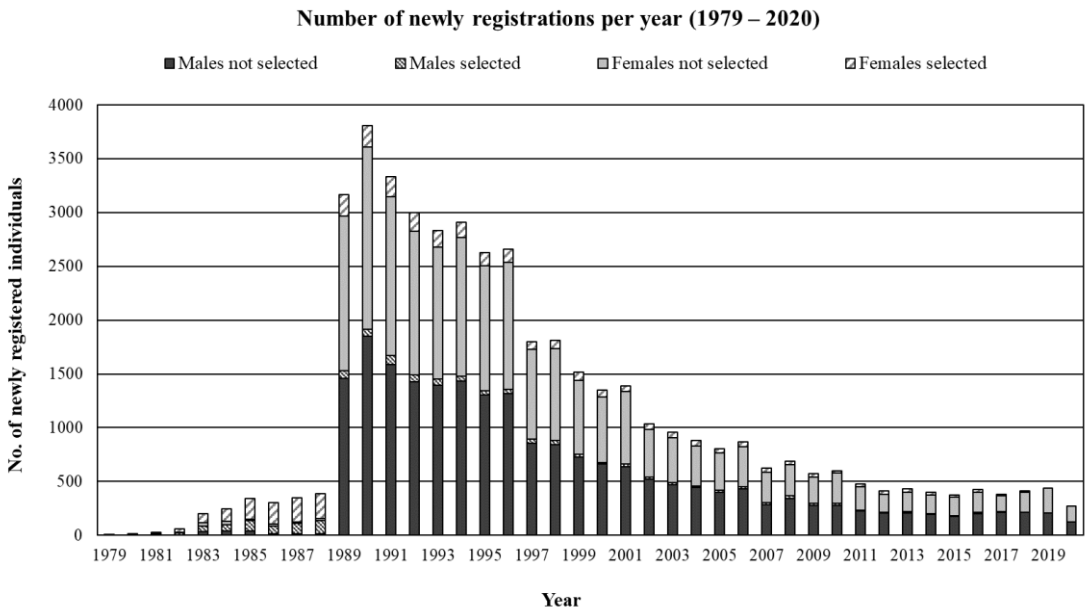

Figure 4. *Number of newly registered Bouviers per year between the years 1979-2020, comprising new-born pups and founders that were either selected for breeding or not (yet).*

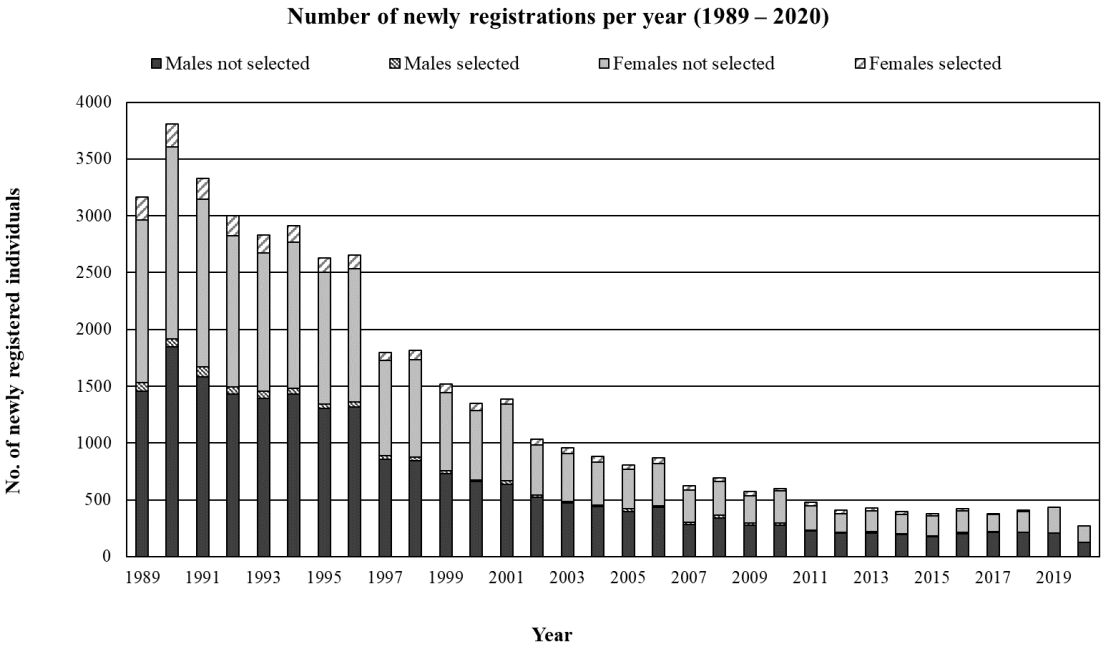

Figure 5. *Number of newly registered Bouviers per year between the years 1989-2020, comprising new-born pups and founders that were either selected for breeding or not (yet).*

The number of pups per litter showed minor fluctuations around the mean (6.07 pups), with the lowest peak in 2014 (see **Figure 6**). While the total number of litters is steadily decreasing (see **Figure 6**). It is hypothesised that as popularity of the breed decreases, so does the total number of litters due to decreased demand of pups of this particular breed.

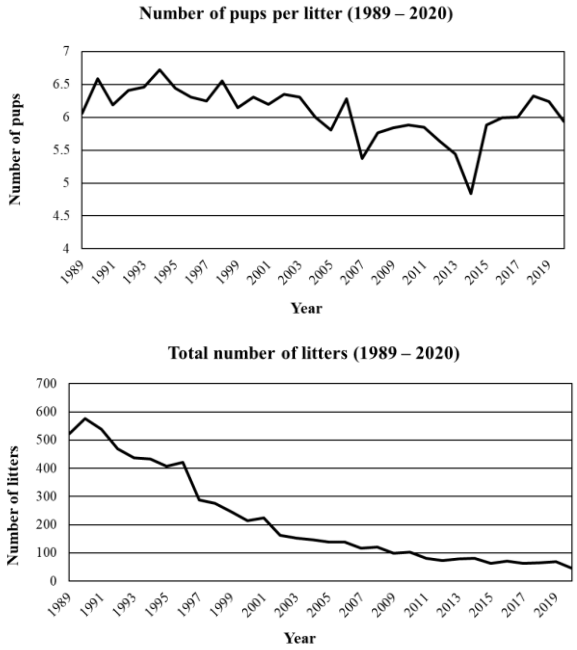

Figure 6. *Number of pups per litter (left) and number of litters per year (right) for the Bouvier from 1989-2020.*

The number of offspring per dam remained relatively consistent over the years, while the number of offspring per sire showed significant fluctuations (see **Figure 7**). The high peaks in 1992, 2000, 2006, 2011 could be due to the use of popular sires, such that the number of progeny in those years (the years of their birth) are higher than average.

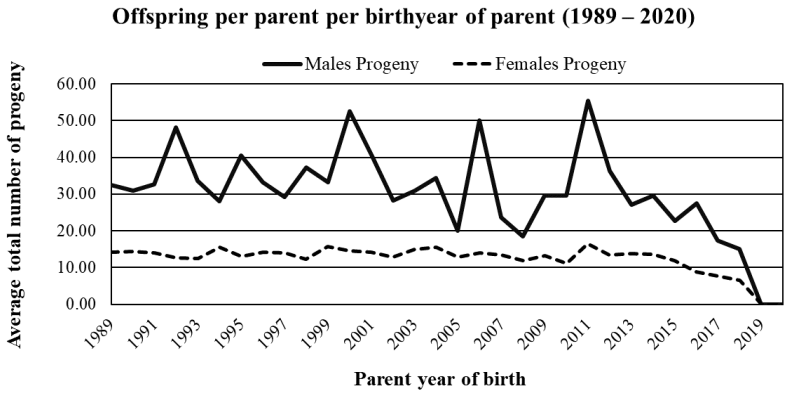

***Figure** 7. Average of the total number of progeny per Bouvier-parent from 1989-2020.*

The mean age of sires and dams at the birth of their progeny is 3.77 and 3.53 years, respectively. The average generation interval for sires and dams is 4.42 and 4.20 years, respectively. From **Figure 8**, it is clear to see that the generation interval is steadily increasing for both sires and dams. This change may be attributed to the decline in breed popularity, resulting in reduced demand for puppies.

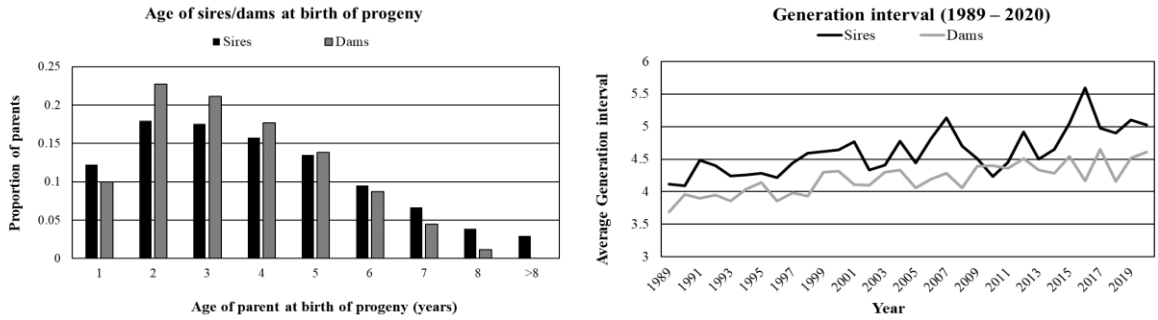

Figure 8. *Age of Bouvier sires and dams at the birth of their progeny (left) and generation interval (right) of the Bouvier per year from 1979 – 2020.*

In 1979, one can clearly see the equal contribution of all 6 sires of that year (all had 16.7% contribution, **Figure 9**). The overall contribution then decreases until 1995 where the contribution of top sires tend to increase. This could be indicative of the use of popular sires towards later years.

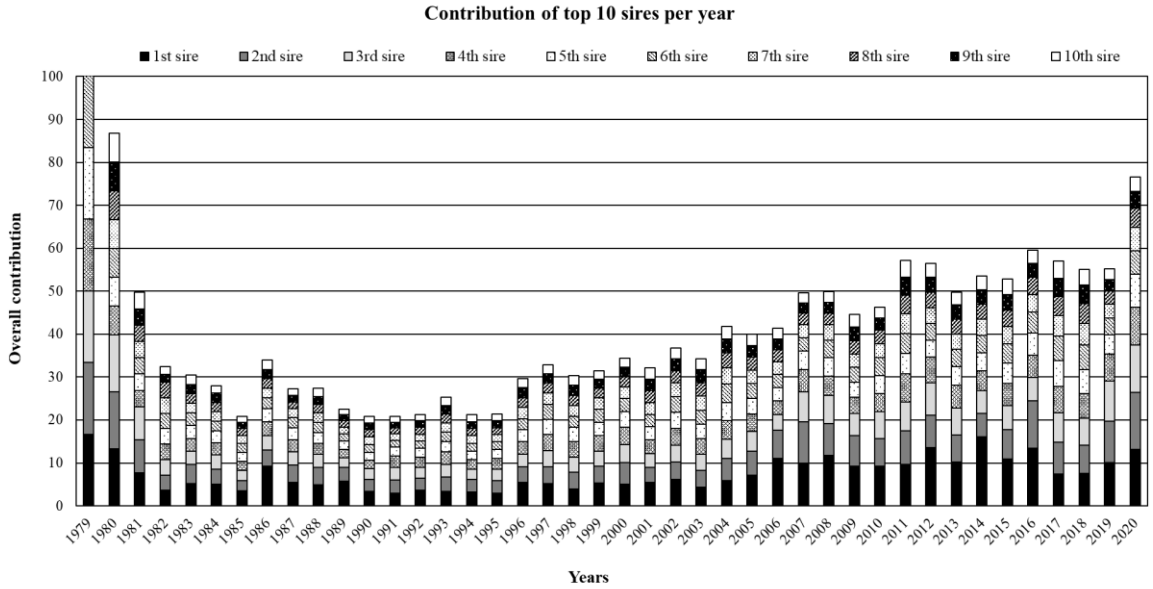

Figure 9. *Contribution of the top 10 Bouvier sires per year from 1979 – 2020.*

Pedigree completeness

During development of the breed, there has been a steady increase in pedigree completeness, ensuring the accuracy of the inbreeding coefficient calculation (See **Figure 10**). It is possible that the sudden decrease in the number of known ancestral generations (3 and 4 generations) around 2005 could be attributed to the importation of external Bouviers into the breeding scheme. When a dog is imported, its entire ancestry may not be included in the pedigree. This can result in a decrease in the known generations despite technological advances.

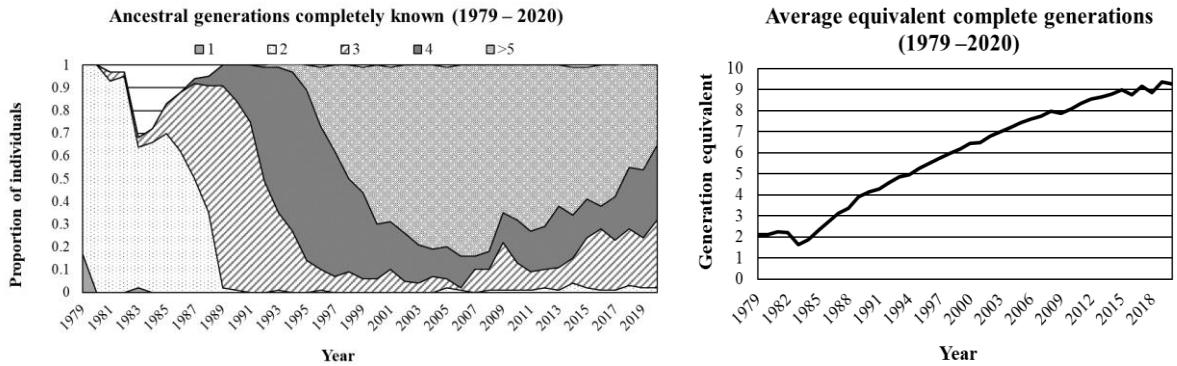

Figure 10. *Pedigree completeness: proportion of Bouviers with x ancestral generations completely known (left) and average equivalent complete generations (right) from 1979-2020.*

Inbreeding

Inbreeding has increased dramatically from 0 to 0.09 over the evolution of the breed (see **Figure 11** and **Figure 12**). However, there is noticeable stabilisation after the peak around 2007. It is assumed that the peak prompted breeders to seek out sires/ dams from external populations since inbreeding awareness increased around 2005. The subsequent plateau in inbreeding can be attributed to the importation of dogs from external populations as evident from the pedigree completeness. Generally, the kinship coefficient (*f*) remains lower than the inbreeding coefficient (*F*) which can be indicative of local inbreeding. The inbreeding rate per ten-year period shows a peak between 1991-2000 followed by a steady decrease over later years (**Figure 13**). The inbreeding rate for the latest period (2011 – 2020) was calculated as 0.45%.

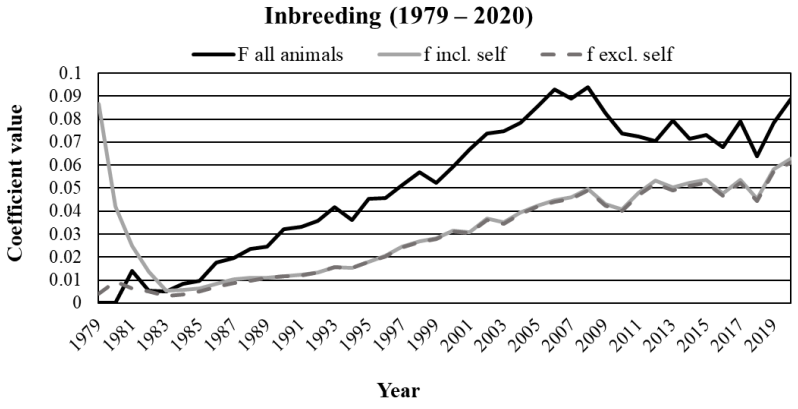

Figure 11*. Inbreeding coefficient (F) and relatedness (f; including and excluding self) in the population of the Bouvier from 1979-2020.*

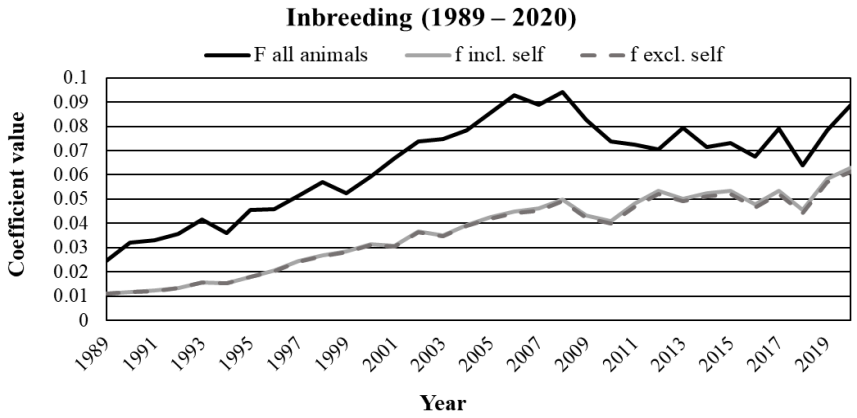

Figure 12. *Inbreeding coefficient (F) and relatedness (f; including and excluding self) in the population of the Bouvier from 1979-2020.*

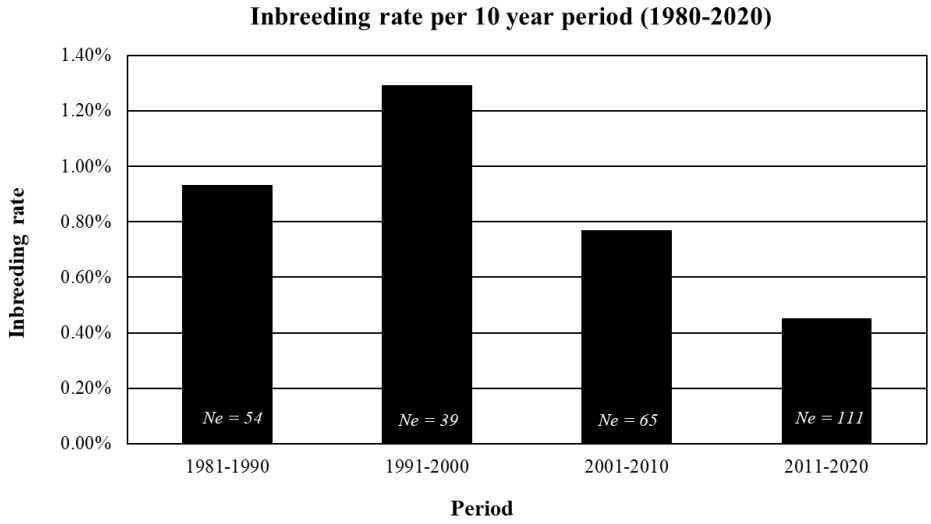

Figure 13. *The rate of inbreeding and inbreeding effective population size in the Bouvier per ten-year period from 1981 to 2020.*

Pedigree simulation results

The input used for the baseline simulation is shown in the appendix (**Table 5**).

Simulation following current restrictions

The simulated Bouvier population's inbreeding rate following the current breeding restrictions was 0.83%, compared to 1.34% without any restrictions (see **Figure 14**). This highlights the importance of implementing the current restrictions.

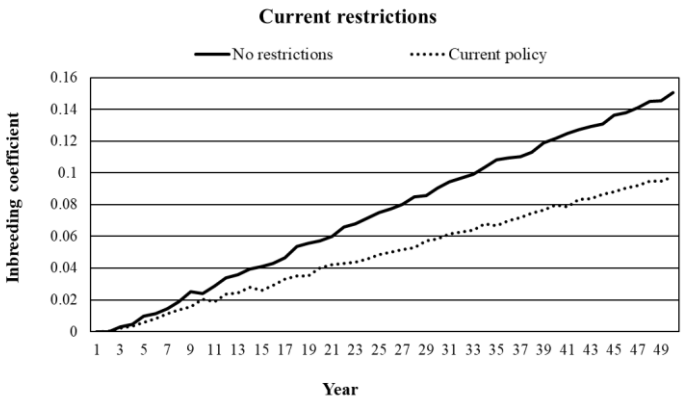

Figure 14. *Effect of the current restrictions on the inbreeding rate.*

Enlarging the breeding population

The effect of enlarging the breeding population size on the inbreeding rate is shown in **Table 6.** By doubling the breeding population size for both sires and dams, there is a significant decrease in the mean inbreeding rate from 0.83% to 0.62%, respectively. The pattern was similar for the mean kinship rates (shown in **Table 7**). Moreover, by a mere 50% increase in the number of males, while keeping the number of females constant, the inbreeding rate decreased to 0.72%.

Table 6. *Effect of increasing the number of available breeding males and females per year on mean inbreeding rate (in %).*

Females available for breeding	**Males available for breeding**[a]						
	Baseline (n = 30)	+5% (n = 31)	+10% (n = 33)	+25% (n = 37)	+50% (n = 45)	+75% (n = 52)	+100% (n = 60)
Baseline (n = 140)	0.83	0.84	0.82	0.78	0.72	0.71	0.67
+ 5% (n = 147)	0.85	0.82	0.83	0.75	0.71	0.69	0.67
+ 10% (n = 154)	0.88	0.81	0.83	0.75	0.71	0.68	0.65
+ 25% (n = 175)	0.81	0.82	0.80	0.75	0.70	0.65	0.68
+ 50% (n = 210)	0.81	0.82	0.79	0.75	0.68	0.66	0.61
+ 75% (n = 245)	0.80	0.80	0.79	0.72	0.70	0.67	0.63
+ 100% (n = 280)	0.79	0.79	0.75	0.73	0.68	0.67	0.62

[a] Sires were restricted to a maximum of 5 litters per year and maximum of 25 litters per life.

Table 7. *Effect of increasing the number of available breeding males and females per year on mean kinship rate (in %).*

Females available for breeding	**Males available for breeding**[a]						
	Baseline (n = 30)	+5% (n = 31)	+10% (n = 33)	+25% (n = 37)	+50% (n = 45)	+75% (n = 52)	+100% (n = 60)
Baseline (n = 140)	0.91	0.91	0.88	0.83	0.78	0.75	0.74
+ 5% (n = 147)	0.92	0.89	0.90	0.82	0.78	0.74	0.73
+ 10% (n = 154)	0.93	0.88	0.88	0.81	0.77	0.73	0.70
+ 25% (n = 175)	0.87	0.90	0.86	0.80	0.76	0.73	0.73
+ 50% (n = 210)	0.89	0.87	0.86	0.82	0.75	0.72	0.70
+ 75% (n = 245)	0.88	0.87	0.86	0.78	0.76	0.73	0.68
+ 100% (n = 280)	0.86	0.85	0.82	0.79	0.75	0.72	0.69

[a] Sires were restricted to a maximum of 5 litters per year and maximum of 25 litters per life.

Breeding restrictions

Enlarging the population by twice as many individuals may be difficult and even impossible, thus it might be worthwhile to constrain top sires. When the number of top sires remained constant, but the number of litters per sires was halved the inbreeding rate decreased to 0.81% (see **Figure 15**). However, the lowest inbreeding rate was achieved when no top sires were present (0.75%).

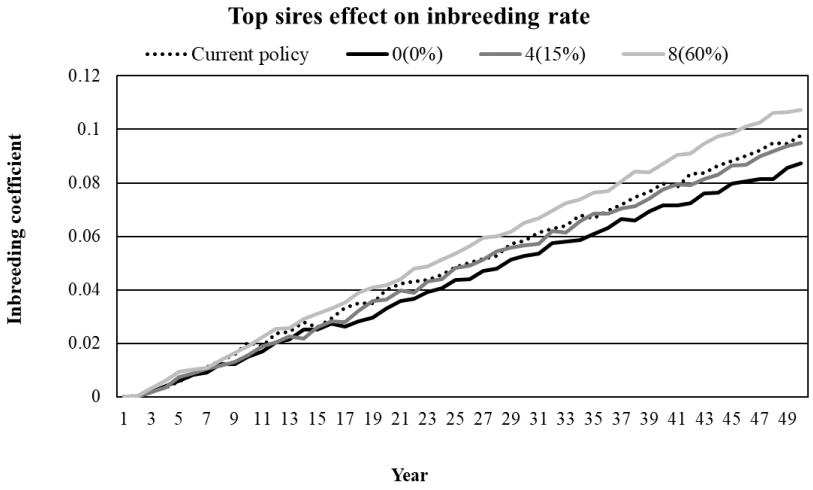

Figure 15*. Effect of changing the number and contribution of top sires on the mean inbreeding rate (in %).*

Sire restrictions were effective in reducing the inbreeding rate in the simulated Bouvier populations, as shown in **Table 8**. When restricting males to 1 litter per sire per life, the lowest inbreeding rate was achieved: 0.71%. Interestingly, increasing or decreasing the number of litters per sire per year had no effect on this value. By decreasing the maximum number of litters per sire per life to 10 and keeping the maximum number of litters per sire per year at 5, one can reach a similar inbreeding rate of 0.74%.

***Table 8**. Effect of restricting the use of sires per year and life on the mean inbreeding rate (in %).*

Max number of litters/ sire/ life	Max number of litters/ sire/ year			
	Baseline	1	5 (current)	10
Baseline	1.34	0.82	0.91	1.24
1	0.71	0.71	0.71	0.71
5	0.79	0.77	0.79	0.79
10	0.95	0.78	**0.74**	0.95
15	0.97	0.81	0.76	0.86
20	0.93	0.82	0.82	0.90
25 (current)	0.98	0.82	0.83	0.94

Interestingly, restricting the number of litters per dam per lifetime increases the inbreeding rate (see **Figure 16**); with 1 litter per dam per life, the inbreeding rate increased to 0.98%. With stricter restrictions, the inbreeding rate increases further, but the generation interval decreases (see **Table 9**). The rationale is that once dams have reproduced, the simulation removes dams that have reached their maximum litters, closely related offspring quickly replace them which leads to higher inbreeding. Furthermore, increasing the number of litters per dam per life to 10 or decreasing it to 3 made no difference on the inbreeding rate; in both cases it was 0.85%.

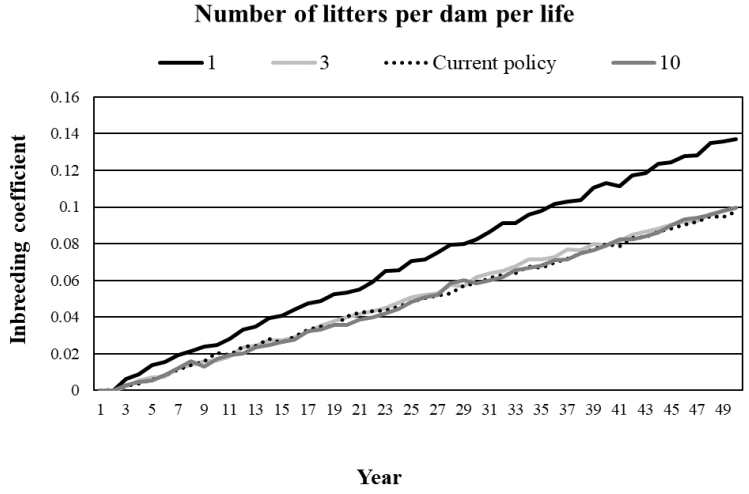

***Figure 16**. Effect of increasing the number of litters per dam per life on the inbreeding coefficient over 50 years of the simulation.*

***Table 9**. Effect of restricting the number of litters per dam per life.*

Parameter	Number of litters per dam per life			
	1	**3**	**5 (current)**	**10**
Inbreeding rate	0.98%	0.85%	0.83%	0.85%
Kinship rate	1.04%	0.92%	0.91%	0.92%
Generation interval	3.33	4.06	4.06	4.05

Coefficient restrictions

In comparison to the current policy, managing matings on the basis of kinship was most effective in decreasing the inbreeding rate (see **Figure 17**). By restricting matings between individuals with high mean kinships, the inbreeding rate decreased from 0.83% to 0.58%. Breeding restrictions based on the parents' kinship had differing effects in the short and long term. Initially, individuals in the population are bred to the least related individuals of that population, lowering the inbreeding rate substantially to 0.06% for the first 10 years (as shown **Table 10**, Period 1 of Minimise parent kinship). However, in the next 40 years the inbreeding rate increased to 0.68% (**Table 10**, Period 5 of Minimise parent kinship). Thus, minimising parent kinship is only truly effective in the short term. Similarly, the combination of constraining the mean kinship as well as minimising the kinship between the breeding individuals had varying short- and long-term effects. Initially, the inbreeding rate decreased substantially to 0.04% for the first 10 years (as shown in **Table 10**, Period 1 of Combination). However, in the next 40 years the inbreeding rate increased to 0.52%, much below the original population (**Table 10**, Period 5 of Combination). To conclude, the current regulations do have a positive effect on the inbreeding rate. However, this could be improved with the implementation of other strategies such as the combined addition of choosing individuals with a low mean kinship for breeding as well as minimising the kinship between the breeding pair.

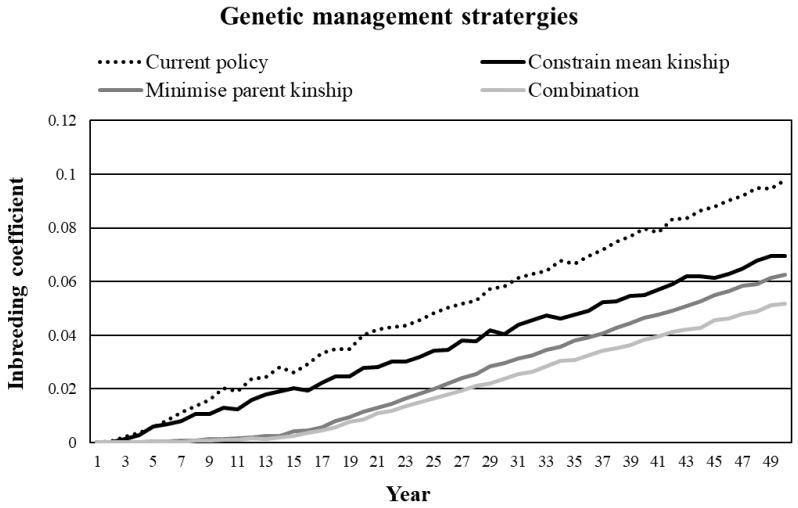

Figure 17. *Effect of alternative genetic management strategies on the inbreeding coefficient over 50 years of the simulation.*

Table 10. *A summary of the inbreeding rates for all periods of both genetic management strategies; minimise parent kinship and combination.*

Genetic management strategy	Period	deltaF	Ne(F)
Minimise parent kinship	Period 1 (1-10 years)	0.06%	891
	Period 2 (11-20 years)	0.42%	119
	Period 3 (21-30 years)	0.73%	68
	Period 4 (31-40 years)	0.68%	74
	Period 5 (41-50 years)	0.68%	74
	Whole period (1-50 years)	**0.63%**	**79**
Combination of minimise parent kinship and mean kinship			
	Period 1 (1-10 years)	0.04%	1355
	Period 2 (11-20 years)	0.31%	163
	Period 3 (21-30 years)	0.53%	94
	Period 4 (31-40 years)	0.53%	94
	Period 5 (41-50 years)	0.52%	97
	Whole period (1-50 years)	**0.49%**	**102**

Analysis of inherited disorders:

Disease prevalence is shown below from the total occurrences of "Not free" individuals per disease from tests done in 2010 – 2020 (**Figure 18**). 447 individuals were tested for each disease: PPM, PHTVL/PHPV, hereditary cataracts, non-congenital cataracts, RD, Distichiasis, CD, PRA, and Entropion. ICAA, a risk factor for Glaucoma, had the highest number of "Not free" individuals: 184 of 299 total tests resulting in a prevalence value of 78%. Due to the variation in sample sizes, the results pertaining to ICAA were omitted from **Figure 18**.

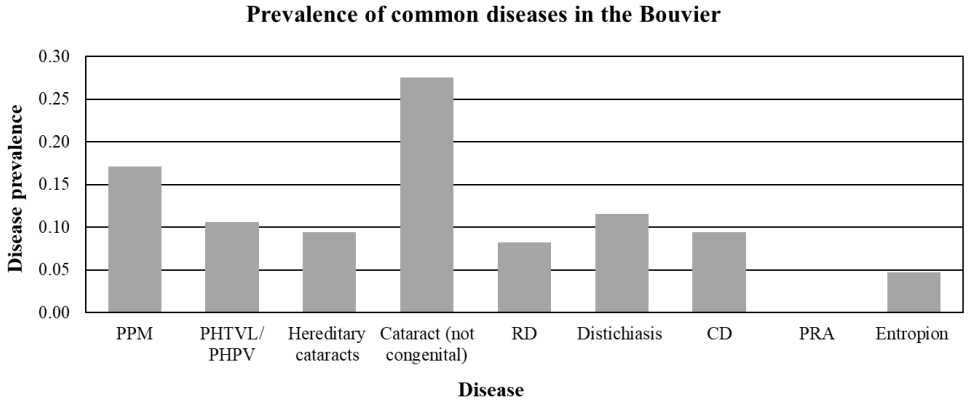

Figure 18*. Prevalence of diseases by total occurrences in the Bouvier population between 2010 and 2020.*

Within the simulation, inbreeding levels were monitored including and excluding diseases included however, for either, there was no significant change. A similar result was found when altering the selection rate of the different diseases (see **Figure 19** below).

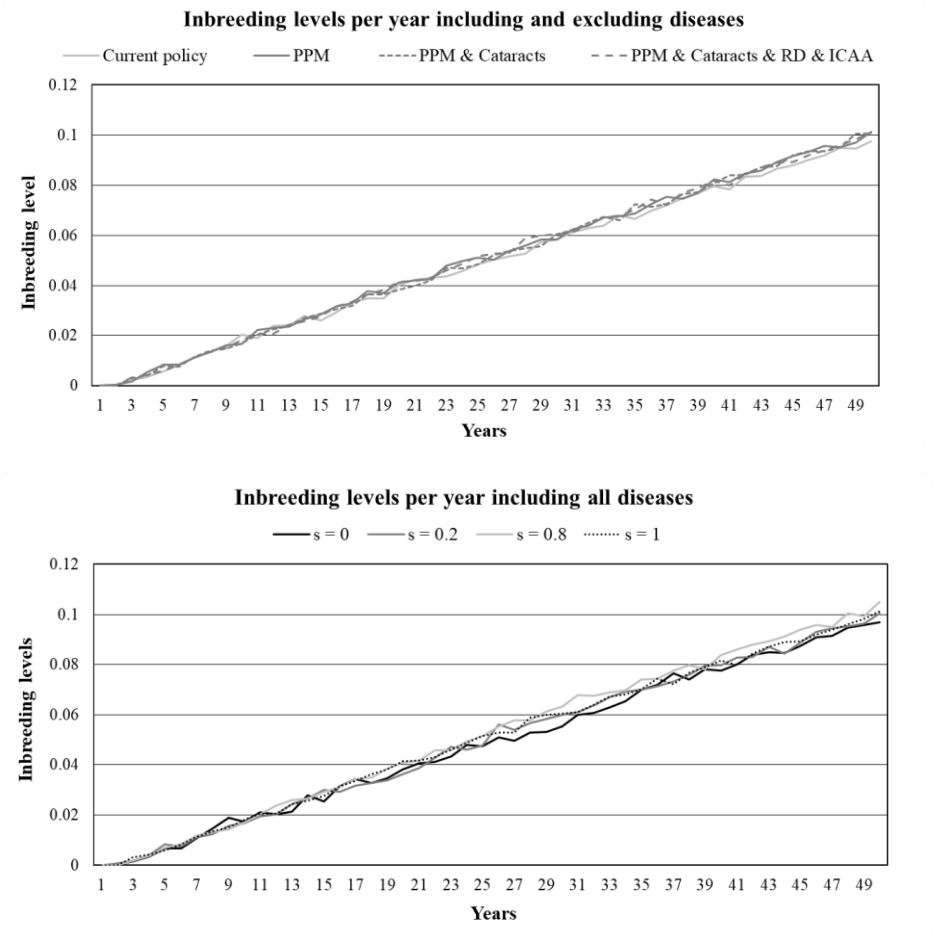

Figure 19. *Inbreeding levels per year including and excluding diseases (top) and with differing levels of selection (bottom).*

The presence of breeding restrictions had no effect on the allele or genotype frequency of a disease (in this case PPM). When selection rate was highest (s = 1), the allele and genotype frequency of the disease with or without breeding restrictions decreased simultaneously (see **Figure 20**).

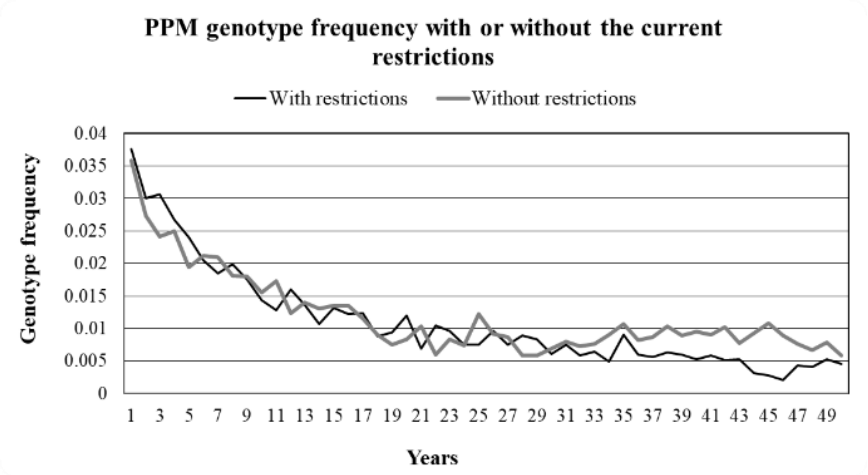

Figure 20. *PPM Genotype frequency with or without the current breeding restrictions.*

Increasing the selection rates, decreased the allele and genotype frequency as the affected individuals were removed from the simulated breeding population (see **Figure 21** and **Figure 22**). When there is no selection, the allele frequency dances around the starting frequency, as the allele is not being actively removed from the population. When selection is at its maximum (s = 1), the allele frequency decreases rapidly until an equilibrium is reached at an allele frequency of 0.05. An explanation for this is that the diseases modelled in Pointer are monogenic recessive and thus, recessive alleles may not always be expressed but may linger in the population. Only homozygous individuals with the recessive allele are removed from the population.

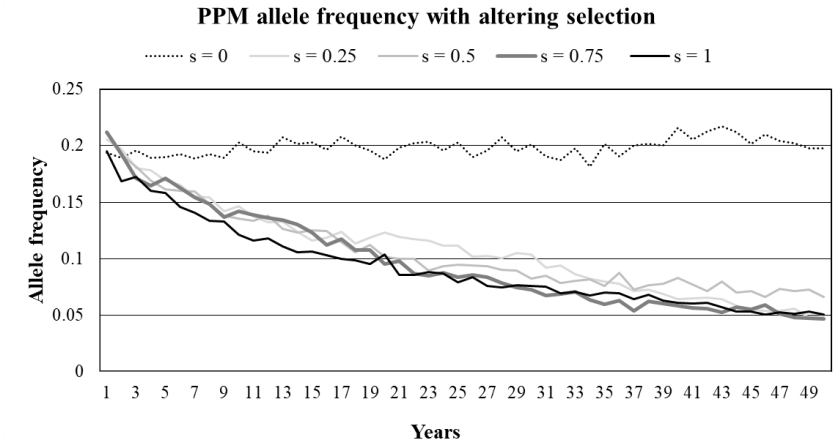

***Figure 21**. The effect of altering selection (s) on the allele frequency of PPM.*

When there is no selection, the genotype frequency increases slightly from the starting frequency (from 0.04 to 0.06). When selection is at its maximum (s = 1), the genotype frequency decreases rapidly until an equilibrium is reached at a frequency of 0.005. This pattern is similar to the above figure and thus is attributed to the monogenic recessive nature of the diseases modelled in Pointer, where recessive alleles may persist in the population due to their lack of expression in heterozygous carriers.

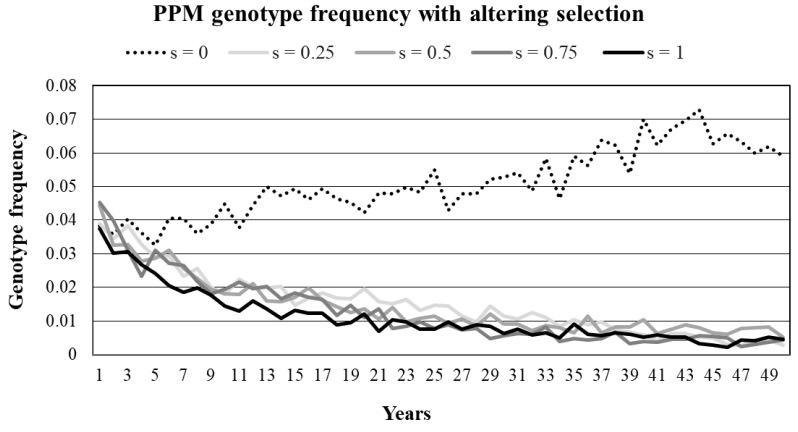

***Figure 22**. The effect of altering selection (s) on the allele frequency of PPM.*

When multiple diseases were included into the program, the effect of selection rate on the allele frequency changed. Nevertheless, the decrease in allele frequency was still proportional to the increase in selection rate i.e., higher selection rate led to a faster decrease in allele frequency. The four investigated diseases included in the simulation were run together and their allele frequencies are shown below (**Figure 23**).

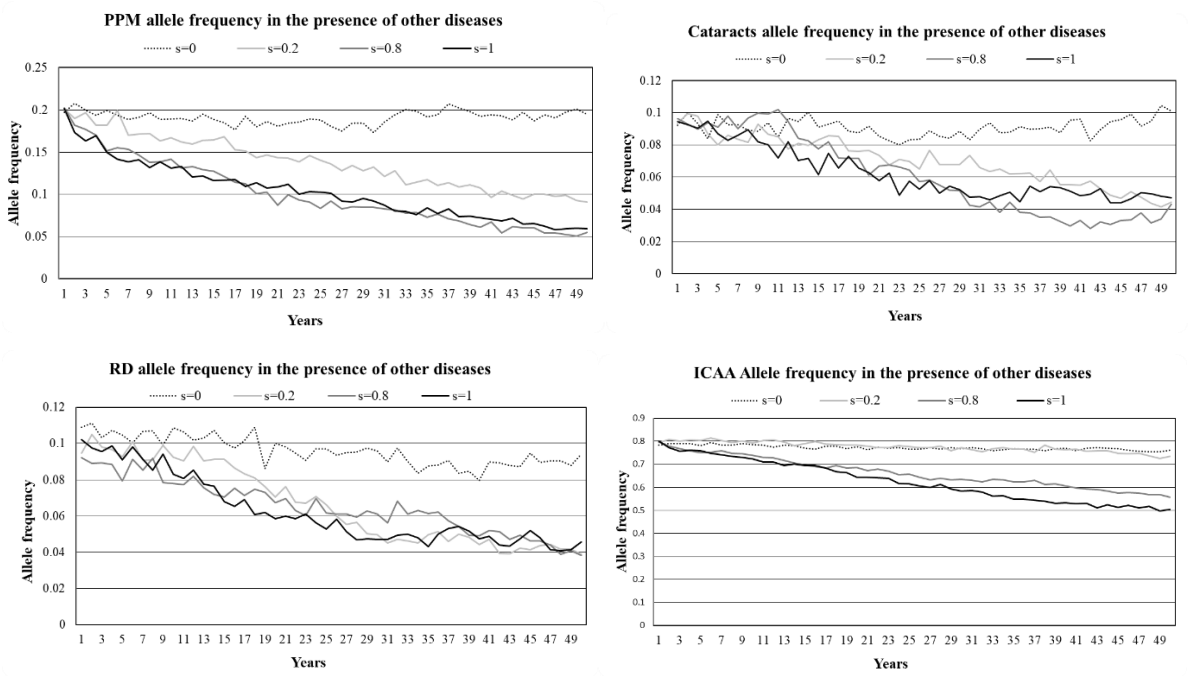

Figure 23. *Allele frequencies of the four included diseases (PPM: top left, Cataracts: top right, RD: bottom left and ICAA: bottom right) with altering selection (s).*

Similarly, the effect of selection rate on genotype frequency varied when multiple diseases were simulated. The same trend is seen that a decrease in genotype frequency was proportional to the increase in selection rate (i.e., higher selection rate led to a faster decrease in genotype frequency). The four investigated diseases included in the simulation were run together and their genotype frequencies are shown below (**Figure 24**).

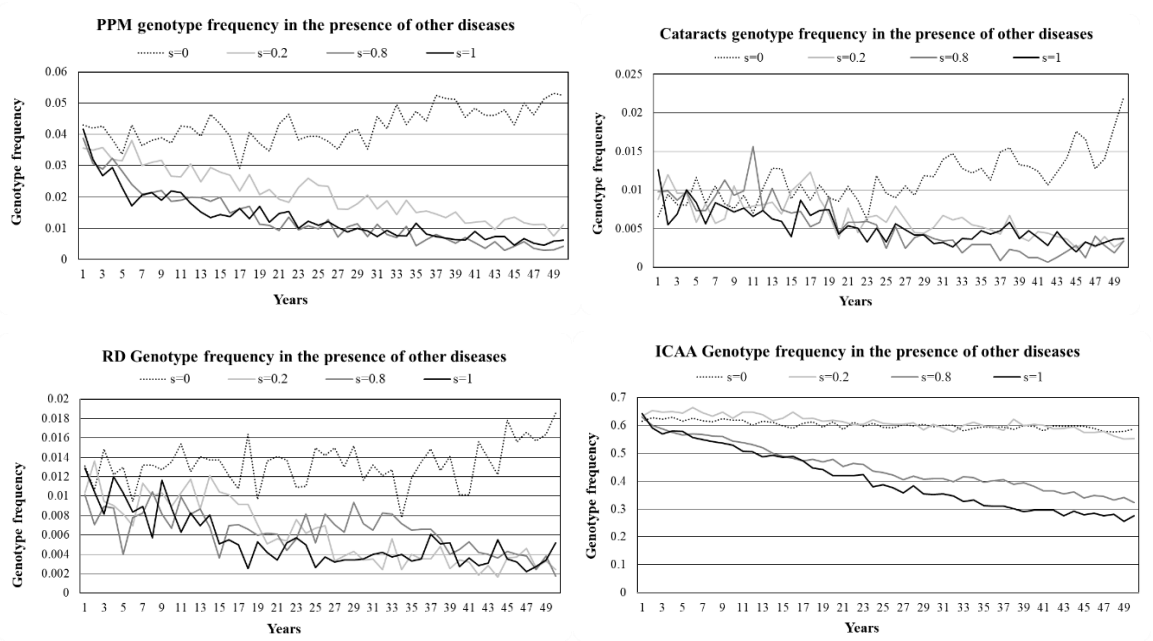

Figure 24. *Genotype frequencies of the four included diseases (PPM: top left, Cataracts: top right, RD: bottom left and ICAA: bottom right) with altering selection (s).*

Discussion

The Bouvier breed has experienced a pronounced demographic decline since its peak in the late 1980s and early 1990s. Although the average litter size among females remains relatively constant at approximately six offspring, the frequency of litters has exhibited a consistent decline. This decrease can be attributed to a corresponding fade in the breed's popularity (**Figure 5**). After an examination of the number of progeny per parent, trends suggest the use of popular sires (**Figures 7** and **9**) which increases the chances of mating close relatives thereby increasing the probability of inbreeding. Additionally, by breeding certain individuals repeatedly one creates a genetic bottleneck where a disproportionate amount of genetic material is passed to the following generations, increasing the breeds susceptibility to certain diseases.

The introduction of computerized dog records has prompted an increase in pedigree completeness within most dog breeds, the Bouvier breed included. Nonetheless, a notable decline in pedigree completeness is evident circa 1999. This reduction is explained by the importation of more Bouviers from abroad – starting approximately in the year 2005, whose comprehensive ancestral records beyond grand-parents have not been incorporated into the existing pedigree database.

The level of inbreeding within the Bouvier breed has undergone a steep incline from an initial level of 0 to 0.09 over the course of the breed's evolutionary history (**Figure 11**). However, stabilization emerges around 2007. This inflection point is most likely attributed to heightened breeder awareness concerning the consequences associated with inbreeding. However, this could also be due to pedigree incompleteness. This trend is not unique to the Bouvier breed but is observed across various dog breeds, including those with reliable pedigree records (Lewis et al., 2015).

This rate of inbreeding serves as an indicator of the potential risks associated with genetic similarity, encompassing both advantageous and deleterious outcomes. In accordance with established guidelines, such as those advocated by the Food and Agriculture Organization (FAO), it is recommended that the rate of inbreeding should ideally be maintained below 1%, with a preferable target threshold of less than 0.5%. When no breeding restrictions are enforced, the inbreeding rate, as predicted by the Pointer Software, escalates significantly to 1.34%. This highlights the need to – at least – maintain the current breeding restrictions in reducing inbreeding within the Bouvier population. When including the existing breeding restrictions, the inbreeding rate stands at 0.83%. The calculated inbreeding rate for the period 2011 – 2020 stood at 0.45% (**Figure 13**), the time period on which the pointer analysis was calculated on. It is highly probable that 0.45% is an underestimation due to the high levels of pedigree incompleteness. When selecting against disease prevalence, an increase in the inbreeding rate is possible, as the strategy may involve favouring matings among closely related individuals, given that

only certain familial lines exhibit resistance to the disease in question, and less individuals can be used for breeding. It is important to note that the inbreeding rates projected by Pointer at the start may significantly underestimate real-world inbreeding levels, as the simulation operates under the assumption that all individuals within the starting breeding population are unrelated, which is not the case in actuality.

Multiple simulation iterations were run to explore the effects of various alterations to the existent breeding restrictions. Among the array of modifications tested, several demonstrated a marked efficacy in lessening inbreeding rates. These included the elimination of top sires, the limiting of males to sire only one litter per individual per lifetime, and the reduction of the maximum allowable litters per sire per lifetime. However, the most beneficial modification, yielding the most reduction in inbreeding rates, was by breeding individuals with low mean kinship and with their lowest kinship counterpart. This composite approach had varying affects in the short and long term in that the inbreeding rate decreased substantially to 0.04% for the first 10 years and decreased the inbreeding rate to 0.52% for the following 40 years, as visually depicted in **Figure 17**.

After conducting a comprehensive analysis of disease prevalence within the population, it was evident that cataracts emerged as the most prevalent condition. ICAA, known as a predisposing factor for glaucoma, exhibited notably high prevalence values in 299 tests, with 184 occurrences categorized as 'Not free', resulting in an incidence rate of 78%. This presented a significant challenge for breeding associations, who aimed to reduce the number of infected individuals in the breeding population. The dilemma lays in the exceptionally high prevalence values, which implies that a substantial portion of the population might need to be removed. Consequently, they posed a critical question: How many individuals with the disease could be safely removed without triggering an increase in the inbreeding rate?

To address this question, a series of computational simulations were conducted using the Pointer software. These simulations aimed to model the removal of diseased individuals from the breeding population. The figure below demonstrates the stepwise reductions in the breeding population, ranging from 100% (representing the original population size) to 75%, 50%, 25%, 15%, and finally, 10% of the original population (**Figure 25**).

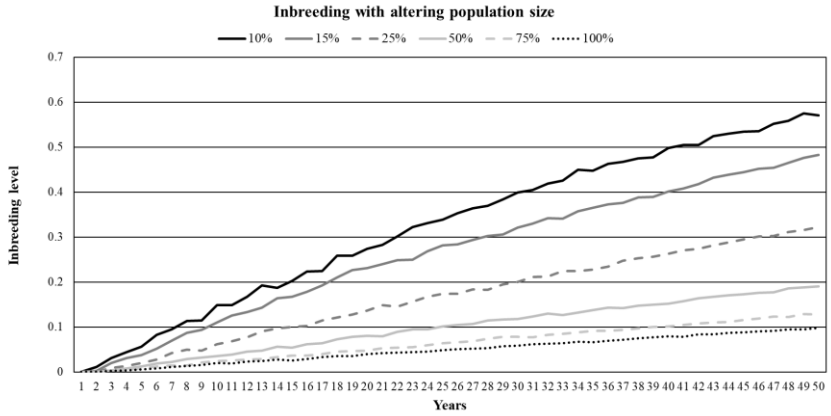

***Figure 25**. The effects of decreasing the population on the inbreeding rate.*

The inbreeding rate of the original population size computed by Pointer stood at 0.83%. However, this rate exhibited a significant increase, reaching 1.1%, when the breeding population was reduced to 75% of the original population. Given the advisory recommendations of the FAO, it is strongly recommended that breeding organizations refrain from reducing the existing breeding population. The sensible course of action would involve the preservation of a high breeding population size, coupled with a cautious selection of individuals for breeding purposes, focusing on untapped genetic resources that have not yet been employed in the breeding program, e.g. those animals with a low mean kinship.

By incorporating the combination of mean kinship and minimisation of parent kinship as a pivotal component within the breeding scheme, which achieves a degree of flexibility concerning the permissible levels of inbreeding. By using this approach, it is anticipated that the rate of inbreeding can be mitigated to allow selection (removal of individuals from the breeding population); thereby serving as a countermeasure against the potential increase in inbreeding, when coupled with strict breeding restrictions.

Once this is accomplished, the next question relates to the selection of the particular disease to be targeted for removal from the population. This choice is influenced by the preferences of the breeding associations and hinges on factors such as disease severity, prevalence, age of onset, and genetic background. For clarification, I present an illustrative and crude priority list below (**Table 11**). The order of priority for addressing specific diseases within the Bouvier breed is based on an evaluation of their severity and prevalence. In this prioritization, I have considered both the potential impact on the health and well-being of the breed's individuals and the frequency with which these diseases occur within the population. At the forefront of this priority list are conditions with both a high prevalence and severe consequences for affected dogs. It is important to keep in mind that this list is intended solely as a reference and should be viewed as an adaptable framework, open to amendments based on the collective negotiations and insights of the association members.

Multiple methods of scoring disease priority exist that breeding associations can look to when developing priority of diseases. The Generic Illness Severity Index for Dogs (GISID) is a sophisticated scoring system designed to assess the duration and severity of inherited canine diseases using information extracted from veterinary and scientific literature (Collins, Asher et al. 2011). Each condition is evaluated, taking into account factors such as diagnosis, treatment, complications, and behavioural implications. Conditions with the highest severity scores (as per GISID) and the highest prevalence are of utmost concern from an animal welfare perspective. With this technique the association can prioritize the assessment and mitigation of disorders that pose the greatest risks to the breed's health and wellbeing. However, it is important to note that ongoing research and data collection efforts are necessary to refine this approach and make more accurate assessments of disease severity and prevalence, ensuring the continued improvement of Bouvier des Flandres breed health.

Another technique is the Breed Disorder Welfare Impact Scores (BDWIS) that are an adaptation of the GISID. The advantage of using BDWIS is that the duration of the disorder as a proportion of the dogs life is taken into account where: BDWIS = prevalence x severity x proportion of life afflicted. Collins, Asher et al. (2011), elaborate further on how the exact values are calculated where prevalence is a measure of the number of affected individuals out of the total population size and severity is a measure of the current GISID score out of the maximum GISID score. The proportion of life afflicted is measured by the mean breed lifespan – mean age of onset out of the mean breed lifespan. It is crucial to investigate these criteria for all diseases thoroughly, as there can be an inherent bias in the risk assessment based on the availability of information.

Lastly, Welfare Adjusted Life Years (WALY) is a recent concept derived from the human Disability-Adjusted Life Year framework (Summers, O'Neill et al. 2019). This metric places significant importance on the duration of a disorder as a fundamental aspect of evaluating the overall welfare impact. It takes into consideration both the time lived with impaired welfare resulting from a specific cause and the years of life lost due to premature death caused by that same factor. In essence, WALY quantifies the combined effects of compromised well-being and life expectancy reduction associated with a particular condition or cause, offering a comprehensive measure of its impact on overall welfare. It is recommended that the breeding associations explore these methods with veterinarians and animal breeding scientists to make informed decisions regarding disease management in order to prioritize diseases that impact the breed's health and welfare.

***Table 11**. Priority list of diseases contingent upon considerations of severity, prevalence, and age of onset.*

Disease	Priority	Remarks
Hereditary Cataracts	Highest	Hereditary cataracts has a relatively high prevalence (27%) and can lead to visual impairment or blindness within a few weeks to a few months since birth. Cataracts are a significant concern due to their potential severity and impact on vision.
Primary Glaucoma	High	Primary glaucoma, measured as ICAA, presents an extremely high prevalence (80%)* and has potential to cause irreversible vision loss, especially if left untreated. Although, symptoms only present after 7 years.
Distichiasis	Moderate	Distichiasis, although less prevalent (11%), can cause corneal irritation and discomfort. While it may not lead to complete blindness, it still affects the dog's quality of life and warrants attention.
PPM	Moderate	PPM, with a prevalence of 17%, can pose a threat to vision, especially in severe cases. While not as prevalent as some other conditions, its potential impact on vision places it in the moderate priority category.
Corneal Dystrophy	Low	Corneal dystrophy, with a prevalence of 9%, can cause ocular discomfort and reduced light refraction but is not as severe as conditions leading to blindness. It falls into the low priority category due to its relatively lower prevalence and less severe consequences.
RD	Low	RD has a prevalence of 8% and can lead to retinal detachment and other issues, but it is less common and less severe than some other conditions on the list. Therefore, it is given a lower priority.
Entropion	Low	Entropion, with a prevalence of 4%, primarily causes ocular irritation but is not a condition that leads to vision loss. While it should be addressed, it is considered lower priority due to its milder nature.
PHTVL/PHPV	Low	PHTVL/PHPV has a prevalence of 10%, and its age of onset is undetermined. While it should be monitored, its lower prevalence and lack of clear age of onset place it in the low-priority category.
PRA	Lowest	PRA is listed as having a prevalence of 0% in Bouviers, which makes it the lowest priority. However, it should still be monitored, but its absence in the breed reduces its priority.

*Prevalence values out of 299 tests with 184 "Not free" occurrences, reaching an incidence rate of 80%.

Another means of selection in which the dissemination of polygenic traits (and possibly possessing harmful alleles) can be mitigated is by using EBVs. One of the primary advantages of using EBVs is their potential to enhance breeding programs by providing a more accurate prediction of an individual's genetic merit. EBVs also allow breeders to reduce the risk of undesirable genetic outcomes by selecting dogs with superior genetic potential. However, difficulties arise due to the limited knowledge of the genetic background and the absence of established heritability and genetic correlation information specific to the Bouvier breed. Determining heritabilities and genetic correlations is essential to calculate accurate EBVs. These parameters are crucial for assessing the reliability and usefulness of EBVs, ensuring that breeding decisions are based on robust genetic data rather than incomplete or inaccurate information. Further measures can be taken to refine selection criteria; however, it is important to highlight that the following suggestions will result in a long and complex project, requiring many years of research:

1. Firstly, investing in genetic research is paramount; identifying the genetic mechanisms of the aforementioned Bouvier health issues (eye diseases in particular) is crucial. If possible, make a comprehensive inventory of diseases that affect the Bouvier including their prevalence and severity as well as genetic mechanisms, mode of inheritance, heritabilities, genetic correlations for polygenic traits, and age of onset that are scientifically sound. Collaborating with geneticists and veterinarians to unravel the precise genetic mechanisms of prevalent diseases is vital.
2. Once known, establish a standardized health screening program that includes genetic testing for hereditary diseases. Breeding animals should undergo rigorous testing for the genetic markers, prioritizing those free from disease – or at minimal risk – for breeding.
3. Once a comprehensive disease inventory is established, and standardised tests are readily available, reassess the priorities of disease based on new scientifically sound information.
4. Identifying dogs with low mean kinships and a healthy track record in order to diversify the gene pool while adhering to breed standards.
5. Advanced statistical methods should be employed to calculate EBVs, considering health, temperament, and conformity to breed standards.
6. A comprehensive health index could be developed in which dogs are ranked in terms of health where aforementioned health screening results are recorded and publicized for each individual. This arbitrary value should be considered when selecting new mates for breeding such that you prioritize pairs with low mean kinships and high health index scores. These could work similarly to EBVs but focus on dog individual health.
7. Mating with the new programme that considers mean kinships, health index scores and their estimated breeding values.

Lastly, collaboration and education are crucial; breeders should be informed about genetic health and modern tools, while data sharing and transparency should be encouraged. The report written by the ACT proved that breeding values are not fully understood by the breeding association members, and requires clear explanations to overcome this knowledge gap. Long-term planning for reducing hereditary diseases and ethical breeding practices is central to the organization's mission. In pursuit of genetically healthier Bouvier dogs, these strategies can be combined to promote both breed integrity and canine well-being.

Conclusion

The Bouvier breed is facing significant challenges related to declining population size, inbreeding, and the prevalence of hereditary diseases. Addressing these issues will require a multi-faceted approach that combines genetic research, responsible breeding practices, health monitoring, and collaboration within the breeding community. Maintaining a balanced approach to the selection process concerning health issues and genetic diseases is of paramount importance. Excessive stringency in these aspects can engender adverse consequences, chiefly an elevated risk of inbreeding and long-term health detriments. By taking proactive steps and prioritizing the health and well-being of the breed without compromising inbreeding rates, the Bouvier can move towards a more sustainable and genetically diverse future. It is my hope that these recommendations will guide breeders and associations in their efforts to ensure the long-term health and vitality of the Bouvier des Flandres.

Closing remarks

I extend my heartfelt appreciation to my supervisor, Jack Windig, whose guidance, expertise, and support have been instrumental in shaping this study. Your mentorship has been invaluable, and I am deeply grateful for the knowledge and insight you have shared with me throughout this process.

I would also like to acknowledge the significant contributions and collaboration of the breeding associations, Boe4 and the Nederlandse Bouvier Club. Your dedication to the betterment of the Bouvier des Flandres breed and your willingness to provide access to valuable data and expertise have been integral to the success of this research.

In closing, I am motivated by the belief that the insights and guidelines presented in this paper will serve as a valuable resource for breeders, enthusiasts, and organizations dedicated to the welfare and preservation of the Bouvier des Flandres breed. I hope that our collective efforts will contribute to the continued health, vitality, and genetic diversity of this remarkable breed.

Thank you all for your support, and I look forward to the continued collaboration and progress in the field of canine genetics and breeding.

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Appendix

Table 2. *Summary of inherited eye diseases of the Bouvier and their breeding rules.*

Disease	Age of onset	Prevalence	Possible mode of Inheritance	Breeding rules
HD	Varying		Polygenic	● HD-C: only with HD-A ● HD-D/E: breeding prohibited
ED	Varying		Polygenic	● Grade 1: only with free individual ● Grade 2/3: breeding prohibited
PPM	>6 weeks	17.1305%	Autosomal recessive/ autosomal dominant/ polygenic	● Iris-iris: only with free individual ● Iris-cornea/ Iris-lens: breeding prohibited
PHTVL/ PHPV	Undetermined	10.5762%	Autosomal incomplete dominant	● Grade I: only with free individual ● Grade II-VI: breeding prohibited
Hereditary cataracts	Few weeks to months	Congenital: 9.4597% Non-congenital: 27.5795%	Autosomal recessive/ dominant	● Cortical, nucleus or post. pol.: breeding prohibited ● Low priority: only with free individuals
RD	2 – 3 months	8.1923%	Autosomal recessive/ X-linked	● Focal/multifocal: only with free individual ● Geographic: only with free individual ● Total: breeding prohibited (incl. parents & offspring)
Distichiasis	0.3 – 8.9 years	11.5857%	Autosomal dominant	● Only with free individuals ● Severe: breeding prohibited
Corneal dystrophy	Varying	9.4597%	Sex linked recessive	● Breeding prohibited
PRA	Early: 2 – 6 weeks Late: 2 – 5 years	0%	Autosomal recessive	● Breeding prohibited (incl. parents & offspring)
Entropion	4 – 7 months	4.7298%	Polygenic	● Only with free individuals
Microphthalmia	Undetermined	-	Autosomal recessive	● Breeding prohibited
Primary Glaucoma	± 7 years	Marked by ICAA ‘not free”: 78.4465%*	Polygenic	● Mild/ moderate ICAA: only with free individuals ● Severe ICAA: prohibited from breeding

*Prevalence values out of 299 tests with 184 “Not free” occurrences, reaching an incidence rate of 80%.

Table 5. *Input values baseline simulation. Values were based on the years 2014 - 2019.*

Parameter		**Baseline input values**		**Alternatives**					
Number of simulated years		50							
Number of simulated runs		25							
Number of breeding males		30		5%	10%	25%	50%	75%	100%
Number of breeding females		140		5%	10%	25%	50%	75%	100%
Number of litters per year		70							
Number of champion sires (offspring %)		4 (31%)		0 (0%)	4 (15%)	8 (60%)			
Fraction of parents per age (y)		Sires	Dams						
	1	6	4						
	2	16	20						
	3	18	22						
	4	15	20						
	5	13	16						
	6	13	10						
	7	8	7						
	8	6	1						
	9	4	0						
	10	1	0						
Fraction of litters per litter size									
	1	21							
	2	8							
	3	6							
	4	7							
	5	8							
	6	10							
	7	13							
	8	10							
	9	8							
	10	5							
	11	3							
	12	1							
Age dam at first litter (months)[a]		24							
Max. number litters per dam/ life		No res		1	3	5			
Max. number of litters per sire/ year		No res		1	5	10			
Max. number of litters per sire/ life		No res		1	5	10	15	20	25
Max. number of sons per sire		No res							
Max. kinship allowed between parents		No res							
Max. inbreeding allowed per animal		No res							
Constrain mean kinship		Off		On					
Minimise kinship between parents		Off		On					

[a]As given by the current breeding policy of Boe4.
No res: No restriction.