Inbreeding and outcrossing in the Wetterhoun

'Binne'

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BSc thesis Animal Breeding and Genetics

1. Summary

This research analyses the Wetterhoun population structure and future possibilities regarding the management of inbreeding and relatedness. Data was provided by the NVSW (Nederlandse Vereniging voor Stabij- en Wetterhounen: Dutch Association for Staby dogs and Wetterhouns). The aim of this research was to inform the NVSW about the current population status and provide recommendations to keep the inbreeding at an acceptable rate in the future. The research was conducted by answering three research questions about the current population structure, inbreeding and relatedness, analysing the outcrosses that the NVSW executed to reduce inbreeding and simulating different genetic management methods.

The population structure was analysed using the Retriever software. It showed that in the last 20 years there were on average around 100 pups born per year and around 17 litters per year. The average litter size was 6 pups. Around 19% of the males and 25% of the females in the pedigree were used in breeding. The share of the top ten sires has been around 85% and data was available for five generations or more for most of the dogs in the pedigree.

The current rate of inbreeding is -1.51%. This is mostly explained by five outcrosses that were done in the period from 2014 until 2021. Outcrossing provided replenishment of genetic diversity and decreased the rate of inbreeding so well that it was negative, which means that the average level of inbreeding has been decreasing in the population. This is good to sustain a healthy population. Before the outcrosses, the rate of inbreeding was 1.32%, which exceed the recommended maximum of 1% by the FAO (Food and Agriculture Organization).

Simulations were executed with the Pointer software to simulate different genetic management strategies. Excluding mating between animals that share a common ancestor in different numbers of past generations provided a decrease in rate of inbreeding, but only on the short-term. Also, minimizing the kinship between father and mother and constraining the mean kinship with the remainder of the breed showed a decrease in the rate of inbreeding. But the rate of inbreeding was below 1% only on the short-term and exceeded this 1% on the long-term. Therefore, outcrosses are needed at least once every six years, which yields a simulated rate of inbreeding of 0.96%. To keep the inbreeding rate below 0.5%, this study recommends that at least one outcross needs to be done every year, in which case the rate of inbreeding is simulated to be 0.17%.

2. Samenvatting

In dit onderzoek is de Wetterhoun populatie structuur en toekomstmogelijkheden met betrekking tot beheer van inteelt en verwantschap geanalyseerd. De NVSW (de Nederlandse Vereniging voor Stabij- en Wetterhounen) leverde de data voor dit onderzoek. Aan de hand van drie onderzoeksvragen, biedt dit onderzoek de NVSW inzage in de huidige status van de populatie en advies te geven over toekomst mogelijkheden om het inteeltpercentage acceptabel te houden voor de populatie. De onderzoeksvragen richtten zich op de huidige populatie structuur, inteelt en verwantschap, het analyseren van het inkruisingen van andere rassen die gedaan zijn en het simuleren van verschillende methodes om inteelt te verminderen.

De populatie structuur is geanalyseerd door de Retriever software. Dit liet zien dat er in de afgelopen 20 jaar gemiddeld 100 puppy's per jaar worden geboren komend uit ongeveer 17 nesten per jaar met een gemiddelde nestgrootte van zes puppy's. Ongeveer 19% van de reuen en 25% van de teven waren gebruikt in de fok. Het aandeel van de top tien reuen was ongeveer 85% en voor een groot deel van de populatie is data beschikbaar voor vijf of meer generaties.

Het huidige inteeltpercentage is -1.51%. Dit komt vooral door de vijf inkruisingen met andere rassen die gedaan zijn door de NVSW tussen 2014 en 2021. Deze inkruisingen hebben voor nieuw genetisch materiaal gezorgd. Daarmee is het inteeltpercentage gedaald naar een negatieve waarde. Dit betekent dat de gemiddelde inteelt tussen gefokte dieren vermindert. Dit is een goede methode om de populatie gezond te houden. Voordat de inkruisingen met andere rassen waren gedaan, was het inteeltpercentage 1.32%. Dit overschrijdt het maximale toegestane inteeltpercentage opgesteld door de FAO (Food and Agriculture Organization; Nederlands: Voedsel- en Landbouworganisatie) van 1%.

Simulaties zijn gedaan met behulp van Pointer software dat verschillende methodes om inteelt te verlagen simuleerden. Het uitsluiten van paringen tussen dieren die een gemeenschappelijke voorouder delen over verschillende aantallen generaties resulteerde in een afname van het inteeltpercentage, maar alleen op korte termijn. Ook het minimaliseren van de verwantschap tussen de ouderdieren en het beperken van de gemiddelde verwantschap met het overgebleven deel van de populatie bood een vermindering van het inteelt percentage. Maar deze methodes bieden geen oplossing op de lange termijn. Het inteeltpercentage blijft boven de 1%. Daarom is het inkruisen van een ander ras nodig. Op zijn minst elke zes jaar om op een inteeltpercentage van 0.96% uit te komen. Om het inteeltpercentage onder de 0.5% te houden is het inkruisen van een ander ras elk jaar nodig, wat een inteeltpercentage van 0.17% oplevert.

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3. Introduction

Dogs are very popular in Europe (HealthforAnimals, 2022). They were domesticated around 15,000 years ago from wolves. During the Victorian era, dogs breeds were developed and bred for preferred characteristics (Lewis & Windig, 2017). The Wetterhoun was bred to be a robust and strong dog to hunt otters and polecats. The breed is part of the waterdogs and has a water repelling astrakhan coat. It is a Dutch breed originating from the lake areas of the province of Friesland (*Nederlandse Vereniging Voor Stabij- En Wetterhounen*, n.d.). Pedigree breeding started a history of inbreeding in the Wetterhoun (Schipper, 2019).

Breeds, like the Wetterhoun, are closed. This means that dogs of other breeds are not allowed in the studbook. The breeders can only mate with animals that are in the studbook. This ensures the breed's characteristics, but the breeding population is therefore limited. This eventually causes related animals to be matched for breeding. If the parents are related, the offspring is inbred. How inbred an animal is, is determined by the level of inbreeding. The level of inbreeding is how closely related the relatives are across the sire and dam lines. Inbreeding can happen in different ways. It can happen because the breeder selects animals that display preferred traits. If these traits are heritable, it is likely those animals are related, because they both share the same preferred trait. An example for this is when a popular sire, for example a champion, and its relatives are used a lot to produce offspring in the population of a breed (Lewis & Windig, 2017). Breeders like to mate their dam with this popular sire because it passes its genes along to the offspring. This causes less genetic variation in the population and all the offspring of this sire to be half siblings. If this offspring is selected for breeding, the genetic variation among them is small and the relatedness among them is high and therefore the inbreeding will be high.

Another cause for low genetic variation can be a small population size. In that case the breeders have no choice but to breed with related animals, which will increase the inbreeding rate in each subsequent generation. The rate of inbreeding is the increase in average inbreeding level in a population from one generation to the next. The Food and Agriculture Organization (FAO) states that the inbreeding rate should be monitored and kept below a rate of 0.5 to 1% (FAO, 2013). High rates of inbreeding can cause autosomal recessive disorders to be expressed in a population (Summers et al., 2010). If the level of inbreeding increases, there will be genetic drift. Genetic drift causes allele frequencies to change in random direction while selection for specific traits causes change in a specific direction. Especially small populations are more vulnerable for big changes in allele frequencies (Honnay, 2013). An important parameter to monitor this is the rate of inbreeding as it determines the average level of inbreeding in a population from one generation to the next. In the Wetterhoun, inbreeding has been a problem in the past. Data was recorded from 1944 onwards. The rate of inbreeding per generation from 1954 to 2011 exceeded 1%. From 2012 to 2018 the inbreeding rate per generation decreased to -0.66%. This decrease was mostly caused by the outliers caused by the outcross programme (Schipper, 2019).

One of the methods to decrease inbreeding is outcrossing. Outcrossing is mating an animal from an, often inbred, breed with an animal of a donor breed (Windig & Doekes, 2018). This donor breed is often like the inbred breed to ensure that the characteristics of the offspring are as close as possible to the original breed. If the parents (P) are not related, the offspring (F1) has an inbreeding level of zero. After the first cross (F1) breeders can choose to either breed the F1 with the inbred breed or breed the F1 with another donor breed. When F1 is crossed with the inbred breed, the offspring (F2) is called the backcross. The backcross offspring may show more of the original breed's characteristics, but its relatedness with the individuals of the population is therefore increased. Outcrossing is an effective and relatively fast method to decrease the level of inbreeding, but it is only temporary and there is a risk of losing breed characteristics (Lewis & Windig, 2017). Outcrossing can be done with different amounts of backcrosses, possible repeats of outcrosses and the size of outcross can vary. Different breeds and populations should have different outcross methods, so that they are appropriate for the functionality and aim of the breed. The NVSW (Nederlandse Vereniging voor Stabij- en Wetterhounen; English: Dutch association of the Staby- and Wetterhoun) used specifically breeds that does not look like the Wetterhoun. The NVSW used outcrossing to decrease the inbreeding in the Wetterhoun, but this has not been evaluated yet.

To gain information about the population structure, outcrosses that were done and which outcross level(s) have which results, the research questions in this research are:

- What are the current population structure, relatedness and inbreeding in the Wetterhoun population?
- How many outcrosses have been done and how successful were they regarding the litter size of the outcross offspring? And with which breeds were these outcrosses done?
- What will the future expectations be with different breeding policies and levels of outcrossing?

4. Material & methods

4.1. Pedigree analysis

To answer the research questions, data was provided by the NVSW.

The first data file contained information on the unique ID, sex, name, breed, sire, dam, and date of birth for each animal registered in the pedigree. In total there were 2788 males and 2747 females in the database, 5520 dogs were classified as 'WETTERHOUN' and 15 dogs as 'WH LOOK ALIKE', which represent dogs that confirm to the breed standard of the Wetterhoun, but do not have a pedigree. For 19 dogs the father and for 18 dogs the mother was unknown. The data file contained information from January 1942 until December 2022. There were eight dogs without a date of birth. Before 1951, less than 12 pups were born per year. Therefore, the years 1943 until 1951 were not analysed.

For assessing the status of the population of the Wetterhoun, Retriever software was used (Windig & Hulsegge, 2021). Retriever used the following information: ID, IDFather, IDMother, Sex, Date of birth. It extracts information per year from the pedigree data on the number of pups born, number of litters and litter size, number of sire and dams, litters per sire, pups per sire, sires with the most offspring, generation interval, completeness of the pedigree and inbreeding and kinship. The results were obtained from the Retriever text file and copied in Microsoft Excel to create figures.

To calculate the inbreeding rate, equation 1 can be used. Where ΔF is the rate of inbreeding per year, F_t is the level of inbreeding in year t and F_{t-1} is the level of inbreeding in year in the previous year.

$$\Delta F = \frac{F_t - F_{t-1}}{1 - F_t} \quad (1)$$

To calculate the rate of inbreeding over a longer period of time, equation 2 can be used. Equation 1 can be rewritten as equation 2, where ΔF is the rate of inbreeding per year, F_{t-x} is the inbreeding coefficient of x years ago from t and L is the generation interval.

$$\Delta F = 1 - \left(\frac{1 - F_t}{1 - F_{t-x}}\right)^{\frac{L}{x}} \quad (2)$$

To obtain a linear function of F_t against t, equation 3 can be written with a natural log transformation, where F_x is the average inbreeding coefficient over period x and ΔF is the inbreeding rate per generation. This was multiplied by the average generation interval to obtain the inbreeding rate per year.

$$\ln(1 - F_x) \approx -\Delta F_x + \ln(1 - F_0) \quad (3)$$

To calculate the kinship rate, equation 4 was used which is derived in the same way as equation 3. The f is the kinship level including the self-kinship and Δf is the kinship rate per generation. This was multiplied by the average generation interval to obtain the kinship rate per year.

$$\ln(1 - f_x) \approx -\Delta f_x + \ln(1 - f_0) \quad (4)$$

4.2. Current outcross analysis

The second data file contained information about the breeding pairs with the sire and dam registration ID, paring date, litter date, relationship percentage and litter size with number of males and females. It was unknown how the relationship percentage was calculated, therefore it was not included in the analysis. The breeds that the NVSW used for the outcrosses were purposely chosen to not look like the Wetterhoun to decrease relatedness.

To investigate the success of the outcrosses that have been done by the NVSW, the number of outcrosses, the breeds that were used, the litter size and the male/female distribution were evaluated. To extract this information, the filter function of Microsoft excel was used. To test if the mean litter sizes of the Wetterhoun-Wetterhoun pairs and Outcross pairs significantly differ, a two-sample t-test (assuming equal variances) was executed. With H_0: mean litter size difference is zero ($\mu_d=0$) and H_a: litter size of Wetterhoun with Wetterhoun is lower than litter size of outcross litter ($\mu_d<0$). With a one-sided p-value and $\alpha=0.05$.

To test if the mean litter sizes of the Wetterhoun-Wetterhoun pairs and Wetterhoun-WH look alike pairs significantly differ, also a two-sample t-test (assuming equal variances) was executed. With H_0: mean litter size difference is zero ($\mu_d=0$) and H_a: Difference in litter size of Wetterhoun-Wetterhoun and Wetterhoun-WH look alike is not zero ($\mu_d\neq0$). With a two-sided p-value and $\alpha=0.05$.
The ratio of males and females will also be evaluated. With H_0: proportion of males is equal to the proportion of females and H_a: proportion of males and females are not equal. Also, with a two-sided p-value and $\alpha=0.05$.

4.3. Effect outcross in the future

To evaluate the possible outcomes of different set ups of outcrosses in the future, Pointer software was used for the simulation of genetic management (Windig & Hulsegge, 2021). For the input of Pointer, information about the number of breeding males and females, number of litters per year, the contribution of the top sires, age structure of the parents and litter size of the Retriever output of the pedigree analysis was used. Information about the breeding policy was provided on the website of the NVSW (*Werkwijze FAC Wetterhoun*, n.d.).

Two sets of simulations were done only using animals of the Wetterhoun population. One of the sets was done by excluding mating between animals with common ancestors over different numbers of past generations. The Pointer default input, which represents the simulation without a breeding policy, was used to study the effect of inbreeding of the different policies. And the other set of simulations was done with different breeding policies regarding the kinship. Here, the NVSW breeding policy without generations excluded was used to study the effect of inbreeding of the different policies.

Information from the summary of the population data of Retriever results was used to provide input for the simulations. This summary was based on data from 2016 until 2021. The share of the top sires was used of which 41% was of the top four sires. The litter size distribution from the summary was used but adjusted (table 1). The litter size of one pup was at 8% which was not realistic and was changed to 1%. Particularly in the beginning of the pedigree, only the animals that were used in breeding were registered. As a result, not all animals of a litter were registered in the studbook. This explains why the percentage of litters with one offspring was larger and was therefore changed. Litter sizes 11 and 12 were treated as 11 or more. This adjusted distribution (table 1) was used for all simulations. The Retriever summary also provided information about the distribution of the age of the parents (table 2). There was 1% of the sires that was 11 years of age, this was removed and 1% was added to nine years of age. The number of litters per year was determined by the average of 2016 until 2022 which was 17 litters per year. The amount of breeding males was set to 17. This was determined by the number of litters, where a sire can either mate with zero, one or two dams per year. This is randomly determined by the Pointer software. The sires can not mate with more than 2 dams, because this is not permitted by the NVSW. The NVSW determined that dams can only have a litter every two years. Therefore, the amount of breeding females is set on twice the number of litters, which is 34. The NVSW also provided that females should at least be 18 months when they have their first litter. All simulations were done for 100 years and with 25 repeats.

Table 1. Litter size distribution

Litter size	Fraction (%)	Adjusted fraction (%)
1	8	1
2	7	3
3	6	6
4	10	6
5	11	10
6	14	16
7	15	20
8	13	20
9	9	13
10	5	2
11	2	3
12	1	0

Table 2. Age distribution of sires and dams

Age	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**
Sires	1	17	30	15	15	11	9	1	1
Dams	1	19	20	34	15	8	3	0	0

4.3.1. Simulation without outcross

The inbreeding level and rate were calculated for two different methods, only using the animals within the Wetterhoun population. The rate of inbreeding with its minimum and maximum was studied for years 0 until 20 and 20 until 100. To study the effect on inbreeding on the long term, the rate of inbreeding was calculated for years 20 until 100. Table 3 represents the values for a situation without a breeding policy and the NVSW breeding policy and was used in both sets of simulations. The rates of inbreeding were calculated by equation 2 for the first 20 years and last 80 years of the simulation.

Table 3. Default input and NVSW breeding policy in Pointer

	Pointer default/ no breeding policy	NVSW breeding policy
Maximum number of females serviced per male/ year	1000	2
Maximum number of litters per female/ life	20	3
Maximum number of females serviced per male/ life	1000	6
Maximum number of sons selected as breeding male per father	1000	6
Maximum kinship allowed between parents	1.000	1.000
Maximum inbreeding allowed per animal	1.000	1.000

4.3.1.1. Exclude mating between animals with common ancestors over different numbers of past generations

One of the sets was executed by excluding mating between animals with common ancestors over different numbers of past generations. With this method, pairs are excluded if animals share a common ancestor within a number of past generations. The rate of inbreeding was calculated for without a breeding policy and with the NVSW breeding policy with different numbers of generations excluded. This was done for zero, one, two and three generations.

4.3.1.2. Different breeding policies regarding kinship

The other set of simulations was executed with different breeding policy regarding the kinship. The first was by constraining the mean kinship with the remainder of the breed. Here, the mean kinship for the population was calculated. After this, the average mean kinship with all other breeding animals in the breed for sires and dams was calculated and only animals that have a lower mean kinship than the average for its sex were included in the breeding population. The animals that exceeded the average mean kinship were excluded from the breeding population. The second was by minimising the kinship between father and mother. The female was matched to the least related male in the population. The third was a combination of both. These were executed with the NVSW breeding policy of table 3 and with zero generations excluded. The inbreeding rate and level of these breeding policies were compared to the inbreeding rate and level when only the NVSW breeding policy was applied with zero generations excluded.

4.3.2. Simulations with outcross

The outcrosses were simulated with the Pointer software. The number of years was 100 and the number of repeats 25. The information of the Retriever output was used. So, the top four champion sires produce 41% of the offspring, litter size is distributed as in table 1, age is distributed as in table 2 and at an age of 18 months a female is able to have her first litter. The NVSW breeding policy as in table 3 with no generations excluded was used in all simulations.

Two breeds have been simulated: the Wetterhoun and a donor breed. Total number of breeding males was 67 and breeding females was 134. The numbers of litters per year was 67. In the 'population structure' tab, it was specified that there were 17 Wetterhoun males, 50 donor males, 34 Wetterhoun females and 100 donor females. To simulate the level of inbreeding without outcross, the first simulation was executed without exchange between groups. To simulate the outcrosses, fertilization across groups in numbers was used. To simulate an inbred breed, there was no fertilization until year 51. The outcrosses that were simulated are: one every year, one every two years until one every seven years. From the results of the simulations, the level of inbreeding for the Wetterhoun and the donor breed and the generation intervals were analysed. The rate of inbreeding is calculated by equation 3 for years zero until 50 and 51 until 100.

5. Results

5.1. Pedigree analysis

5.1.1. Number of pups per year of birth

Before 1951, the number of pups per year was below 12 (figure 1). Between 1951 and 1973, the number of pups born per year increased from 12 up to a peak of 163. From 1973, it decreased from 163 to around 60 between 1985 and 1995 with a minimum of 37 pups per year. From 1995, the number of pups per year increased to around 100 pups per year. From 2013 to 2022, it varied from 70 to 145 pups per year.

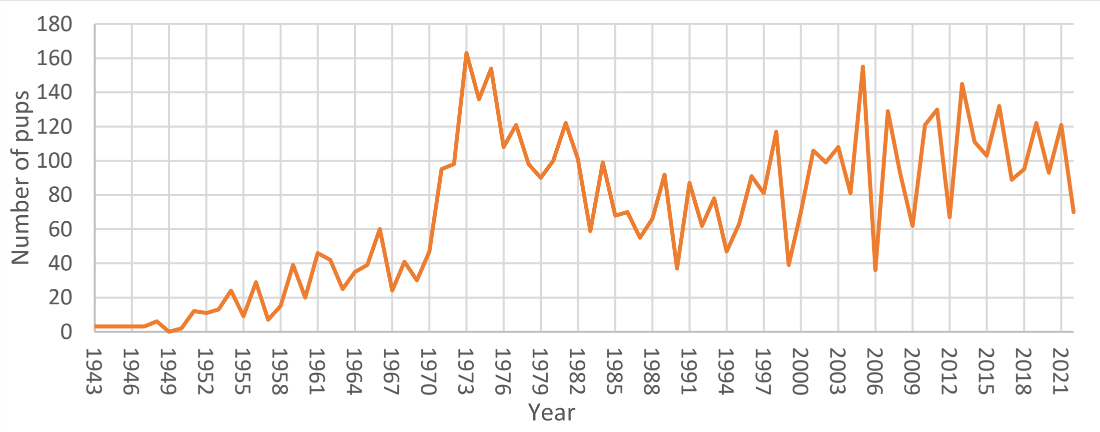

Figure 1. Number of pups born per year.

5.1.2. Litters and litter size

From 1951 until 1969, the number of litters per year increased from around 3 to around 6 (figure 2). Between 1969 to 1973, the number of litters per year increased to 27. Between 1973 and 1994, the number of litters decreased to around 10 litters per year with an exception in 1981 where there were 24 litters born. From 1994 to 2013, the number of litters per year increased to around 18 with a minimum of 7 and a maximum of 25 litters per year. From 2013 to 2022, the number of litters stayed around 16 litters per year with a maximum of 25 and a minimum of 14.

The litter size fluctuated around 5 to 7 pups between 1967 to 2022 (figure 2). Before 1967, there has been more variation of litter size per year.

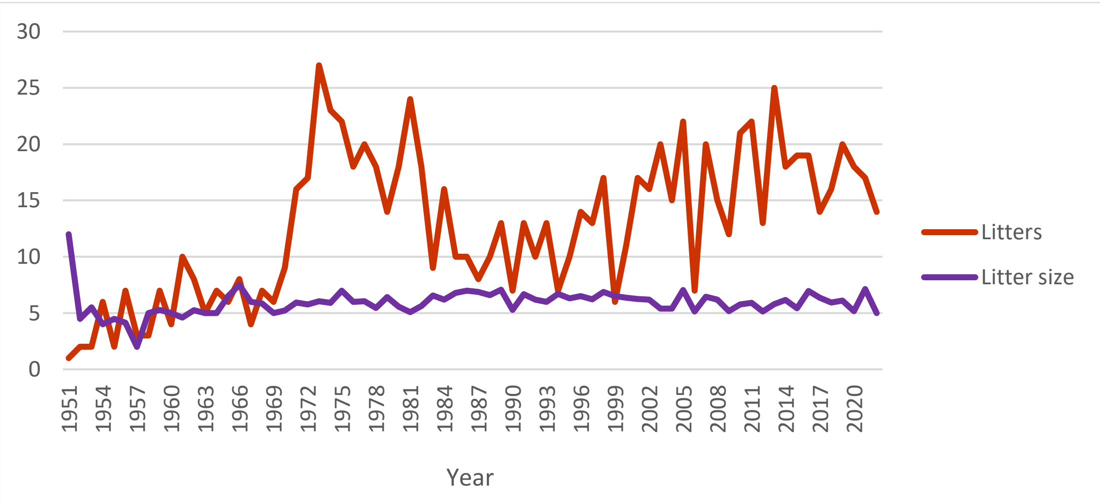

Figure 2. Number of litters per year and mean litter size per year.

5.1.3. Animals used in breeding

From 1951 until 2022, 4647 animals were not used for breeding and 856 animals were used for breeding (figure 3). Of which 329 out of 2446 males and 527 out of 2728 females were used for breeding. From birth years 2020, 2021 and 2022, the animals were still too young to be used in breeding explaining why there were no sires and dams in these years (yet).

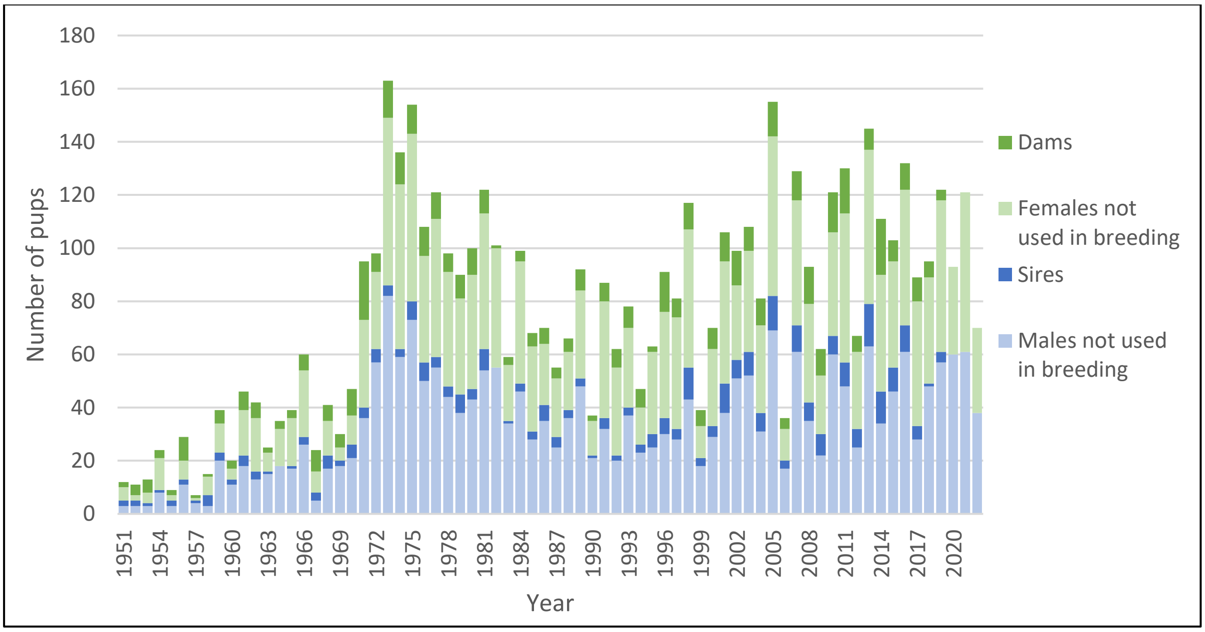

Figure 3. Number of pups per year of birth; males not used in breeding, sires, females not used for breeding, dams.

The percentage used for breeding in dams was often higher than in sires (figure 4). For both male and female, there was a lot of variation in the percentages that were used in breeding before 1967. The number of males used in breeding was around 25% with a maximum of 57.1% and a minimum of 0%. Between 1967 and 1975, the percentage of males used in breeding decreased from around 25% to around 12%. Between 1975 and 1993, this percentage stayed around 12%. From 1993 until 2019, the number of males used in breeding slowly increased to around 19%.

The number of females used in breeding before 1967 was around 35% with a maximum of 66.7% and a minimum of 12.5%. Between 1967 and 1975, the percentage of females used in breeding decreased from around 35% to around 17%. From 1975 until 1993, the percentage of females used in breeding remained around 17%. Between 1993 and 2019, the percentage slowly increased to 25%.

The animals of the birth years 2020, 2021 and 2022 were still too young to be used in breeding. This explains why the percentages used in breeding were 0%.

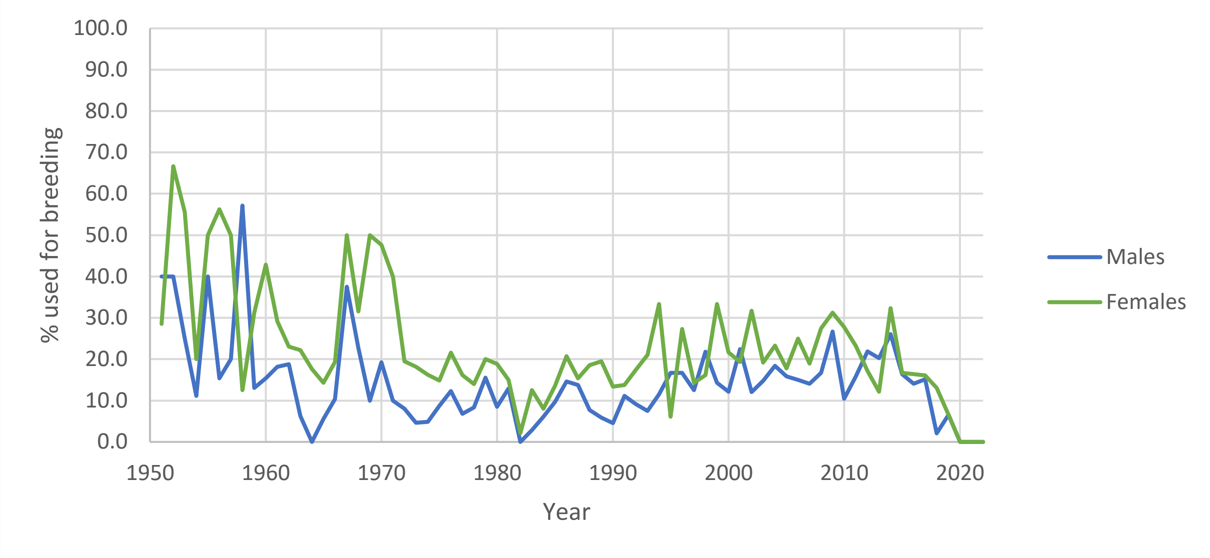

Figure 4. Percentage used for breeding in males and females.

5.1.4. Sires

The number of litters per sire per year fluctuated around 2 from 1951 until 1980 (figure 5). From 1980 to 1991, the number of litters per sire did not exceed 2 and had a mean of around 1.4 litters per sire per year. Between 1991 and 2002, it exceeded 2 litters per sire three times with a mean around 1.6. From 2002 until 2022, the number of litters per sire was around 1.2 litters per sire.

The number of pups per sire was dependent on the number of litters per sire and on the litter size. Therefore, the graph generally displays the same shape as the litters per sire, but with less variance.

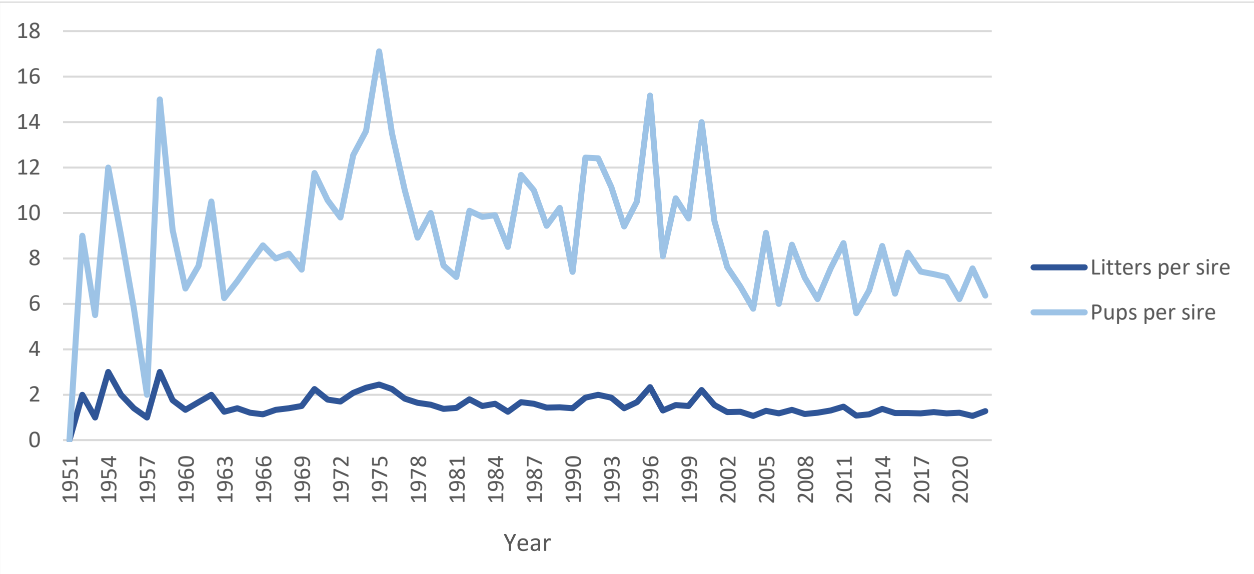

Figure 5. Number of litters per sire per year and number of pups per sire per year.

The number of sires with offspring per year increased from 0 to 17 sires from 1951 until 1981 (figure 6). From 1981 until 1995, it decreased from 17 to around 6 sires. Between 1995 and 2021, the number of sires increased to a value of 16 sires. In 2013, the number of sires was 22. The number of sires per year showed a lot of variation between years.

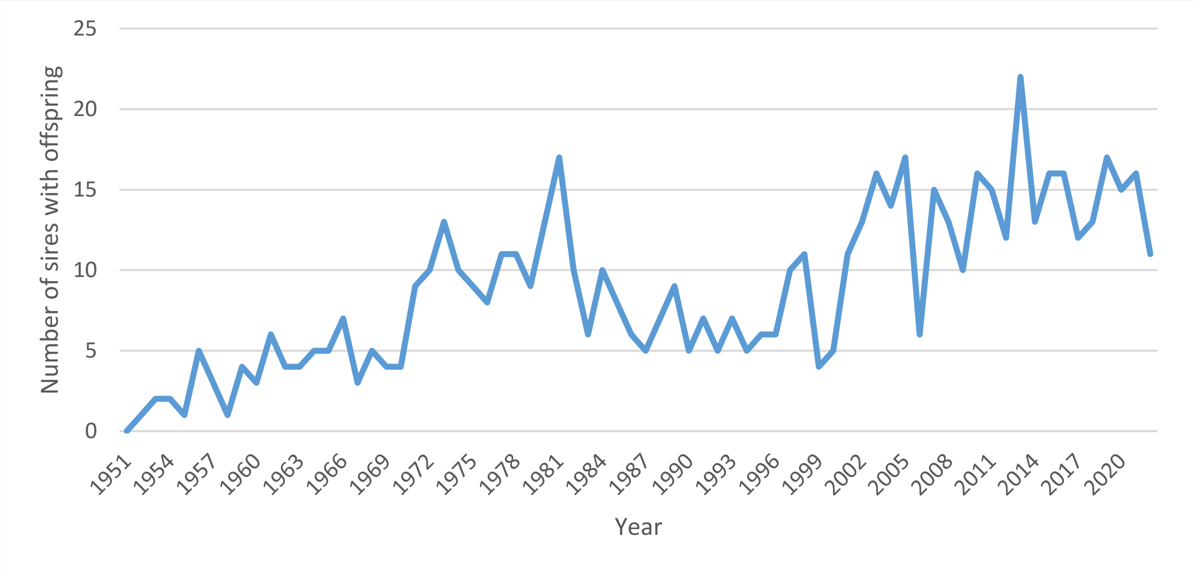

Figure 6. The number of sires with offspring per year.

5.1.5. Sires with the most offspring

Between 1951 and 1999, the share of the top 10 sires increased (figure 7). With only the top 6 sires used in 1987, 1990, 1992 and 1994. And only the top 5 sires used in 1999. From 1999 until 2022 the share of the top 10 sires decreased to an average of around 80% per year. However, in this period, there has been some variation.

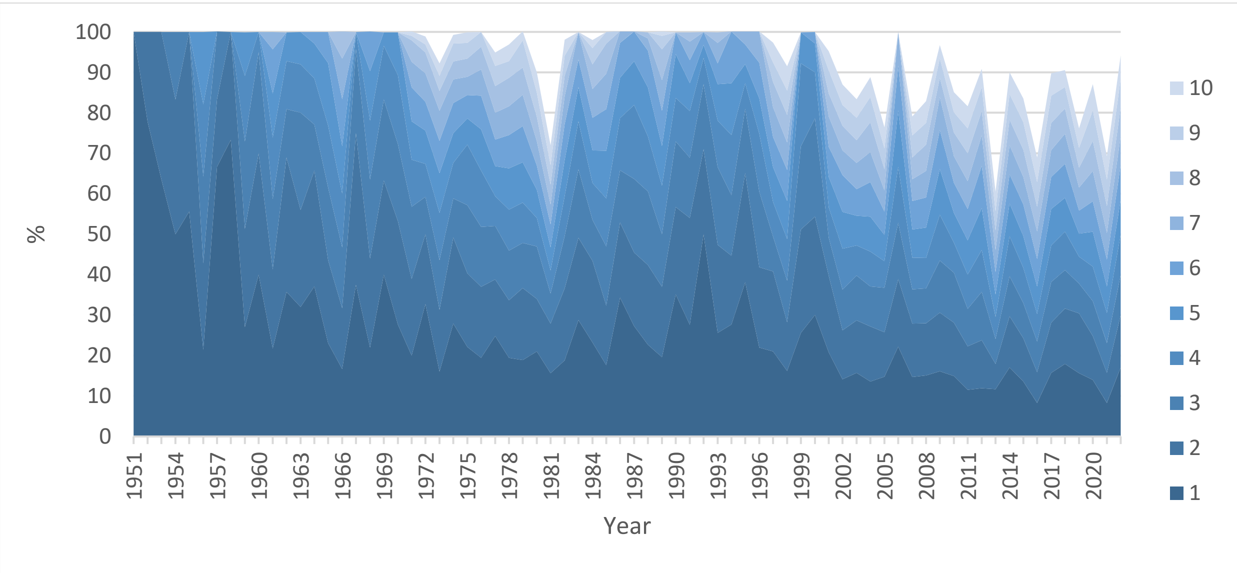

Figure 7. The share in % of the top 10 sires in the pedigree per year.

5.1.6. Generation interval

Between 1951 and 1971, the generation interval fluctuated a lot for both sires and dams, with a maximum of 8.24 and minimum of 2.24 years for sires and a maximum of 5.87 and minimum of 2.45 year for dams (figure 8). For sires, the generation interval from 1971 to 2022 was around 4.5 years. The generation interval for dams in this period was around 4.4. Dams showed more fluctuations per year than the sires.

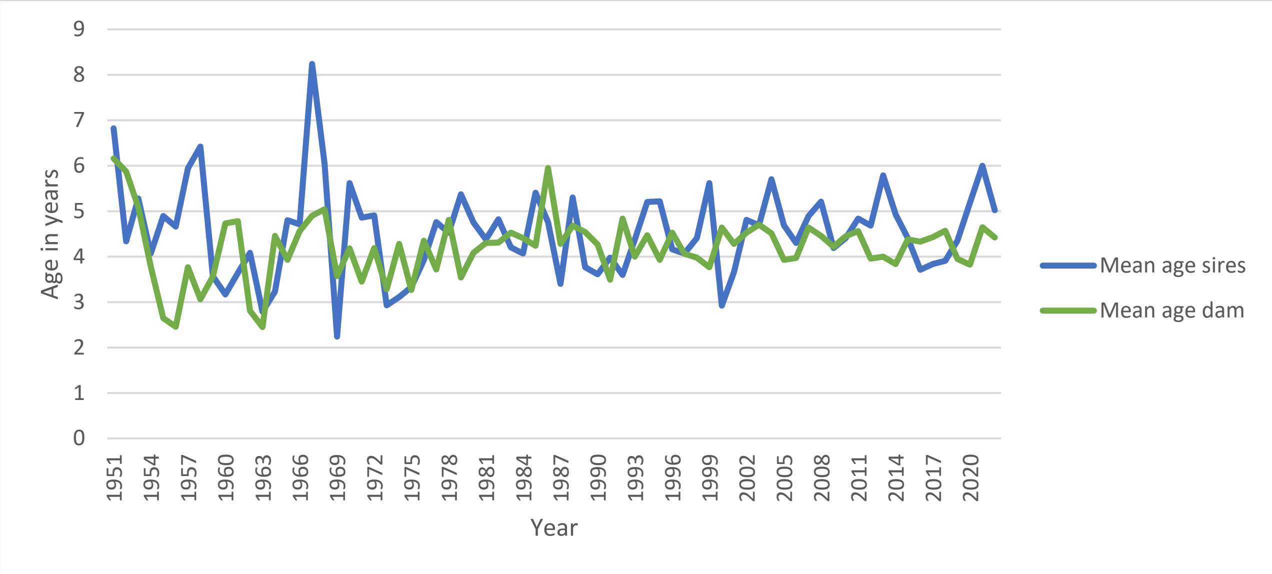

Figure 8. Mean generation interval for sires and dams.

5.1.7. Completeness of the pedigree

Since 1951, there was at least one generation known of the animals registered in the pedigree. From 1956 until 1984, the completeness of the pedigree increased from at least two generations known to five or more generation known (figure 9). Between 1984 and 2013, all dogs had five or more generations known. From 2013 until 2022, five or more generations were known in most years, but not in the years 2014, 2017, 2019 and 2022. In these years outcrosses were done by the NVSW. This was further assessed in the next part of the results at 'Outcrosses analyses.

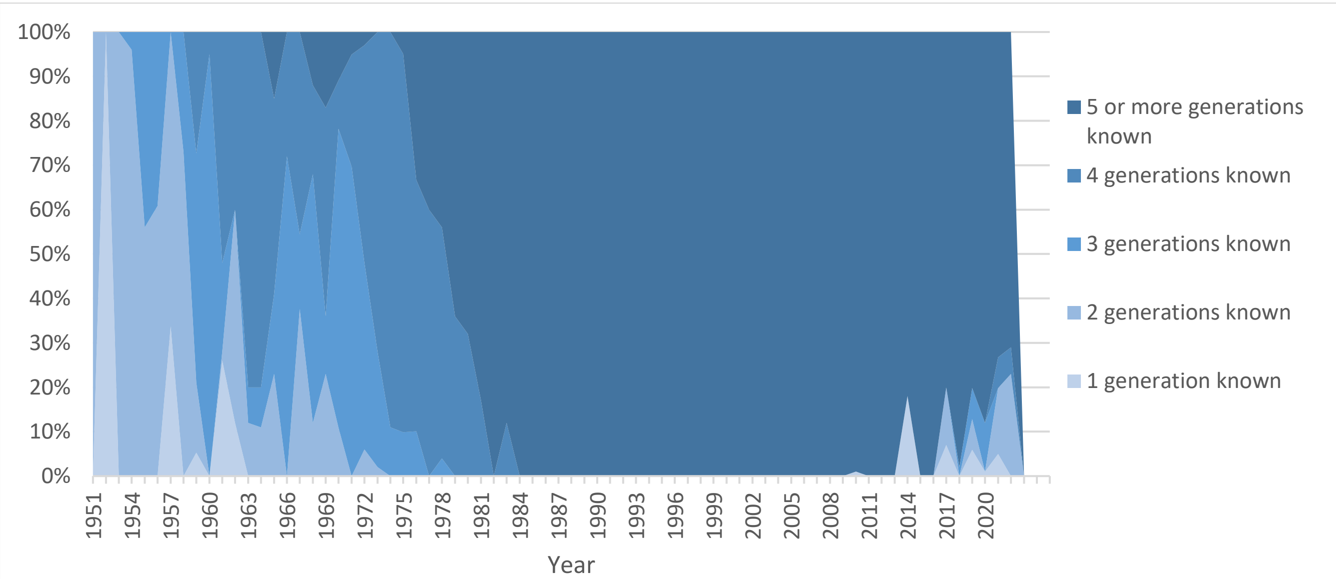

Figure 9. The completeness of the pedigree.

5.1.8. Inbreeding and kinship

From 1951 to 1971, the level of inbreeding showed a lot of variation, with a maximum of 22.6% and a minimum of 0% (figure 10). Between 1971 and 2013, the level of inbreeding has gradually increased from 22.5% to 35.1% with a low variation. From 2013 until 2022, the level of inbreeding showed a large variance. There were sudden drops in level of inbreeding in 2014, 2017, 2019 and 2021. In these years, outcrosses have been done.

The kinship represented the level of inbreeding in next generation and showed similarity with the level of inbreeding (figure 10). Between 1951-1971, the kinship showed a lot of variation with a maximum of 32.7% and a minimum of 11.3%. From 1971 to 2013, the kinship gradually increased from 23.8% to 37.1% with low variation. From 2013 until 2022, the kinship, just like the level of inbreeding, had large variations.

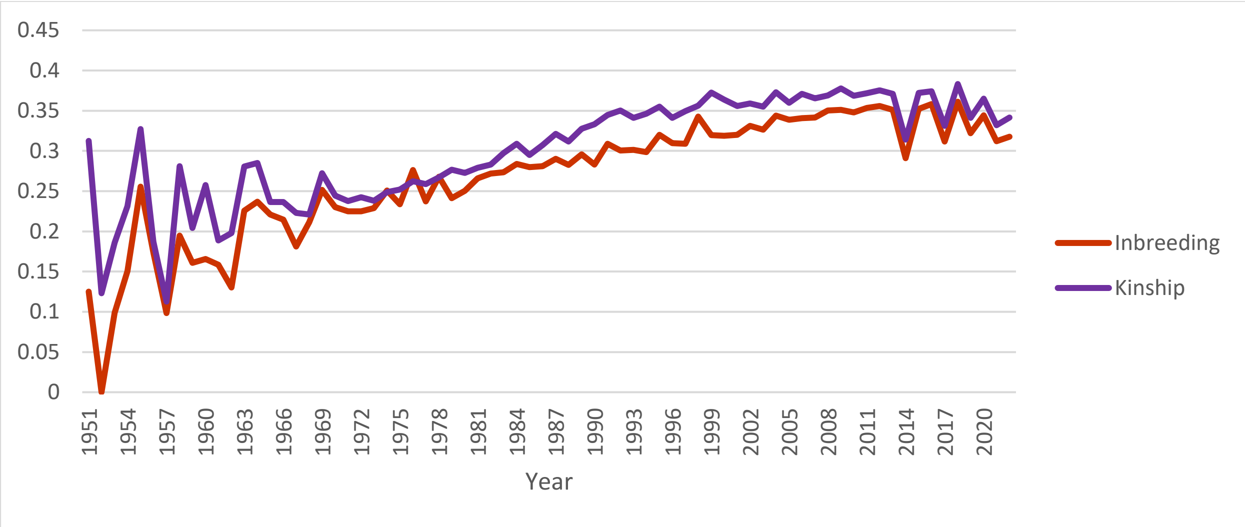

Figure 10. The level of inbreeding and kinship per year.

The rate of inbreeding per year in the period from 1951 until 1970 was 0.83% (figure 11). Using the generation interval calculated earlier, the inbreeding rate per generation for this period was 3.66% (table 4). From 1970 until 1985, the rate of inbreeding was 0.50% per year and 2.12% per generation. In the period from 1985 until 1995, the rate of inbreeding was 0.45% per year and 2.00% per generation. The rate of inbreeding for the period 1995 until 2004 was 0.34% per year and 1.48% per generation. From 2004 until 2012, the rate of inbreeding was 0.29% per year and 1.32% per generation. From 1951 until 2012, the rate of inbreeding has been positive. This changed in the period from 2012 to 2022, where the rate of inbreeding was -0.34% per year and -1.51% per generation. The negative rate of inbreeding resembled a decrease in the level of inbreeding. In the period from 2012 to 2022, five outcrosses have been done. These were the outliers that can be seen in figure 11.

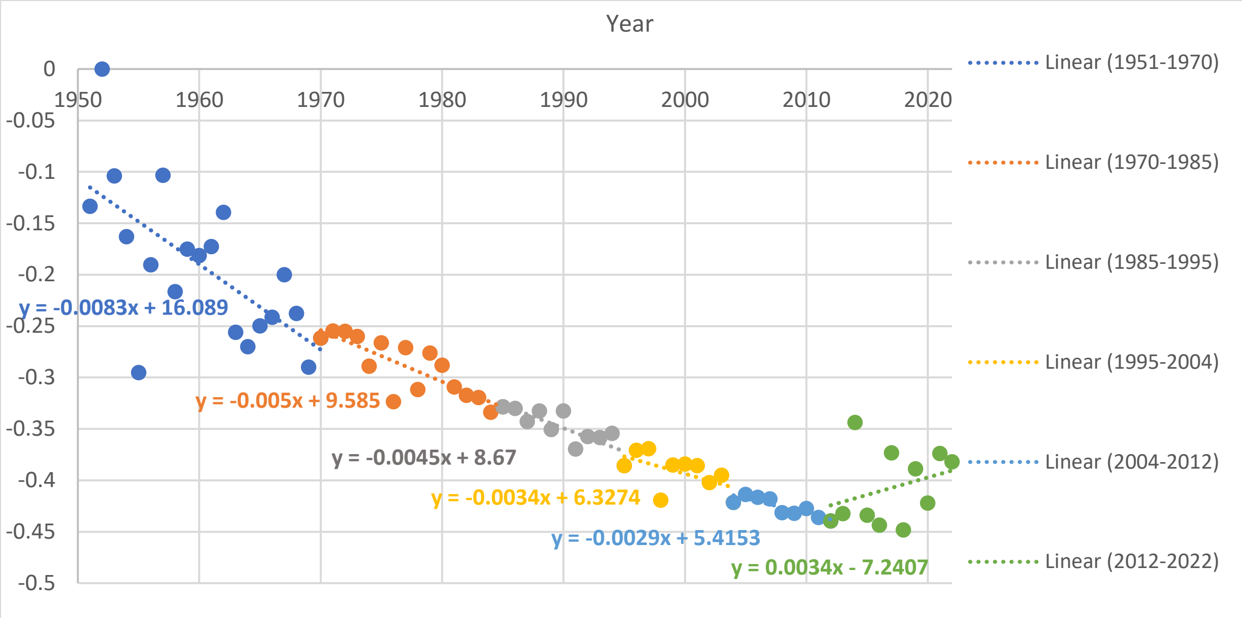

Figure 11. The ln(1-F) is on the y-axis, where F is the level of inbreeding. The slopes of the formulas represent the minus rate of inbreeding per year for different time periods

Table 4. Rate of inbreeding and kinship per generation for different periods.

Period	Inbreeding rate per generation (%)	Kinship rate per generation (%)
1951-1970	3.66	0.12
1970-1985	2.12	2.62
1985-1995	2.00	3.68
1995-2004	1.48	1.16
2004-2012	1.32	0.57
2012-2022	-1.51	-1.41

5.2. Current outcrosses analyses

5.2.1. Breeds used for outcross

There have been five outcrosses with different breeds (table 5). Of which all females are Wetterhoun, and the males are another breed.

Table 5. Male and female breed, litter date and number of offspring of outcross pairs.

Male Breed	Female Breed	Litter Date	Offspring (male, female)
WH OC Labrador	Wetterhoun	27/03/2021	6 (4, 2)
WH OC Barbet	Wetterhoun	09/07/2019	7 (4, 3)
WH OC Airedale	Wetterhoun	11/11/2017	6 (3, 3)
WH OC Big Poodle	Wetterhoun	28/12/2014	9 (6, 3)
WH OC Labrador	Wetterhoun	20/11/2014	11 (2, 9)

5.2.2. Wetterhoun look alike

There have been ten breeding pairs with a Wetterhoun look alike female and Wetterhoun male (table 6).

Table 6. Male and female breed, litter date and number of offspring.

Male Breed	Female Breed	Litter Date	Offspring (male, female)
Wetterhoun	WH Look alike	10/04/2020	1 (0, 1)
Wetterhoun	WH Look alike	15/11/2018	6 (6, 0)
Wetterhoun	WH Look alike	21/04/2018	3 (2, 1)
Wetterhoun	WH Look alike	24/04/2017	8 (3, 5)
Wetterhoun	WH Look alike	01/11/2016	8 (6, 2)
Wetterhoun	WH Look alike	14/01/2016	5 (4, 1)
Wetterhoun	WH Look alike	09/11/2014	1 (0, 1)
Wetterhoun	WH Look alike	24/09/2014	3 (2, 1)
Wetterhoun	WH Look alike	28/03/2014	4 (2, 2)
Wetterhoun	WH Look alike	23/09/2013	1 (0, 1)

5.2.3. Litter size

The mean litter size of Wetterhoun with Wetterhoun crosses was 5.962 pups, the outcross pairs produced a mean litter size of 7.800 pups. The two-sample t-test showed a t-statistics of -1.521 and a one-sided p-value of 0.064.

The mean litter size of Wetterhoun with Wetterhoun crosses was 5.962 pups, the Wetterhoun look alike female mated with a Wetterhoun pairs produced a mean litter size of 4 pups. The two-sample t-test showed a t-statistics of 2.287 and a two-sided p-value of 0.022.

5.2.4. Sex distribution

The Wetterhoun with Wetterhoun pairs had a total offspring of 5437, of which 2734 male and 2703 female. Rounded, this resembled a 50/50 ratio.

The outcross pairs had a total offspring of 39, of which 19 male and 20 female. Rounded, this gave a 49/51 ratio, in line with the 50/50 ratio in Wetterhoun-Wetterhoun pairs.

The Wetterhoun with Wetterhoun look alike pairs had a total of 40 offspring, of which 25 male and 15 female. This gave a 62.5/37.5 ratio. This was different from the 50/50 ratio of the Wetterhoun-Wetterhoun pairs.

5.3. Simulations: future effects of different genetic management

5.3.1. No outcross

5.3.1.1. Exclude mating between animals with common ancestors over different numbers of past generations

In the simulation, applying no breeding policy showed the highest level of inbreeding after 100 years: 0.46 (figure 12). Applying the current breeding policy of the NVSW reduced the inbreeding level to 0.30 and excluding matings of animals with common ancestors reduced levels even further. Excluding common ancestors over three generations showed the lowest level of inbreeding (figure 12). When no breeding policy was applied the inbreeding rate was 2.09% for the first 20 years and 2.34% for the last 80 years (table 7). This decreased to 0.54% for the first 20 years and 1.14% for the last 80 years when the NVSW policy was applied with three generations excluded. The more generations that were excluded, the lower the rate of inbreeding, especially in the first 20 years. The first 20 years represented the short-term effect on the inbreeding rate. When one or more generations were excluded, the inbreeding rate was below 1% for the first 20 years. The last 80 years, which represented simulations of the long-term effect, showed a higher rate of inbreeding.

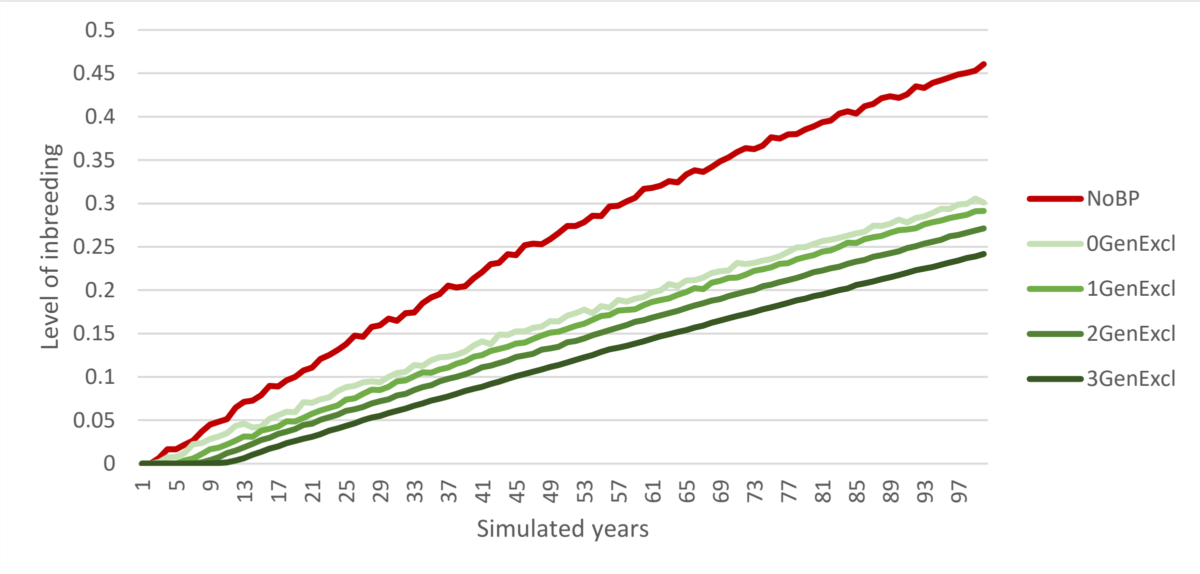

Figure 12. Level of inbreeding for different numbers of generations excluded; no breeding policy (NoBP) and NVSW breeding policy with different numbers of generations excluded (GenExcl).

Table 7. Rate of inbreeding per generation when excluding matings between animals with common ancestors in different numbers of past generations.

Breeding policy	**Rate of inbreeding (min – max) in % Years 0-20**	**Rate of inbreeding (min – max) in % Years 21-100**
No breeding policy	2.09 (1.90-2.28)	2.34 (2.30-2.40)
NVSW + zero generations excluded	1.34 (1.24-1.44)	1.32 (1.30-1.33)
NVSW + one generation excluded	0.99 (0.91-1.07)	1.31 (1.30-1.32)
NVSW + two generations excluded	0.84 (0.78-0.89)	1.24 (1.23-1.24)
NVSW + three generations excluded	0.54 (0.51-0.57)	1.14 (1.13-1.15)

5.3.1.2. *Different breeding policies regarding kinship*

The level of inbreeding with NVSW breeding policy without other policies was with 0.30 after 100 years the highest (figure 13). The lowest inbreeding level was when constraining the mean kinship with the remainder of the breed and minimizing the kinship between father and mother (0.221).

Minimizing the kinship between father and mother showed lower levels of inbreeding in the beginning but after around 57 years it showed higher levels of inbreeding compared to constraining the mean kinship with the remainder of the breed.

The NVSW breeding policy showed a rate of inbreeding of 1.34% for the first 20 years and 1.32% for the last 80 years (table 8). Constraining the mean kinship with the remainder of the breed and minimizing the kinship between father and mother reduced the rate of inbreeding to 0.55% in the first 20 years and 0.99% in the last 80 years. For both methods, the rate of inbreeding was lower in the first 20 years than in the last 80 years.

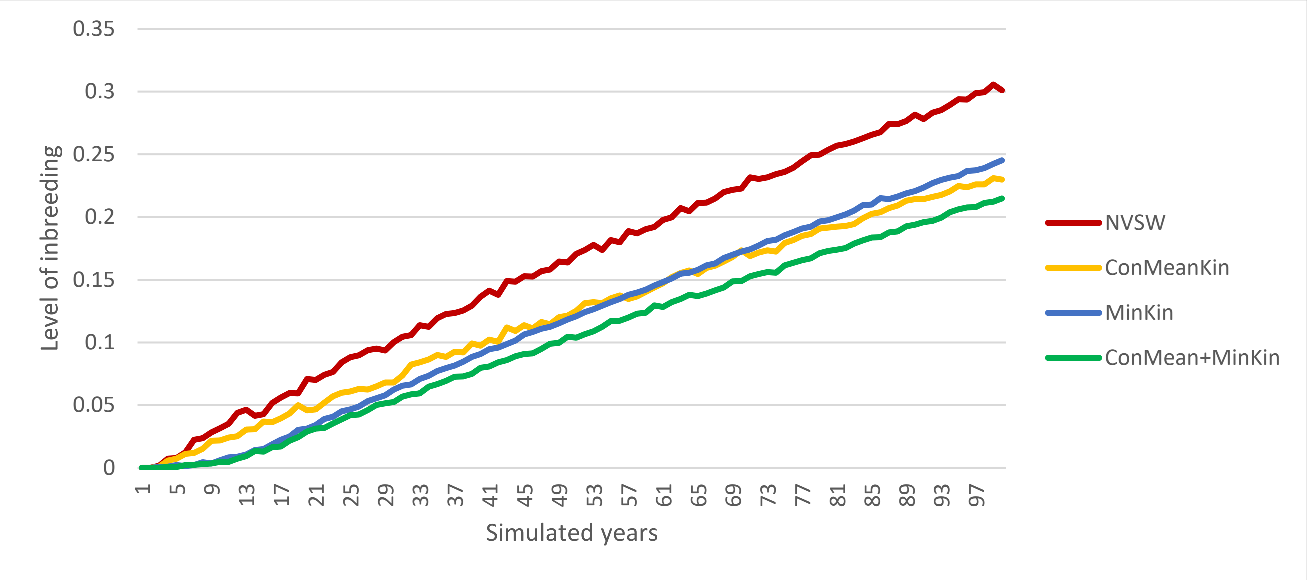

Figure 13. Level of inbreeding for different kinship policies: only NVSW breeding policy (NVSW), constrain mean kinship with remainder of the breed (ConMeanKin), minimize kinship between father and mother (MinKin) and the combination of both (ConMeanKin+MinKin).

Table 8. Rate of inbreeding per generation for different breeding policies and years regarding kinship.

Breeding policy	**Rate of inbreeding (min – max) in % Years 0-20**	**Rate of inbreeding (min – max) in % Years 21-100**
NVSW breeding policy with zero generation excluded	1.34 (1.24-1.44)	1.32 (1.30-1.33)
Constrain mean kinship with remainder of breed	0.87 (0.79-0.96)	1.01 (1.00-1.01)
Minimise kinship between father and mother	0.59 (0.56-0.63)	1.17 (1.16-1.18)
Constrain mean kinship with remainder of breed and minimise kinship between father and mother	0.55 (0.49-0.61)	0.99 (0.99-1.00)

5.3.2. Outcross

The level of inbreeding was highest when no outcross was used and lowest when there is one outcross every year (figure 14). The level of inbreeding for all simulation increased similarly from year 0 until 50, therefore years 0 until 30 have not been shown in figure 14. The level of inbreeding decreased in the years when outcrosses were done, but increased when there were no outcrosses done. In the years without outcrossing in one outcross every seven years, the level of inbreeding increased similarly to when no outcrosses were done. The level of inbreeding only decreased in the years when the outcrosses were executed. When one outcross was done every two years, the level of inbreeding showed the most fluctuation.

The rate of inbreeding was between 1.21 and 1.28% in years 0 until 50 (table 9). In these years, no outcrosses had been done in all simulations; the first outcross in all simulations was done in year 51. The rate of inbreeding was highest for no outcross at 1.26% and lowest for one outcross every year at 0.17%. The rate of inbreeding with one outcross every year was below 0.5% and until one outcross every seven years below 1%. The more frequent an outcross was done, the smaller the difference in the rate of inbreeding was.

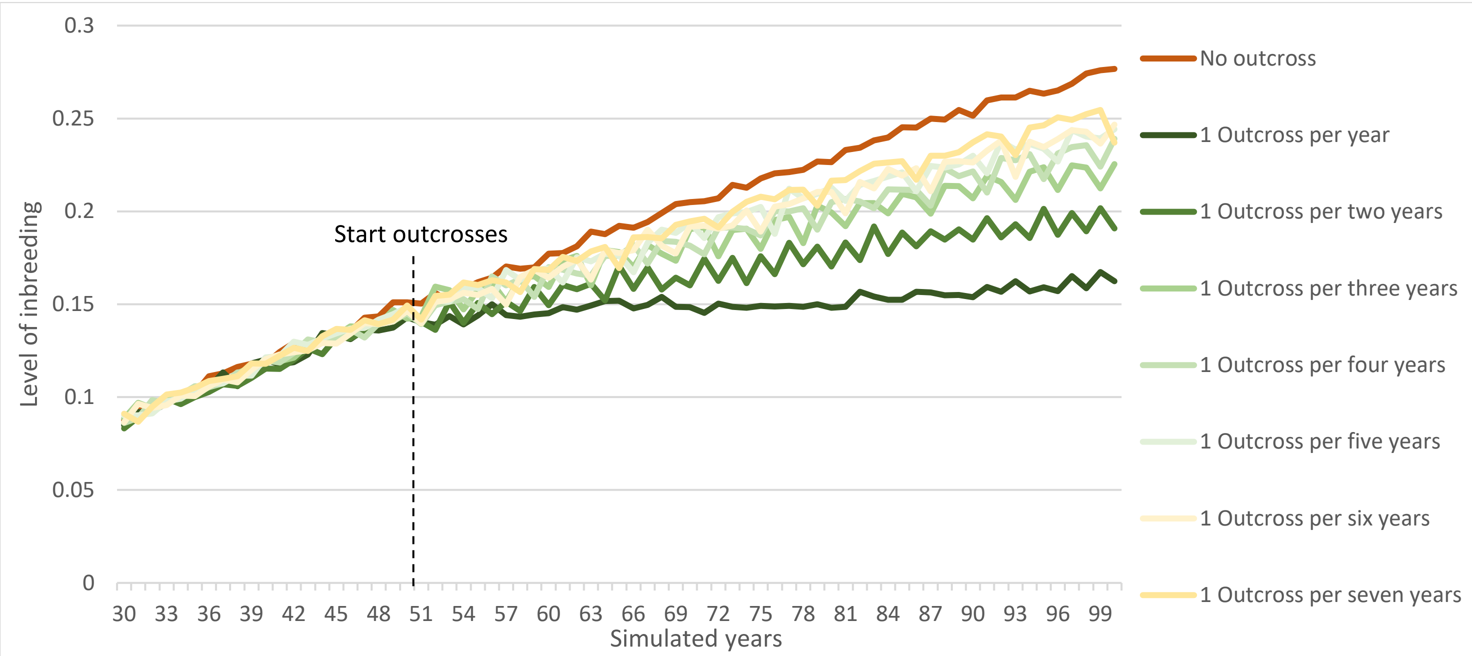

Figure 14. Level of inbreeding of the Wetterhoun population for different outcross methods. All outcrosses started in year 51.

Table 9. Inbreeding rates per generation in % with minimum and maximum for different outcross frequencies.

	Before outcross (years 0-50)	**With outcross (years 51-100)**
No outcross	1.28 (1.09-1.62)	1.26 (1.02-1.47)
One outcross per year	1.23 (1.03-1.49)	0.17 (-0.32-0.51)
One outcross per two years	1.21 (1.03-1.48)	0.52 (0.20-0.80)
One outcross per three years	1.24 (1.03-1.49)	0.71 (0.36-1.13)
One outcross per four years	1.25 (1.02-1.51)	0.86 (0.42-1.41)
One outcross per five years	1.26 (1.03-1.55)	0.93 (0.48-1.13)
One outcross per six years	1.24 (1.03-1.46)	0.96 (0.64-1.32)
One outcross per seven years	1.25 (1.00-1.53)	1.02 (0.72-1.50)

6. Discussion

The Wetterhoun has had a history of inbreeding from 1951 until 2012 where the rate of inbreeding exceeded the FAO recommended 1% (FAO, 2013). High rates of inbreeding are common among dog breeds, but it has been relatively higher in the past in the Wetterhoun (Giessen, 2022). In the years 2014, 2017, 2019 and 2021 five outcrosses have been done with the Wetterhoun with different breeds, which resulted in a negative rate of inbreeding, meaning that the level of inbreeding had decreased. This research showed that minimizing the mean kinship between the father and mother and constraining the kinship with the remainder of the breed decreases the rate of inbreeding, but not below the FAO recommended 0.5% to state that the breed is genetically stable. Outcrosses are needed to keep the rate of inbreeding below 0.5-1% to ensure that the population can sustainably exist. Outcrossing can be combined with minimizing mean kinship between the father and mother to decrease the rate of inbreeding even more. Having a larger breeding population can also decrease the rate of inbreeding (Lewis & Windig, 2017; Schipper, 2019; Windig & Oldenbroek, 2015).

6.1. Pedigree analysis

Earlier research on the Wetterhoun was done, showing similar results (Schipper, 2019). Both pedigree analyses showed that the rate of inbreeding exceeded the FAO recommended 1% from 1951 until 2012. This rate of inbreeding decreased from 3.66% in 1951 until 1970 to 1.32% in 2004-2012. From 2000, the level of inbreeding in multiple breeds in the UK also decreased (Lewis et al., 2015), the Wetterhoun fits in this trend. In this research for the Wetterhoun it can be seen that the number of sires with offspring increased and the share of the top 10 sires decreased around 2000 until 2022. This indicates a decrease in the genetic diversity loss, averting the popular sire syndrome (Lewis et al., 2015; Lewis & Windig, 2017).

From 2014-2021 five outcrosses were done which introduced new genetic material and decreased the rate of inbreeding. These outcrosses were the only method that really decreased the rate of inbreeding which could be because the Wetterhoun only has a small population size.

6.2. Outcross analysis

From 2012 until 2022, the rate of inbreeding was a negative 1.51%, which was a result of the five outcrosses that have been done by the NVSW. Two outcrosses were done in 2014 and one was done in 2017, 2019 and 2021. Some of the donor breeds were chosen by the NVSW deliberately not look like the Wetterhoun to decrease the relatedness between the mated animals even more. The breeds that were used were the Labrador retriever, Barbet, Airedale terrier and the Poodle. These outcrosses showed a mean litter size of 7.8 pups, while the Wetterhoun pairs showed a mean litter size of 5.96. This could not be proven to be significantly different because of the small sample size of 5 outcrosses. That litter size is negatively affected by high level of inbreeding was also shown in the Bernese Mountain dog, German shepherd (Leroy et al., 2015) and German spitz dog (Andrade et al., 2021). If more outcrosses are done, it would be interesting to analyse the litter size again and compare it to the Wetterhoun pairs. The male/ female ratio of the outcross litters shows a 49/51 ratio, which is approximately the 50/50 ratio observed in Wetterhoun pairs.

The NVSW also used Wetterhoun look-alikes in breeding. The related data that was provided by the NVSW was missing some litter sizes. In the data set of all Wetterhoun animals, the parent combination was looked up and showed offspring numbers. The mean litter size of the Wetterhoun with Wetterhoun look-alike pairs was 4 pups, which significantly differs from the mean litter size of the Wetterhoun pairs. Also, the male/female ratio does not resemble 50/50. These results could have been caused by the missing data, where dogs are probably not registered in the pedigree, but do exist. To investigate the real litter sizes, it is advised to ask the NVSW to provide the missing data.

6.3. Simulations without outcross

This research shows that if the breeding policy is not changed, the inbreeding rate will not go below 1%. It was shown by Schipper that if the breeding policy is not changed, the number of pups born per year will be zero at year 42 (Schipper, 2019). Schipper used a maximum kinship between the parent of 10% and if no parent combination met this condition, no animals are mated and subsequently no new animals are born. Although this research has a slightly different approach, it shows that without changing the breeding policy or genetic replenishment with outcrosses, this population will cease to exist.

Excluding matings with common ancestors over different numbers of generations decreased the rate of inbreeding on the short term (year 0-20). With inbreeding rates of 0.54% up to 0.99% when matings with common ancestors were excluded over three respectively one generation. However, in the long term (years 21-100), the rates of inbreeding all exceeded 1%. Exclusion of matings also decreased the inbreeding for the short term in other breeds like Saarloos and Newfoundlander, but also showed higher values when looking at the long term (Giessen, 2022). The possibility to choose parents that exclude matings for different number of common ancestors was limited. Due to the small population size, it was not possible meet this requirement at one point and the level of inbreeding increased. This caused little effect on the long term.

When constraining the mean kinship with the remainder of the breed, the rate of inbreeding was 0.87% for the first 20 years but increased to 1.01% in the last 80 years. Minimising the kinship between the father and the mother decreased the rate of inbreeding to 0.59% for the first 20 but increased to 1.17% for the last 80 years. Only when these two methods were combined, the mean rate of inbreeding was 0.99% for the last 80 years and could be helpful on the long term. But among the 25 repeats some inbreeding rates were 1.00%, so outcrosses were needed to ensure the rate of inbreeding to be below 1%. The decrease in rate of inbreeding when using kinship strategies was also shown for other breeds (Giessen, 2022; Windig & Oldenbroek, 2015).

To decrease the rate of inbreeding in the Wetterhoun, the number of females mated per male can be decreased (Giessen, 2022). This is also observed to decrease the rate of inbreeding in the Dutch 'Schapendoes' (Stilting, 2018). Other strategies that were showed to decrease the inbreeding rate are decreasing the share of the top sires, decreasing the number of sons used for breeding per sire and increasing the number of litters per dam (Windig & Oldenbroek, 2015). The optimal contribution selection can be used to find what the lowest level and rate of inbreeding is possible (Giessen, 2022). This method mathematically calculates the share every dog in the population should have to achieve the lowest rate of inbreeding possible. This method however can pose difficulties when applying it to the breeding population because due to its complexity, it is difficult to explain to breeders and dog owners. It would be interesting to calculate the optimal contribution selection and investigate what the minimum level and rate of inbreeding can be for the Wetterhoun.

When no outcross was done, the rate of inbreeding was 1.26% for the last 50 years of the simulation. One outcross every year resulted in an inbreeding rate of 0.17% per year, but due to the small breeding population of the Wetterhoun it can influence the breed characteristics. To keep the inbreeding rate below 1%, at least one outcross needs to be done every six years. It would be interesting to combine different frequencies of outcrosses with minimizing the kinship between the father and the mother to investigate what the effect of this is regarding the inbreeding rate.

The breeding policy of the NVSW stated that the relatedness should not be higher than 10% over 4 generations (*Werkwijze FAC Wetterhoun*, n.d.). Pointer software cannot simulate this, that is why everything except this precise rule was categorized as 'NVSW breeding policy'. The best approximation of this breeding policy is when matings with common ancestors over three generations are excluded. The first common ancestor in this case is only allowed to be at four generations ago. Every generation half of the genes are passed from that ancestor, so the relatedness of these mated animals is 0.5^4=0.0625. This research showed that the mean rate of inbreeding for this was 1.14%, which exceeded the recommended 1% by the FAO.

6.4. Simulations with outcross

Outcrossing is used to increase the genetic diversity by introducing unrelated animals from another breed (Windig & Doekes, 2018). The donor breeds provide new genetic materials, but also have a different appearance. The offspring of the mated animals represent characteristics from both breeds.

The NVSW chose different breeds for the outcrosses. If the used donor breed of the outcross looks like the Wetterhoun, the offspring is likely to represent at least some characteristics of the Wetterhoun. Similar breeds are still unrelated to the inbred breed because they have been bred separately for decades. If a breed is chosen that looks very different to the Wetterhoun, the offspring may represent less characteristics of a Wetterhoun.

Backcrossing can be done to ensure that the breed sustains its appearance. With backcrossing, the offspring of an outcross mating is crossed with the inbred breed causing that offspring to represent more of the breed's preferred characteristics. However, with reintroducing animals from the inbred breed, the inbreeding rate increases, which was also shown in the Saarloos Wolfdog (Windig & Doekes, 2018). To profit the most from an outcross, it is best to keep the number of backcrosses low. Using a similar donor breed does not mean that those animals relate to the receiving breed. Therefore, it is preferred to use a donor breed that has similar appearance to the recipient breed to sustain the breed's characteristics.

6.5. Conclusion

The rate of inbreeding in the Wetterhoun has been too high until 2012 where outcross program replenished the genetic diversity of the population and inbreeding levels and rate decreased. Breeding policies without outcross do not sufficiently decrease the rate of inbreeding on the long term. These policies include excluding matings of common ancestors of different generations, constraining the mean kinship with the remainder of the breed and minimizing the kinship between the father and the mother. Outcross is needed to keep the Wetterhoun population genetically stable, at least one outcross every six years to not exceed an inbreeding rate of 1%.

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