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# Managing inbreeding and relatedness in the Schapendoes

Jesse Minnema

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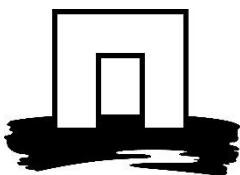
BSc thesis Animal Breeding and Genomics (YAS80312)

January 2024

Supervisors/examiner:

Jack Windig

Thesis: Animal Breeding and Genomics



**WAGENINGEN**  
UNIVERSITY & RESEARCH

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## 1. Summary

Currently, the breeding organization 'De Nederlandse Schapendoes' has been using the mean kinship policy since 2018. Recently, questions have been raised regarding the use of an outcross. Some members question whether the use of mean kinship is enough, and if outcrosses are needed to lower the inbreeding of the Schapendoes. Therefore in this thesis, advice is given to the breeding organization regarding the use of an outcross.

First, the status of the breed was analyzed using the software Retriever. Results showed that currently the Schapendoes is doing well. The current inbreeding rate of the Schapendoes is 0.11% per generation, which is far under the FAO level of 1.0% (preferably 0.5%). The average inbreeding coefficient and kinship are just above 0.29 and has been relatively stable since 2017. Besides that, the average litter size has been stable. Breeding association De Nederlandse Schapendoes has to remain putting effort in keeping the inbreeding low. If the association stops using current measures in the breeding policy, the inbreeding rate may rise again and the status of the breed could worsen.

After simulations were run using the software Pointer, it can be said that the higher the number of outcrosses and the lower the interval, the more the inbreeding values are lowered. If only a couple are done over a long time span, the effect is very small. Performing one outcross after 50 years resulted in a 0.003 lower average inbreeding coefficient. Simulating the ideal usage of mean kinship showed that many outcrosses have to be performed to achieve equal values. Performing outcrosses also changes the phenotypic traits of the Schapendoes. An outcross would be best suited in the case of an emergency or if the breeding association is open for large phenotypic changes in the breed.

From the results it can be concluded that there are no clear indicators suggesting the need of an outcross in the Schapendoes. An outcross would be most suitable in case of emergency or if changes in phenotype are accepted. When compared to the use of Mean Kinship, a very large number of outcrosses has to be done with a small interval to achieve similar results. Therefore my advice to the breeding association of the Schapendoes is as follows: try to use the Mean Kinship policy correctly and as much as possible. The current use of the mean kinship policy can most likely be improved, based on the results. If this is used correctly, the breed can be kept in great condition without altering the phenotype. I would not recommend the use of an outcross at this moment as there are no indicators suggesting that it is necessary and it would change the characteristics of the Schapendoes. However, if litter size significantly decreases in the future or if more genetic defects arise, outcrossing could be a solution to these problems.

## Samenvatting (Dutch Summary)

Momenteel hanteert fokvereniging 'De Nederlandse Schapendoes' sinds 2018 het mean kinship beleid. Recent zijn er echter vragen over het gebruik van een outcross. Sommige leden vragen zich af of het gebruik van mean kinship voldoende is, of dat er outcrosses gedaan moeten worden om de inteelt van de Schapendoes te verminderen. Daarom is er in dit onderzoek advies gegeven aan de vereniging met betrekking tot het gebruik van een outcross.

Eerst werd de status van het ras geanalyseerd door middel van de software Retriever. Uit de analyse van de stamboom bleek dat de Schapendoes het goed doet. Het ras heeft een inteeltcoëfficiënt en kinship van 0.29. Deze waarde is relatief stabiel gebleven sinds 2017. De inteelttoename is 0,11% en dus ver onder het FAO level van 1% (0.5% bij voorkeur). Daarnaast is de gemiddelde worpgrootte stabiel. Het gaat dus goed maar fokvereniging De Nederlandse Schapendoes moet zich blijven inzetten om de inteelt laag te houden. Als de vereniging stopt met het hanteren van de huidige maatregelen in het fokbeleid, kan de inteeltgraad weer stijgen en kan de status van het ras verslechteren.

Nadat er simulaties zijn uitgevoerd met behulp van de software Pointer, kan worden gesteld dat hoe hoger het aantal uitkruisingen en hoe lager het interval, hoe meer de inteeltwaarden worden verlaagd. Als er maar enkele outcrosses worden gedaan over een langere periode, is het effect erg klein. Het uitvoeren van één outcross na 50 jaar resulteerde bijvoorbeeld in een 0,003 lagere gemiddelde inteeltcoëfficiënt. Het simuleren van het ideale gebruik van gemiddelde verwantschap toonde aan dat er veel outcrosses moeten worden uitgevoerd om gelijke waarden te bereiken. Het uitvoeren van outcrosses verandert ook de fenotypische eigenschappen van de Schapendoes. Een outcross zou het meest geschikt zijn in geval van nood of als de fokkerij openstaat voor grote fenotypische veranderingen in het ras.

Uit de resultaten kan worden geconcludeerd dat er geen duidelijke indicatoren zijn die wijzen op de noodzaak van een outcross in de Schapendoes. Een outcross zou het meest geschikt zijn in geval van nood of als veranderingen in het fenotype worden geaccepteerd. Vergeleken met het gebruik van Mean Kinship moet er met een klein interval een zeer groot aantal outcrosses worden gedaan om vergelijkbare resultaten te bereiken. Daarom is mijn advies aan de fokkerijvereniging van de Schapendoes als volgt: probeer het Mean Kinship beleid correct en zoveel mogelijk toe te passen. Op basis van de resultaten kan het huidige gebruik van het mean kinship beleid hoogstwaarschijnlijk worden verbeterd. Als dit op de juiste manier wordt gebruikt, kan het ras in goede conditie worden gehouden zonder het fenotype te veranderen. Het gebruik van een outcross zou ik op dit moment niet aanraden, omdat er geen indicatoren zijn die erop wijzen dat dit nodig is en omdat outcrosses de uiterlijke eigenschappen van de Schapendoes zouden veranderen. Als de worpgrootte in de toekomst echter aanzienlijk afneemt of als er meer genetische defecten ontstaan, kan uitkruising een oplossing voor deze problemen zijn.

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## 2. Introduction

### Inbreeding

Inbreeding is the production of offspring by individuals who are related genetically. This is often the case in pedigree dog breeds as these studbooks are closed, meaning breeders can only mate dogs that are inside the studbook. This may fix certain desired traits and characteristics genetically, but also makes the pool of potential mates smaller and thus makes mating related individuals inevitable. When these related individuals produce offspring, the offspring is more likely to receive identical copies of genes, meaning a higher homozygotic allele pair frequency and a higher detrimental allele pair frequency. Because of the increase in homozygotic allele pairs, expression of recessive genetic defects is more likely to occur in these offspring (Minter et al., 2021). Inbreeding can pose a threat to pedigree dogs even if the population is large. This is because in most dog breeds only a small number of males, the best performers, are used repeatedly for breeding. This is called the popular sire syndrome and this decreases the size of the gene pool used for breeding (Leroy & Baumung, 2011). Ideally, every sire in a breed should have an equal share in breeding. Inbreeding can lead to inbreeding depression, where high levels of homozygosity is seen and results in, for example, lower fertility (Windig et al., 2022) and fitness (Doekes et al., 2021). Research showed that in dogs, offspring of unrelated parents lived about 1.2 years longer than size-matched purebred offspring on average (Yordy et al., 2019). Inbred individuals are usually less healthy than noninbred individuals and therefore inbreeding should be avoided as much as possible (Minter et al., 2021).

Genetic drift is a mechanism in which allele frequencies of a population change randomly over generations because of chance (Honnay, 2013). If inbreeding levels increase, genetic drift will increase too. Because genetic drift is completely random, it can increase the frequency of detrimental alleles. If the inbreeding rate and genetic drift are extremely high, these alleles may even become fixated. Genetic drift is largest in small populations or a small gene pool, as they are more vulnerable for changes. Genetic drift can therefore be connected to the popular sire syndrome.

The inbreeding coefficient ( $F$ ) is a measure used to calculate the probability that an individual inherits two identical alleles from its parents due to common ancestry. The more related the male and female are, the higher the inbreeding coefficient of the offspring. Furthermore, we look at the inbreeding rate ( $\Delta F$ ), which is the increase in average inbreeding level from one generation to the next. Because the inbreeding rate is non-linear, it is expressed relative to how far the population is away from full inbreeding (Oldenbroek & Van der Waaij, 2015). According to The Food and Agriculture Organization (FAO) the inbreeding rate should be below 1,0% (preferably 0,5%) as the size of the rate of inbreeding gives an indication of the decrease in genetic diversity and the risk of inbreeding depression (Oldenbroek & Van der Waaij, 2015).  $\Delta F$  is more important than  $F$ . First of all, because  $F$  is dependent on the length of the pedigree and therefore it is hard to compare breeds and animals with different pedigree lengths. Secondly, there is selection (natural and artificial) against the negative effects of inbreeding. However, if  $\Delta F$  is too high there is not enough time for selection and thus the negative effects of inbreeding, like disorders, will show.

### Outcross

There are multiple ways to avoid or decrease inbreeding. One of them is called outbreeding, which is the use of an individual from outside the breed which will be a less or even unrelated individual, called an outcross. The outcross introduces new genetic material into a population's gene pool which will increase heterozygotic allele pair frequency and thus lower the amount of genetic defects. The use of an outcross can therefore be a relatively fast method to decrease inbreeding levels for highly inbred populations, where genetic diversity is too low and genetic disorders are too common. Sometimes an outcross can be the only way to avoid extinction of the breed, if their inbreeding values are extremely high. As said before, high inbreeding levels can lead to a decrease in fertility. In research about the Wetterhoun it was shown that with the use of an outcross, litter size can be increased in inbred populations (Boering, n.d.).

Despite the positive effects, the use of an outcross is often unwanted and generally not allowed in the breeding of purebred dogs as the desired traits and characteristics of a purebred dog get diluted. Additionally, the use of an outcross can introduce unwanted alleles. Currently, breeding organizations are allowed to use an outcross in a purebred breed, only if a long-term breeding-plan is set up. In 2016, a breeding organization of the Saarloos Wolfhond started using an outcross because of high inbreeding rates, low litter size and an increase in genetic disorders. Improvement after the use of an outcross was seen as litter size increased, inbreeding rate was reduced and there were less genetic disorders (Prosman, 2018).

When an outcross is used, often the offspring is bred with purebred dogs for multiple generations to regain the desired traits. This is called backcrossing, which will eventually lower the initial effect of the outcross. Each backcross lowers the effectiveness of the outcross by  $*0.5$  and therefore a balance must be found between the number of outcrosses and backcrosses. Research has shown that outcrossing has limited short-term effect unless outcross is done continuously (Windig & Doekes, 2018). Other popular ways to decrease inbreeding are applying breeding restrictions, increasing population size, and the constraining of mean kinship in selection of dogs used for breeding.

Estimation of Mean Kinship (MK) has already been implemented by the Nederlandse Schapendoes in their genetic management since 2018, after receiving advice (Stilting, 2018). Mean kinship is a measure of genetic relatedness/similarity of an individual to the population. By selecting individuals with a low mean kinship as parents relatively unrelated animals will be present in the next generation and, eventually the inbreeding rate is expected to be smaller compared to not selecting for low mean kinship. However, mean kinship is a dynamic value and will increase and decrease after each mating in the population and thus should be monitored constantly.

### The Schapendoes

The Nederlandse Schapendoes is a long-haired dog breed, its studbook originating from 1947 when a small group of breeders wanted to reintroduce an old Dutch breed. Because of his agility, weight and jumping qualities the Schapendoes was used for herding and protecting sheep after the 2<sup>nd</sup> world war. In 1971 the Nederlandse Schapendoes was officially acknowledged as a breed. Throughout the seventies, eighties and nineties the Schapendoes has spread across Europe and Canada.

At this moment, the breed has a low risk of genetic diseases. The most common one is Progressive Retinal Atrophy (PRA), a disease where cells of the eye deteriorate over time, eventually leading to blindness. However, there is concern in the breeding organization whether current measures to restrict inbreeding are enough or that an outcross is needed. This study looks at the current inbreeding status and relatedness in the Schapendoes, and simulates the use of an outcross to give breeding advice to the organization.

## 2.1 Aim of paper and the research questions

The aim of this thesis is to give breeding advice to 'Vereniging De Nederlandse Schapendoes' about the use of an outcross in the Schapendoes. Analyzing the pedigree and comparing different outcross scenarios are used to answer the following research questions:

- What is the current inbreeding rate?
  - o How does the current inbreeding rate compare to the inbreeding rate 5 years ago?
  - o What effect did the adaptation of the breeding policy have that was made 5 years ago?
- Is the use of an outcross necessary for the Schapendoes breed?
  - o Are there indicators that suggest an outcross is needed in the Schapendoes? (litter size, amount of genetic defects, inbreeding values etc.)
  - o What are the positive and negative effects of an outcross?
  - o How do different scenarios of outcross-usage compare to each other?

### 3. Material and Methods

#### 3.1 The data

The data was provided by 'Vereniging de Nederlandse Schapendoes' in an Excel sheet. The data included data of 32,176 dogs from one population, divided over different subpopulations (countries). The first analysis was done without subpopulations. Data errors were corrected if possible or removed, for example, the studbook showed a 42 year old female which is unrealistic. To calculate the mean kinship of the fathers, a column was added named 'MK Fathers' and with the excel formula '=VERT.ZOEKEN' (or =VLOOKUP in English) the mean kinship of the fathers was obtained. The second analysis included subpopulations. The dogs for each subpopulation were selected on "RegistrationNumber" in the datafile. The criteria "OwnerCountry" and "BreederCountry" could also be used, but both had a lot of missing/empty data. To assess the differences between subpopulations, 6 subpopulations were analyzed. The 5 largest countries each became a subpopulation. The rest of the dogs were grouped in the subpopulation 'other'. The different subpopulations, together with the number of dogs in that subpopulation, are shown in table 1.

Table 1 Subpopulation sizes in July 2023

<b><i>Subpopulation</i></b>	<b><i>Number of dogs</i></b>
<b>NHSB; The Netherlands</b>	14278
<b>SSK + SE; Sweden</b>	3418
<b>LOF; France</b>	3405
<b>VDH(/IGS); Germany</b>	3168
<b>FI + FIN; Finland</b>	2098
<b>Other;</b>	5808

#### 3.2 Pedigree analysis (retriever)

For the analysis of the current population status, Retriever software was used (Windig & Oldenbroek, 2015). The following information in the datasheet is used in this research: ID, Name, Sex, Date of birth, IDFather, IDMother and Color. Most of the figures in the results are from 1980 onwards, as this is the first year where more than 100 dogs were born. However, figures relating inbreeding and pedigree completeness contain data from 1967 onwards. This is chosen because from 1967 onwards the number of births per year has always been higher than one. As the Schapendoes population has already been analyzed before in 2018 (Stilting, 2018), the results are mainly be focused on the last five years.

Inbreeding rate was assessed as this is one of the most relevant inbreeding indicators. This parameter was visualized in a graph. The inbreeding rate ( $\Delta F$ ) is the rate of inbreeding per year ( $t$  = time) and can be calculated using the following formula:

$$\ln(1-Ft) = -\Delta Ft \text{ (Perez-Enciso, 1995)}$$

Inbreeding can lead to lower fertility and higher prereproductive mortality (Khlal & Khoury, 1991). Therefore changes in litter size can indicate an inbreeding depression. This indicator will therefore be visualized in a graph. If for example, the litter size is decreasing rapidly, then an outcross might be necessary. Also, the amount of males and females used for breeding in the whole population will be assessed and visualized. As said before, in the ideal situation every male used for breeding should have an equal share in breeding. However, this is often not the case and can have a large effect on inbreeding rate.

### 3.3 Outcross analysis (pointer)

To assess the effects of an outcross, Pointer software was used to simulate the Schapendoes population. Pointer is a simulation program where different scenarios can be created, relating an outcross (Windig & Oldenbroek, 2015). The input of Pointer contained information received from Retriever and information from the Schapendoes breeding policy, obtained from the website of Vereniging de Schapendoes (*Verenigingsfokreglement – schapendoes.nl*, n.d.).

The most relevant information for Pointer of the Schapendoes breeding policy is the following:

- The same combination of parents is allowed 2 times
- A sire has to be 21 months old to start mating
- A sire is allowed to have 3 litters in a period of 2 year (24 months) in the Netherlands. Outside of the Netherlands, a sire is allowed to have 3 litters at maximum
- A bitch has to be 24 months old to start mating
- A bitch is not allowed to be mated after 72 months of age if she has not given birth before. If she has given birth before, she is not allowed to be mated after 108 months of age

Three types of simulations were done. First, simulations without an outcross and without kinship constraints to show the expected values if no intervention was done. For the input of pointer this meant it contained only the values retrieved from retriever (Table 2). Second, a simulation without an outcross but with kinship constraints was done to show the expected inbreeding rate if no change is made in the current breeding policy of the Schapendoes. For the input of pointer this means that in the header 'Breeding policy' the options 'constrain mean kinship with remainder of breed' and 'minimize kinship between father and mother' were enabled. Third, simulations with different outcross scenarios are done to show the effects of an outcross. An outcross was added under the tab 'Population structure'. As each backcross lowers the effectiveness of the initial outcross by 0.5x, a balance must be found between the amount of outcrosses and backcrosses. Therefore, different amount of outcrosses were simulated. Also, the interval of an outcross was set to different values. An overview of the different scenarios simulated in this research is given in table 3. By comparing the three different sets of simulations, the effect of mean kinship and the effect of the outcross can be shown clearly.

After analyzing the pedigree with retriever, the input for the simulations contained the age structure of the parents. The output of retriever showed rounded fractions and the fraction combined resulted in a total of only 98% of the fathers and 99% for the mothers. Therefore 2% of the father and 1% of the mothers had been lost in the rounding of retriever. However, without a 100% age structure of the parents, pointer would not run. Therefore the missing percentages were added to the most suitable ages according to the already existing curve. For the fathers this means year 3 and year 5 were upped by one percent. For the mothers this meant year 4 was upped by one percent.



Table 2 General input pointer

<b>Program</b>	
Number of years:	50 / 75
Number of repeats	50
<b>Population size</b>	
Number of breeding males	106
Number of breeding females	166
Number of litters per year	135
.. champion sires .. % of the offspring	4 ; 14
<b>Biological data</b>	
Age structure males year 1; year 2; ~ year 11	3; 17; 20; 18; 13; 9; 7; 5; 4; 3; 1
Age structure females year 1; year 2; ~ year 11	2; 15; 23; 23; 15; 13; 8; 1; 0; 0; 0
Age female first litter (months)	24
Litter size 1 newborn; 2 newborn ...	5; 5; 7; 10; 14; 18; 17; 12; 8; 3; 1
<b>Breeding policy</b>	
Exclude mating ..	1 generation

Table 3 Overview of different simulated scenarios

	Amount of outcrosses	Interval in years	Amount of backcrosses
Scenario 1	1	50	0
Scenario 2	5	50	0
Scenario 3	20	50	0
Scenario 4	1	5	0
Scenario 5	5	5	0
Scenario 6	20	5	0

## 4. Results

### 4.1 Retriever analysis whole population

The number of ancestral generations completely known increased to 5 from 1967 to 1989 (figure 1). Since 1990 almost all dogs have more than 5 known generations. However, if we look at the average generation equivalent in figure 2, it shows a constant increase in known generations. Since 2018, the average generation equivalent has increased from 15.6 to 16.4 in 2022.

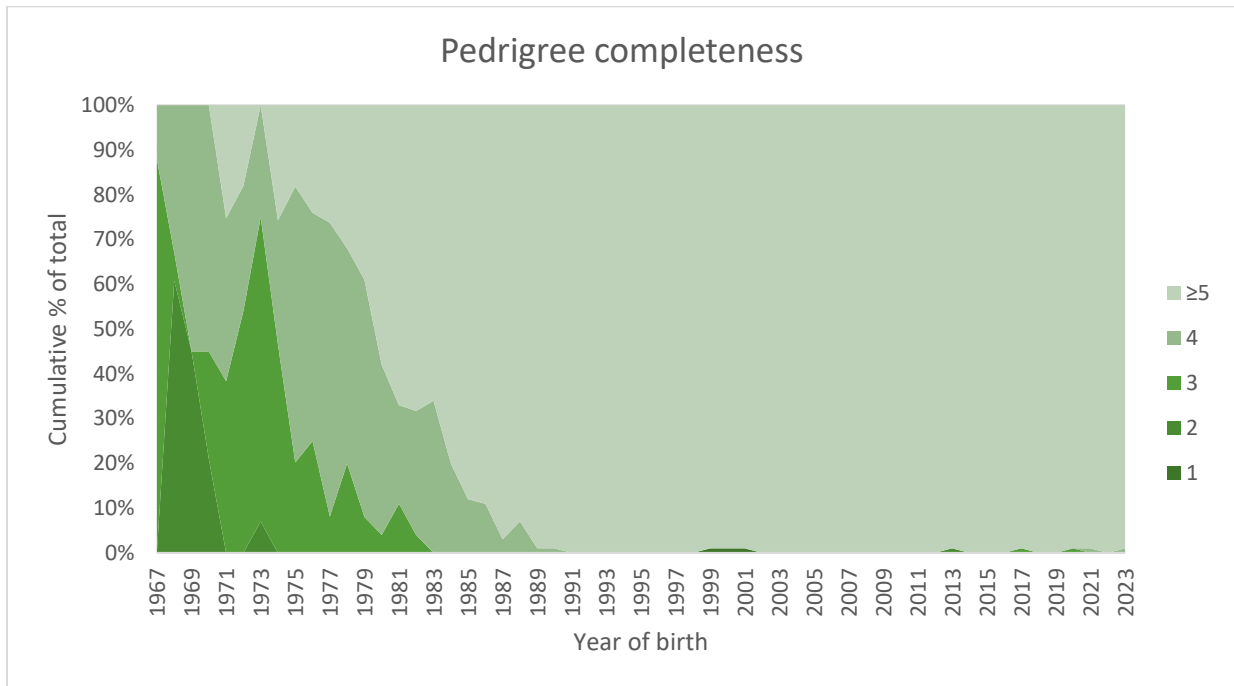


Figure 1 Percentage of dogs with a number of ancestral generations fully known per year of birth

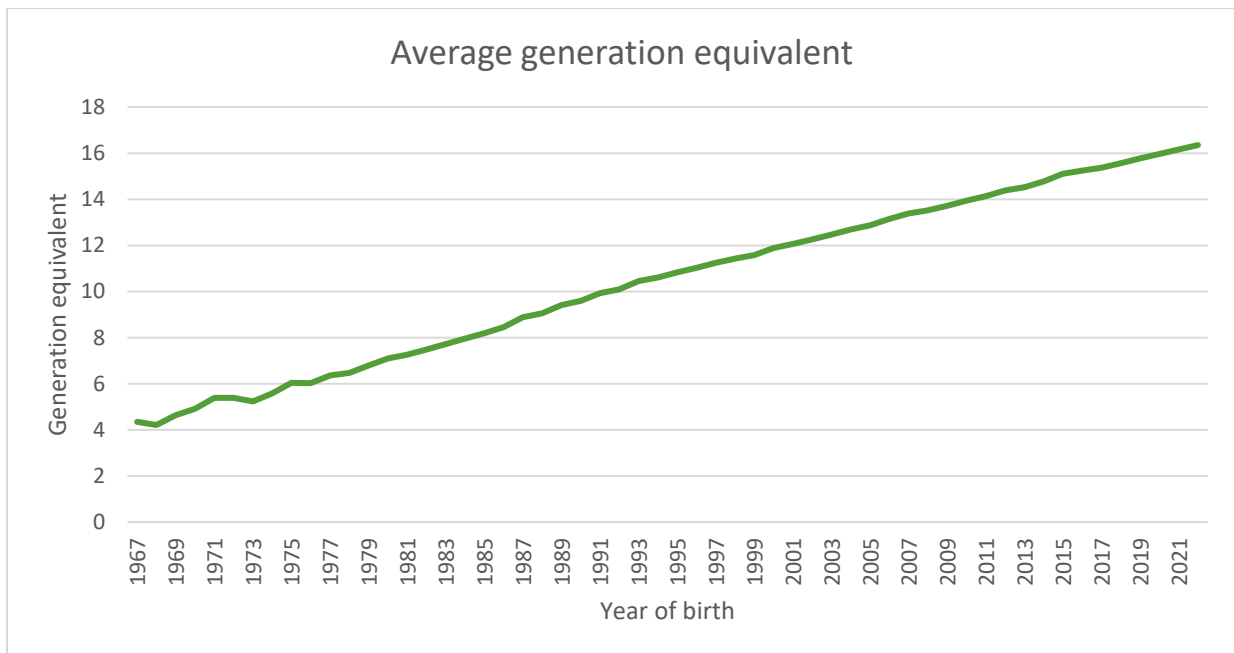


Figure 2 Average generation equivalent of dogs born in a certain year

The number of dogs born per year gradually increased since 1980 but stabilized at around 900-1000 in 2002 (figure 3). From 2016 to 2020 the number of dogs born per year decreased to between 600-800. In 2021 however, an increase to around 900 can be seen. Thus, Figure 3 shows a lot of fluctuation in the last 6 years. The number of animals used for breeding also slightly decreased. In 2013, 15.8% of females and 9.6% of males have later been used for breeding. In 2018, 12.6% of females and 9.5% of males have later been used for breeding. This coincides with the decrease in total born dogs.

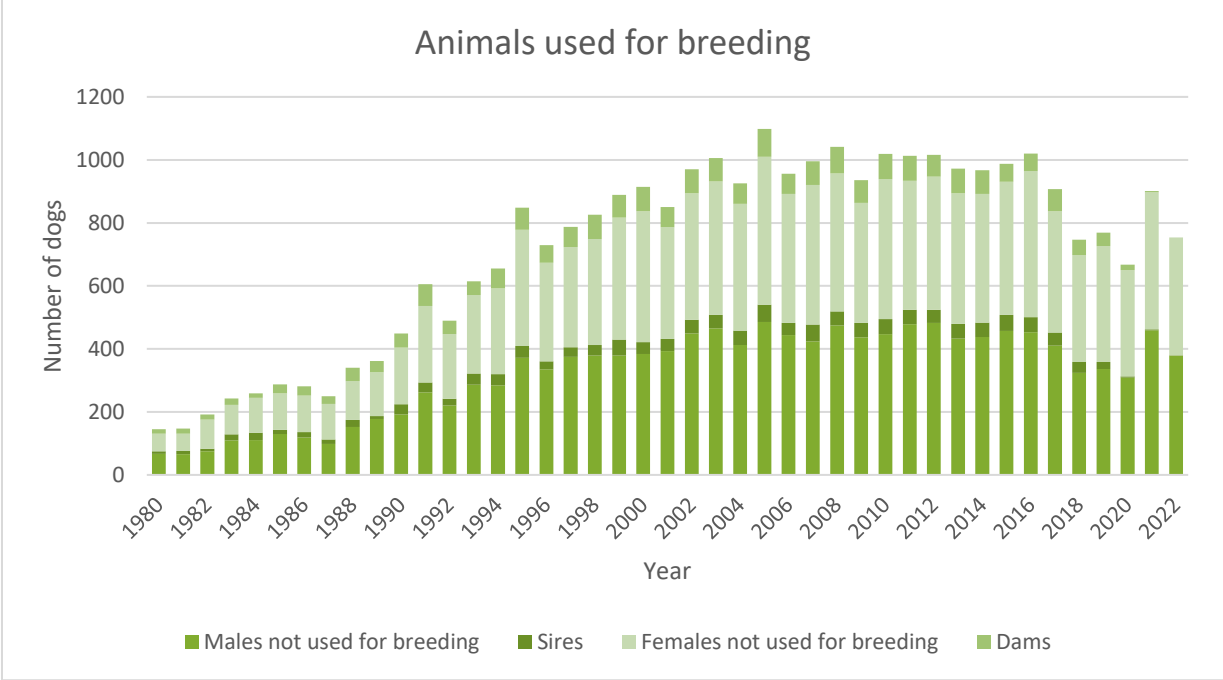


Figure 3 Number of breeding and non-breeding animals born per year

Figure 4 shows an increasing number of sires from 1980 to 2012 after which it stabilized around 110-120 sires. However, from 2016 to 2020 a decrease in number of sires can be observed, followed by an increase of 35.5% in 2021 compared to 2020. In 2022 the number of sires decreased again with 28.7% when compared to 2021. Again, like the total number of dogs born, there has been fluctuation in the last 6 years.

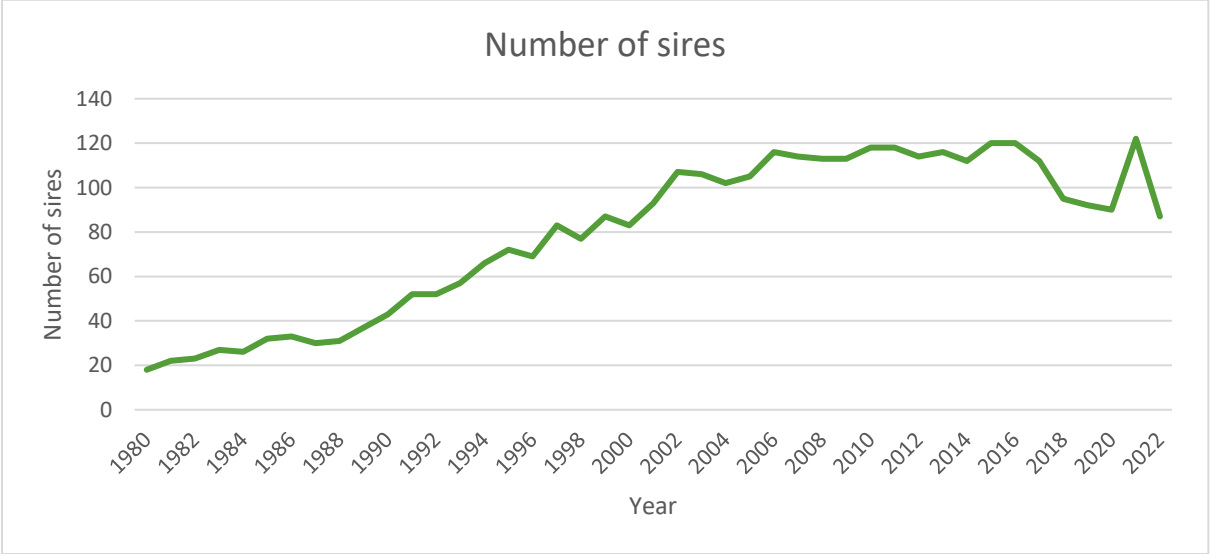


Figure 4 number of sires each year

The average litter size (figure 5) has been relatively stable between 5.5 and 6.5 for the last 39 years. From 2018 to 2022 the average litter size made a relatively large increase, but it is still below 6.5 and stable.

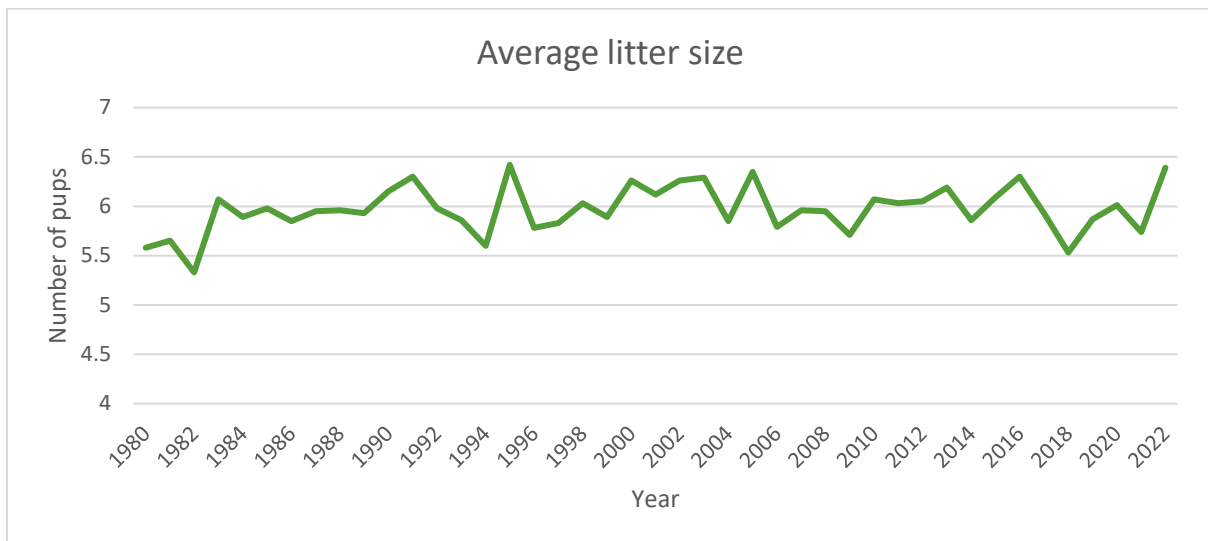


Figure 5 Average litter size per year

The number of litters increased gradually from 1980 to 2002 after which it stabilized for about 14 years (figure 11). From 2016 to 2020 however, the amount of litters has decreased by 31.5%. From 2020 to 2021 the amount of litters increased again. This coincides with the decrease in amount of fathers and decrease in animals born, found earlier.

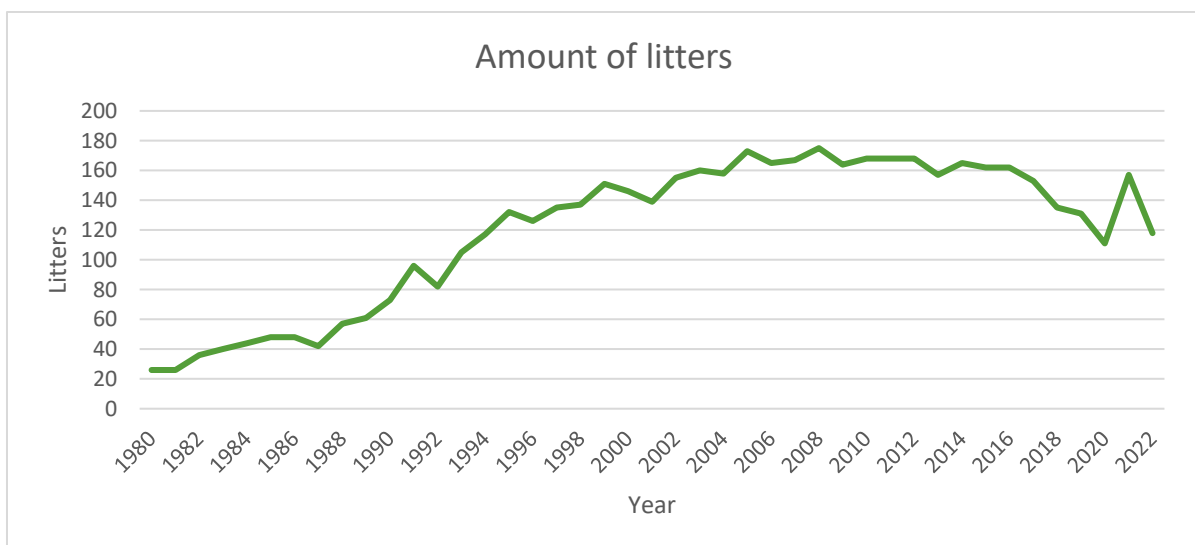


Figure 6 Amount of litters per year

In 1980 the top 10 sires contributed to about 80% to the next generation (figure 7). This contribution decreased until 2002 after which it stabilized around 20-30%. As the number of sires each year is around 100, ideally every sire would have about a 1% contribution to the next generation. In this case it is not close to 1% and thus it shows sires are not randomly chosen. The distribution among these 10 sires has small changes. In 2016, sire number 1 contributed only 0.5% more than the rest of the top 10 sires on average. In 2022, sire number 1 contributed 1.2% more than the rest of the top 10 sires on average.

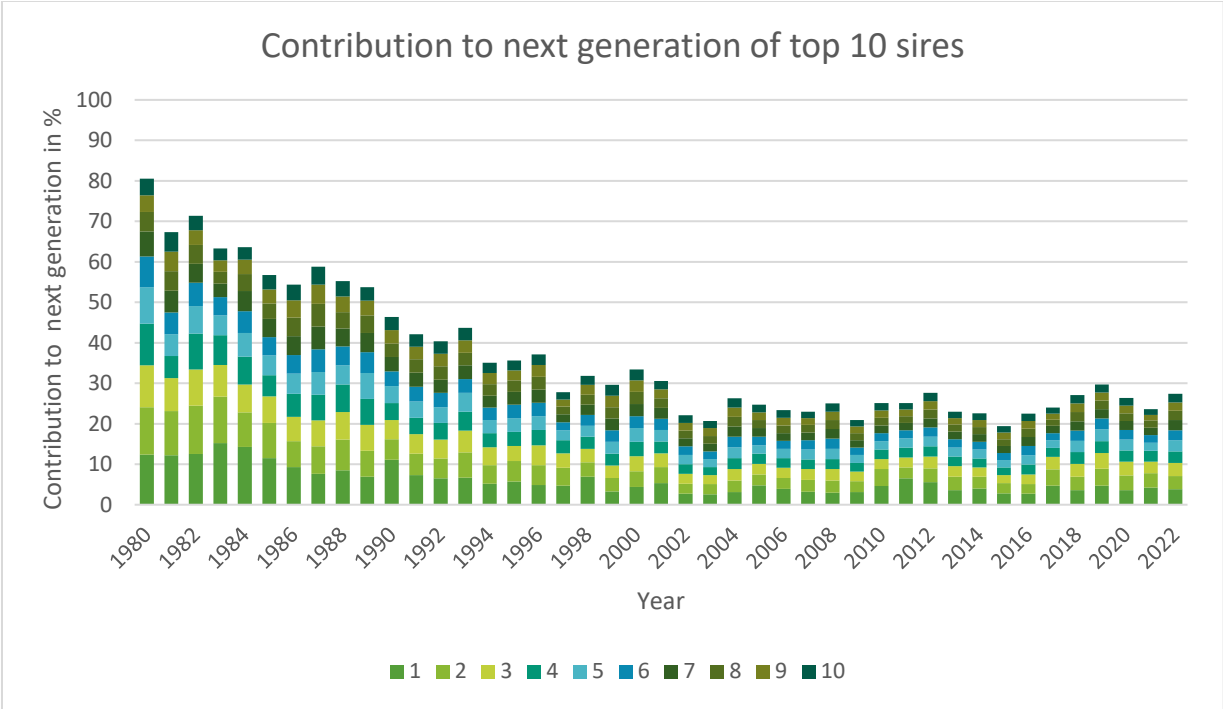


Figure 7 Contribution in percentage of top 10 sires to the next generation

The generation interval is the average age of the sire and dam when their litter is born and can be seen in figure 8. The age of father and mother has some fluctuation. The generation interval of both was stable between 3.5 and 4.5 from 1980 to 1995. After 1995 there was a slight increase after which the generation interval stabilized between 4.5 and 5.5. However, from 2018 to 2022, the average age of the fathers has increased 24.8% while the average age of mothers remained nearly the same.

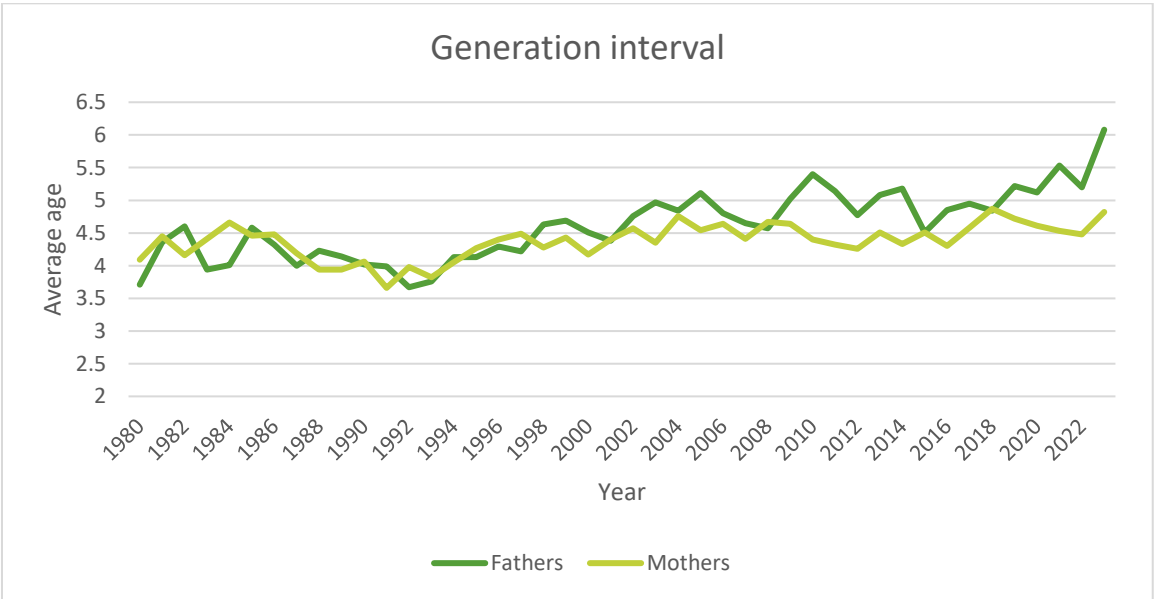


Figure 8 Generation interval per year

The inbreeding coefficient and kinship increased slowly and did not differ a lot from each other (figure 9). Currently both values are just below 0.30%. If we look at the changes from 2002 to 2022, the inbreeding coefficient increased 5.3% and the kinship increased 5.5%. Both values were unstable before 1997 but have stabilized since then. In the last 5 years no large changes are visible.

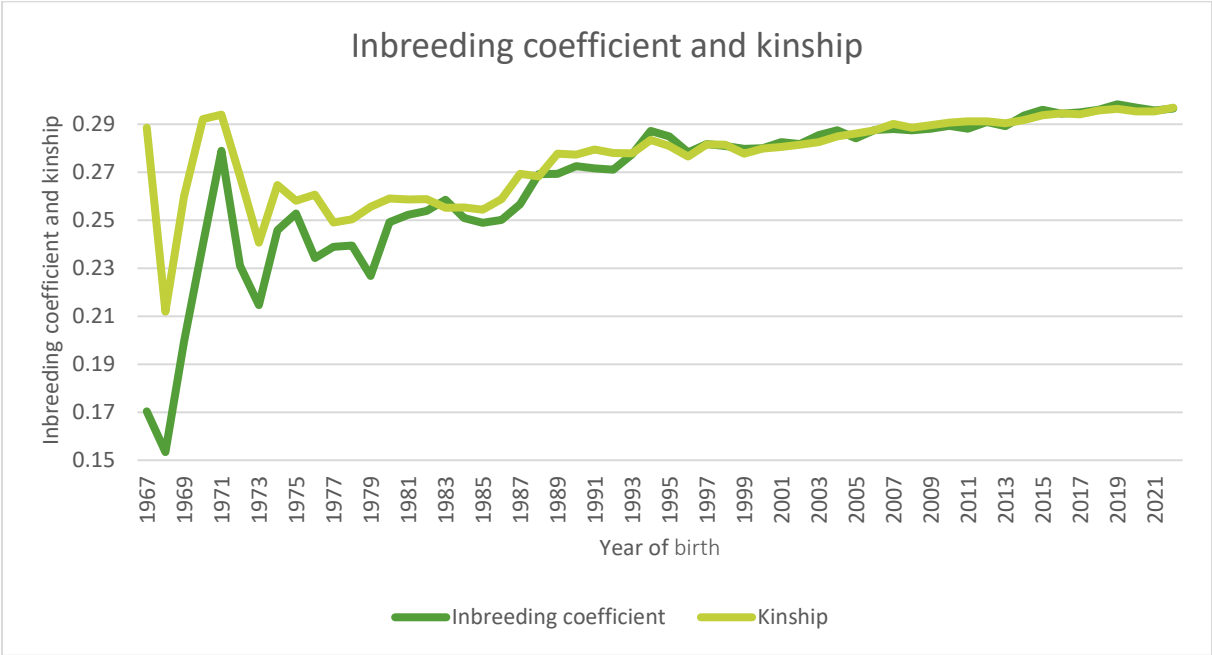


Figure 9 Inbreeding coefficient and kinship per year

The inbreeding rate was estimated for 4 periods (figure 10). A clear overview of the different inbreeding rates per period is given in table 4. As can be seen from the table, the inbreeding rate has decreased.

Table 4 Inbreeding rate per period of time

Period (year)	Inbreeding rate
1967 – 1980	2.59%
1980 – 1998	1.36%
1998 – 2017	0.51%
2017 – 2022	0.11%

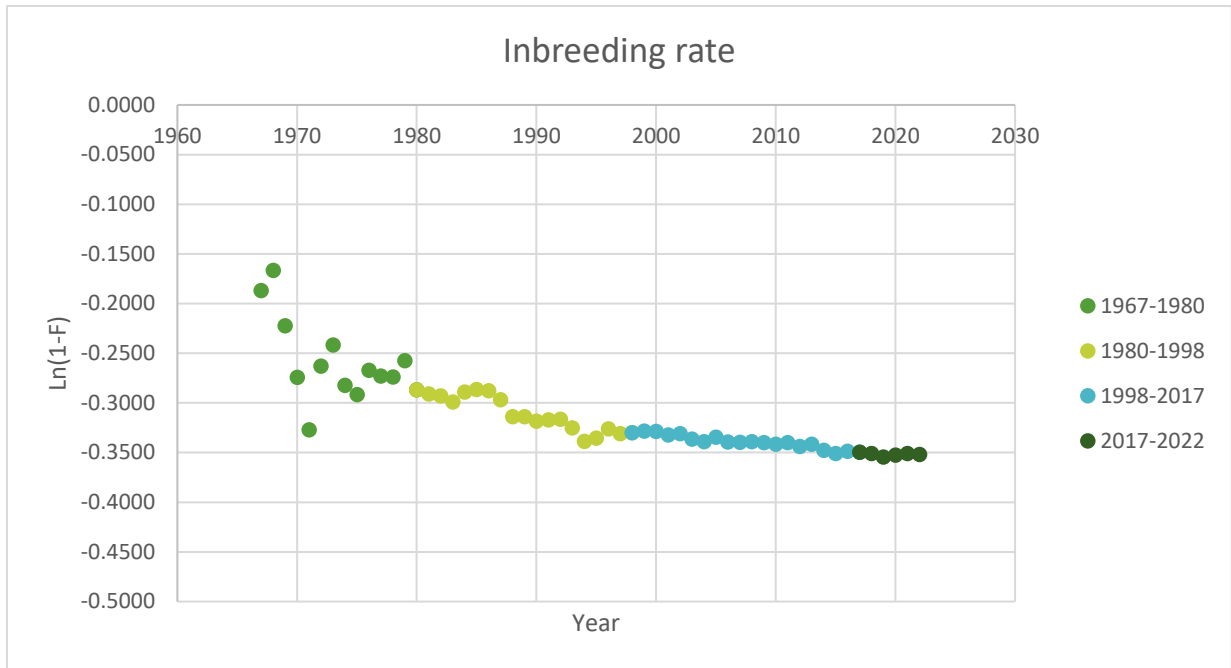


Figure 10 Natural logarithm of 1-F, plotted against time in years. (based on a generation interval of 4.57 years)

The mean kinship of the fathers is calculated and visualized for 2022 and 2023 in figure 11, figure 12 and table 4. The difference between the average mean kinship of fathers in 2022 and 2023 is 0.001 and thus no large change can be observed.

Table 5 Mean kinship fathers 2022 and July 2023

Mean kinship fathers	
2022	0.294
2023	0.295

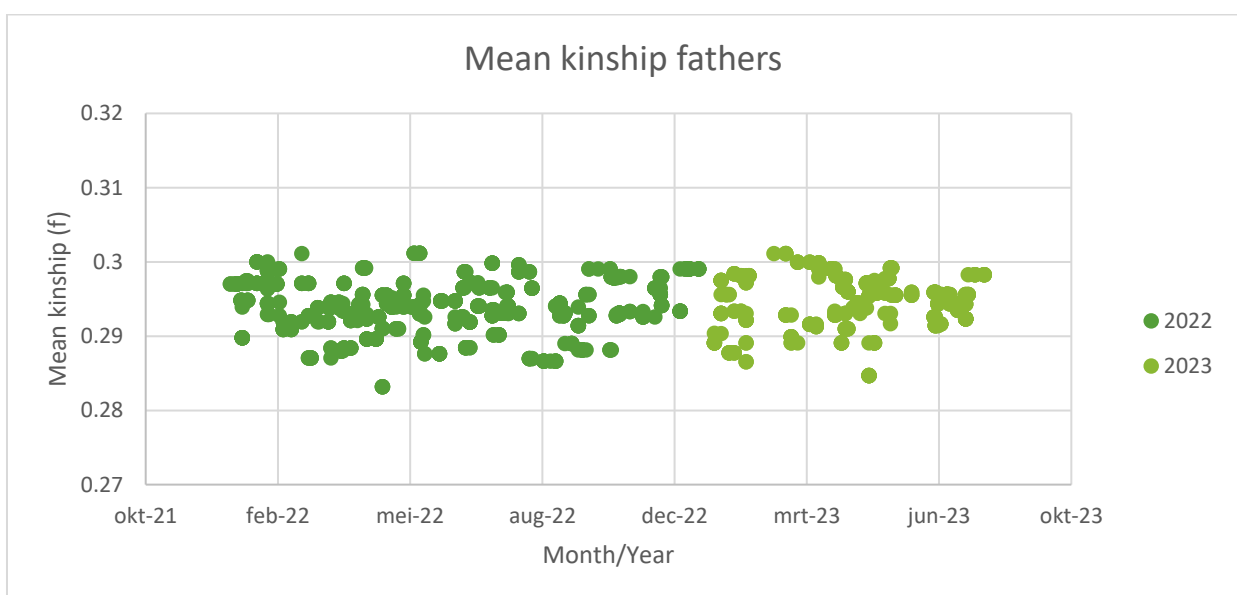


Figure 11 Mean kinship fathers 2022 and July 2023 with trendline

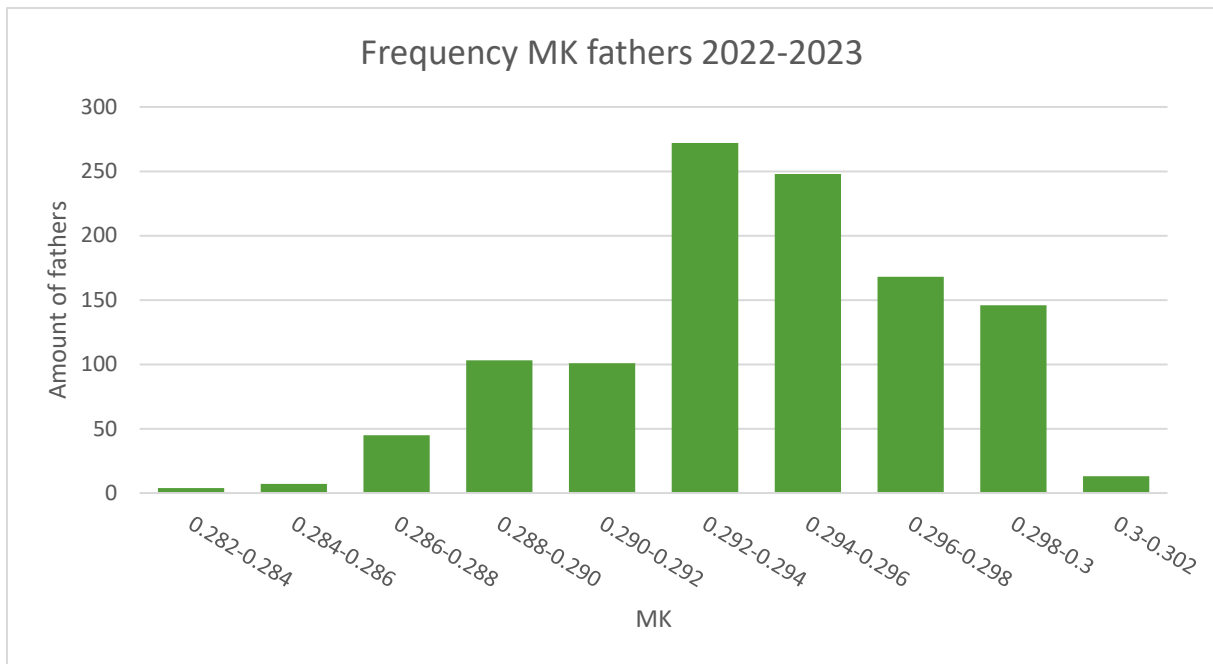


Figure 12 MK fathers 2022 - July 2023

As we can see from figure 13, the mean kinship of the fathers slightly higher for both 2010-2023 and 2022-2023 when compared to the mothers. However, mean kinship is a dynamic value and this can change in the future.

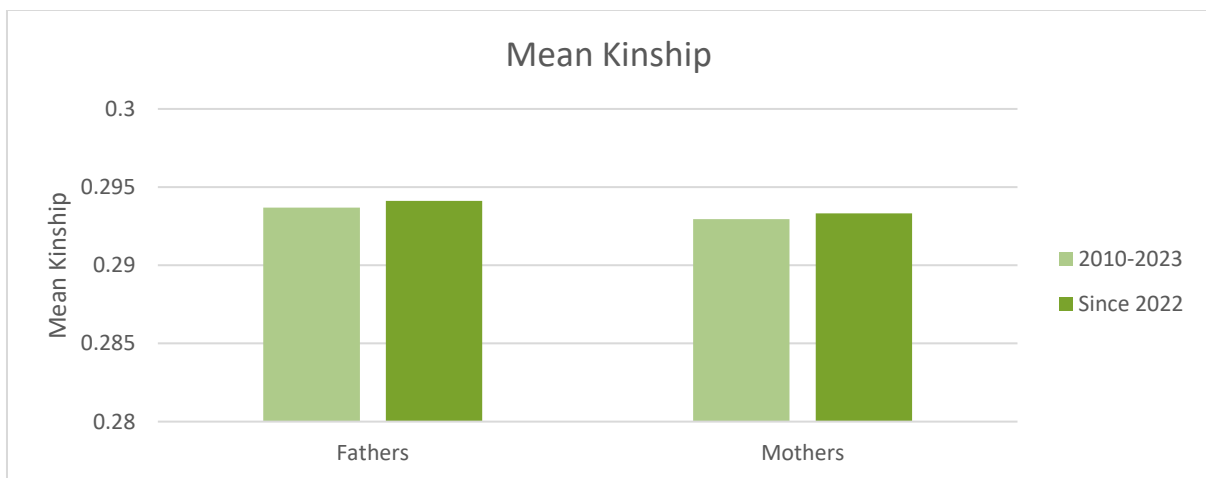


Figure 13 Mean kinship of whole population, fathers and mothers sorted by years



## 4.2 Retriever analysis subpopulations

In figure 14 the subpopulations are shown in percentages of the total population. When compared to 2017 (Stilting, 2018) in figure 15, there are some changes. The Netherlands had a 30% share in 2017 which has increased to 44% in 2023. With a 44% share of the total population, The Netherlands is still the largest subpopulation in 2023. In 2017, Sweden had a 22% share but has now decreased to 11% of the total population. Other subpopulations have had small changes in the last 5 years.

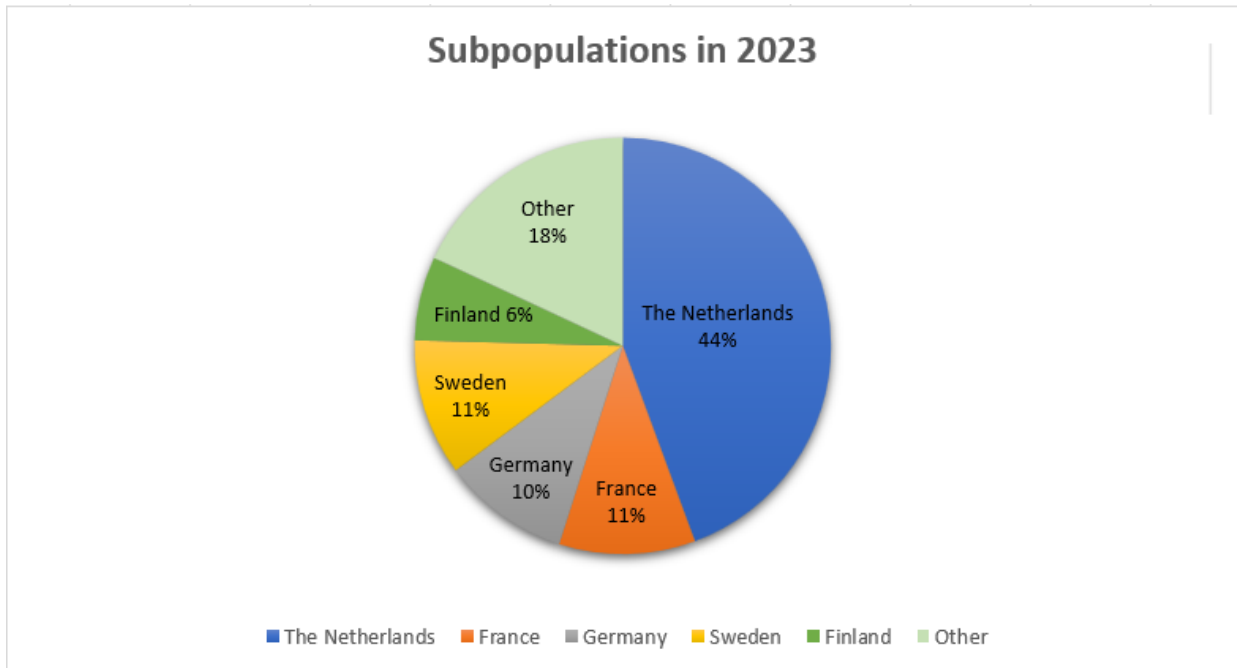


Figure 14 Subpopulations in % for 2023

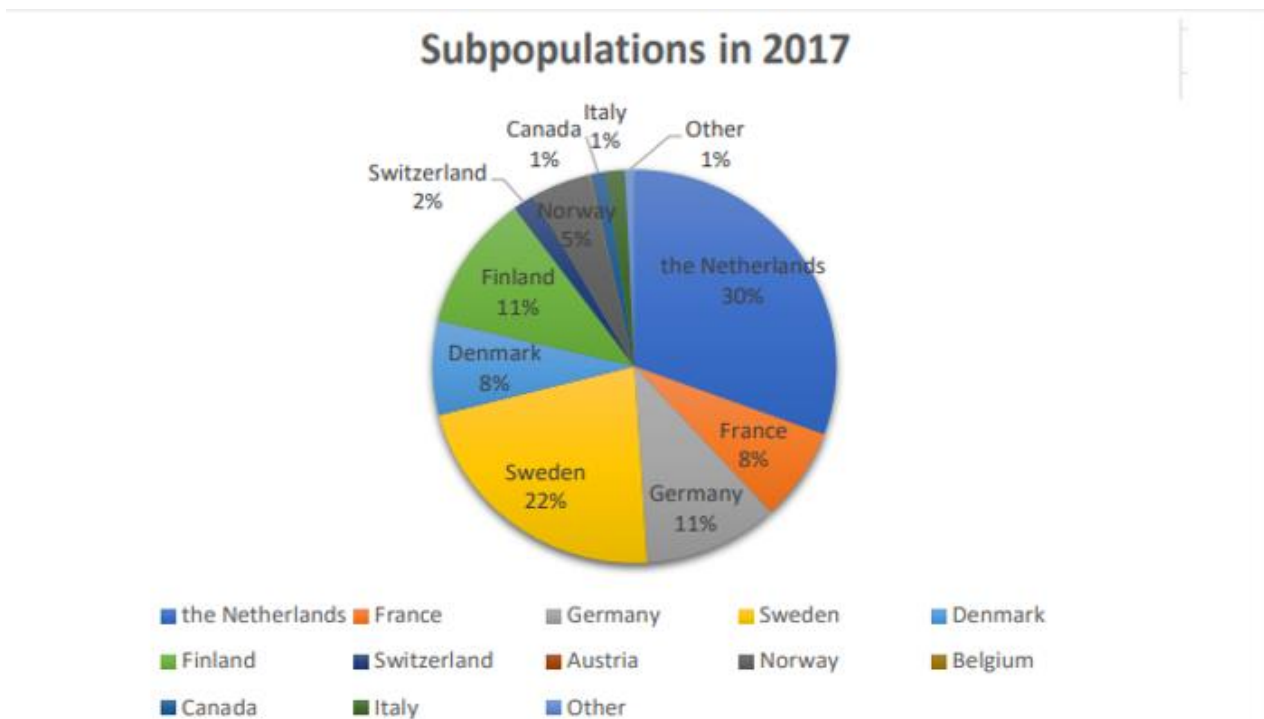


Figure 15 Subpopulations in % for 2017 copied from: (Stilting, 2018)

In figure 16 inbreeding coefficient and kinship are visualized for each subpopulation. The Netherlands has the largest period of time and shows little difference between kinship and the inbreeding coefficient. However, The Netherlands show a slight increase line for both values. Sweden shows slight difference between kinship and inbreeding coefficient. This difference decreased over time and is now almost equal. Germany and Finland show similar lines with noticeable difference between kinship and inbreeding coefficient in the beginning. The values of both subpopulations have stabilized over time and are now close and parallel to each other. Subpopulation France shows changing differences between kinship and inbreeding coefficient. From around 1994 to 2007 both values were very close. From 2008 however, the values have diverted from each other.

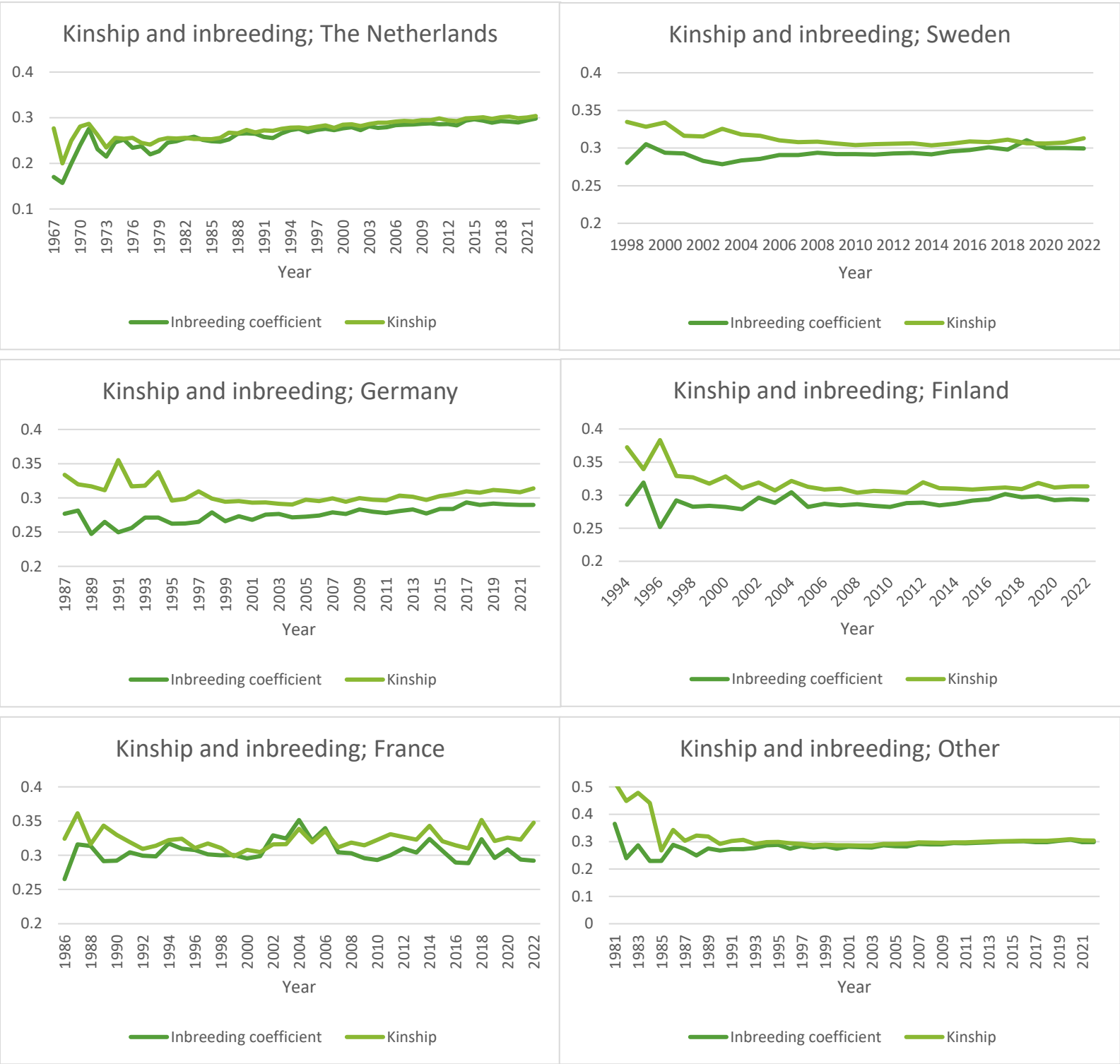


Figure 16 Inbreeding coefficient and kinship for 5 largest subpopulations and other

Figure 17 clearly show the difference in inbreeding coefficient of the different subpopulations. Most subpopulations show instability in the beginning of their data and stabilize over time. France however shows instability for a longer period when compared to the other subpopulations. From 2018 to 2023 all subpopulations seem to stabilize to about equal values.

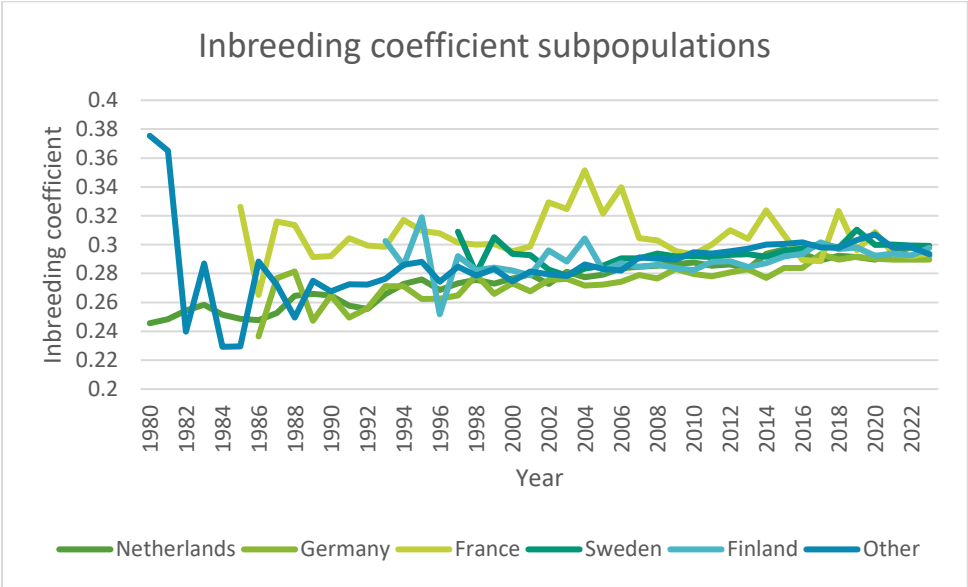


Figure 17 Inbreeding coefficient subpopulations

## 4.3 Pointer simulations

### 4.3.1 Simulation without outcross, comparing the use of kinship constraints to no constraints

The variation of the inbreeding coefficient after 50 years in all 50 simulations without mean kinship constraints is 0.04, ranging from 0.057 – 0.097 (figure 18). The variation of the inbreeding coefficient after 50 years in all 50 simulations when mean kinship constraints are used is 0.007, ranging from 0.023 – 0.030 (figure 19). A noticeable difference is that with the use of mean kinship the average inbreeding coefficient starts increasing after about 19 years while without the use of mean kinship it increased after 3 years.

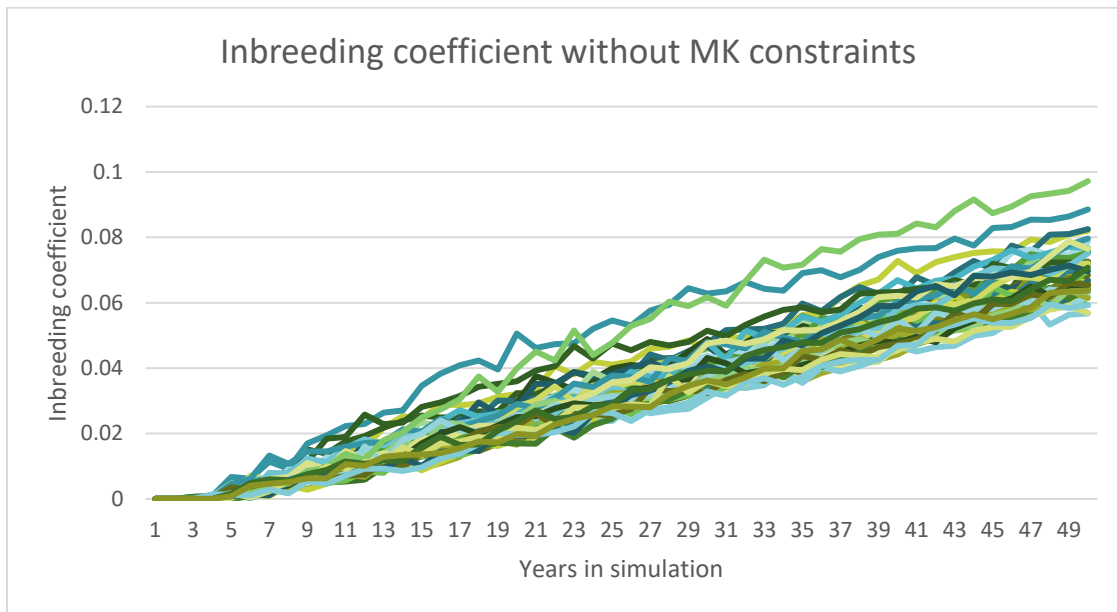


Figure 18 Inbreeding coefficient of 50 simulations without MK constraints

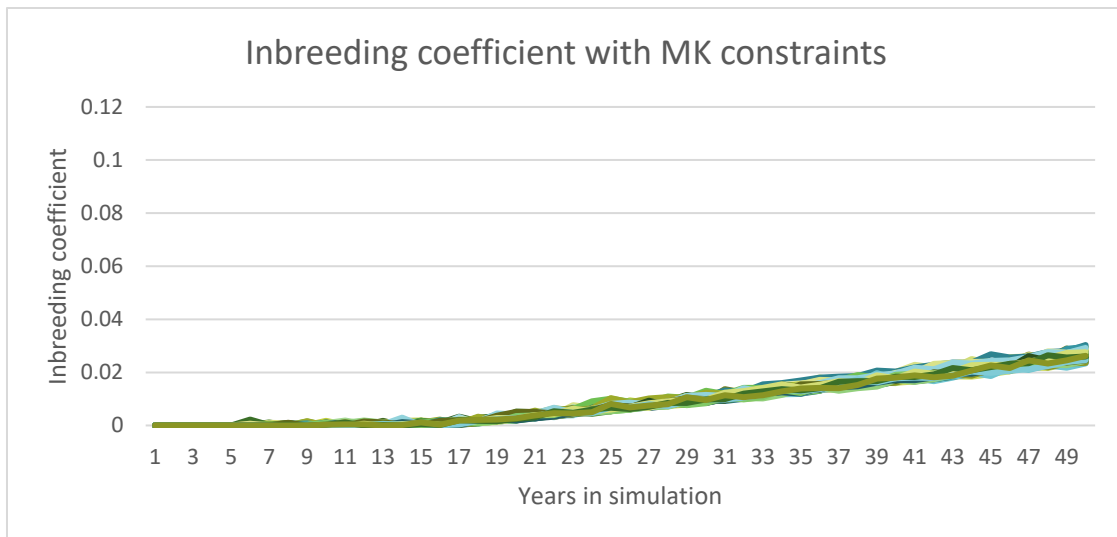


Figure 19 Inbreeding coefficient of 50 simulations with MK constraints

The mean of the inbreeding coefficient with MK constraint is noticeably lower at 0.025 than the inbreeding coefficient without MK constraints, 0.067 (figure 20)

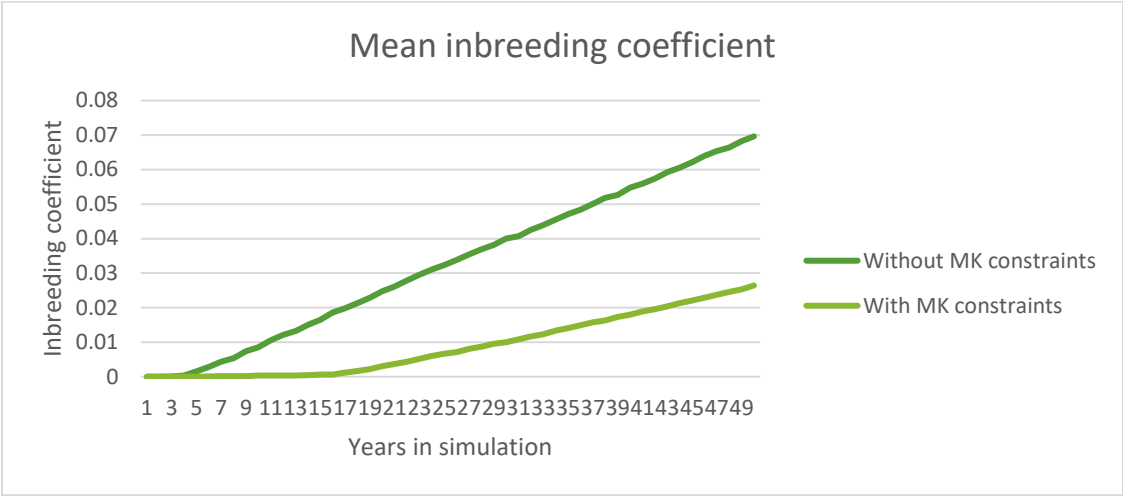


Figure 20 Mean inbreeding coefficient of 50 simulations with and without MK constraints

4.3.2 Simulation with outcross

If we compare the scenarios in figure 21 with each other, we can clearly see the changes when the number of outcrosses is increased. With 1 outcross after 50 years, the inbreeding coefficient is decreased by only 0.003. When number of outcrosses is increased to 5, after 50 years (scenario 2), the decrease in inbreeding coefficient is noticeably larger. The level of inbreeding coefficient is 0.126 and is thus lower after 75 years of simulation. When the number of outcrosses is increased further to 20, (scenario 3) the decrease is again larger. The inbreeding coefficient after 75 years is 0.118.

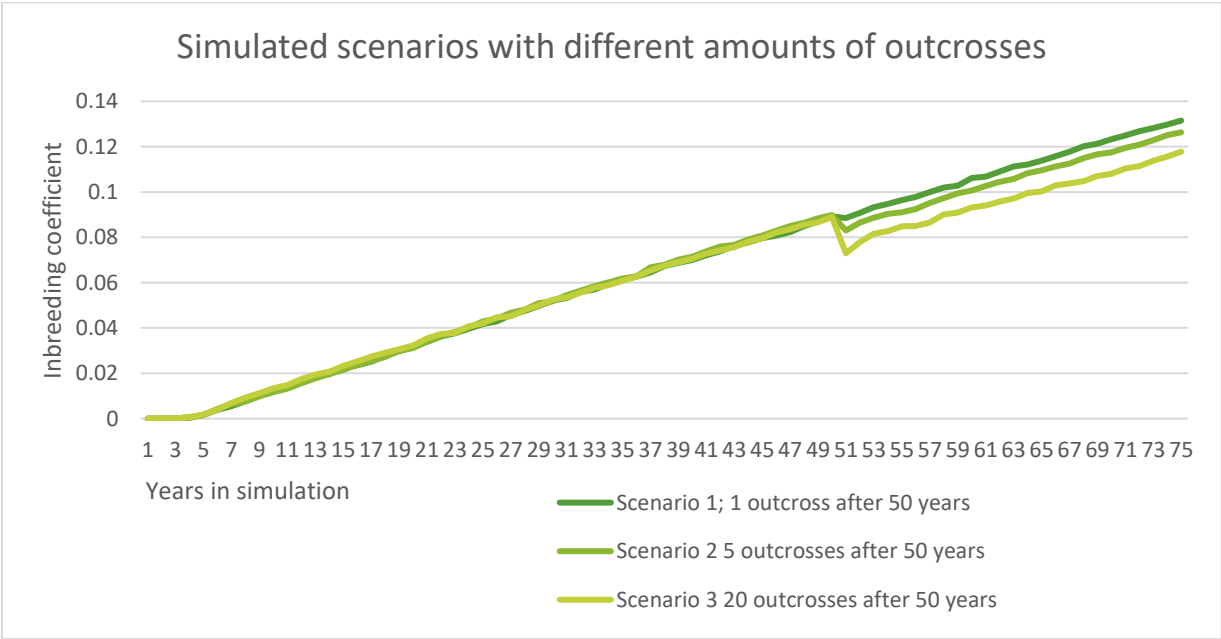


Figure 21 Pointer simulation, scenario 1 (1 outcross after 50 years), 2 (5 outcrosses after 50 years) and 3 (20 outcrosses after 50 years)

In scenario 4, the interval is lowered. When we look at figure 22, we can see the effect of a decreasing interval, together with the increase in performed outcrosses. The inbreeding coefficient after 75 years is 0.131 in scenario 1 (figure 20) and in scenario 4 it is 0.116 (figure 21). In scenario 5 and 6, outcrosses are increased again with the lower interval. In scenario 3 (figure 20), the inbreeding coefficient after 75 years is 0.118 and in scenario 6 it is 0.073 (figure 21). In scenario 6 it is clear that the inbreeding coefficient is lowest when the number of outcrosses is large and the interval is small. Note, however, that backcrosses have not been performed in these simulations.

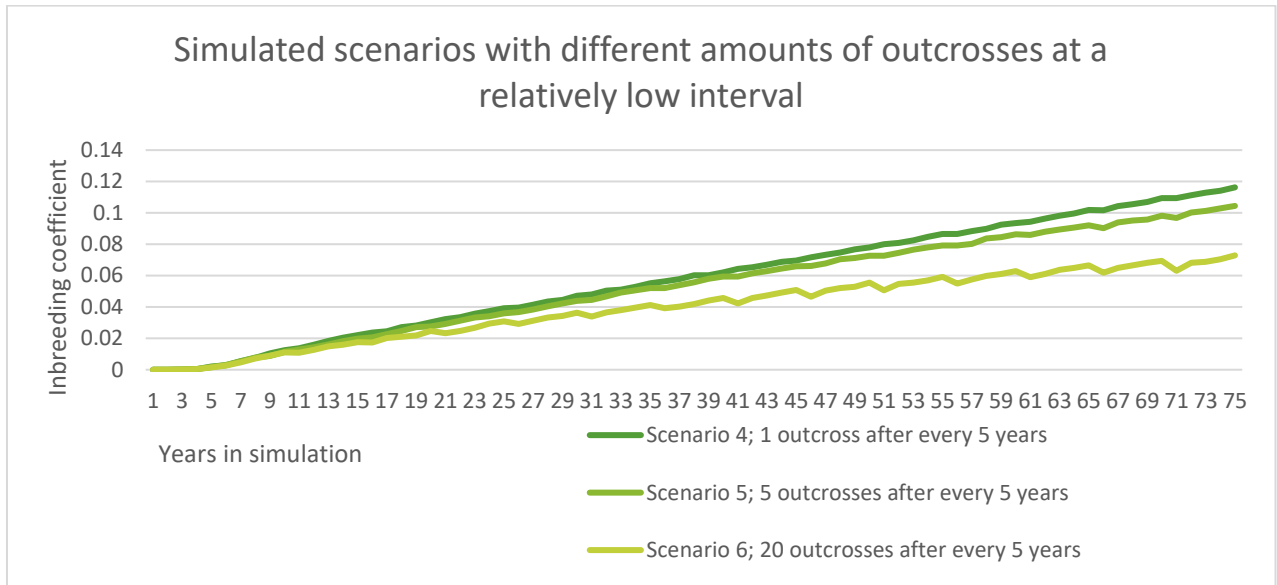


Figure 22 Pointer simulation, scenario 4 (1 outcross every 5 years), 5 (5 outcrosses every 5 years) and 6 (20 outcrosses every 5 years)

In table 6 we can see the inbreeding rate in scenario 1, 2 and 3 barely changes. Performing more outcrosses shows a lower inbreeding rate but it is only a 0.07% change. Scenario 4, 5 and 6 have a significantly lower inbreeding rate than scenario 1, 2 and 3. Achieving a low inbreeding rate is thus possible by performing more outcrosses with a small interval.

Table 6 Inbreeding rate in % of different scenarios

Scenario (outcrosses;interval)	Inbreeding rate year 1 to 50 in %	Inbreeding rate year 52 to 75 in %
1 (1;50)	0.91	0.91
2 (5;50)	0.93	0.89
3 (20;50)	0.91	0.83
		Inbreeding rate year 1 to 75 in %
4 (1;5)		0.78
5 (5;5)		0.70
6 (20;5)		0.46

## 5. Discussion

### Pedigree analysis

The current inbreeding rate of the Schapendoes is 0.11%. Compared to the inbreeding rate of 0.51% between 1998 and 2017, the inbreeding rate has notably dropped. It is unclear if this is due to the addition of Mean Kinship in the breeding policy. In table 4 it was calculated that the mean kinship of fathers in 2022 was 0.294 and in 2023 0.295. In the frequency diagram (figure 12), it is shown that there are quite a large number of fathers with a mean kinship of 0.282 – 0.292. The use of the mean kinship policy could therefore likely be improved as fathers with relatively high mean kinship are still used for breeding. The inbreeding coefficient and kinship are both just above 0.29 which is almost equal to the inbreeding coefficient and kinship in 2017. These values have thus been stable since 2017. It was expected that with using mean kinship that the inbreeding coefficient would decrease or be equal. If the mean kinship was not used, the inbreeding coefficient and inbreeding rate would likely be higher (Stilting, 2018)

In a study on the effect of inbreeding, the inbreeding coefficient of 227 different breeds were calculated (Bannasch et al., 2021). The inbreeding coefficient was calculated using heterozygosity levels in DNA analyses. This value cannot be directly compared with the inbreeding coefficient calculated in this thesis because of a different method. However, they did calculate an adjusted inbreeding coefficient to make the values comparable to the method of using the studbook. The Schapendoes was included in the research and had an (adjusted) inbreeding coefficient of 0.294. The average inbreeding coefficient among these breeds was 0.249. The inbreeding coefficient of the Schapendoes was thus higher than the average of the other breeds. If we compare the adjusted inbreeding coefficient, 0.294, to the inbreeding coefficient of this thesis, 0,29, comparable results can be seen.

Study on the inbreeding rate of all breeds recognized by the UK Kennel Club (common and rare breeds), showed a common trend (Lewis et al., 2015). In the early period (1980's) of all breeds, inbreeding rate was high. From 2000 onwards, the inbreeding rate tended to decline. This is possibly caused by the increased interest in inbreeding and the health of breeds. After analyzing the inbreeding rate for the Schapendoes in this thesis, comparable results are seen. From 1967 to 1980 the Schapendoes had an inbreeding rate of 2.59% and from 1980 to 1998 it was 1.36%. From 1998 to 2017 it was 0.51% and from 2017 to 2022 it was 0.11%.

What is noticeable however, is the fluctuation in the total number of dogs born per year from 2018 to 2022. A relatively large decrease was visible. If this decrease in dogs born per year is related to the addition of Mean Kinship in the breeding policy of the Schapendoes is unclear. The increasing amount of rules and guidelines could be a reason for breeders to breed outside of the pedigree. Meaning the offspring are not officially from the Schapendoes breed. Another possible explanation could be the influence of fashion (social influence). Ghirlanda et al. (2013) showed that fashion has been more important for breed numbers than function (behavior, longer life, genetic disorders etc.). If other breeds become popular, or for example large breeds or long-haired breeds become less popular, the demand of the Schapendoes might decrease.

Among the different subpopulations two noticeable differences were seen. First, the inbreeding coefficient and kinship of the different subpopulations have all stabilized over time except for France. France is one of the largest subpopulations but both values have been relatively unstable. Second, in most subpopulation the kinship was noticeably higher than the inbreeding coefficient. This could be explained by the fact that there is still exchange between the different populations. This is because dogs in different subpopulation will be less related to each other compared to dogs inside the same subpopulation. As the kinship is an estimation calculated within each subpopulation, importation of dogs from another subpopulation with a lower kinship will lead to an inbreeding coefficient with a lower value than the within subpopulation kinship. because of exchange between subpopulations the inbreeding coefficient has a lower value than the kinship.

## Outcross analysis

With the results of the pedigree analysis the indicators for a possible need of an outcross can be assessed. As the average litter size and the inbreeding values show no concerning changes or values, it suggests there is currently no large and irreversible inbreeding depression. Therefore the use of an outcross is not necessary. Also, the relatively low occurrence of genetic defects does not suggest the use of an outcross. An example of using outcrossing can be seen in the Saarlooswolfhond (Prosmán, 2018). The inbreeding coefficient and inbreeding rate in this breed were extremely high when compared to other breeds. As a result of the high inbreeding rate and coefficient, the litter size decreased to a point where a litter contained only 1 or 2 pups, versus 10 to 12 pups before the high inbreeding rates, and the breed was heading towards extinction. The association implemented an outcross program and the breed is improving, which can be seen by the lowered inbreeding rate and increased litter size. To tackle the expected phenotypic changes, they used dog breeds that have some similarities in appearance while being unrelated.

Based on simulations with different numbers of outcrosses, it can be concluded that a small number of outcrosses or outcrosses with a long interval would have barely any effect. The larger the number of outcrosses and the smaller the interval, the lower the inbreeding coefficient. The problem arising with doing a lot of outcrosses is the phenotypic change that the Schapendoes would undergo. Backcrosses can be used to minimize the phenotypic change but this will be at the expense of the outcross effect on inbreeding (Windig & Doekes, 2018). In this research backcrosses were not implemented in the simulations, while they have a large impact depending on how many backcrosses are performed. Each backcross will lower the effect of the outcross on inbreeding with 50%. For example, after three backcrosses the effect of the outcross will be only 12.5% of the original effect the outcross would have. Research on the use of different outcross strategies showed that an outcross can decrease the frequency of lethal alleles and can thus decrease the frequency of genetic defects (Windig & Doekes, 2018). The same research also noted that an outcross can be useful for certain strategies but an outcross had a limited effect on inbreeding rate and inbreeding coefficient after the outcross. Especially when backcrosses were performed after the outcross, the effect on inbreeding levels was small. In this thesis similar results were found. From this it can be said that an outcross would be best suited in the case of an emergency or if the breeding association is open for large phenotypic changes in the breed. In other words, the breeders have less interest in what the dog looks like.

The inbreeding coefficient was also simulated with and without the use of Mean Kinship in the breeding policy. In a situation where Mean Kinship is used ideally, the inbreeding rate is lower than with the use of 20 outcrosses every 5 years. In reality however it would be hard to copy the results of this mean kinship simulation. But, if there is put effort in the use of low Mean Kinship dogs when breeding, the effect would most likely be similar or even better than the use of 20 outcrosses every 5 years. A lower inbreeding would then be achieved without causing phenotypic changes. It should be noted that these results are based on simulations and that in practice results may differ. To lower the impact of the randomization, 50 runs were performed after which the mean was calculated.



## 5.1 Conclusion

The aim of this thesis is to give advice to the breeding association of the Nederlandse Schapendoes. From the results of this thesis it can be concluded that the Schapendoes breed is doing decently well. The breed does have an above average inbreeding coefficient when compared to other breeds but the inbreeding rate is low. The breeding association has to continue the effort to keep the inbreeding rate low. If the association stops using certain measures in the breeding policy, the inbreeding rate may rise again and the breed could worsen. Regarding the use of an outcross, it can be concluded that there are no indicators suggesting a severe inbreeding depression and thus the use of an outcross is not necessary. An outcross would be most suitable in a case of emergency or if changes in phenotype are acceptable. When compared to the use of mean kinship, a very large amount of outcrosses has to be done with a small interval to achieve similar results. Therefore my advice to the breeding association of the Schapendoes is as follows: try to use the Mean Kinship policy as much as possible and in a correct way. If this is used correctly, the breed can be kept in a genetically good condition without altering the phenotype. I would not recommend the use of an outcross at this moment as there are no indicators suggesting that it is necessary. However, if litter sizes significantly decrease in the future or if more genetic defects arise, outcrossing could be a solution to these problems.

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