ORIGINAL ARTICLE

WILEY *Journal of* Animal Breeding and Genetics

Reducing inbreeding rates with a breeding circle: Theory and practice in Veluws Heideschaap

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Funding information
Ministry of agriculture, the Netherlands,
Grant/Award Number: WOT 03-003-056

Abstract

Breeding circles allow genetic management in closed populations without pedigrees. In a breeding circle, breeding is split over sub-populations. Each sub-population receives breeding males from a single sub-population and supplies breeding males to one other sub-population. Donor-recipient combinations of sub-populations remain the same over time. Here, we derive inbreeding levels both mathematically and by computer simulation and compare them to actual inbreeding rates derived from DNA information in a real sheep population. In Veluws Heideschaap, a breeding circle has been in operation for over 30 years. Mathematically, starting with inbreeding levels and kinships set to zero, inbreeding rates per generation (ΔF) initially were 0.29%–0.47% within flocks but later converged to 0.18% in all flocks. When, more realistically, inbreeding levels at the start were high and kinship between flocks low, inbreeding levels immediately dropped to the kinship levels between flocks and rates more gradually converged to 0.18%. In computer simulations with overlapping generations, inbreeding levels and rates followed the same pattern, but converged to a lower ΔF of 0.12%. ΔF was determined in the real population with a 12 K SNP chip in recent generations. ΔF in the real population was 0.29%, based on markers to 0.41% per generation based on heterozygosity levels. This is two to three times the theoretically derived values. These increased rates in the real population are probably due to selection and/or the presence of dominant rams siring a disproportionate number of offspring. When these were simulated, ΔF agreed better: 0.35% for selection, 0.38% for dominant rams and 0.67% for both together. The realized inbreeding rates are a warning that in a real population inbreeding rates in a breeding circle can be higher than theoretically expected due to selection and dominant rams. Without a breeding circle, however, inbreeding rates would have been even higher.

KEYWORDS
computer simulation, DNA analysis, genetic conservation, local breeds, sheep

1 | INTRODUCTION

Numerically small populations often suffer from high inbreeding rates. These are generally associated with decline in fitness and occurrence of genetic defects, that are often lethal (Lynch & Walsh, 1998; Roff, 1997). In captive populations, effective population size can be managed by choosing breeding animals based on their kinship. In the long run, the most effective strategy is to minimize mean kinship of the population rather than kinship of mating pairs only (Frankham,

Briscoe, & Ballou, 2002). Management based on kinships requires detailed knowledge of relatedness between all animals in the population, and the possibility to decide which animals may mate and how often. However, for populations kept in flocks, herds or other groups such as livestock grazing nature reserves and zoo populations of fish in aquaria, this requires a large effort and is often not possible.

An alternative for kinship-based genetic management is rotational mating schemes for populations divided in sub-populations. Several forms exist, but one of the most efficient and easy to implement is a breeding circle (Windig & Kaal, 2008). In this scheme, the first sub-population always provides sires for the second sub-population, the second always to the third sub-population, and so on, until the last sub-population provides sires for the first sub-population. Rams born in a sub-population are never used as a sire within that sub-population and each sub-population always uses sires from the same donor sub-population. Thus, an animal in sub-population n has a father in sub-population $n-1$ and a mother in sub-population n. Both paternal granddam and maternal grandsire originate from flock n, so their parents may be the same. In other words, the most recent common ancestor can only be a great-grandparent. Inbreeding is restricted because although sires originating from the same sub-population are used each year, their sires stem from yet another sub-population.

Breeding circles have been analysed theoretically and it has proved to be effective. Nomura and Yonezawa (1996) provided a mathematical framework to analyse rotational mating schemes with sub-populations of equal size and non-overlapping generations. They concluded that breeding circles are effective in reducing inbreeding rates in the long run and especially if not more than six large sub-populations are involved. Computer simulations confirmed the effectiveness of breeding circles (Mucha & Komen, 2016), although no direct comparison has been made between results from mathematical calculations and computer simulations. Computer simulations showed that breeding circles are also effective in populations with overlapping generations and unequal sub-population sizes (Windig & Kaal, 2008) and in the presence of selection (Windig, Eding, Moll, & Kaal, 2004). Inbreeding rates for breeding circles in life populations, however, have never been assessed, probably due to a lack of pedigree recording. A similar rotational mating scheme in Moghani sheep from Iran, however, was evaluated with pedigree records and proved to be effective (Mokhtari, Miraei-Ashtiani, Jafaroghli, & Gutierrez, 2015).

This paper aims to compare the effectiveness of a breeding circle in reducing inbreeding rates as assessed by mathematical calculations, computer simulations and in real life by DNA analysis. To do so, the population of Veluws Heideschaap is analysed. In this population, a breeding circle has been in operation since the 1980s. Mathematical calculations and computer simulations are performed, using the flock sizes of the real population. Autosomal DNA of two age cohorts, the oldest ewes and young rams, were used to determine the realized inbreeding rate in the live population.

2 | MATERIAL AND METHODS

2.1 | Breed and breeding circle

Veluws Heideschaap is an old Dutch breed known since the Middle Ages. It was used to roam the extensive moors on unfertile sandy soils in the centre-east of the Netherlands (Veluwe and adjacent areas). It provided meat and wool, but its main function was to provide manure for fertilizing soil. After the arrival of artificial fertiliser, the breed declined and became almost extinct around 1960. It has recovered somewhat, because there is a need for sheep grazing nature reserves and a willingness to conserve cultural heritage. Veluwe is the largest forest area in the Netherlands interrupted by large moors, and provides habitat for large numbers of rare animal and plant species. Nowadays, eight of the moors are grazed by flocks of Veluws Heideschaap and there are approximately 1,500 animals in these eight flocks (Figure 1).

By the end of the 1970s, inbreeding problems became apparent and genetic management was needed. There was no pedigree recording, which is very difficult to realise in a large flock with multiple breeding rams, anyway. In collaboration with the Dutch Rare breeds trust (SZH), a breeding circle was established, which has been used continuously since. The order of the circle in Veluws Heideschaap (Figure 1) has remained the same, but some flocks were added or removed. There is some variation in flock size (Table 1), but all flocks add approximately 1 ram per 20 ewes, at the start of the breeding season.

2.2 | Theoretical calculations

Nomura and Yonezawa (1996) worked out the change in inbreeding over time for a breeding circle in case of non-overlapping generations and equally sized sub-populations (further on called flocks). In that case, the expected inbreeding coefficient in a generation is the same in each flock as are kinships between any two flocks in a generation at the same "distance" in the circle. Consequently, one vector of average kinships and inbreeding coefficients is sufficient to describe the genetic relations between the different flocks. In practice, however, flock sizes are seldom equal. Therefore a matrix is needed with each off-diagonal cell containing the kinship between two different flocks, and the diagonal containing the within-flock kinships. Here, we work out how the average kinship and inbreeding coefficients within and between flocks relate to those in the previous generation, for flocks of different sizes, based on the work of Nomura and Yonezawa (1996).
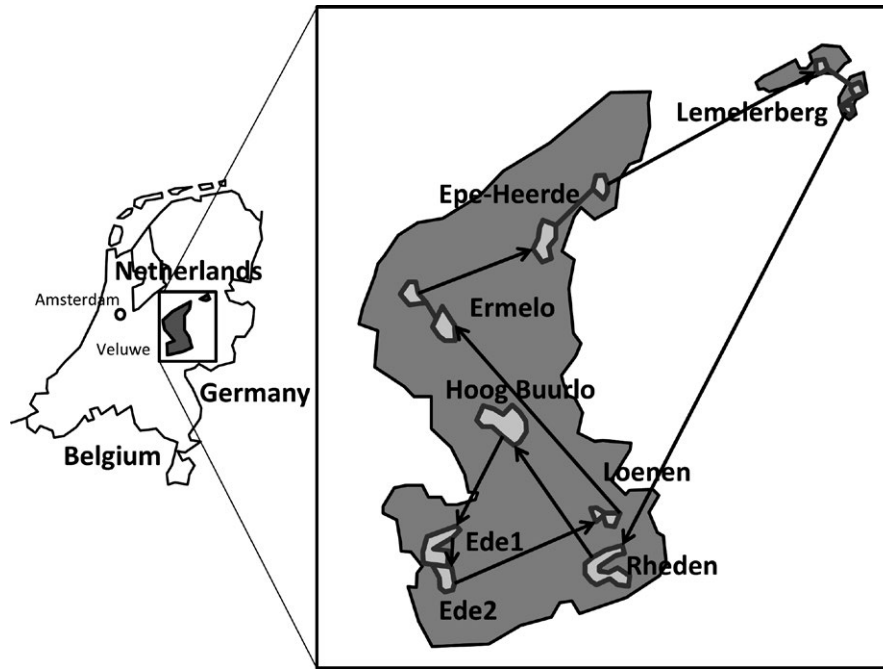
FIGURE 1 Location of flocks of Veluws Heideschaap. Left location of the Veluwe, wooded region in the Netherlands. Right detailed map of the Veluwe, wooded area dark grey, heath fields grazed by a flock of Veluws Heideschaap in light grey, black arrows indicate ram circle, pointing from donor flock of rams to recipient flock

TABLE 1 Flocks of Veluws heideschaap and their size in 2015 and theoretically expected inbreeding rate under random mating

Flock	N_m	N_f	N_e	ΔF_{exp}	F_{20}
Loenen	9	175	34	1.46%	0.244
Ermelo	14	271	53	0.94%	0.164
Epe-Heerde	7	141	27	1.87%	0.302
Lemelerberg	7	110	26	1.90%	0.305
Rheden	9	140	34	1.48%	0.246
Hoog-Buurlo	7	147	27	1.87%	0.301
Ede1	14	279	53	0.94%	0.164
Ede2	9	170	34	1.46%	0.244
Total	76	1,433	289	0.17%	0.032

Note. N_e: effective population size (estimated from N_m and N_f); N_f: number of ewes; N_m: Number of rams; ΔF_{exp}: expected inbreeding rate; F_{20} expected inbreeding level after 20 generations of breeding without exchange between flocks (used as starting level for some simulations)

The average kinship between two flocks is given by

$$f_{x,y(t)} = \frac{1}{4}\left(f_{x,y(t-1)} + f_{x,y-1(t-1)} + f_{x-1,y(t-1)} + f_{x-1,y-1(t-1)}\right) \quad (1)$$

where $f_{x,y(t)}$ is the average kinship between flocks x and y ($x \neq y$) in generation t and $(f_{x,y-1})_{t-1}$ is the average kinship between flocks x and $y-1$ in the preceding generation $(t-1)$. Flock $y-1$ is the flock preceding flock y in the breeding circle, in other words the donor flock of the fathers for y. Similarly, $x-1$ is the father donor flock for flock x, while the mothers originate from the flocks x and y

themselves. In some cases, x can be equal to $y-1$, or y equal to $x-1$, when the mother donor flock is the same as the father donor flock. In these cases, the kinship is the within-flock kinship, excluding self-kinships, because animals in the two different flocks will never have the same parents, since in the one case the parental flock provides fathers and in the other case mothers. To be precise, kinships should then only include male–female kinships, but since there is no reason to expect that these are on average different from male–male and female–female kinships one can use average flock kinships excluding self-kinships.

In the case of the average kinship within a flock, animals may share the same father and/or mother. In that case, an extra term is added to include the contribution of self-kinships of parents to inbreeding and relatedness in the next generation. A self-kinship is equal to $\frac{1}{2}(1 + F(t))$, which has to be multiplied by $1/N_e$ (N_e is the effective population size) to get the contribution to the within-flock kinship in the next generation. N_e is under random mating given by:

$$\frac{1}{N_e} = \frac{1}{4n_m} + \frac{1}{4n_f} \quad (2)$$

With n_m and n_f being the number of males and females in the flock. In a breeding circle, fathers and mothers originate from a different flock with possibly a different F. Consequently, the contribution of self-kinships to within-flock kinship in the next generation becomes:

$$\frac{1}{8n_m}\left(1 + F_{x-1}\right) + \frac{1}{8n_f}\left(1 + F_x\right) \quad (3)$$

n_m in this case is the number of males selected from flock $x-1$ and used in flock x for breeding, which may be different from the number of males used for breeding in flock $x-1$. For the average kinship within a flock not due to self-kinship of the parents formula 1 applies, but since x and y are the same population $f_{x,y}$ becomes f_x, $f_{x,y-1}$ and $f_{x-1,y}$ both become $f_{x,x-1}$ and $f_{x-1,y-1}$ becomes f_{x-1}:

$$f_{x(t)} = \frac{1}{8n_{mx}}\left(1 + F_{x-1(t-1)}\right) + \frac{1}{8n_{fx}}\left(1 + F_{x(t-1)}\right)$$
$$+ \left(1 - \frac{1}{N_{e,x}}\right)\left(\frac{1}{4}f_{x(t)} + \frac{1}{2}f_{x,x-1(t-1)} + \frac{1}{4}f_{x-1(t-1)}\right) \quad (4)$$

The average inbreeding coefficient in flock x is given by

$$F_{x(t)} = f_{x,x-1(t-1)} \quad (5)$$

The calculations were performed for the breeding circle in operation in Veluws Heideschaap. The formulas above were implemented in an excel worksheet and average inbreeding and kinship level within and between flocks calculated for 150 generations. Inbreeding rate was calculated each generation by

$$\Delta F = \frac{F_t - F_{t-1}}{1 - F_{t-1}} \quad (6)$$

2.3 | Calculations in Veluws Heideschaap

Calculations were done using the flock sizes of Veluws Heideschaap, with initial inbreeding levels and kinships of 0. Realistically, however, it is more likely that levels within flocks are high and kinship levels between flocks low, due to limited amounts of exchange between flocks before the ram circle was started. Therefore, calculations were repeated with initial inbreeding and kinship levels above 0. Initial inbreeding level within flocks was set to the level expected after 20 generations without exchange, using $F_{20} = 1 - (1 - \frac{1}{2N_e})^{19}$ (Falconer & Mackay, 1996; Table 1). Note, that instead of raising to the power of 20, $1 - \frac{1}{2N_e}$ was raised to 19, because in populations with separate sexes inbreeding is delayed with one generation (Gutierrez, Cervantes, & Goyache, 2009). The initial kinships between flocks were set to 0.05. Inbreeding rates were calculated per year and over a 6-year period, to enable the comparison with the observed inbreeding rates in the real population determined by sampling animals with an age difference of 6 years (see below). The 6-year inbreeding rate calculations were done at the start of the breeding circle for year 2–8, the first years skipped because no inbreeding occurs until year 3, and for year 30–36, because that is about the period that the ram circle in Veluws Heideschaap existed at the time animals were sampled to determine inbreeding rates in the real population. Note, however, that in the calculations no overlapping generations could be taken into account, and that consequently all animals live for one year, in contrast to real life and the simulations.

2.4 | Simulation of inbreeding rates

To predict inbreeding rates and the effect of a breeding circle with overlapping generations, simulations were performed with the computer. Simulations are an extension of simulations performed to determine the effect of selection for scrapie resistance in Dutch sheep breeds (Windig et al., 2004). Details of the simulation software can be found in Windig and Oldenbroek (2015). In brief, a population is set up as close as possible to the real situation. In this case, the situation of 2015 with eight flocks was simulated (Table 1) in the computer. Rams were always recruited from the same donor flock, not being the own flock, and culled after 1 year, as in the real population. Ewes stayed in their own flock and reached a maximum age of 9 years. Age distribution of mothers was as in the real population with 28% of the mothers being 1 year and 20%, 15%, 12%, 12%, 6%, 3%, 2%, 1% aged, respectively, 2–9 years. 90% of the ewes lambed each year. A 40% of the litters were twins. Population size was kept constant by increasing each year the age of each animal by 1, and removing the excess of animals in each year class compared to the age distribution at the start (Table 1). Removed animals were replaced by lambs of the same sex born the previous year, and selected male lambs were transferred to the recipient flock. All other lambs were removed from the population. Litters are born by randomly choosing mothers from available ewes and pairing them at random to one of the available breeding males. Mothers had only one litter per year, males mated multiple times. Sex of lambs was assigned at random.

Inbreeding and relatedness in the initial population started at 0, and inbreeding and relatedness of all animals was tracked during the simulation. Hundred years were simulated, and repeated 25 times to determine variation in inbreeding rates. The number of repetitions was chosen to be 25, following a trial run in which 200 repetitions were used, and the average did not change anymore after 25 repetitions. We compared inbreeding rates for a 6-year period at the start of the breeding circle (year 2–8) and with a later period (year 30–36), approximately the age of the breeding circle in the real population, using

$$\Delta F_{t-x,t} = \left(1 - \left(\frac{1 - F_t}{1 - F_{t-x}}\right)^{\frac{L}{x}}\right) \quad (7)$$

With $\Delta F_{t-x,t}$ being the inbreeding rate on a generation basis between year t and x years earlier (in our case 6 years), and L the average generation interval in that period, calculated as the average age of the parents (9). In our case, the generation interval was 2.02 years.

As in the calculations, inbreeding rates were also determined when levels did not start at 0. The situation used in the calculations (initial average inbreeding levels within flocks of using $F_{20} = 1 - (1 - 1/2N_e)^{20-1}$ and average kinships between flocks of 0.05), could not be mimicked by simulating 20 years without exchange between flocks, due to overlapping generations and because some exchange was needed to reach kinship levels between flocks of 0.05. With trial and error, it was found that 50 years of simulation with 93% of the lambs sired by rams from their own flocks and 1% sired by rams from each of the other seven flocks, came close to the initial levels used in the calculations.

2.5 | Inbreeding rate determined by DNA typing

2.5.1 | DNA sampling

To determine inbreeding rates in the real population, DNA was sampled and typed from life animals in 2015. In each flock, six females were sampled born 6 years ago (thus in 2009) and six males born in 2015 and not yet selected for breeding. In this way, the inbreeding rate in the past 6 years can be determined by comparing heterozygosity levels between sampled ewes and rams. DNA samples were taken from nasal mucus of the sheep. Twelve samples per flock were taken, resulting in a total of 96 samples. DNA was extracted from the samples by VHL Genetics and genotyped by DNA-Genotek with a 12 k SNP chip. Shepherds gave permission for their animals to be sampled in the study. Sampling of animals was performed by taking non-invasive swabs of nasal mucus of the sheep. Under Dutch law (Wod 18/12/2014) following the EU directive 2010/63/EU, these are not considered as animal testing.

2.5.2 | DNA edits

Six samples with a call rate <95% were eliminated. Further editing steps comprised deleting SNP with call rate <95%, GenCall score ≤0.20 and GenTrain score ≤0.55. Missing genotypes were imputed using Beagle with 20 iterations (Browning & Browning, 2009). The imputation was carried out for each chromosome independently. The mean r^2 value for the accuracy of imputation provided by Beagle was 0.98. Next, a MAF (minor allele frequency) threshold of 0.02 was applied and SNPs deviating widely from HW equilibrium were removed using a Chi^2 test with $p < 0.0001$. Finally, only autosomal markers were used and the 371 SNPs on the X-chromosome were removed as well, since these cannot be heterozygous in males. In total, 1724 SNPs were removed from the 12,785 SNPs on the DNA chip, resulting in 11,061 SNPs.

2.5.3 | Calculating inbreeding rate from DNA

Inbreeding levels for individuals were calculated from the DNA results in three ways: (a) using heterozygosity levels, (b) using marker estimated relationships and (c) using rows of homozygosity. Inbreeding rate from heterozygosity levels was estimated using:

$$\Delta F = 1 - \left(\frac{H_{2015}}{H_{2009}}\right)^{\frac{L}{6}} \tag{8}$$

with H_{2015} being the fraction heterozygous loci of the sampled animals born in 2015 and H_{2009} the fraction heterozygous loci of the animals sampled in 2009, and L the generation interval (2.02) in that period.

Inbreeding level of individual animals was estimated using the software package calcGRM, following the method of Yang et al. (2010) using:

$$F_{\text{Yang}} = \frac{1}{N} \sum_i \frac{x_i^2 - (1 + 2p_i)x_i + 2p_i^2}{2p_i(1 - p_i)} \tag{9}$$

where N is the number of loci, and at each locus i, x_i is the individual genotype coded as 0, 1 or 2 (homozygote, heterozygote and the other homozygote, respectively, thus the number of one of the allele variants) and p_i is the frequency of the allele for which the homozygous genotype is coded as 2 at locus i. The allele frequency p_i is calculated over all individuals of 2009 and 2015 together. For a locus with an allele frequency of 0.5, this results in a value of 1 for homozygotes and −1 for heterozygotes. Homozygotes of rare alleles receive higher values, while homozygotes of common alleles receive values close to 0.

ROH-based inbreeding (F_{ROH}) was defined as the proportion of the genome of an individual covered by long uninterrupted series of homozygous SNPs. These ROHs were identified using PLINK 1.9 (Chang et al., 2015) with the following criteria: (a) a minimum physical length of 3.33 Mb, (b) a minimum of 20 consecutive homozygous SNPs with no heterozygous SNPs allowed in between, and (c) a maximum gap of 500 kb between two consecutive SNPs. The minimum length of 3.33 Mb was chosen to match a pedigree depth of 15 generations, approximately the time a breeding circle is in operation in Veluws Heideschaap. Given the genetic distance of approximately 1 cM per Mb and the average length of 1/(2G) M for ROH derived from a common ancestor G generations ago (Fisher, 1954), the F_{ROH} was expected to capture inbreeding since the start of the breeding circle. The latter two criteria were used to prevent calling of (potentially false positive) ROH in regions with low SNP density. F_{ROH} was calculated for each individual as the fraction of the autosome in ROH (McQuillan et al., 2008):

$$F_{ROH} = \frac{\sum_{m=1}^{N(ROH)} L_{ROH(m)}}{L_{tot}} \quad (10)$$

where $N(ROH)$ is the total number of ROH in the individual, $L_{ROH(m)}$ is the length of the mth ROH and L_{tot} is the length of the autosome covered by SNPs (i.e., the autosome length minus the summed length of gaps longer than 500 kb).

Inbreeding rate was calculated for both F_{Yang} and F_{ROH} as in formula 8 but with heterozygosity replaced by 1 minus the average inbreeding level:

$$\Delta F = 1 - \left(\frac{1 - F_{2015}}{1 - F_{2009}}\right)^{\frac{L}{6}} \quad (11)$$

2.6 | Extra simulations

Extra simulations were performed to help explain differences in results of the calculations, simulations and the real population. These simulations were identical to the simulations described above for the situation where inbreeding levels at the start differed between flocks (Table 1) and kinships between flocks were around 0.05, except for the parameters under investigation. Inbreeding rates were determined between year 30 and 36, similar to the age of the breeding circle at the time of sampling DNA.

To determine the influence of overlapping generations, the age of the ewes was restricted to 1 year, which was already the case for the rams. Because of the unequal sex ratio of the breeding animals (76 rams, 1,433 ewes) and the small litter size (1 or 2 lambs) not enough female lambs were produced to replace all breeding ewes in a year, when the sex ratio was equal of the newborn lambs. Therefore, the sex ratio for lambs was changed to 90% female, 10% male. So this was an unrealistic situation purely done to see the influence of overlapping generations on the inbreeding rate.

To determine the influence of unequal contributions of breeding rams to the next generations (some dominant rams may fertilize the largest part of the flock), the simulations were repeated with 90% of the offspring sired at random by two dominant males per flock, and 10% sired at random by the remaining rams in the flock. To determine the influence of selection for scrapie resistance, a single gene was added to the simulations. This gene had at the start of the simulations one allele at a frequency of 15%, the frequency of the scrapie-resistant ARR allele in the real population at the start of the selection programme in the real population. The first 30 years no selection was performed, so that in year 30 animals carrying the ARR allele were on average more related to each other than to the other animals. From year 30 onwards, only homozygous ARR males were used for breeding, the genotype of

the females was ignored, as in the real population. The frequencies in year 30 differed by random drift. Therefore, six genes were simulated, and the gene with its frequency closest to 15% in year 30 was then used for selection. The frequency chosen for selection did not differ more than 2% from the frequency of 15% in year 30. The simulation with selection was done twice, once without dominant rams and once with dominant rams. Number of lambs born reduced in the first years of selection but later recovered once more ARR rams were available. In two cases of the simulations with both selection and dominant rams, the population went extinct because no homozygous ARR males were available. These two simulations were ignored, so that the average for selection with dominant rams is based on 23 runs instead of 25.

3 | RESULTS

3.1 | Theoretical calculations

When no animals are exchanged between flocks, the calculations indicate that inbreeding levels increase considerably. Estimated effective population sizes within flocks are all below 50 or just above (Table 1), and inbreeding rates vary from 0.94% to 1.90% (Equivalent to an effective population size of 53 to 26). Estimated average inbreeding levels within flocks after 20 generations of mating within flocks range from 0.164 for the largest flocks to 0.305 for the smallest flock. On the other hand, if mating could be fully random in the entire population (i.e., no flocks an unrealistic case), the effective population size is estimated to be 289, the inbreeding rate 0.17% and the inbreeding level after 20 generations to be 0.003.

When rams are exchanged according to a ram circle theoretical inbreeding levels and rates are smaller, compared to no exchange between flocks. When all kinship and inbreeding levels start at 0.00 inbreeding, levels start to increase in the third generation due to matings between uncles and nieces. Initially, the increase in inbreeding levels differs between flocks. After 10 generations, inbreeding levels vary from 0.024 (flock Epe-Heerde) to 0.031 (flock Ede 1; Figure 2). The latter flock is preceded in the ram circle, by the four flocks with the least number of rams, the former by the 4 flocks with the largest number of rams. After about 40 generations, increases in inbreeding levels are identical in different flocks, and variation in inbreeding levels between flocks remains the same as does the rank. After 150 generations, inbreeding levels vary from 0.248 to 0.252.

When inbreeding levels in generation 0 differ between flocks, they immediately drop in generation 1 to the kinship level between flocks for all flocks (i.e., in our calculations to 0.05). In the following generations, they increase in a more or less oscillating pattern, with the rank of flocks constantly changing (Figure 3). In generation 10 they vary from 0.103 (flock Lemelerberg) to 0.112 (flock Loenen). After 40
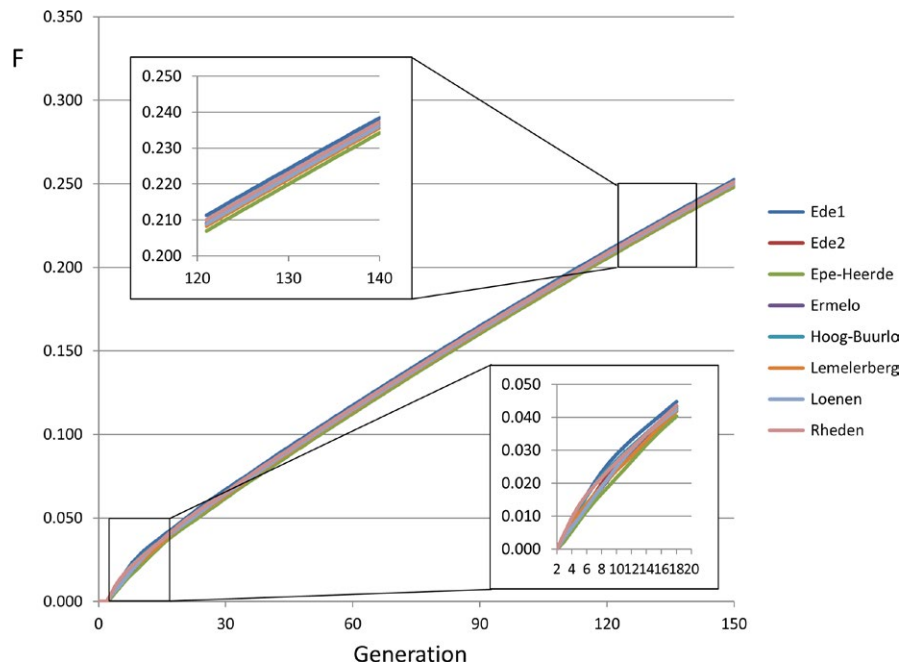
FIGURE 2 Average inbreeding level within flocks estimated with theoretical calculations for 150 generations, starting at zero. Inbreeding levels and kinships at start are zero, generations are not overlapping, flock sizes as in real population (Table 1) [Colour figure can be viewed at wileyonlinelibrary.com]

generations, ranks of inbreeding level of flocks are identical to the ranks for the calculations starting at inbreeding levels of 0. Increase is then the same in all flocks and in generation 150 inbreeding levels vary from 0.305 to 0.309.

Inbreeding rates (ΔF) initially vary widely (Figure 4). For the calculations starting at inbreeding levels of 0, the inbreeding rate between generation 5 and 6 varies from 0.30% to 0.47%. ΔF varies more for the calculations starting at different inbreeding levels in generation 0. In the first generation, all ΔF are negative when the levels drop from initial levels (between 0.16 and 0.30, Table 1) to 0.05 in all flocks, from generation 5 to 6, they vary from −0.36% to +0.68%. For both sets of calculations, inbreeding rates converge to the same value of 0.18% for all flocks (Figure 3). The level of 0.18% for all flocks was reached after 30 generations when calculations started at an inbreeding level of 0, and at generation 75 when initial levels varied.

3.2 | Simulation of inbreeding rates

The pattern of between flock inbreeding levels and rates, initially varying and later converging, found in the calculations was confirmed in the simulations. When averaged over the 25 runs of the simulations they showed the same pattern as the calculated inbreeding levels, except that fluctuations between years were less marked, probably due to overlapping generations. Because generations spanned several years (on average 2.02 years), the inbreeding level increased less per year in the simulations compared to the calculations which

assumed a generation length of 1. Within single runs of the simulations, inbreeding levels and changes of the levels were far more irregular than in the calculations.

Average inbreeding rates in the simulations were lower than in the calculations, even when corrected for generation length. When inbreeding and kinship levels started at zero inbreeding rates over a six-year period (the period used in the real population) varied between flocks from 0.26% to 0.31% in the calculations, and from 0.11% to 0.15% in the simulations between year 2 and 8 (Table 2). Variation between flocks almost disappeared when the inbreeding rate was estimated between year 30 and 36 (about the age of the breeding circle in the real population). In the calculations, they varied from 0.18% to 0.19%, in the simulations from 0.12% to 0.15%. These numbers are the average of the 25 runs in the simulations. In individual runs, the variation was considerably larger. At the start, rates vary from 0.03% to 0.23%, at year 30/36 from 0.04% to 0.23% (Table 2).

When the inbreeding and kinship levels did not start at 0, inbreeding levels in year 1 immediately dropped to the between flock kinship level of 0.05, followed in the calculations by an increase to around 0.12 in year 2. Inbreeding rates in the calculations for year 2–8 varied between flocks from −0.30% to 0.33%. In the simulations, the inbreeding levels increased more slowly after year 1 and took longer to converge across flocks compared to the calculations. From year 2 to 8, inbreeding rates varied from 0.14% to 0.87% across flocks, and within flocks from −0.85% to 1.43% (Table 2). From year 30 to 36, inbreeding rates were similar in the simulations starting
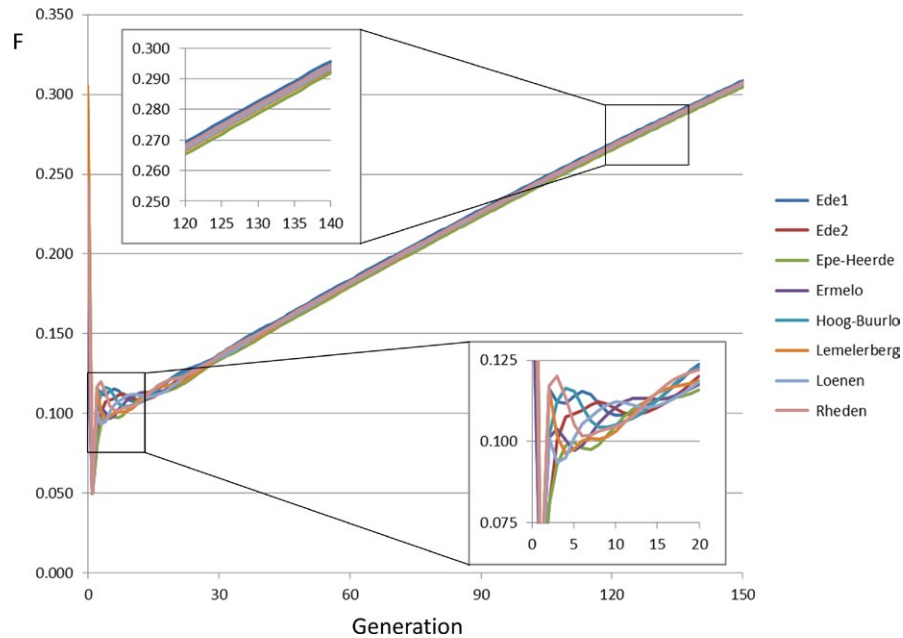
FIGURE 3 Average inbreeding level within flocks estimated with theoretical calculations for 150 generations, starting at high levels. Inbreeding levels start at level expected after 20 generations breeding within flocks (Table 1) and kinship levels at start are 0.05. Generations are not overlapping, flock sizes as in real population (Table 1) [Colour figure can be viewed at wileyonlinelibrary.com]
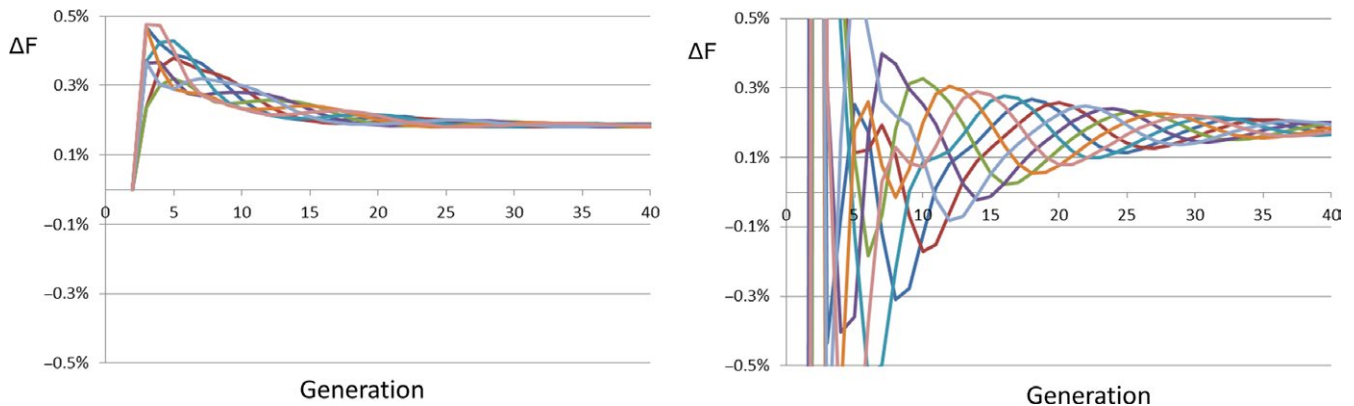

FIGURE 4 Inbreeding rates per flock per generation estimated with theoretical calculations for 40 generations. Inbreeding levels and kinships start at 0 (left panel) or inbreeding levels start at level expected after 20 generations breeding within flocks (Table 1) and kinship levels at start are 0.05. Generations are not overlapping, flock sizes as in real population (Table 1) [Colour figure can be viewed at wileyonlinelibrary.com]

at inbreeding levels of 0 or starting above 0, the latter being slightly more variable, varying from 0.10% to 0.15% between flocks and within flocks between runs varying from −0.03% to 0.28%. In conclusion, variation in inbreeding rates after 30 years of a breeding circle between flocks was considerable less than variation between runs, and on average lower than inbreeding rates estimated with calculations.

3.3 | Inbreeding rate determined by DNA typing

Heterozygosity levels differ between animals born in 2009 and 2015 in all flocks. The percentage of heterozygous loci

of animals born in 2009 was 40.0% while it was 39.5% for animals born in 2015, thus 1.23% less heterozygous animals in 6 years. The difference in heterozygosity between birth years was significant ($F = 78.8$, $p = 0.00$), but not between flocks ($F = 0.971$, $p = 0.46$), nor the interaction between birth year and flock ($F = 0.53$, $p = 0.80$). When the inbreeding rate was estimated from the reduction in heterozygosity levels at the population level it was 0.20% on a yearly basis and 0.41% at a generation basis.

Inbreeding levels estimated from markers show the same pattern as heterozygosity levels: significant differences between birth years, no significant differences between flocks. The average inbreeding level for animals born in 2009 was

TABLE 2 Inbreeding rates per flock and for the total population, estimated by calculations and simulations. Simulation results are average of 25 runs, with between brackets minimum and maximum of the runs

Flock	Calculations (%)		Simulations (%)	
	$\Delta F_{2,8}$	$\Delta F_{30,36}$	$\Delta F_{2,8}$	$\Delta F_{30,36}$
Start 0[a]				
Loenen	0.31	0.18	0.14 (0.08−0.21)	0.12 (0.01−0.20)
Ermelo	0.30	0.18	0.14 (0.06−0.20)	0.15 (0.04−0.27)
Epe-Heerde	0.27	0.19	0.15 (0.08−0.23)	0.13 (0.04−0.23)
Lemelerberg	0.29	0.19	0.11 (0.03−0.19)	0.14 (0.05−0.24)
Rheden	0.31	0.19	0.12 (0.06−0.18)	0.13 (0.06−0.21)
Hoog-Buurlo	0.32	0.19	0.11 (0.07−0.15)	0.13 (0.07−0.19)
Ede1	0.34	0.18	0.11 (0.04−0.17)	0.13 (0.04−0.18)
Ede2	0.31	0.18	0.12 (0.08−0.21)	0.13 (0.06−0.19)
Total	0.30	0.19	0.13 (0.11−0.14)	0.13 (0.11−0.16)
Start F_{20}[b]				
Loenen	0.33	0.18	0.87 (0.44−1.29)	0.11 (0.03−0.20)
Ermelo	0.08	0.16	0.80 (0.07−1.41)	0.13 (0.03−0.25)
Epe-Heerde	0.17	0.16	0.17 (−0.85−1.43)	0.15 (0.05−0.27)
Lemelerberg	−0.01	0.17	0.14 (−0.71−1.07)	0.14 (−0.03−0.28)
Rheden	−0.30	0.19	0.14 (−0.68−0.89)	0.11 (−0.02−0.18)
Hoog-Buurlo	−0.15	0.20	0.34 (−0.31−1.02)	0.11 (0.03−0.20)
Ede1	−0.06	0.21	0.49 (−0.01−1.10)	0.10 (−0.03−0.23)
Ede2	0.21	0.20	0.57 (0.02−0.91)	0.10 (0.03−0.18)
Total	0.04	0.18	0.50 (0.25−0.67)	0.12 (0.08−0.17)

Notes. $\Delta F_{2,8}$ = inbreeding rate at the start of the breeding circle between year 2 and 8; $\Delta F_{30,36}$ inbreeding rate between year 30 and 36 (= age of the breeding circle in the real population);
[a]Start 0: initial inbreeding and kinship levels were 0 [b]Start F_{20} initial inbreeding levels were comparable to 20 years breeding within flocks without exchange, and initial kinship levels were 0.05.

−0.012, for animals born in 2015 it was −0.003. Estimated inbreeding rate from these numbers was 0.15% on a yearly basis and 0.29% on a generation basis. The average inbreeding level estimated from ROH was 0.0680 in 2015 and 0.0594 in 2009. When the inbreeding rate was estimated from the increase in F_{ROH} levels at the population level, it was 0.15% on a yearly basis. And 0.31% at a generation basis.

3.4 | Extra simulations

When the simulation was performed without overlapping generations, the inbreeding rate between year 30 and 36 was on average 0.17% (Table 3), higher than the 0.12% of the simulation with overlapping generations but very similar to the value of 0.18% found in the calculations. When the simulation with overlapping generations was repeated with dominant rams, the average inbreeding rate of the population was on average 0.38% and varied from 0.26% to 0.49% between runs (Table 3), thus larger than without dominant rams, but comparable to the real population. Likewise, the simulation with selection for scrapie resistance resulted in an inbreeding rate of on average 0.35%, varying from 0.18% to 0.43%. When selection and dominant rams were

combined the inbreeding rate was on average 0.67% and varied between 0.41% and 1.27%. The effect of dominant rams and selection was thus more or less additive and combined the inbreeding rate was larger than in the real population, but with considerable variation so that the minimum is close to the value observed in the real population.

4 | DISCUSSION

Both the theoretical calculations and computer simulations show that a breeding circle is an effective way to reduce inbreeding rates. Without a breeding circle, or other exchange between flocks, rates within flocks are expected to vary between 0.94% and 1.90%, while with exchange between flocks using a breeding circle, expected inbreeding rates reduce to 0.18% for all flocks in the long term, only slightly above the 0.17% expected for fully random mating in one big population without flock structure. The inbreeding rates estimated in the real population are higher with 0.29%, 0.31% and 0.41% from ROH, marker estimated inbreeding coefficients, and heterozygosity levels respectively.

The inbreeding rate in the real population estimated from DNA typed animals differed slightly according to the

TABLE 3 Inbreeding rates in extra simulations, average of 25 runs and value of minimum–maximum run. Simulations started with initial inbreeding levels at a level comparable to 20 years breeding within flocks without exchange, and kinship levels of 0.05

Simulation	$\Delta F_{30,36}$	Min.–Max.
Standard (%)[a]	0.12	0.08–0.17
No overlapping generations (%)[b]	0.17	0.13–0.21
Dominant rams (%)[c]	0.38	0.26–0.49
Selection (%)[d]	0.35	0.18–0.43
Selection + Dominant rams (%)[e]	0.67	0.41–1.27

Notes. $\Delta F_{30,36}$ = inbreeding rate of total population between generation 30 and 36.

[a]Simulation as in Table 2 with overlapping generations, equal chance for each ram in a flock to inseminate ewes and no selection [b]As standard, but without overlapping generations [c]As standard but with 2 rams per flock inseminating 90% of the ewes in the flock [d]As standard but with selection for rams carrying the scrapie resistant allele ARR, starting at a frequency of 0.10 [e]As selection but with 2 rams per flock inseminating 90% of the ewes in the flock.

method used. The method used to calculate kinships from the markers corrects for allele frequencies, taking into account that alleles with a high frequency in the population are less likely to be identical by decent than alleles with a low population frequency. This produces estimates relative to a base level, which by using current population frequencies is the current population. For the heterozygosity based estimate, the highest estimate for inbreeding rate, all differences in alleles are weighted equally across loci, and hence the base level lies much further back in time. Estimates based on ROH, the lowest estimate for the inbreeding rate, only take into account alleles in rows of homozygosity, which are due to common ancestors in recent generations. This estimate became even lower when longer ROHs were used (results not shown). In summary, estimated inbreeding rates are higher when inbreeding due to more ancient ancestors is taken into account. Despite these differences, the conclusion of higher inbreeding rates in the real population compared to the expected inbreeding rates holds for all three estimates.

The calculations and simulations assume an equal chance of mating for each male in a flock and no selection. Both conditions are probably not met in the real population. Shepherds of the flocks indicated that it is likely that one or two rams dominate in the flocks and provide the majority of matings in a year. Furthermore, between 2009 and 2015 the birth years of the animals the samples were taken from, selection for scrapie resistance was in operation. The extra simulations do show that both selection and the presence of dominant rams increase the inbreeding rates to roughly the same levels as in the real population. When selection and dominant rams are combined inbreeding rates increase even more, but also become more unpredictable.

The difference between the theoretical inbreeding rates and realised inbreeding rates in the real population stress once more that theoretical estimates may be misleading. In general effective population, sizes estimated from numbers of animals in population are much higher than those estimated from genomic data (Leroy et al., 2013; Schmeller & Merila, 2007). Main reason is variation in the contribution of sires to the next generation, with frequently some dominant sires that contribute a lot to the next generation, and many sires only a small amount. Simulations should take that into account, but data to quantify the extra variation above random mating, can be hard to collect if no pedigree is recorded.

Breeding circles resemble wildlife populations with a herd structure, where young males leave their natal herd while females remain. (Chesser, 1991) modelled inbreeding and kinships for such systems and found that kinship among females within groups accumulated over generations faster than inbreeding. Our calculations for breeding circles show similar results. For example, after 150 years inbreeding levels within flocks were on average 0.301, while kinship levels within flocks were on average 0.320. Results from simulations also show higher inbreeding levels relative to kinship within flocks. Likewise, the average heterozygosity of animals in 2015 was 40.0%, while the expected within flock heterozygosity was 38.5%. This also explains why inbreeding levels estimated from markers are negative. Negative inbreeding levels indicate that observed inbreeding levels are on average lower than expected inbreeding levels based on marker frequencies.

When comparing formula 4 for within-flock kinships with formula 1 for across flock kinships, it is immediately clear that the former contains extra terms involving the inbreeding level in the previous generation. Some of the other terms refer to the within kinship levels in the previous generation. In other words, inbreeding level within flocks accumulates at a lower rate than kinship levels because self-kinships (and to some extent within-flock kinship levels) do not contribute to inbreeding due to avoiding mating animals within flocks.

Inbreeding rates in the simulations were lower than in the calculations. The main difference between the simulations and calculations is that overlapping generations are not taken into account in the calculations. When life span was increased in simulations and overlap between generations became longer, inbreeding rates decreased both on a yearly base and on a generation base. This is caused by the presence and reproduction of older and less inbred and related animals in the flocks, when life span is longer.

The inbreeding rate in the real population estimated from DNA typed animals differed from the estimate from heterozygosity levels and the estimate from marker estimated inbreeding levels. The main difference between the three methods is that in the latter estimates are corrected for allele frequencies,

taking into account that alleles with a high frequency in the population are less likely to be identical by decent than alleles with a low population frequency. This produces estimates relative to a base level, which by using current population frequencies is the current population, hence negative estimates for the inbreeding level are possible. For the heterozygosity based estimate, all differences in alleles are weighted equally across loci, and hence the base level lies much further back in time. Despite these differences, the conclusion of considerably higher inbreeding rates in the real population compared to the expected inbreeding rates holds for both heterozygosity based and marker-based estimates.

Theoretical estimations of inbreeding rates, either by computation or simulation generally start assuming unrelated and non-inbred individuals, although in real life this is seldom the case. This can be justified, however, by the fact that kinship and inbreeding levels are always relative to a certain base. For example, when they are estimated from a pedigree the base is the inbreeding/kinship level for the founder individuals. Inbreeding rates will generally not be affected by the choice of the base generation, provided that sufficient time has passed since the base generation. Here, we showed that for breeding circles the assumption of equal kinship levels has large consequences in the initial generations. Instead of an increase in inbreeding levels after generation 2, inbreeding levels drop sharply in generation 2 when initial kinship levels are higher within flocks than across flocks (Figure 3), and in the following generations inbreeding rates vary between negative and positive values (Figure 4 and Table 2). Nomura and Yonezawa (1996) concluded that circular breeding, similar to the ram circle evaluated here, was less effective in reducing inbreeding rates than cyclical mating in which exchange between sub-populations change each year. They based this conclusion on higher inbreeding rates in initial generations for breeding circles compared to other systems. In the long run, breeding circles are more effective but it took up to generation 141 or more depending on population sizes, before rates were below circular mating systems. However, their calculations started with inbreeding levels and kinships set to 0. If calculations would have started more realistically with high kinship levels within flocks and low kinship levels between flocks inbreeding levels would have dropped in the first generation.

A particular advantage of breeding circles is that they are relatively easy to implement. There is no need for a pedigree, the only important thing to know for each animal is the flock of birth (Mucha & Komen, 2016). Furthermore, each flock owner only needs to arrange exchange with its donor flock and its recipient flock, and the circle can be designed so that geographical distance between donor and recipient flocks is minimised (Windig & Kaal, 2008). In case of a global population, such as is the case for some zoo populations, one may operate several circles simultaneously (e.g., one on every continent) and exchange animals once every 5 years or so between the circles. Effects of such a multilevel scheme need to be researched yet. The simulation program used here can be easily adapted for such a system of exchange. Further aspects that may be explored are combinations of breeding circles with other conservation schemes such as using semen stored in gene banks (Colleau & Avon, 2008) and optimal contribution schemes (Meuwissen, 1997; Mucha & Komen, 2016).

5 | CONCLUSION

The effectiveness of breeding circles reported in literature (Derochambeau & Chavalet, 1985; Farid, Makarechian, & Strobeck, 1987; Nomura & Yonezawa, 1996; Windig & Kaal, 2008) is extended to sub-populations of different size and overlapping generations in this study. In contrast to previous studies, we show that breeding circles are immediately effective in more realistic populations where animals at the start are inbred and related, especially within flocks. Interestingly, inbreeding rates converge to the same value for all flocks despite differences in number of animals. Thus, in a breeding circle flocks with a small number of animals benefit from larger flocks. Realized inbreeding rates in Veluws Heideschaap were higher than those derived mathematically and in simulations. If selection or a disproportionate fraction of females fertilized by a few males within a flock are added to the simulations, inbreeding rates in simulations are in agreement with realized inbreeding rates. All estimates of inbreeding rate in Veluws Heideschaap are below 0.5%, which is considered a sustainable level. However, the higher inbreeding rates in the real population compared to the theoretical estimates are a warning that one cannot solely rely on theoretical estimates to evaluate sustainability of genetic management.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest regarding the publication of this article

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