

Effects on inbreeding of different strategies aimed at eliminating scrapie sensitivity alleles in rare sheep breeds in The Netherlands

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Abstract

The Dutch scrapie eradication programme aims at the exclusive use of homozygous ARR/ARR breeding rams by the end of 2004. As a consequence, breeds with a small population size and a low frequency of the ARR allele may suffer unacceptable losses of genetic diversity and high inbreeding levels. We simulated three breeding strategies to assess their efficacy in eliminating scrapie sensitive alleles and their effect on inbreeding levels. Under mild selection, both homozygous and heterozygous ARR rams were used indiscriminately. Under moderate selection, homozygous ARR rams were used preferably but they were supplemented with heterozygous rams when necessary. Under severe selection, only homozygous rams were used. Severe selection mimics then the proposed eradication programme. Simulations were carried out with allelic frequencies and population parameters of existent rare breeds in The Netherlands. With severe selection all simulated breeds showed unacceptably high inbreeding rates (> 0.5% per year). For some breeds, moderate selection resulted in acceptable inbreeding rates, while for other breeds only mild selection resulted in acceptable rates. The frequency of the ARR allele after 5 years of selection was only slightly lower with moderate selection than with severe selection (0.8% lower on average), but it was clearly lower with mild selection. Based on these simulations, we propose a selection programme where with low frequencies of the ARR allele, mild selection is used initially. Once the ARR frequencies rise to a particular value, the switch can be made to moderate selection. The population size (e.g. below 750, 750 to 3750 and above 3750 ewes) determines the frequency of the ARR allele at which the switch can be made (33%, 25% and 10%, respectively). With even higher ARR frequencies (above 70%, 50% and 33%, respectively) the regime can be changed to severe selection.

Keywords: control programmes, genetics, inbreeding, rare breeds, scrapie.

Introduction

Scrapie is a fatal infectious neurodegenerative disease for which susceptibility is associated with polymorphisms in the ovine prion protein (*PrP*) gene. The polymorphisms occurring most frequently are those at codons 136 (A/V), 154 (R/H) and 171 (Q/R/H), where each letter represents the amino acid coded at the indicated codon (Belt *et al.*, 1995). The VRQ allele is significantly associated with a high susceptibility to scrapie (Hunter *et al.*, 1994), while the ARR allele is significantly associated with resistance to natural and experimental infections with scrapie and bovine spongiform encephalopathy (BSE) (Foster *et al.*, 2001). In breeds where the VRQ allele is rare or absent, the wildtype ARQ allele is

associated with increased susceptibility to scrapie (Clouscard *et al.*, 1995; Bossers *et al.*, 1996; Drögemüller *et al.*, 2001).

The Dutch scrapie eradication programme (the 'accelerated scrapie eradication programme 2002') is based on these genetic susceptibility variants. The goal is to eliminate scrapie by selecting for the ARR/ARR genotype. The programme was brought into action as a precautionary measure because the presence of BSE in the sheep population can not currently be excluded (Bruce *et al.*, 2002). The programme indicates the obligatory and exclusive use of ARR/ARR breeding and multiplier sires by the end of 2004 (Vellema, 2002). An important

provision is that additional inbreeding due to *ARR/ARR* selection will remain acceptable.

Inbreeding occurs when two related animals produce offspring (Falconer and Mackay, 1996) and generally has negative effects (Hedrick and Kalinowski, 2000). Reported effects include reduced growth, reduced milk production, increased susceptibility to diseases and increased occurrence of congenital defects. Congenital defects are already present in the Veluws Heideschaap breed and they dramatically increased following a population bottleneck during the outbreak of the foot and mouth disease in The Netherlands (G. Pastink, personal communication). Inbreeding also leads directly to a reduction in genetic variation (Wiener *et al.*, 1992) which reduces the effect of selection, either artificial selection for production traits or natural selection for adaptation to the (changing) environment (Lynch and Walsh, 1998). In the common Texel sheep breed, breeding values for weight at 135 days have been found to be slightly lower for *ARR/ARR* rams than for non-*ARR/ARR* rams and breeding values for number of lambs born have been found to be slightly higher for *ARR/ARR* rams than for non-*ARR/ARR* rams (Brandsma *et al.*, 2004). In small breeds, larger effects are expected due to genetic drift. It is thus essential that inbreeding should be kept to a minimum. However, small population sizes and high selection pressure leads to an increase in inbreeding (Meuwissen and Woolliams, 1994). The guidelines of Food and Agriculture Organization (FAO) for the management of small populations recommend that the rate of inbreeding per generation (ΔF) should be below 1% (FAO, 1998).

In this paper the effect on inbreeding levels of selection on scrapie genotypes was analysed by computer simulation for several rare Dutch sheep breeds.

Material and methods

Populations

Data of existing populations were used to simulate selection on scrapie genotypes. With this purpose, data on eight breeds with small population size (< 5000 animals) were assembled. Animals within these breeds had been genotyped by the Taqman-method. This method screens for three mutually exclusive allelic variants *ARR*, *AHQ* and *VRQ*, while all other alleles are called *ARQ**. *ARQ** includes predominantly the wildtype *ARQ*, but will also contain all other alleles not specifically screened for, including *ARH* (Bossers *et al.*, 1999). The genotype data were obtained from the Dutch Animal Health Service database. Information on population structure was also extracted from this database, supplemented with information from breeding organizations where necessary.

Simulations

Simulations were used to predict inbreeding levels and allele frequencies across years of selection against susceptible alleles. Populations were set up with the same structure as the breeds. Population size, sex ratio (Table 1), and composition of age groups (Table 2) in the breeding season 2002/2003 were used to form the initial generation. Each individual received two *PrP* alleles (*ARR*, *AHQ*, *ARQ** or *VRQ*), with probabilities determined by the allele frequencies in 2002/2003 (Table 1). All animals were assumed to be unrelated in the starting generation. All breeds had one breeding season per year. Ewes to produce litters for the next breeding season were selected at random from the ewes present in the population. Consequently, at each breeding season, ewes of different ages produced litters (i.e. generations overlapped). Each year the number of ewes selected was the same as the number of litters in the breeding season 2002/2003. Litter size was one or two with probabilities determined by the

Table 1 Breeds and population parameters used in the simulations (population parameters are based on the breeding season 2002/2003 at the start of the control programme)

Breed	No. of ewes	No. of rams	Mean no. ewes per ram	Max. no. ewes per ram	No. lambs per ewe	No. litters per year	Allele frequencies			
							<i>ARR</i>	<i>AHQ</i>	<i>ARQ*</i>	<i>VRQ</i>
Skudde	350	300	10	50	1.37	150	0.171	0.150	0.679	0.000
Schoonebeeker	450	300	8	75	1.30	180	0.064	0.016	0.743	0.029
Veluws Heideschaap	1100	250	40	100	1.34	190	0.132	0.031	0.816	0.049
Mergellander	1550	200	12	75	1.48	375	0.251	0.000	0.693	0.056
Kempisch Heideschaap	1900	200	50	120	1.29	300	0.267	0.010	0.711	0.021
Drents Heideschaap	2200	300	12	75	1.36	225	0.128	0.038	0.750	0.084
Hampshire Down	2215	900	8	75	1.38	600	0.712	0.000	0.284	0.004
Fries Melkschaap	3750	500	11	100	1.49	675	0.102	0.050	0.848	0.000

Table 2 Age composition of the breeds, expressed as percentage of rams and percentage of ewes in each age class

Breed	Age of rams (years)					Age of ewes (years)				
	1	2	3	4	> 5	1	2	3	4	> 5
Skudde	35	34	17	10	0	36	26	21	11	6
Schoonebeeker	68	32	0	0	0	38	23	9	9	21
Veluws Heideschaap	68	32	0	0	0	44	26	12	11	7
Mergellander	69	31	0	0	0	42	25	11	10	12
Kempisch Heideschaap	68	32	0	0	0	40	23	10	10	17
Drents Heideschaap	50	20	15	8	7	20	16	15	15	34
Hampshire Down	44	13	12	8	23	20	17	17	13	33
Fries Melkschaap	25	25	25	13	12	35	35	20	7	3

frequencies in 2002/2003. The number of rams selected varied according to the selection regime (see below). Offspring received at random one of the two alleles of the father and at random one of the two alleles of the mother. Relationships of the offspring with all previously born animals and their inbreeding coefficients were computed using standard procedures (Lynch and Walsh, 1998, p. 763). Each year, the number of animals culled was the same as the number of animals born. The probability of being culled was determined by the age composition of the breed (Table 2).

Selection regimes

Three selection regimes were simulated: mild, moderate and severe. Under all regimes, selection took place by using rams with at least one *ARR* allele to inseminate ewes. The regimes differed in to what extent the use of heterozygous rams was allowed. Under mild selection, no distinction was made between homozygous and heterozygous *ARR* rams and both were used without preference. For each breed, the number of ewes inseminated each year was the same as in 2002. The number of rams used to inseminate ewes was also constant across years and it was determined by the parameters given in Table 1. Under moderate selection, homozygous *ARR* rams were used preferentially. The number of ewes per ram was the same as with mild selection. When there were not enough homozygous *ARR* rams to produce the target number of litters, heterozygous rams were also used. Under severe selection, only homozygous *ARR* rams were used. Since there were fewer rams available, they generally had to inseminate more ewes than under mild and moderate selection. The number of ewes/ram was set to 80% of the maximum number of ewes inseminated by a single ram in 2002. When there were not enough homozygous rams to produce the target number of litters, fewer litters were produced. The severe selection scheme is the scheme followed in the Dutch scrapie eradication programme.

For each combination of breed and selection regime, 25 simulations with 5 years of selection were run, and the parameters reported are the average of the 25 runs. Since the initial relationships were set to zero, inbred individuals can only be born after the second round of selection. Each year t (starting at $t = 2$), the average inbreeding coefficient (F_t) was determined for individuals born in that year. The rate of inbreeding (ΔF) was determined each year by the equation $\Delta F_t = (F_t - F_{t-1}) / (1 - F_{t-1})$ (Falconer and Mackay, 1996). At $t = 5$, the overall inbreeding rate over the 5 years was computed as $\Delta F_{1-5} = 1 - (1 - F_5)^{1/4}$. Both the maximum ΔF_t (generally ΔF_2) and ΔF_{1-5} are reported. The maximum acceptable inbreeding rate was assumed to be 0.5%, which is more conservative than the figure of 1.0% recommended by FAO (1998). This conservative limit is justified because our estimates may well underestimate the rates observed in real populations (see below). Allele frequencies are reported both after one round of selection and after four rounds. For the Schoonebeeker breed, simulations were run for 50 years in order to investigate the long-term effects of different breeding strategies.

Extra simulations

In the simulations described above, a number of assumptions were made that may lead to biased predicted inbreeding levels. Inbreeding levels at the starting generation were set to zero. This will generally influence only the average F and not ΔF in subsequent years. The exception is when rams selected in the first round tend to be more related than unselected rams, which is not unlikely in the case of selection for (homozygous) *ARR* rams. Extra simulations using actual relationships at the start of selection were run for the Fries Melkschaap breed. This breed was chosen because pedigree information was reasonably accurate (unlike other breeds where pedigree information was incomplete).

A second assumption in the simulations was that all selected rams and ewes had the same probability of pairing with each other. In practice, the distribution of a breed over different flocks will lead to varying probabilities of pairing certain rams and ewes. If rams are used preferentially within their own flock they may be paired with more related ewes than expected with random pairing. Also, in practice, breeding schemes aim to reduce inbreeding by using rams of other flocks. We simulated the breeding schemes that are in operation for the Schoonebeeker and the Veluws Heideschaap breeds. These breeds are kept in several large flocks. In the Schoonebeeker breed, a group of three flocks are involved in a joint breeding programme. In this programme each flock only uses rams from the other two flocks and the flock providing rams alternates between years. In the Veluws Heideschaap breed, all nine flocks are involved in a joint breeding programme. Here flock 1 uses rams from flock 2, flock 2 uses rams from flock 3, flock 3 uses rams from flock 4 and so on, and flock 9 uses rams from flock 1.

Results

Under severe selection, all examined breeds, except the Hampshire Down breed, experienced a high rate of inbreeding (Table 3). When the average $\Delta F_{1.5}$ was taken as the criterion for acceptable rates, then the Mergellander breed also showed rates below 0.5%. However, the results in Table 3 are the average of 25 simulations. Thus there is roughly a 50% chance that the populations will have higher inbreeding rates than those indicated. In the Mergellander breed, $\Delta F_{1.5}$ was above 0.5 in 11 of the 25 simulations, and the maximum $\Delta F_{1.5}$ was 0.59%. The inbreeding rate was generally highest at $t = 2$ (Figure 1), especially for the rarer breeds. The average maximum ΔF_t with severe selection over all the breeds (except the Hampshire Down breed) was 1.22%. The frequency of the ARR allele in animals born after 5 years of severe selection was for all breeds above 0.65 and the average over breeds was 0.79. Excluding the breeds Drents Heideschaap and Hampshire Down, the frequency of ARR/ARR animals in the whole population (i.e. the pool of selection candidates), after 5 years of

Table 3 Effects of different selection regimes on the rate of inbreeding and the frequency of the ARR allele after 5 years of selection †

Breed	Selection regime	Max. ΔF_t ($\times 100$)	$\Delta F_{1.5}$ ($\times 100$)	Freq. ARR/ARR in population	Freq. ARR in new born animals
Skudde	mild	0.47 (0.33–0.64)	0.38 (0.29–0.50)	0.219 (0.196–0.246)	0.468 (0.421–0.500)
	moderate	0.66 (0.39–1.39)	0.45 (0.31–0.69)	0.502 (0.449–0.568)	0.822 (0.780–0.888)
	severe	1.76 (1.51–3.08)	1.48 (0.99–1.90)	0.522 (0.485–0.571)	0.835 (0.805–0.857)
Schoonebeeker	mild	0.66 (0.33–0.99)	0.46 (0.29–0.70)	0.130 (0.101–0.160)	0.369 (0.273–0.400)
	moderate	1.02 (0.85–2.38)	0.86 (0.44–1.76)	0.235 (0.201–0.253)	0.715 (0.529–0.776)
	severe	2.21 (1.65–2.75)	1.27 (0.70–1.89)	0.245 (0.211–0.263)	0.720 (0.554–0.806)
Veluws Heideschaap	mild	0.39 (0.26–0.56)	0.32 (0.23–0.49)	0.298 (0.270–0.318)	0.563 (0.538–0.588)
	moderate	0.95 (0.64–2.29)	0.70 (0.42–1.56)	0.406 (0.368–0.436)	0.801 (0.766–0.832)
	severe	1.62 (0.98–3.06)	1.22 (0.76–2.17)	0.415 (0.392–0.429)	0.807 (0.783–0.824)
Mergellander	mild	0.25 (0.19–0.31)	0.16 (0.13–0.21)	0.284 (0.258–0.308)	0.523 (0.480–0.567)
	moderate	0.30 (0.23–0.67)	0.21 (0.16–0.25)	0.402 (0.381–0.423)	0.778 (0.755–0.803)
	severe	0.51 (0.40–0.86)	0.45 (0.33–0.59)	0.406 (0.384–0.430)	0.782 (0.766–0.832)
Kempisch Heideschaap	mild	0.35 (0.27–0.56)	0.28 (0.21–0.49)	0.277 (0.247–0.301)	0.563 (0.539–0.590)
	moderate	0.48 (0.46–0.76)	0.41 (0.31–0.57)	0.340 (0.319–0.358)	0.812 (0.770–0.838)
	severe	0.93 (0.78–1.60)	0.89 (0.62–1.29)	0.342 (0.322–0.361)	0.816 (0.779–0.837)
Drents Heideschaap	mild	0.16 (0.12–0.20)	0.12 (0.09–0.16)	0.062 (0.054–0.088)	0.426 (0.338–0.564)
	moderate	0.33 (0.26–0.57)	0.24 (0.13–0.42)	0.100 (0.094–0.108)	0.663 (0.640–0.695)
	severe	0.73 (0.55–1.45)	0.55 (0.36–0.93)	0.111 (0.108–0.127)	0.661 (0.628–0.702)
Hampshire Down	mild	0.03 (0.01–0.09)	0.02 (0.00–0.07)	0.564 (0.515–0.600)	0.758 (0.732–0.784)
	moderate	0.10 (0.06–0.15)	0.05 (0.03–0.10)	0.712 (0.690–0.733)	0.912 (0.889–0.930)
	severe	0.10 (0.06–0.15)	0.05 (0.03–0.10)	0.712 (0.690–0.733)	0.912 (0.889–0.930)
Fries Melkschaap	mild	0.10 (0.06–0.15)	0.07 (0.04–0.10)	0.139 (0.119–0.159)	0.476 (0.407–0.544)
	moderate	0.30 (0.20–0.57)	0.20 (0.14–0.27)	0.235 (0.201–0.253)	0.781 (0.747–0.799)
	severe	0.77 (0.64–1.37)	0.58 (0.38–0.99)	0.262 (0.247–0.272)	0.801 (0.787–0.820)

† Selection regimes: severe selection = exclusive use of ARR/ARR rams, moderate selection = use of ARR/ARR rams supplemented with ARR/X rams if not enough ARR/ARR rams are available, mild selection = use of both ARR/ARR and ARR/X rams indiscriminately. Max. ΔF_t = maximum rate of inbreeding ($\times 100$) per year, $\Delta F_{1.5}$ = rate of inbreeding ($\times 100$) over 5 years. Numbers in bold indicate unacceptably high (> 0.5%) inbreeding rates. Freq. ARR/ARR in population = frequency of homozygous ARR animals in the whole population after 5 years of selection. Freq. ARR in new born animals: allelic frequency of ARR for animals born in the last year. Numbers in brackets indicate the maximum and the minimum over replicates.

selection ranged from 0.25 to 0.52. The Drents Heideschaap breed had a lower frequency (0.11) because animals tend to remain longer in the population than in other breeds. The Hampshire Down breed had a higher frequency (0.71) because of its high initial ARR frequency.

With moderate selection, the frequency of the ARR allele after 5 years of selection was on average only 0.008 below the frequency under severe selection. Initially, ARR frequencies were higher with severe selection, but moderate selection tended to catch up

after the 2nd year (Figure 1). This is because the difference between severe and moderate selection is only that with the latter, ARR/ARR rams are supplemented with heterozygous ARR/X rams if the number of ARR/ARR rams is insufficient. Thus if the frequency of ARR is high, the two regimes converge. This is why in the Hampshire Down breed (with its high initial ARR frequency) no difference between moderate and severe selection was found. ΔF was considerably lower with moderate selection than with severe selection. This is because, in contrast with severe selection, moderate selection does not

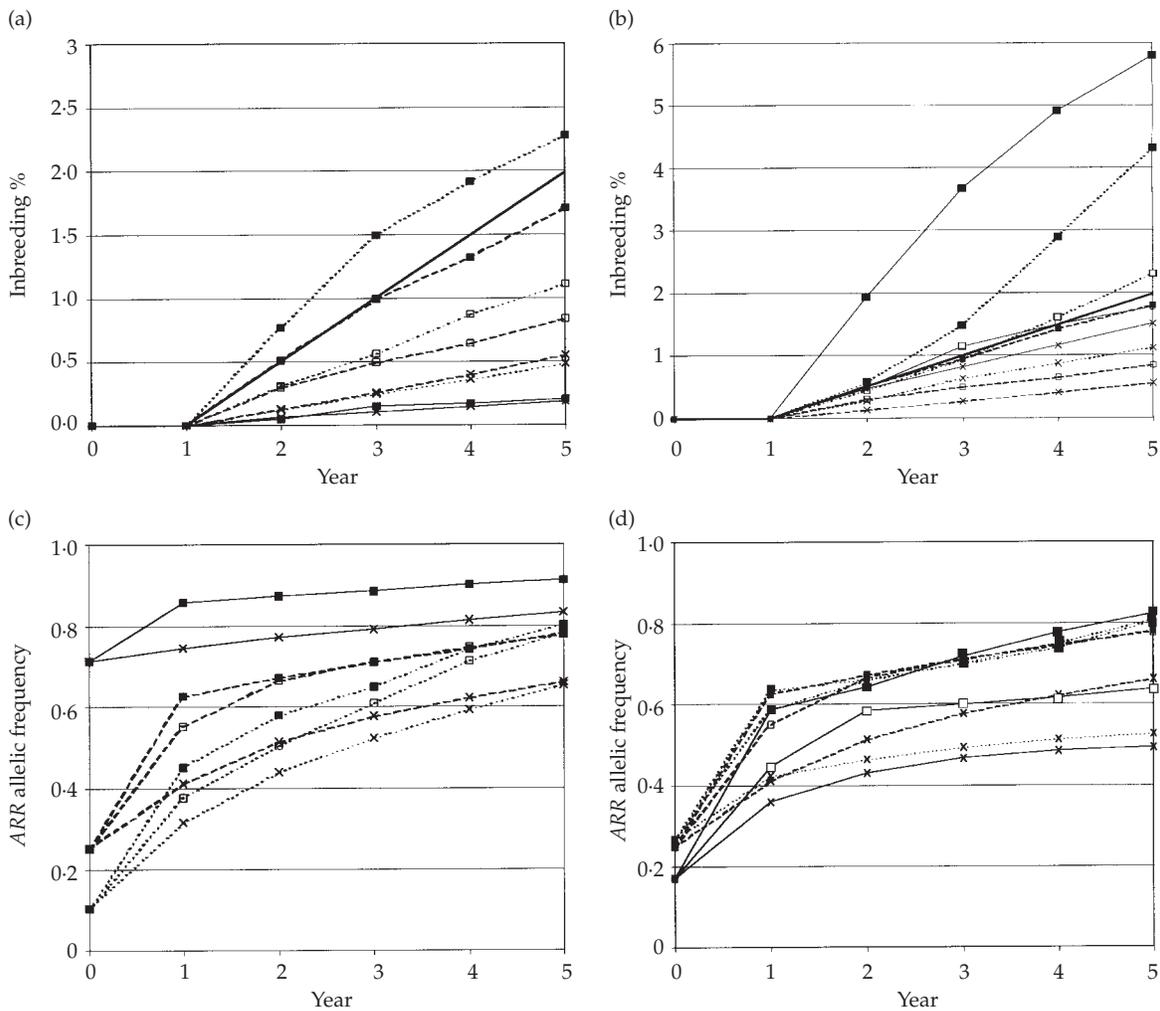


Figure 1 Inbreeding levels ((a) and(b)) and frequency of the ARR allele in new born animals ((c) and (d)) for three breeds differing in ARR frequency in the base generation ((a) and (c)) [Fries Melkschaap (0.10), Mergellander (0.25) and Hampshire Down (0.71)], and for three breeds differing in population size ((b) and (d)) [Skudde (650), Mergellander (1750) and Kempisch Heideschaap (2100)]. Each breed is shown under three selection regimes: severe ■, moderate □ and mild ×. Thick lines indicate an inbreeding rate of 0.5% per year (acceptable).

Table 4 Effects of different selection regimes on the rate of inbreeding and the frequency of the *ARR* allele after 5 years of selection, assuming that an inbreeding restriction is in operation (Schoonebeeker and Veluws Heideschaap breeds) or that animals at the start of selection are not unrelated (Fries Melkschaap breed)†

Breed	Selection regime	Max. ΔF_t ($\times 100$)	ΔF_{1-5} ($\times 100$)	Freq. <i>ARR/ARR</i> in population	Freq. <i>ARR</i> in new born animals
Schoonebeeker	mild	0.33 (0.21–0.93)	0.23 (0.15–0.33)	0.118 (0.088–0.162)	0.361 (0.333–0.390)
	moderate	0.65 (0.34–1.06)	0.37 (0.27–0.51)	0.249 (0.197–0.326)	0.531 (0.494–0.601)
	severe	2.07 (1.09–4.61)	0.99 (0.62–1.51)	0.326 (0.252–0.393)	0.599 (0.533–0.665)
Veluws Heideschaap	mild	0.15 (0.04–0.37)	0.09 (0.02–0.14)	0.075 (0.060–0.091)	0.342 (0.313–0.387)
	moderate	0.37 (0.16–0.44)	0.24 (0.07–0.32)	0.157 (0.139–0.172)	0.441 (0.398–0.487)
	severe	0.43 (0.21–0.63)	0.36 (0.13–0.42)	0.166 (0.146–0.186)	0.469 (0.421–0.512)
Fries Melkschaap	mild	0.17 (0.12–0.33)	0.12 (0.08–0.25)	0.139 (0.119–0.159)	0.476 (0.407–0.544)
	moderate	0.42 (0.31–0.72)	0.30 (0.24–0.68)	0.235 (0.201–0.253)	0.781 (0.747–0.799)
	severe	1.56 (1.32–6.06)	1.04 (0.85–2.01)	0.262 (0.247–0.272)	0.801 (0.787–0.820)

† See Table 3.

rely on a single or a few *ARR/ARR* rams in the first generations of selection. The three rarest breeds (Skudde, Schoonebeeker and Veluws Heideschaap) still showed ΔF values above 0.5% with moderate selection. Under mild selection, ΔF_{1-5} was still lower, but then *ARR* frequencies clearly dropped. For the Schoonebeeker breed and for the Skudde and Kempisch Heideschaap breeds in some cases, the maximum ΔF_t remained on average above 0.5% even under mild selection.

Population decline occurred for most breeds under severe selection because not enough *ARR/ARR* rams were available to inseminate all ewes. The decline was absent in the Hampshire Down breed because of its high initial *ARR* frequency. For the Skudde and Drents Heideschaap breeds, declines were rare (observed in less than four of the 25 simulations) and small (less than 20%). For the other breeds, except the Schoonebeeker breed, the population decline was on average between 10% and 20%. Population declines were observed only in the first year of selection, after which the population fully recovered. No population decline occurred for these breeds with mild selection. For the Schoonebeeker breed, a more serious decline was observed. Under severe selection, a reduction of on average 69.3% occurred and in four of the 25 simulations no *ARR/ARR* rams were available after 1 year of selection. Under mild selection, the population decline was on average 4.7%.

For the two breeds for which an inbreeding restriction is in operation (Schoonebeeker and Veluws Heideschaap), the different selection regimes were also evaluated while keeping the current breeding structure in place as much as possible. The result was that both the inbreeding levels and the *ARR* frequencies decreased compared with simulations without the inbreeding restriction (Table 4). Both ΔF_{1-5} and maximum ΔF_t values for the

Schoonebeeker breed were on average below 0.5% for mild selection. For some simulations, ΔF_t reached values above 0.5%. For the Veluws Heideschaap breed, ΔF values remained below 0.5% for all selection regimes, except in a few simulations under severe selection, where maximum ΔF_t reached 0.63%.

The results for the Schoonebeeker breed over 50 years of selection are shown in Figure 2 in more detail. The main difference between the different selection regimes occurred in the first few years of selection. Especially under severe selection there was a strong increase in inbreeding in the first 3 years. After the initial increase, selection regimes had an inbreeding rate in later years ($t > 10$) comparable with that with no selection (e.g. the slope of the lines was about the same). This is because by the time the *ARR*-frequency is high, selection for the *ARR*-allele starts to resemble random culling of animals. The stronger the selection was, the more variable the inbreeding rate was. An exception was with mild selection in later years where the rate of inbreeding was slightly more variable than with moderate selection. As expected, severe selection reached high *ARR* frequencies earlier than the other selection regimes, although the difference from that with moderate selection was minimal. The average time until fixation was 31.5 years with severe selection and 32.4 years with moderate selection. With mild selection, *ARR* frequencies were more variable and did not reach fixation within the 50 years of selection. A combination of mild selection in the first years and moderate to severe selection in later years seems to be a sensible strategy to apply in practice. One could start with moderate selection, but some simulations showed high ΔF_t in the initial years (up to 0.93%), which were avoided when mild selection was used in the first 3 years. One also has to follow the inbreeding restriction regime strictly as any breach will increase inbreeding levels. Thus in order to be

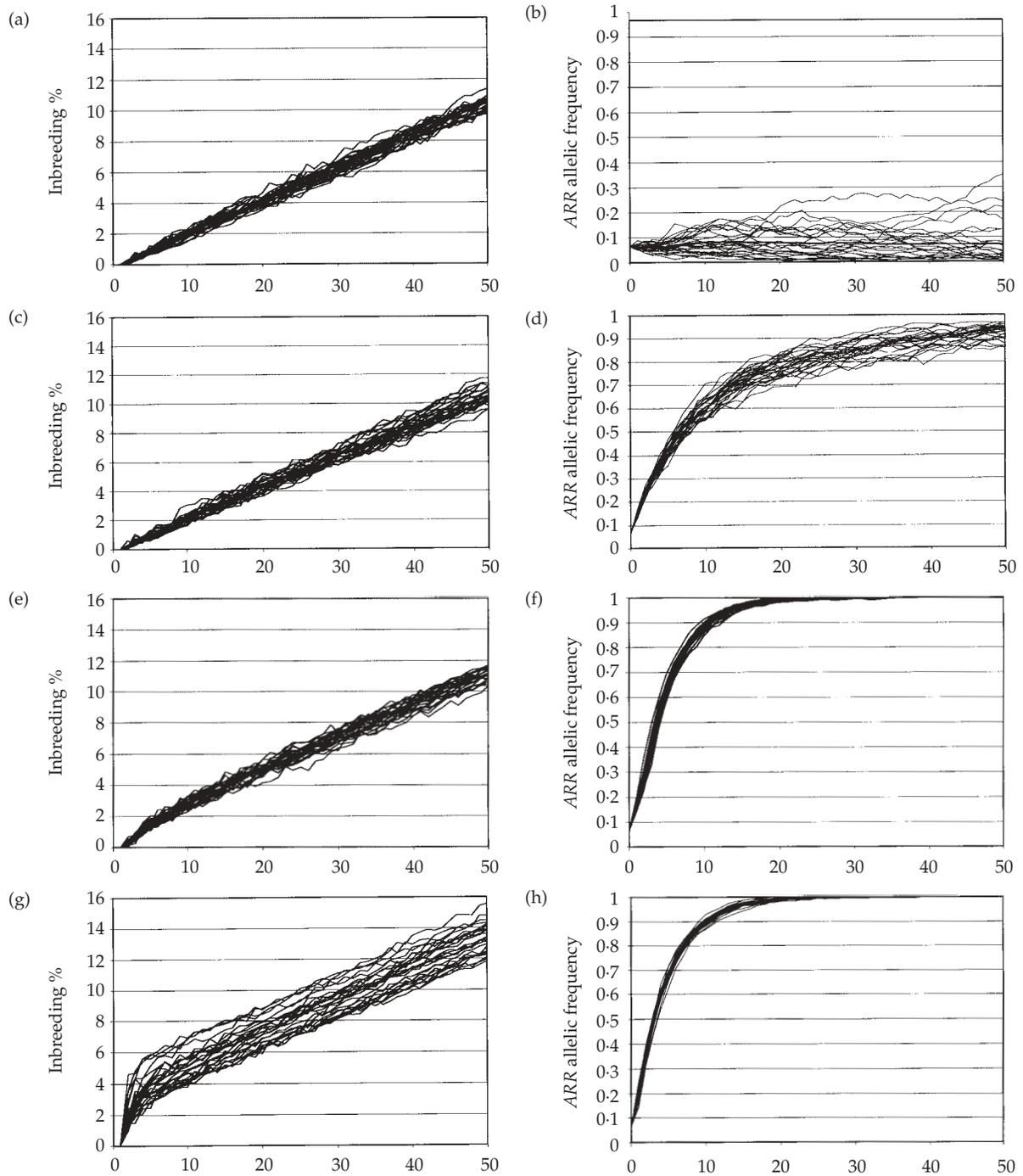


Figure 2 Inbreeding levels ((a), (c), (e) and (g)) and frequency of the *ARR* allele in new born animals (b), (d), (f) and (h)) for the Schoonebeeker breed over 50 years of selection. (a), (b): no selection; (c), (d): mild selection (both heterozygous and homozygous *ARR* rams used), (e), (f): moderate selection (preferential use of homozygous *ARR* rams), (g), (h): severe selection (exclusive use of *ARR* rams). Each line represents results from one simulation.

Table 5 Average co-ancestry of animals with and without ARR alleles in 2003 for the Fries Melkschaap breed (ARR/ARR = homozygous ARR animals, ARR/X = heterozygous ARR animals, X/X = animals without ARR alleles)

	ARR/ARR	ARR/X	X/X
ARR/ARR	0.1358		
ARR/X	0.0366	0.0512	
X/X	0.0003	0.0002	0.0077

on the safe side, mild selection seems the regime of choice to start with.

For the Fries Melkschaap breed, the effect of the degree of relationship between the animals at the start of the selection was also evaluated. Animals with ARR alleles were more closely related to each other than animals without ARR alleles (Table 5). All animals with ARR alleles could be traced back to three ancestors about six generations ago. The high relationship between ARR/ARR animals has consequences for the inbreeding levels, especially under the severe selection regime. When compared with simulations that did not take initial relationships into account, ΔF almost doubled to, on average, 1.04% after the first 5 years of selection (maximum 1.56%) with severe selection. With mild and moderate selection, the ΔF remained on average below 0.5% when initial relationships were taken into account.

General advice

The selection regime for eliminating scrapie susceptible alleles has to be based on population size and frequency of the ARR allele in order to maintain inbreeding levels at an acceptable level. Based on the results in this paper we have set up a rule of thumb

Table 6 General advice to be used as a rule of thumb for which different selection regimes should be applied in order to maintain inbreeding levels and loss of genetic diversity at acceptable levels†

No. of ewes	Advised selection regime		
	Mild	Moderate	Severe
<750	<0.33 (<10%)	0.33–0.70 (10–50%)	>0.70 (>50%)
751–3750	<0.25 (<5%)	0.25–0.50 (5–25%)	>0.50 (>25%)
≥3751	<0.10 (<1%)	0.10–0.33 (1–1%)	>0.33 (>10%)

† The table shows allele frequencies and approximate frequencies of homozygous ARR/ARR rams (in% in brackets) at which a particular selection regime should be followed. Severe selection = exclusive use of ARR/ARR rams, moderate selection = use of ARR/ARR rams supplemented with ARR/X rams if not enough ARR/ARR rams are available, mild selection = use of both ARR/ARR and ARR/X rams indiscriminately.

that can be used to decide which selection regime to use (Table 6). This advice had to be simple and easy to use in practice. Hence, breeds were divided into three categories based on the number of ewes. The two rarest breeds (Skudde and Schoonebeeker) had clearly higher inbreeding rates than the other breeds (see Tables 3 and 4 and Figure 1). Based on the number of ewes in their populations, a value of 750 ewes was set up as the lower threshold. The higher threshold was set up at 3750 ewes (five times the lower threshold which is about the number of ewes in the Fries Melkschaap breed) based on the fact that with moderate selection a few simulations for the Fries Melkschaap breed showed a maximum inbreeding rate of just above 0.5% (i.e. it is at a switching point between mild and moderate selection).

Breeds were also divided into three categories based on their ARR frequencies. For the larger breeds (> 3750) the Fries Melkschaap was used again to set the threshold for choosing between mild and moderate selection. This resulted in a threshold at an ARR frequency of 0.10. The threshold for choosing between moderate and severe selection was set up to achieve a pool of potential rams for siring the next year's lambs of about 75 rams or more. For a breed with 3750 ewes and 750 rams (the number of rams is on average about 20% the number of ewes), this means that the frequency of ARR/ARR rams has to be at least 0.10 before severe selection can be applied, and this corresponds to an ARR frequency of about 0.33. When applied to a breed of 750 ewes, the same reasoning leads to a 0.50 frequency of ARR/ARR rams, corresponding to an ARR frequency of about 0.70. With mild selection, both homozygous and heterozygous rams are used. A frequency of 0.50 rams, needed for a population with 150 rams, with at least one ARR allele corresponds to a frequency of 0.70 non-ARR alleles and to a frequency of 0.09 ARR/ARR animals. The frequency of ARR/ARR rams for choosing between mild and moderate selection for the smallest population size was therefore set to 0.10. The thresholds for populations between 750 and 3750 ewes were set about halfway between the thresholds for smaller and larger populations, leading to the advice given in Table 6.

Following the advice in Table 6, the selection regime will change when the ARR frequencies increase due to selection. Such a dynamic selection strategy has the advantage that when the ARR frequencies increase more slowly (or more quickly) than expected, the selection regime changes accordingly, thus avoiding unexpectedly high inbreeding rates. The results of this strategy are depicted for the Schoonebeeker in Figure 3. It can be seen that the

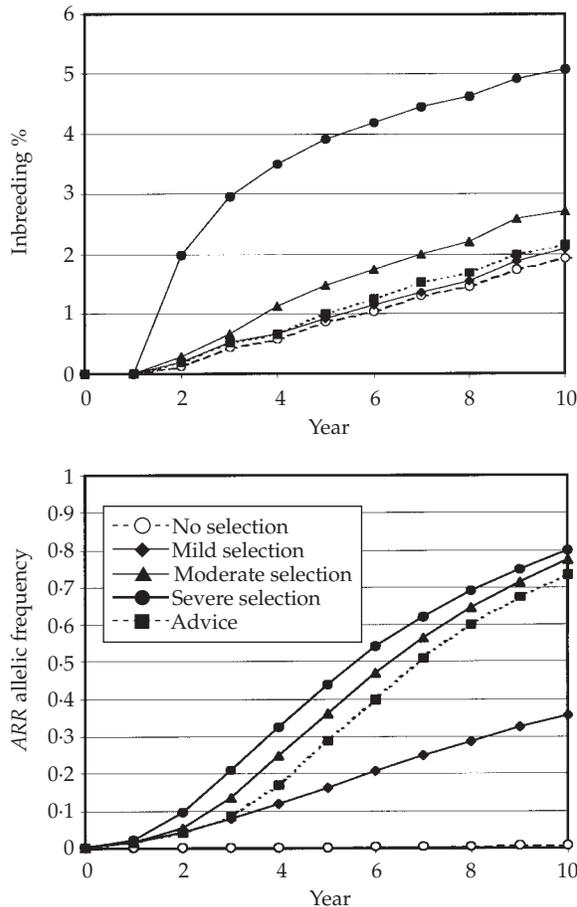


Figure 3 Effect of different selection regimes on (a) inbreeding levels and (b) frequency of the *ARR* allele for the Schoonebeeker breed. With selection following the advice (see Table 6), the regime is changed depending on the frequencies of the *ARR* allele.

increase in inbreeding with this strategy is only small, compared with the situation without selection. The frequency of the *ARR* allele is, however, only slightly less than under severe selection after 10 years of selection. With this strategy, fixation of the *ARR* allele was reached after on average 36.8 years compared with 31.5 and 32.4 years with a fixed severe and moderate selection, respectively.

Discussion

The effects of breeding for scrapie resistance on inbreeding can be severe. For all rare breeds examined in this paper, except the Hampshire Down breed, severe selection (exclusive use of homozygous *ARR* rams) produces inbreeding rates above 0.5% per year. Moreover, most breeds will also suffer a

population decline under severe selection because there are not enough homozygous *ARR* rams available to inseminate all ewes. These two effects combined would increase the probability of extinction of these rare breeds to unacceptably high levels.

The inbreeding levels predicted in this paper probably underpredict real values. In breeds with low frequencies of the *ARR* allele it is likely that the alleles originate from one or few common ancestors. Selection for the *ARR* alleles will then lead to even higher inbreeding levels than those indicated here, because equal co-ancestries between all animals (whether having *ARR* alleles or not) were simulated. Indeed the one breed for which we had a reliable pedigree, the Fries Melkschaap, showed that inbreeding levels were almost doubled when relationships were taken into account. It is likely that in other breeds with low *ARR* frequencies, animals having one or two *ARR* alleles will also have common ancestors. Indeed for the Mergellander breed it is suspected that all *ARR* alleles originate from a single ram (W. de Haan, personal communication).

All selected sires were assumed to produce the same number of offspring. However, this will not be generally the case as differences in fertility may exist and some rams may be used preferentially. This can lead to higher inbreeding levels, if the offspring of a preferred ram also produces more offspring. One may, however, also use variable numbers of offspring to its advantage, by increasing the number of offspring of rams that show a low average relationship to the rest of the population. The optimized contribution algorithm (Meuwissen, 1997) follows this approach, and at the same time optimizes selection. Sonesson *et al.* (2003) evaluated this approach for the elimination of single gene genetic defects. Provided that a reliable pedigree is present, optimal contribution will accelerate the increase of the *ARR* allele at the same or a lower rate of inbreeding than that obtained following the advice of this paper.

Scrapie eradication programmes are being set up all over Europe (e.g. Barillet *et al.*, 2002; Detwiler and Baylis, 2003). *PrP* genotype frequencies have been assessed in a large number of breeds (e.g. Bossers *et al.*, 1999; Drögemüller *et al.*, 2001; O'Doherty *et al.*, 2002; Palhière *et al.*, 2002) and strategies for scrapie eradication in large commercial breeds have been modelled (Kao *et al.*, 2001; Arnold *et al.*, 2002; Sonesson *et al.*, 2003). Our research shows that, in rare breeds, attention to inbreeding is of vital importance. If the most straightforward selection

strategy (i.e. exclusive use of homozygous *ARR* rams) is rigidly implemented these breeds will suffer the risk of extinction. A more careful selection strategy that initially allows the use of heterozygous *ARR* rams as well, will avoid unacceptably high inbreeding levels and lead to the same levels of resistant sheep, albeit some years later. At the same time, the loss of genetic diversity within breeds through genetic drift caused by selection of a few individuals can be also avoided.

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