The use of clones in dairy cattle breeding

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The use of clones in dairy cattle breeding

Imke J.M. de Boer

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Abstract

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The aim of this thesis was to determine a breeding scheme that optimally uses large scale production of genetically identical individuals (clones) in dairy cattle. Such a breeding scheme should optimize the continuous genetic improvement of the breeding population (genetic response), and the selection of genetically superior cows to produce cloned embryos (clonal response), so as to disseminate high merit breeding material to milk producers. The effect on the genetic and clonal response of testing several clones per genotype in a nucleus breeding scheme was studied using simulation models, assuming a fixed number of cows tested each year. The breeding scheme was optimized by comparing genetic and clonal response for various breeding designs. Unlike for semen, the use of cloned embryos can exploit both additive and dominance genetic effects. To fully exploit dominance effects, theory on prediction of individual dominance effects in populations with inbreeding was studied extensively. Subsequently, relevant factors were determined that influence market share of commercially available cloned embryos compared to semen. In addition, ethical aspects of the use of clones in dairy cattle production were discussed.

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Stellingen

1 Een methode, die rekening houdt met inteeltdepressie maar veranderingen in dominantie-(co)varianties als gevolg van inteelt negeert, resulteert in zuivere en nauwkeurige schattingen van dominantie-effekten en is praktisch toepasbaar voor grote populaties met inteelt.
Dit proefschrift

2 Een nucleusfokprogramma met korte generatie-intervallen is, bij toepassing van *in vitro* produktie van embryo's, optimaal wanneer jaarlijks minder koeien dan stieren geselekteerd worden.
Dit proefschrift

3 De conclusie van Teepker en Smith (1989) en De Boer en Van Arendonk (1991) dat, gegeven een vaste testcapaciteit, de produktie van betrouwbare kloonlijnen leidt tot een afname van de genetische vooruitgang is onjuist.
Teepker, G. and C. Smith. 1989. Anim. Prod. 49:49-62. De Boer, I.J.M. and J.A.M. Van Arendonk. 1991. Anim. Prod. 53:1-9.
Dit proefschrift

4 Ondanks de discussie over het gebruik van het infinitesimal model voor de simulatie van kwantitatieve kenmerken lijken huidige alternatieve modellen even discutabel.

5 Uitgebreide voorlichting over biotechnologie geeft mensen een kennisachtergrond waartegen incidentele media-berichten geplaatst en afgewogen kunnen worden, maar leidt niet tot een snellere acceptatie van biotechnologie.
Hamstra, A.M. en M.H. Feenstra, 1989; SWOKA, Instituut voor Konsumentenonderzoek, Den Haag.

6 Het toekennen van een patent op een dier strookt niet met het erkennen van diens intrinsieke waarde.

7 Het terugdringen van de bevolkingsaanwas in ontwikkelingslanden en het terugdringen van de consumptie in 't rijke Noorden zijn de belangrijkste ecologische uitdagingen.
Jonathon Porritt. In: Red de Aarde. 1991. Dorling Kindersley Limited, London.

8 De import van grote hoeveelheden goedkoop krachtvoer uit ontwikkelingslanden voor de voeding van onze landbouwhuisdieren getuigt van weinig respect ten opzichte van ontwikkelingslanden.

9 Het refereren van anonieme artikelen bevordert een objectieve beoordeling.

10 All scientists are equal but some are more equal than others.

Proefschrift van Imke J.M. de Boer. The use of clones in dairy cattle breeding. Wageningen, 16 september 1994.

Voorwoord

Een promotie is een soort Nijmeegse vierjaarse. Voor deelname heb je inschrijfgeld nodig. Met dank aan Holland Genetics kon ik van start gaan. Bij de start kreeg ik een 'ruwe' routebeschrijving. In het begin ben je nog wat onzeker over de exact te volgen route en moet je je eigen tempo nog bepalen. Johan, jij hebt me zelfvertrouwen gegeven om mijn eigen route uit te stippelen en een lekker ritme te vinden. Bedankt hiervoor en speciaal voor de bloemen uit Australië (ook jij Thea)! Johan, jij was lid van het verzorgende team, dat speciaal voor mijn vierjaarse werd samengesteld om mij onderweg te adviseren hoe de eindstreep te halen. Ook Pim, Julius en Theo hiervoor hartelijk dank.

Tijdens de uren durende wandelingen heb ik met mijn 'lotgenoten' Ant, Henk, Luc, Marco, Ron en Sijne héél wat afgekletst (en gemaild). Bedankt dame en heren voor jullie gezelligheid en steun! Luc en Sijne, bedankt voor het delen van een kamer waarin de sfeer steeg naarmate de spanning toenam. Voor wie de vierjaarse nog niet is afgelopen, succes ermee! Ook heb ik tijdens deze wandelingen veel andere, leuke mensen ontmoet. Enkelen wil ik hier graag noemen. Ada, bedankt voor de gezellige 'klets- en rookpauzes' op het secretariaat. Ina, thanks for the inspiring period working together. Mik(i)e, bedankt voor je hulp en altijd zonnige humeur.

Langs de lijn ben ik door veel mensen aangemoedigd. Pappa en mamma bedankt voor jullie interesse en steun. Aan jullie draag ik dit proefschrift op. Lieve Geert, bedankt voor je steun door dik en dun. We zijn er tot nu toe goed in geslaagd 'ons werk' en het 'verwennen' van de tweeling te combineren, ook al heeft het ons al menig pondje gekost. We hebben, ook tijdens de afronding van dit proefschrift, altijd voldoende tijd vrij gemaakt voor elkaar. Ik hoop dat ons dat in de komende jaren ook lukt.

Ook voor een Wageningse vierjaarse geldt: de laatste loodjes wegen het zwaarst. Net als tijdens de laatste kilometer door de 'beruchte Annastraat' heb ik ook nu de mentale steun nodig van goede vrienden. Ron, je was mijn maatje op de vakgroep. Gelukkig zorgde je voordat je naar de zon vertrok voor een goede vervanger. Richard, ik ben blij dat ik een aantal jaren geleden via de feestcommissie aan je 'gekoppeld' werd. Leuk dat ik jullie beiden, en niet te vergeten Corine en Mieke, ontmoet heb!

Na afloop van mijn vierjaarse, zet ik mijn 'vertrouwde' schoenen op de plank en trek een paar nieuwe aan. Ik hoop van harte dat ik bij de sectie Dierlijke Produktie Systemen, de prettige samenwerking met iedereen zal kunnen voortzetten.

Imke

Contents

General introduction

Dairy cattle breeding organisations currently sell mainly semen from sires of high genetic merit to producers to breed the next generation of genetically improved milking cows. For continuous improvement of commercially available semen, breeding organisations select and breed animals of high genetic merit. The reproductive rate of animals has a large impact on selection and breeding of animals, and the way genetic material can be disseminated from the breeding population (nucleus) to the commercial population (commercial). It affects genetic response, resulting from selection and breeding of animals, through selection intensity, selection accuracy, generation interval and rate of inbreeding. Technologies affecting reproductive rate of animals include artificial insemination (AI), multiple ovulation and embryo transfer (MOET), *in vitro* production of embryos (IVP) and production of genetically identical individuals (cloning). In this thesis, the influence of cloning on genetic response and on dissemination was studied. Before the topics of this thesis are introduced more explicitly, an overview of the effect of AI, MOET, and IVP on genetic response and on dissemination is given.

Artificial insemination

Introduction of AI in dairy cattle breeding removed the reproductive constraint from bulls and increased genetic response. With AI, fewer sires were needed as parents for the next generation and each sire had a larger number of offspring dispersed over many herds. Consequently, sire selection intensity increased and sire evaluation became more accurate through extensive use of daughter information. Improved techniques to dilute and freeze semen allowed further increase in the number of offspring per sire, and might therefore increase sire selection intensity. Implementation of these improved techniques, however, hardly affected genetic response because of the larger number of sires required to control inbreeding rate. On the other hand, implementation had a large effect on dissemination due to large scale use of genetically superior sires.

Hence, in a progeny testing scheme, sires selected on progeny test results are used for both breeding and dissemination. A cow descending from either nucleus or commercial cows is eligible for breeding when at least one lactation record is available. Genetic response in a progeny testing scheme is limited by long generation intervals and low reproductive rates for females. Response in a progeny testing scheme with long fixed generation intervals can be increased by selecting breeding individuals on BLUP (best linear unbiased prediction) of their breeding values, irrespective of their age (James, 1987; Meuwissen, 1989). As shown by Meuwissen (1989), this increase in response is due to shorter generation intervals for females and for males.

Multiple ovulation and embryo transfer

Introduction of reproductive techniques such as multiple ovulation, embryo recovery and embryo transfer increased reproductive rate for donor cows. With an increased reproductive rate for females, a progeny testing scheme with optimal generation intervals can be characterized by selection of young individuals descending largely from nucleus individuals. This increase in reproductive rate, enlarged the difference in genetic merit between nucleus and commercial individuals (Meuwissen, 1991). When reproductive rate for females is very high, the optimal progeny testing scheme resembles a closed multiple ovulation and embryo transfer (MOET) nucleus scheme (Nicholas, 1979). In MOET nucleus schemes, each selection candidate is a member of a large family that might contain full sibs, paternal half sibs and maternal half sibs. Cows eligible for selection are evaluated on information of ancestors as well as on information of female full sibs, half sibs or own performance records when available. Sires are no longer selected for breeding on progeny test results, but rather they are selected on pedigree information and information of female full and half sibs when available. Semen of genetically superior sires, selected irrespective of their age, is used for dissemination.

The increase in genetic response of a MOET nucleus scheme, compared to a progeny testing scheme with optimal generation intervals, is mainly due to an increase in selection intensity on females. With shorter generation intervals and extensive use of family information, however, inbreeding rate will also increase. Impact of MOET on breeding schemes has been studied extensively and reviewed by Dekkers (1992).

***In vitro* production of embryos**

The number of embryos per superovulated cow is low and variable, and a fraction of cows fail to produce transferable embryos in any one flush (Leitch *et al.*, 1990). These limitations seriously restrict the use of MOET in dairy cattle breeding schemes. At present, *in vitro* production of embryos (IVP) allows production of viable embryos at a large scale (for review see Rath, 1993). With IVP factorial mating designs, with an equal number of sires and dams (Woolliams and Wilmut, 1989), or hierarchical designs, with fewer dams than sires, might become feasible. Factorial designs, with an equal number of sires and dams, result in higher genetic response than factorial or hierarchical designs with fewer sires than dams at a similar level of inbreeding (Woolliams and Wilmut, 1989). Until now, designs with fewer dams than sires have not been studied. These designs, however, might result in a higher genetic response at a similar level of inbreeding. Moreover, with IVP both semen and embryos could be used extensively for dissemination. This would

decrease the difference in genetic merit between nucleus and commercial individuals.

Cloning

Cloning is the production of genetically identical individuals. Testing several clones for each genotype might increase genetic response to selection by increasing selection accuracy. With a fixed number of cows tested each year, however, testing several clones per genotype means testing fewer genotypes. Decreasing the number of genotypes eligible for selection might affect selection intensity and inbreeding rate. Inbreeding might reduce the mean phenotypic performance of individuals (Falconer, 1989) and complicates the genetic covariance structure of the population. Hence, the question whether testing several clones of each genotype really increases genetic response to selection is of interest.

Cloning might not only affect the genetic response, but cloned embryos from desirable genotypes might, in addition to semen, be used for dissemination. In dairy cattle only female genotypes are of interest, because only cows can be used for milk production. Unlike for semen, cloned embryos can exploit both additive and non-additive genetic effects. Non-additive genetic variance results from interactions between genes at the same locus (dominance) or at different loci (epistasis). In this thesis, only dominance effects were considered. To exploit additive and dominance variance in cloned embryos, accurate prediction of additive and dominance genetic merit of female genotypes eligible for commercial cloning is required.

Large scale use of cloned embryos requires optimal selection of cows with the highest additive plus dominance genetic merit for commercial cloning (clonal selection). Designs optimal for genetic and clonal selection might differ. For continuous improvement of commercially available clones, however, genetic response and clonal response should be optimized simultaneously.

Once the breeding scheme optimal for genetic response and clonal response has been determined, breeding organisations will be interested in the market share of commercially available cloned embryos compared to commercially available semen. The expected market share will be a very important factor for the introduction of cloning by a dairy cattle breeding organisation. Therefore, relevant factors influencing this market share should be known.

For commercial use of cloned embryos there are two requirements: (i) production of a large number of genetically identical individuals (commercial clone lines) through nuclear transfer (as explained later in this introduction) and (ii) production of clones from frozen embryos. The latter requirement would allow testing various female genotypes for traits of interest. Subsequently, stored frozen embryos from females selected for traits of interest can be cloned extensively to

generate a large number of commercially available embryos. Small successes for both steps have been reported (Bondioli, 1992). To study the potential role of cloning, it was assumed throughout this thesis that it is possible to produce a large number of embryos *in vitro*, to clone both fresh and frozen embryos and to produce a large number of genetically identical individuals using nuclear transfer. A summary of relevant embryo technologies is given at the end of the introduction.

Aim

The aim of this thesis was to determine a breeding scheme which optimally uses large scale production of genetically identical individuals (clones). Such a breeding scheme should optimize both the annual response to additive genetic selection (genetic response) and the response to selection of the best female genotype(s) for large scale production of commercially available cloned embryos (clonal response). In this thesis, the effect of testing clones on underlying components of genetic and clonal responses was determined. For this purpose, a better understanding was needed of methodologies to predict an individual's additive and dominance effect in populations with inbreeding. Once the optimal breeding scheme was determined, relevant factors influencing market share of commercially available cloned embryos compared to semen were determined.

Consider selection for a single trait associated with lactation, which is referred to as milk efficiency. The breeding goal for genetic selection is additive genetic merit for milk efficiency. The breeding goal for selection of the best female genotype for commercial cloning, however, is additive plus dominance genetic merit for milk efficiency.

In Chapter 1, we addressed the question of whether the optimal breeding design differed for genetic and clonal selection. Therefore, genetic and clonal responses were maximized independently by varying the mating design in a closed dairy cattle adult nucleus in which 256 or 1024 cows were tested each year, using deterministic simulation. The effect of inbreeding on genetic or clonal response to selection was not simulated. The mating design was characterized by the number of full sibs, maternal and paternal half sibs and the number of clones tested per genotype.

For optimal clonal selection, accurate prediction of additive and dominance effects was required. In the absence of inbreeding, prediction of additive and dominance effects is straightforward (Henderson, 1985). Rate of inbreeding in a closed nucleus, however, is expected to differ between schemes testing different numbers of clones per genotype. If dominance is present, inbreeding might reduce

the mean phenotypic value of the population, a phenomenon known as inbreeding depression (Falconer, 1989). In addition, inbreeding complicates the genetic covariance structure of a population with both additive and dominant gene action (Gillois, 1964; Harris, 1964; Jacquard, 1974; Cockerham and Weir, 1984). Two methods to predict additive and dominance effects in populations with inbreeding have been suggested. One method accounts for inbreeding depression by including the inbreeding coefficient as a covariate in the model, while ignoring changes in the genetic covariance structure of the population (Kennedy *et al.*, 1988). The second method accounts for all changes in mean and genetic covariance with inbreeding (Smith and Mäki-Tanila, 1990). In Chapter 2, we examined the approximate method of Kennedy *et al.* (1988) for several populations by comparing estimated additive and dominance effects for individuals with their corresponding simulated values. In Chapter 3, we analyzed properties of the exact method of Smith and Mäki-Tanila (1990) and presented stochastic simulation results to compare the approximate and the exact method. The approximate method can be implemented for livestock populations of moderate size, whereas implementation of the exact method in livestock populations is not feasible computationally.

A stochastic model simulating both additive and dominance gene action, while accounting for inbreeding, was developed to study the combination of genetic and clonal response in a closed nucleus scheme. At first, the genetic response, corrected for inbreeding, was optimized without cloning, assuming an efficient technique for *in vitro* production of embryos. Hierarchical and factorial designs with fewer sires than dams, with an equal number of sires and dams, or with fewer dams than sires were compared for their genetic response corrected for inbreeding. Results are given in Chapter 4. In Chapter 5, we described the effect on genetic and clonal response of testing clones at the expense of dams, sires, full or half sibs in a design optimal in the absence of cloning. Hence, a breeding scheme was determined that resulted in production of reliable commercial clone lines, now and in future generations.

In Chapter 6, relevant factors influencing marketability of cloned embryos versus semen were determined for the optimal design. Marketability of cloned embryos was assessed as the proportion of replacement commercial cows belonging to a commercial clone line. A commercial cow was inseminated if the net returns from her expected offspring were higher than net returns from a contemporary commercial clone.

Before the use of clones can be introduced in dairy cattle production, ethical aspects of the use of clones have to be considered. In Chapter 7, we discussed ethical aspects on use of clones in combination with *in vitro* production of embryos in dairy cattle breeding.

Description of relevant embryo technologies

In vitro production of embryos. The technique to obtain a large number of embryos *in vitro* involves four components: (i) recovery of oocytes through follicle aspiration, (ii) *in vitro* maturation of oocytes, (iii) *in vitro* fertilization of oocytes, and (iv) *in vitro* culture of embryos until transfer. Before oocyte collection, a cow is anaesthetized epidurally and sedated. Subsequently, oocytes are collected by ultrasound-guided aspiration of visible follicles through the vagina (Pieterse *et al.*, 1988). After collection, oocytes are matured and fertilized *in vitro* and obtained embryos are co-cultured with a bovine oviduct epithelial cell monolayer or in conditioned medium (for review see Rath, 1993). An embryo that develops to the morula or blastula stage can be transferred non-surgically to a recipient cow. Such an embryo can be used also as donor embryo in the process of cloning.

At present, on average, 15 oocytes can be obtained per collection using follicle aspiration (Van der Schans *et al.*, 1992). Oocytes can be collected twice a week for at least 3 months (Kruip *et al.*, 1993), resulting in an average of 390 oocytes per cow. The average number of oocytes collected per session, however, differs significantly between cows (Van der Schans *et al.*, 1992). On average, 25% of collected oocytes develop to transferable embryos (Van der Schans *et al.*, 1992) and 40% of these transferred embryos result in a pregnancy (Kruip *et al.*, 1993). Hence, the current overall efficiency of IVP is 10%, which means that about 10% of all collected oocytes (about 39 of 390) will result in birth of a calf.

Cloning techniques. Two methods have been developed to clone dairy cattle embryos: (i) splitting (Willadsen, 1982) and (ii) nuclear transfer (Willadsen, 1986). Two or four identical embryos can be obtained by splitting an embryo at any stage from two-cell to early blastocyst (approx. an 8-day old embryo). After splitting, each half/quarter must be placed in an artificial zona pellucida for its protection. Split parts obtained from morulae (approx. 5- to 6-day old embryos) and blastocysts, however, survive transfer into a recipient without a zona. Each embryo must be at the morula or blastula stage before it can be transferred non-surgically to a recipient cow. Development of a two-cell embryo to these stages, for example, can be achieved either in a temporary recipient (sheep or rabbit) or in culture.

The splitting procedure has two main limitations. First, repeated splitting is not possible. If a morula is split in half, the path of development of each split half is unaffected. As a result, each half of the embryo forms a blastocysts at the time that the intact blastocyst would have been formed. Secondly, normal development of split parts does not occur if the embryo is split into more than four parts, apparently because each part of the embryo contains too few cells for normal development. The maximum number of identical embryos that can be obtained by splitting is biologically limited to four.

These two limitations of embryo splitting were overcome by the nuclear transfer technique (Figure 1). A recipient oocyte is formed by enucleating a secondary oocyte (oocyte in metaphase-II, Figure 1a). Enucleation is done by drawing the oocyte's chromosomes into a pipette. At the same time, the zona pellucida of the donor embryo is removed and individual cells are separated (disaggregation, Figure 1b). Subsequently, one donor cell is transferred to the enucleated secondary oocyte by placing it behind its zona, against the cytoplast (Figure 1c), and by passing electric currents to cause fusion of the cell and the cytoplast (electrofusion, Figure 1d). The reconstituted oocyte begins to develop as though it were newly fertilized and can be transferred to a recipient cow after development in culture (Figure 1e) to the morula or blastula stage. The newly developed morula can either be transferred (Figure 1f) or used as donor embryo again, a process called repeated cloning (Figure 1g).

An alternative for repeated cloning is use of embryonic stem cells (ESC) as donor nuclei for nuclear transfer (Figure 1h). Embryonic stem cells come from the inner cell mass of an embryo and should be cultured *in vitro* without differentiation (Evans and Kaufman, 1981). Keefer *et al.* (1993) showed that bovine inner cell mass cells isolated from expanded blastocysts are pluripotent; that is they show normal embryonic and fetal development after transfer to enucleated oocytes. The observed pluripotency of these inner cell mass cells is encouraging for the development of embryonic stem cell lines in bovine. Until now, however, bovine stem cell-like colonies have not been isolated.

In theory, nuclear transfer has the potential to produce an unlimited number of identical genotypes. Until now, however, overall success rates of cloning have been quite low. This is typical of technologies that consist of many consecutive steps. Barnes *et al.* (1990) reported a 94% recovery of embryos that were cultured in the sheep oviduct for 5 to 6 days. Observation of recovered embryos showed that fusion of donor nuclei and cytoplast had occurred in 88-93% of the embryos. The percentage of viable embryos depended on whether the embryos were frozen (14%) or fresh (22%). The percentage of viable embryos resulting in a pregnancy varied from 25 to 30%. Only 85% of these pregnancies resulted in birth of a live calf.

Starting with 32 nuclei from a donor morula, 30 cells are recovered on average. From these 30 cells, on average, 6.6 cells are viable and useful for transplantation, resulting in an average of 1.98 pregnancies and, consequently, in 1.7 calves. Results on repeated cloning have been very limited. Bondioli (1992) reported that two generations of nuclear transfer resulted in 2.77 pregnancies per embryo, implying that repeated cloning is still less efficient than cloning an original donor embryo. Results of *in vitro* culture of cloned embryos have not been found in the literature.

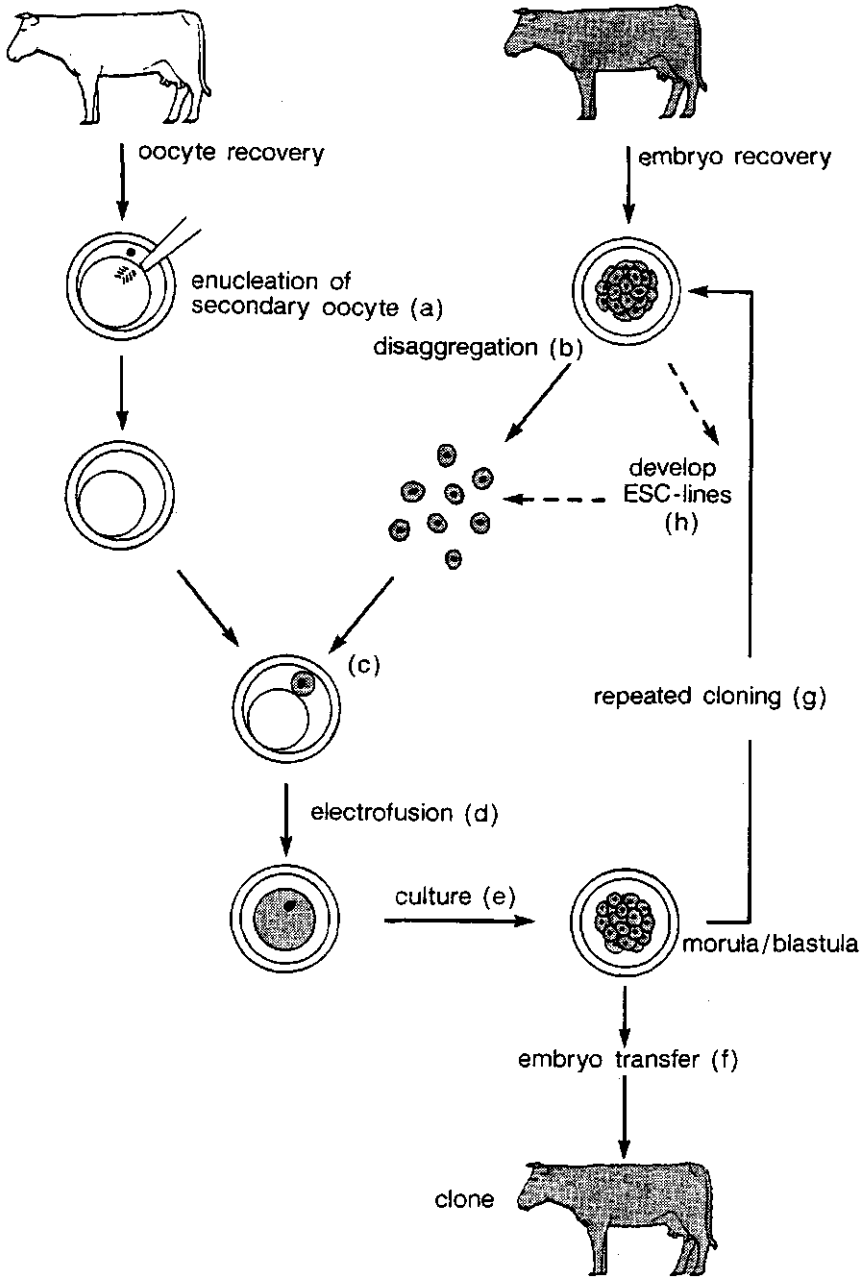

Figure 1. Cloning of embryos through nuclear transfer

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Chapter 1

Genetic and clonal responses in closed dairy cattle nucleus schemes

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* Note: the opportunity is also taken to correct errors in published results. Conclusions were unaffected by corrections

Abstract

The additive genetic response per generation and the genetic superiority of female genotype(s) selected for commercial cloning (clonal response) were maximized for a closed adult nucleus scheme, in which 256 or 1024 cows were tested each year, by varying the mating design (number of clones, full sibs and half sibs). Responses were corrected for effects of finite sample size and correlated index values on selection intensity, and for variance reduction due to selection. Genetic response was always maximal when only one individual was tested per genotype. Maximal genetic response varied from 0.153 to 0.508 phenotypic standard deviations (σ_p) per generation, and equalled 0.67 to 0.89 of the corresponding uncorrected prediction. Reduction in response was largest when intensity and accuracy of male selection were high. In a design optimal for clonal selection, 1 to 32 clones and a maximum number of full sibs were tested per genotype. Maximal clonal response varied from $0.279\sigma_p$ to $2.514\sigma_p$, and increased with the heritability, the intra-clone correlation, the intensity of clonal selection and the number of cows tested each year. Reduction in maximal clonal response, which varied from proportionally 0.02 to 0.18, was smallest when dominance variance was high. Testing 1024 instead of 256 cows each year, increased maximal genetic response by 23% to 142%, while increases in maximal clonal response varied from 11% to 36%. With selection of only one male per full sib family, designs which maximized the genetic or clonal response were different in all situations studied. Differences were largest when the heritability and the intra-clone correlation were low, and clonal selection intensity was high. Without a restriction on male selection, designs optimal for genetic or clonal selection differed, unless dominance variance and intensity of clonal selection were both low.

Key words: breeding programmes, cloning, dairy cattle

Introduction

Cloning of cattle from embryos has been achieved by nuclear transfer (Bondioli *et al.*, 1990). For the production of reliable commercial clone lines, two further steps are required. One is repeated cloning of embryos, which would allow the production of large numbers of genetically identical individuals (clone lines). The other is further cloning from frozen embryos, which would allow testing of a set of genotypes for the traits of interest. After testing, stored embryos from selected superior genotypes can be used for the production of commercial clone lines (Smith, 1989). Successes for both steps have been reported (Bondioli *et al.*, 1990).

Application of clones in dairy cattle breeding programmes will probably result in the establishment of a nucleus and a commercial tier. The nucleus will be responsible for the continuous genetic improvement of the breeding stock and for the production of female genotypes eligible for commercial cloning. Cows, belonging

to one or a few commercial clone lines, will be used for milk production in the commercial tier. All nucleus cows will be tested for milk efficiency during their first lactation. Subsequently, several distinct bulls and cows will be selected for breeding (genetic selection), while only a few female genotypes will be selected to be cloned for commercial use (clonal selection). Cloning can be used to increase the accuracy of both genetic and clonal selection and to disseminate genetic superiority to the commercial population by embryos rather than by semen. Several studies have found no increase in genetic progress when cloning was used to increase the accuracy of selection (Teepker and Smith, 1989; Woolliams, 1989). The potential advantage of cloning, therefore, lies in the fast dissemination of superior tested genotypes in the commercial population.

Teepker and Smith (1989) predicted the genetic response and the genetic superiority of female genotypes selected for commercial cloning in a closed nucleus, for a fixed test capacity and a fixed number of parents. Optimal mating designs (number of half sibs, full sibs and clones) for genetic and clonal responses were found to be different, unless the intra-clone correlation was very high. Maximal clonal responses varied from 1.2 to 2.7 phenotypic standard deviations, increasing with the intra-clone correlation. Responses, however, were computed assuming an infinite sample size, uncorrelated selection index values between selection candidates and no variance reduction due to selection and inbreeding. Several authors have shown that the genetic response corrected for finite sample size, correlations between selection index values, and variance reduction due to selection and inbreeding equals only 0.39 to 0.91 of the corresponding uncorrected prediction (Juga and Mäki-Tanila, 1987; Ruane and Thompson, 1989; Meuwissen, 1989 and 1991).

In this study, maximal genetic and clonal response will be determined by varying the mating design. Moreover, intensities of both genetic and clonal selection, the test capacity, the heritability level and the intra-clone correlation of milk efficiency will be varied. Effects of finite sample size and correlated selection index values on selection intensity, and variance reduction due to selection will be taken into account.

Method

Reproduction techniques

It was assumed that techniques will be available to clone both fresh and frozen bovine embryos, to sex embryos and to produce a large number of genetically identical individuals.

Population structures

Genetic and clonal responses were determined for a closed breeding herd with discrete generations, in which a fixed number of cows (T) was tested for milk efficiency during their first lactation. Either 256 or 1024 test places were considered representing testing of cows on one central test station or on commercial farms, respectively. Four populations, differing in the number of distinct sires and dams selected for breeding the subsequent generation, were studied (Table 1). Population 1 consisted of a breeding herd of 32 sires and 32 dams ($N_s=N_d=32$). Two alternative populations, in which selection among males was more intense, were studied to determine the impact of selection intensity on correcting genetic and clonal responses for finite sample size, correlated index values and selection disequilibrium: population 2 and 3 in which $N_s=16$ and $N_s=4$, respectively. In population 1, 2 and 3, the number of breeding animals used each generation is limited and inbreeding rates will be relatively high. Therefore, population 4 was examined, in which inbreeding will be less important because $N_s=32$ and $N_d=256$.

Given T and the number of selected animals for both breeding and commercial cloning, maximal genetic and clonal responses were computed by varying the mating design. The next generation of individuals can be produced by mating each dam to one sire only (hierarchical design), or by mating each dam to several sires (factorial design). In each population, dams could be mated to one, two, four or at maximum eight (M_d) different sires. These might be realistic mating designs, knowing that *in vitro* production of transferable embryos is possible (Leibfried-Rutledge *et al.*, 1989).The number of dams a sire was mated to was calculated as $M_s = (N_d\ M_d)/N_s$. The number of embryos in each full sib family was varied from 2 to 16, 50% of them being males and 50% females (N_{fs}). Given T and the total number of distinct female genotypes tested ($N_d\ M_d\ N_{fs}$) the number of clones (K) tested per genotype can be computed as: $K=T/(N_d\ M_d\ N_{fs})$. In all alternatives the number of distinct female genotypes selected for the production of commercial clone lines could be either 1 or 10.

Table 1. Number of distinct sires and dams selected for breeding in each population

	Population			
	1	2	3	4
Sires (N_s)	32	16	4	32
Dams (N_d)	32	32	32	256

Selection

Genetic and clonal selection were both based on overall economic merit for milk production, defined as milk efficiency. Milk efficiency was assumed to have a heritability (h^2) of 0.10, 0.25 or 0.40 and was only measurable in females. When h^2 was 0.10 or 0.25, the intra-clone correlation (t_c) could be 1, 2 or 3.2 times the h^2. The latter value was considered to be able to compare results with Teepker and Smith (1989). With a h^2 of 0.40, t_c could be only 1 or 2 times the h^2. The intra-clone correlation gives the proportion of the phenotypic variance which clones have in common. This common variance consists of additive genetic, dominance and epistatic variance and all non-genetic variance (e.g. environmental variance) common to clones. In this study, all non-additive genetic variance common to clones was treated as dominance variance.

Selection index theory was used to calculate the accuracy of both genetic and clonal selection. The breeding goal for genetic and clonal selection differed, and equalled additive genetic merit (A) and clonal merit (C) for milk efficiency, respectively. Clonal merit, which is total genetic merit, included dominance as well as additive genetic effects. Sources of information used to compute selection index values were the same for both types of selection, but differed between bulls and cows (Table 2). Bulls were selected on records of their dam, full sibs, maternal and paternal half sibs, each genotype having K clones. Cows were moreover selected on own first performance and the performance of their clones. Information on grandams, and on half sibs or full sibs of sires and dams was not used. Including this information would only slightly increase accuracy of genetic and especially clonal selection.

Prediction of genetic and clonal responses

The clonal response (C) was determined as:

$$C = i_{C(t)} r_{IC} \sigma_C \qquad [1]$$

where $i_{C(t)}$ equals the selection intensity of female genotypes selected for commercial cloning assuming a finite sample size and correlated index values, r_{IC} is the accuracy of clonal selection, and σ_C is the total genetic standard deviation.

The genetic response per generation (A) was computed as:

$$A = \frac{1}{2}\{ (i_{Ad(t)} r_{IAd} \sigma_A) + (i_{As(t)} r_{IAs} \sigma_A) \} \qquad [2]$$

where $i_{Ad}(t)$ and $i_{As}(t)$ are finite selection intensities for dam and sire selection in nucleus breeding, respectively; r_{IAd} and r_{IAs} are accuracies of genetic selection for dams and sires, and σ_A is the additive genetic standard deviation.

Table 2. Number of records for different sources of information used to estimate additive genetic merit and clonal merit of cows and bulls[a]

	Source of information				
	Self	Dam	Full sibs	Maternal half sibs	Paternal half sibs
Cows	K	K	K (N_{fs}-1)	K N_{fs} (M_d-1)	K N_{fs} (M_s-1)
Bulls	-	K	F N_{fs}	K N_{fs} (M_d-1)	K N_{fs} (M_s-1)

[a] K is the number of clones tested per genotype; N_{fs} is the number of females per full sib family; M_d (M_s) is the number of matings per dam (sire)

Selection intensities assuming finite sample size and correlated index values were approximated for bulls and cows separately as (Rawlings, 1976):

$$i_{(t)} = i_{(0)}(1 - t_{avg})^{\frac{1}{2}} \qquad [3]$$

here is $i_{(t)}$ the approximated selection intensity assuming finite sample size and correlated index values; $i_{(0)}$ the approximated selection intensity assuming finite sample size (Burrows, 1972) and t_{avg} the average correlation between index values of selection candidates. For dams, t_{avg} was calculated as:

$$t_{avg} = \frac{(N_{fs}-1)t_{fs} + (M_d-1)N_{fs}t_{Mhs} + (M_s-1)N_{fs}t_{Phs}}{(N_d M_d N_{fs}-1)} \qquad [4]$$

where t_{fs}, t_{Mhs} and t_{Phs} represent correlations between selection index values of female full sibs, maternal and paternal half sibs, respectively. Without a restriction on the number of males selected per full sib family, t_{avg} of bulls was computed using [4]. However, when only one male could be selected per full sib family, t_{avg} was calculated using [4] with $N_{fs}=1$. The correlation between selection index values of two relatives (t_{rel}) was computed as:

$$t_{rel} = \frac{\mathbf{b'Rb}}{\mathbf{b'Pb}} \qquad [5]$$

where **b** is a n×1 vector of weighting factors for the n information sources used in the index; **R** is a n×n matrix of covariances between phenotypic information sources of the two relatives; **P** is a n×n matrix of covariances between phenotypic information sources of the same individual.

Variance reduction due to selection on additive genetic merit

Reductions in genetic and phenotypic (co)variances due to selection on estimated additive genetic merit were accounted for in calculating accuracies of genetic and clonal selection, additive and total genetic standard deviations and intra-class correlations between family selection index values. The general formula used for reduction of (co)variances due to selection is given by Cochran (1951) as:

$$\sigma^{*}_{AB} = \sigma_{AB} - \frac{\sigma_{AI}\sigma_{BI}i_{\infty}(i_{\infty} - x)}{\sigma^{2}_{I}} \qquad [6]$$

where σ_{AB}, σ^{*}_{AB} is the covariance between A and B before and after selection, respectively; σ_{AI}, σ_{BI} is the covariance between A and B with selection index before selection; σ^{2}_{I} is the variance of selection index before selection; i_{∞} is the standardized selection differential assuming an infinite sample size and x is the truncation point of the normal distribution corresponding to i_{∞}.

In formula [6], the standardized selection differential and the corresponding truncation point in an infinite population were used. Therefore, variance reduction due to selection will be underestimated. The effect of this underestimation on the steady state genetic gain, however, will be small (Meuwissen, 1990).

Reduced additive genetic variance between selected sires and dams in generation t, referred to as $\sigma^{2}_{Ad}{}^{(t)}$ and $\sigma^{2}_{As}{}^{(t)}$, was computed separately using formula [6]. The additive genetic variance between their progeny in generation t+1 ($\sigma^{2(t+1)}_{A}$) was computed as:

$$\sigma^{2(t+1)}_{A} = \frac{1}{4}\sigma^{2(t)}_{Ad} + \frac{1}{4}\sigma^{2(t)}_{As} + \sigma^{2}_{Aw} \qquad [7]$$

where σ^{2}_{Aw} is the within-full-sib family additive genetic variance, which is unaffected by selection when inbreeding is ignored (Bulmer, 1980). Equilibrium variances, which were reached after eight generations of selection, were used to determine required parameters. Total genetic and phenotypic variances between unselected individuals in generation t+1, were predicted assuming that dominance (σ^{2}_{D}) and environmental variances were unaffected by selection. The covariance between full sibs in generation t+1 was computed as: $\frac{1}{4}\sigma^{2}_{Ad}{}^{(t)} + \frac{1}{4}\sigma^{2}_{As}{}^{(t)} + \frac{1}{4}\sigma^{2}_{D}$. Covariances between maternal and paternal half sibs in generation t+1 equal $\frac{1}{4}\sigma^{2}_{Ad}{}^{(t)}$ and $\frac{1}{4}\sigma^{2}_{As}{}^{(t)}$, respectively. The variance of the mean performance of selected dams and the covariance of this mean with the breeding goal were also computed using [6].

Results

Maximal genetic and clonal responses

Maximal genetic and clonal responses were determined in each population by varying the mating design. To illustrate the strategy used to compute maximal responses, genetic and clonal responses and their underlying components are given in Table 3 and 4 for all mating designs studied in population 3 (N_s=4 and N_d=32), assuming $h^2=t_c=0.25$.

The genetic response was maximal ($A=0.400\sigma_p$) when each dam was mated to the maximum number of sires ($M_d=4=N_s$), the number of females per full sib family was two ($N_{fs}=2$) and only one clone was tested per genotype ($K=256/(32x4x2)=1$). Testing more clones per genotype reduced selection intensity relatively more than it increased accuracy of female selection.

The clonal response was maximal when each dam was mated to only one sire ($M_d=1$), the number of females per full sib family was one ($N_{fs}=1$), and the maximum number of clones (K=8) was tested per genotype. Testing fewer clones caused a reduction in accuracy of female selection and total genetic standard deviation, which was not compensated by the increase in selection intensity.

Table 3. Accuracy of selection, selection intensity and corresponding genetic response (in σ_p before selection) in population 3 for varying mating designs[a], with $h^2=t_c=0.25$ and T=256

Females

	Accuracy			Selection intensity			Genetic response		
M_d	1	2	4	1	2	4	1	2	4
N_{fs}									
1	0.857	0.749	0.656	0.000	0.755	1.192	0.193	0.297	0.369
2	0.750	0.658	0.583	0.754	1.191	1.525	0.272	0.346	**0.400**
4	0.661	0.585		1.191	1.523		0.322	0.377	
8	0.591			1.525			0.353		

Males

M_d	1	2	4	1	2	4
N_{fs}						
1	0.546	0.448	0.447	1.498	1.723	1.963
2	0.455	0.447	0.451	1.439	1.708	1.964
4	0.456	0.455		1.431	1.709	
8	0.466			1.434		

[a] M_d is the number of matings per dam; N_{fs} the number of females per full sib family; the number of clones tested per genotype ($K=256/(32\ M_d\ N_{fs})$) is the same for all designs in which $M_d\ N_{fs}$ is constant

Table 4. Accuracy of selection, selection intensity and corresponding clonal response[a] (in σ_p before selection) in population 3 for varying mating designs[b], with $h^2=t_c=0.25$ and T=256

	Females								
	Accuracy			Selection intensity			Clonal response		
M_d	1	2	4	1	2	4	1	2	4
N_{fs}									
1	0.857	0.749	0.656	1.986	2.219	2.427	**0.803**	0.739	0.712
2	0.750	0.658	0.583	2.218	2.425	2.607	0.740	0.714	0.686
4	0.661	0.585		2.425	2.605		0.717	0.688	
8	0.591			2.607			0.694		

[a] Assuming selection of one female genotype for commercial cloning

[b] M_d is the number of matings per dam; N_{fs} the number of females per full sib family; K is the number of clones tested per genotype

Table 5. Designs optimal for genetic (A) or clonal selection (C_1)[a] with corresponding maximal (in σ_p before selection) and relative (as proportion of maximum) responses for varying intra-clone correlations (t_c), with $h^2=0.25$ and T=256

Optimal for	t_c	Design[b]			Maximal Response[c]	Relative response	
		M_d	N_{fs}	K		A	C
A	0.25	8	1	1	0.335 (0.76)	1.00	0.78
	0.50	8	1	1	0.335 (0.76)	1.00	0.93
	0.80	8	1	1	0.335 (0.76)	1.00	0.99
C_1	0.25	1	1	8	0.881 (0.91)	0.00	1.00
	0.50	1	4	2	1.440 (0.89)	0.52	1.00
	0.80	1	8	1	2.203 (0.92)	0.59	1.00

[a] C_1 means selection of one female genotype for commercial cloning

[b] M_d is the number of matings per dam; N_{fs} is the number of females per full sib family; K is the number of clones tested per genotype

[c] Values in parentheses are the proportions of uncorrected response

Effect of the intra-clone correlation

Table 5 shows maximal genetic and clonal responses achieved in population 1 ($N_d=N_s=32$) with corresponding optimal mating designs, for varying t_c. Genetic response was maximal when each dam was mated to eight sires ($M_d=8$), which was the maximum number considered, the number of females per full sib family was one ($N_{fs}=1$), and only one clone ($K=1$) was tested per genotype, irrespective of t_c. Maximal genetic response was not affected by t_c, because covariances between full sibs and clones did not occur in the optimal mating design. Clonal response obtained when genetic selection was maximized, assuming selection of one female genotype (C_1), varied from proportionally 0.78 to 0.99 of its maximum value. For example, with $t_c=0.50$ the clonal response was proportionally 0.93 of its maximum value, The maximal response ($C_1=1.440\sigma_p$) would be obtained with $M_d=1$, $N_{fs}=4$ and $K=2$. In designs which maximized the clonal response, K varied from one to eight. Generally, K decreased as t_c increased, resulting in an increased intensity of clonal selection. Because of this increase in selection intensity and a higher total genetic standard deviation (σ_c), clonal responses increased rapidly with increasing t_c. Genetic responses achieved when clonal selection was maximized varied from proportionally 0 to 0.59 of their maximum. From Table 5 it can be concluded that optimal mating designs for clonal and genetic selection are different even if t_c is high.

Effect of intensity of genetic and clonal selection

In populations 2 ($N_s=16$) and 3 ($N_s=4$) fewer sires were selected for breeding than in population 1 ($N_s=32$). In each population the number of female genotypes selected for commercial cloning could be either 1 or 10. Maximal genetic and clonal responses in both populations with corresponding optimal mating designs are given in Table 6, with $h^2=0.25$ and $T=256$. Genetic responses in population 1 to 3 equalled proportionally 0.68 to 0.76 of their uncorrected predictions (Table 5 and 6), while clonal responses equalled 0.73 to 0.96 of uncorrected responses. Reductions in both genetic and clonal response, due to correcting for finite sample size, correlated index values and linkage disequilibrium, were largest in population 3 when dominance was absent ($t_c=h^2$). As t_c increased, reductions in maximal clonal response decreased considerably, while reductions in maximal genetic response hardly changed.

Genetic responses were higher with $N_s=4$ than with $N_s=16$ or 32 due to the higher intensity of male selection, although differences decreased as a result of correcting responses for finite sample size, correlated index values and selection disequilibrium. Clonal responses, however, decreased when N_s decreased, due to the larger reduction of the additive genetic variance and higher correlations between index values of female genotypes eligible for commercial cloning.

Table 6. Maximal genetic (A) and clonal responses (C_n)[a] in population 2 and 3 (in σ_p before selection) with corresponding optimal designs[b] for varying intra-clone correlations (t_c), with h^2=0.25 and T=256

		Population 2				Population 3			
		Design				Design			
Optimal for	t_c	M_d	N_{fs}	K	Response[c]	M_d	N_{fs}	K	Response
A	0.25	8	1	1	0.375 (0.74)	4	2	1	0.400 (0.68)
	0.50	8	1	1	0.375 (0.74)	4	2	1	0.398 (0.68)
	0.80	8	1	1	0.375 (0.74)	4	2	1	0.395 (0.68)
C_1	0.25	1	1	8	0.836 (0.86)	1	1	8	0.803 (0.82)
	0.50	1	4	2	1.418 (0.88)	1	4	2	1.387 (0.86)
	0.80	1	8	1	2.185 (0.91)	1	8	1	2.158 (0.90)
C_{10}	0.25	1	8	1	0.555 (0.79)	1	8	1	0.531 (0.73)
	0.50	1	8	1	1.057 (0.92)	1	8	1	1.029 (0.89)
	0.80	1	8	1	1.670 (0.96)	1	8	1	1.649 (0.95)

[a] C_n means selection of n genotypes for commercial cloning
[b] M_d is the number of matings per dam; N_{fs} is the number of females per full sib family; K is the number of clones tested per genotype
[c] Values in parentheses are the proportions of uncorrected response

Table 7. Maximal genetic (A) and clonal responses (C_n)[a] in all populations for varying intra-clone correlations (t_c), when T=1024 and h^2=0.25, expressed as proportion of the maximum response when T=256

		Population			
Optimal for	t_c	1	2	3	4
A	0.25	1.38	1.34	1.27	2.42
	0.50	1.36	1.32	1.25	2.42
	0.80	1.34	1.30	1.23	2.42
C_1	0.25	1.11	1.11	1.11	1.36
	0.50	1.18	1.18	1.18	1.25
	0.80	1.14	1.14	1.15	1.13
C_{10}	0.25	1.25	1.26	1.27	1.36
	0.50	1.24	1.25	1.26	1.25
	0.80	1.23	1.23	1.23	1.22

[a] C_n means selection of n genotypes for commercial cloning

Selection of 10 instead of 1 commercial clone line (C_{10} versus C_1) reduced clonal responses, due to a reduced intensity of clonal selection, and affected optimal mating designs. The optimal number of clones tested per genotype was lower when more genotypes were selected for commercial cloning each generation.

Effect of test capacity

Maximal genetic and clonal responses in all populations are given in Table 7 for varying t_c, when T=1024. Increasing T gave proportionally 0.23 to 0.38 higher genetic responses in population 1 to 3, while proportional increases in clonal responses varied from 0.11 to 0.27. In population 4 the increases in genetic response was enormously high. This was due to the fact that only one mating design was possible for population 4 when T=256 (M_d=1; N_{fs}=1 and K=1), and the corresponding genetic response was low ($A=0.153\sigma_p$). Increases in clonal response varied from proportionally 0.13 to 0.36. The increase in response with 1024 compared to 256 test places was mainly caused by an increase in selection intensity. The increase in selection intensity, and consequently in response, was greatest when the number of breeding animals or clone lines selected was large.

Designs which maximized genetic selection still tested a maximum number of maternal half sibs and only one clone per genotype. The number of clones tested per genotype in designs optimal for clonal selection changed in a few situations. Reductions in genetic and clonal response increased slightly when T increased, due to a higher selection intensity and a slightly higher accuracy.

Effect of the heritability level

The effect of h^2 on the maximal genetic and clonal response, and the corresponding mating design, is given in Table 8, for population 1 when T=256. Genetic response was always maximal when each dam was mated to the maximal number of sires (M_d=8) and only one clone was tested per genotype (K=1), irrespective of h^2 or t_c. As expected, genetic response increased with h^2.

Designs optimal for clonal selection depended on h^2 and t_c. Generally, K increased when h^2 decreased, resulting in a decrease in clonal selection intensity in optimal designs. The lower selection intensity together with the lower selection accuracy, gave lower maximal clonal responses with h^2=0.10 than with e.g. h^2=0.25. Reductions in maximal genetic response, due to correcting the selection intensity and accounting for selection disequilibrium, varied from proportionally 0.19 to 0.26. Reductions were largest when h^2 was high, due to a higher selection accuracy. Proportional reductions in maximal C_1 varied from 0.09 to 0.10.

Table 8. Genetic (A) and clonal responses (C_1)[a] in population 1 (in σ_p before selection) with corresponding optimal mating designs[b] for varying h^2 and t_c, with T=256

		Design optimal for A				Design optimal for C_1			
h^2	t_c	M_d	N_{fs}	K	Res	M_d	N_{fs}	K	Res
0.10	0.10	8	1	1	0.160	1	1	8	0.460
	0.20	8	1	1	0.160	1	1	8	0.749
	0.32	8	1	1	0.160	1	2	4	1.048
0.25	0.25	8	1	1	0.335	1	1	8	0.881
	0.50	8	1	1	0.335	1	4	2	1.440
	0.80	8	1	1	0.335	1	8	1	2.203
0.40	0.40	8	1	1	0.479	1	1	8	1.191
	0.80	8	1	1	0.479	1	8	1	2.151

[a] C_1 means selection of one female genotype for commercial cloning
[b] M_d is the number of matings per dam; N_{fs} is the number of female full sibs per family; K is the number of clones tested per genotype

Table 9. Maximal genetic (A) and clonal (C_n)[a] responses (in σ_p before selection) with corresponding optimal designs[b] in population 1, with and without a restriction on male selection for h^2=0.25 and T=256

		Restriction				No restriction			
		Design				Design			
Optimal for	t_c	M_d	N_{fs}	K	Response	M_d	N_{fs}	K	Response
A	0.25	8	1	1	0.335	1	8	1	0.363
	0.50	8	1	1	0.335	1	8	1	0.341
	0.80	8	1	1	0.335	8	1	1	0.335
C_1	0.25	1	1	8	0.881	1	1	8	0.881
	0.50	1	4	2	1.440	1	4	2	1.422
	0.80	1	8	1	2.203	1	8	1	2.188
C_{10}	0.25	1	8	1	0.581	1	8	1	0.547
	0.50	1	8	1	1.078	1	8	1	1.058
	0.80	1	8	1	1.684	1	8	1	1.673

[a] C_n means selection of n genotypes for commercial cloning
[b] M_d is the number of matings per dam; N_{fs} is the number of female full sibs per family; K is the number of clones tested per genotype

Effect of restriction on male selection

Male selection was based on information of female full sibs, paternal and maternal half sibs, and until now only one male could be selected per full sib family. To determine the effect of this restriction on male selection, genetic and clonal responses were computed for a situation in which all full sib males were eligible for selection. Maximal responses and corresponding optimal designs obtained with selection of one (restriction) or all (no restriction) male full sibs per family, are given in Table 9 for population 1. Designs which optimized genetic response changed in the situation of no restriction, while designs optimal for clonal selection were unaffected. With a restriction on male selection, designs optimal for genetic or clonal selection still differed, unless the intra-clone correlation and clonal selection intensity were both low.

Discussion and conclusions

In this study the genetic response in the nucleus and the genetic superiority of female genotypes selected to be cloned commercially (clonal response) were maximized by varying the mating design. Responses were corrected for the effects of finite sample size and correlated index values on selection intensity, and for variance reduction due to selection.

Reductions in maximal genetic and clonal response were mainly caused by variance reduction due to selection, and were consequently largest when the intensity and the accuracy of genetic selection were high (e.g. heritability was high; no dominance). For the genetic response, this is in agreement with results of Keller *et al.* (1990) and Ruane and Thompson (1989). For a fixed heritability, an increase in dominance variance hardly affected reductions in maximal genetic response, because dominance decreased selection accuracy only slightly in optimal designs. Reductions in clonal response, however, decreased considerably as dominance variance increased. When dominance variance increases own performance information of female genotypes eligible for commercial cloning becomes relatively more important, consequently correlations between index values decrease. Moreover, dominance variance is not affected by selection (Bulmer, 1980).

With low correlations between index values of selection candidates and/or a large number of animals selected (>10), approximated selection intensities used to determine genetic and clonal responses are satisfactory (Meuwissen, 1991). The average correlation between index values of female and male selection candidates, in designs optimal for genetic selection, varied from 0.011 to 0.134 and from 0.016 to 0.203, respectively. The average correlation between index values of female

genotypes eligible for commercial cloning varied from 0 to 0.134 in optimal designs. Correlations were largest with a high intensity of genetic selection and no dominance. Consequently, genetic and clonal response will be slightly overestimated in those cases. Reductions in genetic and clonal response might increase when variance reduction due to inbreeding and inbreeding depression will be taken into account (Ruane and Thompson, 1989).

With a restriction on male selection, designs which maximized genetic or clonal response were always different, even if the intra-clone correlation was high. The additive genetic response was maximized with a maximum number of half sibs and only one clone per genotype. In designs optimal for clonal selection, a maximum number of full sibs and 1 to 32 clones were tested per genotype. The number of clones tested per genotype was largest when the heritability and the intra-clone correlation were low, 1024 cows were tested each year, and only one female genotype was selected for commercial cloning each generation. Teepker and Smith (1989), who also assumed a restriction on male selection, found that if the intra-clone correlation was high optimal mating designs for genetic and clonal selection were equal. This was true in their particular situation, because only one mating design existed in which one clone was tested per genotype. Correcting genetic and clonal responses for effects of finite sample size and correlated index values on the selection intensity, and linkage disequilibrium did change optimal mating designs for clonal selection in some situations.

Without a restriction on male selection, genetic response was maximal with a maximal number of full sibs, unless the intra-clone correlation was high (0.80). The optimal number of clones tested per genotype was always one. Designs optimal for clonal selection, however, did not change when the restriction on male selection was relaxed. In summary, designs optimal for genetic and clonal selection were still different, unless the intra-clone correlation was less than 0.80 and the intensity of clonal selection was low. Selection of all males per full sib family, however, will increase inbreeding rates considerably when the number of males selected is constant.

In this study milk efficiency was assumed to have a heritability of 0.10, 0.25 or 0.40, which might be a realistic range of heritabilities knowing that milk efficiency is some combination of primary production traits (milk, fat and protein production) and secondary traits (e.g. reproductive traits). The intra-clone correlation was varied from 0.10 to 0.80. The repeatability of milk production (close to 0.50) might be taken as the upper limit for the intra-clone correlation. Although an intra-clone correlation of 0.80 will not be realizable it was used here to gain insight into the situations studied.

In the long term, continuous genetic progress in the nucleus is important, which evidently can be achieved most effectively by testing only one clone per genotype. For the short term, it is important to select the cow with the highest clonal value for milk efficiency. However, using information of only one clone for the selection of commercial clone lines is usually not sufficient. The combination of both types of selection in a continuous breeding programme requires study.

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Chapter 2

Prediction of additive and dominance effects in selected or unselected populations with inbreeding

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Abstract

A genetic model with either 64 or 1600 unlinked biallelic loci and complete dominance was used to study prediction of additive and dominance effects in selected or unselected populations with inbreeding. For each locus the initial frequency of the favourable allele was 0.2, 0.5 or 0.8 in different alternatives, while the initial narrow-sense heritability was fixed at 0.30. A population of size 40 (20 males and 20 females) was simulated 1000 times for five generations. In each generation five males and 10 or 20 females were mated, with each mating producing four or two offspring respectively. Breeding individuals were selected randomly, on own phenotypic performance or such yielding increased inbreeding levels in subsequent generations. A statistical model containing individual additive and dominance effects, but ignoring changes in mean and genetic covariances associated with dominance due to inbreeding, resulted in significantly biased predictions of both effects in generations with inbreeding. Bias, assessed as the average difference between predicted and simulated genetic effects in each generation, increased almost linearly with the inbreeding coefficient. In a second statistical model the average effect of inbreeding on the mean was accounted for by a regression of phenotypic value on the inbreeding coefficient. The total dominance effect of an individual in that case was the sum of the average effect of inbreeding and an individual effect of dominance. Despite a high mean inbreeding coefficient (up to 0.35), predictions of additive and dominance effects obtained with this model were empirically unbiased, for each initial frequency in the absence of selection, and 64 unlinked loci. With phenotypic selection of five males and only 10 females in each generation and 64 loci, however, predictions of additive and dominance effects were significantly biased. Observed biases disappeared with 1600 loci for allelic frequency at 0.2 and 0.5. Bias was due to a considerable change of allelic frequency with phenotypic selection. Ignoring the covariance between additive and dominance effects with inbreeding, and the change in dominance variance due to inbreeding did not significantly bias predictions of additive and dominance effects in selected or unselected populations with inbreeding.

Key words: finite-locus model, dominance, inbreeding, selection

Introduction

Mixed model methodology is used widely in animal breeding. In most applications, however, only additive genetic effects are considered. Accurate prediction of non-additive effects may be important in for example, the accurate prediction of additive genetic merit, selection of clones in plant or animal breeding, or selection of mates based on their specific combining ability (Allaire and Henderson, 1965; DeStefano and Hoeschele, 1992). Non-additive genetic effects

result from interactions between genes at the same locus (dominance) or at different loci (epistasis). In this study only dominance is considered.

In noninbred populations, prediction of dominance effects is straightforward but computationally demanding (Henderson, 1985). The dominance relationship matrix can be computed from the additive genetic relationship matrix, inverted and then applied in mixed model equations. Hoeschele and VanRaden (1991) presented a method to compute directly the inverse of the dominance relationship matrix in noninbred populations. Prediction of additive and dominance effects in noninbred populations requires knowledge of the additive and dominance variance in the base population. Intense selection in finite animal breeding populations will increase average inbreeding levels. Inbreeding complicates the genetic covariance structure of the population. Computation of the genetic covariance between two relatives with arbitrary levels of inbreeding, requires knowledge on three extra genetic parameters: 1) the sum over loci of the squared complete inbreeding depressions, 2) the dominance variance in the completely inbred population, and 3) the covariance between additive and dominance effects in the completely inbred population (e.g. Harris, 1964; Jacquard, 1974). In addition, inbreeding may reduce the mean phenotypic value of the population, referred to as inbreeding depression (Falconer, 1989).

Two methods to predict additive and dominance effects in populations with inbreeding have been suggested. One method accounts for the average effect of inbreeding on the mean by including the inbreeding coefficient as a covariate in the model while ignoring the reduction of base dominance variance due to inbreeding, the increase of dominance variance of completely inbred individuals, and the covariance between additive and dominance effects with inbreeding (Kennedy *et al.*, 1988). An individual's total dominance effect is estimated as the sum of the average effect of inbreeding on the mean and an individual effect of dominance. This method was examined for populations with an average inbreeding coefficient of at most 0.08, selection of the 25% phenotypically best males and all females, and for initial allelic frequencies at 0.5 and 0.8 (Uimari and Kennedy, 1990). In these situations, predictions of additive and dominance effects were empirically unbiased.

The other method to predict additive and dominance effects in populations with inbreeding accounts for all changes in mean and genetic covariance with inbreeding (Smith and Mäki-Tanila, 1990). The exact genetic covariance matrix between additive effects of gametes and dominance effects of gamete pairs existing in animals and other non-existing gamete pairs, the so-called extended genomic matrix (**E** matrix), is formed using tabular rules. To predict additive effects of gametes and dominance effects of (non)-existing gamete pairs, the inverse of **E** is required. Matrix **E** is singular, however, for only two alleles per locus. Prediction

of additive and dominance effects via direct inversion of **E** is not suitable for genetic models with biallelic loci (Smith and Mäki-Tanila, 1990, p.79), which was the model of interest in the present study. Properties of the **E** matrix, and the possibility of predicting individual additive and dominance effects by extracting only those elements that involve animals deserves further study.

In this paper, the impact of level of inbreeding and intensity of selection on prediction of additive and dominance effects will be studied for different allelic frequencies, using the approximate method proposed by Kennedy *et al.* (1988). Simulation at the individual locus level was used to compare predicted additive and dominance effects with corresponding simulated values. Strictly additive genetic models were studied to compare the finite-locus model with the infinitesimal model.

Methods

Simulation

This study followed the simulation strategy of Uimari and Kennedy (1990). The simulated trait was affected by a finite number (64 or 1600) of unlinked, biallelic loci, each with an equal effect, and was measured on males and females. At each locus, the genotypic value of the heterozygote was either intermediate or equal to that of the favourable homozygote. An individual's genetic value was the sum of its genetic values for all loci affecting the trait. A normally distributed environmental deviation was added to each genotypic value such that the narrow-sense heritability was 0.30 in the base generation.

Each simulated population included five generations. The initial generation contained 20 males and 20 females whose genes were randomly chosen from a base population in Hardy-Weinberg proportions and gametic phase equilibrium. For each locus, the frequency of the favourable allele (p) in the base population was 0.2, 0.5 or 0.8 in different simulated populations. Corresponding additive and dominance variances in the noninbred base population at the animal level equalled: 52.43 and 6.55 for p=0.2, 32 and 16 for p=0.5, and 3.28 and 6.55 for p=0.8. To produce progeny, five males and 10 or 20 females were mated, with each mating resulting in two or one offspring of each sex, respectively. Breeding individuals were selected randomly, on own phenotypic performance or such yielding increased inbreeding levels in subsequent generations. For those selected at random or on their own performance, males and females were mated randomly. Increased inbreeding levels in subsequent generations were obtained by maximizing the number of matings between closely related individuals (e.g. full sibs), denoted by full sib mating. Full sib mating was studied to examine the effect of inbreeding on prediction of additive

and dominance effects in the absence of selection. Different intensities of female selection (proportion selected of 100% versus 50%) were used to analyze its effect on prediction of additive and dominance effects.

For each alternative 1000 replicates were examined.

Evaluation

At the end of the last generation, phenotypic information on individuals in all five generations was used to estimate additive and dominance effects, using the known additive and dominance variance of the base population. Statistical models with and without a regression on inbreeding were used to examine the average effect of inbreeding on the mean:

$$y_i = \mu + a_i + d_i + e_i \qquad [a]$$

$$y_i = \mu + a_i + d_i + bF_i + e_i \qquad [b]$$

where y_i is the phenotypic value of animal i, μ is the base population mean, a_i is the additive effect of animal i, d_i is the dominance effect of animal i, b is the regression of the phenotypic value (y_i) on the inbreeding coefficient (F_i) and e_i is the random error term of animal i. For the model with regression, an individual's dominance effect corrected for the average effect of inbreeding ($\hat{d}_i^*$) was predicted as:

$$\hat{d}_i^* = \hat{d}_i + \hat{b}F_i \qquad [1]$$

The regression of phenotypic value on the inbreeding coefficient will account for the average effect of inbreeding on the mean. For a one-locus model with two alleles, the mean of an unselected population with an average inbreeding coefficient F (μ_F), ignoring genetic drift, can be written as (Kempthorne, 1957):

$$\begin{aligned} \mu_F &= (p^2+pqF) \cdot a + (2pq(1-F)) \cdot d + (q^2+pqF) \cdot -a \\ &= \mu_R - 2pqdF = \mu_R + bF \end{aligned} \qquad [2]$$

where μ_R is the mean in the noninbred random mating population (a(p-q)+2pqd); p, q is the frequency of the favourable and unfavourable allele, respectively; a,d, -a is the genotypic value of the favourable homozygote, the heterozygote and the unfavourable homozygote, respectively; F is the average inbreeding coefficient in the population; b is the regression coefficient, which equals the complete inbreeding depression or $\mu_I-\mu_R=-2pqd$, where μ_I is the mean in the completely inbred population which is a(p-q).

For a model with L unlinked, biallelic loci in gametic phase equilibrium, the theoretical value of b equals:

$$b = -2\sum_{\ell=1}^{L} p_\ell q_\ell d_\ell \qquad [3]$$

Mixed model equations used to obtain estimates of additive and dominance effects require the inverse of the additive genetic ($\mathbf{A}^{-1}$) and the dominance genetic ($\mathbf{D}^{-1}$) relationship matrix. The effect of inbreeding was accounted for in the construction of $\mathbf{A}^{-1}$ (Henderson, 1975). Matrix **D** was computed from elements of **A** ignoring inbreeding, and inverted (Henderson, 1985). Matrix $\mathbf{D}^{-1}$ was also obtained directly (Hoeschele and VanRaden, 1991). Results from each strategy were almost identical, but in the present simulations obtaining $\mathbf{D}^{-1}$ directly required more CPU time. As the number of animals increases, however, obtaining $\mathbf{D}^{-1}$ directly will be more efficient than inverting **D**. To obtain estimates of additive and dominance effects, the mixed model equations were solved using iteration on the data (Schaeffer and Kennedy, 1986). Solutions were considered stable when the convergence criterion, which equals the sum of squares of differences in solutions between iterations divided by the sum of squares of the most recent solutions, was less than 10^{-10}.

Estimated additive and dominance effects were compared to corresponding simulated effects. Bias was assessed as the average difference between predicted individual additive and dominance effects and corresponding simulated effects in each generation. Biases observed in subsequent generations varying in average inbreeding level or in simulated populations differing in female selection intensity were compared to examine the average effect of selection and/or inbreeding on prediction of genetic effects. An individual's simulated additive (or dominance) effect was the sum of the simulated additive (or dominance) effects for all loci affecting the trait, which were computed relative to the random mating noninbred base population (Falconer, 1989, p.121). This is consistent with the infinitesimal model which assumes negligible changes in allelic frequency due to selection. Simulated additive or dominance variances in each generation were calculated directly as:

$$\sigma_g^{2(t)} = \frac{1}{n-1}(\mathbf{g}^{(t)\prime}\mathbf{g}^{(t)} - (\bar{\mathbf{g}}^{(t)})^2) \qquad [4]$$

where $\mathbf{g}^{(t)}$ is a vector of simulated additive or dominance effects for n animals in generation t, respectively.

Infinitesimal model versus the finite-locus model

Estimated additive and dominance effects were computed assuming an infinitesimal model. This model supposes an almost infinite number of unlinked loci, each with a small effect, which results in a negligible change in allelic frequency due to selection (Bulmer, 1980). The number of loci in the finite-locus model must be large enough to assess properties of the infinitesimal model, especially stability of allelic frequencies under selection. For a given genetic variance, the expected change in frequency of an allele at one locus, after one generation of selection, is inversely proportional to the square root of the number of loci of equal effect (Crow and Kimura, 1970, p.229). As in Uimari and Kennedy (1990), a genetic model with 64 unlinked loci was examined. The expected initial increase in frequency of the favourable allele for an initial frequency of 0.5 equals 5%, with phenotypic selection of five males and 10 females in each generation. Reducing this expected increase in allelic frequency to about 1% requires 1600 loci. An additional genetic model with 1600 loci was considered, therefore, in which the total genetic variance was unchanged. Consequently, additive and dominance variances at the animal level, and the covariance between additive and dominance effects arising with inbreeding were unaffected.

A strictly additive genetic model was used to examine whether the infinitesimal model could be approximated by a finite number of unlinked loci for five generations, with and without selection. In the absence of selection, the expected additive variance in generation t, assuming an infinitesimal model, was computed as Van der Werf and De Boer (1990):

$$E(\sigma_a^{2(t)}) = \frac{1}{n-1} tr(\mathbf{QA}_t)\sigma_a^2 \qquad [5]$$

where $\mathbf{A}_t$ is the matrix of additive genetic relationships between n animals in generation t and $\mathbf{Q}=(\mathbf{I}-\frac{1}{n}\mathbf{J})$, where $\mathbf{I}$ is an $n \times n$ identity matrix and $\mathbf{J}$ is an $n \times n$ matrix in which all elements equal 1. With selection, the simulated additive variance obtained with the finite-locus model was compared with the simulated variance obtained with an infinitesimal model.

Results and discussion

Additivity at 64 or 1600 loci

Strictly additive genetic models with 64 or 1600 loci were used to compare the finite-locus model with the infinitesimal model. For each locus, the initial frequency of the favourable allele was 0.2, 0.5 or 0.8 in different simulated populations, while the genetic difference among homozygotes equalled 2 or 0.4 with 64 or 1600 loci

respectively. Consequently, the additive genetic variance was dependent on the initial allelic frequency.

Results from five generations of random mating between five randomly chosen males and all 20 females are given in Table 1, for the 64-loci model. The mean simulated additive effect in each generation was close to zero. The variance of simulated additive effects, however, declined as a result of the establishment of covariances between animals and the increase in average inbreeding coefficient. Simulated additive variances agreed well with expected additive variances assuming an infinitesimal model. Predictions of additive effects were empirically unbiased. As expected, the average frequency of the favourable allele was unchanged in the absence of selection.

Phenotypic selection of five males and 10 females in each generation changed the mean simulated additive genetic merit, variance of simulated additive genetic merit, and allelic frequency for both models with 64 and 1600 loci (Tables 2 and 3). Selection increased mean additive genetic merit, while additive variance declined due to the establishment of covariances between animals, the increase of inbreeding, and gametic phase disequilibrium. In addition, additive variance changed as a result of changes in allelic frequency.

Table 1. Mean (μ_a) and variance (σ_a^2) of simulated additive effects, expected additive variance, mean predicted minus simulated additive effects (â-a), and mean frequency of the favourable allele (p), in generations 1, 3 and 5 with random selection and mating, averaged over 1000 replicates for 64 loci, additive gene action, and initial p (p_i) at 0.2, 0.5 and 0.8 (empirical standard error between brackets)[a]

p_i	Gen[b]	μ_a	σ_a^2	$E(\sigma_a^2)$[c]	â-a	p
0.2	1	-0.01 (0.02)	20.60 (0.15)	20.48	0.01 (0.02)	0.20
	3	-0.05 (0.05)	19.15 (0.18)	19.07	0.03 (0.04)	0.20
	5	-0.05 (0.07)	18.09 (0.17)	18.12	0.04 (0.04)	0.20
0.5	1	0.02 (0.03)	32.11 (0.22)	32.00	-0.02 (0.03)	0.50
	3	0.01 (0.06)	29.98 (0.28)	29.80	0.01 (0.05)	0.50
	5	0.12 (0.08)	28.53 (0.26)	28.32	-0.01 (0.05)	0.50
0.8	1	0.02 (0.02)	20.64 (0.15)	20.48	-0.02 (0.02)	0.80
	3	0.01 (0.05)	19.34 (0.19)	19.07	-0.01 (0.04)	0.80
	5	0.08 (0.07)	18.31 (0.18)	18.12	-0.02 (0.04)	0.80

[a] Proportion selected is 25 % for males and 100 % for females

[b] Mean F in generations 1, 3 and 5 is 0.00, 0.03 and 0.08, independent of initial allelic frequency

[c] Expected variance according to formula [5] in text

Table 2. Mean (μ_a) and variance (σ_a^2) of simulated additive effects, mean expected additive variance, mean predicted minus simulated additive effects (â-a), and mean frequency of the favourable allele (p), in generations 1, 3 and 5 of phenotypic selection, averaged over 1000 replicates for 64 loci, additive gene action, and initial p (p_i) at 0.2, 0.5 and 0.8 (empirical standard error between brackets)[a]

p_i	Gen[b]	a	σ_a^2	$E(\sigma_a^2)$[c]	â-a	p
0.2	1	-0.02 (0.02)	20.84 (0.15)	20.40 (0.14)	0.02 (0.02)	0.20
	3	4.85 (0.06)	18.70 (0.18)	16.54 (0.15)	-0.04 (0.04)	0.24
	5	9.35 (0.08)	18.35 (0.18)	15.00 (0.14)	-0.19 (0.05)	0.27
0.5	1	0.01 (0.03)	32.23 (0.23)	31.87 (0.23)	-0.01 (0.03)	0.50
	3	5.74 (0.06)	25.56 (0.24)	25.85 (0.24)	-0.01 (0.05)	0.54
	5	10.67 (0.08)	22.85 (0.22)	23.44 (0.22)	0.02 (0.05)	0.58
0.8	1	0.02 (0.02)	20.55 (0.15)	20.40 (0.14)	-0.02 (0.02)	0.80
	3	4.46 (0.05)	14.31 (0.14)	16.54 (0.15)	-0.03 (0.04)	0.83
	5	7.85 (0.06)	11.78 (0.11)	15.00 (0.14)	0.21 (0.04)	0.86

[a] Proportion selected is 25 % for males and 50 % for females
[b] Mean F in generations 1, 3 and 5 is 0.00, 0.04 and 0.11, independent of initial allelic frequency
[c] Expected variance based on simulation with infinitesimal model

Table 3. Mean (μ_a) and variance (σ_a^2) of simulated additive effects, mean expected additive variance, mean predicted minus simulated additive effects (â-a), and mean frequency of the favourable allele (p), in generations 1, 3 and 5 of phenotypic selection, averaged over 1000 replicates for 1600 loci, additive gene action, and initial p (p_i) at 0.2, 0.5 and 0.8 (empirical standard error between brackets)[a]

p_i	Gen[b]	μ_a	σ_a^2	$E(\sigma_a^2)$[c]	â-a	p
0.2	1	0.01 (0.02)	20.60 (0.15)	20.40 (0.14)	-0.01 (0.02)	0.20
	3	4.70 (0.05)	16.65 (0.16)	16.54 (0.15)	0.02 (0.04)	0.21
	5	8.84 (0.07)	15.59 (0.14)	15.00 (0.14)	-0.04 (0.04)	0.21
0.5	1	-0.01 (0.03)	31.87 (0.23)	31.87 (0.23)	0.01 (0.03)	0.50
	3	5.78 (0.07)	25.83 (0.25)	25.85 (0.24)	0.07 (0.05)	0.51
	5	10.86 (0.09)	23.32 (0.22)	23.44 (0.22)	0.03 (0.05)	0.52
0.8	1	0.05 (0.02)	20.27 (0.15)	20.40 (0.14)	-0.05 (0.02)	0.80
	3	4.58 (0.05)	15.94 (0.15)	16.54 (0.15)	0.02 (0.04)	0.81
	5	8.45 (0.07)	14.40 (0.14)	15.00 (0.14)	0.06 (0.04)	0.81

[a] Proportion selected is 25 % for males and 50 % for females
[b] Mean F in generations 1, 3 and 5 is 0.00, 0.04 and 0.11, independent of initial allelic frequency
[c] Expected variance based on simulation with infinitesimal model

Changes in allelic frequency due to selection were consistent with their expectations (Crow and Kimura, 1970, p.229), e.g. both the expected and the realized initial change in average allelic frequency for 64 loci and p=0.5 equalled 5 %. As expected, the increase in average frequency of the favourable allele was greater with 64 than with 1600 loci. Consequently, the additive variance with 1600 loci was closer to the additive variance with an infinitesimal model than the additive variance with 64 loci in later generations.

However, for the 64-loci model predictions of additive effects remained empirically unbiased when the initial frequency was 0.5, whereas with initial frequencies of 0.2 or 0.8 selection produced biased (α=0.05) predictions of additive effects in later generations. This results from the fact that the change of the additive variance due to the change in allelic frequency with selection, which is ignored in mixed model methodology, is relatively larger with extreme than with intermediate initial frequencies (Falconer, 1989).

Biases observed with extreme initial frequencies and 64 loci were reduced when 1600 loci were considered. Increasing the number of loci decreased the average change in allelic frequency due to selection, and as a result, the corresponding change of the additive variance. The genetic model with 64 loci will be used to study prediction of additive and dominance effects in unselected populations, while both 64- and 1600-loci models will be considered in selected populations.

Complete dominance at 64 loci with random selection

After five generations of random or full sib mating between five males and all 20 females in the absence of directional selection, additive and dominance effects were predicted with statistical model [a] and [b] for varying initial allelic frequencies (Tables 4 and 5). For each mating strategy and an initial allelic frequency of 0.5, model [a] resulted in empirically biased (α=0.05) predictions of additive and dominance effects in generations with inbreeding (Table 4). The average underestimation of additive effects, however, was slightly smaller than the average overestimation of dominance effects. Hence, total genetic effects were biased upwards. Observed biases for additive and dominance effects increased almost linearly with average inbreeding coefficient (F). Including F as covariate in the model resulted in empirically unbiased predictions of additive and dominance effects for random and full sib mating strategies.

When all loci have two alleles with allelic frequencies of 0.5, however, the covariance between additive and dominance effects, which is ignored in model [b], is zero. This covariance is nonzero if allelic frequencies are different from 0.5 (Harris, 1964).

Table 4. Mean predicted minus simulated additive and dominance effects (empirical standard error between brackets) and mean inbreeding level (F) in generations 1, 3 and 5 of random or full sib mating without selection averaged over 1000 replicates, with complete dominance and initial frequency of favourable allele of 0.5[a]

		Additive effects		Dominance effects		
		Analysis model[b]		Analysis model		
Mating	Gen	[a]	[b]	[a]	[b]	F
Random	1	-0.02 (0.03)	-0.02 (0.03)	0.07 (0.02)	0.01 (0.02)	0.00
	3	-0.65 (0.05)	0.02 (0.05)	0.93 (0.03)	-0.01 (0.02)	0.03
	5	-1.52 (0.05)	-0.02 (0.06)	2.28 (0.03)	0.03 (0.04)	0.08
Full sib	1	0.00 (0.03)	0.00 (0.03)	0.16 (0.02)	0.01 (0.02)	0.00
	3	-3.01 (0.05)	-0.04 (0.06)	3.90 (0.03)	0.05 (0.04)	0.14
	5	-7.07 (0.07)	0.00 (0.09)	8.84 (0.05)	0.00 (0.09)	0.35

[a] Proportion selected is 25% for males and 100% for females
[b] Model [a] without and model [b] with regression on inbreeding

Table 5. Mean predicted[a] minus simulated additive and dominance effects (empirical standard error between brackets) in generations 1, 3 and 5 of random or full sib mating without selection averaged over 1000 replicates, with complete dominance and initial allelic frequency of 0.2 or 0.8[b]

		Additive effects		Dominance effects	
		Frequency		Frequency	
Mating	Gen	0.2	0.8	0.2	0.8
Random	1	0.02 (0.04)	-0.01 (0.01)	0.00 (0.01)	-0.01 (0.01)
	3	0.04 (0.06)	-0.01 (0.02)	0.01 (0.02)	-0.01 (0.01)
	5	0.06 (0.07)	0.00 (0.02)	0.03 (0.04)	-0.01 (0.02)
Full sib	1	0.00 (0.04)	0.00 (0.01)	0.00 (0.01)	-0.01 (0.01)
	3	0.02 (0.07)	0.00 (0.02)	-0.03 (0.04)	-0.02 (0.02)
	5	0.09 (0.12)	0.04 (0.03)	-0.12 (0.12)	-0.06 (0.03)

[a] Statistical model [b] with regression on inbreeding
[b] Proportion selected is 25% for males and 100% for females

Therefore, initial frequencies of 0.2 and 0.8 were considered (Table 5). Average inbreeding coefficients in these cases were equal to those given in Table 4. Ignoring the covariance between additive and dominance effects with inbreeding and the change in dominance variance due to inbreeding, did not significantly bias predictions of additive and dominance effects in unselected populations with inbreeding (Table 5).

Complete dominance at 64 or 1600 loci with phenotypic selection

After five generations of random mating between the phenotypically five best males and all 20 females, additive and dominance effects were predicted with statistical model [a] and [b]. Mean predicted minus simulated additive and dominance effects in generations one, three, and five are given in Table 6 for 64 loci and an initial allelic frequency of 0.5. For model [a], predicted additive and dominance effects in generations with inbreeding were biased (α=0.05) by about the same amount as with random selection of males and females. For model [b] and phenotypic selection, predicted dominance effects in generation five were slightly biased (α=0.05).

With selection of 10 instead of 20 females in each generation and 64 unlinked biallelic loci, model [b] resulted in significantly biased (α=0.05) predictions of both additive and dominance effects in generations with inbreeding (Table 7). Observed biases might be due to ignoring the covariance between additive and dominance effects and the change in dominance variance due to inbreeding and/or to ignoring changes in allelic frequency in simulated and estimated additive and dominance effects. With 1600 unlinked biallelic loci and an initial allelic frequency of 0.5, predictions of additive and dominance effects were empirically unbiased. When the initial allelic frequency is 0.5, however, and changes in average allelic frequency due to selection are small, the covariance between additive and dominance effects is negligible. The absolute covariance is largest with an initial allelic frequency around 0.2 (Harris, 1964). Increasing the number of loci with an initial frequency of 0.2 also decreased observed biases considerably, although predicted dominance effects remained slightly higher than corresponding simulated effects in generation five. Increasing the number of loci decreased the average change in allelic frequency due to selection, while additive and dominance covariances were unaffected. Moreover it reduced possible skewness of the genetic distribution (Mäki-Tanila and Kennedy, 1986). Consequently, ignoring both the covariance between additive and dominance effects, and the change in dominance variance due to inbreeding, did not significantly bias predictions of additive and dominance effects in selected populations with inbreeding.

Table 6. Mean predicted minus simulated additive and dominance effects (empirical standard error between brackets), mean inbreeding level (F) and mean frequency of favourable allele (p) in generations 1, 3 and 5 of phenotypic selection, averaged over 1000 replicates, with complete dominance, 64 loci and an initial p of 0.5[a]

	Additive effects		Dominance effects			
	Analysis model[b]		Analysis model			
Gen	[a]	[b]	[a]	[b]	F	p
1	-0.01 (0.03)	-0.01 (0.03)	0.05 (0.02)	-0.01 (0.02)	0.00	0.50
3	-0.68 (0.05)	0.00 (0.05)	0.92 (0.02)	0.01 (0.03)	0.03	0.53
5	-1.52 (0.06)	-0.08 (0.06)	2.34 (0.03)	0.10 (0.04)	0.08	0.55

[a] Proportion selected is 25% for males and 100% for females
[b] Statistical model [a] with and model [b] without regression on inbreeding

Table 7. Mean predicted[a] minus simulated additive and dominance effects (empirical standard error between brackets) and mean frequency of favourable allele (p) in generations 1, 3 and 5 of phenotypic selection averaged over 1000 replicates, with complete dominance and 64 or 1600 loci[b]

	64 loci			1600 loci		
Gen[c]	Additive	Dominance	p	Additive	Dominance	p
			Initial p is 0.2			
1	0.04 (0.04)	0.01 (0.01)	0.20	-0.01 (0.04)	-0.01 (0.01)	0.20
3	-0.18 (0.07)	0.16 (0.03)	0.24	0.01 (0.06)	-0.02 (0.03)	0.21
5	-0.70 (0.08)	0.78 (0.06)	0.27	-0.04 (0.08)	0.13 (0.05)	0.21
			Initial p is 0.5			
1	-0.01 (0.03)	0.00 (0.02)	0.50	0.01 (0.03)	-0.03 (0.02)	0.50
3	-0.13 (0.05)	0.14 (0.03)	0.54	0.01 (0.05)	0.02 (0.03)	0.51
5	-0.21 (0.06)	0.34 (0.05)	0.58	0.01 (0.06)	0.02 (0.04)	0.51

[a] Statistical model [b] with regression on inbreeding
[b] Proportion selected is 25% for males and 50% for females
[c] Mean F in generations 1, 3 and 5 is 0.00, 0.04 and 0.11, respectively, independent of the allelic frequency

Observed biases with selection were mostly due to ignoring allelic frequency changes in simulated and estimated additive and dominance effects.

Uimari and Kennedy (1990) also concluded that including inbreeding as a covariate in the model of analysis resulted in empirically unbiased predictions of additive and dominance effects in selected and unselected populations with inbreeding. The maximum average inbreeding coefficient in their simulation, however, equalled 0.08, which is much lower than the maximum of 0.35 in the present study. They did not observe significant bias in selected populations due to instability of allelic frequencies because only males were selected in each generation and the number of replicates was smaller.

Neglecting the effect of inbreeding on genetic covariances associated with dominance, however, might result in considerable over- or underestimation of individual additive and dominance effects in each generation, although predictions are on average unbiased. Comparing accuracies of prediction of additive and dominance effects obtained with the approximate method (Kennedy *et al.*, 1988) and the exact method (Smith and Mäki-Tanila, 1990) will give information about prediction error variance of additive and dominance effects.

Estimated and theoretical value of the average effect of inbreeding

Mean predicted regression coefficients obtained with statistical model [b] are given in Table 8 for populations with and without selection, varying initial allelic frequency, and 64 loci. In addition, the theoretical value of the regression coefficient in the absence of selection is given, which is computed according to equation [3]. Estimated regression coefficients obtained with model [b] corresponded well with theoretical coefficients in the absence of selection. With selection, however, equation [3] can not be used to determine the theoretical value of the regression coefficient, because the population is neither in Hardy-Weinberg (Falconer, 1989) nor in gametic phase equilibrium (Bulmer, 1980). Due to selection against the unfavourable homozygote, the frequency of this genotype will decrease. Therefore, the estimated regression coefficient will decrease when the initial frequency of the favourable allele is smaller than 0.5 and increase when it is larger than 0.5, as can be seen in Table 8.

Table 8. Theoretical and mean predicted regression coefficients (empirical standard error between brackets) obtained with statistical model with regression on inbreeding in selected and unselected populations for varying initial allelic frequency p_i and 64 loci

	No selection			
p_i	Theoretical b[a]	Random mating	Full sib mating	Selection
0.2	-20.48	-20.09 (0.49)	-20.67 (0.28)	-23.80 (0.51)
0.5	-32.00	-31.43 (0.39)	-31.98 (0.24)	-31.18 (0.40)
0.8	-20.48	-20.10 (0.17)	-20.65 (0.12)	-15.31 (0.15)

[a] Theoretical value of regression coefficient b according to equation [3]

Table 9. Mean additive (μ_a) and dominance effects (μ_d), mean variances (σ^2_a, σ^2_d), and the mean covariance (σ_{ad}) in generations 1, 2, 3, 4 and 5, simulated with the finite-locus model or the infinitesimal model for full sib mating, averaged over 1000 replicates (empirical standard error between brackets)[a]

Gen[b]	μ_a	μ_d	σ^2_a	σ^2_d	σ_{ad}
		Simulation with finite-locus (64) model			
1	0.00 (0.03)	-0.01 (0.02)	32.16 (0.23)	16.00 (0.12)	-0.06 (0.12)
2	0.05 (0.06)	0.00 (0.02)	30.35 (0.29)	15.94 (0.12)	-0.07 (0.12)
3	0.06 (0.09)	-4.45 (0.03)	29.31 (0.32)	21.58 (0.15)	0.20 (0.15)
4	-0.04 (0.13)	-7.86 (0.04)	25.19 (0.29)	18.88 (0.14)	0.10 (0.13)
5	-0.07 (0.15)	-11.25 (0.05)	22.55 (0.25)	16.91 (0.14)	0.18 (0.13)
		Simulation with infinitesimal model			
1	-0.05 (0.03)	0.01 (0.02)	31.85 (0.23)	16.13 (0.12)	0.01 (0.11)
2	-0.10 (0.06)	0.02 (0.02)	29.95 (0.29)	16.00 (0.12)	-0.06 (0.12)
3	-0.03 (0.09)	-4.38 (0.03)	28.82 (0.30)	22.18 (0.16)	0.01 (0.14)
4	-0.04 (0.13)	-7.84 (0.03)	25.47 (0.29)	20.68 (0.15)	-0.09 (0.14)
5	0.04 (0.15)	-11.22 (0.03)	22.17 (0.24)	20.51 (0.16)	-0.04 (0.13)

[a] Proportion selected is 25% for males and 100% for females

[b] Mean F in generations 1, 2, 3, 4 and 5 was 0.00, 0.00, 0.14, 0.25, and 0.35, in both the finite-locus model and the infinitesimal model

Simulation of dominance with the infinitesimal model

The present and previous simulations (Uimari and Kennedy, 1990) of populations with additive and dominance gene action and inbreeding, have used finite-locus (64 or 1600 loci) models. In the absence of inbreeding, recurrence equations relating offspring genetic merits to parental values exist, and these allow the simulation of additive and dominance effects with the infinitesimal model. An individual's additive genetic effect is simulated as the average of its parental values plus Mendelian sampling, while an individual's dominance effect is a function of its sire-dam combination effect plus Mendelian sampling (Hoeschele and VanRaden, 1991). Unlike a finite-locus model, the infinitesimal model does not require assumptions on the number of loci, the number of alleles per locus and corresponding allelic frequencies, and the genetic values of all possible genotypes at a locus. However, required recurrence equations to simulate additive and dominance effects in populations with inbreeding are currently not available.

Results indicate that including the average effect of inbreeding on the mean and ignoring the effect of inbreeding on genetic covariances associated with dominance gave empirically unbiased predictions of additive and dominance effects in selected and unselected populations with inbreeding. This concept might be used to approximate the simulation of additive and dominance effects with an infinitesimal model. An individual's dominance effect ignoring inbreeding (d_i) was simulated as its sire-dam combination effect plus Mendelian sampling, where a sire-dam combination effect is a function of combination effects of the sire with the parents of the dam, the dam with the parents of the sire, and among parents combination effects (Hoeschele and VanRaden, 1991). To simulate the total dominance effect (d_i^*) of an individual (equation [1]) the average effect of inbreeding on the mean is required. Given the underlying genetic model, the value of the regression of phenotype on inbreeding can be computed when changes in allelic frequency due to selection are ignored.

To compare both approaches of simulating dominance effects in populations with inbreeding, five generations of full sib mating between five males and all 20 females were simulated with a finite (64 loci) and an infinitesimal model. Mean and variances of simulated additive and dominance genetic effects are given in Table 9, for an initial allelic frequency of 0.5. Mean additive and dominance genetic merit and additive variance agreed well in both simulations. As expected mean additive genetic merit was close to zero in the absence of selection, while mean dominance effect declined linearly with the inbreeding coefficient. Reduction of the additive variance was due to establishment of covariances between individuals and the increase in average inbreeding coefficient. In the finite-locus model, inbreeding decreased dominance variance while variation in inbreeding coefficient resulted in

an increase in dominance variance. The infinitesimal model ignores changes of dominance variance due to inbreeding. Consequently, with an initial allelic frequency of 0.5 simulated dominance variance was too high in later generations, while the average dominance effect was correctly simulated. In addition, the infinitesimal model ignores the covariance between additive and dominance effects with inbreeding. This covariance is, however, zero when the initial allelic frequency is 0.5 (Table 9). Thus, simulation of additive and dominance effects with the approximate infinitesimal model accounts for the average effect of inbreeding on the mean, while ignoring its effect on genetic covariances associated with dominance. Assumptions on the actual number of loci and alleles, corresponding allelic frequencies, and genetic values of genotypes possible at each locus, however, are not required.

Conclusions

A statistical model containing individual additive and dominance effects, but ignoring changes in mean and genetic covariances associated with dominance due to inbreeding, resulted in significantly biased predictions of both effects. Bias increased almost linearly with the inbreeding coefficient.

A statistical model accounting for the average effect of inbreeding on the mean, while ignoring its effects on genetic covariances associated with dominance, resulted in empirically unbiased predictions of additive and dominance effects in selected and unselected populations with inbreeding, for varying initial allelic frequencies at 64 or 1600 unlinked, biallelic loci.

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Chapter 3

Genetic evaluation methods for populations with dominance and inbreeding

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Abstract

The effect of inbreeding on mean and genetic covariance matrix for a quantitative trait in a population with additive and dominance effects is shown. This genetic covariance matrix is a function of five relationship matrices and five genetic parameters describing the population. Elements of the relationship matrices are functions of Gillois'(1964) identity coefficients for the four genes at a locus in two individuals. Equivalence of path coefficient method (Jacquard, 1966) and tabular method (Smith and Mäki-Tanila, 1990) to compute the covariance matrix of additive and dominance effects in a population with inbreeding is shown. The tabular method is modified to compute relationship matrices rather than the covariance matrix, which is trait dependent. Finally, approximate and exact best linear unbiased predictions (BLUP) of additive and dominance effects are compared using simulated data with inbreeding but no directional selection. The trait simulated was affected by 64 unlinked biallelic loci with equal effect and complete dominance. Simulated average inbreeding levels ranged from zero in generation one to 0.35 in generation five. The approximate method only accounted for the effect of inbreeding on mean and additive genetic covariance matrix, whereas the exact method accounted for all of the changes in mean and genetic covariance matrix due to inbreeding. Approximate BLUP, which is computable for large populations where exact BLUP is not feasible, yielded unbiased predictions of additive and dominance effects in each generation with only slightly reduced accuracies relative to exact BLUP.

Key words: best linear unbiased prediction, dominance, inbreeding

Introduction

Genetic variation may be composed of additive and non-additive variance. Non-additive genetic variation includes dominance variance, resulting from interaction between genes at the same locus, and epistasis, resulting from interaction between genes at different loci. Genetic covariance between individuals in a random mating, noninbred population for quantitative traits is a well-defined linear function of the genetic variance components (Cockerham, 1954) assuming small contributions from many unlinked loci.

Inbreeding may reduce the mean phenotypic value of a population, a phenomenon referred to as inbreeding depression (Falconer, 1989). Inbreeding also complicates the genetic covariance structure of a population. Genetic covariance between inbred relatives in a population with additive and dominance gene action but without epistasis can be modelled as a linear function of additive and dominance variance in an infinite random mating base population, and additional genetic parameters. Extra parameters are: dominance variance and covariance between additive and dominance effects in a completely inbred population with allelic

frequencies identical to those in the base population (Gillois, 1964; Harris, 1964; Jacquard, 1974) and, in certain settings, the sum over loci of squared effects of complete inbreeding depression (Gillois, 1964; Harris, 1964; Jacquard, 1974; Cockerham and Weir, 1984). Genetic covariance between inbred relatives is the sum of the genetic parameters each multiplied by a different coefficient of relationship. Coefficients of relationship are functions of probabilities that any of the four genes at the same or two different loci in two individuals are identical by descent. Two basic methods are used to compute additive relationships, a path coefficient method (Wright, 1921) and a tabular method (Emik and Terrill, 1949). More generally, the genetic covariance matrix of a population with additive and dominance variation but without epistasis can be computed from path coefficients (Jacquard, 1966) or from a tabular method (Smith and Mäki-Tanila, 1990).

In this paper, genotypic variance as a special case of genotypic covariance in a population with additive and dominance gene action and inbreeding is rederived first, with additive and dominance effects defined in an infinite, random mating base population. Different settings lead to slightly different formulae for genotypic variance, which are reviewed and related.

Subsequently, formulae for genetic covariance are used to model phenotypic performance of a population for a quantitative trait via a mixed linear model including effect of inbreeding depression and a covariance matrix among individual additive and dominance effects. Equivalence of the path coefficient (Jacquard, 1966) and tabular method (Smith and Mäki-Tanila, 1990) to compute the covariance matrix is shown and used to derive a modified tabular method. The latter computes relationship matrices, which must be formed only once for a given population. The covariance matrix among additive and dominance effects depends on values of the genetic parameters, and hence would have to be recomputed for each trait in a given population by the method of Smith and Mäki-Tanila (1990).

Finally, approximate and exact Best Linear Unbiased Prediction of additive and dominance effects are compared using data simulated with the individual locus model of De Boer and Van Arendonk (1992). Approximate BLUP (best linear unbiased prediction) only accounts for effects of inbreeding on mean and additive genetic covariance, while exact BLUP accounts for all changes in mean and genetic covariance with inbreeding. Approximate BLUP can be implemented for livestock populations of moderate to large size, where exact BLUP is not feasible.

Theory

Genetic variance

Genotypic covariances among individuals, in which additive and dominance variation and inbreeding were taken into account, have been derived by several authors. If the situations considered are limited to those where a trait was affected by several to many loci and additive and dominance effects were defined in an infinite, random mating base population, four different settings can be found. The assumptions appear to be:

(i) many finite subpopulations derived in an identical fashion from an infinite base population (Gillois, 1964; Chevalet and Gillois, 1977);
(ii) a large population derived from an infinite random mating base by some system of inbreeding with no selection, unlinked loci, and all individuals having identical inbreeding coefficients (Harris, 1964);
(iii) as (ii) but with variation among individuals in inbreeding coefficients (Cockerham and Weir, 1984);
(iv) one particular finite population (Chevalet, 1971).

In setting (i), genotypic variance represents total genetic variance across lines (Chevalet and Gillois, 1977; Falconer, 1989). For (ii) or (iii), genotypic variance may represent total genetic variance among unrelated individuals with common or average inbreeding level F.

Common to all four settings is the derivation of genotypic covariance based on identity coefficients. Identity coefficients refer to the possible identity modes pertaining to the four genes at the same locus in two individuals (Figure 1), or at two different loci (Cockerham and Weir, 1984). An identity coefficient represents the probability of a particular identity mode. The four settings differ in the definition of identity coefficients and in the types of identity modes that need to be considered.

Following Chevalet (1971), let S_k^ℓ represent an indicator variable for identity mode k (k=1,...,15) pertaining to the four genes at locus ℓ in two individuals, and let δ_k represent the probability of this identity mode. Then, δ_k equals the frequency of identity mode k in the limit, or:

$$\delta_k = \lim_{n\to\infty} \{ \frac{1}{n} [S_k^\ell(u_1) + S_k^\ell(u_2) + ... + S_k^\ell(u_n)] \} \qquad [1]$$

where $S_k^\ell(u_i)$ equals 1 if identity mode k is realized at locus ℓ and 0 otherwise, and u_i represents a pair of individuals (covariance) or one individual (variance). In setting (i), u_i is a (pair of) individual(s) in subpopulation i.

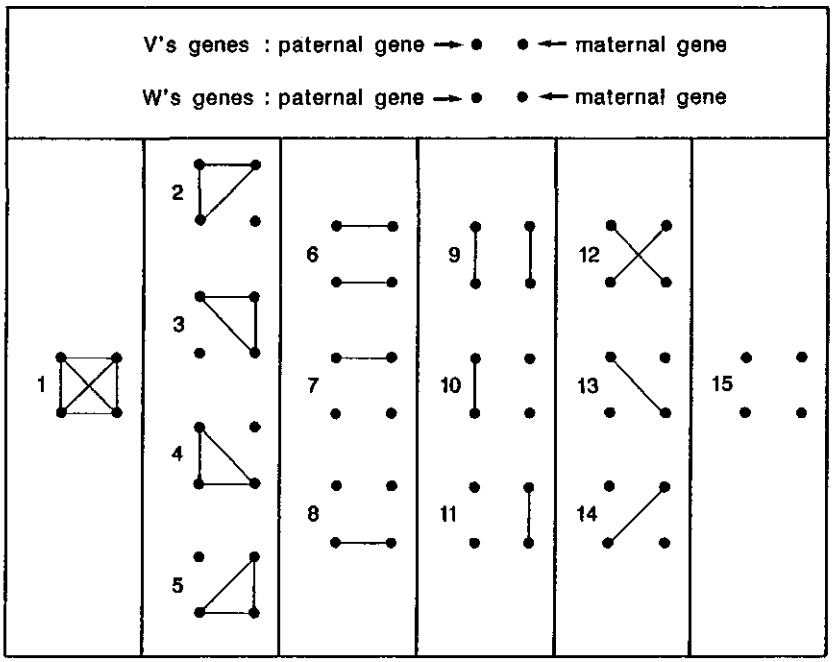

Figure 1. Fifteen possible identity modes between the paternal and the maternal gene of individual V and the paternal and the maternal gene of individual W at a particular locus. Genes identical by descent are connected by a line (reproduced from Jacquard, 1974, p.105)

In settings (ii) and (iii), u_i may represent n independent pairs or n unrelated individuals in a large population. In setting (iv), δ_k is defined differently as:

$$\delta_k = \lim_{n\to\infty} \{ \frac{1}{n} [S_k^1(u) + S_k^2(u) + ... + S_k^n(u)] \} \qquad [2]$$

where u represents a pair of individuals in one particular finite population, and δ_k is the limit taken over n independent loci. Setting (iv) requires the assumption of a large number of loci and a small ratio of largest to smallest contribution relative to number of loci (Chevalet, 1971). The other settings are general with respect to number of loci and size of their contributions.

For genotypic variance, u represents an individual, and identity modes other than 1, 9 and 12 cannot be realized (Figure 1). Hence, only S_1^ℓ, S_9^ℓ and S_{12}^ℓ are random variables, which are redefined as $F^\ell(u)=S_1^\ell(u)$ and $1-F^\ell(u)=S_9^\ell(u)+S_{12}^\ell(u)$. If alleles at locus ℓ in individual u are identical by descent (i.b.d.), $F^\ell(u)$ equals 1, else $F^\ell(u)$ is zero.

The common starting point in all four settings is to define genotypic value at locus ℓ in an infinite, random mating base population (G_b^ℓ) as the sum of breeding value (BV_b^ℓ) and dominance deviation (D_b^ℓ), or:

$$G_b^\ell = BV_b^\ell + D_b^\ell \qquad [3]$$

Expectation of G_b^ℓ with respect to the distribution of alleles for a given individual u is:

$$E^{p\ell|u}(G_b^\ell) = F^\ell(u)\, E^{p\ell}_{ibd}(G_b^\ell) + [\,1-F^\ell(u)\,]\, E^{p\ell}_{not\ ibd}(G_b^\ell) \qquad [4]$$

where $E^{p\ell}_{ibd}$ denotes the expectation conditional on the alleles being i.b.d., and p_ℓ is the frequency of the favourable allele at locus ℓ. In the absence of inbreeding ($E^{p\ell}_{not\text{-}ibd}$) the expectation of dominance effects is zero, whereas the expectation of additive effects is zero with ($E^{p\ell}_{ibd}$) and without inbreeding ($E^{p\ell}_{not\ ibd}$) (Jacquard, 1966). As a result, equation [4] reduces, e.g., for a biallelic locus to:

$$E^{p\ell|u}(G_b^\ell) = F^\ell(u)\,[\,p_\ell\, D_{11}^\ell + q_\ell\, D_{22}^\ell\,] = F^\ell(u)\Delta_i^\ell \qquad [5]$$

where D_{ij}^ℓ is dominance deviation of genotype ij at locus ℓ in the base, $q_\ell = 1-p_\ell$, and $\Delta_i^\ell = -2p_\ell q_\ell d_\ell$ is the complete inbreeding depression at locus ℓ. Variance of G_b^ℓ with respect to the distribution of alleles for a given individual u is:

$$Var^{p\ell|u}(G_b^\ell) = E^{p\ell|u}[(G_b^\ell)^2] - [E^{p\ell|u}(G_b^\ell)]^2 \qquad [6]$$

Using

$$E^{p\ell|u}[(G_b^\ell)^2] = F^\ell(u)E^{p\ell}_{ibd}[(G_b^\ell)^2] + [1-F^\ell(u)]E^{p\ell}_{not\ ibd}[(G_b^\ell)^2]$$

with for a biallelic locus,

$$E^{p\ell}_{ibd}\,[(G_b^\ell)^2] = p_\ell\,(\,BV_{11}^\ell + D_{11}^\ell\,)^2 + q_\ell\,(\,BV_{22}^\ell + D_{22}^\ell\,)^2$$

and

$$E^{p\ell}_{not\ ibd}\,[(G_b^\ell)^2] = p_\ell^{\,2}\,(\,BV_{11}^\ell + D_{11}^\ell)^2 + 2p_\ell q_\ell\,(\,BV_{12}^\ell + D_{12}^\ell)^2 + q_\ell^{\,2}\,(\,BV_{22}^\ell + D_{22}^\ell)$$

in equation [6] yields:

$$Var^{p\ell|u}(G_b^\ell) = [1+F^\ell(u)]\sigma^2_{ar(\ell)} + [1-F^\ell(u)]\sigma^2_{dr(\ell)} + F^\ell(u)\sigma^2_{di(\ell)} + 2F^\ell(u)\sigma_{adi(\ell)} \qquad [7]$$

where omitting subscript ℓ,

$$\sigma^2_{ar} = p^2\, BV^2_{11} + 2pq\, BV^2_{12} + q^2\, BV^2_{22} = 2pq[a + d(q-p)]^2 = 2pq\alpha^2$$

[8]

$$\sigma^2_{dr} = p^2\, D^2_{11} + 2pq\, D^2_{12} + q^2\, D^2_{22} = (2pqd)^2$$

[9]

$$\sigma^2_{di} = p^2\, D^2_{11} + q^2\, D^2_{22} - (pD_{11} + qD_{22})^2 = 4pq(p^3 + q^3)d^2 - (2pqd)^2$$

[10]

$$\sigma_{adi} = p\, BV_{11}\, D_{11} + q\, BV_{22}\, D_{22} = 4pq(p-q)\alpha d$$

[11]

where $\alpha = a + d(q-p)$, and a and d are genotypic values of the favourable homozygote and the heterozygote, respectively (Falconer, 1989, p. 112). Variances σ^2_{ar} and σ^2_{dr} are additive and dominance variances at locus ℓ in the base, and σ^2_{di} and σ_{adi} are dominance variance and covariance between additive and dominance effects among homozygotes, or in a complete inbred population with the same allelic frequency as the base. Values of inbreeding depression (Δ_I) and of the (co)variances defined in equations [8] to [11] are given in Figure 2 for varying allelic frequency. Figure 2 shows that the relative importance of the (co)variance components changes strongly with allelic frequency.

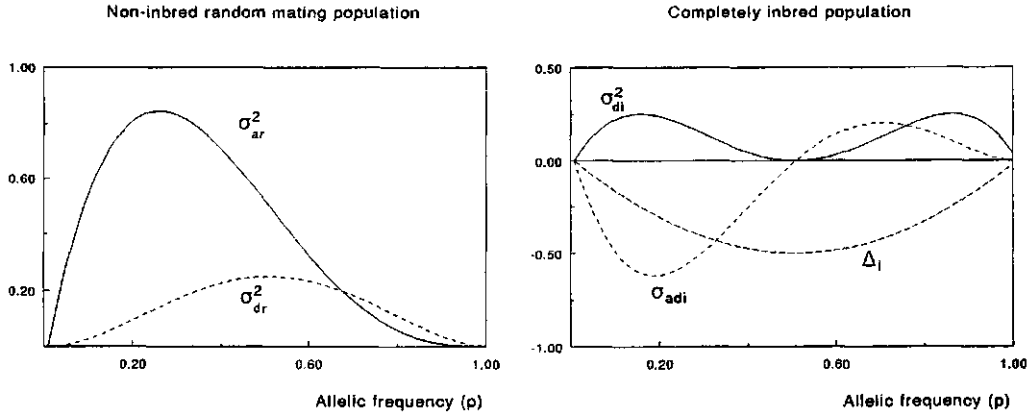

Figure 2. Magnitude of the components of genetic variance (symbols defined in text) at a biallelic locus with complete dominance (a=d=1) as a function of allelic frequency p

In settings (i), (ii) and (iii), expectations are taken jointly with respect to the distribution of allelic frequencies and u, with u representing independent subpopulations or unrelated individuals. Using

$$E^{p\ell,u}(.) = E^{u}[E^{p\ell|u}(.)]$$

the joint expectation of G_b^{ℓ} for a biallelic locus is:

$$E^{p\ell,u}(G_b^{\ell}) = E^{u}[F^{\ell}(u)](p_{\ell}D_{11}^{\ell} + q_{\ell}D_{22}^{\ell}) = F\Delta_i^{\ell} \qquad [12]$$

where $F=\delta_1$ is the inbreeding coefficient. Similarly, the variance of G_b^{ℓ} is:

$$\begin{aligned}
\mathrm{Var}^{p\ell,u}(G_b^{\ell}) &= E^{u}[F^{\ell}(u)]E^{p\ell}_{ibd}[(G_b^{\ell})^2] + E^{u}[1-F^{\ell}(u)]\,E^{p\ell}_{not\ ibd}[(G_b^{\ell})^2] - F^2(\Delta_i^{\ell})^2 \\
&= F\,E^{p\ell}_{ibd}[(G_b^{\ell})^2] + (1-F)E^{p\ell}_{not\ ibd}[(G_b^{\ell})^2] - F^2(\Delta_i^{\ell})^2 \\
&= (1+F)\sigma^2_{ar(\ell)} + (1-F)\sigma^2_{dr(\ell)} + F\sigma^2_{di(\ell)} + F(1-F)(\Delta_i^{\ell})^2 + 2F\sigma_{adi(\ell)}
\end{aligned} \qquad [13]$$

If several biallelic loci contribute to the genotypic value for a trait, the total genotypic value, denoted as G_b, then equals $\Sigma_{\ell}G_b^{\ell}$. The mean of G_b is derived as:

$$E^{p\ell,u}(G_b) = F\sum_{\ell=1}^{L}\Delta_i^{\ell} = F\Delta_I \qquad [14]$$

where L is number of loci. The variance of G_b is:

$$\begin{aligned}
\mathrm{Var}^{p\ell,u}(G_b) &= E^{p\ell,u}[(\sum_{\ell=1}^{L}G_b^{\ell})^2] - F^2(\Delta_I)^2 \\
&= \sum_{\ell=1}^{L}E^{p\ell,u}[(G_b^{\ell})^2] + \sum_{\ell\neq}^{L}\sum_{\ell'}^{L}E^{p\ell,u}(G_b^{\ell}G_b^{\ell'}) - F^2(\Delta_I)^2 \\
&= (1+F)\sum_{\ell=1}^{L}\sigma^2_{ar(\ell)} + (1-F)\sum_{\ell=1}^{L}\sigma^2_{dr(\ell)} + 2F\sum_{\ell=1}^{L}\sigma_{adi(\ell)} \\
&+ F\,[\sum_{\ell=1}^{L}\{p_{\ell}(D_{11}^{\ell})^2 + q_{\ell}(D_{22}^{\ell})^2\}] + \sum_{\ell\neq}^{L}\sum_{\ell'}^{L}E^{p\ell,u}(G_b^{\ell}G_b^{\ell'}) - F^2(\Delta_I)^2 \\
&= (1+F)\sigma^2_{AR} + (1-F)\sigma^2_{DR} + 2F\sigma_{ADI} + F\sigma^2_{DI} \\
&+ F\Delta_I^2 - F^2(\Delta_I)^2 + \sum_{\ell\neq}^{L}\sum_{\ell'}^{L}E^{p\ell,u}(G_b^{\ell}G_b^{\ell'})
\end{aligned} \qquad [15]$$

where,

$$\Delta_I^2 = \sum_{\ell=1}^{L} (\Delta_i^\ell)^2 \quad \text{and} \quad (\Delta_I)^2 = (\sum_{\ell=1}^{L} \Delta_i^\ell)^2 \qquad [16]$$

and

$$\sum_{\ell\neq}^{L}\sum_{\ell'}^{L} E^{p\ell,u}(G_b^{\ell}G_b^{\ell'}) = \sum_{\ell\neq}^{L}\sum_{\ell'}^{L} E^{u}[F^{\ell}(u)F^{\ell'}(u)]\, E^{p\ell p\ell'}(G_b^{\ell}G_b^{\ell'})$$

$$= \sum_{\ell\neq}^{L}\sum_{\ell'}^{L} E^{u}[F^{\ell}(u)F^{\ell'}(u)]\,(p_\ell G_{11}^{\ell} + q_\ell G_{22}^{\ell})(p_{\ell'} G_{11}^{\ell'} + q_{\ell'} G_{22}^{\ell'})$$

$$= F^{-}\sum_{\ell\neq}^{L}\sum_{\ell'}^{L} \Delta_i^\ell \Delta_i^{\ell'} = F^{-}[(\Delta_I)^2 - \Delta_I^2] \qquad [17]$$

$$\text{with} \quad F^{-} = E^{u}[F^{\ell}(u)F^{\ell'}(u)] \qquad [18]$$

Hence, using [17] in [15]:

$$\text{Var}^{p\ell,u}(G_b) = (1+F)\sigma_{AR}^2 + (1-F)\sigma_{DR}^2 + F\sigma_{DI}^2 + 2F\sigma_{ADI} + F(1-F)\Delta_I^2 + (F^{-}-F^2)[(\Delta_I)^2 - \Delta_I^2] \qquad [19]$$

From [18], F^{-}-F^2 is the covariance among $F^{\ell}(u)$ and $F^{\ell'}(u)$. Let $F^{\ell}(u)$ be composed of its expected value and a residual or $F^{\ell}(u)=F+R^{\ell}(u)$. Hence, F^{-}-F^2 = $\text{Cov}(F^{\ell}(u),F^{\ell'}(u))$ may be partitioned as σ_F^2 + $\text{Cov}(R^{\ell}(u),R^{\ell'}(u))$. For unlinked loci the last term is equal to zero, and F^{-}-F^2=σ_F^2, with σ_F^2 representing variation among individuals in inbreeding coefficient. If σ_F^2 is zero and loci are unlinked, then F^{-}=F^2, hence the last term in the right-hand-side of [19] is zero.

Equation [19] is general and holds for any number of alleles per locus (Cockerham and Weir, 1984). Equation [19] is obtained in setting (iii), while for settings (i) and (ii) σ_F^2=0, and hence genotypic variance equals equation [19] but without the last term of equation [19]. For setting (iv), genotypic mean is:

$$E(G_b) = \sum_{\ell=1}^{L} E^{p\ell|u}\,[\,F^{\ell}(u)\,]\,E_{ibd}^{p\ell}(G_b^{\ell}) \simeq F\Delta_I$$

with a proof of this approximation given by Chevalet (1971). Similarly, genotypic variance is approximated as:

$$\mathrm{Var}(G_b) = \sum_{\ell=1}^{L} \mathrm{Var}^{p\ell|u}(G_b^{\ell}) \simeq (1+F)\sigma^2_{AR} + (1-F)\sigma^2_{DR} + F\sigma^2_{DI} + 2F\sigma_{ADI}$$

which is equation [19] with the last two terms omitted.

Genetic covariance between relatives

Equation [19] represents the special case of the genetic covariance of an individual with itself. A general formula for the genetic covariance between individuals V and W with arbitrary inbreeding coefficients is (Cockerham and Weir, 1984):

$$\sigma_{GvGw} = a_{vw}\sigma^2_{AR} + dr_{vw}\sigma^2_{DR} + di_{vw}\sigma^2_{DI} + c_{vw}\sigma_{ADI} + c_{wv}\sigma_{ADI} + u_{vw}\Delta_I^2 + t_{vw}\,[(\Delta_I)^2 - \Delta_I^2]$$

where [20]

a_{vw} is the additive genetic relationship between individuals V and W;

dr_{vw} is the relationship between individuals V and W due to dominance variance in the base population;

di_{vw} is the relationship between individuals V and W due to dominance variance in the completely inbred population;

c_{vw} is the relationship between the additive effect of individual V and the dominance effect of individual W;

c_{wv} is the relationship between the additive effect of individual W and the dominance effect of individual V;

u_{vw} is the relationship between individuals V and W due to the sum of squared inbreeding depressions; and

t_{vw} is the relationship between individual V and W due to component $(\Delta_I)^2$-Δ_I^2.

Genotypic covariance derived in settings (i) and (ii) (Gillois, 1964; Harris, 1964; Jacquard, 1974) does not include the last term of [20]. Table 1 shows the equivalence of equation [20] and the equations given by Harris (1964, p.1329), Jacquard (1974, p.135) and Cockerham and Weir (1984, p.160). Genotypic covariance in setting (iv) is equal to [20] with the last two terms omitted (Chevalet, 1971; Chevalet and Gillois, 1977).

Table 1. Equivalence between parameterizations of genetic covariance used in this paper and in Harris (1964, p.1329), Jacquard (1974, p.135) and Cockerham and Weir (1984, p.160)

	This paper	Harris (1964)[a]	Jacquard (1974)	Cockerham & Weir (1984)
Covariances	σ^2_{AR}	σ^2_{AR}	V_A	σ^2_A
	σ^2_{DR}	σ^2_{DR}	V_D	σ^2_D
	σ^2_{DI}	σ^2_{DI}	$V_H - D^2_H$	D^*_2
	σ_{ADI}	σ_{ADI}	$2Cov_H(A,D)$	$2D_1$
	Δ^2_I	D^2_I	D^2_H	H^*
	$(\Delta_I)^2-\Delta^2_I$	-	-	H^2-H^*
Relationship coefficients	a_{vw}	$2r_{vw}$	$2\Phi_{vw}$	$2\theta_{vw}$
	dr_{vw}	u_{vw}	Δ_7	$2(\Delta_{v+w}-\delta_{vw})$
	di_{vw}	t_{vw}	Φ_4	δ_{vw}
	$c_{vw}+c_{wv}$	$s_{vw}+s_{wv}$	$\Phi_3+\Phi_4$	$2(\gamma_{vw}+\gamma_{vw})$
	u_{vw}	$t_{vw}+v_{vw}-F_vF_w$	$\Delta_2-F_vF_w+\Phi_4$	$\Delta_{v+w}-F_vF_w$
	t_{vw}	-	-	$\Delta_{vw}-F_vF_w$

[a] Formula [26] on page 1329 from Harris (1964) is based on a one locus-model

The additive genetic relationship between individuals V and W, a_{vw}, equals twice the probability that a gene taken at random from V is identical by descent (i.b.d.) to a gene taken at random from W. This occurs ¼ of the time for identity modes 10, 11, 13, and 14 in Figure 1, ½ of the time for identity modes 2, 3, 4, 5, 9, and 12, and always for identity mode 1, or:

$$a_{vw} = 2[\delta_1 + \tfrac{1}{2}(\delta_2+\delta_3+\delta_4+\delta_5+\delta_9+\delta_{12}) + \tfrac{1}{4}(\delta_{10}+\delta_{11}+\delta_{13}+\delta_{14})] \qquad [21]$$

Dominance relationship dr_{vw} equals the probability that each gene in V is i.b.d. to a different gene in W and genes in the same individual are not i.b.d., an event represented by identity modes 9 and 12, or:

$$dr_{vw} = \delta_9 + \delta_{12} \qquad [22]$$

Dominance relationship di_{vw} is the probability that all four genes in V and W are i.b.d., or:

$$di_{vw} = \delta_1 \qquad [23]$$

Relationship c_{vw} equals the probability that a gene taken at random from V is i.b.d. to both genes in W, involving identity modes 1, 4, and 5, or:

$$c_{vw} = \delta_1 + \frac{1}{2}(\delta_4+\delta_5) \quad [24]$$

Similarly:

$$c_{wv} = \delta_1 + \frac{1}{2}(\delta_2+\delta_3) \quad [25]$$

From results in Jacquard (1974, p.135) and Table 1:

$$u_{vw} = (\delta_1+\delta_6-F_vF_w) \quad [26]$$

where F_v and F_w are the inbreeding coefficients of individuals V and W respectively, which can also be expressed in terms of the identity coefficients (Jacquard, 1974, p.109), or

$$F_v = \delta_1 + \delta_2 + \delta_3 + \delta_6 + \delta_7 \quad \text{and} \quad F_w = \delta_1 + \delta_4 + \delta_5 + \delta_6 + \delta_8$$

Relationship coefficient t_{vw} was defined by Cockerham and Weir (1984) (see Table 1) and depends on identity coefficients involving genes at two different loci.

Equation [20] reduces to equation [19] when considering covariance of a individual V with itself. In this case the only non-zero identity coefficients are $\delta_1=F_v$ and $(\delta_9+\delta_{12})=1-F_v$.

Genetic covariance matrix for a quantitative trait

A linear model for phenotypic measurements of individuals for a quantitative trait with additive and dominance variance includes additive and dominance genetic values, with dominance values partitioned into the effect of inbreeding depression plus dominance effects, and systematic environmental effects, or:

$$\mathbf{y} = \mathbf{X}\boldsymbol{\beta} + \mathbf{Z}\mathbf{a} + \mathbf{Z}(\mathbf{f}\Delta_I+\mathbf{d}) + \mathbf{e} \quad [27]$$

where **ß** is a vector of fixed environmental effects, **a** is a vector of random additive effects, **d** is a vector of random dominance effects, **X** and **Z** are known incidence matrices, **f** is a known vector of inbreeding coefficients F, Δ_I is the fixed effect of the complete inbreeding depression, and **e** is a vector of random residuals.

Mean and covariance matrix of **y** are $E(\mathbf{y})=\mathbf{X}\boldsymbol{\beta} + \mathbf{Z}\mathbf{f}\Delta_I$ and $Var(\mathbf{y}) = [\ \mathbf{Z}, \mathbf{Z}]\ \mathbf{G}\ [\ \mathbf{Z}, \mathbf{Z}\ ]' + \mathbf{I}\sigma_e^2$ with error variance σ_e^2, respectively, and **G** is the covariance matrix of $[\ \mathbf{a}', \mathbf{d}'\ ]'$, or:

$$Var\begin{bmatrix}\mathbf{a}\\ \mathbf{d}\end{bmatrix} = \mathbf{G} = \begin{bmatrix}\mathbf{A}\sigma_{AR}^2 & \mathbf{C}\sigma_{ADI}\\ \mathbf{C}'\sigma_{ADI} & (\mathbf{D}_R\sigma_{DR}^2 + \mathbf{D}_I\sigma_{DI}^2 + \mathbf{U}\Delta_I^2)\end{bmatrix} \quad [28]$$

where **A** is a matrix of additive genetic relationships (a_{vw}), **C** is a matrix of

relationships between additive and dominance effects (c_{vw} and c_{wv}), $\mathbf{D}_R$ is a matrix of relationships due to the dominance variance in the base population (dr_{vw}), $\mathbf{D}_I$ is a matrix of relationships due to dominance variance in the completely inbred population (di_{vw}), and $\mathbf{U}$ is a matrix of relationships due to the sum of squared inbreeding depressions (u_{vw}).

Covariance matrix [28] does not include the last term in the right-hand-side of [19] or [20], because variation among individuals in their inbreeding coefficients, and hence in inbreeding depression, is eliminated by partitioning dominance effects into $\mathbf{f}\Delta_I$ and $\mathbf{d}$ in [27]. Treating f as a random variable rather than a known constant, $Var(\mathbf{f}\Delta_I) = \sigma_F^2 (\Delta_I)^2$. If number of loci, L, tends to infinity and Δ_i is constant across loci, $(\Delta_I)^2 = L^2\Delta_i^2$ and $(\Delta_I)^2 - \Delta_i^2 = L(L-1)\Delta_i^2 \simeq L^2\Delta_i^2$. Hence, $(\Delta_I)^2 \simeq (\Delta_I)^2 - \Delta_i^2$. The term $\mathbf{U}\Delta_i^2$ should be dropped if [28] represents the covariance matrix of genetic effects of individuals in one particular finite population.

Matrix **G** can be computed with a path coefficient method (Jacquard, 1966) and a tabular method (Smith and Mäki-Tanila, 1990). Both methods will be described briefly and their equivalence will be shown.

Path coefficient method

The path coefficient method of Jacquard (1966) determines the genetic covariance between two individuals V and W by computing probabilities of all identity modes in Figure 1. The following steps are required: 1) Find all common ancestors of V and W; 2) Determine all possible paths of origin of their four genes at a locus; 3) Determine the probability of each path; 4) For each path, determine the various identity modes and their probabilities; 5) Sum the probabilities by identity mode across paths.

The path coefficient method is useful for single and simple pedigrees, but is not suitable for computing the genetic covariance matrix of a large population.

Tabular method

The tabular method of Smith (1984) and Smith and Mäki-Tanila (1990) determines the exact genetic covariance structure in a population using an extended genomic table following Harris (1964) and Gillois (1964). The extended genomic table, denoted by **E**, contains first moments or expected values of additive effects of gametes and dominance effects of gamete pairs in its first row and column, except for the first element that is equal to one, and second moments of all effects in its remaining rows and columns. Elements of **E** are computed using recursive rules of Smith and Mäki-Tanila (1990, p.71-72). An initial list of gametes and gamete pairs includes all gametes and gamete pairs represented in individuals of a population. Smith and Mäki-Tanila (1990) give an algorithm to form the list of gametes and

gamete pairs, which adds ancestral gamete pairs and produces an ordering required to compute **E** recursively.

After absorption of the first row and column, **E** represents a matrix of covariances, which may be partitioned into a submatrix of covariances among additive effects of gametes, a submatrix of covariances among additive effects of gametes and dominance effects of gamete pairs, its transpose, and a submatrix of covariances among dominance effects of gamete pairs.

Equivalence between path coefficient and tabular methods

Equivalence between path coefficient (Jacquard, 1966) and tabular method (Smith, 1984; Smith and Mäki-Tanila, 1990) will be shown for each submatrix of **E** separately.

Covariance between additive effects. The additive genetic relationship between individual V with paternal and maternal gametes i and j, and individual W with paternal and maternal gametes k and m, is computed from **E** as (Smith, 1984):

$$a_{vw} = \frac{[\ E(a_i,a_k) + E(a_i,a_m) + E(a_j,a_k) + E(a_j,a_m)\ ]}{\sigma^2_{AR}} \qquad [29]$$

where $E(a_i,a_k)$ is the element in **E** corresponding to row a_i and column a_k, which is the second moment or covariance between additive effects of gamete i (a_i) and k (a_k). Second moments equal covariances because the expected value of a gamete's additive effect is zero. The additive genetic covariance between gametes i and k is equal to the probability that a gene in gamete i is i.b.d. to another gene at the same locus in gamete k, denoted by $P(i\equiv k)$, times the additive variance among gametes or $\frac{1}{2}\sigma^2_{AR}$ (Smith and Allaire, 1985). Hence:

$$a_{vw} = \tfrac{1}{2}[\ P(i\equiv k) + P(i\equiv m) + P(j\equiv k) + P(j\equiv m)\ ] \qquad [30]$$

Each probability in equation [30] may be computed by adding all probabilities of identity modes (Figure 1) containing the particular identity, e.g. for $i\equiv k$:

$$\begin{aligned} P(i\equiv k) &= P(i\equiv j\equiv k\equiv m) + P(i\equiv j\equiv k\not\equiv m) + P(i\equiv k\equiv m\not\equiv j) + P(i\equiv k\not\equiv j\equiv m) \\ &\quad + P(i\equiv k\not\equiv j\not\equiv m) = \delta_1 + \delta_2 + \delta_4 + \delta_9 + \delta_{10} \end{aligned} \qquad [31]$$

Use of equation [31] and similar identities in equation [30] yields the additive relationship in terms of identity coefficients given in equation [21].

Furthermore, the probabilities of gene identities in equation [30] can be computed recursively, using the following rules. Let $i \geq k$, if i is a descendant of k. Base gametes do not have any known parental gametes. If i and k are base gametes:

$$P(i \equiv k) = 1 \quad \text{if } i = k \text{ , else } P(i \equiv k) = 0 \qquad [32]$$

If i is not a base gamete, and x and y are the parental gametes of i, then for $i \neq k$:

$$P(i \equiv k) = P(x \equiv k)\, P(i = x) + P(y \equiv k)\, P(i = y) = \tfrac{1}{2}[\, P(x \equiv k) + P(y \equiv k)\,] \qquad [33]$$

where, e.g. $P(i = x)$ is the probability that a gene in gamete i is a copy of a gene at the same locus in parental gamete x. For $i = k$:

$$P(i \equiv k) = P(i \equiv i) = \tfrac{1}{2}[\, P(x \equiv x) + P(y \equiv y)\,] = 1 \qquad [34]$$

Equations [32], [33] and [34] are analogous to recurrencies for covariances or second moments among additive effects of gametes presented by Smith and Mäki-Tanila (1990, p.71).

Covariance between additive and dominance effects. The relationship coefficient between additive effect of individual V with gametes i and j, and the dominance effect of individual W with gametes k and m, c_{vw}, is computed from **E** as:

$$c_{vw} = \frac{[\, E(a_i, d_{km}) + E(a_j, d_{km})\,]}{\sigma_{ADI}} \qquad [35]$$

where $E(a_i, d_{km})$ is the second moment or covariance between the additive effect of gamete i (a_i) and dominance effect of gamete pair km (d_{km}). Similar to the covariance among additive effects of gametes, $Cov(a_i, d_{km})$ equals the probability that a gene at a particular locus in gamete i is i.b.d. to both genes at the same locus in gamete pair km, denoted by $P(i \equiv k \equiv m)$, times $\frac{1}{2}\sigma_{ADI}$. Then, the relationship coefficient between the additive effect of individual V and the dominance effect of individual W can be written as:

$$c_{vw} = \tfrac{1}{2}[\, P(i \equiv k \equiv m) + P(j \equiv k \equiv m)\,] \qquad [36]$$

Both probabilities in equation [36] can be written in terms of identity coefficients (Figure 1), yielding the additive-dominance relationship coefficient in equation [24]. For example, $P(i \equiv k \equiv m)$ can be expressed as:

$$P(i\equiv k\equiv m) = P(i\equiv j\equiv k\equiv m) + P(i\equiv k\equiv m\not\equiv j) = \delta_1 + \delta_4 \qquad [37]$$

The probabilities of gene identities in equation [36] can again be computed recursively. Let $i\geq k\geq m$. If i is a base gamete, implying that k and m are also base gametes, then:

$$P(i\equiv k\equiv m) = 1 \quad \text{if } i=k=m \quad \text{else} \quad P(i\equiv k\equiv m) = 0 \qquad [38]$$

If i has known parental gametes x and y, then for $i\neq k\neq m$:

$$\begin{aligned} P(i\equiv k\equiv m) &= P(x\equiv k\equiv m)\ P(i=x) + P(y\equiv k\equiv m)\,P(i=y) \\ &= \tfrac{1}{2}[\ P(x\equiv k\equiv m) + P(y\equiv k\equiv m)\ ] \end{aligned} \qquad [39]$$

and for i=k:

$$P(i\equiv k\equiv m) = P(i\equiv m) = \tfrac{1}{2}[\ P(x\equiv m) + P(y\equiv m)\ ] \qquad [40]$$

Recurrence [39] was given in Harris (1964, p.1322), and recurrencies [38], [39] and [40] are given by Smith and Mäki-Tanila (1990, p.71) for second moments rather than identity probabilities.

Covariance between dominance effects. The covariance between dominance effects of individuals V (d_{ij}) and W (d_{km}) is the element of **E** pertaining to gamete pairs ij and km, which is by definition:

$$Cov(d_{ij},d_{km}) = \sum_{\ell=1}^{L}[\ E(d_{ij}^{\ell}\ d_{km}^{\ell}) - E(d_{ij}^{\ell})\ E(d_{km}^{\ell})\ ] \qquad [41]$$

where d_{ij}^{ℓ} is the dominance effect of gamete pair ij at locus ℓ with $d_{ij}=\Sigma_{\ell}d_{ij}^{\ell}$, L is number of loci, $E(d_{ij}^{\ell})$ is the first moment or expected value of d_{ij}^{ℓ}, and $E(d_{ij}^{\ell}d_{km}^{\ell})$ is the second moment among dominance effects of gamete pairs ij and km at locus ℓ.

The first moment of d_{ij}^{ℓ} is equal to the probability that the genes in gamete pair ij at locus ℓ are i.b.d. times the inbreeding depression at locus ℓ, or $E(d_{ij}^{\ell}) = P(i\equiv j)\Delta_i^{\ell}$. Then the part of equation [41] pertaining to first moments becomes:

$$\sum_{\ell=1}^{L} E(d_{ij}^{\ell})\ E(d_{km}^{\ell}) = P(i\equiv j)P(k\equiv m)\sum_{\ell=1}^{L}(\Delta_i^{\ell})^2 = P(i\equiv j)P(k\equiv m)\,\Delta_I^2 \qquad [42]$$

Probabilities in equation [42] can be obtained from identity coefficients, e.g.:

$$\begin{aligned} P(i\equiv j) &= P(i\equiv j\equiv k\equiv m) + P(i\equiv j\equiv k\not\equiv m) + P(i\equiv j\equiv m\not\equiv k) \\ &+ P(i\equiv j\not\equiv k\equiv m) + P(i\equiv j\not\equiv k\not\equiv m) \\ &= \delta_1 + \delta_2 + \delta_3 + \delta_6 + \delta_7 = F_v \end{aligned} \quad [43]$$

Similarly, $P(k\equiv m)=F_w$, hence equation [42] can be rewritten as:

$$\sum_{\ell=1}^{L} E(d_{ij}^{\ell})\, E(d_{km}^{\ell}) = F_v\, F_w \Delta_I^2 \quad [44]$$

Each second moment in equation [41] is a weighted sum of dominance variance in the noninbred base population, the dominance variance in the completely inbred population, and sum of squared inbreeding depressions, or:

$$\begin{aligned} E(d_{ij}^{\ell}, d_{km}^{\ell}) &= [P(i\equiv k\not\equiv j\equiv m) + P(i\equiv m\not\equiv j\equiv k)]\, \sigma^2_{dr(\ell)} \\ &+ P(i\equiv j\equiv k\equiv m)\ \{\sigma^2_{di(\ell)} + (\Delta_i^{\ell})^2\} + P(i\equiv j\not\equiv k\equiv m)\ (\Delta_i^{\ell})^2 \end{aligned} \quad [45]$$

where $\sigma^2_{dr(\ell)}$, $\sigma^2_{di(\ell)} + (\Delta_i^{\ell})^2$, and $(\Delta_i^{\ell})^2$ are second moments conditional on the four identity cases above. Second moments for all other identity cases (Figure 1) are zero due to relationships (Harris, 1964):

$$\sum_{i=1}^{s} p_i d_{ij} = \sum_{j=1}^{s} p_j d_{ij} = 0$$

where s is the number of alleles per locus. Summation over loci yields:

$$\begin{aligned} \sum_{\ell=1}^{L} E(d_{ij}^{\ell}, d_{km}^{\ell}) &= [P(i\equiv k\not\equiv j\equiv m) + P(i\equiv m\not\equiv j\equiv k)]\, \sigma^2_{DR} + P(i\equiv j\equiv k\equiv m)\ \sigma^2_{DI} \\ &+ [P(i\equiv j\equiv k\equiv m) + P(i\equiv j\not\equiv k\equiv m)]\, \Delta_I^2 \end{aligned} \quad [46]$$

Expressing the probabilities in equation [46] as identity coefficients (Figure 1) and combining equations [46] and [44] in equation [41] yields:

$$Cov(d_{ij}, d_{km}) = (\delta_9 + \delta_{12})\sigma^2_{DR} + \delta_1\sigma^2_{DI} + (\delta_1 + \delta_6 - F_v F_w)\Delta_I^2 \quad [47]$$

with coefficients equal to those in equations [22], [23] and [26].

The probabilities in equation [46] were referred to as "four-way coefficients" and "two-pair coefficients de parenté" by Harris (1964), and recursive rules for their computation were given.

In conclusion, extracting elements from **E** to compute additive, additive-dominance, and dominance relationship coefficients is equivalent to computing these relationships from identity coefficients evaluated with the path coefficient method as defined in equations [21] to [26]. Recursive computation of **E**, however, requires use of the genetic parameters σ^2_{AR}, σ^2_{DR}, σ^2_{DI}, σ_{ADI}, and Δ^2_I. This approach becomes inefficient if the same population is analyzed for several traits with different genetic parameter values, or when parameter values are unknown and, if possible, estimated iteratively. Hence, a modified tabular method will be presented next.

Modified tabular method

Relationship coefficients can be computed from probabilities of gene identities by descent as shown in equations [30], [36], [42], and [46]. Hence, instead of computing a matrix with first and second moments (**E**), matrices with the required types of probabilities of gene identities may be computed recursively. A tabular method then consists of two main steps:

1 Form five matrices including the following probabilities from a list of gametes and gamete pairs, respectively. Let ij and km represent gamete combinations of two individuals.
$\mathbf{M}_1=\{P(i\equiv k)\}$; $\mathbf{M}_2=\{P(i\equiv k\equiv m)\}$; $\mathbf{M}_3=\{P(i\equiv k\neq j\equiv m)+P(i\equiv m\neq j\equiv k)\}$;
$\mathbf{M}_4=\{P(i\equiv j\equiv k\equiv m)\}$; $\mathbf{M}_5=\{P(i\equiv j\equiv k\equiv m)+P(i\equiv j\neq k\equiv m)-P(i\equiv j)P(k\equiv m)\}$

2 Compute relationship matrices in equation [28]. Matrix **A** may be obtained from $\mathbf{M}_1$ but is computed more efficiently using the well-known tabular method described by Henderson (1976), **C** is obtained from $\mathbf{M}_2$ using equation [36], $\mathbf{D}_R=\mathbf{M}_3$, $\mathbf{D}_I=\mathbf{M}_4$, and $\mathbf{U}=\mathbf{M}_5$.

Step (1) consists of the following sub-steps:

1.1 Form ordered lists of gametes and gamete pairs, respectively. The list of gametes includes paternal and maternal gametes of all individuals. The list of gamete pairs includes those in all individuals, and ancestor pairs are added with the algorithm of Smith and Mäki-Tanila (1990, p.70).

1.2 Form $\mathbf{M}_1$ for all gametes i and k using equations [32], [33], and [34], or form **A** directly.

1.3 Form $\mathbf{M}_2$ for all gametes i and gamete pairs km. Identify rows of $\mathbf{M}_2$ by the ordered list of gametes, and write the parental gametes on the left of each gamete. Identify the columns of $\mathbf{M}_2$ by the ordered list of gamete pairs, and write the parental gamete pairs above each pair. Parental gamete pairs of gamete pair km are xm and ym, if $k\geq m$ and x and y are parental gametes of k.

Then, fill the matrix by proceeding from left to right within row and from top to bottom within column using the following rules.

(a) If $i \geq k$, and
- i is a base gamete, use equation [38]
- i is not a base gamete, and
  - $i \neq k$ and $k \neq m$, use equation [39]
  - $i = k$ and $k \neq m$, use equation [40]
  - $i = k = m$, $M_2(i,km) = 1$

(b) If $i < k$, and
- k is a base gamete, $M_2(i,km) = 0$
- k is not a base gamete, and
  - $k \neq m$, $M_2(i,km) = \frac{1}{2}[M_2(i,xm) + M_2(i,ym)]$
  - $k = m$, $M_2(i,km) = \frac{1}{2}[M_2(i,xx) + M_2(i,yy)]$

1.4 To form $\mathbf{M}_3$, $\mathbf{M}_4$, and $\mathbf{M}_5$, identify rows and columns by the ordered list of gamete pairs. For gamete pairs ij and km, let $i \geq j$ and $k \geq m$. Computing only the lower half of the matrix implies that $i \geq k$. Then $M_3(ij,km)$ may be computed with the following rules.

(a) If i is a base gamete, $M_3(ij,km) = 1$ if $i = k$, $j = m$, $i \neq j$, and $k \neq m$, and zero elsewhere.

(b) If i is not a base gamete:
- $i \neq j$, and
  - $i \neq k$, $M_3(ij,km) = \frac{1}{2}[\, M_3(xj,km) + M_3(yj,km)\,]$
  - $i = k$, $M_3(ij,km) = \frac{1}{2}[\, M_3(xj,xm) + M_3(yj,ym)\,]$

- $i = j$, and
  - $i \neq k$, $M_3(ij,km) = \frac{1}{2}[\, M_3(xx,km) + M_3(yy,km)\,]$
  - $i = k$, $M_3(ij,km) = \frac{1}{2}[\, M_3(xx,xx) + M_3(yy,yy)\,]$

1.5 To form $\mathbf{M}_4$, apply the rules in (1.4), except replace (a) with: if i is a base gamete, $M_4(ij,km) = 1$ if $i = j = k = m$, and zero elsewhere.

1.6 To form $\mathbf{M}_5$, include one extra row and column containing $P(i \equiv j)$ for any gamete pair ij. The remaining rows and columns contain $P(i \equiv j \equiv k \equiv m) + P(i \equiv j \neq k \equiv m)$.

(a) Start with the first column, using recurrences [32], [33], and [34].

(b) Compute remaining rows and columns of the lower triangular matrix with rules in (1.4), except replace (a) with: if i is a base gamete, $M_5(ij,km) = 1$ if $(i = j)$ and $(k = m)$, and zero elsewhere.

(c) Adjust each element in all rows and columns except the first by $M_5(ij,km) = M_5(ij,km) - M_5(1,km)M_5(ij,1)$. Delete first row and column.

Results

Numerical example for computation of covariance matrix

A parent-offspring mating, depicted in Figure 3 with individuals identified by letters A, B and C, will be used to illustrate path coefficient and tabular methods for computing genetic covariances among individuals.

Path coefficient method. Computing the genetic covariance between individuals B and C requires evaluation of all 15 identity coefficients pertaining to the four genes in B and C at any locus. The four genes are defined as the paternal (4) and maternal (3) gene in B and the paternal (5) and maternal (6) gene in C. Identity coefficients are computed in five steps as follows: 1) The only common ancestor of B and C is A; 2) Genes 1, 2, and 3 in Figure 3 are base genes. There are two paths of origin of the four genes of interest (4,3,5,6). In both paths gene 3 is a base gene, and genes 4 and 5 derive from the base genes in A. The paths differ in the origin of gene 6 in C. In the first path, gene 6 is inherited from individual A through individual B, implying that 4 and 6 are copies of the same ancestral gene in A. In the second path, gene 6 is a copy of gene 3 in B; 3) The probability of each path of origin is ½; 4) The four genes of interest (4,3,5,6) can be copies of the base genes 1, 2, and 3 in different ways, e.g. (4=1,3=3,5=1,6=1).

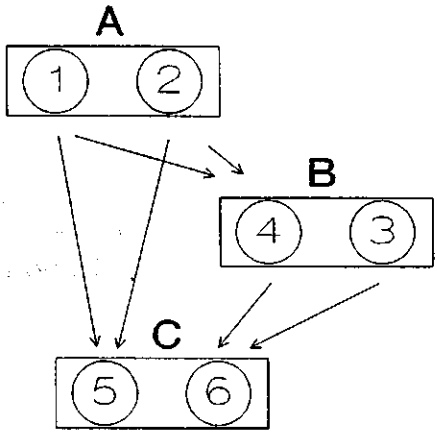

Figure 3. Parent-offspring mating with animals represented by **blocks** and **letters**, paternal gametes as **left-** and maternal gametes as **right-numbered circles within blocks**

Table 2. Probabilities of identity modes (IM) pertaining to the four genes of individuals B and C in Figure 3 computed with the path coefficient method of Jacquard (1966)

Path[a]	Prob. Path	Origin genes B		Origin genes C		IM[b]	Prob. IM
		4	3	5	6		
1	½	A	B	A	A		
		1	3	1	1	4	1/4
		1	3	2	1	13	1/4
		2	3	1	2	13	1/4
		2	3	2	2	4	1/4
2	½	A	B	A	B		
		1	3	1	3	9	1/4
		1	3	2	3	11	1/4
		2	3	1	3	11	1/4
		2	3	2	3	9	1/4

[a] Origins of genes (4,3,5,6) are A,B,A,A, for path 1 and A,B,A,B for path 2, respectively
[b] Number of identity mode as presented in Figure 1

All possibilities are listed in Table 2 by path of origin. The case (4=1,3=3,5=1,6=1) has probability $P(4=1)\times P(3=3)\times P(5=1)\times P(6=1|4=1) = ½\times 1\times ½\times 1 = ¼$, and is represented by identity mode 4 of Figure 2 (first line for path 1 in Table 2). Identity modes and their probabilities for all other cases are given in Table 2). Multiplication of probability of path and probability of identity mode and summing by identity mode in Table 2 yields the only nonzero identity coefficients $\delta_4=(½\times ¼)+(½\times ¼)=¼$, $\delta_9=¼$, $\delta_{11}=¼$, and $\delta_{13}=¼$.

From the coefficients of identity, relationship coefficients are computed via [21] through [26], or $a_{BC}=¾$, $dr_{BC}=¼$, $di_{BC}=0$, $c_{BC}=⅛$, $c_{CB}=0$, and $u_{BC}=0$. Consequently the genetic covariance between individuals B and C is:

$$\sigma_{G_BG_C} = \frac{3}{4}\sigma^2_{AR} + \frac{1}{4}\sigma^2_{DR} + \frac{1}{8}\sigma_{ADI} \qquad [48]$$

Tabular method. To compute the genetic covariance matrix between individuals B and C from the pedigree in Figure 3 with the modified tabular method, lists of gametes and gamete pairs, respectively, must be created first. The list of gametes included the gametes indexed 1, 2, 3, 4, 5, and 6. The list of gamete pairs initially contains the pairs existing in individuals A, B, and C, or 21, 43, 65. Subsequently, starting with the gamete pair of the youngest individual, 65, ancestral gamete pairs are added into the list: 54 and 53 for 65, 42 and 41 for 54, 32 and 31 for 53, 22 and 21 for 42, and 11 for 41, yielding the ordered list 11, 21, 22, 31, 32, 41, 42, 43, 53, 54, 65.

Matrices $\mathbf{M}_1$, $\mathbf{M}_2$, $\mathbf{M}_3$, $\mathbf{M}_4$, and $\mathbf{M}_5$ were computed from the two lists and rules (1.1) to (1.6) stated earlier, and are given in Tables 3 to 7.

The additive genetic relationship between individuals B (43) and individual C (65) is computed from $\mathbf{M}_1$ in Table 3 as $\frac{1}{2}[M_1(3,5) + M_1(3,6) + M_1(4,5) + M_1(4,6)]=\frac{3}{4}$. The relationship coefficient between the additive effect of B and the dominance effect of C is computed from $\mathbf{M}_2$ in Table 4 as $\frac{1}{2}[M_2(3,65) + M_2(4,65)] = \frac{1}{8}$. The relationship between the dominance effect of B and the additive effect of C is computed from $\mathbf{M}_2$ in Table 4 as: $c_{BC} = \frac{1}{2}[M_2(43,5) + M_2(43,6)] = 0$. The relationship between the dominance effects of individuals B and C due to σ^2_{DR} is element (43,65) in $\mathbf{M}_3$ of Table 5, hence $dr_{BC}=\frac{1}{4}$. The relationships due to σ^2_{DI} and due to Δ^2_I are the corresponding elements in $\mathbf{M}_4$ of Table 6 and $\mathbf{M}_5$ of Table 7, respectively, hence $di_{BC}=u_{BC}=0$. The total genetic covariance between B and C is therefore that given in [48]. The matrix of genetic covariances between additive and dominance effects of all individuals in Figure 3 is given in Table 8.

Table 3. Matrix $\mathbf{M}_1 = \{ P(i \equiv k) \}$ in the modified tabular method for the list of gametes from the pedigree in Figure 3. For gametes identifying rows, paternal and maternal gametes are given on the left

			1	2	3	4	5	6
		1	1					
		2	0	1				
		3	0	0	1			
1	2	4	1/2	1/2	0	1		
1	2	5	1/2	1/2	0	1/2	1	
4	3	6	1/4	1/4	1/2	1/2	1/4	1

Table 4. Matrix $\mathbf{M}_2 = \{ P(i \equiv k \equiv m) \}$ in the modified tabular method for the lists of gametes and gamete pairs from the pedigree in Figure 3. Parental gametes or gametes pairs are given on the left for gametes and on the top for gamete pairs, respectively

		11 21	21 22	31 32	31 32	41 42	53 54
		11	21 22 31 32	41 42 43 53	54	65	
	1	1	0 0 0 0	1/2 0 0 0	1/4	1/8	
	2	0	0 1 0 0	0 1/2 0 0	1/4	1/8	
	3	0	0 0 0 0	0 0 0 0	0	0	
1 2	4	1/2	0 1/2 0 0	1/2 1/2 0 0	1/2	1/4	
1 2	5	1/2	0 1/2 0 0	1/4 1/4 0 0	1/2	1/4	
4 3	6	1/4	0 1/4 0 0	1/4 1/4 0 0	1/4	1/4	

Table 5. Matrix $\mathbf{M}_3 = \{ P(i \equiv k \neq j \equiv m) + P(i \equiv m \neq j \equiv k) \}$ in the modified tabular method for the list of gamete pairs from the pedigree in Figure 3. For gamete pairs identifying rows, parental gamete pairs are given on the left

			11	21	22	31	32	41	42	43	53	54	65
		11	0										
		21	0	1									
		22	0	0	0								
		31	0	0	0	1							
		32	0	0	0	0	1						
11	21	41	0	1/2	0	0	0	1/2					
21	22	42	0	1/2	0	0	0	0	1/2				
31	32	43	0	0	0	1/2	1/2	0	0	1			
31	32	53	0	0	0	1/2	1/2	0	0	1/2	1		
41	42	54	0	1/2	0	0	0	1/4	1/4	0	0	1/2	
53	54	65	0	1/4	0	1/4	1/4	1/8	1/8	1/4	1/2	1/4	3/4

Table 6. Matrix $\mathbf{M}_4 = \{ P(i \equiv j \equiv k \equiv m) \}$ in the modified tabular method for the list of gamete pairs from the pedigree in Figure 3. For gamete pairs identifying rows, parental gamete pairs are given on the left

			11	21	22	31	32	41	42	43	53	54	65
		11	1										
		21	0	0									
		22	0	0	1								
		31	0	0	0	0							
		32	0	0	0	0	0						
11	21	41	1/2	0	0	0	0	1/2					
21	22	42	0	0	1/2	0	0	0	1/2				
31	32	43	0	0	0	0	0	0	0	0			
31	32	53	0	0	0	0	0	0	0	0	0		
41	42	54	1/4	0	1/4	0	0	1/4	1/4	0	0	1/2	
53	54	65	1/8	0	1/8	0	0	1/8	1/8	0	0	1/4	1/4

Table 7. Matrix $\mathbf{M}_5 = \{ P(i \equiv j \equiv k \equiv m) + P(i \equiv j \neq k \equiv m) - P(i \equiv j)P(k \equiv m) \}$ in the modified tabular method for the list of gamete pairs from the pedigree in Figure 3. For gamete pairs identifying rows, parental gamete pairs are given on the left

			11	21	22	31	32	41	42	43	53	54	65
		11	0										
		21	0	0									
		22	0	0	0								
		31	0	0	0	0							
		32	0	0	0	0	0						
11	21	41	0	0	0	0	0	1/4					
21	22	42	0	0	0	0	0	-1/4	1/4				
31	32	43	0	0	0	0	0	0	0	0			
31	32	53	0	0	0	0	0	0	0	0	0		
41	42	54	0	0	0	0	0	0	0	0	0	1/4	
53	54	65	0	0	0	0	0	0	0	0	0	1/8	3/16

Table 8. Genetic covariance matrix between additive (a) and dominance (d) effects of individuals A, B and C in Figure 3

	a_A	a_B	a_C	d_A	d_B	d_C
a_A	σ^2_{AR}	$1/2\ \sigma^2_{AR}$	$3/4\ \sigma^2_{AR}$	0	0	$1/8\ \sigma_{ADI}$
a_B		σ^2_{AR}	$3/4\ \sigma^2_{AR}$	0	0	$1/8\ \sigma_{ADI}$
a_C			$5/4\sigma^2_{AR}$	0	0	$1/4\ \sigma_{ADI}$
d_A				σ^2_{DR}	0	$1/4\ \sigma^2_{DR}$
d_B					σ^2_{DR}	$1/4\ \sigma^2_{DR}$
d_C						$3/4\ \sigma^2_{DR} + 1/4\ \sigma^2_{DI} + 3/16\ \Delta^2_I$

Prediction of additive and dominance effects from simulated data

Data were simulated with an individual locus model described by De Boer and Van Arendonk (1992). The simulated trait was affected by 64 unlinked biallelic loci with complete dominance (a=d=1) and no epistasis, and was measured on both males and females. A normally distributed environmental deviation was added to each genotypic value based on a broad sense heritability (H^2) of 0.2 or 0.5 in the base generation. Each simulated population included five generations. The initial generation contained 20 males and 20 females, whose genes were randomly chosen according to Hardy-Weinberg proportions and gametic phase equilibrium. Frequency of the favourable allele in the base generation was 0.2, 0.5 or 0.8 at all loci. In each generation, five randomly chosen males and all 20 females were mated, with each mating producing one male and one female offspring. Each of the five males was mated to its full sib and three related females with the result that inbreeding levels increased from 0 to 0.35 in generation five. For each combination of heritability and allelic frequency, the simulated population was replicated 1000 times. In each replicate, average predicted minus simulated additive and dominance effects and correlation between predicted and simulated effects were computed within generation and averaged across replicates.

Data from all five generations were used to predict additive and dominance effects with exact and approximate BLUP. Mixed model equations (MME) for exact BLUP were based on model [27] with $X\beta$ replaced by 1μ, where μ was the mean in the base generation, and with genetic covariance matrix in [28]. Approximate MME were also based on model [27], but the genetic covariance matrix was approximated by:

$$\mathbf{G}^{*} = \begin{bmatrix} \mathbf{A}\sigma^2_{AR} & 0 \\ 0 & \mathbf{D}\sigma^2_{DR} \end{bmatrix}$$

where **D** is a dominance relationship matrix computed by ignoring inbreeding. For both approximate and exact MME, the total dominance effect of an individual V was predicted as $\hat{d}_v + \hat{\Delta}_1 F_v$.

Average predictions of additive and dominance effects in each population and generation from both approximate and exact BLUP were empirically unbiased, which is expected and consistent with results from De Boer and Van Arendonk (1992). Mean predicted minus simulated additive effects in each generation ranged from -0.04 to 0.09, with corresponding standard errors of 0.06 and 0.12. Mean predicted minus simulated dominance effects in each generation ranged from -0.12 to 0.05, with corresponding standard errors of 0.12 and 0.04. Mean empirical accuracies of predicted additive and dominance effects in generations 1, 3, and 5 are given in Table 9, for varying H^2 and initial allelic frequency. Level of H^2 did not clearly affect differences in accuracy of predicted additive effects between both methods.

Table 9. Mean empirical accuracies of predicted additive and dominance effects in generations 1, 3 and 5, averaged over 1000 replicates, for approximate and exact BLUP, for a broad sense heritability of 0.20 and 0.50, and varying initial allelic frequency p_i[a]

		Broad heritability of 0.20				Broad heritability of 0.50			
		Approximate		Exact		Approximate		Exact	
p_i	Gen	Add	Dom	Add	Dom	Add	Dom	Add	Dom
0.2	1	0.482	0.144	0.482	0.145	0.704	0.238	0.705	0.240
	3	0.528	0.450	0.529	0.463	0.704	0.494	0.705	0.509
	5	0.413	0.366	0.414	0.387	0.592	0.393	0.595	0.427
0.5	1	0.427	0.257	0.427	0.258	0.623	0.412	0.623	0.413
	3	0.511	0.575	0.511	0.578	0.667	0.637	0.668	0.641
	5	0.439	0.543	0.440	0.549	0.604	0.599	0.606	0.610
0.8	1	0.315	0.358	0.316	0.360	0.460	0.570	0.461	0.571
	3	0.464	0.624	0.472	0.634	0.593	0.758	0.601	0.765
	5	0.479	0.604	0.487	0.633	0.610	0.760	0.623	0.787

[a] Accuracy of prediction was computed as the correlation between predicted and simulated values; average inbreeding coefficients in generations 1, 3 and 5 were 0.00, 0.14 and 0.35

For initial allelic frequencies of 0.2 and 0.5, empirical accuracies of predicted additive effects were almost identical. For p=0.8, however, additive effects were predicted with a slightly higher accuracy with the exact than with the approximate method in generations with inbreeding. By comparison with Figure 2, it appears that there is a noticeable difference in accuracy of predicted additive effects between the exact and the approximate method only if dominance variance is large relative to additive variance (p=0.8), whereas a large covariance between additive and dominance effects (p=0.2) has little impact. Differences in accuracy of predicted dominance effects were larger than for additive effects, but still quite small. The difference was largest for p=0.2 and H^2=0.5 in generation five. For p=0.2, dominance variance is small relative to the additive variance, σ^2_{DI} is much larger than σ^2_{DR}, and the covariance between additive and dominance effects is most important. For p=0.5, accuracies of predicted dominance effects were almost identical. For p=0.8, differences between both methods were larger for H^2=0.2 than for H^2=0.5.

Discussion

Inbreeding changes mean and genetic covariance structure of a population. With inbreeding, genetic covariance remains a sum of products of relationship coefficients and (co)variance components. In addition to additive and dominance variance in an infinite, random mating base population, extra parameters required are dominance variance and covariance between additive and dominance effects in the completely inbred population with allelic frequencies equal to those in the base population, and sum over loci of effects of inbreeding depression and squared effects of inbreeding depression (e.g. Gillois, 1964; Harris, 1964; Jacquard, 1974; Cockerham and Weir, 1984).

A mixed linear model for a phenotype of a quantitative trait with additive and dominance variation includes additive effect, expected value of dominance effect or effect of complete inbreeding depression times inbreeding coefficient, and dominance effect beyond inbreeding depression. Although there is an argument over the existence of a genetic model with an infinite number of loci in gametic phase equilibrium and directional dominance (Robertson and Hill, 1983; Smith and Mäki-Tanila, 1990), the two real and unsolved issues are whether the linear model can adequately describe data on a trait affected by a finite number of loci, in particular with selection, and whether all required genetic parameters can be estimated from real data. The present and previous simulations (Uimari and Kennedy, 1990; De

Boer and Van Arendonk, 1992) showed that predictions of additive and dominance effects were empirically unbiased in unselected or selected populations with inbreeding, for a trait with a finite number of biallelic loci. Estimation of all required genetic parameters has been addressed by Chevalet and Gillois (1977).

Implementation of the mixed model with additive and dominance effects and the exact genetic covariance matrix in [28] for large populations is currently not feasible. Computation of **G** requires calculation of five matrices with different probabilities of gene identities. The maximum order of these matrices is determined by the number of gametes and gamete pairs. The number of required gamete pairs will increase with the number of generations in the data, and decrease due to inbreeding. For the simulated population with 200 individuals after generation five the number of gamete pairs was 1326. Size of these matrices for large populations and potential improvements in efficiency have not yet been investigated. More importantly, $\mathbf{G}^{-1}$ is required in MME and was computed by first creating **G** and subsequently inverting it, which is not feasible for large populations.

Smith and Mäki-Tanila (1990) presented a method for direct computation of $\mathbf{E}^{-1}$, the inverse of an extended genetic covariance matrix, which could be used in MME predicting additive effects of gametes and dominance effects of gamete pairs. This approach, however, could not be used for the simulated data, because **E** is singular for biallelic loci, i.e. $\mathbf{E}^{-1}$ does not exist although $\mathbf{G}^{-1}$ exists. The singularity is caused by a linear relationship among additive effects of base gametes i and dominance effects of gamete pairs ii due to the identity $\sigma^2_{ar}\sigma^2_{dr}=\frac{1}{2}\sigma^2_{adi}$ for two alleles.

Simulations of populations with inbreeding and additive and dominance variation have used individual loci models. Under the infinitesimal model, total genetic effects are normally distributed. A method for generating total additive and dominance effects taking full account of the covariance structure in [28] is not available. In the absence of inbreeding, recurrence equations exist which allow generating an offspring's additive effect as average effect of sire and dam plus Mendelian sampling (e.g. Quaas, 1988). An offspring's dominance effect is generated as sire-dam combination effect plus Mendelian sampling, and sire-dam combination effect is generated from combination effects of sire with parents of dam, dam with parents of sire, and among parents (Hoeschele and VanRaden, 1991). These recurrences also permit computing $\mathbf{A}^{-1}$ (Henderson, 1976; Quaas, 1988) and $\mathbf{D}^{-1}$ (Hoeschele and VanRaden, 1991) directly. However, they do not generate all genetic relationships among inbred animals and their close relatives, e.g. covariances among additive and dominance effects are ignored.

Approximate BLUP accounts only for the effect of inbreeding on mean and additive covariance but is computationally feasible for large populations. Approximate predictions of additive and dominance effects had only slightly reduced

accuracies relative to exact BLUP for traits affected by a finite number of loci and inbreeding.

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Chapter 4

Additive response to selection adjusted for effects of inbreeding in a closed dairy cattle nucleus assuming a large number of gametes per female

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Abstract

Inbreeding leads to reduction of the additive variance, whereas inbreeding depression reduces the performance of milk producing cows in both the nucleus and the commercial population. In this study, the cumulative additive genetic response to 30 years of selection corrected for variance reduction due to inbreeding and inbreeding depression in the commercial cow population (denoted as expected phenotypic level or P) was evaluated in a closed (1024 cows tested per year) dairy cattle nucleus scheme, assuming a large number of gametes available per female. No dominance effects were simulated nor estimated in the nucleus. Various hierarchical and factorial designs with fewer sires than dams, an equal number of sires and dams, or even a larger number of sires than dams were compared for P. The trait considered was overall economic merit for milk production with a heritability of the unselected base population of 0.30. Sires and dams were selected on their animal model estimated additive effect for the trait considered at either 15 or 27 months of age. All full sibs were available for selection. In the absence of inbreeding depression, a complete factorial scheme with more sires than dams resulted in the highest P. With increasing inbreeding depression, the optimal number of sires increased relatively more than the optimal number of dams. Increasing the number of sires decreased inbreeding relatively more than increasing the number of dams, and resulted in a relatively higher P. This is due to the fact that correlations between estimated additive effects of male selection candidates are higher than between those of female selection candidates.

Key words: nucleus scheme, dairy cattle, *in vitro* production of embryos, inbreeding

Introduction

For dairy cattle, simulation studies (for review see Dekkers, 1992) predicted that MOET (multiple ovulation and embryo transfer) nucleus schemes could offer improved rates of genetic progress over progeny testing schemes. In these studies the number of gametes available per cow was considerably smaller than the number of gametes available per bull. Hence, both hierarchical (see e.g. Nicholas and Smith, 1983) and factorial designs (Woolliams, 1989; Ruane, 1991) in which the number of sires was smaller than the number of dams have been considered.

In vitro production of embryos may offer the potential for increasing the number of offspring available per cow. This technology involves four components: recovery of oocytes, *in vitro* maturation of oocytes, *in vitro* fertilization of oocytes and culture of embryos (Leibfried-Rutledge *et al.*, 1989). With this technique, factorial designs with an equal number of sires and dams, or even hierarchical designs with fewer dams than sires might become feasible. Woolliams and Wilmut (1989) were the first to investigate potential genetic responses in a complete

factorial dairy cattle adult nucleus scheme with an equal number of sires and dams. The potential benefits of such a scheme in both response and inbreeding were shown by comparing a complete factorial design with 4 sires and 36 dams with a complete factorial design with 12 sires and 12 dams. The latter resulted in a 11% higher response and a 13% lower inbreeding rate. Genetic responses in complete factorial juvenile schemes with an equal number of sires and dams were studied by Kinghorn *et al.* (1991). To date, designs with fewer dams than sires have not been studied (e.g. 36 sires and 4 dams). These designs might result in higher genetic response at a similar level of inbreeding.

In computing genetic response, Woolliams and Wilmut (1989) and Kinghorn *et al.* (1991) ignored variance reduction due to inbreeding. In addition, the effect of inbreeding on the performance of milking cows was not considered. Nucleus breeding schemes and more intense selection using best linear unbiased prediction (BLUP), will cause increased inbreeding levels in dairy cattle populations (Wray, 1989). In a dairy cattle population with only one nucleus, the genetic change and the inbreeding rate in the nucleus will control the genetic and inbreeding level in the commercial cow population. Inbreeding leads to reduction of the additive variance, whereas inbreeding depression reduces the performance of milk producing cows in both the nucleus and the commercial cow population. A reduction in performance of commercial cows will result in reduced benefits for dairy farmers. A reduction in milking performance of nucleus cows is, however, economically not very important. Goddard and Smith (1990a) suggested to use the economic value of the genetic response, adjusted for additive variance reduction due to inbreeding and the effect of inbreeding depression on the performance of commercial cows, to determine the optimal breeding scheme. In this way, an attempt was made to weigh the genetic response of a breeding scheme versus its inbreeding level.

The objective of this study is to evaluate genetic response corrected for both effects of inbreeding in a closed dairy cattle nucleus scheme, assuming a large number of gametes per cow. Various hierarchical and factorial designs with fewer sires than dams, an equal number of dams and sires, or even a larger number of sires than dams were compared for their corrected genetic response. A stochastic model described by De Boer and Van Arendonk (1994) was used to determine corrected genetic responses in each alternative studied.

Methods

Simulation model

A stochastic model was used to simulate a closed dairy cattle nucleus herd. Validation of the simulation model is described by De Boer and Van Arendonk

(1994). A nucleus in which each year 1024 cows were tested was considered. Meuwissen (1990) showed for a closed adult nucleus scheme that genetic gain increases only marginally when more than 1000 cows are tested each year. The trait considered was overall economic merit for milk production and was assumed to be measurable in females only. This trait was simulated assuming an infinitesimal model with additive gene action (Bulmer, 1980). The heritability in the unselected base population was 0.30. Unit of simulation was one year.

In the starting situation, each age class contained offspring from N_s founder sires and N_d founder dams. To reduce computational requirements, founder individuals were derived from an unrelated, selected base population. The number of founder sires and dams equalled the number of sires and dams in subsequent generations. Based on Schrooten and Van Arendonk (1992), a genetic difference between founders generating two consecutive age groups of 0.12 units σ_p was used. The estimated additive effect of the i^{th} founder of age group j ($\hat{a}_{ij}$) was simulated as:

$$\hat{a}_{ij} = \mu_j + RND_{tr} r_i' \sigma_A' \qquad [1]$$

where μ_j is the additive genetic mean of the j^{th} age group, and RND_{tr} is a random number from a truncated standard normal distribution. The truncation point corresponded to selection of N_s founder sires or N_d founder dams out of 1024 selection candidates. The additive variance in the selected base population is denoted as $\sigma_A^{2\prime}$ and equalled 0.70 $\sigma_A^{2(t=0)}$ (following Schrooten and Van Arendonk, 1992), where $\sigma_A^{2(t=0)}$ is the additive variance before selection which equals $h^{2(t=0)}$ assuming an initial phenotypic variance of one. Accuracies of sire and dam selection (r_i'), corrected for variance reduction due to selection (Dekkers, 1989), were taken at 0.92 and 0.61, corresponding to evaluation of sires on 100 progeny records and dams on 3 lactation records, respectively.

Given the estimated additive effect, the true additive effect of the i^{th} founder of age group j (a_{ij}) was computed by adding a prediction error:

$$a_{ij} = \hat{a}_{ij} + RND_n \sqrt{(1-r_i'^2)}\, \sigma_A' \qquad [2]$$

where RND_n is a random number from a standard normal distribution.

Subsequently, founder sires and dams were mated at random under hierarchical or factorial designs to produce first generation offspring. The additive effect of these offspring and offspring born in subsequent generations was computed as:

$$a_i = \frac{1}{2}(a_s + a_d) + m_a \quad [3]$$

where a_i, a_s, a_d are additive effects of animal i, its sire s and dam d, respectively, and m_a is a Mendelian sampling term. The latter was taken at random from a normal distribution with mean zero and variance $\frac{1}{2}(1\text{-}F)\sigma_A^{2(t=0)}$, where F is the average inbreeding coefficient of the parents. Inbreeding coefficients were determined as described by Tier (1990). A cow's record at 90 days of the first lactation (27 months) was simulated as:

$$y_i = a_i + e_i \quad [4]$$

where a_i, e_i is the additive and environmental effect of the first record from cow i. No further lactations were considered. Extension of part lactation records was used, which enables the use of part lactation records in the genetic evaluation, and consequently selection of cows at 27 months of age. Extended records were assumed to have a genetic correlation of one with complete lactation and equal variance.

An individual animal model including genetic groups (Quaas, 1988; Westell *et al.*, 1988) was used to estimate additive effects. Mixed model equations were solved using iteration on the data in combination with Gauss-Seidel iteration. The ratio of the sum of squared differences in solutions between iterations over the sum of squares of the most recent solutions was used as convergence criterion, as suggested by Schaeffer and Kennedy (1986). Solutions were considered stable when the convergence criterion was less than 10^{-6}.

Selection of breeding individuals was based on their estimated additive effects. Sires and dams were eligible for selection at either 15 or 27 months of age. Although estimated additive effects were the same for full sib males and for full sib 15-month old females, no restriction on full sib selection was applied. Selection of, e.g. all males in a full sib family in combination with a higher number of sires results in higher genetic responses at similar inbreeding levels than selection of only one male full sib per family (Ruane, 1991; Meuwissen, 1990).

Different schemes were generated by varying the number of sires used annually (N_s), the number of dams used annually (N_d), the number of matings per dam per year (M_d), the number of matings per sire per year (M_s), and the number of full sibs of each sex per family (N_{fs}). Schemes were compared on the basis of the expected phenotypic level after 30 years of selection adjusted for both effects of inbreeding. The number of cows tested each year was 1024. Hence, $1024 = N_d \cdot M_d \cdot N_{fs} = N_s \cdot M_s \cdot N_{fs}$. Limited by computer requirements, all alternatives were run for 100 replicates and statistics were averaged over replicates.

Computation of expected phenotypic level (P_b)

The expected phenotypic level (P_b in units σ_p) of a particular design was computed as:

$$P_b = A - b\,F \qquad [5]$$

where A (in units σ_p) is the cumulative genetic response corrected for additive variance reduction due to inbreeding, b is the depression in performance per unit of inbreeding, and F is the final inbreeding level. Three levels of b (inbreeding depression) were considered: 0, 0.25% and 1% of the mean per 1% inbreeding. Assuming a coefficient of variation of economic merit of milk production of 0.12 given by Balaine *et al.* (1981), this corresponds to 0, 0.021 σ_p, and 0.083 σ_p depression per 1% inbreeding.

Results

Illustration of results

To illustrate results obtained with the model, trends in additive genetic level and inbreeding level of embryos are given in Figure 1a and 1b respectively, for two designs described in Table 1. The designs studied differed in inbreeding level. In the low inbreeding design, $N_s=128$, $N_d=128$, $M_d=8$, $M_s=8$, $N_{fs}=1$, while in the high inbreeding design $N_s=16$, $N_d=16$, $M_d=1$, $M_s=1$, $N_{fs}=64$. The high inbreeding design had a much higher inbreeding rate because fewer sires and dams were selected annually (16 vs 128). In addition, correlations between estimated additive effects of selection candidates were higher in the high than in the low inbreeding design.

The number of founder sires and dams selected in order to generate animals in all age classes in the starting situation equalled the number of sires and dams in subsequent generations. Therefore, the smaller the number of sires and dams, the higher the initial additive genetic level of the embryos. As a result, the initial additive genetic level of the embryos was higher for the high than for the low inbreeding design (Figure 1a).

Due to the build up of parental information, the difference in annual genetic response between the low and the high inbreeding design was small in the first few years. Subsequently, the additive genetic level in the low inbreeding design increased almost linearly in time. In the high inbreeding design, annual genetic response seemed to be unaffected by inbreeding up to an inbreeding coefficient of embryos of 0.37. The corresponding average parental inbreeding coefficient will, however, be lower than 0.37. As the average inbreeding coefficient of embryos increased above 0.37 (year 10), annual genetic response declined each year as a consequence of inbreeding.

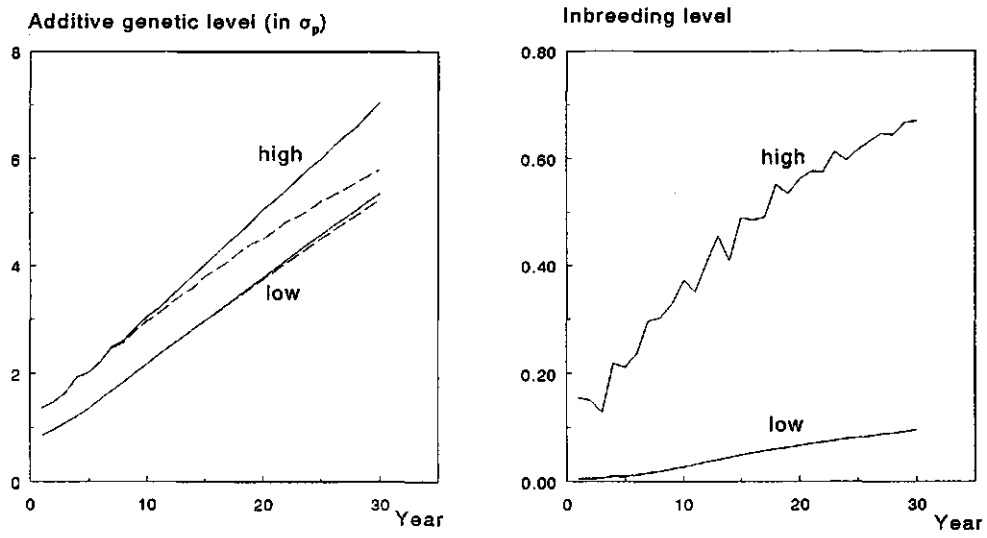

Figure 1. Trends in additive genetic level and inbreeding level of embryos in two designs: (i) the low inbreeding design (low) with $N_s=N_d=128$, $M_s=M_d=8$ and $N_{fs}=1$, (ii) the high inbreeding design (high) with $N_s=N_d=16$, $M_d=M_s=1$ and $N_{fs}=64$. Solid lines show simulated additive genetic levels ignoring variance reduction due to inbreeding. Dotted lines show simulated additive genetic levels accounted for variance reduction due to inbreeding

This is consistent with results from Belonsky and Kennedy (1988) and Meuwissen (1989). As a result, the difference in additive genetic level between the high and the low inbreeding design decreased after 10 years of selection.

For computation of P_b of each alternative design, the genetic response between year 1 and 30 was considered. Hence, differences in initial additive genetic level between various alternatives did not affect computation of P_b. Instead of the average annual genetic response, the cumulative genetic response between year 1 and 30 was used. At high levels of inbreeding the annual response declines each year as a consequence of inbreeding. An average annual genetic response might be obtained by dividing the cumulated response between year 1 and 30 by 30.

Comparison of designs with no inbreeding depression

Table 1 shows expected phenotypic levels in the absence of inbreeding depression (P_0) for various designs with an equal number of sires and dams (varying from 128 to 16).

Table 1. Expected phenotypic levels in the absence of inbreeding depression (P_0=A) and final inbreeding levels (F) for various breeding designs[a]

Design						
N_s	N_d	M_s	M_d	N_{fs}	P_0=A	F
128	128	1	1	8	4.339 (0.018)	0.146 (0.004)
128	128	2	2	4	4.384 (0.017)	0.120 (0.003)
128	128	4	4	2	4.404 (0.016)	0.106 (0.003)
128	128	8	8	1	4.433 (0.018)	0.096 (0.002)
64	64	1	1	16	4.800 (0.022)	0.330 (0.009)
64	64	2	2	8	4.853 (0.021)	0.251 (0.006)
64	64	4	4	4	4.871 (0.018)	0.190 (0.004)
64	64	8	8	2	4.950 (0.017)	0.166 (0.004)
64	64	16	16	1	5.008 (0.018)	0.158 (0.004)
32	32	1	1	32	4.777 (0.041)	0.644 (0.014)
32	32	2	2	16	5.004 (0.033)	0.543 (0.012)
32	32	4	4	8	5.157 (0.027)	0.421 (0.011)
32	32	8	8	4	5.293 (0.026)	0.350 (0.010)
32	32	16	16	2	5.400 (0.026)	0.312 (0.008)
32	32	32	32	1	5.443 (0.023)	0.292 (0.007)
16	16	1	1	64	4.688 (0.039)	0.670 (0.012)
16	16	2	2	32	4.876 (0.040)	0.656 (0.013)
16	16	4	4	16	4.980 (0.037)	0.639 (0.012)
16	16	8	8	8	5.243 (0.037)	0.584 (0.012)
16	16	16	16	4	5.456 (0.034)	0.528 (0.012)

[a] standard errors between brackets

Hierarchical designs (M_d=1; M_s=1) resulted in lower P_0 and higher F than factorial designs (M_d>1; M_s>1). Testing more half sibs instead of full sibs will decrease the average correlation between estimated additive effects of selection candidates. As a result, inbreeding coefficients decreased and selection intensities increased moving from a hierarchical to a factorial design. Both effects will increase cumulative genetic response. Sire and dam selection accuracies, however, decreased by replacing full sib by half sib information (Ruane, 1991). Despite the lower selection accuracy, factorial designs resulted in higher cumulative genetic responses (P_0=A) than hierarchical designs. The difference in P_0 between factorial and hierarchical designs increased as the number of sires and dams decreased. For example, with N_s=N_d=128, P_0 increased from 4.339 to 4.433 moving from a hierarchical to a factorial design with maximal M_s (=M_d). With N_s=N_d=16, P_0 equalled 4.688 and 5.456 in the hierarchical and the complete factorial design

($N_s=N_d=M_s=M_d$), respectively. This can be explained by the fact that correlations between estimated additive effects of selection candidates in a hierarchical design were much higher with $N_s=N_d=16$ than with $N_s=N_d=128$. When $N_s=N_d=16$, $M_s=M_d=1$ and $N_{fs}=64$, all sires descended from one full sib family. When $N_s=N_d=128$, $M_s=M_d=1$ and $N_{fs}=8$, 16 different full sib families were selected. Moving from a hierarchical to a factorial design decreased the average correlation between estimated additive effects of selection candidates relatively more when $N_s=N_d=16$ than when $N_s=N_d=128$.

Results from varying the number of sires at the expense of the number of matings per sire, or varying the number of dams at the expense of the number of matings per dam are given in Figure 2. The design with $N_s=64$, $N_d=64$, $M_s=16$, $M_d=16$ and $N_{fs}=1$ was used as reference. In this design, P_0 was 5.008 and F equalled 0.158. Decreasing N_s from 64 to 32 or 16 while increasing M_s from 16 to 32 or 64, resulted in a proportionately 0.03 and 0.04 higher P_0, respectively. Inbreeding was proportionately 0.50 and 1.19 higher. Decreasing N_d from 64 to 32 or 16 while increasing M_d from 16 to 32 or 64, resulted in proportionately 0.06 and 0.11 higher P_0, respectively. Inbreeding was proportionately 0.25 and 0.69 higher.

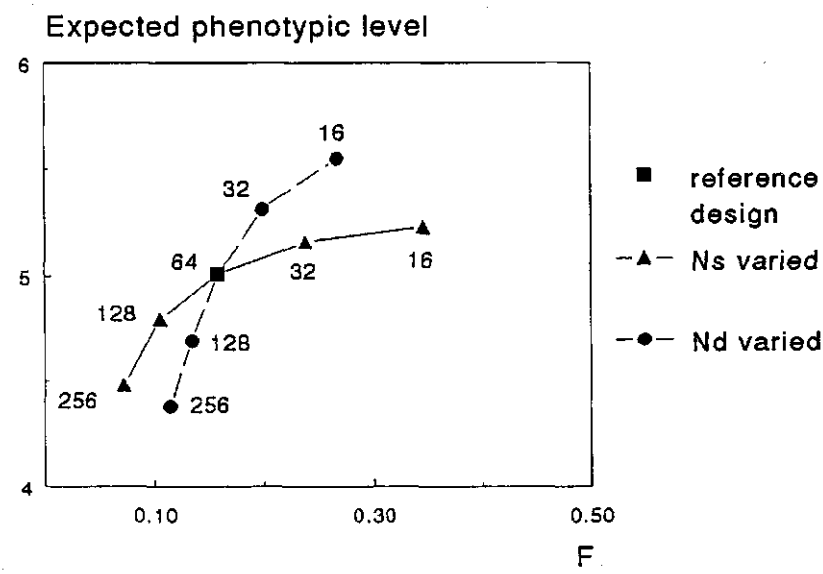

Figure 2. Expected phenotypic levels in the absence of inbreeding depression ($P_0=A$) versus corresponding inbreeding levels (F) of embryos for various designs. The mark (■) represents the reference design: $N_s=N_d=64$, $M_s=M_d=16$ and $N_{fs}=1$. Subsequently, N_s was varied at the expense of M_s, and N_d was varied at the expense of M_d

When N_s equalled 128 or 256 while M_s was 8 or 4, P_0 was proportionately 0.96 and 0.89 of P_0 in the reference design, while F was proportionately 0.67 and 0.45 of F in the reference design. When N_d equalled 128 or 256 and M_d was 8 or 4, P_0 was proportionately 0.94 and 0.87 of P_0 in the reference design, respectively. Inbreeding was proportionally 0.85 and 0.72 of F in the reference design.

Hence, as shown in Figure 2, P_0 was higher and F was lower when, e.g. N_s=64 and N_d=32 (no. sires > no. dams) than when N_s=32 and N_d=64 (no. sires < no. dams). This is due to the fact that the majority of the dams was selected once their own production record was available (27 months), while sires have no own information. As a result, correlations between estimated additive effects of sires are higher than correlations between estimated additive effects of dams. Consequently, use of 32 sires will result in higher F and therefore lower P_0 than use of 32 dams. In addition, high correlations between selection criteria of eligible candidates reduce P_0 due to reduced selection intensities. The relative reduction in selection intensity is, however, larger with N_s=32 than with N_s=64 (Meuwissen, 1991).

Comparison of designs at various levels of inbreeding depression

Results from increasing N_s while decreasing M_s or decreasing N_d while increasing M_d (or N_{fs} if M_d is maximal) in factorial designs from Table 1 with maximal M_s (=M_d), are in Table 2.

In the absence of inbreeding depression (b=0) a complete factorial design with 32 sires and 16 dams and two full sibs of each sex per family, resulted in the highest P_0 (P_0=A). This design, however, also resulted in a high average inbreeding level of embryos transferred in year 30 of 0.37. Table 2 shows that with an inbreeding depression of 0.25% of the mean (b=0.25) this complete factorial design is no longer optimal. The design which resulted in the highest $P_{0.25}$ is N_s=64, N_d=16, M_d=64, M_s=16 and N_{fs}=1. With an increased level of inbreeding depression (b=1), the breeding design with maximum P_1 was N_s=256, N_d=32, M_d=32, M_s=4 and N_{fs}=1.

As inbreeding depression becomes relatively more important the optimal number of sires increased relatively more than the optimal number of dams to decrease the average inbreeding coefficient. Increasing the number of sires decreased inbreeding relatively more than increasing the number of dams, because correlations between estimated additive effects of male selection candidates are higher than between those of female candidates.

Table 2. Expected phenotypic levels (P_b) and final inbreeding levels of embryos (F) for various designs, assuming no inbreeding depression (P_0), 0.25% ($P_{0.25}$) or 1 % (P_1) depression of the mean per 1% inbreeding[a]

Design					Expected phenotypic level			
N_s	N_d	M_s	M_d	N_{fs}	P_0	$P_{0.25}$	P_1	F
256	64	4	16	1	4.480 (0.017)	4.330	3.881	0.072 (0.002)
128	64	8	16	1	4.793 (0.020)	4.574	3.917	0.105 (0.003)
64	64	16	16	1	5.008 (0.018)	4.679	3.692	0.158 (0.004)
64	32	16	32	1	5.312 (0.018)	4.899	3.355	0.198 (0.004)
64	16	16	64	1	5.547 (0.024)	***4.991***	3.323	0.267 (0.006)
64	8	8	64	2	5.545 (0.029)	4.805	2.586	0.355 (0.008)
64	4	4	64	4	5.356 (0.032)	4.454	1.747	0.433 (0.010)
512	32	1	32	1	4.566 (0.015)	4.414	3.956	0.073 (0.001)
256	32	4	32	1	4.885 (0.017)	4.665	***4.005***	0.106 (0.002)
128	32	8	32	1	5.110 (0.019)	4.812	3.916	0.143 (0.004)
64	32	16	32	1	5.312 (0.018)	4.899	3.662	0.198 (0.004)
32	32	32	32	1	5.443 (0.023)	4.835	3.010	0.292 (0.007)
32	16	16	32	2	***5.617*** (0.028)	4.842	2.518	0.372 (0.010)
32	8	8	32	4	5.577 (0.028)	4.627	1.777	0.456 (0.012)
128	16	2	16	4	5.284 (0.025)	4.781	3.271	0.242 (0.006)
64	16	4	16	4	5.431 (0.026)	4.781	2.828	0.312 (0.008)
32	16	8	16	4	5.534 (0.024)	4.701	2.203	0.400 (0.010)
16	16	16	16	4	5.458 (0.034)	4.356	1.057	0.528 (0.012)

[a] Standard errors (S.E.) between brackets; S.E. of $P_{0.25}$ and P_1 might be approximated by summing S.E. of P_0 and F

Discussion

In this paper, following Goddard and Smith (1990a), the cumulative genetic response corrected for variance reduction due to inbreeding and for inbreeding depression in commercial animals, was evaluated in various closed dairy cattle nucleus schemes. The effect of inbreeding on reproductivity and mortality of nucleus individuals (Falconer, 1989) was, however, ignored. Including an effect of inbreeding on reproduction and mortality will reduce the number of nucleus candidates available for selection, resulting in reduced selection differentials. With *in vitro* production of embryos, however, losses due to reduced fertilization and embryo mortality might be overcome by increasing the numbers of embryos produced. In the situation that commercial animals are crossbred, e.g. situation in pig and

chicken breeding, a design with the highest genetic response corrected for variance reduction due to inbreeding only, is nearly optimal. In the situation with only one breeding population, however, the genetic response and the inbreeding rate in the nucleus will control the genetic response and the inbreeding level in the commercial population. When non-additive genetic variance for the trait of interest exists, inbreeding will reduce performance of commercial animals. As a result, benefits for dairy farmers will be reduced. For optimization of breeding designs, cumulative genetic responses should be corrected for depression in performance of commercial animals.

Although in this paper the cumulative genetic response was corrected for depression in performance of commercial animals, no dominance effects were simulated nor estimated in the nucleus. Simulation of dominance effects and inbreeding depression as proposed by De Boer and Van Arendonk (1992) would hardly affect selection of breeding individuals on their estimated additive effects. In their simulation a cow's production record included a dominance effect and was corrected for inbreeding depression, while changes in dominance-related (co)variances due to inbreeding were ignored. A linear model with an additive and dominance effect for each individual and a linear regression on inbreeding, resulted in accurate and empirically unbiased estimates of additive effects (De Boer and Van Arendonk, 1992). Therefore, the same individuals would be selected on estimated additive effect with or without dominance gene action. To reduce computing requirements, dominance effects and inbreeding depression were not simulated nor estimated in the present paper.

To date, good estimates of depression of economic merit of milk production are not available. Estimates of inbreeding depression could be obtained using the approximate animal model including additive and dominance effects with a regression on inbreeding as described by De Boer and Van Arendonk (1992). However, information in the data to predict additive and dominance effects in dairy cattle populations is limited. Goddard and Smith (1990a) have attempted to quantify the depression in economic merit of milk production from inbreeding at about 0.25% of the mean per 1% inbreeding, assuming a coefficient of variation of 0.12 (Balaine *et al.*, 1981). In this paper the value of inbreeding depression was varied from 0, 0.25% to 1% of the mean per 1% inbreeding. The latter value may be favourable, since variance of response and hence risk associated with a breeding program is dependent on level of inbreeding. Incorporation of risk aversion might be approximated by an inflated level of inbreeding depression. In addition, Meuwissen and Woolliams (1994) argued that optimization of economic efficiency after Goddard and Smith (1990a) is less effective for inbreeding reduction than preventing a decline in fitness by natural selection.

The level of inbreeding depression, the cumulative genetic response and the inbreeding level of a nucleus scheme determine the expected phenotypic level in a population. The inbreeding level of a nucleus scheme depends among others on the size and the openness of the nucleus. An increase in nucleus size will reduce the inbreeding level. By opening the nucleus and allowing animals from the commercial cow population to enter the scheme, inbreeding will be reduced. In black and white cows, however, animals entering the nucleus will to a certain extent be related to nucleus individuals, because one Holstein population is dominating the breeding population. As a consequence, the effect of opening the nucleus is smaller than when, e.g., unrelated individuals from another nucleus program are introduced. The effect of enlarging or opening the nucleus on the cumulative genetic response corrected for inbreeding could be approximated by considering a lower effect of inbreeding depression. However, this would be difficult to quantify.

In this paper, sire and dam selection intensities and mating designs were varied to obtain high genetic responses at lower inbreeding levels. Another strategy applied to obtain a high genetic response at lower levels of inbreeding was selection of complete male and 15-month old female full sib ships. Using all, e.g., male full sibs alternatively instead of only one full sib per family reduces the rate of inbreeding without adversely affecting response (Ruane, 1991). Thus, if all male full sibs are available for selection (N_{fs}), selection of N_s sires means selection of N_s/N_{fs} full sib groups. Other strategies proposed to maintain genetic responses at lower levels of inbreeding are (i) minimization of coancestry relations between matings of selected breeding individuals (De Roo, 1988) or simultaneously optimization of selection and mating (Toro and Pérez-Enciso, 1990), and (ii) changing the selection criterion, e.g. changing the relative weight in selection index given to between and within family information (Dempfle, 1990, Toro and Pérez-Enciso, 1990) or correction of an individual's estimated additive effect for the average relationship between its possible mates (Goddard and Smith, 1990b). Recently, Woolliams and Meuwissen (1993) suggested to correct estimated additive effects of selection candidates for their prediction error variance and for prediction error covariances of previously selected individuals, and hence reduce the variance in response (and thus inbreeding) of the breeding design. De Roo (1988) showed that mating of selected individuals that are least related only postpones and not prevents accumulation of inbreeding. Simultaneously optimization of selection and mating gave similar results (Toro and Pérez-Enciso, 1990). Further research is needed to see if use of especially the last group of methods will result in maintained genetic responses at lower inbreeding rates.

Large scale use of *in vitro* production of embryos (IVP) results in nucleus breeding schemes in which it is optimal to have fewer dams than sires. Using

ultrasound guided transvaginal follicle aspiration, on average 15 oocytes can be obtained per collection (Van der Schans *et al.*, 1992). Oocytes can be collected twice a week for about 3 months (Kruip *et al.*, 1993). With 25% of the oocytes developing to transferable embryos (Van der Schans *et al.*, 1992) and a pregnancy rate of 40% (Kruip *et al.*, 1993), 10% of these oocytes will result in a live calf. Hence, 39 offspring per cow might be realistic at this moment. The number of offspring per cow needed in designs of interest in this paper varied from 32 to 256. With some improvement of the IVP technique, these designs might become realistic in the near future. With an efficient IVP technique, the intensity of sire and dam selection is no longer determined by their reproductive capacity, but only by the information available to determine the selection criterion.

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Chapter 5

Combining the genetic and clonal response in in a closed dairy cattle nucleus scheme

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Abstract

Designs testing clones in a closed nucleus, in which 1024 cows are tested each year, were compared for their additive genetic response to selection (genetic response) and their genetic superiority of female genotype(s) selected for commercial cloning (clonal response), using stochastic simulation. Clones were tested at the expense of dam or sire families, matings per dam (sire), or full sibs per family. The reference design maximized the genetic response corrected for inbreeding in the absence of cloning. The trait considered was overall economic merit for milk production, which was simulated assuming an approximate infinitesimal model with both additive and dominant gene action. Bulls and cows eligible for breeding were selected on their animal model estimated additive genetic effect at either 15- or 27-months of age. Female genotypes eligible for commercial cloning were selected on their estimated total genetic effect at 27-months of age. All (fe)male full sibs were available for selection. With only additive gene action, testing clones at the expense of sire families, matings per dam or full sibs per family reduced genetic response, while it increased clonal response and inbreeding. Testing clones at the expense of dam families, however, added to both the genetic and clonal response without increasing inbreeding. When eight clones were tested at the expense of dam families, the genetic response and the final genetic level of commercially available cloned embryos were maximal. Accuracy of clonal selection equalled 0.83. With dominant gene action, however, testing two clones at the expense of dam families maximized the final genetic level of cloned embryos, irrespective of the level of inbreeding depression (accuracy of 0.72). Reliable commercial clone lines can be produced now and in future generations by testing clones at the expense of dam families.

Key words: breeding programs, cloning, dairy cattle

Introduction

Recently, several studies have focused on use of clones in dairy cattle breeding. It has been pointed out that cloning is of potential use for either increasing the additive genetic response (genetic response) or for rapidly increasing the average genetic level of the commercial population. The latter can be achieved by selling cloned embryos of genetically superior female genotypes selected from the nucleus to dairy farmers (Van Vleck, 1981; Nicholas and Smith, 1983; Smith, 1989; Teepker and Smith, 1989; Woolliams, 1989; De Boer and Van Arendonk, 1991; Colleau, 1992). The set of constraints on the total number of embryos transferred or cows tested, or on the number of bulls and cows selected for breeding in various studies has a major impact on obtained benefits of cloning.

With no limits on the total number of embryos transferred, cloning increased the genetic response in a closed MOET (multiple ovulation and embryo transfer)

scheme due to increased female selection accuracy (Nicholas and Smith, 1983; Smith, 1989). Schemes optimal for genetic selection will also result in optimal selection of female genotype(s) for commercial cloning, denoted as clonal selection.

For a given number of cows tested per year and a fixed number of sires and dams, however, maximal genetic response was obtained by maximizing selection intensity with testing only one clone per genotype (Woolliams, 1989; Teepker and Smith, 1990; De Boer and Van Arendonk, 1991). Schemes that maximized clonal response, however, tested multiple copies of fewer genotypes (Teepker and Smith, 1990; De Boer and Van Arendonk, 1991), assuming a realistic intra-clone correlation. Hence, for a given test capacity and a fixed number of sires and dams, designs optimal for genetic selection and those optimal for clonal selection were different. For example, in a situation presented by De Boer and Van Arendonk (1991), clonal response in designs optimal for genetic selection equalled 78% to 99% of its maximum, whereas genetic response in designs optimal for clonal selection varied from 0 to 59% of its maximum.

When only the number of embryos transferred was fixed, testing clones at the expense of sire or dam families could increase genetic response. Testing clones at the expense of sire families increased genetic response in hierarchical designs in combination with a low heritability (Woolliams, 1989). Recently, Colleau (1992) found that testing clones at the expense of dam families increased genetic response in hierarchical mixed (i.e. with bull progeny testing) MOET schemes. Testing clones at the expense of sire or dam families might, however, increase inbreeding and decrease dam and sire selection intensities. De Boer and Van Arendonk (1994) maximized genetic response corrected for inbreeding in a closed dairy cattle nucleus in the absence of cloning. They concluded that selecting fewer sires resulted in lower genetic response at a similar level of inbreeding than selecting fewer dams. This was because correlations between estimated additive genetic effects of males were higher than between those of females. Therefore, with sire evaluation on ancestors, half or full sibs information, testing clones at the expense of dam families might result in a higher genetic response at a similar level of inbreeding than testing clones at the expense of sire families.

In deterministic studies of Woolliams (1989) and Colleau (1992) only the genetic response was computed. As a result, no information was available on consequences of testing clones at the expense of sire or dam families on clonal response. The aim of this paper was to compare the genetic and clonal response for alternative designs in a closed dairy cattle nucleus scheme using stochastic simulation. Designs considered tested clones at the expense of dam or sire families, matings per dam or full sibs per family. Additive genetic variance reduction due to inbreeding and inbreeding depression were taken into account.

At first, the stochastic model was validated by comparing simulated responses with previously obtained deterministic results (De Boer and Van Arendonk, 1991) adjusted for inbreeding in a closed adult nucleus.

Model description

General

The simulation was set up to allow any structure of a closed nucleus herd in terms of number of sires, dams, full sibs per family, matings per dam (sire) and clones tested per genotype. Unit of simulation was one year. The trait considered was overall economic merit for milk production, and was assumed to be measurable in females only. Milk efficiency was simulated assuming an approximate infinitesimal model with both additive and dominant gene action, but without epistasis. Bulls and cows eligible for breeding were selected on their animal model estimated additive genetic effect at either 15- or 27-months of age. Female genotypes eligible for commercial cloning were selected on their estimated total genetic effect at 27-months of age. No additional culling was considered (type, fertility). It was assumed that techniques were available to produce a large number of transferable embryos from each cow, to clone both fresh and frozen embryos, to sex embryos, and to produce a large number of genetically identical embryos.

The simulated period was separated into two phases of 5 and 20 years, respectively. The design simulated in the initial five years maximized the cumulative additive genetic response corrected for variance reduction due to inbreeding and inbreeding depression as determined by De Boer and Van Arendonk (1994), and did not differ between alternatives studied. In the second phase alternative designs were considered that tested clones at the expense of sire or dam families, matings per dam (sire) or full sibs per family.

Simulation

In the initial phase, each age class contained offspring from N_s different founder sires and N_d different founder dams. To reduce computational requirements, founder individuals were derived from an unrelated, selected base population. The number of founder sires and dams equalled the number of sires and dams used in subsequent generations. Based on Schrooten and Van Arendonk (1992), a genetic difference between founders generating two consecutive age groups of 0.12 units σ_p was used. The estimated additive genetic effect of the i^{th} founder of age group j ($\hat{a}_{ij}$) was simulated as:

$$\hat{a}_{ij} = \mu_j + RND_{tr} r_i' \sigma_A' \quad [1]$$

where μ_j is the additive genetic mean of the j^{th} age group and RND_{tr} is a random number from a truncated standard normal distribution. The truncation point corresponded to selection of N_s founder sires or N_d founder dams out of 1024 selection candidates. From Schrooten and Van Arendonk (1992), the additive variance in the selected base population, denoted as $\sigma_A^{2'}$, was taken at 0.70 $\sigma_A^{2(t=0)}$, where $\sigma_A^{2(t=0)}$ is the additive variance before selection which is $h^{2(t=0)}$ (see Table 1) assuming an initial phenotypic variance of one. Accuracies of sire and dam selection (r_i'), corrected for variance reduction due to selection (Dekkers, 1989), were taken at 0.92 and 0.61, corresponding to evaluation of sires on 100 progeny records and dams on 3 lactation records, respectively.

Given the estimated additive effect, the true additive effect of the i^{th} founder of age group j (a_{ij}) was computed by adding a prediction error:

$$a_{ij} = \hat{a}_{ij} + RND_n \sqrt{(1-r_i^{2'})}\, \sigma_A' \quad [2]$$

where RND_n is a random number from a standard normal distribution.

A founder's dominance effect was taken at random from a normal distribution with mean zero and variance σ_D^2, assuming no reduction of dominance variance due to selection on estimated additive genetic effects.

Subsequently, founder sires and dams were mated at random under a factorial design to produce first generation offspring. The additive effect of these offspring and offspring born in subsequent generations was computed as:

$$a_i = \tfrac{1}{2}(a_s + a_d) + m_a \quad [3]$$

where a_i, a_s, a_d are additive genetic effects of animal i, its sire s and dam d, respectively, and m_a is a Mendelian sampling term. The latter was taken at random from a normal distribution with mean zero and variance $\frac{1}{2}(1-F)\sigma_A^{2(t=0)}$, where F is the average inbreeding coefficient of the parents. Inbreeding coefficients were determined as described by Tier (1990).

An individual's dominance effect, corrected for the average effect of inbreeding on the mean (d_i^*), was simulated as:

$$\begin{aligned} d_i^* &= d_i + bF_i \\ &= f_{S*D} + \delta_i + bF_i \end{aligned} \qquad [4]$$

where d_i is the dominance effect of individual i ignoring inbreeding, b is the regression coefficient on inbreeding, and F_i is the inbreeding coefficient of individual i. The regression coefficient on inbreeding, which equals the complete inbreeding depression (De Boer and Hoeschele, 1993), was taken at 0 or 1% of the mean per 1% inbreeding. Assuming a coefficient of variation of milk efficiency of 0.12 (Balaine *et al.*, 1981), this corresponds to 0 or 0.083 units σ_p, respectively. Following Hoeschele and VanRaden (1991), d_i was simulated as the sum of f_{S*D} and δ_i, where f_{S*D} is the average dominance effect of many hypothetical full sibs from sire S and dam D, the so-called S * D combination effect and δ_i is the individual's deviation from the S * D combination effect due to Mendelian sampling. Variation of S * D combination effects is equal to the covariance among full sibs due to dominance, i.e. $\frac{1}{4}\sigma_D^2$. Hence, individual Mendelian sampling terms (δ_i) were taken at random from a normal distribution with mean zero and variance $\frac{3}{4}\sigma_D^2$.

A S * D combination effect was simulated as (Hoeschele and VanRaden, 1991):

$$f_{S*D} = \frac{1}{2}(f_{S*SD}+f_{S*DD}+f_{SS*D}+f_{DS*D}) - \frac{1}{4}(f_{SS*DD}+f_{DS*DD}+f_{SS*SD}+f_{DS*SD}) + m_d \qquad [5]$$

where f_{X*Y} is the combination effect between individual X and Y with SS as sire of sire, SD as sire of dam, DS as dam of sire, DD as dam of dam, and m_d is a Mendelian sampling term taken at random from a normal distribution with mean zero and variance $1/16\sigma_D^2$.

For first generation animals, however, grandparents are unknown. A S * D combination was therefore taken at random from a normal distribution with mean zero and variance $\frac{1}{4}\sigma_D^2$. For second and later generation individuals both parents and grandparents are known, and [5] can be used to simulate S * D combination effects. Unknown combinations of grandparents were taken at random from a normal distribution with mean zero and variance $\frac{1}{4}\sigma_D^2$, while unknown parent-grandparent combination effects were determined according to the following relationship (Hoeschele and VanRaden, 1991):

$$f_{S*DD} = \frac{1}{2}(f_{SS*DD}+f_{DS*DD}) + m_d \qquad [6]$$

where m_d is a Mendelian sampling term taken at random from a normal distribution with mean zero and variance $\frac{1}{8}\sigma_D^2$.

A cow's record at 90-days of the first lactation (27 months) was simulated as:

$$y_i = a_i + d_i^* + e_i \quad [7]$$

where a_i and e_i is the additive genetic and environmental effect of the first record of cow i, and d_i^* is the dominance effect of the first record of cow i simulated according to [4]. No further lactations were considered. Extension of part lactation records was used which enables the use of part lactation records in the genetic evaluation and consequently selection of cows at 90 days of lactation. Extended records were assumed to have a correlation of one with complete lactation records and equal variance.

Animal model evaluation

An individual animal model was used to estimate additive and dominance effects. In matrix notation, the model used to analyze the data can be written as:

$$\mathbf{y} = \mathbf{1}\mu + \mathbf{ZQg} + \mathbf{Za} + \mathbf{Zd} + \mathbf{Zf}b + \mathbf{e} \quad [8]$$

where **y** is a vector with first lactation records; **1** is a vector in which all elements equal 1; μ is the population mean; **Z** is a matrix relating records to random additive or dominance effects; **Q** is a matrix relating individuals to genetic groups; **g** is a vector with genetic group effects; **a** is a vector with additive effects; **d** is a vector with dominance effects, corrected for inbreeding depression; b is the regression coefficient on inbreeding, which equals the complete inbreeding depression; **f** is a vector with inbreeding coefficients and **e** is a vector with environmental effects.

First and approximate second moments (De Boer and Van Arendonk, 1992; De Boer and Hoeschele, 1993) of [8] are given as:

$$\begin{aligned} E(\mathbf{y}) &= \mathbf{1}\mu + \mathbf{ZQg} + \mathbf{Zf}b \\ \sigma_y^2 &= \mathbf{Z'AZ}\sigma_A^2 + \mathbf{Z'DZ}\sigma_D^2 + \mathbf{I}\sigma_E^2 \end{aligned} \quad [9]$$

where **A** is the additive genetic relationship matrix (Henderson, 1975), **D** is the dominance genetic relationship matrix ignoring inbreeding (Hoeschele and VanRaden, 1991), σ_A^2, σ_D^2 and σ_E^2 are the additive, dominance and environmental variances, respectively.

Genetic groups were used to account for genetic differences between founder individuals of different age groups (Quaas, 1988; Westell *et al.*, 1988). The individual's final estimated additive effect equalled a weighted average of contributing genetic group estimates plus its own estimated additive effect.

To obtain the inverse of the dominance relationship matrix the procedure

described by Hoeschele and VanRaden (1991) was used. In this procedure an individual's dominance effect is partitioned into a S * D combination effect and a Mendelian sampling term. The inverse of a matrix with relationships between both combination effects and individuals dominance effects is computed directly (matrix $\mathbf{D}^{*-1}$). To reduce computer requirements, interactions among ancestors more than two generations removed from an animal with data were discarded.

Cloned animals were treated as repeated observations on one genotype where clones have no environmental effects in common (Falconer, 1989, p.139).

The mixed model equations (MME) solved are:

$$\begin{bmatrix} \mathbf{1'1} & \mathbf{1'ZQ} & \mathbf{1'Z} & \mathbf{1'Z} & 0 & \mathbf{1'Zf} \\ \mathbf{Q'Z'1} & \mathbf{Q'Z'ZQ} & \mathbf{Q'Z'Z} & \mathbf{Q'Z'Z} & 0 & \mathbf{Q'Z'Zf} \\ \mathbf{Z'1} & \mathbf{Z'ZQ} & \mathbf{Z'Z+A^{-1}}\alpha & \mathbf{Z'Z} & 0 & \mathbf{Z'Zf} \\ \mathbf{Z'1} & \mathbf{Z'ZQ} & \mathbf{Z'Z} & \mathbf{Z'Z+D^{11}}\beta & \mathbf{D^{12}}\beta & \mathbf{Z'Zf} \\ 0 & 0 & 0 & \mathbf{D^{21}}\beta & \mathbf{D^{22}}\beta & 0 \\ \mathbf{f'Z'1} & \mathbf{f'Z'ZQ} & \mathbf{f'Z'Z} & \mathbf{f'Z'Z} & 0 & \mathbf{f'Z'Zf} \end{bmatrix} \begin{bmatrix} \hat{\mu} \\ \hat{\mathbf{g}} \\ \hat{\mathbf{a}} \\ \hat{\mathbf{d}} \\ \hat{\mathbf{s}} \\ \hat{\mathbf{b}} \end{bmatrix} = \begin{bmatrix} \mathbf{1'y} \\ \mathbf{Q'Z'y} \\ \mathbf{Z'y} \\ \mathbf{Z'y} \\ 0 \\ \mathbf{f'Z'y} \end{bmatrix} \quad [10]$$

where $\alpha = \sigma_E^2/\sigma_A^2$, $\beta = \sigma_E^2/\sigma_D^2$, $\mathbf{A}^{-1}$ is the inverse of the additive relationship matrix, **s** is a vector with combination effects, and

$$\mathbf{D}^{*-1} = \begin{bmatrix} \mathbf{D}_{11} & \mathbf{D}_{12} \\ \mathbf{D}_{21} & \mathbf{D}_{22} \end{bmatrix}^{-1} = \begin{bmatrix} \mathbf{D}^{11} & \mathbf{D}^{12} \\ \mathbf{D}^{21} & \mathbf{D}^{22} \end{bmatrix}$$

where $\mathbf{D}^{11}$, $\mathbf{D}^{12}$, $\mathbf{D}^{21}$ and $\mathbf{D}^{22}$ are submatrices of $\mathbf{D}^{*-1}$ with 1 and 2 pertaining to dominance and combination effects.

After transformation of MME according to Westell *et al.* (1988), MME were solved using iteration on the data (Schaeffer and Kennedy, 1986) by a Gauss-Seidel algorithm. The ratio of the sum of squared differences in solutions between iterations over the sum of squares of the most recent solutions was used as a convergence criterion, as suggested by Schaeffer and Kennedy (1986). Solutions were considered stable when this convergence criterion was less than 10^{-6}.

Bulls and cows were selected for breeding (i.e. genetic selection) on their estimated additive genetic effect (â) at either 15 or 27 months of age. Bulls and 15-month old cows were evaluated on ancestors, full and half sib information when available. Although estimated additive genetic effects were identical for full sib males and 15-month old females, no restriction on full sib selection was applied.

Selection of, e.g., all males in a full sib family in combination with a higher number of sires results in a higher genetic responses at a similar level of inbreeding than selection of only one male full sib per family (Meuwissen, 1990; Ruane, 1991).

Female genotypes eligible for commercial cloning were selected on their estimated total genetic effect at 27-months of age. From [4] and [10], the estimated total genetic effect of cow i was computed as $\hat{a}_i + \hat{d}_i + \hat{b}F_i$.

Parameters

The simulated closed nucleus herd is characterised by the parameters given in Table 1. In each replicate, the genetic and clonal response and their underlying components were determined.

Additive genetic response to selection in generation t was computed as the difference between the average simulated additive genetic effect of individuals born in generation t+1 and t. Clonal response in generation t was determined as the difference between the average total genetic value of female genotypes selected for commercial cloning and the average total genetic value of all females eligible for clonal selection in generation t. Both genetic and clonal response decline each year as a consequence of additive genetic variance reduction due to inbreeding. For comparison of alternative designs, genetic response was cumulated, while clonal response was averaged over the simulated period. Average rather than cumulated clonal response was computed because clonal response does not accumulate over generations but reflects a selection differential.

Analysis of variance (Searle, 1971, Chapter 6 and 7) was used to compute (co)variances of true and estimated additive and total genetic effects. These (co)variances were used to determine underlying components of response, such as sire and dam selection accuracies and intensities, and the standard deviation of the breeding goal.

Average inbreeding coefficient was determined for cows and bulls selected for breeding, female genotypes selected for commercial cloning, and newborn offspring. Simulations were run for several replicates (see Table 1) and statistics were averaged over replicates.

Validation of simulation

The simulation program was validated by comparing simulated genetic and clonal responses with deterministic predictions for all designs considered in a previously studied closed adult nucleus (De Boer and Van Arendonk, 1991). Parameters characterizing this scheme are given in Table 1.

Table 1. Parameters characterising the closed nucleus herd

Parameter definition		Model validation[a]	Optimization[b]
h^2	heritability	0.25	0.30
d^2	% variance due to dominance	0-0.25	0-0.20
t_c	intra-clone correlation	h^2+d^2	h^2+d^2
T	# cows tested each year	256	1024
year	# years simulated	30	30
N_s	# sires per period	32	N_s
N_d	# dams per period	32	N_d
M_d	# matings per dam per period	1...8	M_d
M_s	# matings per sire per period	1...8	M_s
N_{fs}	# female full sibs per family	$T/(32 \cdot M_d)$	$T/(N_d \cdot M_d)$
K	# clones tested per genotype	$T/(32 \cdot M_d \cdot N_{fs})$	$T/(N_d \cdot M_d \cdot N_{fs})$
Number of replicates computed		350	100
Selected founder population		no	yes
Initial period without cloning		no	yes
Restriction on full sib selection		yes	no

[a] Parameters used to validate the stochastic simulation model
[b] Parameters used in optimizing a design with cloning

The deterministic model was changed to account for the effect of inbreeding on the additive genetic variance. The correct level of inbreeding could not be predicted deterministically and was therefore taken from stochastic simulation. As in the stochastic model, deterministic prediction ignored the effect of inbreeding on dominance related (co)variances. In addition, inbreeding depression was not simulated.

The additive genetic variance in generation t ($\sigma_A^{2(t)}$) used in deterministic prediction was adjusted for inbreeding as (formula [A13] in appendix A):

$$\sigma_A^{2(t)} = \frac{1}{4}(1 - k_s r_{IHs}^{2(t-1)})\sigma_A^{2(t-1)} + \frac{1}{4}(1 - k_d r_{IHd}^{2(t-1)})\sigma_A^{2(t-1)} + \frac{1}{2}(1 - F_{t-1})\sigma_A^{2(t=0)} \qquad [11]$$

where $r_{IHs}^{2\,(t-1)}$ and $r_{IHd}^{2\,(t-1)}$ are accuracies of sire and dam selection in generation t-1, respectively; $k_x = i_x(i_x - x_x)$ with i_x as the infinite selection intensity of sires (x=s) or dams (x=d) and x_x as the corresponding truncation point. The average inbreeding level in generation t-1 is denoted as F_{t-1}.

Selection intensities used in deterministic prediction were adjusted for population size and structure following Burrows (1972) and Rawlings (1976), respectively. Accuracies of selection were computed using selection index theory. Bulls were selected on records of their dam, full sibs, maternal and paternal half

sibs whereas cows were in addition selected on their own 90-days lactation record, each genotype having K clones.

Results

Validation of simulation

Genetic response. Stochastically obtained genetic response to one round of selection (cA_1) is given in Table 2 for different designs in the absence of dominance. In addition, Table 2 shows differences in cA_1 and its underlying components between stochastic and deterministic simulation. Stochastic predictions of cA_1 were always within 2% of deterministic predictions. Predictions of underlying components of stochastically obtained cA_1 were very accurate when compared to deterministic predictions. Absolute differences in male and female selection intensities, and the additive genetic standard deviation between both simulation approaches were always less than 1%. Stochastic simulation, however, resulted in slightly lower accuracies of dam and especially sire selection than deterministic simulation. This was due to fitting a fixed effect (formula [8]) in stochastic simulation, whereas selection index theory used in deterministic prediction assumes fixed effects to be known (Henderson, 1973).

Stochastically obtained cumulative genetic response to 1 (cA_1) or 10 (cA_{10}) rounds of selection and its difference relative to deterministic prediction is given in Table 3 for various designs when $h^2=t_c=0.25$ or $h^2=0.25$ and $t_c=0.50$. In most cases, stochastic prediction of cA_1 and cA_{10} was within 2% of deterministic prediction. Generally, stochastically computed cA_{10} was higher than the deterministic result. This was because unlike deterministic simulation, stochastic simulation used information on all ancestors to determine selection accuracies. As a result, stochastic selection accuracy was higher than the deterministic accuracy in second and later generations.

Clonal response. Stochastically obtained clonal response to selection of 1 (C_1) or 10 (C_{10}) female genotypes is given in Table 4, for various designs when $h^2=t_c=0.25$ or $h^2=0.25$ and $t_c=0.50$. In the absence of dominance ($t_c=h^2=0.25$), the difference in C_1 and C_{10} between both simulation strategies varied from -7.4% to 2.7%, and from -2.4% to 1.5%, respectively. Largest difference in both C_1 and C_{10} was observed with a maximum number of full sibs ($N_{fs}=8$) and only one identical individual tested per genotype ($K=1$). These large differences were due to overprediction of the intensity of clonal selection. As pointed out by Meuwissen (1991), this overprediction is largest with a high correlation between selection criteria of eligible candidates and intense selection. Both phenomena are maximal when $N_{fs}=8$ and $K=1$.

Table 2. Stochastically obtained genetic response to one round of selection denoted as cA_1 (prediction error between brackets), and the difference in cA_1 and its components (i_x=selection intensity; R_{IHx}=selection accuracy of females (x=f) and males (x=m); σ_a=additive genetic standard deviation) relative to deterministic prediction (%) for various designs[a] with $h^2=t_c=0.25$

Design				Differences (%) relative to deterministic prediction					
M_d	N_{fs}	K	cA_1	cA_1	i_f	R_{IHf}	i_m	R_{IHm}	σ_a
1	1	8	0.000 (0.002)	0.0	0.0	-0.2	0.0	-2.0	-0.5
1	2	4	0.155 (0.003)	0.7	0.7	-0.2	0.0	-2.1	0.5
1	4	2	0.218 (0.003)	-1.5	0.4	-1.2	0.0	-2.2	-0.1
1	8	1	0.255 (0.003)	-1.7	-0.8	-0.6	0.0	-1.1	0.2
2	1	4	0.250 (0.003)	0.0	0.3	-0.4	0.5	-1.7	0.1
2	2	2	0.311 (0.003)	-0.6	0.8	-0.9	0.9	-1.5	0.0
2	4	1	0.346 (0.004)	0.1	-0.5	-0.7	0.9	-1.3	0.2
4	1	2	0.363 (0.004)	-0.3	0.0	-0.5	0.6	-0.8	0.5
4	2	1	0.396 (0.004)	0.8	0.3	-0.4	0.6	-1.3	0.1
8	1	1	0.431 (0.004)	-0.1	0.1	-0.4	0.0	-0.7	0.1

[a] M_d is the number of matings per dam; N_{fs} is the number of female full sibs; and K is the number of clones tested per genotype

Table 3. Stochastically obtained cumulative genetic response to 1 (cA_1) or 10 (cA_{10}) rounds of selection, and its difference relative to deterministic prediction (diff in %) for varying designs[a] with $h^2=0.25$ and $t_c=0.25$ or 0.50

Design			$t_c=0.25=h^2$				$h^2=0.25$ $t_c=0.50$			
M_d	N_{fs}	K	cA_1	Diff	cA_{10}	Diff	cA_1	Diff	cA_{10}	Diff
1	1	8	0.000	0.0	0.008	0.8	0.000	0.0	-0.002	0.8
1	2	4	0.155	0.7	1.378	1.2	0.131	0.1	1.185	1.2
1	4	2	0.218	-1.5	1.929	1.0	0.197	-1.1	1.752	1.0
1	8	1	0.255	-1.7	2.196	-0.7	0.241	-0.8	2.088	-0.7
2	1	4	0.250	0.0	2.021	1.6	0.211	-2.7	1.778	1.6
2	2	2	0.311	-0.6	2.513	0.8	0.290	0.4	2.338	0.8
2	4	1	0.346	0.1	2.763	0.3	0.331	0.8	2.678	0.3
4	1	2	0.363	-0.3	2.843	1.4	0.340	0.0	2.691	1.4
4	2	1	0.396	0.8	3.070	0.7	0.389	0.5	3.032	0.7
8	1	1	0.431	-0.1	3.311	0.8	0.431	-0.1	3.274	0.8

[a] M_d is the number of matings per dam, N_{fs} is the number of female full sibs, and K is the number of clones tested per genotype

Table 4. Stochastically obtained response to selection of 1 (C_1) or 10 (C_{10}) female genotypes for commercial cloning averaged over the last 17 simulation years, and its difference relative to deterministic prediction for various designs[a] with h^2=0.25 and t_c=0.25 or 0.50

Design			t_c=0.25				t_c=0.50			
M_d	N_{fs}	K	C_1	Diff	C_{10}	Diff	C_1	Diff	C_{10}	Diff
1	1	8	0.877	0.5	0.474	0.6	1.380	1.7	0.732	-0.2
1	2	4	0.810	1.2	0.528	1.5	1.431	1.1	0.918	-0.3
1	4	2	0.766	-1.5	0.565	1.2	1.425	-0.9	1.026	-0.6
1	8	1	0.700	-7.4	0.565	-2.4	1.390	-0.9	1.062	-1.0
2	1	4	0.791	2.7	0.507	1.3	1.406	0.5	0.901	-0.9
2	2	2	0.735	0.1	0.529	0.2	1.418	0.6	1.006	-0.6
2	4	1	0.672	-3.7	0.527	-1.2	1.337	-1.5	1.028	-0.8
4	1	2	0.722	0.3	0.520	0.5	1.410	0.9	1.000	-0.3
4	2	1	0.660	-2.4	0.514	-0.6	1.324	-0.5	1.014	-0.2
8	1	1	0.655	-1.3	0.506	-0.3	1.311	0.1	0.993	-0.9

[a] M_d is the number of matings per dam; N_{fs} is the number of female full sibs; and K is the number of clones per genotype

Testing more maternal half sibs instead of full sibs or testing more clones per genotype decreased the correlation between selection criteria of female genotypes eligible for commercial cloning. As a consequence, the difference in intensity of clonal selection between both approaches decreased, which decreased the observed difference in C_n. Dominance also reduced the correlation between selection criteria of eligible genotypes. Therefore, the difference in C_1 or C_{10} between both simulation approaches was smaller with t_c=0.50 than with t_c=0.25, and varied from -1.7% to 1.7% and from -1% to -.2%, respectively.

Illustration of results in the absence of dominance

In the absence of dominance a design in which N_s=32, N_d=16, M_s=16, M_d=32, N_{fs}=2 and K=1 was simulated in the initial five years in all alternatives studied. For illustration, detailed results of two extreme designs in the second simulation period of 20 years are presented. The first design was equal to that simulated in the initial five years (N_s=32, N_d=16, M_s=16, M_d=32, N_{fs}=2 and K=1). In the second design eight clones were tested at the expense of dam families (N_s=32, N_d=2, M_s=2, M_d=32, N_{fs}=2 and K=8).

The additive genetic level of nucleus embryos and the best female genotype selected for commercial cloning are given in Figure 1, for both designs. Cloning of embryos was first considered in year six. As a consequence, tested female genotypes for commercial cloning were not available until year nine.

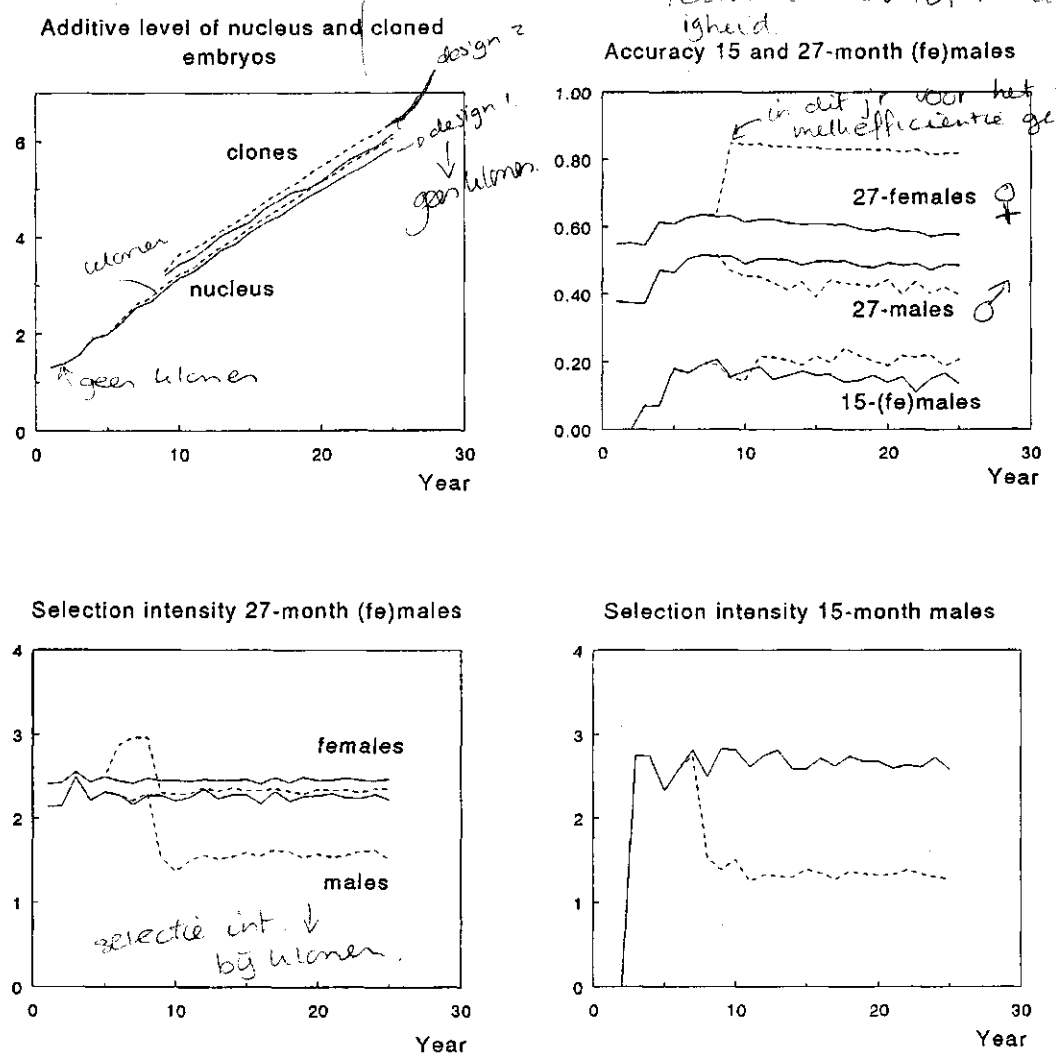

Figure 1. Additive genetic level of nucleus embryos and tested cloned embryos, and (fe)male selection accuracies and intensities for two designs: (i) solid line: no cloning and (ii) dashed line: cloning at the expense of dam families

Figure 1 shows that the average additive genetic level of cloned embryos available in year nine is higher than the average additive genetic level of nucleus embryos. The annual change in additive genetic level of cloned embryos is, however, identical to the annual change in additive genetic level of nucleus embryos. This

illustrates the importance of the annual additive genetic response for the long term total additive genetic level of clones. Both genetic and clonal response were higher with eight than with one clone(s) tested per genotype.

To explain observed genetic and clonal response, underlying selection intensities and accuracies are given in Figure 1. Analysis of variance was used to compute (co)variances required to determine these underlying components. This technique is theoretically correct in the first generation, while it is an approximation in later generations.

Testing clones increased selection accuracy of both 15-month old individuals and 27-month old females. Selection accuracy of 27-month old females increased in year nine, which was the first year in which clones were tested for milk efficiency. From year 11, the majority of 15-month old individuals derived from dams evaluated on clonal information, which explains the observed increase in selection accuracy of 15-month old individuals. Selection accuracy of 27-month old males decreased slightly as more clones and less genotypes were tested. Records on two genetically identical female full sibs contribute less information for breeding value estimation of their male full sib than records on two different female full sibs.

Testing clones decreased the number of 15-month old and 27-month old genotypes of each sex from 1024 to 128 in year eight and nine, respectively. As a result, selecting the same number of sires reduced selection intensity for 15-month and 27-month old males in year eight and nine, respectively (Figure 1). The majority of dams (>90%) was selected at 27 months of age, and therefore only selection intensity of 27-month old dams could be computed accurately. The selection intensity of 27-month old dams increased temporarily in year six, seven and eight, because two instead of 16 dams were selected out of 1024 genotypes. Whereas in subsequent generations, two dams were selected out of 128 genotypes. Due to the smaller population size, selection intensity was lower with selection of two out of 128 genotypes than with 16 out of 1024 genotypes.

Testing clones increased 15-month old male accuracy, while 27-month old male accuracy decreased (Figure 1). Generation interval for males decreased from 22.9 to 19.3 months. Generation interval for females increased from 26.2 to 26.8 months, because cloning increased 27-month old female selection accuracy relatively more than 15-month old female selection accuracy.

The cumulative genetic response (cA) equalled 5.852 in the absence of cloning, while the final level of inbreeding (F) was 0.333. Testing clones, however, increased cA to 6.062. The increase in especially female selection accuracy did offset the decrease in male selection intensity. The use of fewer dams with cloning, however, hardly affected the final level of inbreeding (F=0.335). Testing clones reduced the correlation between estimated additive genetic effects of selection candidates and

increased generation interval for females slightly. Both effects reduced inbreeding and did offset the use of fewer dams.

Responses to clonal selection of one female genotype were averaged over the last 17 simulation years and equalled 0.839 and 0.922 without and with cloning, respectively. Clonal response was defined as the difference between the average total genetic value of the female genotype selected for commercial cloning and all females eligible for clonal selection. In Figure 1, the additive genetic level of nucleus embryos rather than 27-month old females eligible for commercial cloning is given. Consequently, the difference in additive genetic level of nucleus and cloned embryos equals clonal responses presented minus three times the annual additive genetic response.

Genetic and clonal response in the absence of dominance

Genetic response. Table 5 shows the cumulative genetic response (cA), the clonal response to selection of n genotypes (C_n) and the final level of inbreeding (F) for different designs studied in the absence of dominance. In the initial five simulation years of each alternative $N_s=32$, $N_d=16$, $M_s=16$, $M_d=32$, $N_{fs}=2$ and $K=1$. This reference design maximized cA corrected for variance reduction due to inbreeding in the absence of cloning (De Boer and Van Arendonk, 1994). In the second 20 simulation years, clones were tested at the expense of the number of sires or dams, matings per dam (sire) or full sibs per family.

Testing clones at the expense of matings per dam (M_d) or female full sibs per family (N_{fs}) reduced cA and decreased F. The former is in line with earlier results (De Boer and Van Arendonk, 1991). For a given number of cows tested each year, and a fixed number of sires and dams, testing clones reduced sire and dam selection intensity relatively more than it increased accuracy of female selection.

Testing clones at the expense of sire families (N_s) decreased cA and increased F. In this case, testing clones hardly affected sire selection intensity, while it reduced dam selection intensity. Dam selection intensity reduced because the same number of dams were selected out of fewer genotypes. The increase in selection accuracy for females did not offset losses in dam selection intensity, male selection accuracy, and increased inbreeding.

Testing clones at the expense of dam families (N_d) increased cA slightly. The increase in selection accuracy for females did offset the decrease in selection accuracy and intensity for males. The final level of inbreeding was hardly affected. As pointed out earlier, cloning reduced the correlation between estimated additive effects of female selection candidates and increased the generation interval for females. Both effects reduced inbreeding and did offset use of fewer dams.

Table 5. Cumulative genetic response (cA), response to clonal selection of n genotypes (C_n) and final inbreeding level (F) of embryos (empirical standard errors between brackets) obtained in various designs considered in the second 20 simulation years without dominance. Design simulated in initial five years: N_s=32, N_d=16, M_s=16, M_d=32, N_{fs}=2 and K=1

Design[a]						Response			
N_s	N_d	M_s	M_d	N_{fs}	K	cA	C_1	C_{10}	F
32	16	16	32	2	1	5.852 (.027)	0.839 (.044)	0.703 (.021)	0.333 (.009)
		Cloning at N_d							
32	8	8	32	2	2	5.938 (.023)	0.892 (.047)	0.720 (.020)	0.317 (.009)
32	4	4	32	2	4	6.015 (.025)	0.928 (.038)	0.717 (.017)	0.326 (.008)
32	2	2	32	2	8	6.062 (.034)	0.922 (.039)	0.690 (.015)	0.335 (.007)
		Cloning at N_s							
16	16	16	16	2	2	5.852 (.028)	0.873 (.050)	0.717 (.021)	0.355 (.009)
8	16	16	8	2	4	5.729 (.030)	0.899 (.044)	0.707 (.020)	0.412 (.009)
4	16	16	4	2	8	5.587 (.031)	0.893 (.039)	0.669 (.019)	0.468 (.010)
		Cloning at M_d							
32	16	8	16	2	2	5.776 (.022)	0.902 (.043)	0.736 (.022)	0.277 (.008)
32	16	4	8	2	4	5.648 (.021)	0.957 (.042)	0.751 (.019)	0.252 (.006)
32	16	2	4	2	8	5.445 (.022)	1.011 (.041)	0.749 (.015)	0.254 (.007)
		Cloning at N_{fs}							
32	16	16	32	1	2	5.786 (.026)	0.898 (.051)	0.731 (.020)	0.283 (.007)

[a] N_s (N_d) is the number of sires (dams), M_d (M_s) is the number of matings per dam (sire), N_{fs} is the number of female full sibs and K is the number of clones tested per genotype

Testing clones at the expense of sire families, however, reduced the average correlation between male selection criteria only slightly and decreased the generation interval for males. Consequently, testing clones at the expense of sire families increased the final level of inbreeding.

Clonal response. As expected, testing more than one genetically identical individual per genotype increased C_n. Maximal C_1 was obtained when eight clones were tested at the expense of M_d. The total genetic level of cloned embryos after 20 years, which is proportional to the sum of cA and C_n, was however highest when eight clones were tested at the expense of N_d.

Table 5 shows that selection of 10 instead of 1 commercial clone line reduced clonal response. This reduction in response is due to a reduction in intensity of clonal selection. In addition, selection of 10 instead of 1 commercial clone line affected the optimal design. The optimal number of clones tested per genotype

decreased when more commercial clone lines are needed. Maximal C_n was obtained with eight or with four clones tested per genotype for C_1 and C_{10}, respectively. This is in agreement with earlier deterministic results (De Boer and Van Arendonk, 1991). The total genetic level of commercially available cloned embryos after 20 years of selection was highest when testing eight clones at the expense of N_d. The difference in total genetic level of selected cloned embryos when testing four or eight clones at the expense of N_d was, however, very small. Testing eight clones per genotype might be preferable due to the higher accuracy of clonal selection (0.83 vs 0.75). On the other hand, variation of cA was considerable higher with eight than with four clones tested per genotype.

In conclusion, testing clones at the expense of dam families resulted in maximal cA and the highest final genetic level of commercially available cloned embryos. Therefore, in the absence of dominance, reliable commercial clone lines can be produced now and in future generations by testing clones at the expense of dam families.

Genetic and clonal response with dominance

Similarly as without dominance, the reference design simulated in the initial phase of all alternatives with dominance maximized cA corrected for variance reduction due to inbreeding and inbreeding depression. As determined by De Boer and Van Arendonk (1994), this design is characterized as $N_s=256$, $N_d=32$, $M_s=4$, $M_d=32$, $N_{fs}=1$ and $K=1$. Limited by computer requirements, only testing clones at the expense of dam families was studied. Inbreeding depression was assumed equal to 0 or 1% of the mean per 1% inbreeding, respectively. Results are given in Table 6.

Genetic response. Testing two clones per genotype did not affect cA, while testing four clones per genotype decreased cA. As expected, cA was unaffected by the assumed level of inbreeding depression. The final level of inbreeding increased slightly as more clones were tested at the expense of dam families.

Clonal response. Clonal response was higher with than in the absence of dominance variance (Table 6 vs Table 5). The higher response was mainly due to exploitation of dominance variance. In addition, clonal response was higher with than without presence of inbreeding depression. The latter can be explained as follows. Female genotypes eligible for commercial cloning were selected at 27 months of age on their total estimated genetic effect. From [4] and [10], the estimated total genetic effect of cow i was computed as $\hat{a}_i+\hat{d}_i+\hat{b}F_i$. If $\hat{b}$ is non-zero, inbreeding depression will decrease the expected mean of total genetic effects of all genotypes eligible for commercial cloning.

Table 6. Cumulative additive response (cA), response to clonal selecting the best genotype (C_1), the final genetic level of the best genotype for commercial cloning (Clonal level), and the final level of inbreeding (F) of embryos (empirical standard errors between brackets) obtained in various designs considered in the second phase of the simulation assuming $d^2=0.20$ and inbreeding depression of 0 or 1% of the mean per 1% inbreeding. Design simulated in initial five years: $N_s=256$, $N_d=32$, $M_s=4$, $M_d=32$, $N_{fs}=1$ and $K=1$.

Design[a]						No inbreeding depression			
N_s	N_d	M_s	M_d	N_{fs}	K	cA	C_1	Clonal Level	F
256	32	4	32	1	1	4.879 (.016)	1.529 (.065)	5.904 (.050)	0.092 (.002)
256	16	2	32	1	2	4.866 (.016)	1.680 (.056)	6.032 (.045)	0.098 (.003)
256	8	1	32	1	4	4.737 (.017)	1.742 (.066)	6.021 (.042)	0.109 (.002)
						Depression 1% of the mean per 1% inbreeding			
256	32	4	32	1	1	4.894 (.016)	1.685 (.069)	5.364 (.048)	0.091 (.002)
256	16	2	32	1	2	4.877 (.016)	1.788 (.068)	5.425 (.045)	0.097 (.002)
256	8	1	32	1	4	4.736 (.017)	1.862 (.069)	5.318 (.037)	0.110 (.002)

[a] N_s (N_d) is the number of sires (dams), M_d (M_s) is the number of matings per dam (sire), N_{fs} is the number of female full sibs and K is the number of clones tested per genotype

Results show that when inbreeding depression (b) is nonzero, average F of genotypes selected for commercial cloning equalled only 69% to 71% of average F of all eligible candidates. Therefore, genetic superiority of selected genotypes was higher with inbreeding depression due to exploitation of differences in the expectation of an individual's total genetic effect.

In conclusion, due to the definition of clonal response, a decrease in the expectation of an individual's total genetic effect does increase clonal response, whereas it will decrease the expected final genetic level of genotypes selected for commercial cloning (Table 6). Therefore, with inbreeding depression the final genetic level of cloned embryos is not proportional to cA and C_n. The final genetic level of commercially available cloned embryos is, in addition to the genetic and clonal response, given in Table 6. Table 6 shows that the final total genetic level of cloned embryos was highest when two clones were tested per genotype and inbreeding depression was absent.

Discussion

Comparison of stochastic versus deterministic results

The stochastic simulation model was validated by comparing obtained results with deterministic predictions for a previously studied adult nucleus scheme (De Boer and Van Arendonk, 1991). Differences in cumulative additive genetic response

(cA) between both simulation approaches were always less than 2%, while differences in clonal response varied from 0.1% to 7.4%.

Keller *et al.* (1990) compared stochastically obtained cA versus deterministic prediction in a hierarchical adult nucleus, in the absence of cloning and dominance. Differences in cA after ten generations of selection between both simulation approaches were generally within $\pm 1\%$. In the present study, the difference in cA between both simulation approaches equalled -0.7% for this particular situation. Keller *et al.* (1990), however, used a slightly different formula for deterministic prediction of the additive genetic variance (formula [A9] in appendix A). Unlike the formula used in the present study (formula [11]), formula [A9] accounts for the effect of selecting a finite number of sires and dams (Appendix A). In computation of genetic response, however, the effect of selecting a finite number of sires and dams is generally taken into account by correcting the selection intensity and was therefore omitted in [11] (Meuwissen, 1991). The difference between [A9] and [11] is, however, small when the number of sires and dams is large as was the case in their study. This is, however, not true in general.

Ruane (1989) used formula [A12] for deterministic prediction of cA in a hierarchical adult nucleus, in the absence of dominance and cloning. The difference in cA after six generations of selection between both simulation approaches was at maximum 4%. Use of formula [A12] in the present study also resulted in larger differences in cA after ten generations of selection between both simulation approaches, varying from +0.8 to +5.1%. Especially with more intense selection and higher inbreeding, formula [A12] underpredicted genetic response. In the absence of selection, formula [A12] and [11] can shown to be equivalent (appendix A), whereas with selection both formulas can give different results.

Comparison of genetic and clonal response in alternative designs

In the present paper, dominance variance of milk efficiency was considered either absent or assumed to explain 20% of the phenotypic variance (d^2). To date, dominance variance estimates for milk production traits in dairy cattle are limited. VanRaden (1989) estimated d^2 for milk and fat yield using an sire-maternal grandsire model and a tilde hat approach to restricted maximum likelihood (REML). For milk and fat yield $\hat{d}^2$ were small and equalled 0.04 and 0.03, respectively. Standard errors on $\hat{d}^2$ were not reported. Using the same procedure Fürst and Sölkner (1994) estimated d^2 and the relevance of additive by additive variance (a^2) for milk yield, fat and protein percentage, using first, second and third lactations records of three different populations. Only results of the purebred population are presented here. For milk yield, fat and protein percentage, $\hat{d}^2$ varied from 0.04 to 0.09, from 0.02 to 0.06 and from 0.06 to 0.11, respectively. Corresponding standard

errors of prediction varied from 0.06 to 0.08, from 0.10 to 0.12 and from 0.11 to 0.14. For milk yield, $\hat{a}^2$ varied from 0.04 to 0.15 (0.08-0.11), while for fat and protein percentage $\hat{a}^2$ was negligible.

Tempelman and Burnside (1991) estimated d^2 for milk and fat yield in four subsets of data using an animal model and REML. For milk yield, $\hat{d}^2$ ranged from 0.08 to 0.22, and was significant in only one subset. For fat yield, $\hat{d}^2$ varied from 0.08 to 0.49 and was significant in three subsets. Approximate standard errors for $\hat{d}^2$ varied from 0.08 to 0.19 for milk yield, and from 0.15 to 0.20 for fat yield.

Obtained standard errors on estimated variances show that datasets used contained a relatively small number of dominance (Tempelman and Burnside, 1991; Fürst and Sölkner, 1994) and additive by additive relationships (Fürst and Sölkner, 1994). In addition, in the data set of Tempelman and Burnside (1991), dominance variance estimates could be biased because records of cows born after embryo transfer were used.

Using the same procedure, Wei and Van der Werf (1993) found a dominance variance for egg number in poultry of, on average, 0.15. The approximate standard error on $\hat{d}^2$ was, however, considerably smaller (between 0.04 and 0.06) due to more dominance relationships in poultry data.

Hence, the d^2 of 0.20 used in this study might be seen as an upper limit. Lowering d^2 will increase the optimal number of clones tested per genotype and reduce total genetic level of commercially available cloned embryos.

An approximate infinitesimal model with both additive and dominant gene action was used to stochastically simulate genetic and clonal response for various designs. The model accounted for the effect of inbreeding on the mean and the additive genetic variance. The effect of inbreeding on dominance related (co)variances was ignored (De Boer and Hoeschele, 1993), such as the arising of a covariance between additive and dominance effects (σ_{ADI}) with inbreeding and the change of dominance variance with inbreeding. The influence of ignoring these changes in (co)variances on obtained clonal response depends on allelic frequency of all loci affecting the trait. For example, assume that a trait is affected by a large number of biallelic loci with an equal effect and the same frequency for the favourable allele (p). When p=0.5, σ_{ADI} is zero, and as a result ignoring this covariance does not affect clonal response. With extreme allelic frequencies, σ_{ADI} is large (De Boer and Hoeschele, 1993; Figure 2). When p=0.20, σ_{ADI} is negative and selection on estimated additive effects will reduce the total genetic variance. Ignoring σ_{ADI} will then result in overprediction of clonal response. When p=0.80, however, σ_{ADI} is positive. As a result, ignoring σ_{ADI} will result in underprediction of clonal response.

Methods to increase additive genetic response while maintaining inbreeding at

a constant rate have been suggested, e.g. changing the BLUP selection criterion before selection (Dempfle, 1990; Goddard and Smith, 1990; Woolliams and Meuwissen, 1993). In the absence of dominance, cloning at the expense of dam families increased cA slightly without increasing F. For example, the scheme which maximized cA in the absence of cloning (5.852) is $N_s=32$, $N_d=16$, $M_d=32$, $M_s=16$, $N_{fs}=2$ and $K=1$ (De Boer and van Arendonk, 1994). Testing two clones ($K=2$) at the expense of dam families ($N_d=8$) increased cA to 5.938 (0.023), while F decreased slightly from 0.333 to 0.317. Selection of 8 dams and testing four female full sibs ($N_{fs}=4$), however, resulted in cA of 5.850 (0.032), while F increased to 0.421. This is consistent with results from Colleau (1992) in mixed MOET schemes.

In the absence of dominance, cloning at the expense of dam families not only added to cA but also maximized total genetic level of commercially available cloned embryos after 20 years of selection. In the optimal design, eight clones were tested per genotype and accuracy of clonal selection was 0.83. Therefore, in the absence of dominance reliable commercial clone lines can be produced now and in future generations by testing clones at the expense of dam families. The genetic level of commercially available cloned embryos will be higher than the average genetic level of all nucleus embryos (Figure 1). The genetic level of the embryos from the best sire-dam combination might, however, be higher than the level of the best available cloned embryos. Cloned embryos are obtained from 27-month old female genotypes tested for milk efficiency, while the additive level of nucleus embryos increased three times the annual additive response during this testing period. Accuracy of clonal selection is, however, considerably higher (0.83) than selection accuracy of available nucleus embryos (0.21). Unlike the lower selection accuracy of untested nucleus embryos, it might be interesting for a breeding organisation to sell *in vitro* produced embryos from the best available nucleus cow-sire combination, instead of cloned embryos from the best available female genotype.

With dominance, testing clones at the expense of dam families decreased genetic response slightly, whereas it increased clonal response considerably. The final genetic level of commercially available cloned embryos after 20 years of selection was highest when two clones were tested per genotype (accuracy of clonal selection of 0.72) and inbreeding depression was absent. The cumulative genetic response equalled 99% of its maximum in this situation. Therefore, also with dominance, reliable commercial clone lines can be produced now and in future generations by testing clones at the expense of dam families. Dominance can be exploited efficiently in producing tested cloned embryos for commercial use. Consequently, with dominance the genetic level of cloned embryos will be higher than that of untested embryos from the best sire-dam combination. As a result, selling cloned embryos instead of *in vitro* produced embryos would be more

profitable for a breeding organisation when dominance variance is high.

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Appendix A

Derivation of between family variance corrected for inbreeding and selection

Situation with random selection (Goddard, 1992, pers. comm.). Consider families of large and equal size prior to selection. The true additive genetic value of individual i, a_i, can be written as $\frac{1}{2}(a_s+a_d)+m_a$, where a_s and a_d is the additive genetic value of its sire and dam and m_a is Mendelian sampling. The additive family mean then equals: $\frac{1}{2}(a_s+a_d)$, while the generation mean can be given as $\frac{1}{2}(\bar{a}_s+\bar{a}_d)$. The between family variance ($\sigma^{2\,(t)}_{Ab}$) in generation t is a function of (co)variances in generation t-1:

$$\begin{aligned}\sigma^{2(t)}_{Ab} &= V(\tfrac{1}{2}(a_s+a_d)-\tfrac{1}{2}(\bar{a}_s+\bar{a}_d))\\ &= \tfrac{1}{4}V(a_s)+\tfrac{1}{4}V(a_d)+\tfrac{1}{2}Cov(a_s,a_d)-\tfrac{1}{2}Cov(a_s,\bar{a}_s)-\tfrac{1}{2}Cov(a_s,\bar{a}_d)\\ &- \tfrac{1}{2}Cov(a_d,\bar{a}_s)-\tfrac{1}{2}Cov(a_d,\bar{a}_d)+\tfrac{1}{4}V(\bar{a}_s)+\tfrac{1}{4}V(\bar{a}_d)+\tfrac{1}{2}Cov(\bar{a}_s,\bar{a}_d)\end{aligned} \qquad [A1]$$

Required (co)variances of generation t-1 can be given as:

$$\begin{aligned}V(a_s) &= V(a_d) = (1+F_{t-1})\,\sigma^{2(t=0)}_A\\ Cov(a_s,a_d) &= Cov(a_s,a_s') = Cov(a_d,a_d') = 2F_t\sigma^{2(t=0)}_A\\ Cov(a_s,\bar{a}_s) &= V(\bar{a}_s) = \tfrac{1}{S}(1+F_{t-1}+2(S-1)F_t)\,\sigma^{2(t=0)}_A\\ Cov(a_d,\bar{a}_d) &= V(\bar{a}_d) = \tfrac{1}{D}(1+F_{t-1}+2(D-1)F_t)\,\sigma^{2(t=0)}_A\\ Cov(\bar{a}_s,\bar{a}_d) &= 2F_t\sigma^{2(t=0)}_A\end{aligned}$$

where F_t is the average inbreeding level in generation t which equals half the average additive genetic relation in generation t-1, S and D are the number of sires and dams, respectively and $\sigma^{2(t=0)}_A$ is the additive variance in the unselected base population. Substituting these (co)variances into [A1] gives:

$$\sigma^{2(t)}_{Ab} = \tfrac{1}{2}(1-\tfrac{1}{2S}-\tfrac{1}{2D})(1+F_{t-1}-2F_t)\,\sigma^{(t=o)}_A \qquad [A2]$$

Subsequently, the within family variance in generation t ($\sigma^{2\,(t)}_{Aw}$) can be written as:

$$\sigma^{2(t)}_{Aw} = \tfrac{1}{2}(1-F_{t-1})\,\sigma^{(t=0)}_A \qquad [A3]$$

The total variance in generation t ($\sigma^{2(t)}_A$) then equals:

$$\sigma_A^{2(t)} = \frac{1}{2}(1-\frac{1}{2S}-\frac{1}{2D})(1+F_{t-1}-2F_t)\sigma_A^{2(t=o)} + \frac{1}{2}(1-F_{t-1})\sigma_A^{2(t=0)} \qquad [A4]$$

Formula [A4] can be shown to be equivalent to formulas given by Keightley and Hill (1987), Keller *et al.* (1990) and Verrier *et al.* (1991), by taking (co)variances in generation t around the mean of generation t-1 instead of the base population. Taking (co)variances of generation t around the mean of generation t-1 results in:

$$\begin{aligned}
V(a_s) &= V(a_d) = \sigma_A^{2(t-1)} \\
Cov(a_s, a_d) &= Cov(a_s, a_s') = Cov(a_d, a_d') = 0 \\
Cov(a_s, \overline{a_s}) &= V(\overline{a_s}) = \frac{1}{S}\sigma_A^{2(t-1)} \\
Cov(a_d, \overline{a_d}) &= V(\overline{a_d}) = \frac{1}{D}\sigma_A^{2(t-1)} \\
Cov(\overline{a_s}, \overline{a_d}) &= 0
\end{aligned}$$

Substituting these (co)variances into [A1] gives:

$$\sigma_{Ab}^{2(t)} = \frac{1}{2}(1-\frac{1}{2S}-\frac{1}{2D})\sigma_A^{2(t-1)} = \frac{1}{4}(1-\frac{1}{S})\sigma_A^{2(t-1)} + \frac{1}{4}(1-\frac{1}{D})\sigma_A^{2(t-1)} \qquad [A5]$$

and the total additive genetic variance in generation t equals:

$$\sigma_A^{2(t)} = \frac{1}{4}(1-\frac{1}{S})\sigma_A^{2(t-1)} + \frac{1}{4}(1-\frac{1}{D})\sigma_A^{2(t-1)} + \frac{1}{2}(1-F_{t-1})\sigma_A^{2(t=0)} \qquad [A6]$$

To confirm that [A4] and [A6] are equivalent, consider (Crow and Kimura, 1970, p.102)

$$F_t = F_{t-1}(1-2\Delta F) + \Delta F(1+F_{t-2}) \qquad [A7]$$

With (Crow and Kimura, 1970, p.103),

$$\Delta F = \frac{1}{8S} + \frac{1}{8D} \qquad [A8]$$

Formula [A5] and [A2], and hence [A6] and [A4], can shown to be equivalent for random mating. Substituting $\sigma_A^{2(t-1)}$ in [A5] by $\sigma_A^{2(t-1)}$ as defined in [A4], and substituting F_t in [A2] by $F_{t-1}(1-2\Delta F) + \Delta F(1+F_{t-2})$ as defined in [A7] shows that [A5] and [A2] are equivalent.

Situation with directional selection. The between family variance we are interested in is, however, the between family variance in a situation with directional selection. To account for the reduction of the variance due to selection we would like to express the variance in generation t as a function of the variance in generation

t-1. The variance between selected sires ($V(a_s)^*$) and dams ($V(a_d)^*$) in generation t, according to Bulmer (1980), is:

$$V(a_s)^* = (1 - r_{IHs}^{2(t-1)} k_s)\,\sigma_A^{2(t-1)}$$

$$V(a_d)^* = (1 - r_{IHd}^{2(t-1)} k_d)\,\sigma_A^{2(t-1)}$$

where $r_{IH}^{2(t-1)}$ is the selection accuracy and k=i(i-x) where i is the infinite selection intensity and x the corresponding truncation point. To compute required covariances, the relationship between selected sires and dams is generally assumed to be equal. This implies ignoring covariances between selected sires and dams ($Cov(a_s,a_d)^*$), between selected sires ($Cov(a_s,a_s')^*$) and between selected dams ($Cov(a_d,a_d')^*$), or:

$$Cov(a_s,a_d)^* = Cov(a_s,a_s')^* = Cov(a_d,a_d')^* = Cov(\overline{a_d},\overline{a_s})^* = 0$$

and therefore:

$$Cov(a_s,\overline{a_s})^* = V(\overline{a_s})^* = \frac{1}{S}(1 - r_{IHs}^{2(t-1)} k_s)\,\sigma_A^{2(t-1)}$$

$$Cov(a_d,\overline{a_d})^* = V(\overline{a_d})^* = \frac{1}{D}(1 - r_{IHd}^{2(t-1)} k_d)\,\sigma_A^{2(t-1)}$$

Substituting these (co)variances into [A6] gives:

[A9]

$$\sigma_A^{2(t)} = \frac{1}{4}(1-\frac{1}{S})(1-r_{IHs}^2 k_s)\sigma_A^{2(t-1)} + \frac{1}{4}(1-\frac{1}{D})(1-r_{IHd}^2 k_d)\sigma_A^{2(t-1)} + \frac{1}{2}(1-F_{t-1})\,\sigma_A^{2(t=0)}$$

as used by Keller *et al.* (1990) and Verrier *et al.* (1991). Alternatively, Woolliams *et al.* (1994) presented a method to predict $\sigma_A^{2(t)}$ including different relationships between selected parents.

Before [A4] can be corrected for the effect of selection, [A4] is expressed as a function of $\sigma_A^{2(t-1)}$, assuming $\sigma_A^{2(t-1)}=(1-F_{t-1})\sigma_A^{2(t=0)}$:

$$\sigma_A^{2(t)} = \frac{1}{2}(1-\frac{1}{2S}-\frac{1}{2D})(1+F_{t-1}-2F_t)/(1-F_{t-1})\,\sigma_A^{2(t-1)} + \frac{1}{2}(1-F_{t-1})\,\sigma_A^{2(t=0)} \qquad [A10]$$

and, subsequently corrected for the effect of selection, giving:

$$\sigma_A^{2(t)} = \tfrac{1}{4}(1-\tfrac{1}{S})(1-r_{IHs}^2 k_s)(1+F_{t-1}-2F_t)/(1-F_{t-1})\sigma_A^{2(t-1)} + \tfrac{1}{4}(1-\tfrac{1}{D})(1-r_{IHd}^2 k_d)(1+F_{t-1}-2F_t)/(1-F_{t-1})\sigma_A^{2(t-1)} + \tfrac{1}{2}(1-F_{t-1})\sigma_A^{2(t=0)} \quad [A11]$$

Selection intensities as defined by Hill (1976) and Rawlings (1976) are standardized by the variance between estimated additive values in an "infinite" population and are adjusted for the effect of selection of a finite number of sires and dams. Hence, for prediction of the additive genetic response (i r_{IH} σ_A) the effect of a finite number of dams is accounted for in the selection intensity (i) and should be omitted in computation of σ_A (Meuwissen, 1991). Therefore, the following two formulas were used to verify the stochastic simulation model. At first:

$$\sigma_A^{2(t)} = \tfrac{1}{4}(1-r_{IHs}^2 k_s)(1+F_{t-1}-2F_t)/(1-F_{t-1})\sigma_A^{2(t-1)} + \tfrac{1}{4}(1-r_{IHd}^2 k_d)(1+F_{t-1}-2F_t)/(1-F_{t-1})\sigma_A^{2(t-1)} + \tfrac{1}{2}(1-F_{t-1})\sigma_A^{2(t=0)} \quad [A12]$$

which is identical to the formula given by Wray (1989) and used by Ruane (1989) to compare stochastic versus deterministic simulation. The second formula equals:

$$\sigma_A^{2(t)} = \tfrac{1}{4}(1-r_{IHs}^2 k_s)\sigma_A^{2(t-1)} + \tfrac{1}{4}(1-r_{IHd}^2 k_d)\sigma_A^{2(t-1)} + \tfrac{1}{2}(1-F_{t-1})\sigma_A^{2(t=0)} \quad [A13]$$

Chapter 6

Market share for semen and cloned embryos in dairy herds

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Abstract

Cloning in dairy cattle breeding enables a faster dissemination of superior genetic breeding material to milk producers using cloned embryos from desirable genotypes (commercial clone lines). The proportion of replacement cows belonging to a commercial clone line each year is an indication for the market share of cloned embryos compared to semen. In this paper, relevant factors affecting market share of cloned embryos were studied using deterministic simulation. To produce the next generation commercial cows, the nucleus provided semen from the best sire (15- or 27-months of age) and cloned embryos from the best 27-month old female genotype. The breeding scheme considered in the nucleus was as determined by De Boer and Van Arendonk (1994). Selection was for a single trait associated with lactation. A commercial cow was inseminated if net returns from her expected offspring were higher than net returns from an expected contemporary clone. Net returns of an expected offspring were computed as net milking returns (milk returns - feed costs), which were assumed directly related to the genetic value, minus the costs required to breed this expected offspring (i.e. purchase price of semen or a cloned embryo). If not inseminated, a cow could be used for implantation of a cloned embryo. Relevant factors influencing the market share of cloned embryos were: the difference in genetic merit between cloned embryos and available semen ($\Delta_{Gc\text{-}As}$), the annual additive genetic response achieved in the nucleus (A), and the difference (δ_c) in costs required to breed an offspring from either insemination or implantation (e.g. difference in purchase price between cloned embryos and semen). In addition, market share was affected by characteristics of the commercial cow population before use of clones: e.g. use of sexed or unsexed semen, or generation interval of commercial cows. Increasing $\Delta_{Gc\text{-}As}$ and A increased the market share of clones, whereas increasing δ_c decreased the market share of clones. All cows belong to commercial clone lines when δ_c was less than $\frac{1}{2}\Delta_{Gc\text{-}As}+A(1+\frac{1}{2}n_\ell)$, where n_ℓ is the number of lactations of the youngest age class available for selection. When δ_c was larger than $\frac{1}{2}\Delta_{Gc\text{-}As}+A(1+\frac{1}{2}n_\ell)$, market share decreased stepwise until finally no clones were used in the commercial cow population. Market share of cloned embryos influenced the average genetic level and the genetic uniformity of the commercial cow population.

Key words: clones, market share, milk production

Introduction

Cloning is the production of genetically identical individuals. Testing several clones for each genotype at the expense of testing dam families in a closed dairy cattle nucleus, in which a fixed number of cows are tested each year, hardly affects annual genetic response. However, the genetic superiority of female genotype(s) selected for commercial cloning (clonal response) increases considerably (De Boer and Van Arendonk, 1994). Therefore, the main advantage of cloning is a faster

dissemination of genetically superior breeding material to the milk producers using cloned embryos from desirable genotypes.

A producer's decision either to inseminate a cow by a tested sire or to implant a cloned embryo from a tested female genotype into a cow will depend on factors determined by the breeding scheme considered in the nucleus, such as the difference in genetic level between available semen and cloned embryos. In addition, e.g., the difference in purchase price between cloned embryos and semen will affect this decision. For a breeding organisation selling semen and/or cloned embryos, it is important to know relevant factors that determine the market share of available cloned embryos versus available semen.

Economic considerations regarding use of embryo transfer to produce replacement commercial cows have been based on expected extra milk returns, assuming transfer of available embryos into all commercial cows (Jansen, 1978; McDaniel and Cassell, 1981; Van Vleck, 1981). Similarly, expected extra milk returns from implantation of cloned embryos into all commercial cows also might be determined. The decision to inseminate a cow or to implant a cloned embryo into a cow, however, might differ between cows. In this study, a cow was inseminated if net returns of her expected offspring were higher than net returns from a contemporary commercial clone. In this way, each year the proportion replacement cows belonging to a commercial clone line, which indicates the market share of commercially available cloned embryos, was computed. The aim of this paper was to study relevant factors influencing the market share of commercially available cloned embryos.

Model description

General

The dairy cattle population considered was divided into two tiers: a breeding population (nucleus) and the milk producing population (commercial). The nucleus provided both semen from tested sires and cloned embryos from superior female genotypes (commercial clone lines) to produce the next generation commercial cows. The breeding scheme considered in the nucleus was as determined by De Boer and Van Arendonk (1994), and was assumed to be in equilibrium with respect to its genetic response. Selection was for a single trait associated with lactation. This trait can be envisioned as an individual production trait or as a total merit, which combines several characteristics.

At first, a commercial cow population, which was in equilibrium with respect to its genetic response in the absence of cloning, was simulated deterministically.

Simulation was carried out on an annual basis. Offspring were born from insemination of selected cows by the best available nucleus sire. Cows had their first offspring at two years of age and subsequently at one-year intervals. To accelerate reaching an equilibrium genetic response, the founder population was created as a population under selection.

Once annual genetic response in the commercial tier was stabilised, both semen and cloned embryos were provided by the nucleus. Cows born from either insemination or implantation were used for milk production in the commercial. The proportion of replacement cows belonging to a commercial clone line each year (i.e. market share of cloned embryos) was determined by comparing net returns of expected offspring born from either insemination or implantation. A commercial cow was inseminated if net returns from her expected offspring were higher than net returns from an expected contemporary clone. Net returns of an expected offspring were computed as net milking returns (milk returns - feed costs), which were assumed directly related to the genetic value, minus the costs required to breed this expected offspring (X_{insem}, X_{clone}). For example, costs required to breed an offspring from insemination (X_{insem}) are determined by the purchase price of semen and the pregnancy rate after insemination. Hence, a cow was inseminated with nucleus semen if:

$$\begin{aligned} \tfrac{1}{2}\,[\hat{A}_s + \hat{A}_d\,] - X_{insem} &> \hat{G}_c - X_{clone} \\ \tfrac{1}{2}\,[\hat{A}_s + \hat{A}_d] &> \hat{G}_c - (X_{clone} - X_{insem}) \\ \tfrac{1}{2}\,[\hat{A}_s + \hat{A}_d] &> \hat{G}_c - \delta_c \end{aligned} \qquad [1]$$

where, $\hat{A}_d$ is the estimated additive genetic effect of the cow inseminated, $\hat{A}_s$ is the estimated additive genetic effect of the best available nucleus sire, $\hat{G}_c$ is the estimated total genetic effect of the best available commercial clone line, and δ_c is the difference in costs required to breed an offspring from insemination and costs required to breed an offspring from implantation (e.g. difference in purchase price between semen and cloned embryos). Both $\hat{A}_s$ and $\hat{G}_c$ are determined by the breeding scheme considered in the nucleus, and expressed in units σ_p of net milking returns. Various values for δ_c (expressed in units σ_p) were studied because at present δ_c is difficult to quantify, as shown in the discussion. From [1], it can be seen that a cow was inseminated if her estimated additive genetic value ($\hat{A}_d$) met the following requirement:

$$\hat{A}_d > 2[\hat{G}_c - \delta_c] - \hat{A}_s \qquad [2]$$

Selection of commercial cows

As given in [2], a cow was only inseminated if her estimated additive genetic effect ($\hat{A}_d$) was above a specified truncation point. Cows available for insemination either descended from a nucleus sire or were adult clones. To determine $\hat{A}_d$ of a cow, commercial cows were divided into age classes, and age classes were subdivided into age subclasses. Cows within an age (sub)class were assumed to have an equal amount of information to compute $\hat{A}$. Age classes were defined according to origin (born from insemination or implantation) and age of cow. Newborn clones were grouped into one age class because their values for $\hat{A}$ were equivalent; therefore, no age subclasses needed to be distinguished. For clones, $\hat{A}$ was determined by, and therefore taken from, the breeding scheme considered in the nucleus.

An age class with offspring born from insemination was divided into age subclasses according to age of dam at mating, because age of dam influences the amount of pedigree information. The variance of $\hat{A}$ for the j^{th} age class ($\sigma^2_{\hat{A}j}$) was determined according to Meuwissen (1989):

$$\sigma^2_{\hat{A}j} = \sum_{k=1} w_{jk} \sigma^2_{\hat{A}jk} + \sum_{k=1} w_{jk} \mu^2_{jk} - (\sum_{k=1} w_{jk} \mu_{jk})^2 \qquad [3]$$

where $\sigma^2_{\hat{A}jk}$ is the variance of $\hat{A}$ in age subclass jk, μ_{jk} is the mean of age subclass jk, and w_{jk} is the relative number of animals in age subclass jk. Selection index theory (Hazel, 1943) was used to compute $\sigma^2_{\hat{A}jk}$. Information sources used were: estimated breeding values of the sire and dam, own performance records when available, and paternal half sib or full sib records when available. Detailed information to compute $\sigma^2_{\hat{A}jk}$ is given in Appendix A. Subsequently, according to [2] cows were selected for insemination across age classes using the algorithm described by Ducrocq and Quaas (1988). For each age group j, the fraction selected, the corresponding standardized selection intensity and the truncation point were derived. Information required by the procedure was: the overall selected fraction, the relative size of each age group and the mean and standard deviation of $\hat{A}$ per age group.

Computation of true mean and variance

The mean genetic level of newborn clones was taken from the nucleus breeding scheme. Newborn clones are genetically identical, giving zero variance between true and estimated additive genetic effects of clones in one age class.

The mean additive genetic level of cows born from insemination (A_0) was determined as:

$$A_o = \frac{1}{2} [A_s + \{ \sum_j w_j(\mu_j + i_j \sigma_{\hat{A}j}) \}] \quad [4]$$

where A_s is the true additive genetic value of the selected sire, w_j is the relative contribution of dam age class j to newborn individuals and i_j is the standardized selection intensity of selected dams in age class j (which is zero for selected adult clones). The variance between true additive genetic effects of cows born from insemination (σ^2_{Ao}) was:

$$\sigma^2_{Ao} = \frac{1}{4}\sigma^{2*}_{As} + \frac{1}{4}\sigma^{2*}_{Ad} + \sigma^2_{ms} \quad [5]$$

where σ^{2*}_{As} and σ^{2*}_{Ad} are the additive variances between selected sires and dams, respectively, and σ^2_{ms} is the Mendelian sampling variance. The additive variance between selected sires was computed as $(1-r^2_{IHs}k_s)\sigma^2_{An}$, where σ^2_{An} is the additive variance in the nucleus, r^2_{IHs} is the accuracy of sire selection (see Table 1), $k_s = i_\infty(i_\infty - x) = 0.94$, with i_∞ as the infinite sire selection intensity and x as the corresponding truncation point. The variance between selected dams (σ^{2*}_{Ad}) was computed as:

$$\sigma^{2*}_{Ad} = \sum_j w_j \sigma^{2*}_{Adj} + \sum_j w_j [\mu_j + i_j \sigma_{\hat{A}j}]^2 - (\sum_j w_j [\mu_j + i_j \sigma_{\hat{A}j}])^2 \quad [6]$$
$$= \sigma^2_{Aw} + \sigma^2_{Ab}$$

where the first term is the genetic variance within dam age groups after selection (σ^2_{Aw}) and the last two terms represent genetic variance between dam age groups (σ^2_{Ab}).

Results

Example population with only one age group

To illustrate the model, we simulated a population with discrete generations in which only commercial cows with one lactation (27-months of age) were available for insemination. In this population, each cow was assumed to generate one female replacement. Additive genetic variance was the only source of genetic variance. Relevant information on the breeding scheme considered in the nucleus is in Table 1.

Table 1. Required parameters of the breeding scheme considered in the nucleus (De Boer and Van Arendonk, 1994)

Description	Value
Additive variance corrected for selection	$0.20\sigma_p^2$
Annual additive response (A)	$0.20\sigma_p$
Accuracy of sire selection (r_{IHs})	0.258
Generation interval for sires (L_δ)	2.358
Genetic superiority of sires over their selection candidates (S_δ)	$0.29\sigma_p$
Accuracy of clonal selection (r_{IHc})	0.83
G_c-A_s=$\Delta_{Gc\text{-}As}$ [a]	$0.47\sigma_p$

[a] where G_c is the true clonal genetic value and A_s is the true sire additive genetic value

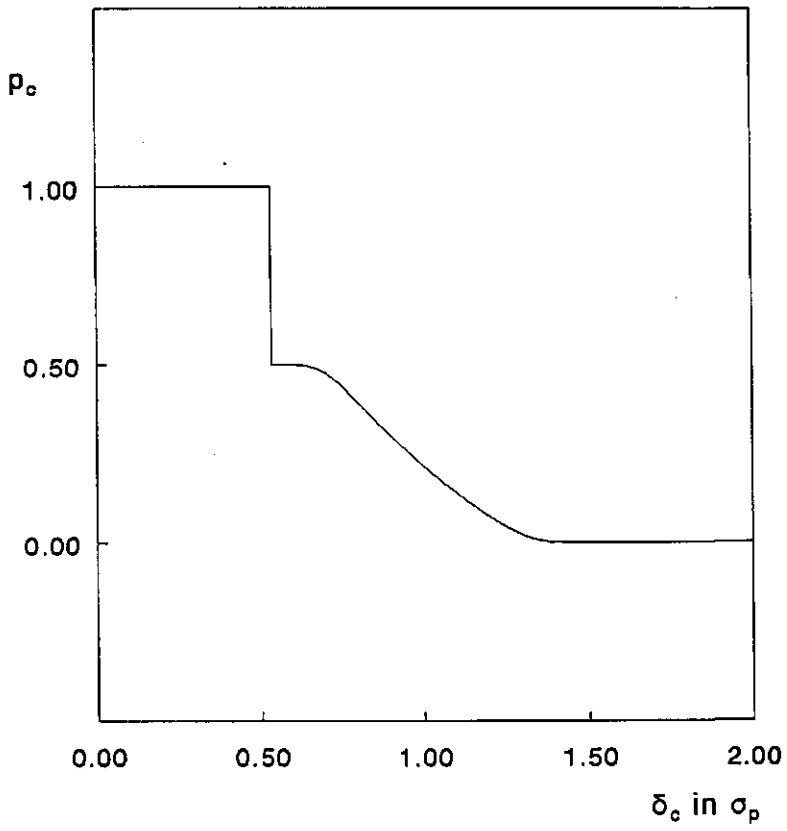

Figure 1. Proportion of newborn offspring belonging to commercial clone lines (p_c) each year for varying δ_c (expressed in phenotypic standard deviations of net milking returns or σ_p) in the example population with only one age group, where δ_c is the difference in costs required to breed an offspring from either insemination or implantation

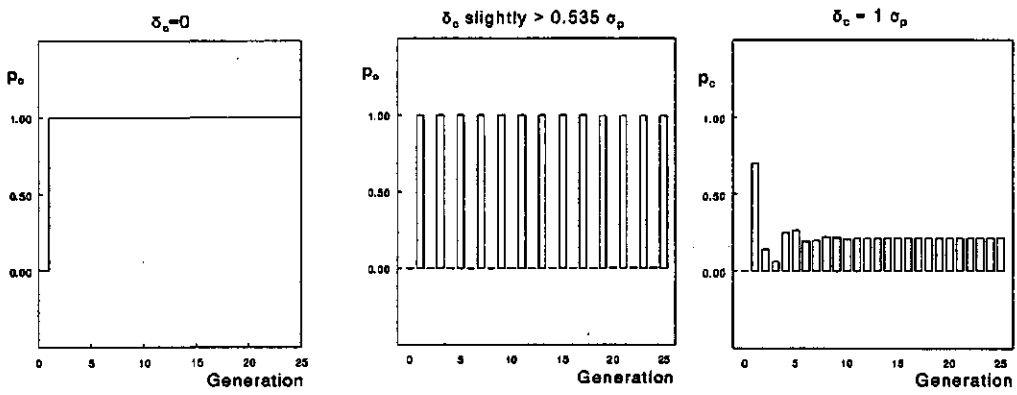

Figure 2. Proportion of newborn offspring belonging to commercial clone lines each generation for three values of δ_c (=difference in costs to breed an offspring from either insemination or implantation, expressed in phenotypic standard deviations of net milking returns or σ_p) in the example population with one age group, $\delta_c=0$, δ_c is slightly larger than $0.535\sigma_p$ and $\delta_c=1\sigma_p$

Equilibrium or average values of the proportion replacement cows that belong to a commercial clone line (p_c), for various values of δ_c is in Figure 1. When δ_c is less than $0.535\sigma_p$, all replacement cows are clones and $p_c=1$. When δ_c is slightly larger than $0.535\sigma_p$, p_c equals 0.5. As δ_c increases further, the number of cows which are inseminated increased so that p_c decreased. Finally, all newborn offspring were the result of inseminating commercial cows. To explain observed results in Figure 1, Figure 2 gives p_c in subsequent generations, when $\delta_c=0$, when δ_c is slightly larger than $0.535\sigma_p$, and when $\delta_c=1\sigma_p$.

When $\delta_c=0$, all replacement cows belong to the commercial clone line ($p_c=1$). This can be explained as follows. Let's denote the expected genetic value of the best commercial clone line at present as G_c. From Table 1, the expected additive genetic value of the best nucleus sire at that time then equals $G_c-0.47\sigma_p$, denoted as $G_c-\Delta_{Gc-As}$. Cows available for insemination are either adult clones or born from insemination. The expected genetic value of a 27-month old commercial clone equals G_c-3A, where A is the annual genetic response ($A=0.20\sigma_p$, see Table 1). As a result, the expected genetic value of an offspring born from inseminating a 27-month old clone is:

$$\frac{1}{2}(G_c - \Delta_{Gc-As}) + \frac{1}{2}(G_c - 3A) = G_c - \frac{1}{2}\Delta_{Gc-As} - \frac{3}{2}A \qquad [7]$$

From formula [1] and [7] it can be concluded that a 27-month old commercial clone will only be inseminated if:

$$\delta_c > \tfrac{1}{2}\Delta_{Gc-As} + \tfrac{3}{2}A \qquad [8]$$

Hence, a 27-month old commercial clone was not inseminated until $\delta_c > \frac{1}{2}\cdot 0.47\sigma_p + 1\frac{1}{2}\cdot 0.20\sigma_p > 0.535\sigma_p$.

Similarly, the fraction of 27-month old cows born from insemination, that will be selected for insemination, can be determined. Following Guy and Smith (1981), the expected value of an 27-month old cow born from insemination can be written as $A_s-\lambda+L_{\delta}\cdot A-S_{\delta}-L_{\varphi}\cdot A+S_{\varphi}$ (Appendix B), where λ is the genetic lag between the nucleus and commercial in the absence of cloning ($\lambda=0.782\sigma_p$), L_{δ} (L_{φ}) is the average age of sires (dams) when their offspring are born ($L_{\delta}=2.358$; $L_{\varphi}=3$), and S_{δ} (S_{φ}) is the genetic superiority of sires (dams) over their contemporary mean ($S_{\delta}=0.29\sigma_p$). The expected value of an offspring born from insemination of such a 27-month old cow is:

$$\tfrac{1}{2}(G_c-\Delta_{Gc-As})+\tfrac{1}{2}(G_c-\Delta_{Gc-As}-\lambda+L_{\delta}\cdot A-S_{\delta}-L_{\varphi}\cdot A+S_{\varphi})= \qquad [9]$$

$$G_c-\Delta_{Gc-As}-\tfrac{1}{2}[\,\lambda-L_{\delta}\cdot A+S_{\delta}+L_{\varphi}\cdot A-S_{\varphi}\,]$$

From formula [1] and [9] it can be concluded that a 27-month old cow born from insemination will only be inseminated if her expected genetic superiority (S_{φ}), is above:

$$S_{\varphi} > 2\Delta_{Gc-As}+\lambda+L_{\varphi}A-L_{\delta}A+S_{\delta}-2\delta_c \qquad [10]$$

Thus, a 27-month old cow born from insemination is only inseminated if her expected genetic superiority over her contemporary mean is larger then $2.14\sigma_p-2\delta_c$. The expected genetic superiority of an individual cow is determined by her estimated additive genetic effect. Knowing that $\delta_c=0$ and $\sigma_{\hat{A}}=0.226$, the fraction of 27-month old cows born from insemination, whose genetic superiority is larger than $2.14\sigma_p$ was computed and seemed negligible. In conclusion, when $\delta_c=0$ neither an adult commercial clone nor a cow born from insemination will be inseminated. As a result, $p_c=1$.

When δ_c is slightly larger than $0.535\sigma_p$, p_c is 1.0 in the first generation. The fraction of 27-month old cows born from insemination, whose expected genetic superiority was larger than $1.07\sigma_p$ ($2.14\sigma_p-1.07\sigma_p$), was negligible. According to [8], all 27-month old commercial clones were inseminated in generation two and $p_c=0$. As a consequence of inseminating clones in generation two, the genetic lag between

the nucleus and commercial tier equalled $-\frac{1}{2}\Delta_{Gc\text{-}As}+1\frac{1}{2}A+L_{\delta}\cdot A\text{-}S_{\delta}=0.247\sigma_p$. Use of this genetic lag in [10] shows that a cow was inseminated if her expected genetic superiority was larger than $0.535\sigma_p$. Knowing that $\sigma_{\hat{A}}=0.156$, 0.0003 of all cows were inseminated so that $p_c=0.9997$. In generation four, again only commercial clones were inseminated so that $p_c=0.0003$. Hence, when δ_c is slightly larger than $0.535\sigma_p$, cloned embryos and semen were used alternatively and p_c is on average 0.50.

At higher values of δ_c, the proportion of cows born from insemination, which were themselves inseminated, increased and p_c stabilized quickly at an equilibrium value less than 0.50. For example, when $\delta_c=1\sigma_p$, p_c stabilized at $0.215\sigma_p$ (Figure 2).

Population with more than one age group

No dominance. Results will be described for a population with overlapping generations and cows available for insemination at 15 (35%), 27 (30%), 39 (20%), 51 (10%) or 63 (5%) months of age. Each year, 40% of all cows produced a female replacement after either insemination or implantation. Additive genetic variance was the only source of genetic variance. The breeding scheme considered in the nucleus was the same as in the example population. Annual genetic response in the nucleus (A), the difference ($\Delta_{Gc\text{-}As}$) between the expected genetic level of the best commercial clone line (G_c) and the expected additive value of the best available sire (A_s), accuracy of sire (r^2_{IHs}) and clonal selection (r^2_{IHc}), and the generation interval of the sire (L_{δ}) are in Table 1. The genetic lag between the nucleus and commercial in the absence of cloning was $0.678\sigma_p$ (Appendix B).

Figure 3 shows equilibrium values of p_c for varying levels of δ_c. When $\delta_c<0.435\sigma_p$ all replacement cows belong to the commercial clone line ($p_c=1$). From generalization of [7] and [8], we can see that a commercial clone will only be inseminated if:

$$\delta_c > \tfrac{1}{2}\Delta_{Gc\text{-}As} + A\,(1 + \tfrac{1}{2}n_t) \qquad [11]$$

where n_t is the number of lactations of the clone considered for insemination. Hence, 15-month old commercial clones that do not yet have a lactation record, i.e. $n_t=0$, will not be inseminated until $\delta_c>0.435\sigma_p$. Insemination of 15-month old clones is observed in Figure 3 as a decrease in p_c when δ_c is $0.435\sigma_p$. Similarly, p_c decreased when δ_c was $0.535\sigma_p$, $0.635\sigma_p$, $0.735\sigma_p$ and $0.835\sigma_p$. These decreases were due to inseminating 27-month ($n_t=1$), 39-month ($n_t=2$), 51-month ($n_t=3$) and 63-month ($n_t=4$) old commercial clones, respectively. The decrease in p_c was less clear, however, for older commercial clones. This is because a dairy herd consists of ,e.g., relatively less 63-month old (5%) than 15-month (35%) clones.

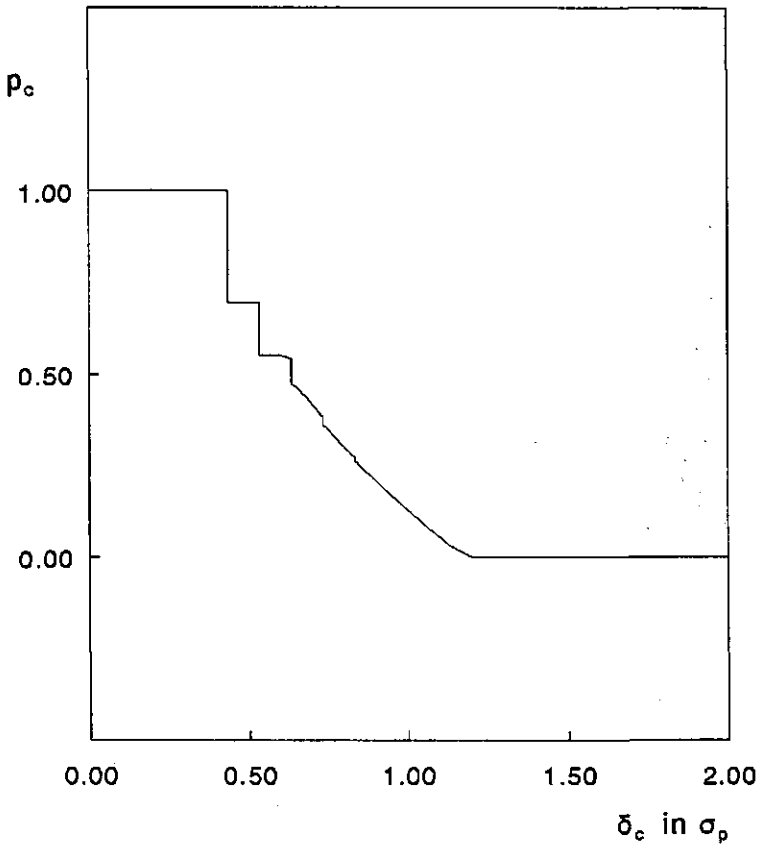

Figure 3. Proportion of newborn offspring belonging to commercial clone lines (p_c) each year for varying δ_c (= difference in costs required to breed an offspring from either insemination or transplantation expressed in phenotypic standard deviations of net milking returns or σ_p) in the population with overlapping generations, when $\Delta_{Gc\text{-}As}=0.47\sigma_p$ and $A=0.20\sigma_p$

Situation with dominance variance. With dominance variance, the difference between the expected genetic merit of selected commercial clone lines and nucleus sires might increase considerably (De Boer and Van Arendonk, 1994), whereas annual genetic response will only slightly decrease. For example, when dominance variance equals 20% of the phenotypic variance and inbreeding depression is absent $\Delta_{Gc\text{-}As}=1.09\sigma_p$ and $A=0.17\sigma_p$ (De Boer and Van Arendonk, 1994). With 1% depression of the mean per 1% inbreeding, however, $\Delta_{Gc\text{-}As}$ is only $0.59\sigma_p$. In this study, consequences of dominance were illustrated by varying levels of $\Delta_{Gc\text{-}As}$ and A. The effect of doubling $\Delta_{Gc\text{-}As}$ and/or halving A is shown in Figure 4.

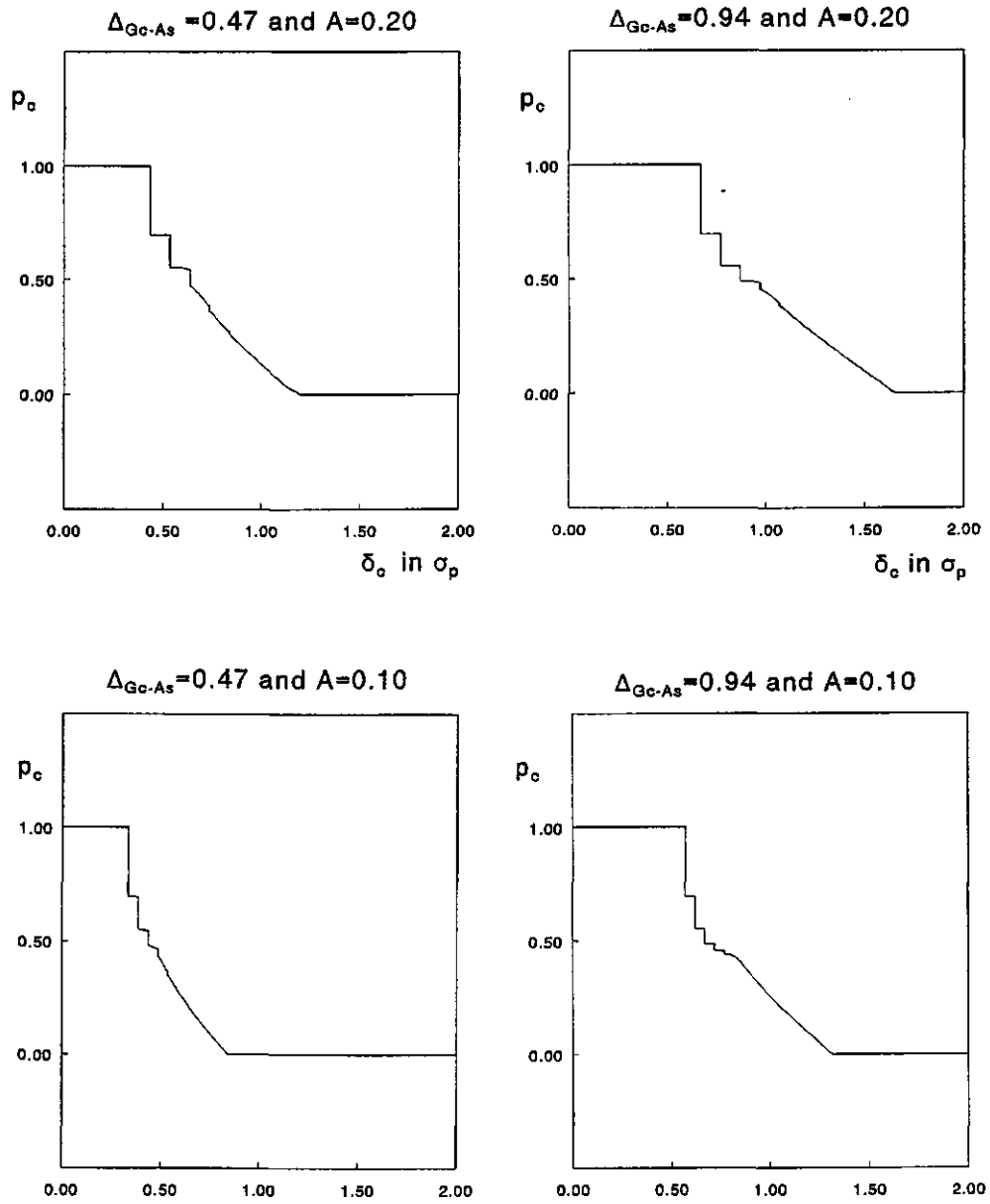

Figure 4. Proportion of newborn offspring belonging to commercial clone lines (p_c) each year for varying δ_c (= difference in costs required to breed an offspring from either insemination or implantation) in the population with overlapping generations, when Δ_{Gc-As}=0.47 or 0.94 and A=0.20 or 0.10 (expressed in phenotypic standard deviations of net milking returns or σ_p)

As expected from [11], doubling $\Delta_{Gc\text{-}As}$ shifted the graph to the right. As a result, p_c was higher at a similar level of δ_c. Halving A shifted the graph to the left, and consequently p_c was lower at a similar level of δ_c. Both doubling $\Delta_{Gc\text{-}As}$ and halving A resulted in higher p_c at a similar level of δ_c. This suggests that doubling $\Delta_{Gc\text{-}As}$ was relatively more important than halving A; this is because $\Delta_{Gc\text{-}As}$ is generally larger than A. In conclusion, with both additive and dominant gene action p_c will probably be higher at similar levels of δ_c than with only additive gene action.

Maximal increase in genetic level due to using clones. The main advantage of cloning is a faster dissemination of genetically superior breeding material to the commercial population. The maximum increase in genetic level of the commercial cow population can be computed as follows.

From Appendix B, the genetic lag between the nucleus and commercial in the absence of cloning ($\lambda_{no\ clones}$) is $L_{\delta}\cdot A\text{-}S_{\delta} + L_{\varphi}\cdot A\text{-}S_{\varphi}$, where L_{δ} (L_{φ}) is the generation interval for sires (dams) and S_{δ} (S_{φ}) is the genetic superiority of sires (dams) over their selection candidates. In the extreme situation that all commercial cows are clones ($p_c=1$), the genetic lag between the nucleus and commercial (λ_{clones}) is $L_{\varphi}\cdot A\text{-}S_{\varphi}$, where L_{φ} is the average generation interval of female genotypes selected for commercial cloning ($L_{\varphi}=3$) and S_{φ} is the genetic superiority of these female genotypes over their contemporary mean. From De Boer and Van Arendonk (1994), S_{φ} equals $0.922\sigma_p$ without dominance and equals $1.680\sigma_p$ with dominance and no inbreeding depression. As a result, when $p_c=1$ the genetic level of the commercial is higher than the genetic level of the nucleus.

The maximal increase in genetic level of the commercial population can be given as $\lambda_{no\ clones} - \lambda_{clones}$ and equals $1.0\sigma_p$ without dominance or $1.74\sigma_p$ with dominance. Expressed in annual genetic response, this maximal increase in genetic level is 5 or 10 times A, without or with dominance.

Discussion

In this paper, relevant factors influencing the market share of cloned embryos versus semen were studied. Market share, which was computed as the proportion of replacement cows belonging to a commercial clone line each year (p_c), was affected by: the difference in genetic merit between cloned embryos and available semen ($\Delta_{Gc\text{-}As}$), the annual additive genetic response achieved in the nucleus (A), and the difference (δ_c) in costs required to breed an offspring from either insemination or implantation (e.g. difference in purchase price between cloned embryos and semen). In addition, market share was affected by characteristics of the commercial cow population before use of clones: e.g. use of sexed or unsexed semen, or generation interval of commercial cows.

Each year semen from the best nucleus sire (15- or 27-months old) and cloned embryos from the best female genotype (27-months old) were available to breed a new generation of commercial cows. Due to technical limitations of nuclear transfer, however, the number of live clones from one genotype might be considerably smaller than the number of offspring from one sire. When the number of clones per genotype is limited, more than one commercial clone line can be used to produce replacement cows. The effect on p_c of selection of more commercial clones lines might be approximated by decreasing $\Delta_{Gc\text{-}As}$. A decrease in $\Delta_{Gc\text{-}As}$ will result in a decrease in p_c at a similar level of δ_c. For example, in the absence of dominance, the average genetic merit of the five best commercial clone lines is 68% of the genetic merit of the best commercial clone line (Unpublished results, De Boer and Van Arendonk, 1994). As a result, $\Delta_{Gc\text{-}As}$ will decrease by 32%. In addition, the decline in p_c at specific levels of δ_c will be stepwise due to differences in genetic merit between available commercial clone lines. For example, in the absence of dominance, the expected difference in genetic merit between the best and the worst commercial clone line of the five is $0.3\sigma_p$. As a result, increasing the number of commercial clone lines used will result in smoothening the graph.

In this study, an offspring from insemination of a commercial cow was either a male or a female. In future, however, it might be possible to sex semen. Use of sexed semen, would mean that all calves born from insemination are females. As a result, fewer cows have to be selected for insemination to obtain the same number of female replacement calves. This will result in lower p_c at similar levels of δ_c because the average genetic merit of newborn female calves will be higher when using sexed semen than when using unsexed semen.

As stated previously, the difference in costs required to breed an offspring from either insemination or transplantation (δ_c) does affect p_c. The value of δ_c is determined by, e.g., the difference in purchase price between semen and cloned embryos, and the difference in pregnancy rate after insemination or implantation. Various values of δ_c were studied, because at present δ_c is difficult to quantify. Nuclear transfer might theoretically result in production of an unlimited number of clones. Until now, however, overall success rates of nuclear transfer have been low. As reported by Bondioli (1992), only an average of 1.98 pregnancies were obtained after cloning one 32-cell embryo. Results of a second generation of nuclear transfer were even lower. Therefore, use of clones in dairy cattle production currently is not possible. As a result, the purchase price of cloned embryos and the pregnancy rate after implantation of cloned embryos into commercial cows are unknown. At the start of using clones, however, costs to breed a newborn clone are expected to be higher than costs to breed an offspring from insemination.

In addition to differences in purchase price and pregnancy rate other factors

not directly related to δ_c, might affect p_c, e.g. positive or negative consequences of a genetically more uniform population. The effect on p_c of such factors might be demonstrated by determining their effect on δ_c. Negative consequences of an increased genetic uniformity of the commercial population might be demonstrated by an increase in δ_c. Quantification of this increase in δ_c, however, is difficult because literature on negative consequences of a genetically more uniform population is absent. For example, an increased genetic uniformity might give problems with formation of groups. All natural and interrelated groups seem to be stable because of differences in individual behaviour. Whether only environmental differences in individual behaviour might be enough for normal formation of groups is unknown (Wiepkema, 1990). In addition, the increase in risk of disease spreading due to an increase in genetic uniformity is unknown. The effect on p_c of positive consequences of a genetically more uniform population (e.g. standardization of housing and milking conditions) might be demonstrated by a decrease in δ_c.

Before quantifying consequences of a genetically more uniform population, however, the uniformity of a population with clones should be assessed. Knowing p_c, we can quantify genetic uniformity of the population as the average genetic relationship between commercial cows eligible for selection. The effect of p_c on genetic uniformity is shown in Figure 5.

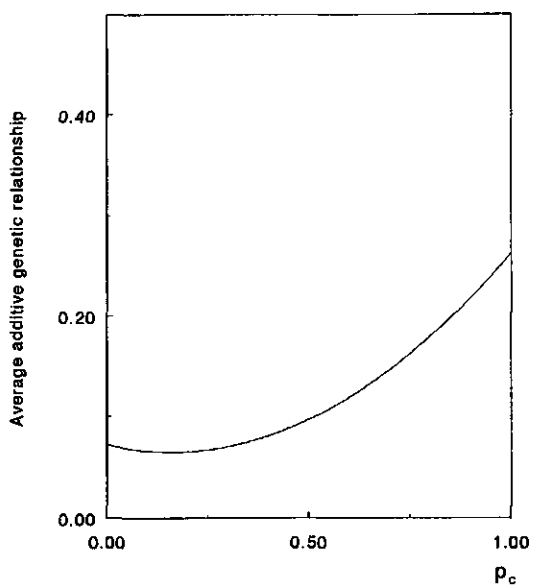

Figure 5. Genetic uniformity in the commercial populations, assessed as the average additive genetic relationship between cows, for varying p_c (=proportion of newborn offspring belonging to commercial clone lines each year)

Simultaneous use of cloned embryos and semen for dissemination at first decreased the additive genetic relationship between commercial cows. This decrease was because commercially available semen and cloned embryos were assumed unrelated. When p_c was less than about 0.35, genetic uniformity did not increase. When p_c was larger than 0.35, however, genetic uniformity increased considerably. In conclusion, moderate use of cloned embryos in the commercial does not seriously affect genetic uniformity, whereas large-scale use does increase genetic uniformity considerably. Consequently, only with large-scale use of cloned embryos, it is necessary to assess positive and negative effects of an increased genetic uniformity of the commercial population.

For a comparison of net returns of an expected offspring from either insemination or implantation, the value of these offspring as dams for future generations was assumed equivalent. However, as the relative importance of dominance in the total genetic merit of a clone increases, the value of this clone as dam for future generations decreases. This value is relevant in the situation that cows are still inseminated. This effect can be incorporated by increasing δ_c.

To obtain one female replacement cow, twice as many cows have to be inseminated on average than are required as recipients for implantation. Use of cloned embryos requires fewer cows for replacement. As a result, relatively more cows can be used for other purposes, e.g. beef production. In the situation of beef production, additional cows can either be inseminated by beef sires or used as recipients for implantation of cloned beef embryos. Hence, two factors do affect p_c when using unsexed semen: (i) the difference in costs required to breed a beef calf (X_{beef}) or a male offspring from insemination by a milking sire (X_{milk}), and (ii) the additional benefit of a more desirable beef calf. Their effect will be demonstrated by determining their effect on δ_c. If $X_{beef}<X_{milk}$, δ_c will decrease, whereas if $X_{beef}>X_{milk}$, δ_c will increase. The additional benefit due to production of a more desirable beef calf will decrease δ_c. The overall effect of using relatively more cows for beef production will probably decrease δ_c.

Finally, in deciding on the use of clones in dairy cattle production, also ethical aspects of cloning in dairy cattle production should be evaluated. It might be that as a result of this clones are not used in dairy cattle production. A similar result arises with a very large value for δ_c (e.g. $30\sigma_p$).

When ignoring all the above mentioned factors that might affect δ_c, we can assess the difference in costs between the purchase price of a cloned embryo and semen. For this, we assumed a pregnancy rate after both insemination and implantation of 65%. Selection was for a single trait associated with lactation, which can be taken as net-profit-index (INET) (Dommerholt and Wilmink, 1986). INET is a combination of breeding values for milk, fat and protein yield with a heritability

of 0.30 and an additive genetic standard deviation of 115 Dutch guilders (Bovenhuis and De Boer, 1992). As shown in Figure 3, all replacement cows will belong to a commercial clone line when δ_c is less than $0.435\sigma_p$ or less than 91.3 Dutch guilders per newborn offspring per lactation. With an average of 3.5 lactations per cow and a pregnancy rate of 65%, this is less than 208 Dutch guilders per amount of semen required for insemination. When the difference in purchase price between semen and cloned embryos exceeds 567 guilders, no clones will be used in the commercial cow population. Thus, for commercial application of clones, the difference in purchase price between a cloned embryo and semen should be less than 567 guilders. With dominance, the difference in purchase price between a cloned embryo and semen might be higher, depending on the increase in $\Delta_{Gc\text{-}As}$ due to exploitation of dominance.

Acknowledgements

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Appendix A

Computation of the variance of Â for individuals in an age subclass

Information sources used to compute the estimated additive effect for individual i in age subclass jk were: the estimated breeding value of the sire (1) and dam (2) of individual i, own performance records of individual i when available (3), and records on either paternal half sibs or full sibs when available (4). The matrix with covariances between information sources (**P**) equals:

$$\begin{bmatrix} r^{2*}_{IHs}\sigma^{2*}_{As} & 0 & \frac{1}{2}r^{2*}_{IHs}\sigma^{2*}_{As} & \frac{1}{2}r^{2*}_{IHs}\sigma^{2*}_{As} \\ 0 & r^{2*}_{IHd}\sigma^{2*}_{Ad} & \frac{1}{2}r^{2*}_{IHd}\sigma^{2*}_{Ad} & (\frac{1}{2}r^{2*}_{IHd}\sigma^{2*}_{Ad}) \\ \frac{1}{2}r^{2*}_{IHs}\sigma^{2*}_{As} & \frac{1}{2}r^{2*}_{IHd}\sigma^{2*}_{Ad} & \sigma^{2}_{Pi} & \sigma_{Pi,Pr} \\ \frac{1}{2}r^{2*}_{IHs}\sigma^{2*}_{As} & (\frac{1}{2}r^{2*}_{IHd}\sigma^{2*}_{Ad}) & \sigma_{Pi,Pr} & \sigma^{2}_{Pr} \end{bmatrix} \quad [1]$$

where $r^{2}_{IHs}{}^{*}$ ($r^{2}_{IHd}{}^{*}$) is the evaluation accuracy in sires (dams) updated for additional information that became available between time of parental selection and evaluation of animal i, $\sigma^{2}_{As}{}^{*}$ ($\sigma^{2}_{Ad}{}^{*}$) is the additive variance between selected sires (dams), σ^{2}_{Pi} is the phenotypic variance of the average performance of animal i, $\sigma_{Pi,Pr}$ the covariance between the average performance of animal i and her relatives (either paternal half sibs or full sibs) and σ^{2}_{Pr} is the average performance of the relatives.

The matrix with covariances between the information sources used and the breeding goal (**G**) can be written as,

$$\begin{bmatrix} \frac{1}{2}r^{2*}_{IHs}\sigma^{2*}_{As} \\ \frac{1}{2}r^{2*}_{IHd}\sigma^{2*}_{Ad} \\ \sigma^{2}_{Ajk} \\ \frac{1}{2}r^{2*}_{IHs}\sigma^{2*}_{As} + (\frac{1}{2}r^{2*}_{IHd}\sigma^{2*}_{Ad}) \end{bmatrix} \quad [2]$$

where σ^{2}_{Ajk} is the true additive genetic variance between individuals in age subclass jk. Elements between brackets in **P** or **G** are only non-zero when relatives are full sibs. For example, in an age subclass with animals born from insemination of adult clones, relatives are full sibs.

Sire selection accuracy updated for additional daughter information ($r^{2}_{IHs}{}^{*}$) was assumed to equal 0.90. Similarly, the accuracy of the additive genetic value of a cloned individual selected for breeding was assumed to be equal to 0.90. Dam selection accuracy was updated for additional own performance records, as described for an example situation. Suppose a dam was selected at 27 months of age and her

offspring evaluated at 15-months of age. Between the time of dam selection and offspring evaluation, two additional dam records are produced (p_2+p_3). Subsequently, sources of information to compute adjusted dam selection accuracy are: (1) dam's estimated additive effect ($\hat{A}_d$) at time of selection, based on parental breeding values, her first lactation record (p_1) and first lactation records of her female relatives, and (2) the average of the second and third dam performance ($\bar{p}=\frac{1}{2}(p_2+p_3)$). The matrix with covariances between information sources ($\mathbf{P}_d$) can be written as:

$$\begin{bmatrix} (1-k_d)r^2_{IHd}\sigma^2_{\hat{A}d} & (1-k_d)\sigma_{\bar{p},\hat{A}d} \\ (1-k_d)\sigma_{\bar{p},\hat{A}d} & \sigma^2_{\bar{p}}-\dfrac{(\sigma_{\bar{p},\hat{A}d})^2\ k_d}{\sigma^2_{\hat{A}d}} \end{bmatrix} \qquad [3]$$

where r^2_{IHd} is dam accuracy before selection, $\sigma^2_{\hat{A}d}$ is the additive variance between unselected dams, $\sigma_{\bar{p},\hat{A}d}$ is the covariance between the average additional dam performances ($\bar{p}$) and $\hat{A}_d$, $\sigma_{\hat{A}d}$ is the variance of $\hat{A}_d$, and $k_d=i_\infty(i_\infty-x)$ where i_∞ is the infinite dam selection intensity and x the corresponding truncation point.

The matrix with covariances between information sources and the breeding goal ($\mathbf{G}_d$) is:

$$\begin{bmatrix} (1-k_d)\sigma_{\hat{A}d,a} \\ \sigma_{\bar{p},a}-\dfrac{(\sigma_{\bar{p},\hat{A}d}\sigma_{a,\hat{A}d})\ k_d}{\sigma^2_{\hat{A}d}} \end{bmatrix} \qquad [4]$$

where $\sigma_{a,\hat{A}d}$ is the covariance between the additive genetic value of the dam and $\hat{A}_d$. Adjusted dam selection accuracy was then computed as,

$$r^{*}_{IHd} = \sqrt{\frac{\mathbf{b}_d'\mathbf{P}_d\mathbf{b}_d}{\sigma^{2*}_{Ad}}} \qquad \text{where} \quad \mathbf{b}_d = \mathbf{P}_d^{-1}\mathbf{G}_d \qquad [5]$$

Finally, the variance of $\hat{A}$ within an age subclass was computed using selection index theory and corrected for correlations between estimated additive effects as:

$$\sigma^2_{\hat{A}jk} = (1-t)\,\mathbf{b}'\mathbf{P}\mathbf{b}$$

$$\text{where } \mathbf{b} = \mathbf{P}^{-1}\mathbf{G} \quad \text{and} \quad t = \frac{\mathbf{b}'\mathbf{R}\mathbf{b}}{\mathbf{b}'\mathbf{P}\mathbf{b}}$$

where $\mathbf{R}$ is a matrix with covariances between phenotypic information sources of two relatives in age subclass jk.

Appendix B

Genetic lag between nucleus and commercial (Guy and Smith, 1981)

Following Guy and Smith (1981), the genetic lag between the nucleus and the commercial tier (λ) can be computed as:

$$N\text{-}C = N - \tfrac{1}{2}[(N\text{-}L_{\delta}A + S_{\delta}) + (C - L_{\varphi}A + S_{\varphi})] \qquad [B1]$$

$$\lambda = N\text{-}C = (L_{\delta}A - S_{\delta}) + (L_{\varphi}A - S_{\varphi})$$

where, N and C are average genetic levels of individuals born into the nucleus and commercial, respectively, L is the average age of parents when their offspring are born, A is the annual additive genetic response, S is the genetic superiority of parents over their contemporary mean.

Population with discrete generations

Required parameters to compute λ were taken from the breeding scheme considered in the nucleus as determined by De Boer and Van Arendonk (1994) (e.g. $L_{\delta}=2.358$, $S_{\delta}=0.29\sigma_p$ and $A=0.20\sigma_p$) and from the deterministically simulated commercial cow population. In the example population with discrete generations, without cloning all cows were selected at 27-months of age. Therefore $L_{\varphi}=3$ and $S_{\varphi}=0$ and hence $\lambda=0.7816\sigma_p$.

From the genetic difference between available nucleus sires and selected commercial cows, the genetic merit of selected cows can be written as

$$\begin{aligned} A_s\text{-}A_d &= [(N\text{-}L_{\delta}A + S_{\delta}) - (C - L_{\varphi}A + S_{\varphi})] \\ &= \lambda - L_{\delta}\cdot A + S_{\delta} + L_{\varphi}\cdot A - S_{\varphi} \\ A_d &= A_s - \lambda + L_{\delta}\cdot A - S_{\delta} - L_{\varphi}\cdot A + S_{\varphi} \end{aligned} \qquad [B2]$$

Population with overlapping generations

As in the population with discrete generations, required parameters to compute λ in a situation with overlapping generations were taken from De Boer and Van Arendonk (1994) as: $L_{\delta}=2.358$, $S_{\delta}=0.29\sigma_p$ and $A=0.20\sigma_p$. For various alternatives studied in this situation, average female generation interval (L_{φ}), genetic superiority of selected dams (S_{φ}) and the average standard deviation of estimated additive effects ($\sigma_{\hat{A}}$) are given in Table B1.

An alternative is characterized by values for annual additive response (A), the difference in total genetic merit between available cloned embryos and semen ($\Delta_{Gc\text{-}As}$) and the proportion of cows required for replacement or p (i.e. the use of unsexed or sexed semen).

Table B1. Parameters required to compute the genetic lag between the nucleus and commercial population ($L_{♀}$: average generation interval of commercial cows; $S_{♀}$=average genetic superiority of commercial cows; $\sigma_{\hat{A}}$=average standard deviation of estimated additive effects of commercial cows) for alternative populations with overlapping generations. Alternatives are characterized by the assumed annual genetic response (A), the difference in genetic merit between cloned embryos and semen ($\Delta_{Gc\text{-}As}$) and the proportion of commercial cows selected for replacement (p).

Alternative			Parameters		
A	$\Delta_{Gc\text{-}As}$	p	$L_{♀}$	$S_{♀}$	$\sigma_{\hat{A}}$
0.20	0.47	0.80	2.86	0.075	0.215
0.20	0.94	0.80	2.86	0.075	0.215
0.10	0.47	0.80	2.94	0.077	0.206
0.10	0.94	0.80	2.94	0.077	0.206
0.20	0.47	0.40	2.41	0.135	0.170

Chapter 7

Ethical aspects of the use of clones in dairy cattle breeding

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" It is evident that few people subject their ethical beliefs to rational analysis, but that in no way diminishes the importance of such an analysis (Mepham, 1993) "

General introduction

In this thesis, the technical aspects of the use of cloning in dairy cattle breeding were studied. Cloned embryos can be used to improve dissemination of superior genetic material from the nucleus breeding stock to milk producing commercial cows. This improved dissemination will reduce the difference in genetic merit between the nucleus and commercial population. The annual genetic improvement of commercially available cloned embryos and, therefore, of the milk producing population, however, will be almost the same as the genetic change in the absence of cloning. Thus, commercial use of clones in dairy cattle production will increase the genetic level of the commercial population only once. This increase, however, will be permanent if clones remain to be used.

In deciding to use cloning in dairy cattle production, ethical aspects of cloning should be evaluated also. This is not only my personal opinion, but in future assessment of ethical aspects of new animal biotechnology will be required officially in the Netherlands. In 1993, a Animal Health and Welfare Act was passed, which states that various, in future documents to be specified animal biotechnology techniques, will not be permitted unless approved by the Minister of Agriculture, Nature-management and Fisheries. Consent will only be given if there are (1) no unacceptable effects on health and welfare of the animals and (2) there are no ethical objections. The Minister will install an independent board to review the ethical aspects of the relevant biotechnology and to advice on the ethical acceptability of the technique.

Following EU-terminology, biotechnology is defined as: "All the techniques that use or cause changes in biological material (such as animal or plant cells or cell lines, enzymes, plasmids and viruses), micro-organisms, plants and animals; or that cause changes in an organic material by biological means". According to this definition, making beer from grains using live yeast cells, for instance, is biotechnology because it causes changes in organic material (grains) by biological means (using live yeast cells). Breeding of animals is biotechnology because it uses and causes changes in their genotype. Hence, biotechnology is already very old and not always controversial. New biotechnologies, however, which directly operate on the embryonic or DNA-level, are controversial and might be seen as a break of trend. According to a Dutch advisory committee on ethical questions concerning

animal biotechnology, which was installed in 1989 and which reported on these issues in 1990, three groups of animal biotechnology-techniques should not be permitted unless consequences on animal health and welfare, and ethical objections have been assessed. These groups are (i) transgenesis (ii) embryo manipulation as cloning and formation of chimaeres and (iii) administration of substances obtained as a result of biotechnology.

In conclusion, an evaluation of the possibilities of cloning in dairy cattle production is not complete without taking account of ethical issues involved. In this chapter, ethical aspects of the use of clones in combination with *in vitro* production of embryos in dairy cattle breeding will be discussed. First, however, by way of general introduction, aspects relevant in an ethical discussion are described to make the reader familiar with some methods and principles of ethical evaluation.

Ethical theory

Schroten (1992) gave the following popular definition of ethics: " Ethics is about systematic reflection on morals and morality, i.e. on what we ought (not) to do and why (not)." This reflection is done by systematic reasoning based on moral theory. The two most important approaches in ethical theory are (i) the utilitarian approach and (ii) the deontological approach. Each approach is based on a different normative theory, namely utilitarianism and deontology. Each theory will be explained briefly.

Utilitarianism is a moral theory in which there is only one criterion of what is morally right, wrong or obligatory: the criterion of utility. Utilitarianism prescribes us to always pursue that action which results in the greatest balance of 'good' consequences over 'bad', e.g., good over evil, pleasure over misery (Frankena, 1973). Consider the following example, taken from Van Willigenburg *et al.* (1993). To test a new medicine that might reduce pain for patients having cancer, a hundred experimental rats will have a slow and extremely painful death. Based on the utilitarian approach, it might be morally right to test this medicine because one can argue that the good consequences outweigh the bad ones. The medicine will reduce pain in many human beings suffering from cancer (good) whereas only one hundred rats have to suffer (bad).

One problem of utilitarianism is that consequences have to be qualified, quantified and balanced against each other. It might be difficult to balance for example bad consequences for health and welfare of experimental rats versus good consequences for health and welfare of human beings. Is it allowed to use a million experimental rats to test a medicine that would only decrease pain for a small group of human beings suffering from cancer?

A second problem with this theory is that this reasoning can result in conclusions that are for many people morally unacceptable. Again consider the example of rats and cancer patients. As an alternative to spending money to develop a medicine against pain, the money could be spent for screening people having a high risk of developing cancer. The alternative may save more human lives than developing a medicine against pain. When we compare only consequences of various alternative actions, we might better invest the money in screening programs. This means, however, that people having cancer might suffer a lot of pain which is morally unacceptable.

This last objection is one reason why another approach, the so-called deontological one, has a strong appeal. In deontological theories, decisions on moral (un)acceptability of an action do not depend (only) on the good or bad consequences of this action, but depend (also) on moral principles that are not necessarily related to the consequences of the action. Consider again the example taken from Van Willigenburg *et al.* (1993) on testing a human medicine against cancer using experimental rats. Based on utilitarianism, it might be best to invest money in prevention of cancer by screening people with a high risk of developing cancer. This would mean, however, that people suffering from cancer would have a lot of pain, which is contrary with the moral principle "not to harm human beings". In deontological theory, investing money in cancer prevention instead of in developing medicine against pain might therefore be morally unacceptable.

In practice, however, it is often very difficult to sustain an ethical position solely on deontological principles, because these principles might conflict. For example, assume we believe that we should not harm human beings and therefore we should invest money in developing medicine against pain for cancer patients. A result of developing new medicine, however, is that we will harm animals. Harming animals is in conflict with the moral principle that we should not harm animals. Hence, deontological decision-making will also require balancing of good over bad.

A more cautious and less theory-driven approach of ethical decision-making is suggested by Van Willigenburg and Heeger (1989), and referred to as the 'network-model'. A network model is a variant of a reflective-equilibrium model, as originally suggested by Rawls (1972). It starts with determining fundamental principles that together constitute a framework for the detection of ethical issues in a given situation. In terms of ethical relationships between human beings four principles have originally been defined by Beauchamp and Childress (1983): beneficence, non-maleficence, justice and autonomy. According to Mepham (1993), these can be translated as: kindness, harmlessness, fairness and freedom. In the context of animal biotechnology, the following four fundamental moral principles

concerning our relationship with animals are suggested by Van Willigenburg *et al.* (1993):

(1) the principle of beneficence,
(2) the principle of non-maleficence,
(3) the principle of justice,
(4) the principle of respect for the integrity of animals.

Based on a Dutch advisory committee concerning animal biotechnology, which reported in 1990, two additional principles are considered in this discussion:

(5) the principle of irreversibility
(6) the principle of democratic control

Moral principles

The principle of beneficence and non-maleficence

According to the principle of beneficence, we should promote animal health and welfare, whereas according to the principle of non-maleficence, we should not harm animals. Here harm is defined as pain, suffering, discomfort, illness and poor welfare (Rutgers, 1993). A principle of non-maleficence points to consequences of a particular action, and seems to point at the direction of a utilitarian approach. It is called a deontological norm, however, because we consider it as setting a limit to our actions; that is, we will not accept a particular great, predefined, harm to animals regardless of the goal.

To illustrate both principles, consider the following examples from Van Willigenburg *et al.* (1993), which relate to human beings. Based on the principle of non-maleficence, I should not drive when I am drunk, because I could more easily harm other people. This does not imply that if I do not drive when I am drunk, I have done a benefit. Taking a pedestrian who got sick to a hospital is based on the principle of beneficence and not on the principle of non-maleficence.

The principle of justice

In our justice towards animals, two levels can be distinguished (Rutgers, 1993):

(1) Equal treatment of animals of comparable species.
(2) Fair distribution of good (benefits) over evil (costs) between humans and animals.

The former level can be illustrated with the following example taken from Rutgers (1993). Generally, cats are anaesthetized when castrated, whereas male piglets are not anaesthetized when castrated. It is generally assumed that cats and pigs are similar in their perception and experience of pain. Consequently, the principle of justice requires equal treatment of both cats and pigs during castration.

The second level of the principle of justice is of major importance for animal production. It means, for example, that the good received from the production of food from animal origin should be in fair proportion with the bad experienced by those animals, such as possible curtailment of animal welfare. To apply this second level of the principle of justice, we have to balance the interests of animal and humans. Linskens *et al.* (1990) distinguished fundamental and non-fundamental interests. Fundamental interests include everything animals and humans need to survive and to express their species-specific nature. Examples of fundamental interests are eating and drinking, freedom of movement, protection against cold and heat, and having social contact (Rutgers, 1993). All other interests are non-fundamental, e.g. using make-up or production of colouring matters. The way people balance fundamental and non-fundamental interest of animals and humans might differ.

The principle of respect for the integrity of animals

Integrity of the animal is an important concept in the Dutch discussion on research or application of animal biotechnology. This concept, however, is not always clearly defined. In literature, integrity is also referred to as intrinsic value, autonomy or naturality of animals (Vorstenbosch, 1993). As defined by Vorstenbosch (1993), integrity means "wholeness", "intactness", an "unharmed or undamaged" state of something. Several dimensions of integrity should be distinguished: for animals physical and genetic integrity seem of importance. For example, genetic integrity means "intactness" of the genome. Hence, introducing foreign genes into an animal by means of transgenesis damages the genetic integrity of an animal.

Secondly, the subject of integrity should be qualified: humans, animals, plants or an eco-system. Recognizing only the integrity of humans might lead to anthropocentric reasoning, whereas recognizing the integrity of the world as an eco-system might lead to eco-centric reasoning. Respecting the integrity of individual animals is part of a zoocentric ethic.

The strict application of the principle of integrity of an animal might easily lead to conclusions that differ from conclusions we reach by application of the principle of beneficence or non-maleficence. The integrity of an animal might be damaged without damaging its health or welfare. For example, introducing a foreign gene into an animal affects its genetic integrity without necessarily having negative

effects on its health or welfare. Similarly, cloning of embryos affects the integrity of an embryo, whereas health and welfare of the resulting animal might be unaffected. A second point is that integrity of an animal is an attribute of its body or genome. Integrity cannot be attributed to a group of animals, as health can. For instance, general health of animals might be assessed as the percentage of sick animals in a group, whereas 'general' integrity, ranging over several animals, is a strange idea and may even be incoherent (Vorstenbosch, 1993). Integrity is an 'individual-regarding' concept, which is often associated with 'rights of an individual animal' and set against 'well-being' as a 'group-regarding' concept. Because of these concepts, 'integrity' as a criterion for the acceptability of biotechnology, is a discrete yes or no criterion, whereas for example health is a continuous criterion*.

The principle of irreversibility

The principle of irreversibility means that one should act in such a way that one can redress consequences of the action. This implies that all consequences of a particular action need to be assessed, because unforeseen consequences might become irreversible. In practice, however, it is not possible to assess all consequences of an action. This means that introduction of a new biotechnology implies taking a risk. According to the principle of irreversibility, however, this risk should be minimized.

The principle of democratic control

This principle states that biotechnology for the problem areas mentioned require public nature and democratic control. A public debate on biotechnology should be stimulated. Inventory of various ideas via a public debate will give information on formation of a framework for ethical decision-making. In addition, with a public debate, we preclude the possibility that only direct parties and experts can influence ethical decision-making.

Decision-making

Moral decision-making according to the network model means balancing of moral intuitions, moral principles and morally relevant facts so as to make a decision on a particular case. For decision-making on a particular action, we have to consider the following steps (Rutgers, 1993):

*Note: of course, one could state that what counts in ethics is the health and welfare of animals. The concept of welfare does not rule out this 'individual'

(1) explain moral intuitions with regard to the case to be evaluated;
(2) elucidate the case from the perspective of moral principles;
(3) trace morally relevant facts in the case.

Moral intuitions are defined by Van Willigenburg and Heeger (1989) as convictions that arise immediately and spontaneously after hearing the moral problem. Intuitive feelings towards the use of a particular technique might change after knowing all relevant facts. Hamstra and Feenstra (1989), however, showed that it is not true that people think more negatively or more positively about biotechnology after they have been extensively informed. For an ethical discussion, it is important to report moral intuitions, moral principles and to give morally relevant facts.

The final outcome on moral (un)acceptability of an action, however, will differ among people. In moral decision-making, few people will rely on either a utilitarian or a strict deontological approach. The point of view of the Dutch organisation of protection of animals concerning biotechnology is "biotechnology should in principle be prohibited regardless of the importance of the goal, because it affects the integrity of the animal " (Linskens, 1992). This might be seen as strict deontological reasoning using the principle of integrity of animals. The "no, unless" policy of the Dutch government states " biotechnological activities are prohibited, unless (i) it is unreasonable to think that relevant values are violated (e.g. an animal's health, welfare and integrity) or (ii) the goal is so important that violation of these values is overruled" (Brom and Schroten, 1993). Thus, the "no, unless" policy of the Dutch government might be seen as a mixture of both extreme approaches: it balances good and bad consequences, while taking into account deontological principles of beneficence, non-maleficence, justice, the integrity of the animal, irreversibility and democratic control.

Introduction of case: use of clones in dairy cattle breeding

The dairy cattle cow population can be divided into two populations: a breeding population and a milk producing population. Selection and breeding of animals is organised by dairy cattle breeding or AI (Artificial Insemination) organisations. In addition, breeding organisations sell, or disseminate, genetic material (semen) from superior sires to producers as so to breed a new generation of genetically improved milking cows. Thus, a breeding organisation is responsible for (i) the genetic improvement of the breeding population, and for (ii) dissemination of superior genetic material. Genetic improvement of the milk producing population is, therefore, dependent on genetic improvement of the breeding population (genetic response), as achieved by the breeding organisation.

In this thesis, the value of using clones for increasing both the genetic response and dissemination was studied. Results show that using clones does not increase the genetic response considerably. The main advantage of cloning is improved dissemination of superior genetic breeding material to the milk producing population by using cloned embryos in addition to semen. Use of clones in dairy cattle production, however, will increase the additive genetic level of the milk producing population only once. This increase, which is permanent if clones are remained to be used, might be equal to 5 to 10 times the annual genetic response (De Boer and Van Arendonk, 1994b). Subsequently, annual improvement of the milk producing population will be the same as in the absence of cloning (Figure 1).
We have to keep in mind that obtained results describe maximal gains from application of cloning, because we assumed throughout this thesis that it was possible to produce a large number of embryos *in vitro*, to clone both fresh and frozen embryos and to produce a large number of genetically identical individuals using nuclear transfer.

An alternative to using clones in dairy cattle production is not using clones. In that case, milk production efficiency might be increased using current breeding techniques or improved feeding or management techniques.

The **central moral problem** in this case can be put as: is it morally acceptable to generate clones to increase the average genetic level of the milk producing population for relevant or economically important traits ?

Before we can to discuss moral acceptability of this case, we must define which traits are relevant or economically important. Generally, the goal in dairy cattle production is to generate an economically and biologically efficient milk-producing cow. This is a healthy cow that produces a sufficient amount of milk of the desirable quality from available feed, under environmental and animal-friendly conditions. Such a cow will in this discussion be referred to as an efficient milk-producing cow. To generate such an efficient milk-producing cow, in my opinion, is morally acceptable.

For many years, dairy cattle breeding organisations selected mainly on production traits such as milk yield, and fat and protein percentage. Recently, however, we have become aware of that selection on only production traits does not lead to a biologically efficient milk-producing cow. For example, extreme selection on milk yield in dairy cattle decreased the reproductive performance of dairy cows. Therefore, in selection of animals we must not consider only milk production traits, but also, e.g., reproductive traits, health traits, and longevity.

In democratic societies, public acceptability is the final arbiter of the viability of new biotechnologies, particularly those affecting food production (principle of democratic control). Many people seem to be morally concerned about the idea of

creating genetically identical animals. Of 595 people above 16 years of age interviewed in the Netherlands, 339 (57%) had, intuitively, a negative attitude concerning cloning of dairy cattle embryos (Heijs *et al.*, 1993).

Hence, there is considerable antipathy to cloning of dairy embryos. From literature, we have tried to reconstruct moral concerns on use of clones in dairy cattle production:

(1) techniques are likely to be misused on human beings
(2) techniques might have negative effects on animal health and welfare
(3) techniques might interfere with the animal's integrity
(4) genetic variation is likely to diminish
(5) increase of dependence of milk producers on breeding organisations
(6) stimulation of large-scale or more intense milk production and as a result increase of social or environmental problems

A discussion of above mentioned moral concerns follows.

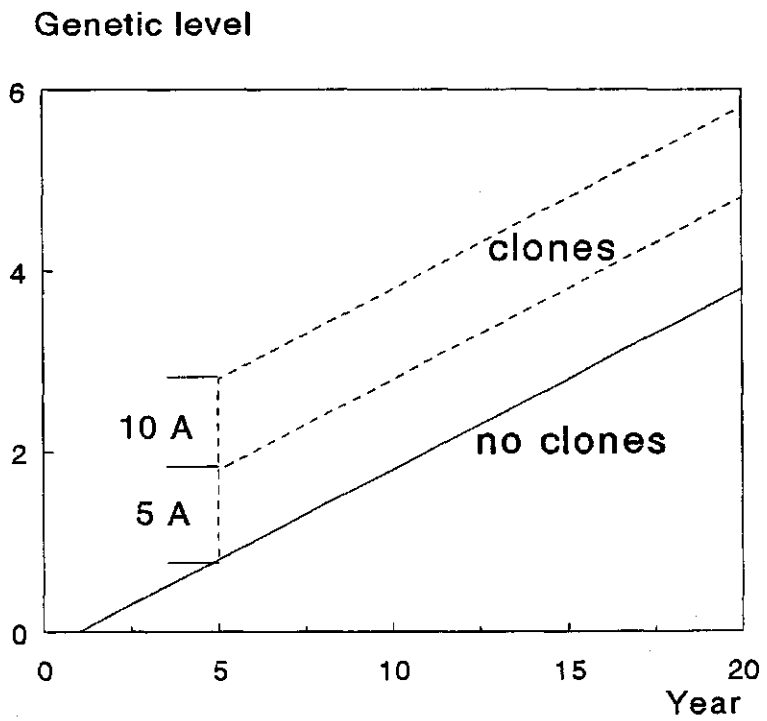

Figure 1. Genetic level of a commercial cow population using only clones (from year 5) or no clones, where A is the annual additive genetic response

Misuse of techniques on human beings

Techniques of *in vitro* production of embryos and cloning of embryos will be discussed here with respect to their (mis)use on human beings are. *In vitro* production (IVP) of embryos consists of several steps: (i) transvaginal oocyte puncturing, (ii) *in vitro* maturation of oocytes, (iii) *in vitro* fertilization of oocytes, and (iv) *in vitro* culture of fertilized embryos till morula or blastula stage (5-7 days old). Techniques are described in more detail in the general introduction (pp. 8-10).

The question here is whether use of clones (as a result of using IVP and cloning techniques) in dairy cattle production will stimulate use of these techniques in human beings. For IVP the situation seems to be the other way around. Before IVP was studied and applied in dairy cattle, the technique had already been used in human medicine. The technique for transvaginal oocyte puncturing in dairy cattle is even derived from human medicine (Rath, 1993), where it has been used routinely with much success for more than 10 years. The goal of IVP in human medicine has been to generate embryos for couples who have not succeeded in conceiving a baby.

Only recently, cloning of human embryos through splitting has been reported by Kolberg (1993). In developing a technique to clone human embryos, knowledge of techniques to clone animal embryos has probably been used. Suggested applications of human cloning include (i) generating multiple embryos for implantation after IVP for couples who produce less than 3 to 5 distinct embryos, which is normally required for IVP, and (ii) increasing the success rate for screening genetic defects of an embryo, through use of a cloned embryo instead of gene amplification.

Thus, both IVP and cloning have been achieved in human beings, although only IVP has been applied. Irrespective of moral (un)acceptability of IVP and cloning in human beings, I believe that, at this stage, use of clones in dairy cattle breeding will not stimulate (mis)use of these techniques in human beings. It is possible now to use these techniques on human beings, which by definition means that it is possible now to misuse these techniques. Misuse of these techniques is independent of whether clones will be used in dairy cattle production.

Animal health and welfare

A moral concern of using IVP and cloning in dairy cattle production is the possible negative effects on animal health and welfare. For each technique, relevant information is given below.

In vitro production of embryos. Success rates of IVP have increased considerably through extensive research (Rath, 1993). Research on anatomical and physical side effects of repeated transvaginal oocyte puncturing during IVP have been limited. Pieterse *et al.* (1991) reported a prolonged oestrus cycle after three

puncture sessions for 7% of all cows, but did not observe follicular cysts or failure to develop a corpus luteum. After slaughtering, ovaries of cows who have had repeated punctures were examined. Some haemorrhagic follicles and a thickening of the ovarian capsule were observed (Pieterse *et al.*, 1991; Van der Schans *et al.*, 1991; Simon *et al.*, 1993). Effects of IVP on animal welfare are not published. It might be expected, however, that during transvaginal oocyte puncturing, when a cow is fixated and anaesthetized, the cow will be stressed and her welfare will be damaged.

Cloning. Success rates for cloning of dairy cattle embryos have been low. Results of effects of cloning embryos on health of live calves are rarely described. The following aspects, however, have been reported by Seidel (1992):

(1) 60-70% of all calves are completely normal.
(2) Perhaps 20-30% of calves are larger than normal (up to twice normal size) at birth without prolonged gestation. After birth by Caesarian section, most large calves survive and, remarkably, develop into normal size animals within a few months. Several explanations for the high birth weight have been suggested. The real causes, however, are still unknown and require further research.
(3) Incidence of abnormalities other than large size, such as joint problems, may exceed 10% of calves.

The current nuclear transfer technique to clone dairy cattle embryos seems to result in health problems of live clones. The real causes for these problems, however, are still unknown. Further research on the effect of cloning on animal health is required. Cloning of dairy cattle embryos is of little practical value before further research has increased efficiency of the technique and decreased possible side effects on health. Effects of cloning on the welfare of live clones are not reported. With a cloning technique that results in a larger number of healthy clones, however, I believe that nuclear transfer does not have to result in welfare problems of live clones.

In conclusion, research on both IVP and cloning has been directed mainly on their technical aspects, and not on effects on animal health and welfare. As a result, little is known about the use of IVP and cloning on animal health and welfare. More extensive research on the effect of IVP and cloning on animal health and welfare is required before these techniques should be used.

Effect on the animal's integrity

The effect of using IVP and cloning on animal integrity will be discussed, were integrity, defined by Vorstenbosch (1993), is: "wholeness", "intactness", an "unharmed or undamaged state" of an animal. According to this definition, respect

for the integrity of an animal is a 'yes' or 'no' criterium. An action either does or does not interfere with the integrity of an animal.

Both IVP and nuclear transfer, in addition to artificial insemination, will further externalise the reproduction process of the cow, which means further interference with the integrity of the animal. In addition, when using IVP and nuclear transfer, the value of a cow depends increasingly on her economical value only (i.e. "instrumentalisatie van de koe"). Thus, use of IVP and cloning does interfere with the integrity of a cow.

A problem with using this principle as a 'yes' or 'no' criterion is that in animal production almost all actions interfere with an animal's integrity. According to this principle, therefore, almost all actions in animal breeding would be morally unacceptable. For a practical application of this principle, we have to determine which interference with an animal's integrity is morally acceptable and which is not. For example, changing an animal's DNA via direct insertion of new material might be seen as morally unacceptable. In that case, IVP and cloning are acceptable. We could also say that embryo manipulation is morally unacceptable. In that case, IVP and cloning are unacceptable, although certain steps of IVP, such as transvaginal oocyte puncturing, are acceptable.

I believe that the value of "the principle of respect for integrity of an animal" should be further studied and developed. I believe that this principle is important because it stresses our moral responsibility toward livestock.

Genetic variation is likely to diminish

From the principle of irreversibility, loss of genetic variation is a moral concern. Before we can discuss loss of genetic variation due to use of clones in dairy cattle production, we first have to define genetic variation at two levels: the genetic variation between breeding individuals (or genetic variation in the nucleus) and the genetic variation between milk producing cows (commercial population).

The nucleus is responsible for a continuous genetic improvement of the commercial cow population, which requires genetic variation. A decrease in genetic variation of breeding individuals, therefore, would be undesirable. Implementation of a nucleus breeding design optimal to exploit cloning does not decrease the genetic variation in the nucleus breeding population (De Boer and Van Arendonk, 1994a).

Genetic variation in the milk producing population will depend on the proportion of replacement cows belonging to commercial clone lines, which is the market share of cloned embryos compared to semen. Market share of cloned embryos is determined by the breeding scheme in the nucleus, e.g. the difference in genetic merit between available cloned embryos and semen, and other factors, such as differences in purchase price of cloned embryos and semen (De Boer and Van

Arendonk, 1994b). When only cloned embryos are used to breed replacement commercial cows, genetic uniformity in the commercial population will increase considerably. In the extreme case of using cloned embryos from **one** female genotype each year, the number of different genotypes on a farm equals the number of age classes present in years. When market share of cloned embryos is less than 35%, however, genetic variation between commercial cows does **not** decrease (De Boer and Van Arendonk, 1994b). This is because commercially available cloned embryos are likely to be unrelated to available semen. In conclusion, moderate use of cloned embryos to breed replacement commercial cows is not expected to increase genetic uniformity of the commercial cow population, whereas large-scale use of cloned embryos is expected to increase genetic uniformity considerably.

The commercial cow population is responsible for efficient milk production, but not for breeding. Losses in genetic variation between milk producing cows, therefore, will not influence possibilities for breeding. An increased genetic uniformity of a population, however, might result in other positive or negative effects. For example, a number of genetically identical cows might cause problems with formation of a group. All natural and interrelated groups seem to be stable because of differences in individual behaviour. The individual development from fertilized egg to adult individual, however, might still differ between clones due to environmental influences (Wiepkema, 1990). These differences in individual behaviour might be enough for normal formation of groups. This is an important aspect on which no information is available, that should be studied for large-scale use of commercial clone lines on dairy herds. In addition, with increased genetic uniformity in a population, individuality of cows might disappear, which can be a moral concern to some people. As with individual behaviour, however, this effect will depend on the genetic influence on 'individuality'.

Another negative effect of increased genetic uniformity in a population might be the higher risk of the spread of disease. An increased genetic uniformity, however, might also have positive effects on, e.g., standardization of milking conditions or the final milk product. Before permitting the large-scale use of clones in dairy cattle production, both positive and negative effects of increased genetic uniformity of a population should be studied.

Dependency of producers on the breeding organisation

At present, producers mainly buy semen from breeding organisations to breed a new generation of genetically improved milking cows. Producers choose semen from among a small group of preselected and tested sires. In addition, a producer selects cows to breed the offspring. Groen *et al.* (1993) showed that different producers pursue different breeding goals, which results in variation in their choice

of semen. Many producers experience choosing semen and selecting cows for breeding as an enjoyable aspect of dairy farming.

With cloning, breeding organisations might sell both semen and cloned embryos to producers for dissemination. Producers will still be able to choose between semen from different sires and, in addition, between embryos from different clone lines. For each cow, a producer has to choose between insemination or implantation. I believe that, with moderate use of clones in dairy production, a producer's dependency on the breeding organisation will not increase compared to the current situation. In the extreme situation of using only cloned embryos for dissemination, a producer will still have the opportunity to choose between different commercial clone lines. As in the current situation, a breeding organisation will offer a variety of commercial clone lines to producers so they can pursue different breeding goals. The flexibility of a producer to change the breeding goal will increase, because genetic material is disseminated more quickly from the nucleus to the commercial. The joy of selecting cows to breed, however, will be lost. In addition, it might be more difficult to buy semen for a producer who does not want to use cloned embryos.

Stimulation of large-scale (more intense) milk production

All producers can buy cloned embryos from a breeding organisations to improve the average genetic level of their cow population, and benefit financially. In contrast to investing in an automatic milking machine, for example, which might only be cost-effective with a large number of cows, the decision of a farmer to buy cloned embryos is independent of population size. In that sense, use of clones in dairy cattle production does not stimulate large-scale milk production.

The question arises whether use of clones results in intensified and large-scale milk production, due to an increase in milk efficiency. This will depend on the goal for which clones are produced. For many years, the goal was to increase output per unit of product, where the main factors were labour and land. Increasing the output per unit of labour or land intensified milk production and increased the scale of production. If we develop clones to increase output per unit of product, therefore, we will intensify milk production and increase the scale of production. However, if we pursue another goal, e.g. increasing output per unit of mineral input, use of clones does not necessarily stimulate intensive and large-scale milk production (Groen, 1994, personal communication).

Hence, use of clones in dairy cattle production does not necessarily imply stimulating intensive, large-scale milk production. On the other hand, only wealthy and progressive breeding organisations might start production of commercial clone

lines, which might result in more power for large breeding organisations, whereas small organisations may disappear.

Moral decision-making

As described previously, moral decision-making means balancing moral intuitions, morally relevant facts and moral principles for each particular case. The final outcome on moral (un)acceptability of an action therefore will differ between people. Different people have different fundamental attitudes towards animals and nature. These can be of overriding influence on the way they balance intuitions, facts and principles in the case of cloning. Following Kockelkoren (1993), the difference in decision-making between people is demonstrated using three types of people (reasoners): the ruler, the steward and the partner/participant. For each type its fundamental attitude towards animals and nature is defined, and a **possible** decision on application of cloning is given. Following the government policy, the final outcome on moral acceptability of a case can be: "yes", "yes, provided that", "no, unless" and "no".

The ruler. The ruler thinks one can arrange nature for human survival. For the ruler, nature is a source of raw material, functional for human use. The ruler only respects the integrity of human beings. Nature has to be conquered, managed and controlled for optimal pleasure. In nature, natural selection based on trial and error generates successful genotypes. Which genotypes are successful depends on the environment, e.g. food supply, the presence of parasites and predators. Biotechnology uses this trial and error process more efficiently, because it uses only preselected genotypes and it generates fewer misfits.

The ruler is dynamic and a trend-breaker, and searches for maximal utility (utilitarianism). While searching for maximal utility, however, the ruler follows juridical and economic rules of democratic society (respect for the principle of democratic control).

New techniques can be used to make and keep the world hospitable for human beings, without needlessly harming other people or other live forms that can feel pain. A new technique is acceptable if its risks are predictable and controllable (respects principle of irreversibility). We should not, however, exaggerate the importance of risks of new biotechnologies, because risks of nature are enormously high. The ruler does agree with humanisation of nature.

The possible increase of large-scale production due to new biotechnologies is no problem as long as it does not affect global biodiversity. If it does affect biodiversity, this can be preserved by keeping wildlife or by creating gene banks.

Decision: **Yes**. A ruler thinks one should use nature as efficiently as possible,

which means that one should pursue maximal utility (as in utilitarianism). Using clones in dairy cattle production results in production of more efficient milking cows and does not needlessly harm animals. For the ruler, increased milk efficiency outweighs possible negative effects of IVP on animal welfare (no needless harm).

Compared to the risks of nature, the risks of using cloning in dairy cattle production are small and acceptable. Genetic diversity is preserved because the diversity of the breeding population is not affected by using clones. Negative effects of increased genetic uniformity of the milk producing population due to large-scale use of clones should not be exaggerated, and are for the ruler acceptable.

The steward. The steward also uses nature for human survival (anthropocentric), but not at any cost. Nature is not just a source of raw materials but must be used with care. The steward not only cares about people but also cares about other forms of life, independent of whether or not they can feel pain.

Biotechnology with animals is acceptable, but not at any cost (deontological reasoning). The problem is the ordering of the intrinsic value of different life forms (the principle of justice). For a steward, fundamental human interests are more important than fundamental animal interests. Generally, fundamental interests of animals are more important than non-fundamental, economic human interests, unless such an economic human interest serves another fundamental human interest. For example, animal experiments to develop human medicine are acceptable, whereas animal experiments to test make-up are unacceptable. Testing make-up only has an economic interest and does not serve another higher fundamental interest. Whether or not a particular economic interest serves a fundamental interests should be determined for each particular case.

In contrast to the ruler, the steward does not consider new biotechnologies as morally neutral. Biotechnology is not morally neutral because it implies further "instrumentalization" (considering something merely as a production machine) of nature, which does not mean, however, that all new techniques should be prohibited. A technique should only be applied if it does not interfere with the homeostasis of various species and larger eco-systems. Hence, integrity is defined at the level of an individual species or of an individual eco-system. The integrity of nature as developed from evolution is very important to the steward.

The steward does not in principal reject humanisation of nature. But humanisation of nature should be done while respecting nature.

Large-scale production and loss of genetic diversity is unwanted, and should not lead to universalisation of landscapes.

Decision 1. **"Yes, provided that"**. The steward still uses nature for human survival but not to any extent. This means that a steward is still weighing good over bad

consequences, but the final outcome on a moral case is restricted by a deontological norm: integrity of nature (a whole eco-system) as developed from evolution. In weighing good over bad consequences, a steward believes that economic human interests are less important than fundamental animal interests, unless economic interests serve a higher fundamental interest. Use of clones in dairy cattle breeding will result in a more efficient milk production and hence contribute to food supply. A more efficient milk production might, according to a steward, be seen as an economic interest that serves a higher fundamental interest, namely food supply. As a result, increasing efficiency of milk production is more important than fundamental interests of animals (e.g. controlling their own reproduction).

The steward, however, does not want to harm other forms of life. Hence, use of IVP and cloning techniques should minimize effects on animal health and welfare. Techniques should be used only after these effects have been studied and minimized. In addition, use of clones in dairy cattle production should not interfere with the integrity of larger eco-systems. Use of clones is only acceptable if it does not result in disordering of homeostasis of other species and larger eco-systems.

2. "**No**". In a situation of surplus milk production, however, a steward might also conclude that a more efficient milk production does not serve a higher fundamental interest, namely food supply. As a result, use of clones is not acceptable.

The partner/participant. The partner believes that nature is a combination of various forms of life, where each form has it own expression and intrinsic value. This means that a partner recognizes the integrity of an animal and an eco-system. This idea of nature does require a respectful treatment of nature, including animals. Human beings differ from other life forms because they not only participate biologically in nature, but they also can decide on their relationship with nature. This freedom is expressed in the role of partnership (or participation) in nature, having respect for other life forms.

People might use new techniques only without forcing other life forms. This means that sometimes human interests are less important than animal interests, even if we talk about fundamental human interests. In a situation of surplus milk production, as in the Netherlands, increasing milk efficiency, is not regarded as a fundamental human interest.

Biotechnologies should operate within the margins of the homeostatic capacity of all elements of nature.

Loss of genetic diversity is not acceptable because interactions between life forms are lost. Biodiversity in agriculture is considered to be of great value.

Decision: **No**. The partner will not accept use of clones in dairy cattle breeding

to increase efficiency of milk production. For the partner, IVP and cloning interfere too much with the integrity of the animal and might affect animal welfare. This does not outweigh a non-fundamental human interest, such as increasing efficiency of milk production in a situation of surplus production. In addition, possible negative effects of increased genetic uniformity of the milk producing population (e.g. problems with group formation, loss of individuality of cows) are unacceptable. Therefore, use of clones in dairy cattle breeding is unacceptable.

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Summary

Dairy cattle breeding organisations sell mainly semen from sires of high genetic merit to producers to breed the next generation of genetically improved milking cows. For continuous genetic improvement of commercially available semen, breeding organisations select and breed animals of high genetic merit. As described in the general introduction, the reproductive rate of animals has a large impact on selection and breeding of animals and on the way genetic material can be disseminated from the breeding population (nucleus) to the commercial population (commercial). In this thesis, the influence of cloning on genetic response and on dissemination was studied.

Cloning is the production of genetically identical individuals (clones). Testing several clones per genotype might affect genetic response through effects on selection accuracy, selection intensity and inbreeding. In addition, cloned embryos from desirable genotypes can be used for dissemination. Unlike for semen, the use of cloned embryos can exploit both additive and non-additive genetic effects. Throughout this thesis, dominance variance was assumed to be the only source of non-additive genetic variance common to clones. To study the potential role of cloning, it was assumed that it is possible to produce a large number of embryos *in vitro*, to clone both fresh and frozen embryos and to produce a large number of clones using nuclear transfer. Cloning of frozen embryos allows testing of various female genotypes for the trait of interest (e.g. milk production) and subsequently cloning stored frozen embryos from selected females.

The aim of this thesis was to determine a breeding scheme that optimally uses large scale production of clones. Such a breeding scheme should optimize both the genetic response in the nucleus and the response to selection of the best female genotype to produce cloned embryos for dissemination (clonal response). The effect of testing several clones per genotype on the underlying components of genetic and clonal response was studied. For this purpose, a better understanding was needed of methodologies to predict an individual's additive and dominance effect in populations with inbreeding. Once the optimal breeding scheme was determined, relevant factors influencing market share of commercially available cloned embryos compared to semen were determined. In addition, ethical aspects of the use of clones in dairy cattle production were discussed.

Selection was for a single trait associated with lactation, denoted as milk efficiency. The breeding goal for genetic selection is additive genetic merit for milk efficiency, whereas the breeding goal for clonal selection equals additive plus dominance genetic merit for milk efficiency.

In Chapter 1, genetic and clonal responses were maximized independent of each other using deterministic simulation, by varying the mating design in a closed dairy cattle adult nucleus in which 256 or 1024 cows were tested each year.

Inbreeding was not simulated. The mating design was characterized by the number of full sibs, maternal and paternal half sibs and the number of clones tested per genotype. Genetic response was always maximal when only one individual per genotype was tested. Testing several clones per genotype at the expense of testing full sibs, paternal or maternal half sibs reduced selection intensity relatively more than it increased accuracy of female selection. Clonal response, however, was maximal with 1 to 32 clones tested per genotype and a maximum number of full sibs. Designs that maximized genetic and clonal response were different unless dominance variance and intensity of clonal selection were both low and unless all male full sibs were available for selection. Differences between designs optimal for genetic and clonal selection were largest when heritability and intra-clone correlation were low and clonal selection intensity was high.

In the deterministic study in Chapter 1, testing clones at the expense of testing sire or dam families was not considered because this was expected to increase inbreeding considerably, and inbreeding was not simulated. Before these alternatives could be studied, a better understanding was needed of methodologies to predict individual additive and dominance effects with inbreeding. Inbreeding might reduce the mean phenotypic performance of individuals and complicates the genetic covariance structure of the population.

In Chapter 2, an approximate method to predict individual additive and dominance effects was studied in (un)selected populations with inbreeding, using a genetic model with 64 or 1600 unlinked biallelic loci, each with an equal effect. This approximate method accounts for inbreeding depression by including the inbreeding coefficient as a covariate in the model while ignoring changes in the genetic covariance structure of the population. Despite a high mean inbreeding coefficient (up to 0.35), predictions of additive and dominance effects were empirically unbiased in the absence of selection. With intense phenotypic selection and only 64 loci, however, predictions of additive and dominance effects were significantly biased. Biases disappeared with the 1600-loci model. Bias was due to a considerable change of allelic frequency with phenotypic selection. Ignoring changes in genetic covariances associated with dominance did not significantly bias average predictions of additive and dominance effects in selected and unselected populations with inbreeding. However, neglecting these changes might result in considerable over- or underprediction of additive and dominance effects of individuals in each generation, although predictions are unbiased on average.

To get more insight into correct prediction of individual additive and dominance effects, theory on prediction of individual additive and dominance effects with inbreeding is described and further developed in Chapter 3. Chapter 3 shows that there is a linear method that results in theoretically exact prediction of additive

and dominance effects with inbreeding. This exact method, however, is not suitable for implementation in large livestock populations, whereas the approximate method can be implemented for large populations. From a comparison of approximate and exact predictions of additive and dominance effects in unselected populations, we concluded that the approximate method yielded unbiased predictions of additive and dominance effects in each generation with only slightly reduced accuracies of selection.

The approximate method, therefore, was used to predict individual additive and dominance effects in a stochastic simulation model that was developed to study testing of clones at the expense of sire and dam families, accounting for inbreeding. In addition, the model used the concept of the approximate method to simulate additive and dominance effects with an infinitesimal model. At first, the stochastic model was used to optimize the cumulative genetic response to 30 years of selection, corrected for effects of inbreeding, in a closed (1024 cows tested per year) dairy cattle nucleus scheme, in the absence of cloning (Chapter 4). It was assumed that the number of gametes available per female was large. Cumulative genetic response (P) was corrected for variance reduction due to inbreeding and inbreeding depression in the commercial cow population. Various hierarchical and factorial designs with fewer sires than dams, an equal number of sires and dams, or more sires than dams were compared for P. Sires and dams were available for selection at either 15 or 27 months of age. All full sibs could be selected. In the absence of inbreeding depression, a complete factorial scheme with more sires than dams resulted in the highest P. With increasing inbreeding depression, the optimal number of sires increased relatively more than the optimal number of dams. Increasing the number of sires decreased inbreeding relatively more than increasing the number of dams, and resulted in a relatively higher P. This is because correlations between estimated additive effects of male selection candidates are higher than between those of female selection candidates.

The stochastic model was used in Chapter 5 to study the effect on the genetic and clonal response of testing clones at the expense of testing full sibs, paternal or maternal half sibs, and sire or dam families. The reference design, as determined in Chapter 4, optimized the genetic response corrected for inbreeding in the absence of cloning. When only additive gene action was considered, testing clones at the expense of testing sire families, matings per dam or full sibs per family reduced genetic response, while it increased clonal response and inbreeding. Testing clones at the expense of testing dam families, however, added to both the genetic and clonal response without increasing inbreeding. When eight clones were tested at the expense of dam families, both the genetic response and the final genetic level of commercially available cloned embryos were maximal. Accuracy of clonal selection

equalled 0.83. With dominance gene action, however, testing two clones at the expense of testing dam families maximized both genetic response and the final genetic level of cloned embryos, irrespective of the level of inbreeding depression (accuracy of 0.72). Hence, reliable commercial clone lines can be produced now and in future generations by testing clones at the expense of testing dam families.

In conclusion, testing clones at the expense of testing dam families is optimal for both long- and short-term clonal selection. A breeding organisation that is going to sell cloned embryos will be interested in the market share of commercially available cloned embryos compared to semen. Results from Chapter 6 show that this market share is determined by: the difference in genetic merit between cloned embryos and available semen ($\Delta_{Gc\text{-}As}$), the annual additive genetic response achieved in the nucleus (A), and the difference (δ_c) in costs required to breed an offspring from either insemination by semen or implantation of a cloned embryo. In addition, market share was affected by characteristics of the commercial cow population before the introduction of clones, i.e. use of sexed or unsexed semen. Increasing $\Delta_{Gc\text{-}As}$ and A increased the market share of clones, whereas increasing δ_c decreased the market share. The market share of cloned embryos influences (i) the increase in genetic level of the commercial due to using clones and (ii) the genetic uniformity of the commercial cow population. The maximal increase of the commercial genetic level due to using clones equals 5 times A without dominance and 10 times A with dominance.

In Chapter 7, ethical aspects of the use of clones in combination with *in vitro* production (IVP) of embryos in dairy cattle breeding were discussed. The approach for moral reasoning that was followed started with determination of six fundamental moral principles that together constitute a framework for detection of ethical issues for a given situation. These moral principles were: the principle of beneficence, the principle of non-maleficence, the principle of justice, the principle of respect for the integrity of the animal, the principle of irreversibility and the principle of democratic control.

On the basis of the principle of non-maleficence, possible negative effects on animal health and welfare are morally relevant facts. Research on both IVP and cloning techniques have been directed mainly on the technical aspects and not on effects on animal health and welfare. As a result, little is known on effects of IVP and cloning on animal health and welfare. More extensive research on the effect of IVP and cloning on animal health and welfare, however, is required before these techniques should be used.

IVP and cloning do interfere with the integrity of the animal. The cow's integrity is infringed upon because the reproduction process is further externalised and because a cow's value depends increasingly on her economical value.

On the basis of the principle of irreversibility, the effect of using clones on genetic diversity of the breeding population is a morally relevant fact. Implementation of a breeding scheme optimal for a situation to exploit cloning does not decrease genetic diversity in the breeding population. Losses in genetic diversity in the commercial cow population due to use of clones are not irreversible. An increased genetic diversity of the commercial cow population, however, might result in other positive or negative effects (e.g. increased risk of disease spreading). Moderate use of cloned embryos for dissemination will not increase genetic uniformity in the commercial population, whereas large-scale use of cloned embryos for dissemination will increase genetic uniformity considerably.

Use of clones increases the genetic level of the commercial cow population only once. This increase, which is permanent when clones remain to be used, is equal to 5 to 10 times the annual genetic response. Use of clones might therefore increase the producer's benefits from milk production (principle of beneficence for the producer).

Moral decision-making means balancing of moral intuitions, moral principles and morally relevant facts in order to make a decision on a particular case. The final outcome on moral (un)acceptability of an action will, therefore, differ between human beings. In Chapter 7, decision making on use of clones was demonstrated using three types of people with different fundamental attitudes towards animals and nature.

Main conclusions of this thesis

- Exact best linear unbiased prediction (BLUP) of individual additive and dominance effects in populations with inbreeding is theoretically possible, but computationally not feasible for large populations.
- Approximate BLUP that accounts for inbreeding depression while ignoring changes in dominance related (co)variances due to inbreeding, is computationally feasible for large livestock populations. Approximate BLUP results in unbiased predictions of individual additive and dominance effects with only slightly reduced accuracies of selection in populations with inbreeding.
- With an efficient technique to produce embryos *in vitro* (IVP), the optimal intensity of sire and dam selection in a breeding program is no longer determined by their reproductive capacity, but by the information available to determine the selection criterion and by inbreeding. Hence, in a dairy cattle breeding program without bull progeny testing, it is optimal to select more sires that dams.

- Given a fixed number of cows tested each year, testing clones at the expense of sire families, half sibs or full sibs does decrease the annual additive genetic response, whereas testing clones at the expense of dam families does not decrease (with dominance) or even slightly increases (without dominance) annual additive genetic response.
- Given a fixed number of cows tested each year, testing clones at the expense of dam families only slightly affects the annual additive genetic response, whereas it maximizes the final genetic level of cloned embryos available for dissemination.
- The main advantage of cloning is a faster dissemination of genetically superior breeding material to the commercial cow population.
- Use of clones instead of semen for dissemination results in a maximal increase of the average genetic level of milking cows of 5 to 10 times the annual genetic response, depending on the amount of dominance variance.
- Market share of cloned embryos compared to semen is, apart from the efficiency of the cloning technique and ethical aspects, determined by: the difference in genetic merit between embryos and semen, the annual additive genetic response, the difference in costs required to breed an offspring from insemination or from implantation of a cloned embryo and characteristics of the commercial cow population (i.e. use of (un)sexed semen).

Samenvatting

Voor de produktie van een nieuwe generatie, genetisch verbeterde melkkoeien kopen melkveehouders voornamelijk sperma van hoogwaardige stieren van fokkerijorganisaties. Via de selektie en het paren van genetisch hoogwaardige dieren zijn fokkerijorganisaties verantwoordelijk voor de kontinue verbetering van dit beschikbare sperma. Zoals beschreven in de algemene inleiding, beïnvloedt het voortplantingsvermogen van dieren de mogelijkheden voor de fokkerij en de manier van doorgeven van hoogwaardig genetisch materiaal van de fokvee- naar de melkproducerende populatie. In dit proefschrift is de invloed van het gebruik van klonen op de genetische vooruitgang en op de doorgifte van hoogwaardig fokveemateriaal naar melkveehouders bestudeerd.

Klonen zijn genetisch identieke dieren. Het testen van meerdere klonen per genotype in de fokveepopulatie kan de genetische vooruitgang beïnvloeden via de nauwkeurigheid en intensiteit van selektie en via de mate van inteelt. Daarnaast kunnen gekloonde embryo's worden gebruikt voor de doorgifte van hoogwaardig fokveemateriaal naar melkveehouders. In tegenstelling tot sperma, kunnen gekloonde embryo's naast additieve effekten eveneens niet-additief genetische effekten benutten bij de doorgifte van genetisch materiaal van de fokvee- naar de melkveepopulatie. In dit onderzoek is de niet-additief genetische variantie, die klonen gemeenschappelijk hebben, beperkt tot dominantie-variantie. Verder is verondersteld dat het technisch mogelijk is een groot aantal embryo's *in vitro* te produceren en zowel verse als ingevroren embryo's ongelimiteerd te klonen. Het klonen van ingevroren embryo's biedt de mogelijkheid tot het testen van diverse koeien in de fokveepopulatie voor economisch belangrijke kenmerken (bijv. melkproduktie), om vervolgens de ingevroren, gekloonde embryo's van alleen de béste koeien te klonen voor commercieel gebruik.

Het doel van dit onderzoek was het ontwerpen van een fokprogramma dat optimaal gebruik maakt van grootschalige produktie van klonen. In zo'n fokprogramma moeten twee responsen geoptimaliseerd worden: de genetische respons in de fokveepopulatie en de klonale respons. De klonale respons is gelijk aan de genetische superioriteit van koeien die geselekteerd zijn voor de produktie van gekloonde embryo's voor commercieel gebruik. Het effekt van het testen van meerdere klonen per genotype op de onderliggende componenten van zowel de genetische als de klonale respons is bestudeerd. Hiervoor was meer inzicht nodig in de methodologie van het schatten van individuele additieve en dominantie-effekten in ingeteelde populaties. Na vaststelling van het optimale fokprogramma zijn de relevante faktoren, die het marktaandeel van klonen ten opzichte van sperma beïnvloeden, bestudeerd. Tevens zijn de ethische aspecten van het gebruik van klonen in de melkveehouderij beschreven en bediscussieerd.

Het fokdoel in dit onderzoek omvat één theoretisch kenmerk dat geassocieerd is met laktatie, genaamd melkefficiëntie. Het fokdoel voor genetische selektie is de additief genetische aanleg voor melkefficiëntie, terwijl het fokdoel voor klonale selektie gelijk is aan de som van de additieve plus de dominantie-aanleg voor melkefficiëntie.

Hoofdstuk 1 beschrijft de resultaten van een maximalisatie van enerzijds de genetische en anderzijds de klonale respons, in een gesloten fokprogramma met 256 of 1024 testplaatsen. De deterministisch berekende respons werd gemaximaliseerd door het vergelijken van verschillende paringsschema's. Effekten van inteelt zijn hierbij buiten beschouwing gelaten. Een paringsschema wordt gekarakteriseerd door het aantal full sibs per familie, het aantal maternale en paternale half sibs, en het aantal geteste klonen per genotype. Dit betekent dat het testen van bijvoorbeeld twéé in plaats van één kloon per genotype alleen mogelijk is wanneer het aantal te testen full sibs per familie (òf het aantal paternale òf het aantal maternale half sibs) wordt gehalveerd.

De genetische respons was altijd maximaal wanneer slechts één individu per genotype werd getest. Het testen van meerdere klonen per genotype ten koste van het aantal full sibs, paternale of maternale half sibs verlaagde de selektie-intensiteit relatief meer dan dat het de nauwkeurigheid van selektie verhoogde. De klonale respons bleek daarentegen maximaal met één of meerdere klonen per genotype en een maximaal aantal full sibs per familie, afhankelijk van de hoeveelheid aangenomen dominantie-variantie. Schema's, die de genetische respons maximaliseerden, verschilden van schema's die de klonale respons maximaliseerden, met uitzondering van situaties waarin zowel de dominantie-variantie als de klonale selektie-intensiteit laag waren èn alle mannelijke full sibs per familie geselekteerd konden worden. De verschillen waren het grootst in situaties waarin zowel de erfelijkheidsgraad als de intra-kloon korrelatie laag waren èn de klonale selektie-intensiteit hoog was.

In de deterministische studie, die is beschreven in hoofdstuk 1, werd het testen van klonen ten koste van het aantal stier- of koefamilies niet bestudeerd, daar deze alternatieven waarschijnlijk zouden leiden tot een hogere inteelt en effekten van inteelt niet in het model waren opgenomen. Het bestuderen van deze alternatieven vereiste meer kennis van methoden voor het schatten van individuele additieve en dominantie-effekten in ingeteelde populaties. Inteelt kan leiden tot een afname van de gemiddelde prestatie van dieren, ook wel inteeltdepressie genaamd, en kompliceert de genetische kovariantie struktuur van een populatie.

Hoofdstuk 2 beschrijft de resultaten van een studie waarin individuele additieve en dominantie-effekten zijn geschat met behulp van een benaderingsmethode. Het hierin gebruikte stochastische simulatiemodel

veronderstelt een genetisch model met 64 of 1600 ongerelateerde loci, met ieder twee allelen en een gelijk effekt op het kenmerk. De benaderingsmethode korrigeert voor inteeltdepressie door inteelt als kovariabele op te nemen in het statistische model, maar houdt géén rekening met veranderingen in de genetische kovariantiestruktuur van de populatie als gevolg van inteelt. Ondanks de hoge gemiddelde inteeltcoefficiënt in de populatie (tot 0.35), bleken schattingen van additieve en dominantie-effekten empirisch zuiver wanneer ouderdieren willekeurig ('random') geselekteerd werden. Schattingen van additieve en dominantie-effekten waren echter onzuiver wanneer ouderdieren op basis van eigen prestatie intens geselekteerd werden èn slechts 64 loci werden verondersteld. Deze onzuiverheid verdween wanneer een genetisch model met 1600 in plaats van 64 loci werd verondersteld. De onzuiverheid werd veroorzaakt door aanzienlijke veranderingen in allelfrequentie als gevolg van de selektie van ouderdieren. Het verwaarlozen van veranderingen in de genetische kovariantiestruktuur van een populatie als gevolg van inteelt leidde niet tot significante onzuiverheid van schattingen van additieve en dominantie-effekten in (on)geselekteerde populaties met inteelt. Het negeren van deze veranderingen kan echter wel resulteren in een verlaging van de nauwkeurigheid van de schattingen van individuele additieve en dominantie-effekten.

Om meer inzicht te krijgen in het schatten van individuele additieve en dominantie-effekten is de hiervoor benodigde theorie beschreven en verder uitgewerkt in hoofdstuk 3. Hoofdstuk 3 laat zien dat er een methode is welke resulteert in BLUP-schattingen (best linear unbiased prediction) voor individuele additieve en dominantie-effekten in ingeteelde populaties. Deze methode is echter niet geschikt voor gebruik in grote datasets, terwijl de benaderingsmethode wel toegepast kan worden in grote populaties. Uit een vergelijking van beide methoden in een ongeselekteerde, ingeteelde populatie bleek dat de benaderingsmethode resulteerde in zuivere schattingen van individuele additieve en dominantie-effekten. De nauwkeurigheid van de schattingen verkregen met behulp van de benaderingsmethode bleek echter iets lager dan de nauwkeurigheid van de BLUP schattingen.

De benaderingsmethode is gebruikt voor het schatten van individuele additieve en dominantie-effekten in een stochastisch simulatiemodel, dat is ontwikkeld voor het bestuderen van alternatieven waarin klonen werden getest ten koste van het aantal stier- of koefamilies. Het simulatiemodel gebruikt de theorie van de benaderingsmethode voor het simuleren van individuele additieve en dominantie-effekten met behulp van een oneindige locus model. Het simulatiemodel is eerst gebruikt voor het optimaliseren van de kumulatieve genetische respons na 30 jaar van selektie in een gesloten fokprogramma (1024 testplaatsen) zonder gebruik van

klonen (hoofdstuk 4). Uitgangspunt hierbij was de mogelijkheid tot produktie van een groot aantal gameten per koe. De kumulatieve genetische respons werd gekorrigeerd voor de afname van de additief genetische variantie als gevolg van inteelt èn voor inteeltdepressie op melkveebedrijven. De kumulatieve respons van verschillende geneste en gekruiste schema's, met minder stieren dan koeien, een gelijk aantal stieren en koeien, en méér stieren dan koeien, zijn vergeleken. Stieren en koeien konden geselekteerd worden op zowel 15 als 27 maanden. Alle mannelijke en vrouwelijke full sibs per familie konden geselekteerd worden. Zonder inteeltdepressie bleek een volledig gekruist schema met méér stieren dan koeien te resulteren in een maximale kumulatieve respons. Met inteeltdepressie steeg in het optimale fokprogramma het aantal stieren relatief sterker dan het aantal koeien. Dit kan als volgt verklaard worden. Een afname van het aantal stieren leidt tot een sterkere inteelttoename dan een vergelijkbare afname van het aantal koeien, en resulteert in een relatief lagere kumulatieve respons. Dit wordt veroorzaakt door de hogere korrelatie tussen geschatte additieve effekten van stieren dan tussen die van koeien.

Het simulatiemodel is vervolgens in hoofdstuk 5 gebruikt voor het bepalen van zowel de genetische als de klonale respons in schema's waarin klonen werden getest ten koste van het aantal stier- of koefamilies, het aantal full sibs per familie of het aantal paternale of maternale half sibs. De berekende responsen werden, net als in hoofdstuk 1, gemaximaliseerd door het vergelijken van verschillende paringsschema's. Een paringsschema wordt hier gekarakteriseerd door het aantal geselekteerde stieren (stierfamilies), het aantal geselekteerde koeien (koefamilies), het aantal full sibs per familie, het aantal paternale en maternale half sibs en het aantal geteste klonen per genotype. Het uitgangsschema, zoals bepaald in hoofdstuk 4, optimaliseerde de kumulatieve genetische respons gekorrigeerd voor inteelt zonder gebruik van klonen. In een situatie met alleen additief genetische variantie, resulteerde het testen van klonen ten koste van het aantal stierfamilies, het aantal full sibs per familie òf het aantal paternale of maternale half sibs, in een afname van de genetische respons, terwijl de klonale respons en de inteelt toenamen. Het testen van klonen ten koste van het aantal koefamilies, daarentegen, verhoogde zowel de genetische als de klonale respons zonder dat de inteelt toenam. De genetische respons en het totale genetische nivo van gekloonde embryo's bleek maximaal wanneer er acht klonen per genotype werden getest ten koste van het aantal koefamilies. De nauwkeurigheid van klonale selektie was in die situatie 0.83. In een situatie met dominantie-variantie bleek de genetische respons en het genetisch nivo van gekloonde embryo's maximaal wanneer twee klonen per genotype werden getest (nauwkeurigheid van klonale selektie van 0.72). Het testen van klonen ten koste van het aantal koefamilies leidt tot de produktie van betrouwbare

kloonlijnen, nu en in de toekomst. Met andere woorden, het testen van klonen ten koste van het aantal koefamilies is optimaal voor zowel de korte als de lange termijn klonale respons.

Een fokkerijorganisatie die gekloonde embryo's wil gaan verkopen is geïnteresseerd in het marktaandeel van deze embryo's en van sperma. De resultaten in hoofdstuk 6 laten zien dat het marktaandeel van gekloonde embryo's werd bepaald door: het verschil in genetisch nivo tussen gekloonde embryo's en sperma ($\Delta_{Gc\text{-}As}$), de jaarlijkse additief genetische vooruitgang in de fokveepopulatie (A), en het verschil in kosten voor het genereren van een kalf via inseminatie met sperma of via transplantatie van een gekloond embryo (δ_c). Een verhoging van $\Delta_{Gc\text{-}As}$ of A resulteerde in een vergroting van het marktaandeel van gekloonde embryo's, terwijl een verhoging van δ_c resulteerde in een verlaging van het marktaandeel. Het marktaandeel werd daarnaast beïnvloed door eigenschappen van de melkveepopulatie vóór de introduktie van klonen, zoals bijvoorbeeld het gebruik van gesekst of ongesekst sperma. Het marktaandeel van gekloonde embryo's beïnvloedde op haar beurt de toename van het genetisch nivo en van de genetische uniformiteit van de melkproducerende populatie. De maximale toename van het genetisch nivo van de melkproducerende populatie was gelijk aan 5 tot 10 maal de jaarlijkse additief genetische vooruitgang. De hoogte van deze stijging was afhankelijk van de hoeveelheid aanwezige dominantie-variantie.

De ethische aspecten van het gebruik van klonen in combinatie met *in vitro* produktie van embryo's (IVP) in de melkveehouderij zijn bediscussieerd in hoofdstuk 7. De gevolgde ethische redeneermethode begint met het vaststellen van zes morele principes die tezamen het raamwerk vormen voor het bepalen van de ethische aspecten in een bepaalde situatie. Deze morele principes zijn: het principe van goed doen, het principe van geen kwaad doen, het principe van rechtvaardigheid, het principe van respect voor de integriteit van het dier, het principe van onomkeerbaarheid en het principe van democratische controle.

Uitgaande van het principe van geen kwaad doen zijn mogelijke negatieve effekten van IVP en klonen op de gezondheid en het welzijn van een dier moreel relevante feiten. Het onderzoek naar zowel IVP als kloningstechnieken richt zich voornamelijk op de technische aspekten en niet op de gezondheids- en welzijnseffekten op het dier. Uitgebreider onderzoek naar zowel de gezondheids- als de welzijnsaspekten van IVP en klonen is echter een vereiste voordat deze technieken toegepast zouden moeten worden.

IVP en klonen doen inbreuk op de integriteit van het dier. De integriteit van de koe, wier eicellen worden gewonnen voor IVP en wier embryo's worden gekloond, wordt geschaad door de sterkere externalisering van het reproduktieproces en door de toenemende 'verdinglijking' van het dier.

Uitgaande van het principe van onomkeerbaarheid is het effekt van het gebruik van klonen op de genetische variatie in de fokveepopulatie een moreel relevant feit. Implementatie van een fokprogramma, dat optimaal gebruik maakt van klonen, leidt niet tot een versnelde afname van de genetische variatie in de fokveepopulatie. Een afname in de genetische variatie op melkveebedrijven is daarentegen niet onomkeerbaar, maar kan wel resulteren in andere positieve of negatieve gevolgen (bijvoorbeeld een verhoogde kans op verspreiding van ziekten). Een beperkt gebruik van gekloonde embryo's op melkveebedrijven leidde niet tot een afname van de genetische variatie op deze bedrijven. Grootschalig gebruik van gekloonde embryo's op melkveebedrijven resulteerde daarentegen wel in een sterke afname van de genetische variatie op deze bedrijven.

Het gebruik van klonen leidde tot een éénmalige verhoging van het genetisch nivo van melkveebedrijven, die gelijk was aan 5 tot 10 maal de jaarlijkse genetische vooruitgang. Het gebruik van klonen kan dus resulteren in een verhoging van de netto melkopbrengsten voor de veehouder (het principe van goed doen voor de boer).

Ethische besluitvorming betekent nu het afwegen van morele intuïties, morele principes en moreel relevante feiten om vervolgens een beslissing te nemen over een bepaalde zaak. De uitkomst omtrent de ethische (on)aanvaardbaarheid van een bepaalde handeling zal daarom verschillen tussen mensen. Hoofdstuk 7 beschrijft een mogelijke ethische besluitvorming voor drie typen mensen die een verschillende grondhouding hebben ten opzichte van dieren en natuur.

Belangrijkste conclusies van dit proefschrift

- Een methode die resulteert in BLUP-schattingen (best linear unbiased prediction) van individuele additieve en dominantie-effekten in ingeteelde populaties is theoretisch toepasbaar, maar computermatig niet bruikbaar voor grote populaties.
- Het voorspellen van individuele additieve en dominantie-effekten in ingeteelde populaties met behulp van een benaderingsmethode is computermatig wel toepasbaar voor grote populaties. Deze benaderingsmethode, die corrigeert voor inteeltdepressie en die veranderingen in de kovariantiestruktuur van een populatie als gevolg van inteelt negeert, resulteert in zuivere schattingen van individuele additieve en dominantie-effekten met een iets lagere nauwkeurigheid dan de BLUP schattingen.

- Wanneer een efficiënte techniek voor de *in vitro* produktie van embryo's beschikbaar is, wordt de optimale intensiteit van selektie van stieren en koeien niet langer bepaald door hun reproduktievermogen, maar door de informatie die beschikbaar is voor het bepalen van het selektiekriterium en door inteelt. In een melkveefokprogramma zonder nakomelingenonderzoek voor stieren blijkt het dan optimaal méér stieren dan koeien te selekteren.
- Uitgaande van een vast aantal te testen koeien per jaar in de fokveepopulatie, resulteert het testen van meerdere klonen per genotype ten koste van het aantal stierfamilies, full sibs, paternale of maternale half sibs in een afname van de genetische respons. Het testen van meerdere klonen per genotype ten koste van het aantal koefamilies resulteert daarentegen in dezelfde (met dominantie) of in een iets hogere genetische respons (zonder dominantie).
- Het testen van meerdere klonen per genotype ten koste van het aantal koefamilies in de fokveepopulatie leidt tot de produktie van betrouwbare kloonlijnen, nu en in de toekomst.
- Het voordeel van klonen is een versnelde doorgifte van hoogwaardig genetisch materiaal van de fokveepopulatie naar melkveebedrijven.
- Het gebruik van gekloonde embryo's in plaats van sperma op melkveebedrijven resulteert in een maximale stijging van het genetisch nivo van deze bedrijven gelijk aan 5 tot 10 maal de jaarlijkse additief genetische vooruitgang. De hoogte van deze stijging is afhankelijk van de hoeveelheid aanwezige dominantie-variantie.
- Het marktaandeel van gekloonde embryo's ten opzichte van sperma wordt naast de efficiëntie van de kloningstechniek en de ethische aspekten, bepaald door: het verschil in genetisch nivo tussen gekloonde embryo's en sperma, de jaarlijkse genetische vooruitgang in de fokveepopulatie, het verschil in kosten voor het verkrijgen van een kalf via inseminatie met sperma of via transplantatie van een gekloond embryo en eigenschappen van de melkveepopulatie vóór de introduktie van klonen (bijvoorbeeld het gebruik van (on)gesekst sperma).

Curriculum vitae

Immelie Johanna Maria (Imke) werd op 26 juli 1966 geboren in Heerlen (Limburg). Na het behalen van het diploma Atheneum-B aan het St. Janscollege te Hoensbroek, begon zij in 1984 met de studie Zoötechniek aan de toenmalige Landbouwhogeschool te Wageningen. In maart 1989 sloot zij haar studie (afstudeervakken Veefokkerij en Gezondheids- en Ziekteleer) met lof af. Van april 1989 tot februari 1990 werkte zij als toegevoegd onderzoeker bij de vakgroep Veefokkerij. In februari 1990 begon zij als AIO (assistent in opleiding) met het promotieonderzoek waarvan het thans voor u liggende proefschrift het resultaat is. Vanaf juni 1994 werkt zij als universitair docent bij de sectie Dierlijke Produktie Systemen, behorende bij de vakgroep Veehouderij van de Landbouwuniversiteit.