MANAGEMENT OF GENETIC DIVERSITY IN SAHIWAL CATTLE BREED IN KENYA

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DECLARATION AND RECOMMENDATION

Declaration

This thesis is my original work and has not, wholly or in part, been presented for an award of a degree or diploma in this or any other university known to me.

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Recommendation

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ABSTRACT

The Sahiwal population in Kenya, which is bred under a closed nucleus, is faced with declining effective population size over the years and rate of inbreeding per generation >1% beyond which it should not exceed for a population to maintain its long-term fitness and viability. This study estimated gene origin statistics, Wright's F-statistics, current and future rates of inbreeding, coancestry and effective population size and genetic gain in lactation milk yield at predetermined rates of inbreeding. The aim was to develop a strategy to manage genetic variability at a predetermined level while achieving desirable genetic progress in the breeding goal. Since the founding population, ancestors contributed equally as shown by the ratio of f_e/f_a of 0.94, which could also be due to lack of effective selection in this population. The ratio of f_g/f_a of 0.63 indicated genetic loss of genetic variability occurred through genetic drift in the population. Predicted rate of inbreeding per generation for individuals with at least 3 and 6 complete generations were 0.82 and 1.19% and Ne of 54 and 35, respectively. Parameters of genetic differentiation (Wright's F-statistics) were F_{IS}=- 0.0071, F_{ST}= 0.0036 and F_{IT} =- 0.0034. Loss in genetic diversity for the whole population was -0.023, an indication of random genetic drift and unequal contribution of founders. When the best 2 sires were mated to the best 210 females disregarding average relationship among them (1, 0), genetic gain of 210 kg was realized accompanied by an average inbreeding and rate of inbreeding per generation of 14.5% and 4%, respectively. Restricting average relationship alone (0, -1), resulted in future rate of inbreeding of 1.6% and average merit of 154 kg. Other restrictions of merit and relationship resulted in intermediate values of rate of inbreeding per generation and genetic gain. A rate of inbreeding per generation of <1% was achieved at the same restriction level (0, -1) but using 8 top sires, which resulted in a rate of inbreeding per generation of 0.9 % accompanied by an average merit of 128.2 kg. To achieve a rate of inbreeding per generation of <1%, the size of the breeding population should be increased to at least 8 bulls and 840 females. Practical implications include improving pedigree recording, opening up the nucleus to include other institutional and private herds, in a dispersed open nucleus scheme and appreciation of the depressed genetic gain in the breeding objective.

Keywords: *effective population size, pedigree, genetic diversity, inbreeding, optimum contribution, average relationship, genetic merit*

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CHAPTER ONE

GENERAL INTRODUCTION

1.1 Background

The Kenya Sahiwal is a dual-purpose cattle breed which originated from Pakistan and India and was imported into Kenya in 1939 (Ilatsia *et al*., 2011a). It is a product of a long-term upgrading programme involving Sahiwal bulls and the Small East African Zebu (SEAZ) dams (Ilatsia *et al*., 2011a). The Sahiwal is preferred by Maasai pastoralists to other breeds due to its high milk production, growth performance, good reproductive ability (Ilatsia *et al*., 2007; Ilatsia *et al*., 2011b) and its adaptive characteristics to low-input production systems (Roessler *et al*., 2010; Ilatsia *et al*., 2011b). In Kenya, the Sahiwal is bred under a closed nucleus breeding programme (CNBP) where performance recording and selection is confined to the nucleus, and the pastoral herds are the main recipients of the resultant genetic superiority (Ilatsia *et al*., 2011b).

Nucleus breeding programmes have been advocated for genetic improvement of cattle in developing countries (Kahi *et al*., 2004) due to their ease of implementation as recording is done in the nucleus. However, CNBPs are associated with high inbreeding levels and low effective population sizes due to increased focus on a few high performing animals often closely related animals (Fernandez *et al*., 2011). High levels of inbreeding are associated with inbreeding depression, reduced genetic variation, increased sampling variance of breeding programmes and emergence of lethal recessive alleles in homozygous form (Meuwissen and Sorensen, 1998; Malhado *et al*., 2013). Difficulties in planning mating systems aimed at controlling future rates of inbreeding due to increased average relatedness (AR) among individuals have also been reported in nucleus breeding programmes (Fernandez *et al*., 2011).

In the Kenyan Sahiwal population, level and rate of inbreeding and AR are on an upward trend (Muasya *et al*., 2011; Kamiti, 2014) and are above the value of 1% per generation recommended by FAO (1998) as a standard beyond which inbreeding levels in breeding populations should not exceed. Consequently, effective population size (Ne) is declining and is currently reported to be 219 (Muasya *et al*., 2011). The reported values of Ne are below the threshold of 500 recommended by Franklin and Frankham (1998) for a population to maintain its long-term viability. Effective population size and inbreeding levels of a population reflect mainly long term effects of selection strategies and are very sensitive to incomplete pedigree information (Boichard *et al*., 1997; Muasya *et al*., 2013). On the other hand, gene origin statistics are less sensitive to pedigree completeness and are useful in accounting for the bottlenecks and explaining random loss of alleles in a population (Boichard *et al.*, 1997). However, these parameters for the Kenyan Sahiwal population are lacking. In view of the declining genetic variability in the Sahiwal, a strategy to maximize genetic gain whereas minimizing the rate of inbreeding in Sahiwal need to be put in place (Carvalheiro *et al*, 2010).

1.2 Statement of the problem

The effective population size for the Sahiwal population in Kenya of 219 is less than the threshold of 500 below which a population looses its evolutionary potential and is unable to maintain its long-term viability. Similarly level and rate of inbreeding are on an upward trend and are above value of 1% recommended per generation as a standard beyond which inbreeding levels should not exceed for a population to maintain its long-term viability. Despite these challenges, parameters that are required to understand the trend in genetic diversity such as gene origin statistics, current and future trend in inbreeding and effective population size have not been estimated. Consequently, a strategy to manage genetic diversity in the Sahiwal cattle breeding programme is lacking.

1.3 Objectives

1.3.1 Broad Objective

The overall goal of this study was to contribute to a sustainable breeding programme for the Sahiwal breed in Kenya through development of a strategy to manage genetic variability while achieving optimal genetic gain.

1.3.2 Specific Objectives

To achieve the goal, the specific objectives were:

i. To estimate the genetic variability using pedigree analysis of the Sahiwal cattle breed in Kenya
ii. To estimate within-population genetic structure and genetic diversity trend for Sahiwal cattle breed in Kenya

iii. To evaluate effect of controlling future rate of inbreeding on expected genetic gain and genetic variability of the Sahiwal cattle breed in Kenya.

1.4 Research questions

Three research questions were formulated to guide in achievement of the study objectives. They included;

i. What is the genetic variability of Kenyan Sahiwal breed?
ii. What is the within-population structure and genetic diversity trend for the Kenyan Sahiwal breed Population?
iii. What is the effect of controlling future rates of inbreeding on expected genetic gain in the Kenyan Sahiwal breed?

1.5 Justification of the study

Assessment of population structure through parameters of gene origin statistics and estimation of future trends of effective population size and inbreeding provided an understanding of the extent of loss of genetic diversity in the Sahiwal population. Evaluation of the effect of constraining future rates of inbreeding on expected genetic gains led to development of a strategy for optimizing genetic gains. It also helped in monitoring current and future trends of genetic diversity of the Sahiwal breeding programme in Kenya. This study has provided a strategy to achieve genetic gain in the breeding objective while at the same time maintaining genetic diversity of the population at levels which ensure the population maintains it fitness and long-term viability.

1.6 Limitation of the Study

The study only considered the effect on milk yield due to loss in genetic diversity Inbreeding affects various animal traits differently. Therefore, results on effect of loss in genetic variability for milk yield may not be generalized for all other traits for Sahiwal population in Kenya.

CHAPTER TWO

LITERATURE REVIEW

2.1 Introduction

The Sahiwal breed cattle in Kenya was introduced in early 1930s when breeding bulls were imported from India and Pakistan. The first bulls were imported from Pusa in India and were only three of them. After 1945, 60 Sahiwal bulls and 10 Sahiwal cows were introduced from Jahangirabad in Pakistan. Another importation of 15 Sahiwal bulls was from Karnal in India in 1964 (Muhuyi *et al.*, 1999). They were imported for improvement of the local Zebu for higher milk production and improved growth performance under low-input production conditions (Ilatsia *et al.*, 2011b). By 1962, there were 2500 Sahiwals in the 13 Livestock Improvement Centres across the country. The most recent importation was 1000 doses of semen originating from six proven Sahiwal bulls from Pakistan in 1991 for use in the Sahiwal Stud at Naivasha and other Sahiwal herds in the country (Muasya *et al.*, 2011). The promising results of this upgrading programme led to the breed gaining preference over the local Zebu among the Maasai pastoralists (Ilatsia *et al.*, 2011a).

2.2 Sahiwal Cattle Breed Characteristics

The breeds are heavily built and their colour ranges from reddish brown to chestnut, a dark brownish colour is common around the hump and neck. Males have colour that darkens towards the head, legs and tails while females maintain the reddish coat colour (Muhuyi *et al.*, 1999). They have a well-developed hump in the cervico-thoracic position. They have height at withers about 1.4 meters for males and 1.2 meters for females (Ilatsia *et al.*, 2007). Females have udders and teats that are large compared to cattle of other *Bos indicus* breeds. The breed has ears that are long and drooping. Skin coat is generally smooth and shiny especially during hot weather conditions. In comparison with other Zebu cattle breeds, Sahiwals are generally docile and of low temperament and this enables them to be milked in the absence of the calf (Ojango *et al.*, 2006; Ilatsia *et al.*, 2007; Ilatsia *et al.*, 2011b).

2.3 Production systems for Sahiwal cattle breed in Kenya

Sahiwal cattle are raised under low-input production systems by both pastoralists and ranchers, a strategy that aims to minimize the potential negative effects of genotype by environment interaction when breeding animals are exchanged (Ilatsia *et al.*, 2011b). Their purpose in low-input production systems is primarily interrelated with the family subsistence and revenue generation through sale of surplus milk and live animals. The major breeding goals of the producers are high milk production, large body size, good fertility and adaptation to local production conditions (Roessler *et al*., 2010).

In Kenya, there has been systematic crossing of purebred Sahiwal bulls with some improved indigenous zebu cows that are used as foundation stock in the grading up and multiplication of the Kenya Sahiwal cattle (Muhuyi, 1997). In dairy ranching which is practised in agro-ecological zones III and IV (highlands, sub-humid and semi-arid areas) with relatively low annual rainfall of 600-800 mm, Sahiwals and their crossbreds with exotic dairy breeds are used (Muhuyi *et al*., 1999). In this system, cattle are grazed extensively on improved and natural pastures with milking cows being supplemented with mineral licks and concentrates. There are also infrastructures that are well developed to facilitate marketing of dairy products, milk and surplus cattle (Muhuyi *et al*., 1999; Ilatsia *et al*., 2011b).

2.4 The Sahiwal cattle breeding programme in Kenya

The National Sahiwal Stud (NSS) was set up in 1962 following a breeding programme developed by the government to consolidate breeding activities by collecting the best Sahiwal cows and bulls from various livestock centres (Roessler *et al*., 2010) . The NSS is a closed nucleus research herd used for improvement of the Kenya Sahiwal cattle breed for milk and meat production. The resultant genetic gain is passed on to pastoralists and other stud herds in form of breeding stock, in form of breeding bulls and recently as semen (Ilatsia *et al.,* 2011b). Compared to other nucleus herds, the NSS keeps relatively good performance and pedigree records, which are used to support selection and management decisions (Ilatsia *et al*., 2011b). Sahiwal cattle nucleus interact with the pastoral communities that keep Sahiwal cattle through exchange of genetic material based on temporal breeding structures established over 45 years ago. These structures were envisaged to serve only on interim basis as more elaborate and systematic plans were contemplated to anchor a more inclusive and sustainable breeding programme (Ilatsia *et al*., 2011a).

2.5 Challenges of Closed Nucleus Breeding Programmes

The current breeding system for Sahiwal cattle breed in Kenya depicts a typical closed nucleus breeding programme (CNBP) where performance recording and selection is confined to the nucleus, and the pastoral herds being the main recipients of the resultant genetic superiority generated in the nucleus herds (Ilatsia *et al*., 2011a). Nucleus breeding programs have been recommended in developing countries as a strategy for genetic improvement of cattle since the countries have inadequate money, expertise and structure required for running effective improvement programme based on artificial insemination (AI) and field recording in the whole population (Smith, 1988; Kahi *et al*., 2004). Such breeding programme does not require expensive infrastructure because recording is only done in the nucleus herd (Kahi *et al*., 2004). Since there are no new genes introduced from outside, the closed nucleus breeding programmes (CNBPs) are associated with low genetic gain in desirable traits (Fuquay *et al*., 2011). CNBPs are thus associated with high levels of inbreeding as compared to open nucleus breeding programme (Ilatsia *et al*., 2011b).

The Sahiwal population in Kenya was reported to have been established from a small number of founders and therefore leading to limitation on the genetic variability since establishment (Muhuyi *et al.*, 1997). Further, the breed has been genetically closed hence subsequent loss in genetic diversity is being experienced (Muasya *et al*., 2011). The average inbreeding level for Sahiwal (0.6%) and the average relatedness (1.9%) with rate of increase in inbreeding of 0.49% and 0.4%, threatens the sustainable utilization of this important resource (Muasya *et al*., 2011). High levels of inbreeding are associated with inbreeding depression, reduced genetic variation, increased sampling variance of breeding programmes and emergence of lethal recessive alleles in homozygous form (Koenig and Simianer, 2006; Mahlando *et al*., 2012). Panetto *et al*., (2010) reported increased inbreeding that caused poorer performance in daily milk yield, age at first calving and calving intervals for Guzerat dairy herd. High levels of inbreeding are also associated with difficulties when planning mating systems to control future rates of inbreeding to increased average relatedness (AR) among individuals (Fernandez *et al*., 2011).

2.6 Measures of genetic diversity

Genetic improvement of various livestock populations with finite population sizes creates a need to strike a balance between selection intensity of a small number of parents in the current generation and maintenance of sufficient genetic diversity for future generations (Biscarini *et al*., 2015). Genetic diversity is assessed by estimating probability of gene origin statistics, inbreeding coefficient and effective population size (Boichard *et al*., 1997).

2.6.1 Effective population size

The effective population size (Ne) is defined as the size of an idealized population which would give rise to the rate of inbreeding, or the rate of change in variance of gene frequencies observed in the population under consideration (Wright, 1931). As such, Ne is a key parameter in conservation and population genetics because of its direct relationship with the level of inbreeding, fitness and the amount of genetic variation loss due to random genetic drift (Ervantes *et al*., 2008). When genealogies are available, the effective population size can be estimated from the increase in inbreeding (ΔF) between two discrete generations according to (Falconer and Mackay, 1996) as $Ne = \dfrac{1}{2\Delta F}$, with $\Delta F = \dfrac{F_t - F_{t-1}}{1 - F_{t-1}}$, where F_t and F_{t-1} are the average inbreeding at t and $t-1$ generations. The intensification in inbreeding is constant for a population that is ideal and of constant size without migration, without mutation and also without selection over discrete generations. In real populations with overlapping generations, the number of males and females is usually different and non-random mating is the rule, making ΔF a difficult parameter to deal with.

Effective population size is the most important criterion to indicate the endangered status of breeds (Ervantes *et al*., 2008). Melka and Schenkel (2010) reported an effective population size of 50 is necessary to withstand effects of inbreeding in a pig population, while a size of 500 is required to sustain the genetic diversity and evolutionary potential of the population for several generations. Maintaining within-breed genetic diversity for the long-term depends very much on the effort to increase effective population size, and balancing the contribution of ancestors to avoiding mating of much-related animals and reducing genetic drift (Cervantes *et al*., 2011). The effective population size has been more often utilized for predictive purposes rather than for investigating realised pedigrees. However, the effective population size can be used to understand the relationship with other pedigree tools (Gutiérrez and Goyache, 2005)

The current Ne for the Kenyan Sahiwal breed has been reported to be 219 according to Muasya *et al.* (2011) and 247 by Kamiti, (2014) which is below 500 that is recommended for a population to maintain long-term genetic viability (Franklin and Frankham, 1998). The most efficient way to increase genetic diversity in Kenyan Sahiwal cattle populations should be directed towards increasing the effective population size of the breed. According to Nagy *et al*. (2014), genetic drift is one of major aspects that affect genetic improvements in populations under selection. As *Ne* decreases, genetic drift reduces genetic variation, raises the likelihood of fixation of lethal alleles, and decreases the efficacy of selection, which in general reduces overall fitness and limit adaptive responses (Hare *et al*., 2011). A small effective population size may cause variations in genetic gain, especially using Best Linear Unbiased Prediction (BLUP) for predicting breeding values (König *et al*., 2010). In order to exploit genetic gain in Sahiwal cattle there is therefore need to develop strategies aiming to increase the population size, to use guided matings to control inbreeding and to avoid intensive use of few individuals.

2.6.2. Probabilities of gene origin

Probabilities of gene origin are useful tools in measuring genetic diversity within breeds even after only a small number of generations (Boichard *et al*., 1997). Changes in the population structure owing to migration, bottlenecks and changes in breeding strategy, are accounted for by comparing the genetic variability in the reference population to that of the founding population (Boichard *et al*., 1997). These include number of founders, effective numbers of founders, effective number of ancestors and founder genomes. Founders are all animals with unknown sire and dam (Lacy, 1989; Boichard *et al*., 1997). Effective numbers of founders is defined as the number of equally contributing founders that is expected to produce the same amount of genetic diversity as that in the population under study (Lacy, 1989). Effective number of ancestors is where the marginal contribution of each ancestor (i.e. the contribution not yet explained by the other ancestors) is considered while the effective number of founder genomes (f_{ge}) analyzing the probability that a given gene present in the founders is still present in the population under study (Lacy, 1989; Boichard *et al*., 1997). When all founders contributes equally, the effective number of founders is equal to the total number of founders (Melka and Schenkel, 2010). The effective number is usually lower than total number of founders because of unequal contributions of founders for populations that have undergone selection (Muasya *et al*., 2013).

Ratios of probability of gene origin statistics help to understand the historical development of a population, since the founder population (Boichard *et al.*, 1997; Muasya *et al*., 2013). The comparison between effective number of founders (f_e) and number of founders (f) helps to validate loss in genetic diversity due to unequal contributions of founders. This could normally occur as a result of increased use of some animals as parents of succeeding the generations (Melka and Schenkel, 2010). Unequal contributions of founders will lead to a direct increase of average inbreeding and coancestry in the population under study. When the ratio of effective number of founders to effective number of ancestors (f_e / f) ratio is small, there is excessive amount of loss of genetic diversity as a result of unequal contribution of the founders (Muasya, *et al*., 2013). In the study by Muasya *et al*. (2013) found a ratio of f_e / f for the Kenyan Holstein-Friesian to be 0.08 which was relatively higher than those reported for Tunisian Holstein (0.02) (Hammami *et al*., 2009), Luxembourg Holstein (0.04) (Hammami *et al*., 2007) and Irish Holstein (0.002) populations (McParland *et al*., 2007).

The ratio of effective number of founders to effective number of ancestors (f_e / f_a) expresses the effect of population bottlenecks (Malhado *et al*., 2012) hence important in describing the history of a population (Boichard *et al*., 1997; Muasya *et al*., 2013). A low ratio means that the population under study has gone through bottlenecks, which results to decrease in genetic variability (Muasya *et al*., 2013). Various studies have reported different ratios for various breeds with Muasya *et al*. (2013) reporting a ratio of 0.69 for the Kenyan Holstein-Friesian which was higher than estimates of 0.15 for Tunisian Holstein-Friesian (Hammami *et al*., 2007) and 0.29 for Danish Holsteins, (Sørensen *et al*., 2005). Other studies have shown loss of genetic diversity due to bottleneck in Slovak Spotted cattle and French Simmental (Danchin-Burge *et al*., 2012), Slovak Pinzgau (Kadlečík *et al*., 2011) and Slovak Holstein (Pavlík *et al*., 2013) populations which had f_e / f_a of 0.33, 036,0.37 respectively.

The effective number of founder genomes to effective number of founder ratio measures the influence of genetic drift. Lower values of the ratio are associated with higher loss of genetic diversity due to genetic drift (Hazuchová *et al*., 2013). Based on this ratio, the effect of random genetic drift has been reported for Brown Swiss (0.07) and Canadienne (0.08) populations (Melka *et al*., 2010). The ratios are lower compared to the Slovak Spotted breed subpopulations which were reported to have ratios of0.15, 0.14 and 0.13 (Hazuchová *et al*., 2013). These ratios are an indication that only a small number of ancestors were needed to explain half of the genetic diversity in the studied subpopulations. In a study by Piccoli *et al*. (2014), it was found that for the four British cattle in Brazil the number of founders and ancestors contributing 50% of the studied population gene pool were 211 and26, 41 and 14, 164 and 25, 79 and 10 Angus, Devon, Hereford and for Shorthorn, respectively. Effective number of founders, ancestors and founder genomes was 470, 68 and 36 for Angus; 89, 33 and 16 for Devon; 289, 59 and 30 for Hereford and 200, 28 and18 for Shorthorn, respectively. Worede *et al*., (2013) reported the effective number of founders and the effective number of ancestors as 141 and 88 respectively for the International brown Swiss Cattle. The f_{ge} indicates the loss of genetic diversity due to both unequal founder contribution and random genetic drift. The f_{ge} / f_e ratio measures the impact of genetic drift excluding the effect of founder contribution on genetic diversity, so that lower ratios are associated with a higher impact of genetic drift. Assessment of genetic diversity using parameters based on the probability of gene origin allows description of population genetic diversity precisely.

Brito *et al.* (2013) used parameters based on the probability of gene origin in the large Brazilian Nellore herd to describe the genetic diversity hence helping to show an overall loss of genetic diversity in the herd. In this study, the effective number of founders, ancestors, and founder genomes of Nellore herd was reported to be decreasing over time, showing an overall loss of genetic diversity. This study reported that one noticeable ancestor contributed 10.6% to the gene pool of the herd, and 30% of this pool was contributed by 31 ancestors which explains the loss of genetic diversity of the breed (Brito *et al*., 2013). For Brazillian Marchiginia and Bonsmara breeds, the number of ancestors explaining 50% of the gene pool and effective population size was reported to be 13 and 97.79 for Brazillian Marchigiana and 41 and 54.57 for Bonsmara, respectively (Santana *et al*., 2012). The estimates indicates decline in genetic variability for both breeds. (Gutiérrez *et al*., 2003) used the same parameters to estimate the genetic structure of eight Spanish breeds of beef cattle as well as Oravcová, (2013) to estimate the genetic variability of the White Shorthaired goat in Slovakia. Therefore the probability of gene origin parameters can be used to characterize the Sahiwal breeding population or detect recent significant changes in the breeding strategy, before their consequences appear in terms of inbreeding increase (Boichard *et al*., 1997).

2.6.3 Inbreeding (F)

Inbreeding can be defined as the probability that two alleles at any locus are identical by descent, and occurs when related individuals mate (Malécot, 1948). The potential undesirable effects of inbreeding is a problem for domestic animals, especially where large populations often stem from a limited number of founding individuals (Malecot, 1948). When the population size is restricted or the number of individuals allowed to breed is fairly small, the allele frequencies change, which results in increased homozygosity and/or losses of alleles (Toro *et al.*, 2011). Genetic drift is the change in the frequency of allele in a population because of random sampling. In a finite population, these effects are accumulating because of sampling of gametes. The changes can be expressed in terms of inbreeding or coancestry. The existence of inbreeding in any breed population is an indication that there is an occurrence of genetic variation in fitness traits (Charleswoth and Willis, 2009).

2.7 Effect of quality of pedigree on estimation of Effective population size, inbreeding and gene origin statistics

Effective population size and inbreeding level are dependent on the depth and quality of pedigree (Gutierrez *et al*., 2003). When the pedigree completeness is low, it leads to overestimation of these two parameters (effective population size and inbreeding level) (Boichard *et al*., 1997). Therefore, the two parameters are not very informative in cases where pedigree is shallow as the number of animals that are inbred and average coefficient of inbreeding are underestimated (Boichard *et al*., 1997). Although gene origin statistics are less sensitive to the quality of the pedigree as compared to inbreeding level and effective population size, the values of inbreeding may be overestimated due to missing information in the pedigree since any individual with one or either two parents unknown is regarded as a founder when computing this parameter (Gutierrez and Goyache, 2005; Muasya *et al*., 2013).

It is common to encounter incomplete pedigrees in which one or both parents of some individuals are unknown and hence the assumption that those individuals are unrelated to the others in the population (founders) would result in a contribution from them that is too high and in sub-optimal levels of genetic diversity (Fernandez *et al*., 2011). Therefore, alternative strategies such as molecular paternity analysis techniques can be employed to identify the correct parent (Martınez and Fernandez, 2008). Use of molecular information in replacement of genealogical data when the pedigree quality is low would allow the same procedures such as optimal contributions and minimum coancestry mating to be implemented (Fernandez *et al*., 2011)

2.8 Measure of loss of genetic diversity

Measures of the loss of genetic diversity can be derived from the effective number of founders, effective number of founder genomes and effective number of non-founders. The amount of genetic diversity (GD) in the reference population accounting for loss of diversity due to genetic drift and unequal founder contribution was calculated as (Lacy 1995):

$$GD = 1 - \frac{1}{2fge} \tag{1}$$

When expressed as 1-GD, the value obtained is the measure of genetic diversity lost in the population since the founder generation due to bottlenecks and genetic drift. It is assumed that the number of founders in the base population is large enough and therefore the genetic diversity in the base population is close to 1 (Tang *et al*., 2013).

2.9 Effects of inbreeding on performance

When genetic relationship between parents increases, the likelihood that pairs of alleles in offspring are copies of a single allele in an ancestor generations back increases and such alleles are said to be identical by descent. Inbreeding produces unsuitable effects on all the traits of importance. Inbreeding depression reduces the realization of genetic gain in general and of health, fertility and the productivity of the dairy cows in particular (Adamec *et al*., 2006; Sørensen *et al*., 2006). However, in the long term the major problem of inbreeding is the expected reduction of future genetic gain because of reduced genetic variation.

Results on the effects of inbreeding on the animals' performance in terms of production, fertility and health can be of striking impact in the long run if there is small sample of sires to be selected per year (Parland *et al*., 2007) . Matings between highly related individuals increase the risk of appearance of lethal recessive genes in the homozygous state like the single recessive gene causing Complex Vertebral Malformation (CVM) (Koenig and Simianer, 2006). Besides from malformed dead born calves, abortion in the early stage of parity is another symptom in the homozygous state. The loss of genetic diversity is associated inbreeding depression in fitness-related traits, the fixation of favorable alleles and inclined fluctuation in selection response (Tang *et al*., 2013). Ten percent increase of inbreeding coefficient has been reported to affect birth weight, weaning weight and 2 years weight in Sahiwal Cattle breed in Thailand (Bullangnoi *et al*., 2012).

2.10 Management of genetic diversity in livestock improvement programmes

Studies have been conducted showing reduced effective population size across various breeds. Muasya *et al*. (2013) reported *Ne* for Kenya Holstein Friesian to be 263 while the effective population size for the Brazillian large herd of Nellore cattle was reported to be 114, 245, and 101 for the time periods 1995-1999, 1999-2003, and 2003-2007 respectively (Brito *et al*., 2013). Worede *et al*. (2013) when evaluating genetic variability of Brown Swiss Population in Sweden reported effective population size in 2004 to be 204. Low effective population size is associated with high levels of inbreeding and difficulties in planned mating systems aimed at controlling future rates of inbreeding to increased average relatedness (AR) among individuals (Fernandez *et al*., 2011). Therefore the best method to reduce inbreeding that is a result of low number of effective population size is by minimizing coancestry mating strategy and prevention of cognate matings. According to Muasya *et al.* (2011), the rate of inbreeding has been estimated at slightly more than 1% per generation in the Kenyan Sahiwal cattle breed, which is in agreement with the rate of inbreeding as estimated by Kamiti (2014). This has increased the need for monitoring the current inbreeding rate, and for tools to control the future rates of inbreeding within populations. According to Brito *et al*. (2013) the analysis of inbreeding under random mating indicated that the mating strategies when used in the herd were slowing down inbreeding rates. Circular mating strategy when applied in synthetic Pannon White rabbit it was found to be generally effective (Nagy *et al*., 2014).

Small and closed populations are always at risk because of a higher loss of genetic diversity and increased rates of inbreeding as a result of small effective population size. Current breeding programmes involves accurate breeding value estimation and use of advanced reproductive technologies. Hence the programmes lead to rapid genetic progress but also lead to the accumulation of inbreeding via heavy impact of a few selected individuals or families (Weigel, 2001). Muasya *et al*., 2013 reported a weak breeding structure for the Kenyan Holstein-Friesian since only small number of herds contributed to the breeding sires. Selection schemes in Holstein dairy cattle are characterized by the widespread use of genetically superior proven AI sires (Koenig and Simianer, 2006).The future genetic progress of Sahiwal Cattle breed in Kenya will depend largely on the availability of adequate genetic variation. Genetic diversity of any breed is required to meet current and future production requirements in different environments, to allow sustained economically important genetic improvement, and to be convenient for adapting rapidly the breeding objectives (Melka and Schenkel, 2010; Tang *et al*., 2013). The rise in inbreeding can be used to derive the realized effective size of a population. This method however is sensitive to pedigree incompleteness although it reflects mainly long term effects of selection choices (Boichard *et al*., 1997). Parameters derived from the probabilities of gene origin are therefore suggested as valuable and corresponding alternative (Muasya *et al*., 2013).

The inbreeding trend is indisputably the tool most regularly used to quantify the rate of genetic drift. Assessing the inbreeding and loss of genetic diversity within a breed is necessary for sustainable development in the long term (Melka and Schenkel, 2010). Fortunately, Sahiwal cattle breed has pedigree information which can be used in evaluating and checking genetic diversity of breed based on the effective population size and the probabilities of gene origin. Effective number of ancestors which is a gene origin statistic parameter will help in accounting for the bottlenecks in Sahiwal cattle pedigree (Boichard *et al*., 1997). According to Piccoli *et al*. (2014) continuous monitoring of inbreeding trends of the breeds through use of parameters derived from probability of gene origin should be guaranteed to permit the long-term conservation of genetic diversity.

Selection of mates is very critical process in management of populations. Once the individuals to be parents of the next generation and the specific number of offspring they will produce are chosen, the way in which the animals are to be mated has to be determined. Mating strategies affect the levels of inbreeding in a population, but have little influence on the short-term level of maintained genetic diversity (a main objective of a conservation programme) (Fernandez *et al*., 2011). There is a two-step process in management of populations: selecting the parents and their contributions and choosing the way to mate them. However, the optimal solution from the first step sometimes cannot be implemented because of physiological and logistic challenges in the mating phase. Hence an alternative approach is to try to take both steps at the same time; that is, the number of offspring an individual is supposed to contribute and with which females he will mate (Fernandez *et al*., 2001). In this case, the variables to optimise are all of the possible combinations of males and females. This approach permits the inclusion of other restrictions such as no full-sibs generated (by restricting to one the number of contributions per couple) (Fernandez *et al*., 2011).

The large accumulated decline in genetic variability in Sahiwal Cattle breed in Kenya indicates the need to improve the current breeding strategy. A strategy that maximises the genetic gain while restricting the rate of inbreeding or the relationships among the candidates to be selected has been applied in German Holstein dairy cattle population (Koenig and Simianer, 2006). The method picks the selected parents and assign genetic contributions to the next generation for each selected candidate. The same strategy has been applied in poultry where contributions from 3 selected sires and hens lines were used to develop specific mating plans to minimize inbreeding in the following generation by applying a simulated annealing algorithm (König *et al*., 2010). The studies found increased genetic gain at the same rate of inbreeding compared to traditional selection schemes. Koenig and Simianer (2006) used strategy to maximize genetic gain while constraining rate of inbreeding in managing inbreeding in the German Holstein dairy cattle population and it was found to address inbreeding concerns on the farm.

The strategy to maximize genetic gain while constraining rate of inbreeding theory using official estimated breeding values for egg production was applied for 3 different commercial White Leghorn lines of a layer breeding programme to find the ideal allocations of hens and sires. All the constraints used in different scenarios enabled higher genetic gain up to 10.9% at the same level of genetic gain when compared with selection schemes that are conventional while ignoring relatedness (König *et al*., 2010). In his study, Oh (2012) showed the strategy to maximize genetic gain while constraining rate of inbreeding algorithm effectively controlled inbreeding and maintained consistent increases in response selection. When compared to the actual breeding programme applied in practice, these strategies have revealed the potential to increase genetic gain under the similar constraint for the rise of average relationship by 13.1% (Schierenbeck *et al*., 2011). Optimum-contribution selection when coupled with restrictions during optimization realizes most of the long-term genetic gain than for the selection without restrictions (Henryon *et al*., 2015). Despite all these benefits associated with this strategy, it is not widely used in practical breeding schemes including the Sahiwal breeding scheme.

In order to maximize long-term genetic gain in Sahiwal cattle, there is need strike the balance between short-term rates of genetic gain and inbreeding. This will help promote long-term genetic gain at rates of inbreeding that do not significantly erode additive genetic variation. The approach will help identify the mating partners to ensure maximum genetic gain at specific constraints on maximum relationship. The approach has been suggested to identify elite matings between preselected individuals to maintain genetic diversity (Schierenbeck *et al*., 2011). Muasya *et al*. (2011) reported average inbreeding level for the Sahiwal population to be 0.6% and the average relatedness as 1.9% and. Inbreeding levels by generation are on as the effective population size is declining (Kamiti, 2014). Due to increasing levels of inbreeding and measures need to be put in place to develop a breeding strategy which integrates management of genetic variability and selection. Therefore optimal genetic contribution selection is a strategy that can be used to manage breeding schemes for populations facing inbreeding problems such as the Kenyan Sahiwal breed.

CHAPTER THREE

ASSESSMENT OF GENETIC VARIABILITY USING PEDIGREE ANALYSIS OF THE SAHIWAL BREED IN KENYA

3.1 Abstract

Pedigree analysis using genealogical information of 18 315 animals born between 1949 and 2008 was done to quantify genetic variability of the Sahiwal population in Kenya. Generation intervals for sire pathways were longer than dam pathways and increased over year periods, from about 4–16 years. The later was due to use of old bulls for breeding in the last 2 year groups and cessation of progeny testing in the year 2000. Average inbreeding level in last year period studied was 1.2 percent. Genetic variability of the population as assessed based on gene origin statistics decreased over the years. The ratio of effective number of founders to founders of 0.06 showed unequal contribution of founders to the reference population. However, since the founding population, ancestors contributed equally as shown by the ratio of fe/fa of 0.94, which could also be due to lack of effective selection in this population. The ratio of fg/fa of 0.63 indicated genetic loss of genetic variability occurred through genetic drift in the Kenyan Sahiwal population. The small number of ancestors (16) that accounted for 50 percent of the total variation in the reference population suggested overuse of a small number of some animals as parents over generations. The smaller ratio of fg/fe compared with fa/fe also confirms loss of genetic variability in the population by genetic drift than bottlenecks. Therefore the breeding strategy for the Sahiwal population in Kenya should incorporate tools that balance rate of genetic gain and the future rate of inbreeding.

Keywords: *effective population size, genealogy, genetic diversity, inbreeding, optimum contribution*

3.2 Introduction

The Sahiwal was first introduced into Kenya in the first half of the twentieth century to improve the performance of the local Zebu cattle for milk production and growth. The original population was composed of 60 bulls and 20 cows, which were put in Livestock Improvement Centres (LICs) in different parts of the country following the recommendation of Meyn and Wilkins (1974). Since then breeding in this herd has been a closed nucleus with external germplasm in form of semen from Pakistan being introduced in 1992. In 1963, after some basic performance evaluations, superior individuals were selected and used to start a breeding herd in Naivasha, Kenya, referred to as the national Sahiwal Stud (NSS). Since then the Sahiwal has been bred under a closed nucleus.

Closed nucleus breeding programmes are popular in developing countries because they are cheaper to implement and run in terms of logistics, financing and expertise required (Kahi *et al.*, 2004). The nucleus is usually composed of high performing animals (Schierenbeck et al., 2011), recording of performance traits is thorough. Best Linear Unbiased Prediction (BLUP) based genetic evaluations and reproductive technologies such as artificial insemination (AI) lead to faster genetic gains by increasing the selection intensity due to use of only a few superior individuals in an entire population. Availability and use of semen from these superior individuals can lead to reduction of genetic variability due to increasing inbreeding levels in livestock herds (Weigel and Lin, 2002). This is more likely to occur in closed nucleus breeding programmes, precipitating erosion of genetic diversity.

Inbreeding level of the Kenyan Sahiwal breed is reported to be above 2 % and increasing in recent years (Muasya *et al.,* 2011; Kamiti, 2014). Effective population size (Ne) was reported to be between 102 and 247 based on complete pedigree information (Muasya *et al.,* 2011; Kamiti, 2014). Whereas the Ne is within recommended levels required for a population to maintain its evolutionary potential (FAO, 1998), the estimates are below the threshold of 500 for any breeding population to maintain its genetic variability in the long term (Franklin and Frankham, 1998). If unchecked, continued reduction in genetic variability would lead to unfavourable effects such as inbreeding depression, emergence of lethal recessive alleles when in homozygous form and increased variance of genetic progress due to chance (Falconer and Mackay, 1996).

Measures of inbreeding and effective population size are useful for long term management of genetic variability and can be used to monitor its trends in cattle breeding programmes. These parameters however, can be over- or underestimated depending on pedigree completeness (Faria *et al.,* 2009; Muasya *et al.,* 2013). Alternative measures of genetic variability which are less sensitive to pedigree completeness are gene origin statistics (Boichard *et al.,* 1997). These parameters are therefore more informative than Ne in describing short-term effects on genetic variability of populations (Boichard *et al.,* 1997). They provide a better understanding of a breed’s history and inform future actions in order to achieve greater genetic gains without loss in genetic diversity (Malhado, *et al.,* 2006) by monitoring and controlling rates of inbreeding (Fernandez *et al.*, 2011). Loss of genetic diversity can occur due to heavy use of afew AI sires (Hammami *et al.*, 2007; Muasya *et al.,* 2013), periodic reductions in population size, unequal contributions of ancestors and changes in the number of contributing males (Boichard *et al.,* 1997). This has been shown to contribute to increased family variance (Faria *et al.,* 2009) making it difficult to interpret estimates of inbreeding and Ne (Boichard *et al.,* 1997). In this Chapter, the genetic variability in the Sahiwal cattle in Kenya is assessed through pedigree analysis.

3.3 Materials and Methods

3.3.1 Materials

Data on pedigree of the Sahiwal cattle in Kenya were obtained from animals born in the National Sahiwal Stud at Kenya Agricultural and Livestock Research Organisation (KALRO) Naivasha and consisted of animals born between 1949 and 2008. Information on these animals included dates of birth and sex of each animal. The genealogy of each animal was traced individually as far back as possible in the birth record book database in order to include all known ancestors and relatives of each individual leading to the creation of a database of 18 315 animals.

3.3.2 Methods

Generation intervals

Generation intervals, or the age of parents when their progeny are born (Falconer and Mackay, 1996) were calculated for the sire–son, sire–daughter, dam–son and dam–daughter pathways.

Pedigree completeness

Parameters estimated to quantify pedigree quality included pedigree completeness index, number of maximum traced, number of complete generations and number complete generation equivalent. The parameters were estimated as follows:

Number of maximum traced generations

Number of generations was calculated as the number of generations separating the offspring from its furthest known ancestor in each path. Ancestors with unknown parents were considered as founders and assigned to generation 0.

Number of complete generations

Number of equivalent generations is the number of generations, *n* separating the individual from the furthest generation where both generation ancestors of the individual are known. This was computed as:

$$GE = \sum_{i=1}^{n} \left(\frac{1}{2}\right)^{2},$$

(2)

where *n* is the number of generations separating the individual to each known ancestor, where both generation ancestors are known.

Number of complete generations equivalents

Complete generation equivalent was computed as the farthest generation for which all ancestors are known. For an individual j, the number of complete generation equivalents, CGE, was calculated as:

$$CGE = \sum_{i-1}^{n_j} \frac{1}{2^{g^{ij}}},$$

(3)

where n_j is the number of ancestors for animal j; g_{ij} is the number of generations separating individual j and its ancestor i (Sölkner *et al*., 1998).

Increase in inbreeding per generation

Increase in inbreeding per generation was calculated based on regression (b) of individual increase in inbreeding over equivalent generations as:

$$\Delta F = \frac{F_t - F_{t-1}}{1 - F_{t-1}},$$

(4)

where F_t and F_{t-1} are the average inbreeding of the i th generation.

Individual increase in inbreeding

Individual increase in inbreeding ΔF_i was calculated as (Gutierrez *et al.*, 2009):

$$\Delta F_i = 1 - \sqrt[CGE-1]{1 - F_i} \, ,$$

(5)

where F_i is the individual coefficient of inbreeding and CGE is the complete generation equivalent (Maignel *et al*., 1996).

Effective population size

Effective population size, Ne is defined as the number of breeding animals expected to cause the actual increase in inbreeding if they contributed equally to the next generation (Falconer and Mackay, 1996). Effective population size, Ne for each generation was calculated based on ΔF as:

$$Ne = \frac{1}{2\Delta F} \, ,$$

(6)

as long as $F_t > F_{t-1}$ to allow for characterise the effect of remote and close inbreeding. For small populations and or with shallow pedigrees, Ne is usually overestimated (Goyache *et al.*, 2003). Additional values of Ne were estimated as the coefficient of regressing individual inbreeding coefficient on number of (i) full generations, (ii) maximum and equivalent generations traced and (iii) complete generation equivalents. The corresponding coefficient of regression represented the increase in inbreeding between any two consecutive generations $(F_t - F_t - 1 = b)$, such that $Ne = \frac{1}{2}b$. Estimates of Ne calculated using full, maximum and equivalent generation are useful in informing lower, upper and real limits of Ne for the population under study.

Probability of gene origin statistics

Number of founders (f) was computed as all individuals in the population with unknown parents and were non-inbred. Also any individual with either parent unknown, that parent was taken as a founder.

Effective number of founders (fe), defined as the number of equally contributing founders expected to produce the same diversity as observed in the reference population (Lacy, 1989) was estimated as:

$$fe = 1 / \sum_{k=1}^{f} q^2 k$$

(7)

where q_k is the probability of a gene origin of the k^{th} founder.

Effective number of ancestors (fa) is defined as the minimum number of ancestors required to explain the complete genetic diversity in a population (Boichard *et al.,* 1997). This parameter was computed as:

$$fa = \frac{1}{\sum_{j}^{a} q_j^2}$$

(8)

where q_j is the marginal contribution of an ancestor j, which is not explained by other ancestors chosen before it. Effective number of ancestors accounts for recent bottlenecks occurring in a population and therefore partially accounts for loss of allelic diversity in descendant population (Boichard *et al*., 1997).

Effective number of founder genomes (f_g) is the number of founders that would give the same level of genetic variability in the population under study with equal representation of founders and no loss of alleles (Ballou and Lacy, 1995). This parameter was computed as twice the average coancestry of the individuals in the reference population.

Mean number of progeny per sire, number of generations, inbreeding coefficient, average relatedness (AR) coefficient, individual increase in inbreeding and average offspring size and probability of gene origin in the Kenyan Sahiwal population were computed for 5 10-year groups as follows: 1(1961–1969); 2(1970–1979); 3(1980–1989); 4(1990–1999) and 5(2000–2008). All parameters were calculated using ENDOG software (Gutiérrez and Goyache, 2005).

3.4 Results

Registered sires and the average progeny per sire and their respective standard deviations are given in Table 1. The coefficient of variation was well over 100% (119 to 136 percent).

Generation intervals for sire pathways were longer than dam pathways and increased over year periods, and ranged from about 8 years to 12 years (Table 2). The long generation interval along the sire pathway was due to use of old sires because progeny testing at the National Sahiwal Stud had ceased since the year 2000. Since breeding at the National Sahiwal Stud in Kenya solely uses AI, only old progeny tested bulls had their semen available. Also as a policy of the stud, progeny tested bulls were mated to elite usually old cows to produce young bulls for progeny testing (Muhuyi *et al*., 1999), partly explaining why the generation intervals were increasing over the year groups.

Table 1: Mean number of progeny per sire and standard deviation in the five year periods in the Sahiwal cattle in Kenya

		Number of progeny per sire	
	Number of sires	Mean	Standard deviation
Period			
1	105	34.2	46.4
2	144	32.4	43.3
3	153	26.8	32.0
4	107	22.9	30.4
5	72	20.6	23.3

Individual inbreeding coefficient, individual AR coefficient, individual increase in inbreeding and average offspring size are given in Table 3. Individual inbreeding level and AR among individuals in the population increased over time from 0.09 and 0.7 percent to 1.2 and 2.1 percent, respectively. Values of effective population size computed by regressing individual inbreeding coefficient over number of full generations, maximum number of generations traced and complete equivalent generations were 102.4, 265.1 and 125.2, respectively.

Table 2: Generation intervals of the four gametic pathways in the five year periods the for the Kenyan Sahiwal population

Period	Gametic pathway			
	Males		**Females**	
	Sire	Dam	Sire	Dam
1	4.1	5.9	4.9	4.6
2	9.5	7.7	7.5	5.9
3	10.1	8.2	8.4	6.4
4	16.2	11.8	8.7	7.5
5	15.7	14.7	12.1	7.4

Probability of gene origin statistics, their ratios and number of ancestors explaining 50 percent of total variations in each year period are shown in Table 4.

3.4 Discussion

Average number of progeny per sire and the respective standard deviation decreased over the years by about 50% and 60%, respectively, from the first to the last year period (Table 1) partly due to a decline in population size over the years (Table 4). Generation intervals for the sire pathways were very long compared to other European breeds, even though zebu cattle generally have delayed age at first calving. This was partly due to use of old sires, born before the year 2000 following the cessation of progeny testing at the NSS. Apart from lengthening generation intervals in the sire pathways, use of few old sires led to reduction in genetic variation and limited or no genetic progress. This was confirmed by Ilatsia *et al.* (2007) who reported lack of genetic progress in milk production and fertility traits for the Kenyan Sahiwal. Other studies which reported long generation intervals for the sire pathways include Faria *et al*. (2009) and Filho *et al.* (2010) for Brazilian zebu breeds. In experimental herds of some Zebu breeds however, shorter generation intervals of 3.7 years in both pathways have been reported (Razook *et al*., 1993; Faria *et al*., 2009). Generation equivalents of the Sahiwal population in the current study were lower than those reported for Brazilian Zebu breeds (Faria *et al*., 2009). Elsewhere, Italian beef breeds were reported to have generation equivalents of about 5 years (Bozzi *et al*., 2006).

Table 3: Number of generations, inbreeding coefficient, average relatedness coefficient, individual increase in inbreeding and average offspring size for the Kenyan Sahiwal population

Year period	Average inbreeding coefficient, %	Average relatedness, %	Individual increase in inbreeding, %	Offspring	Effective population size		Maximum generations	Complete generations	Generation equivalents
					Ne^1	Ne^2			
1	0.09	0.72	0.05	2.97	1088.3	199.3	1.08	0.87	0.97
2	0.25	1.77	0.1	2.09	484.8	150.6	2.76	1.55	2.06
3	0.59	2.19	0.18	1.47	271.0	118.5	4.42	2.22	3.04
4	1.15	2.36	0.28	1.18	175.9	72.0	6.01	2.59	3.85
5	1.19	2.07	0.27	0.23	186	70.3	7.47	2.02	3.96
Overall	0.52	1.72	0.15	1.84	-	-	3.70	1.74	2.48

Ne^1 and Ne^2 represent effective population size computed through regression on individual increase in inbreeding and regression on complete equivalent generations, respectively

Table 4: Characteristics based on probability of gene origin in the Kenyan Sahiwal population

Year group	Parameter								
	N	Founders	Effective number of founders	Effective number of ancestors	Effective number of founder genomes	Ancestors explaining 50% of genetic diversity	$f_a/_{Nf}$	f_e/f_a	f_g/f_a
1	4583	986	118	102	78	41	0.12	0.86	0.76
2	4802	705	51	51	47	19	0.07	1.00	0.92
3	4366	637	42	41	33	14	0.07	0.98	0.80
4	2602	481	40	38	26	14	0.08	0.95	0.68
5	1962	781	49	46	29	16	0.06	0.94	0.63
Overall	18315	1087	59	59	58	22	0.05	1.00	0.98

Inbreeding coefficient of the Sahiwal cattle breed was 1.2 % in the last year period studied with a general increase over the years. This could be attributed to large-scale utilization of few superior sires and use of old bulls from before the year 2000 due to cessation of progeny testing, which also led to an increase in the generation interval and the family size variance (Falconer and Mackay, 1996) and limited effective population size. In the current study, effective population size decreased as inbreeding level increased across the year periods studied (Table 3). A similar trend was reported among the Gyr, Nerole and Guzerat Zebu breed in Brazil (Faria, *et al*., 2009). Average inbreeding coefficients of 0.5 and 1.2 % for the entire population and the reference population, respectively were lower than corresponding values of 7.8 and 10.8 % for the Lidia breed in Spain (Cortés *et al*., 2014), 4.81 % for Burlina breed in Italy (Battagin *et al*., 2010) and 2.82 percent for Gyr dairy cattle in Brazil (Filho *et al*., 2010). Even higher inbreeding coefficients have been reported in other cattle breeds (McParland *et al*., 2007; Bouquet *et al*., 2011; Danchin-Burge *et al*., 2012).

Estimates of inbreeding levels and effective population size are closely related to the quality of pedigree (Gutiérrez *et al*., 2003; Muasya *et al*., 2013). Further evidence is provided by the values of 102.4, 125.2 and 265.1, obtained based on complete, full and complete equivalent generations. According to Goyache *et al.* (2003) these estimates are useful for providing lower, real and upper limits of Ne, respectively, for the population under study since for small populations and or with shallow pedigrees, Ne is usually overestimated. In other Zebu breeds, Ne estimates ranging from 124 in polled Nelore breeds to nine in Sindi breed have been reported (Faria *et al*., 2002). Verneque *et al*. (2006) reported Ne ranging from 16 to 125 for the Dairy Gir, with more recent generations being more inbred, similar to the present study. Muasya *et al.* (2013) found *Ne* ranging from 454 to 263 in Kenyan Holstein–Friesian. Cortés *et al.* (2014) reported *Ne* of 37.5 for the Lidia breed of Spain. The decline in Ne can be attributed to increase in inbreeding coefficient in the population due intense use of few superior sires (Nomura *et al.,* 2001).

Management of genetic variation in selection programmes is important for conservation purposes and short and longterm selection responses (Hill, 2000). Effective population size is an important parameter to monitor when managing genetic diversity in breeding programmes because it reduces genetic variability and depresses performance. An effective population size of 31-250 has been recommended to prevent decline in fitness of a population due to inbreeding depression (FAO, 1998; Meuwissen and Woolliams, 1994) and to maintain mid-term genetic variability. This can be achieved by ensuring that the rate of inbreeding per generation does not exceed a value of 1% beyond which a population begins to lose its fitness (Franklin and Frankham, 1998). Since in most breeding programmes the aim is to maintain genetic variability in the longterm , *Ne* of at least 500 should be targeted (Franklin and Frankham, 1998). In the present study *Ne* in the most recent year group was below the recommended levels, regardless of pedigree completeness (Table 3). Therefore strategies should be put in place to control future rates of inbreeding while achieving genetic progress for traits of economic importance in the Kenyan Sahiwal breed. Nevertheless, controlling future rates of inbreeding by constraining the degree of relatedness of mates is only effective for a few generations (Fernadez *et al.,* 2011). Incorporation of genomic selection in breeding programmes, which is capable of minimising mendelian sampling allows for increasing effective population size (Daetwyler *et al.,* 2007) should be considered.

Effective population size is normally overestimated after a number of generations with incomplete pedigree information, becoming worse as number of generations increase. On the other hand, gene origin statistics are less sensitive to pedigree completeness and are therefore more informative than Ne for describing short term effects on genetic variability of populations (Boichard *et al.,* 1997). In the present study the number of founders (animals without parent information) was high compared to the total population size studied, indicating that current Ne value could be an overestimation. This is seen in the change in Ne from 265.1 to 102.4 when estimated based on full and complete generations traced, respectively.

Gene origin parameters also indicate the loss of genetic variability in the Sahiwal breed. Number of founders, effective numbers of founders, ancestors and founder genomes decreased over the years but increased slightly in the last year group, indicating that loss in genetic variability occurred in the Sahiwal breed. The increase in the last year group could be due to the use of old sires due to cessation of progeny testing at the NSS because of logistical reasons. The effective numbers of founders were much smaller compared with the number of founders. The marginal genetic contribution of the most important ancestor increased, while the number of ancestors required for explaining half of the total variation observed in the Kenyan Sahiwal breed decreased over time. Effective number of founders reported in the current study of 49 in the most recent year group was higher than that of 38 for the Nelore of Brazil (Faria *et al.,* 2009) and 27.8 for Lidia breed in Spain (Cortes *et al.,* 2014) but smaller than 284 for the Gir and 247 for Guzerat cattle breeds (Faria *et al.*, 2009). The effective number of ancestors of 46 found in the current study was lower than 211 and 166 for Gir and Guzerat Zebu breeds (Faria *et al.,* 2009), but higher than 15.8 for the Lidia breed in Spain (Cortes *et al*., 2014). Other studies which have reported lower estimates include Sørensen *et al*. (2005) which found values of 20.6, 23.8 and 34.6, respectively, for Danish Holstein, Jersey and Red breed populations(Bouquet *et al.*, 2011, Danchin-Burgue *et al.,* 2012 and Melka *et al.*, 2013). Effective number of founder genomes of 29 was higher than 12.3 reported for the Lidia breed of Spain (Cortes *et al.,* 2014).

Ratios of parameters of gene origin provide information on the historical development of a population since the founder population (Boichard *et al.,* 2007). The ratio of f_e / N_f describes whether the founders were used in a balanced manner and the extent of pedigree completeness. A low ratio means that the population has gone through bottlenecks and or indicates gaps in pedigree recording. For the most recent year group studied, the ratio of 0.06 was larger than 0.0004 for Nerole but close to 0.5 for both Gir and Guzerat breeds in Brazil (Faria *et al.*, 2009). This implies an unequal contribution of founders to the reference population. From these studies however, the rations are somewhat dependent on population size, since the Nelore has a large number of founders (84452) compared to the Gir (6081) or Guzerat (4980) and pedigree completeness such that lower values are reported for large populations with high levels of pedigree completeness.

The ratio of f_a / f_e is useful when describing the history of a population. The ratio of 0.94 reported in the current study indicates that the Kenyan Sahiwal population has been genetically stable. This ratio is close to 0.89 reported for the Nerole breed but higher than 0.74 and 0.67 for Gir and Guzerat breeds of Brazil (Faria *et al.,* 2009). Other lower values include 0.15 for Tunisian (Hammami *et al.*, 2007) and 0.57 for Spanish Lidia cattle breed (Cortes *et al.,* 2014). The ratio reported in the current study implies a balanced contribution of ancestors to the reference population of the Kenyan Sahiwal breed, which could be due to lack of effective selection in this population (Ilatsia *et al.,* 2007). A ratio of f_g / f_e of 0.59 found in the current study was within the range of 0.4 to 0.68 reported for Brazilian Zebu breeds (Vozzi *et al.,* 2006; Faria *et al.,* 2009) and Spanish Lidia breed (Cortes *et al.,* 2014), indicating loss of genetic variability occurred through genetic drift in the Kenyan Sahiwal population.

The number of ancestors required to explain 50 % of the observed variation in the reference population of 16 was lower than 56, 37 and 41, for Nelore, Gir and Guzerat, respectively, (Faria *et al.,* 2009). In the Kenyan Holstein-Friesian population a value of 89 was reported (Muasya *et* *al.,* 2013). For the Lidia cattle breed of Spain, 6 ancestors were required to explain 50 % of the total variation (Cortes *et al.,* 2014). The small number of ancestors that account for 50% of the total variation in the reference population suggests overuse of a small number of some animals as parents over generations, explaining to some extend the loss in diversity in the Sahiwal population in Kenya. Also, the smaller ratio of f_g/f_e compared to f_a/f_e , implies loss of genetic variability in the population was more by genetic drift than bottlenecks.

In Kenya the Sahiwal is bred under a closed nucleus breeding programme where performance recording and selection is confined to the nucleus, and the pastoral herds being the main recipients of the resultant genetic superiority (Ilatsia *et al.,* 2011b). Nucleus breeding programmes have been advocated for genetic improvement of cattle in developing countries (Kahi *et al.,* 2004) due to their ease of implementation as recording is done in the nucleus. If the current inbreeding levels continue to increase and unchecked and Ne declines, the nucleus may experience a reduction of genetic variability and inbreeding depression for traits of economic importance. There is also a possibility of emergence of lethal recessive alleles in homozygous form and increased sampling variance of breeding programmes (Meuwissen and Sorensen, 1998; Malhado *et al.,* 2013;). Difficulties in planning mating systems aimed at controlling future rates of inbreeding due to increased average relatedness (AR) among individuals may also be experienced (Fernandez *et al.,* 2011). Apart from putting into place strategies to control future rates of inbreeding, new germplasm from other Sahiwal populations such as from India and Pakistan can be introduced in order to widen the population’s genetic base.

3.5 Conclusion

The Kenyan Sahiwal population is derived from a small founding population and has fairly high pedigree completeness. Ratios of gene origin statistics indicate that loss of genetic variability is more through genetic drift than due to inbreeding. Level of inbreeding is most recent year group revealed high inbreeding levels and small effective population size for the breed. Future breeding strategy should aim to control future rates of inbreeding. From the perspective of long-term management of genetic variability, rate of increase in coancestry is most crucial since it uses all currently available information to predict future trend in inbreeding. Therefore the breeding strategy for the Sahiwal population in Kenya should incorporate tools that balance rate of genetic gain and the future rate of inbreeding.

CHAPTER FOUR

WITHIN-POPULATION GENETIC STRUCTURE AND GENETIC DIVERSITY TREND FOR SAHIWAL CATTLE BREED IN KENYA

4.1 Abstract

Pedigree data of 18315 animals born from 1961 to 2008 was obtained from the National Sahiwal Stud at the Kenya Agricultural and Livestock Research Organisation to estimate genetic conservation index (GCI), genetic structure, contribution to total genetic diversity and realized and expected measures of genetic variation. GCI was computed from the genetic contributions of all founders identified. Inference of population structure from pedigree information was done by computing Nei's minimum distance and F statistics. Total genetic diversity contribution was computed as average coancestry in relation to the entire individuals. Endog v4.8 was used to assess all parameters. The parameters of genetic differentiation for the whole population under study (1961-2008) had values of F_{IS} =- 0.0071, F_{ST} = 0.0036 and F_{IT} =- 0.0034. GCI for whole population was 5.93% whereas for individuals with at least three complete generations and the reference population had values of 7.87% and 9.87%, respectively. Loss in genetic diversity for the whole population was -0.023 an indication of random genetic drift and unequal contribution of founders. In the recent population, the rate of inbreeding per generation was 0.79 and rate of coancestry per generation being 0.85 which is slightly below 1% per generation recommended for a breed to maintain long-term genetic viability. The weak breeding structure of the breed is due to reduced herd number leading to fewer breeding males. The problem can be overcome by allowing more animals that are not related in breeding herd. There should be improved pedigree recording for accurate estimation of parameters (Effective population size and Inbreeding)

Keywords: *Differentiation, Pedigree, Population, Sahiwal, Variability*

4.2 Introduction

The Sahiwal cattle is a dual purpose breed that was introduced was introduced into Kenya in 1930s and 1940s from India and Pakistan (Ilatsia *et al*., 2007). The National Sahiwal Stud (NSS) at Naivasha, Kenya is the main breeding station where the population is bred under a closed nucleus. The mandate of the NSS is to breed Sahiwal cattle that are suitable for pure breeding due to its adaptive characteristics to low-input production systems (Ilatsia *et al*., 2011b). The breed is also utilized for crossbreeding for dairy production in a low input-output production system (Roessler *et al*., 2010). Being closed nucleus, intense pedigree and performance recording are done at the nucleus (Muhuyi *et al*., 1999; Ilatsia *et al*., 2007). Genetic gain generated in the nucleus is disseminated to the commercial population through breeding males, semen and replacement heifers.

Performance data and pedigree information have been used widely to measure the genetic structure of most domestic livestock such as dairy and beef cattle, sheep, pigs and horses (Huby *et al*., 2003). This has enabled exploration of genetic structure of domestic animals that have genetic heritage, ecological and socio-economic values to assess their genetic status and risk of extinction (Krupa *et al*., 2015). The Sahiwal cattle breed in Kenya had been reported to have a decreasing effective population size and increasing levels of inbreeding (Muasya *et al*., 2011; Kamiti, 2014) which imply low genetic variability. This has raised concern over the long-term sustainability of the current breeding strategies. The reduced genetic variability could be attributed to increase in average relatedness and ineffective procedures for genetic evaluation for the population (Muasya *et al*., 2011).

Inbreeding levels for the Sahiwal breed are increasing (Dahlin *et al*., 1995; Muasya *et al*., 2009) and have already surpassed the recommended 1% increase per generation for a population to maintain its long-term genetic variability (FAO, 1998). Adverse effects on performance have been reported for the same population (Musingi *et al*., 2018). Therefore there is need to describe the within-population differentiation for the Sahiwal cattle population in Kenya and also determine genetic diversity loss in the breed. This will assist in developing a strategy for sustainable management of genetic diversity and genetic improvement in the population. In this Chapter, the within-population genetic structure and diversity loss or gain necessary when implementing selection programmes is assessed. Their implications on the genetic history of the Kenyan Sahiwal cattle breed over the years of breeding were also evaluated.

4.3 Materials and methods

4.3.1 Materials

Data for this study were obtained from the National Sahiwal Stud at the Kenya Agricultural and Livestock Research Organization, Naivasha. A total of 18315 animals born between 1961 and

2008 were included in the study. Information on each animal included animal identification, date of birth and sex. The genealogy of each animal was traced as far back as possible in the birth record book database, this way all possible ancestors and relatives of each individual were included in the analyses.

4.3.2 Methods

Effective population size

Effective population size (Ne) was calculated as the regression coefficient b of the individual increase in inbreeding on maximum and complete generations (t). If b is the change in inbreeding over two generations, then

$$\mathrm{b} = \mathrm{F}_{\mathrm{t}-}\mathrm{F}_{\mathrm{t}-1} = \frac{\mathrm{F}_{\mathrm{t}} - \mathrm{F}_{\mathrm{t}-1}}{1 - \mathrm{F}_{\mathrm{t}-1}} \tag{9}$$

where F_t and F_{t-1} are the average inbreeding of the i^{th} generation, respectively.

Therefore

$$\mathrm{Ne} = \frac{1}{2\Delta \mathrm{F}} = \frac{1}{2}\mathrm{b}\,. \tag{10}$$

Estimates of *Ne* calculated using maximum and equivalent generations provide guides for upper and real limits of *Ne* for a population.

Individual increase in inbreeding ΔF_i was calculated as

$$\mathit{\Delta} F_i = 1 - CGE\sqrt{1 - F_i} \tag{11}$$

where F_i is the individual coefficient of inbreeding and CGE is the complete generation equivalent (Maignel *et al*., 1996). Complete generation equivalent GCE, defined as the farthest generation for which all ancestors of an individual are known was computed as:

$$CGE = \sum_{i-1}^{n_j} \frac{1}{2^{g^{ij}}} \tag{12}$$

,

where n_j is the number of ancestors for animal *j* and g_{ij}; is the number of generations between an individual j and its ancestor i (Sölkner *et al.*, 1998).

Genetic Conservation Index

The Genetic Conservation Index (GCI) was calculated from the genetic contributions of all established founders in the reference population. The following formula was used according to Alderson (1992).

$$GCI = \frac{1}{\sum p_i^2} \tag{13}$$

where p_i is the proportion of founder *i*'s genes in an animal's pedigree. Estimated this way, GCI is based on the assumption that the objective of a conservation programme is to retain the full range of alleles possessed by the base population. In this respect, the ideal individual would receive equal contributions from all the founder ancestors in the population and, consequently, the higher the *GCI* value the higher the values of an animal for conservation (Alderson, 1992).

Contribution of sub-populations to total diversity

The average coancestry (Malècot, 1948) *f*, over an entire metapopulation of individuals consisting of *n* sub-populations, population *i* with N_i breeding individuals, is:

$$\overline{f} = \frac{\sum_{i.j-1}^{n} f_{ij} N_i N_j}{N_T^2} = \frac{\sum_{i-1}^{n} f_{ii} N_i}{N_T} - \overline{D} = \sum_{i-1}^{n} \frac{N_i}{N_T}\left[f_{ii} - \frac{\sum_{j-1}^{n} D_{ij} N_j}{N_T} \right] \tag{14}$$

F_{ij} being the average pairwise coancestry between individuals of sub-populations *i* and *j*, including all $N_i \times N_j$ pairs; f_{ii} is the average pairwise coancestry within sub-population *i* and D_{ij} is the Nei's minimum genetic distance (Nei ,1987) between sub-populations *i* and *j* computed as $D_{ij}= [(f_{ii} + fj_j)/2] - f_{ij}$. The sub-populations in this study were defined as year group cohorts as follows; 1960-1969, 1970-1979, 1980-1989, 1990-1999 and 2000-2008.

Genetic structure

Genetic structure of the Kenyan Sahiwal population was assessed using F-statistics (F_{IT}, F_{ST}, F_{IS}; Wright, 1978). F_{IT} is the inbreeding coefficient of the individual relative to the whole population; F_{ST} is the average inbreeding of the sub-population relative to the whole population and; F_{IS} is the inbreeding coefficient of the individual relative to its own sub-population (Falconer and Mackay, 1996).

Change in genetic diversity for the Sahiwal population in Kenya

The amount of genetic diversity (*GD*) in the reference population was calculated according to Lacy (1995) as;

$$GD = 1 - \frac{1}{2fge} \tag{15}$$

where f_{ge} is number of equally contributing founders with no loss of founder alleles that would give the same amount of genetic diversity as is presented in the reference population (Caballero and Toro (2000). The measures the genetic diversity (GD) lost in the reference population of the Sahiwal cattle since the founder generation due to both bottlenecks and genetic drift was computed as $1 - GD^*$, where

$$GD^* = 1 - \frac{1}{2fe} \tag{16}$$

where f_e is a measure of founder contributions to the population and is defined as the number of founders with equal contribution, which would give the same amount of genetic diversity that is present in the current population (Lacy, 1989).

The above formula was used to estimate the loss of genetic diversity that occurred in the population due to the unequal contributions of founders before their contributions converged (Caballero and Toro, 2000; Honda *et al*., 2004). For this study, the reference population included animals born between 2000 and 2008.

Realized and predicted effective population size

The realized effective population size that was be calculated as:

$$Ne = \frac{1}{2\Delta Fy.L} \tag{17}$$

where ΔFy is the rate of increase in inbreeding and L is the generation interval. Rate inbreeding per generation was estimated according to the method of VanRaden (1992). The rate of increase in coancestry, Δf is alternatively interpreted as future rate of inbreeding per year (Falconer and Mackay, 1996). Therefore the expected effective population size was calculated by replacing ΔFy with Δf. The coancestry or coefficient of kinship (f) between any two individuals is the probability that any two gametes taken at random, one from each, carry alleles that are identical by descent (Gutiérrez *et al*., 2009; Cervantes *et al*., 2011). The coefficient of kinship provides a measure of the relationship by descent between any two mates. The coancestry of two individuals A and B, whose parents are respectively P and Q; and M and N was estimated as) $f_{AB}= ¼f_{PM}+¼f_{PN}+¼f_{QM}+¼f_{QN}$ (Gutierrez and Goyache, 2005) and the inbreeding coefficient of an offspring between A and B is the coancestry between A and B. Coancestry estimates between mate pairs were compared against predetermined inbreeding levels. All the parameters were computed using the ENDOG V4.8 (Gutierrez and Goyache, 2005).

4.4 Results

Table 5 shows the Sahiwal breed distribution within different levels of inbreeding. Among all animals in the entire pedigree, there were a total of 4493 inbred animals with levels of inbreeding ranging from 0.01 to 26.6 %. The number of inbred animals constituted 24.5 % of the whole population. The highest inbreeding level (26.6%) for an individual was recorded in 2006.

Table 5: Percentage of inbred animals for the Sahiwal cattle in Kenya within different inbreeding levels

Inbreeding class, %	N	%
0	13818	75.5
0<F≥1.0	1696	9.3
1.0<F≥5.0	2407	13.1
5.0<F≥10.0	183	1.0
10.0<F≥20.0	193	1.1
20.0<F≥20.0	14	0.1

The parents' mean age when the offspring were born was 7.1 years for the whole population and 10.2 years for the reference population (Table 6). The age increased as the years progressed while the number of animals in each year cohort reduced over the same time period. The lowest mean age of 4.23 years was recorded for 1960-1969 cohort while the highest (10.23) years was recorded for 2000-2008 cohort. Effective population size estimated from regression on individual increase in inbreeding and complete generation equivalents increased over the years following the trend of inbreeding.

Table 6: Mean age of parents when offsprings are born and effective population size for various year groups for the Sahiwal cattle population in Kenya

Period	No. of animals	Parents Mean age when offsprings are born	Effective population	
			Ne^1	Ne^2
1960-1969	4582	4.23±0.02	190.4	177.2
1970-1979	4802	6.71±0.03	184.4	144.0
1980-1989	4366	7.16±0.04	173.2	111.8
1990-1999	2603	9.70 ±0.07	129.7	66.5
2000-2008	1962	10.23±0.10	140.0	65.7
Overall	18315	7.10 ±0.02	265.9	216.0

**Ne^1 and Ne^2 represent effective population size computed through regression of individual increase in inbreeding on discrete and complete equivalent generations, respectively.*

In Table 7, the current rates of inbreeding and coancestry per generation were used as basis for calculation of the current and future effective population size, respectively. Rate of inbreeding and rate of coancestry per generation increased as more complete generations were considered. Effective population size also decreased with increase in complete generations. The rates of coancestry were higher than the reported rates of inbreeding levels for the reference population (Table 7).

Table 8 shows the number of actual and effective number of founder males which successfully contributed breeding sons to the population up to the sixth generation. Characterization of the number of sires was carried out through the recovery of founders. The number of males that had at least one progeny in the population were 453. When additive genetic relationships were accounted for, this number decreased to 157. The effective number of sires of sire, great grandsires, great grand-grand-sires, founders, effective number of founders decreased over the generations, an indication of low concentration of origin of reproductive animals.

Table 7: Generation interval, rate of inbreeding (%) and rate of increase in coancestry (%) per generation and current and future effective population size for Kenyan Sahiwal cattle breed

Period	Generation Interval	Rate of inbreeding per generation (%)	Rate of coancestry per generation (%)	Ne_c	Ne_f
2000-2008 birth year cohort	11	0.79	0.85	66	65
Individuals with 3 complete generations	9.7	0.82	0.89	59	54
Individuals with 6 complete generations	6.9	1.19	1.36	39	35
Entire population	6.9	0.22	0.26	227	192

Ne_c and Ne_f are current and future effective population sizes, respectively

Table 8: Parameters describing genetically important and effective number of sires for the Sahiwal population in Kenya

	Number of sires	Effective number of sires
Sires	453	157.0
Sires of sire	126	42.0
Great grandsires	52	16.5
Great grand-grand-sires	22	6.1
Actual number of founder sires	5	2.1
Effective number of founder sires	1	1.0

**A founder sire was defined as a sire for whom both or either parent were not known*

The genetic conservation index was assessed from the genetic impact of the identified founders for the population (Figure 1). The mean index for the whole population was 5.9% whereas for the individuals with at least three complete generations and the reference population (2000 to 2008 birth year cohort) the value was 10.4% and 12.9%, respectively. The indices for these two groups increased over the years. Genetic conservation index for animals with at least 3 complete generations was on average 40% greater than that of the entire population, implying that poor pedigree quality can lead to underestimation of this parameter.

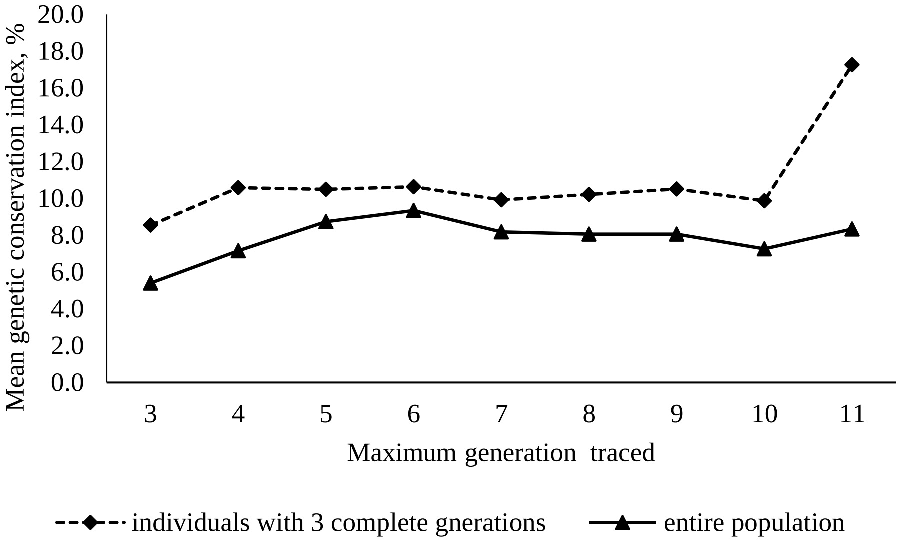

Figure 1: Mean genetic conservation index of the Kenyan Sahiwal population for animals born in 2000-2008 year group

F-statistics were used to assess within-population genetic differentiation from the pedigree (Nei, 1987). The analysis was carried out based on the reference population which was defined as the most recent population (2000-2008 cohort). The reference population had 5 sub-populations with mean coancestry within sub-populations of 0.012 whereas mean coancestry in the meta-population was 0.0086. Self-coancestry, inbreeding and Nei distance were 0.50, 0.0052, and 0.0036, respectively. There was a loss in genetic gain of -0.023 for each cohort from 1960 to 2008. Table 9 shows loss or gain in genetic diversity for the reference population for the various sub-populations. The overall trend in the genetic diversity of the Sahiwal cattle breed (Table 9) indicates that the breed has been losing diversity over the last five decades. It was found that there was loss in genetic diversity for the whole population as well as the reference population (-0.023).

Table 9: New genetic diversity, and loss /gain of diversity (in %) for the reference population (2000-2008)

Subpopulation	Genetic diversity	Internal diversity	Mean distance	Loss or gain
1	0.991	0.091	-0.018	0.074
2	0.989	-0.067	0.017	-0.050
3	0.990	0.050	0.032	0.082
4	0.991	0.153	-0.008	0.145
5	0.987	-0.226	-0.139	-0.366

**The subpopulations were derived from reference group based on the genetic diversity*

4.5 Discussion

All animals that had inbreeding levels above 20% were born in the most recent year group (2000-2008) which explains the increased inbreeding levels in the recent years (Kamiti, 2014; Mwangi *et al*., 2016). Among the inbred animals, majority (62.2 %) had levels of inbreeding above 1%. High inbreeding levels reported in the current study may have been as a result of few bulls being selected every year for breeding (Muhuyi *et al*., 1999). The average mean age of 7.1 years for the whole population was lower than 8 years for Nelore, Gir and Guzerat zebu cattle registered in Brazil (Faria *et al*., 2009). Gutiérrez *et al*., (2003) reported lower estimates for eight Spanish beef cattle breeds with generation intervals ranging from 3.7 to 5.5 years, similar for Chianina, Marchigiana and Romagnola breeds of 5.35, 4.93 and 5.15 years, respectively (Bozzi *et al*., 2006).

The value of 7.1 years for the whole population was lower compared to Brazilian Gyr dairy cattle which 8.41 years (Filho *et al*., 2010).

The effective population size for the whole population was 192 and was much lower for the recent year group (66) which was as a result of increased average inbreeding levels (Muasya *et al*., 2009). The value of 66 was much lower than 138, 122 and 124 for Chianina, Marchigiana and Romagnola breeds, respectively (Bozzi *et al*., 2006) but higher than Danish dairy breeds that ranges from 47 to 53 (Sørensen *et al*., 2005). The future effective population for individuals with high pedigree completeness was below the range of 50 to 500 recommended for sustainable management of genetic diversity (FAO, 1998; Franklin and Frankham, 1998). The previous studies by Kamiti, (2014) and Muasya *et al*. (2011) reported low pedigree completeness which may have contributed to overestimation of the parameter for the entire population. The decline in future effective population size was due to increase in levels of average coancestry in the Sahiwal cattle breed. Similar results were reported by Muasya *et al*. (2013) for the Holstein-Friesian population in Kenya. The effective population size of the most recent cohort was lower than values of 263 Holstein-Friesian cattle populations in Kenya (Muasya *et al*, 2013) and 76 for Canadian Brown Swiss Milking Shorthorn dairy breeds (Melka *et al*., 2013). The depth and quality of the pedigree has been shown to have a correlation to effective population size and inbreeding levels (Gutierrez *et al*., 2003).

The values for rate of inbreeding and coancestry for the most recent year cohort were higher when assessed for the animals with three and six complete generations (0.82 versus 1.19 and 0.89 versus 1.36, respectively). These values were above the limit of 1% recommended for a population to maintain it viability (FAO, 1998). Pedigree completeness for the same population have been reported to have decreased over time from 100% in 1956 to 76.3% in 2008 (Kamiti, 2014). Estimation of these parameters based on better pedigree quality revealed higher rates of inbreeding and rates of coancestry than reported for the entire population. Similar results for other cattle populations were reported by Gutiérrez and Goyache (2005) and Muasya *et al*. (2013). Consequently the future effective population size of 35 was below the range of 50 to 500 recommended for a population to maintain its viability and long-term fitness in the long-term (FAO, 1998; Franklin and Frankham, 1998). This means that the population may not be able to maintain its long-term genetic viability and fitness. A similar trend was been reported for the Kenyan Holstein-Friesian (Muasya *et al*., 2013). Higher values of inbreeding have been reported for Tunisian, Luxembourg and Danish Holsteins for individuals with at least three complete generations (Sørensen *et al*., 2005; Hammami *et al*., 2007). This may be attributed to pedigrees that are deeper and have more historical information as compared to that of the Kenyan Sahiwal population.

Superior animals that are used for breeding ought to get equivalent genetic contribution from the founders in the population if genetic diversity is to be conserved. Therefore, the higher the GCI value the higher the genetic contribution from the founder for an individual (Gutiérrez and Goyache, 2005). Genetic conservation index increased as number of maximum generation increased meaning increase in genetic contribution for individual animals from the founder generation (Gutiérrez and Goyache 2005; Hazuchová *et al*., 2012). Similarly, better pedigree quality improved the estimation of this index. This in conformity to previous studies which reported that estimation of parameters related to genetic diversity of livestock populations were affected by pedigree quality (Gutierrez *et al*., 2003; Muasya *et al*., 2013).

Wright's F parameters for the 2000-2008 cohort were below 1% for the population which is an indication of reduced differentiation (FAO, 1998; Gutiérrez and Goyache., 2005). The wide use of some individual animals within the population may have led to reduce differentiation in the population (Muasya *et al*., 2011). The values for the loss of genetic diversity remained constant over the years and were relatively low compared to that of Slovak Spotted bulls of +0.063 (Hazuchová *et al*., 2012). Lower values have been reported for Holsteins, Milking Shorthorn, Brown Swiss, Guernsey and Ayrshire in Canada (Melka *et al*., 2008). The major reason for reduced genetic diversity in most cattle breeds has been due to genetic drift as a result of small effective population size (Melka *et al*., 2008; Stachowicz *et al*., 2011). Honda *et al.* (2004) also reported a similar decrease in genetic diversity in Japanese Black Cattle as a result of genetic drift. Loss in genetic diversity for the Sahiwal cattle breed in Kenya could be attributed to genetic drift as well as unequal contribution by founders (Muasya *et al*., 2011).

4.6 Conclusion

The Kenyan Sahiwal population breed is characterized by shrinking effective population size and increasing inbreeding levels which in turn has led to systematic loss of genetic diversity. The breeding structure of the breed is weak as a result of reduced effective number of sires contributing breeding males. The problem can be overcome by planned matings and active control of rates of inbreeding by mating animals that are not related in the breeding herd. Pedigree recording should be improved for accurate estimation of measures of genetic diversity.

CHAPTER FIVE

EFFECT OF CONTROLLING FUTURE RATE OF INBREEDING ON EXPECTED GENETIC GAIN AND VARIABILITY IN SAHIWAL POPULATION

5.1 Abstract

The current study assessed the use of average relationship as a means to control future rates of inbreeding in a small cattle closed nucleus and its effect on genetic gain for milk yield as a means of managing genetic variability in livestock improvement programmes. The effect of the number of potential sires and dams on genetic gain and future rate of inbreeding was also assessed. The aim was to strike an ideal balance between genetic gain and loss of genetic variability. A total of 8452 milk yield records of Sahiwal cows from National Sahiwal Stud, Kenya were used to estimate breeding values and 19315 records used to estimate average relatedness of all individuals. The estimated breeding values and genetic relationships were then used to optimize individual genetic contributions between the best two males and all the females in 2000-2008 year group and the best 210 females, and between the best two males and the top 210 females, as well as between the best 4, 6, and 8 males and top, 420, 630 and 840 females based on estimated breeding values for lactation milk yield. Weights on genetic merit and average relationship considered in this study were (1, 0), (1, −300), (1, −500), (1, −1000) and (0, −1). The average relatedness was 2.07% in the 2000-2008 year group, and was 2.06 and 2.07 for females and males, respectively. Inbreeding was also high for the same group (1.19%). When the best sires were selected and used for mating disregarding average relationship with their mates i.e. (0, -1), genetic gain of up to 213 kg was realized accompanied by a rate of inbreeding per generation of 4%. Intermediate levels of restrictions on genetic merit and average relationship (1, -300; 1, -500 and 1, -1000) gave average future rates of inbreeding per generation ranging from 1.1% to 3.2 % and average genetic merit of 137 kg to 209 kg. Restricting average relationship alone i.e. (0, -1), resulted in a future rate of inbreeding of 1.6% and average merit of 154 when top two sires were used for breeding. At the same restriction level but using 8 top sires, the rate of inbreeding per generation was 0.9 % accompanied by an average merit of 128.2 kg. Controlling average relationship between mates resulted in increased genetic variability i.e. lower rate of inbreeding though average merit declined. A rate of inbreeding per generation of <1% is required for a population to maintain its long-term viability. For this level to be attained, the size of the breeding population should be increased.

Practical implications for closed nucleus programmes such as the Sahiwal programme in Kenya include expanding the nucleus to include other institutional and privately owned herds.

Key words: *average relationship, genetic merit, Kenyan Sahiwal, optimum contribution*

5.2 Introduction

Genetic diversity of any breed is required to meet current and future production requirements in different or changing environments. This is to allow sustainable, economically important genetic improvement and to be convenient for adapting rapidly to the existing breeding objectives (Melka and Schenkel, 2010; Tang *et al*., 2013). Maintaining local breed diversity is usually an acknowledged conservation objective as it contributes a lot to farm animal genetic diversity (Gandini *et al*., 2014). Many local breeds such as Sahiwal cattle breed are important as they provide ecosystem services and are associated to specific agro-ecological zones and pastoral social diversity which necessitates their conservation. Local breeds help in maintaining livelihoods for communities living in marginal areas (FAO, 2007). The future genetic progress of any breed will depend largely on the availability of adequate genetic variation.

Small and closed populations are always at risk because of a higher loss of genetic diversity and increased rates of inbreeding as a result of small effective population size (FAO, 1998). In Kenya the Sahiwal is bred under a closed nucleus breeding programme (CNBP) where performance recording and selection is confined to the nucleus, and the pastoral herds are the main recipients of the resultant genetic superiority (Ilatsia *et al*., 2011b). Nucleus breeding programmes have been advocated for genetic improvement of cattle in developing countries (Kahi *et al*., 2004) due to their ease and lower cost of implementation since recording and selection are done in the nucleus only. CNBPs however, are associated with high inbreeding levels and low effective population sizes due to increased focus on a few high performing animals often closely related (Fernandez *et al*., 2011). High levels of inbreeding are associated with inbreeding depression, reduced genetic variation, increased sampling variance of breeding programmes and emergence of lethal recessive alleles in homozygous form (Meuwissen and Sorensen, 1998; Malhado *et al*., 2013). Difficulties in planning mating systems aimed at controlling future rates of inbreeding due to increased average relatedness (AR) among individuals have also been reported in CNBPs (Fernandez *et al*., 2011).

In the Kenyan Sahiwal population, current rate of inbreeding and AR are on an upward trend (Muasya *et al*., 2011; Kamiti, 2014) and is above value of 1% recommended value (FAO, 1998) beyond which a population loses its fitness. As a consequence, effective population size (Ne) is declining and is currently below the threshold of 500 recommended (Franklin and Frankham, 1998) for a population to maintain its long-term viability ((Muasya *et al*., 2011; Kamiti, 2014). Further, a small number of ancestors (16) account for over half of the total variation in the population suggesting overuse of a small number of some animals as parents over generations (Muasya *et al*., 2009; Mwangi *et al*., 2016).

The optimum genetic contribution, a strategy that maximises the genetic gain while restricting the rate of inbreeding or the relationships among the candidates has been developed and applied in large livestock populations (Koenig and Simianer, 2006; König *et al*., 2010). The strategy leads to increased genetic gain at the same rate of inbreeding compared to traditional selection schemes. It is not known however, whether the approach is effective in a CNBP with small population such as that of the Sahiwal cattle in Kenya. In this Chapter, the average relatedness and coancestry of individuals in the Sahiwal herd is estimated. In addition the effect of controlling future rates of inbreeding on expected genetic gain and mate allocation in the Kenyan Sahiwal cattle population is evaluated.

5.3 Methodology

Data for this study were obtained from the National Sahiwal Stud (NSS) at the Kenya Agricultural and Livestock Research Organization (KALRO), Naivasha. Information collected was identification, sire and dam date of birth and sex of each animal. Each individual was required to have at least one complete lactation milk yield. The pedigrees of the animals were traced as far back as possible in the birth record book database. All ancestors and relatives of each individual were included in the analyses. A total of 8452 lactation milk yield records were used to estimate breeding values of all cows and sires with milk yield records at the NSS. Pedigree records of 18315 animals were used to estimate parameters related to inbreeding, average relatedness and to predict future rate of inbreeding.

5.4 Estimation of parameters

5.4.1 Inbreeding coefficient (F) and additive genetic relationship among individuals

The level of inbreeding, F, was calculated as the probability that an individual had two alleles that were identical by descent using the method of VanRaden (1992). In this method, the estimated inbreeding coefficient of an animal with unknown origin is equal to half the average relationship between genetic groups of its phantom parents. It also allows for computation of inbreeding coefficients per generation and takes into account inbreeding level of founders. The algorithm of Colleau (2002) was used to calculate the additive genetic relationships between groups of selected mates.

5.4.2 Future increase in inbreeding per generation

The average coancestry between a group of animals predicts the average inbreeding coefficient in the next generation (Falconer and Mackay, 1996). Therefore the rate of increase in coancestry, Δf, can be interpreted as the future rate of inbreeding per generation. Hence the future rate of inbreeding per generation was calculated as:

$$\Delta f = \frac{1}{2Ne} \tag{18}$$

where Ne is effective population size and Δf is the rate of inbreeding per generation.

5.5 Selection of candidates to be parents of the next generation

The candidates for the study were selected based on their merit for lactation milk yield. The best two sires were selected and each mated to 105 elite females according to Meyn and Wilkins (1974). The number of top males was increased from 2 to 8 in order to determine whether the size of the breeding herd influenced measures of genetic variability. For the different numbers of top sires the mating ratio was maintained at 1:105

5.5.1 Estimation of breeding values

Breeding values for all animals for lactation milk yield were estimated using a single trait repeatability animal model. The mixed model equation for the animal model was as described by Mrode, (2014):

$$\mathbf{Y} = \mathbf{Xb} + \mathbf{Za} + \mathbf{e}$$

(19)

where, **y** = vector of lactation milk yield records; b= vector of fixed effects; **a**= vector of random animal effects; **e** = vector of random residual effects; **X** = incidence matrix relating records to fixed effects; **Z** = incidence matrix relating records to random animal effects.

5.5.2 Optimal genetic contributions

Assuming discrete generations, the contribution to the average relationship, *c* in year *t* as a function of current genetic contributions is: $\mathrm{c} = \mathbf{c}^{'}\mathbf{Ac}$, where *A* is the numerator relationship matrix of candidates for selection in year t−1.

Optimal genetic contributions maximizes a criterion which is:

$$\mathrm{C} = \mathrm{w}_{\mathrm{a}}.\mathbf{c}^{'}.\hat{\mathbf{a}} + \frac{\mathrm{w}_{\mathrm{r}}}{(2\mathrm{L})^{2}}(\mathbf{c}^{'}.\mathbf{A.c} + 2\mathbf{c}^{'}.\mathbf{A.P.v} + \mathbf{v}^{'}\mathbf{P}^{'}.\mathbf{A.P.v})$$

(20)

where **A**, is the numerator relationship matrix, ***â*** a vector of predicted breeding values, **P** previous genetic contributions, ***v*** a lifetime breeding profile (Grundy *et al.* 2000); **c'Ac** and **Pv** are average relationship among breeding animals in the next generation and commited future contributions, respectively; **v'P'APv** and **c'APv** are the average relationship among offspring of animals with committed futire contributions and average relationship among breeding animals and offspring of breeding animals with committed future contributions in the next generation; summation of **c'Ac,** 2**c'APv** and **v'P'APv** gives the average relationship of animals in the next generation. Therefore reducing this expression i.e. **c'Ac +**2**c'APv** + **v'P'APv** results in reduced future rate of inbreeding and increase genetic gain in the long-run (Sonesson and Meuwissen, 2002); w_a is the value of merit and w_r the cost of inbreeding/relationship and ***c*** the vector of genetic contributions to be optimize (Berg *et al.*, 2006). The variation of merit is usually larger than the variation of the average relationship of animals in the next generation; therefore the weight on merit, $\mathrm{w_r}$ is usually larger than that on relationship, $\mathrm{w_a}$. The pairs of weights (w_a, w_r) considered in this study were (1, 0), (1, −300), (1, −500), (1, −1000) and (0, −1) (Sørensen *et al.*, 2008). EVA software was used to optimise individual genetic contributions by optimizing a criterion as defined by Grundy *et al.*

(2000), while breeding values for lactation milk yield were estimated using BLUPF90 software (Misztal et al., 2014).

5.6 Results and Discussion

Estimates of average relatedness (AR) and inbreeding (F) are shown in Table 10. The mean AR of the 2000-2008 population was 2.1%. The value was higher in female than in male animals in 2000-2008 year group (Table 10). The higher AR and F estimates for both males and females could be attributed to use of fewer breeding animals that are often related (Kearney *et al*., 2004).

Table 90: Average relatedness for the Kenyan Sahiwal breed for 2000-2008 year cohort

	2000-2008	**Males**	**Females**
N	1962	1058	904
AR %	2.07	2.06	2.07
F %	1.19	1.15	1.23

AR – Average relatedness; F – Inbreeding Coefficient; N – Number of animals

Average relatedness was higher than 1% for the entire population as well as among male and female groups. When AR reaches high relative values (>1%), matings should be carefully planned, otherwise it would be easy to find matings between individuals showing a certain degree of relatedness (Fernandez *et al*., 2011). AR considers inbreeding and coancestry coefficients and therefore it is a good indicator of inbreeding in a population (Gutierrez *et al*., 2003). Therefore this parameter is beneficial as it guides in using mating animals that have lowest average relatedness values leading to maintenance of genetic diversity for a population. As average relatedness increases, matings should be carried out and planned systematically so as to reduce inbreeding levels. The best mating strategy should target the progeny mating of founders with low values for average relatedness so as to ensure that the founders' original diversity is represented efficiently throughout the generations in that population (Muasya *et al*., 2009).

The mean genetic level, genetic relationship and inbreeding coefficient within groups of best sires and dams for lactation milk yield are given in Table 11. The average mean genetic level was 198.8 and 133.9 kg for selected sires and dams, respectively. Average inbreeding was 1.9% and 2.1% for potential sires and dams, respectively. Average relationship was 31.0% among potential sires and 6.8% for potential dams, and 11.5% between potential sires and dams implying that under random mating inbreeding coefficient would be 5.8%. The average relationship between potential sires and dams was higher than that of 10.9% and 9.5% reported for US and Danish

Holsteins (Weigel and Lin, 2002; Sørensen *et al.*, 2008), German Holstein which was 5.93% (Koenig and Simianer, 2006). This is an indication that breeding animals in the current Sahiwal populations in Kenya are related to an extent similar to relatedness of above 10%.

Table 101: Mean genetic level, inbreeding coefficient (%) and genetic relationship among potential parents (2 sires and 210 dams)

Mean genetic level		Inbreeding coefficient (%)		Genetic relationship (GR)		GR between sires and dams
Potential Sires	Potential Dams	Potential sires	Potential Dams	Potential sires	Potential Dams	
198.8	133.9	1.9	2.1	31.0	6.8	11.5

Optimal genetic contributions were first calculated for the best 2 sires and 210 dams based on lactation milk yield according to the breeding programme developed by Meyn and Wilkins (1974). The number of breeding males was gradually increased while maintain the mating ratio in order to assess the effect of size of breeding population on genetic merit and predicted rate of inbreeding per generation. Table 12 shows the average merit and average relationships for the selected matings along with the expected rate of inbreeding for different weightings on merit and genetic relationship. For weight to merit (1, 0), the average merit for the selected matings was 213.0 and average relationship of 0.05. For weights of 1, -300; and 1, -500 for average merit and relationship, respectively, the resulting average merit was 209.3 kg and an average relationship of 0.05 for both levels. Weights of 1 and -1000 for average merit and relationships led to an average merit and average relationship of 169.0 and 0.045 %, respectively.

When there was weight on average relationship only, average merit was 154.0 kg while average relationship was 0.045%. This showed that, when more weight was put on average relationship as compared to merit, lower average merit and average relationship would be realised. This can be attributed to a more dispersed utilization of sires hence checking on average relationship. When weight was on merit alone, the resulting average merit was high for all weights. High average merit from these weights is due to lack of consideration of pedigree information that is useful in determination of the relationship among the mates. This is similar to the current approach in the National Sahiwal Stud, in Kenya, where focus has been on merit only, leading to a decline of the population’s effective population size (Muasya *et al*., 2009; Kamiti, 2014) due to increased inbreeding.

Table 112: Average merit for lactation milk yield and average genetic relationship among the selected matings for the Kenyan Sahiwal breed for different weights on genetic merit and relationship and size of breeding population

Breeding scheme	Weights	Average merit	Average relationship	Average inbreeding (%)	Predicted rate of inbreeding per generation (%)
2 vs 210	1,0	210.0	0.049	14.51	4.03
	1,-300	209.3	0.048	4.52	3.22
	1,-500	209.3	0.048	4.52	3.22
	1,-1000	169.0	0.046	0.93	1.92
	0,-1	154.0	0.045	0.70	1.60
8 vs 840	1,0	213.0	0.049	14.5	4.1
	1,-300	209.3	0.048	4.52	3.2
	1,-500	190.5	0.046	0.75	2.5
	1,-1000	137.1	0.044	0.26	1.1
	0,-1	128.2	0.044	0.16	0.9

When the number of individuals in the breeding population was increased to 848 (i.e. the top 8 and 840 elite females), average merit was generally lower for all sets of weights while average relationship was similar across the two breeding scenarios. Average inbreeding was similar for the two scenarios for the first two weighting criteria (1, 0 and 1, -300). For weights 1, -500; 1, -1000 and 0, 1, average inbreeding and predicted rate of inbreeding were consistently lower when the top 8 bulls were used for breeding. Predicted rate of inbreeding was 0.9% per generation when restriction was applied on average relationship alone (0, 1)

Average inbreeding was very high (14.5%) when there was no restriction on average relationship but was accompanied by the highest gain in merit (210.0 kg and 213kg for scenario 1 and 2, respectively). When there was restriction on average relationship as well, (1, -1000; and 0, -1) the average inbreeding was 0.9% and 0.7% and 0.3 and 0.2% for scenario 1 and 2, respectively. This is an indication that when pedigree information is used as a basis for mate selection, future inbreeding levels can be controlled leading to maintenance of genetic diversity, while at the same time achieving genetic progress. More importantly the results point out the need to increase the size of the breeding herd if viability and fitness of the population is part of the breeding objective. Females with low relationship to the breeding males can be chosen as parents when large cost is weighted on average relationship relative to merit. This should be done with caution since the dams or sires may have low recorded relationship due to incompletepedigree information. (Sørensen *et al.*, 2008).

For the current Sahiwal cattle breeding programme in Kenya, where only two sires are selected after progeny testing (Meyn and Wilkins, 1974), the predicted rate of inbreeding per generation when only relationship was constrained (0, -1) was above 1% which is recommended for long term maintenance of the breeds (FAO, 1998). This number of breeding males (2) is lower than the 20 to 30 used in other studies evaluating the benefits of optimum genetic contributions in managing genetic diversity (Koenig and Simianer, 2006;Sørensen *et al.*, 2008). In the current study, a rate of inbreeding below 1% per generation was realised when the number of potential bulls and dams was increased to 8 and 840, respectively. The rate of inbreeding per generation of a breeding population should not exceed 1%, if the population is to maintain its longterm viability (FAO, 1998). For selected matings as recommended by Meyn and Wilkins (1974) rates of inbreeding per generation were above 1% for all possible weights on merit and average relationship. Therefore the Sahiwal population will continue to lose genetic variability over time. Mwangi *et al.* (2016) found that the Kenyan Sahiwal population could be losing variability through genetic drift attributed to a small effective population size. Franklin and Frankham (1998) recommended an effective population size of between 500 and 1000 for a population to maintain its fitness in the longterm, translating to a rate of inbreeding per generation of 0.1%.

To achieve the desired level of genetic viability in future generations, the size of the breeding population should be increased to 840 (a mating ratio of 1 bull to 105 females). This approach may be unrealistic for the closed nucleus at the National Sahiwal Stud, Kenya, due to limited resources. Ilatsia *et al* (2011b) recommended opening up the nucleus to include other institutional and private Sahiwal herds in Kenya. Despite the anticipated logistical and cost implications, allowing animals from other herds in to the nucleus has been shown to result into superior genetic gains compared to closed nucleus breeding programmes (Gicheha *et al.*, 2006; Ilatsia *et al.*, 2011b; Rewe *et al.*, 2011). This is due to use of genetically superior sires from diverse populations. An initial step could be to have the potential dams distributed in several herds. Potential sires could then be progeny tested in the dispersed nucleus. An added benefit would be an increase in accuracy of estimated breeding values as well as matching genotype to environment: the latter due to testing in different herd and or climatic conditions. In general, males to be used for breeding should also be selected based on more complete pedigree information and lower average relationship to ensure that the rate of inbreeding per generation doesn’t exceed the recommended level. However, the value of optimal genetic contribution selection could decrease with increased intensive pre-selection of possible parents (Sørensen *et al*., 2008).

5.7 Conclusion

Using optimum genetic contributions, it was possible to optimize the genetic gain, while controlling the rates of inbreeding per generation. Genetic merit was highest when focus was on genetic gain disregarding average relationship among breeding individuals. When the aim was to control future rate of inbreeding there was a reduction in both average merit and average relationship. When only two breeding males were utilised for breeding, future rate of inbreeding per generation was above 1% for all levels of restriction on average relationship among breeding individuals, meaning that the population would lose its viability in the long-term. Increasing the number of top bulls to 8, it was possible to constrain the future rate of inbreeding per generation to below 1%, although less genetic merit was realised. Sustainable genetic improvement and maintenance of genetic variability can be realised for a population to ensure its long-term viability and fitness. The size of the breeding population however, may require to be increased. Practical implications for closed nucleus programmes such as the Sahiwal programme in Kenya include expanding the nucleus to include other institutional and privately owned herds, barring logistical and cost implications for small livestock populations.

CHAPTER SIX

GENERAL DISCUSSION AND CONCLUSIONS

6.1 Aim of the study

There is increasing global concern about the potential long term consequences of loss of domestic animal diversity. Small, closed populations are at risk because of a higher loss of genetic diversity and increased rates of inbreeding (FAO, 1998). The Sahiwal population in Kenya is bred under a closed nucleus and the effective population has been declining over the years which is an indication of a population that is losing its evolutionary potential and which may not be able to maintain its long-term viability. Level and rate of inbreeding are on an upward trend and are >1%; the recommended threshold beyond which a population begins to lose its ability to maintain its long-term viability. The overall goal of this study was to contribute to a sustainable breeding programme for the Sahiwal breed in Kenya through development of a strategy to maintain and manage genetic diversity while achieving desirable genetic gain in the breeding objective. The specific objectives were: i) to determine the population structure and estimate parameters based on gene origin statistics for Kenyan Sahiwal cattle breed, ii) to estimate realized and predicted expected measures of genetic diversity for different year cohorts for the Kenyan Sahiwal population and iii) to evaluate the effect of controlling future rate of inbreeding on expected genetic gain and design a strategy to manage genetic diversity of the Sahiwal breed in Kenya.

This thesis addressed three major research questions which included; i) what is the population structure and trend in genetic variability of Kenyan Sahiwal breed? ii) what are the realized and expected measures of genetic diversity for different year cohorts for the Kenyan Sahiwal Population? and iii) what is effect of controlling future rates of inbreeding on expected genetic gain in the Sahiwal in Kenya?

6.2 Study Methodology

For assessing genetic variability of the Kenyan Sahiwal population, pedigree analysis using genealogical information of animals born between 1949 and 2008 was carried out. The parameters estimated included, generation intervals, pedigree completeness (number of maximum traced generations, number of complete generations and number of complete generation equivalents), increase in inbreeding per generation, individual increase in inbreeding and effective population size. Parameters of gene origin statistics were used to describe the historical genetic variability of the breed since they are less sensitive to the quality of the available pedigree information. The parameters estimated were number of founders (N_f), effective number of founders (f_e), effective number of ancestors (f_a) and effective number of founder genomes (f_g). All parameters were calculated using ENDOG software (Gutiérrez and Goyache, 2005) and were computed for 5 10-birth year groups.

The second objective assessed the within-population genetic structure and genetic diversity loss or gain necessary accrued due to implementing selection programmes to increase genetic breeding stock for selection. Current and future indicators of genetic diversity were also estimated. The implications on the genetic history of the Kenyan Sahiwal cattle breed over the years of breeding was evaluated too. The genetic conservation index was calculated from the genetic contributions of all established founders from the reference population. Genetic structure of the Kenyan Sahiwal population was assessed using F-statistics (F_{IT}, F_{ST}, F_{IS}; Wright, 1978). Contribution of subpopulations to total diversity was computed for the ten year subgroups from 1960 to 2008. The amount of loss of genetic diversity in the reference population (2000-2008) was assessed according to Lacy (1995).

A strategy to control future rate of inbreeding while achieving desirable genetic gain so as to manage genetic diversity was tested based on the optimum genetic contribution. Breeding values for animals for the lactation milk yield were estimated using a single trait repeatability animal model. The candidates for the study were selected based on their merit on milk yield. Two best sires and 210 dams were selected based on their superiority i.e. high estimated breeding values for the milk. Only two males were selected since the National Sahiwal Stud, Kenya normally uses semen from the best two bulls to inseminate the elite herd. In the first scenario, the best two males were mated against all the available males in the 2000-2008 year group. The second scenario involved the best 4, 6 and then 8 males against top 105 females each. The genetic progress in the Sahiwal population for lactation milk yield with levels of restrictions on genetic merit and average relationship of (0, -1), (1, -300), (1, -500) and (1, -1000), respectively.

In order to develop a suitable and sustainable genetic conservation strategy for a breed, it entails an initial step of assessing its existing genetic diversity. This study was initiated to provide comprehensive information on Kenyan Sahiwal cattle population structure so as to develop a sustainable conservation and strategy. There was need for assessment of genetic diversity because the utilization of assisted reproduction technologies, such as artificial insemination used for the breed have been reported to potentially and rapidly lower the genetic diversity of populations (Vasconcellos *et al*., 2003).

6.3 Genetic variability of Sahiwal cattle breed

Genetic variability of the population assessed based on gene origin statistics decreased over the years (Chapter 3). The ratio of effective number of founders to founders of 0.06 showed unequal contribution of founders to the reference population. However, since the founding population, ancestors contributed equally as shown by the ratio of f_e/f_a of 0.94, which could also be due to lack of effective selection in this population. The ratio of f_g/f_a of 0.63 indicated genetic loss of genetic variability occurred through genetic drift in the Kenyan Sahiwal population. The small number of ancestors (16) that accounted for 50 % of the total variation in the reference population suggested overuse of a small number of some animals as parents over generations. Therefore the National Sahiwal Stud should embark on progeny testing to avert use of old bulls over a prolonged period of time. The smaller ratio of f_g/f_e compared to f_a/f_e also confirms that loss of genetic variability in the population was through genetic drift than bottlenecks. Results from this study demonstrated a pressing necessity for developing suitable breeding strategies for the Sahiwal population in Kenya incorporating tools that balance rate of genetic gain and the future rate of inbreeding.

6.4 Genetic structure and within-population variation

The assessment of the within populations genetic variability has received increasing attention over the recent years (Woolliams *et al.* 2002). Considering both conservation and selection, some of the simple demographic parameters have a large impact on the evolution of the genetic variability and largely depend on the management of the population (Goyache *et al.* 2003; Gutiérrez *et al.* 2003; Honda *et al.* 2004). Furthermore, researchers and breeders are concerned with ascertaining the extent to which an unplanned mating policy leads to structuring the populations of the breed under study (Caballero and Toro 2002). In this study (Chapter 4), the parameters of genetic differentiation for the whole population under study (1961-2008) had values of less than 1% which is an indication of reduced differentiation (FAO, 1998; Gutiérrez and Goyache, 2005). The lowered genetic variation for the population may be attributed to wide use of few individual animals within the herd (Muasya *et al*., 2011; Mwangi *et al*., 2016).

The genetic conservation index (GCI) for the whole population was 5.93% whereas for the individuals with at least three generations and the reference population had the values of 7.87%

and 9.87%, respectively. Loss in genetic diversity for the whole population as well as the reference population was reported as -0.023. In the recent population, the rate of inbreeding per generation was 0.79% with the rate of coancestry per generation being 0.85%. The GCI and rates of inbreeding and coancestry per generation for individuals with at least 6 complete generations were 7.87% 0.89% and 1.36%. Predicted rate of inbreeding per generation for individuals with at least 3 complete generations 0.89 translating to an effective population size of 54. The future rate of inbreeding per generation and effective population size for individuals with 6 complete generations was 1.36% and 35, respectively. These values were >1%, implying that the population is losing genetic diversity and may not be viable in the long-term (Franklin and Frankham, 1998). The GCI for whole population was 5.93% whereas individuals with at least three complete generations and the reference population had values of 7.87% and 9.87%, respectively. The parameters of genetic differentiation for the whole population under study (1961-2008) were F_{IS}=- 0.0071, F_{ST}= 0.0036 and F_{IT} =- 0.0034. Loss in genetic diversity for the whole population was -0.023, indicating loss of genetic diversity through random genetic drift and unequal contribution of founders. Improved pedigree completeness led to higher estimates of parameters describing the structure of the population (GCI, Ne_c, Ne_f, ΔF and Δf). Mating should be carried out between mates with low coancestries and also by allowing more animals that are not related into breeding herd. Pedigree recording should be improved for accurate estimation of parameters

6.5 Management of genetic diversity

The main goal of breeding programmes is to maximize genetic gain. Nevertheless, controlling of inbreeding is key, and hence methods of selection have to be designed to manage inbreeding rates (Hinrichs and Wetten 2006). Selection in small populations such as for the Kenyan Sahiwal breed needs to take into account of accurate inbreeding control measures. Optimum Genetic contribution (OGC) selection is effective in controlling the rates of inbreeding while maximizing on genetic gain (Gandini *et al*. 2014). However, this strategy has been tested on large livestock populations ((Koenig and Simianer, 2006; König *et al*., 2010; Hinrichs *et al*., 2014). The OGC was used for the current small population and proved to offer practical means in the management of inbreeding in Sahiwal population. The trends in AR and coancestries were undesirable. The average relatedness reached 2.07% in the most recent year of birth cohort (2000-2008 year group), and was 2.06% and 2.07% for females and males respectively. Inbreeding was found to be high for the most recent year group. Restriction on average relationship, at (1, -1000) and (0, -1) yielded an average inbreeding of 0.9% and 0.7%, respectively (Chapter 5). The rate of change for inbreeding when only average relationship was constrained (0, -1) was slightly above 1% which is recommended for long-term maintenance of genetic viability for a breed (Franklin and Frankham, 1997). This was an indication that the Sahiwal population in Kenya will continue to lose its genetic diversity under the current breeding programme. Increasing breeding bulls to 8 (and 840 females with a mating ratio of one bull to 105 females) succeeded in achieving a rate of inbreeding per generation that was lower than 1%. Increasing the breeding population for Sahiwal breed will most often require specific investments in infrastructure and manpower while also considering other factors such as the need for accurate data recording, genetic evaluation and selection skills and the presence of reproductive technologies such as AI. Despite these challenges, it will lead to use of more genetically superior sires from diverse populations as it will include having potential dams distributed in several herds (Gicheha *et al*., 2006; Ilatsia *et al*., 2011b; Rewe *et al*., 2011).

6.6 Conclusions

i. The Kenyan Sahiwal breed is derived from a small founding population and lose of genetic diversity is through genetic drift.
ii. The breeding structure of the breed is weak as a result of reduced effective number of sires contributing breeding males leading to systematic loss of genetic diversity.
iii. Using optimum genetic contributions it was possible to optimize the genetic gain, while controlling the rates of inbreeding per generation with high genetic merit achieved when focus was on genetic gain while disregarding average relationship.

6.7 Recommendations

i. The breeding strategy for the Sahiwal population in Kenya should incorporate tools that balance rate of genetic gain and the future rate of inbreeding through planned matings and active control of rates of inbreeding by mating animals that are not closely related.
ii. Pedigree recording of the population should be improved for accurate estimation of measures of genetic variability.

iii. The breeding population of the breed requires to be increased by including other institutional or private herds alongside a strategy in place to balance between levels of inbreeding and genetic gain.

iv. Further studies on the effectiveness of managing genetic diversity on the breeding objective for the Sahiwal population in Kenya are needed

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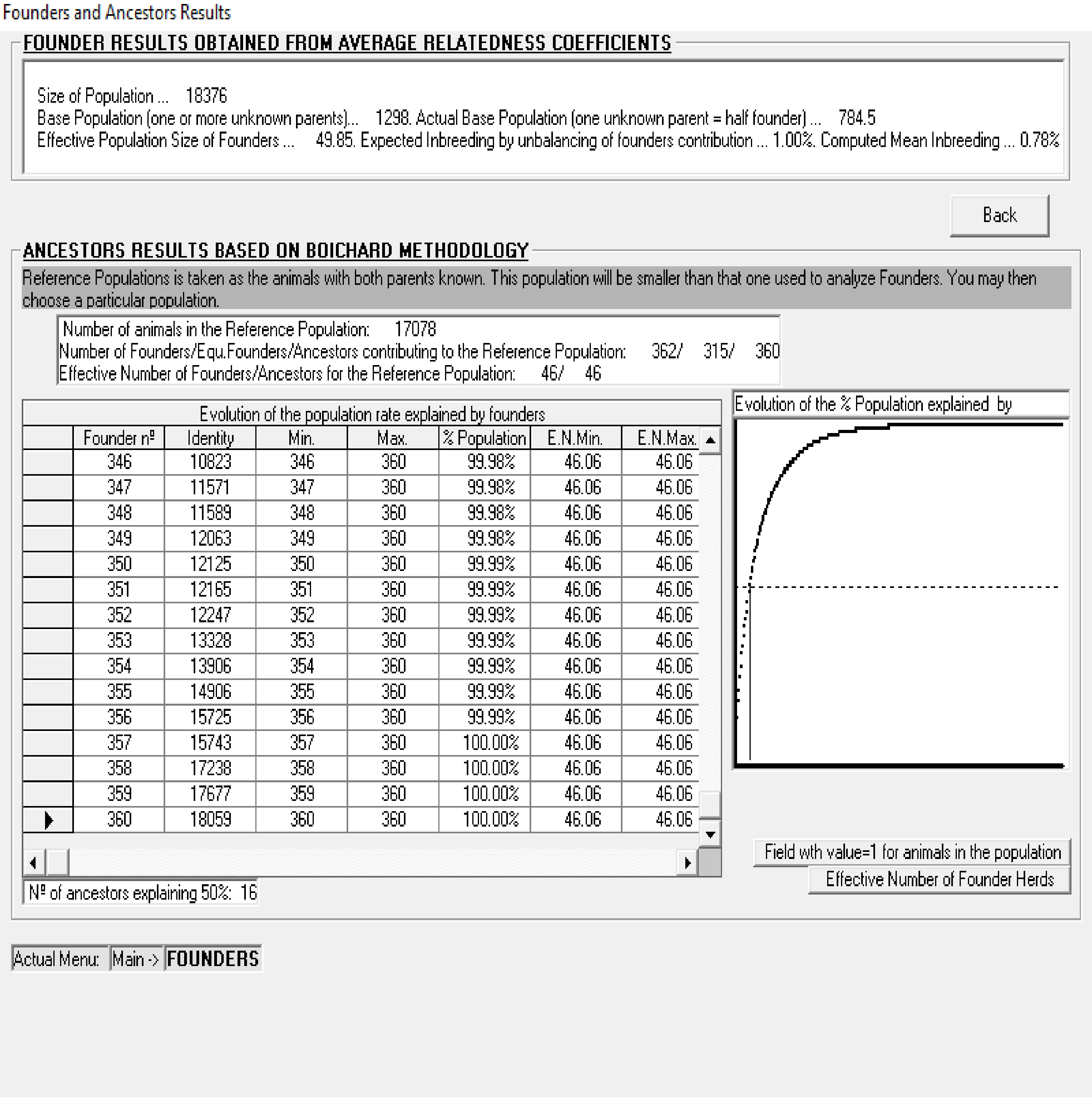
APPENDIX

A section of the results obtained from data analysis for the Sahiwal population in Kenya



Founders and Ancestors Results

FOUNDER RESULTS OBTAINED FROM AVERAGE RELATEDNESS COEFFICIENTS

Size of Population ... 18376
Base Population (one or more unknown parents)... 1298. Actual Base Population (one unknown parent = half founder) ... 784.5
Effective Population Size of Founders ... 49.85. Expected Inbreeding by unbalancing of founders contribution ... 1.00%. Computed Mean Inbreeding ... 0.78%

ANCESTORS RESULTS BASED ON BOICHARD METHODOLOGY

Reference Populations is taken as the animals with both parents known. This population will be smaller than that one used to analyze Founders. You may then choose a particular population.

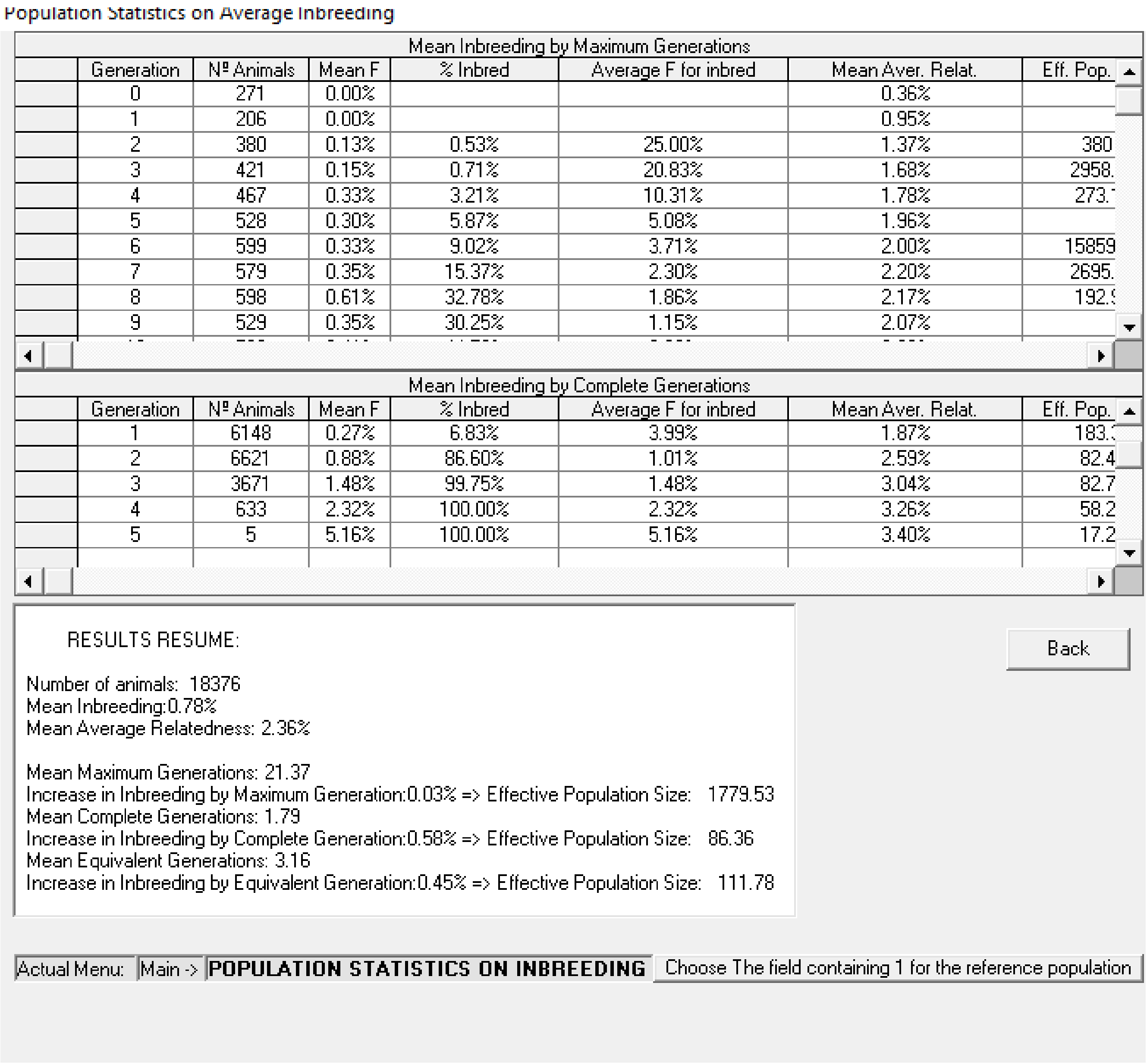
Number of animals in the Reference Population: 17078
Number of Founders/Equ.Founders/Ancestors contributing to the Reference Population: 362/ 315/ 360
Effective Number of Founders/Ancestors for the Reference Population: 46/ 46

Evolution of the population rate explained by founders

	Founder nº	Identity	Min.	Max.	% Population	E.N.Min.	E.N.Max.
	346	10823	346	360	99.98%	46.06	46.06
	347	11571	347	360	99.98%	46.06	46.06
	348	11589	348	360	99.98%	46.06	46.06
	349	12063	349	360	99.98%	46.06	46.06
	350	12125	350	360	99.99%	46.06	46.06
	351	12165	351	360	99.99%	46.06	46.06
	352	12247	352	360	99.99%	46.06	46.06
	353	13328	353	360	99.99%	46.06	46.06
	354	13906	354	360	99.99%	46.06	46.06
	355	14906	355	360	99.99%	46.06	46.06
	356	15725	356	360	99.99%	46.06	46.06
	357	15743	357	360	100.00%	46.06	46.06
	358	17238	358	360	100.00%	46.06	46.06
	359	17677	359	360	100.00%	46.06	46.06
▶	360	18059	360	360	100.00%	46.06	46.06

Nº of ancestors explaining 50%: 16

Evolution of the % Population explained by



Population Statistics on Average Inbreeding

Mean Inbreeding by Maximum Generations

	Generation	Nº Animals	Mean F	% Inbred	Average F for inbred	Mean Aver. Relat.	Eff. Pop.
	0	271	0.00%			0.36%	
	1	206	0.00%			0.95%	
	2	380	0.13%	0.53%	25.00%	1.37%	380
	3	421	0.15%	0.71%	20.83%	1.68%	2958.
	4	467	0.33%	3.21%	10.31%	1.78%	273.
	5	528	0.30%	5.87%	5.08%	1.96%	
	6	599	0.33%	9.02%	3.71%	2.00%	15859
	7	579	0.35%	15.37%	2.30%	2.20%	2695.
	8	598	0.61%	32.78%	1.86%	2.17%	192.
	9	529	0.35%	30.25%	1.15%	2.07%	

Mean Inbreeding by Complete Generations

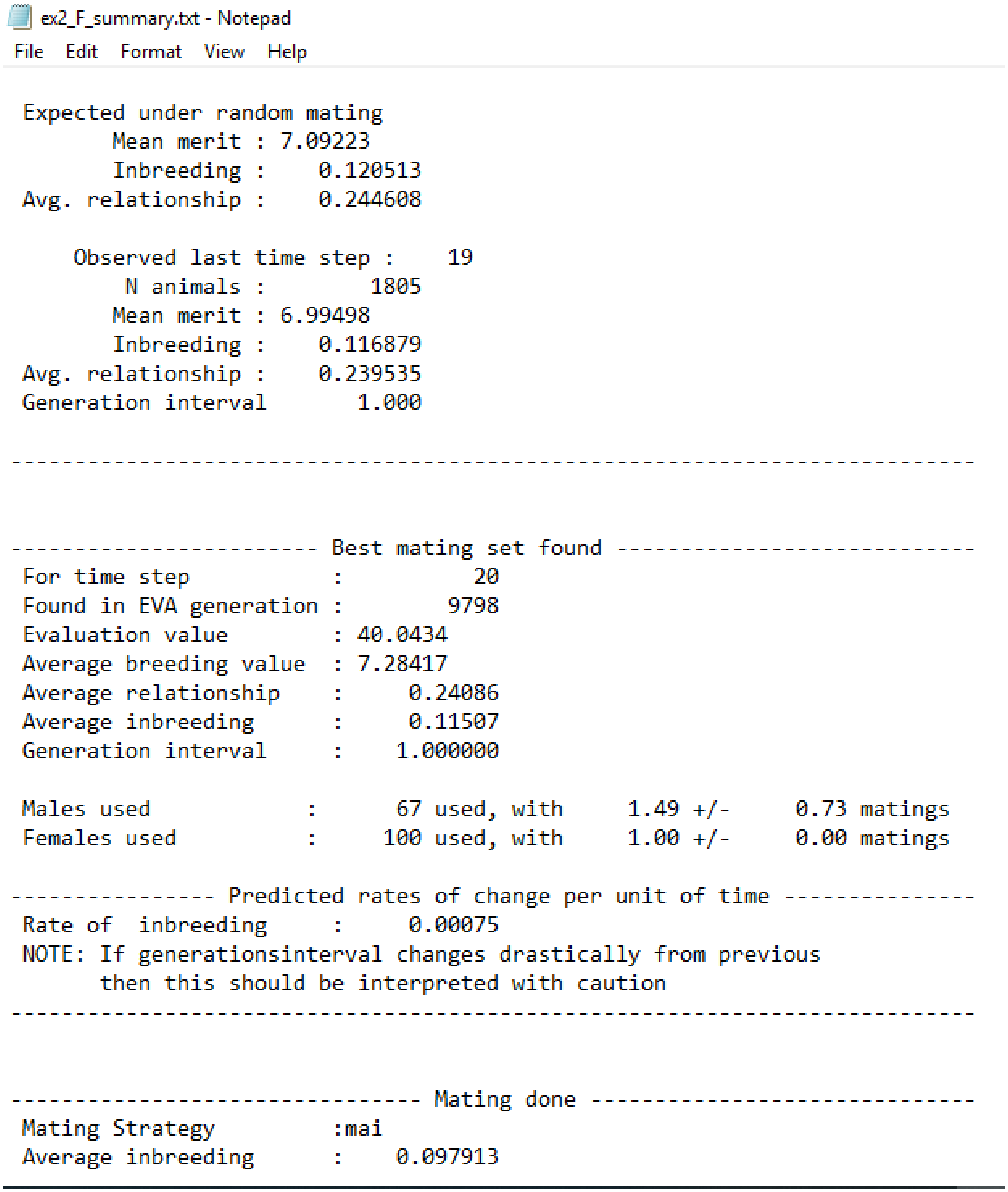
	Generation	Nº Animals	Mean F	% Inbred	Average F for inbred	Mean Aver. Relat.	Eff. Pop.
	1	6148	0.27%	6.83%	3.99%	1.87%	183.
	2	6621	0.88%	86.60%	1.01%	2.59%	82.4
	3	3671	1.48%	99.75%	1.48%	3.04%	82.7
	4	633	2.32%	100.00%	2.32%	3.26%	58.2
	5	5	5.16%	100.00%	5.16%	3.40%	17.2

RESULTS RESUME:

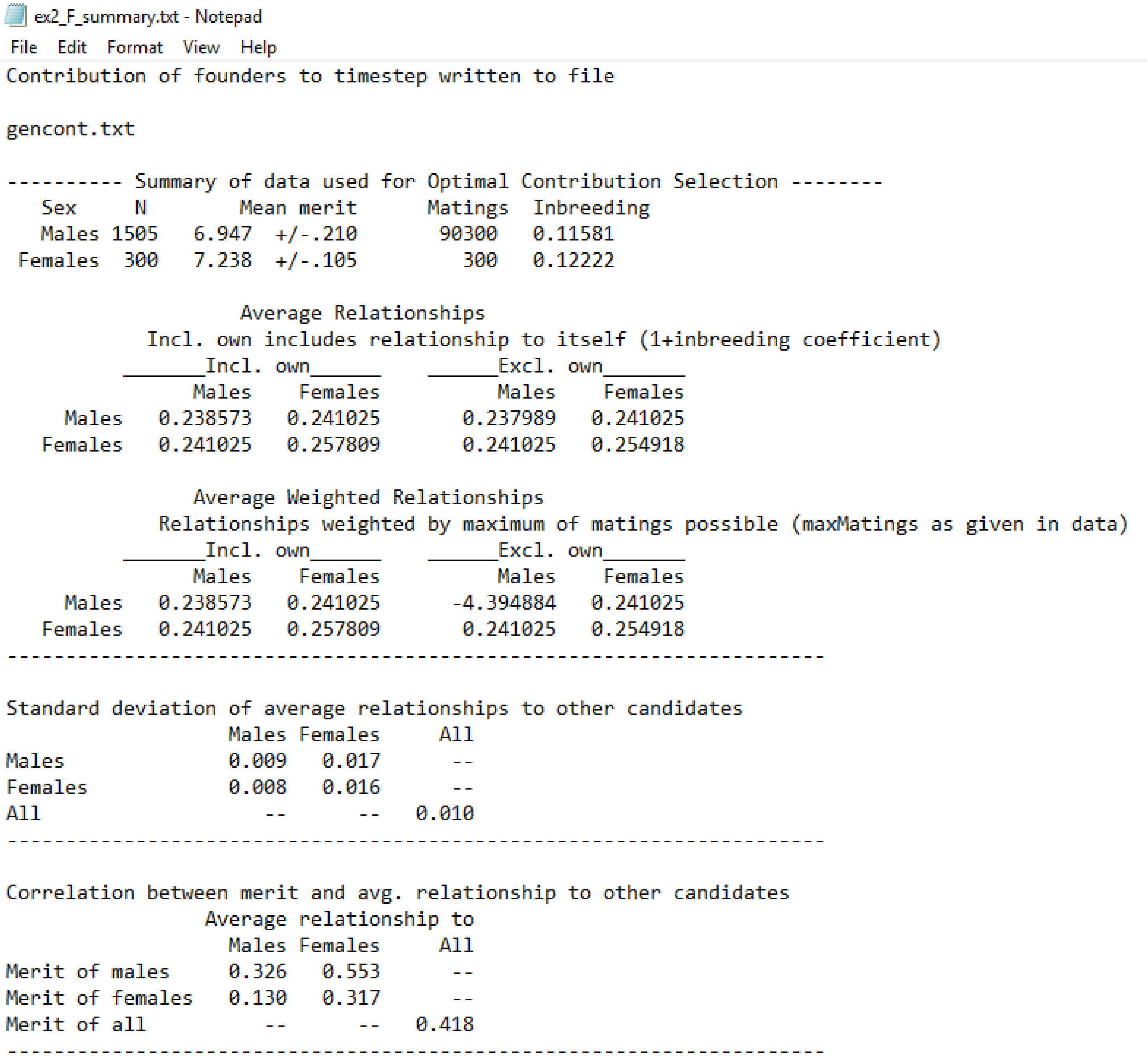
Number of animals: 18376
Mean Inbreeding:0.78%
Mean Average Relatedness: 2.36%

Mean Maximum Generations: 21.37
Increase in Inbreeding by Maximum Generation:0.03% => Effective Population Size: 1779.53
Mean Complete Generations: 1.79
Increase in Inbreeding by Complete Generation:0.58% => Effective Population Size: 86.36
Mean Equivalent Generations: 3.16
Increase in Inbreeding by Equivalent Generation:0.45% => Effective Population Size: 111.78

Actual Menu: Main -> **POPULATION STATISTICS ON INBREEDING** Choose The field containing 1 for the reference population



```
 Expected under random mating
        Mean merit : 7.09223
        Inbreeding :     0.120513
 Avg. relationship :     0.244608

     Observed last time step :    19
         N animals :         1805
        Mean merit : 6.99498
        Inbreeding :     0.116879
 Avg. relationship :     0.239535
 Generation interval       1.000

---------------------------------------------------------------------------



------------------------ Best mating set found ----------------------------
 For time step           :          20
 Found in EVA generation :        9798
 Evaluation value        : 40.0434
 Average breeding value  : 7.28417
 Average relationship    :     0.24086
 Average inbreeding      :     0.11507
 Generation interval     :    1.000000

 Males used            :      67 used, with     1.49 +/-     0.73 matings
 Females used          :     100 used, with     1.00 +/-     0.00 matings

---------------- Predicted rates of change per unit of time ---------------
 Rate of  inbreeding     :     0.00075
 NOTE: If generationsinterval changes drastically from previous
       then this should be interpreted with caution
---------------------------------------------------------------------------



-------------------------------- Mating done ------------------------------
 Mating Strategy         :mai
 Average inbreeding      :    0.097913
```



```
Contribution of founders to timestep written to file

gencont.txt

---------- Summary of data used for Optimal Contribution Selection --------
   Sex     N        Mean merit      Matings  Inbreeding
   Males 1505   6.947  +/-.210       90300   0.11581
 Females  300   7.238  +/-.105         300   0.12222

                    Average Relationships
            Incl. own includes relationship to itself (1+inbreeding coefficient)
          _______Incl. own______    ______Excl. own_______
                Males    Females          Males    Females
     Males   0.238573   0.241025       0.237989   0.241025
   Females   0.241025   0.257809       0.241025   0.254918

                Average Weighted Relationships
             Relationships weighted by maximum of matings possible (maxMatings as given in data)
          _______Incl. own______    ______Excl. own_______
                Males    Females          Males    Females
     Males   0.238573   0.241025      -4.394884   0.241025
   Females   0.241025   0.257809       0.241025   0.254918
----------------------------------------------------------------------

Standard deviation of average relationships to other candidates
                   Males Females     All
Males              0.009   0.017      --
Females            0.008   0.016      --
All                   --      --   0.010
----------------------------------------------------------------------

Correlation between merit and avg. relationship to other candidates
                 Average relationship to
                   Males Females     All
Merit of males     0.326   0.553      --
Merit of females   0.130   0.317      --
Merit of all          --      --   0.418
----------------------------------------------------------------------
```