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GENETIC DIVERSITY AND ADMIXTURE ANALYSIS OF ETHIOPIAN FAT-TAILED AND AWASSI SHEEP USING SNP MARKERS FOR DESIGNING CROSSBREEDING SCHEMES

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Genetic diversity and admixture analysis of Ethiopian fat-tailed and Awassi sheep using SNP markers for designing crossbreeding schemes

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Dedication

This Thesis is dedicated

To

My beloved wife Meseret Abebaw and my lovely son Yohannes Tesfaye:

Abbreviations and Acronyms

AIM	Ancestry informative markers
ARARI	Amhara Regional Agricultural Research Institute
ASBMC	Amed Guya Sheep Breeding and Multiplication Center
BOKU	University of Natural Resources and Life Sciences
CSA	Central Statistical Agency
DBARC	Debre Berhan Agricultural Research Center
DBSBMC	Debre Berhan Sheep Breeding and Multiplication Center
ICARDA	International Center for Agricultural Research on the Dry Areas
ILRI	International Livestock Research Institute
ISGC	International Sheep Genomics Consortium
LD	Linkage disequilibrium
ROH	Runs of homozygosity
SAS	Statistical system analysis
SNP	Single nucleotide polymorphism

Preface

The conception of the idea for this PhD study was when we (me and Prof Johann Sölkner) were observing the physical appearances of Awassi x Menz crossbred sheep under farmer's management in Menz area, Ethiopia. Menz is one of the ICARDA-ILRI-BOKU community based sheep breeding programs site. In one of the two villages in Menz called Negasi Amba, 75% Awassi-Menz crossbred rams have been distributed for a long time to increase the body size of the indigenous sheep breeds in the area. Another village (Chiro) in Wollo is having the same history of crossbreeding. It was extremely interesting to investigate how crosses (composites) of different blood levels perform under smallholder management. Setting optimum level of admixture is one of the prerequisites to implement crossbreeding under smallholders situations.

Ear tissue samples were collected mainly from crossbred ewes and lambs in Negasi-Amba and Chiro. Additional blood samples were obtained from Dr. Tadele Mirkena collected by ICARDA-ILRI-BOKU community based sheep breeding programs from the Negasi-Amba village. Genome DNA was extracted from ear tissue and FTA cards using standard protocols and then genotyped. Additional genotype data were obtained from Prof. Johannes Lenstra, John McEwan and Prof. Elisha Gootwine. The 50KSNP data were used for genetic diversity, population structure, linkage disequilibrium and runs of homozygosity analysis. As an interesting finding of this study as few as ~50 ancestry informative markers (AIMs) were found suffice for the estimation of admixture levels from the crossbred populations. This indicates great opportunity for farmers in developing countries to have access to accurate information at a relatively cheap cost. Admixture level estimates were applied to recommend appropriate levels of Awassi for different areas under farmer's management. Farmer's knowledge to estimate the level of admixture considering the size and morphological characters were highly appreciated.

The output of this thesis is thus helpful in designing or modifying the Awassi sheep crossbreeding program in particular and also would give insight to design breeding program for sheep and other livestock in Ethiopia as well as other developing countries.

Thesis structure

This study was superimposed on the on-going sheep crossbreeding program that has been run by Debre Berhan Agricultural Research Center in Ethiopia since 1997. Improved Awassi (IA) sheep from Israel has been used for the improvement of the productivity of Ethiopian short-fat tailed Menz and Wollo sheep breeds through crossbreeding in two different villages namely Negasi-Amba and Chiro. Three parental populations Menz, Wollo and improved Awassi and two admixed populations Menz x Awassi and Wollo x Awassi were considered for this study.

Description of the study area and genetic and phenotypic characteristics of the parental breeds used for crossbreeding, description of the on-going sheep cross breeding project, details on data collection and analysis are described in materials and methods section.

In the results and discussion section; genetic diversity, population structure, linkage disequilibrium and runs of homozygosity for the parental Menz, Wollo, local Awassi, improved Awassi and some other African, Asian and European breeds based on high density Ovine 50KSNP data were presented and discussed. Detailed information on the ancestry informative markers (AIMs) differentiating the Ethiopian breeds (Menz and Wollo) and improved Awassi were provided. Numbers of top ranked AIMs required for estimating admixture level and their accuracy of using for the estimation of admixture level were studied. Association of individual admixture level with lamb growth and ewe reproductive data was employed and optimum level of admixture based on the current management situation were suggested for the two locations. Regression analysis of was employed to establish relationship between linear body measurement and morphological characters, and SNP based admixture levels.

Finally, concluding remarks were made based on the major results.

Abstract

The aims of the thesis were to select small cost effective set of SNP markers for estimation of admixture level and to identify optimal levels of Awassi admixture for the on-going crossbreeding program that has been run in farmer's villages in the highlands of Ethiopia since 1997. Improved Awassi (IA) sheep from Israel has been used for the improvement of the productivity of Ethiopian short-fat tailed Menz (M) and Wollo (W) sheep breeds through crossbreeding in Negassi-Amba and Chiro villages. High density ~50KSNP markers distributed along the ovine genome were analyzed for M (n=34), W (n=18), local Awassi (LA) (n=24) and IA (n=23) sheep breeds to assess genetic diversity, population structure, linkage disequilibrium (LD) and levels of inbreeding. Awassi level was estimated for admixed individuals using 74 top F_{ST} ranked ancestry informative markers (AIMs). The Awassi admixture level estimates were associated with lamb and ewe performances, body measurements and morphological characters. Spearman's rank correlation analysis was employed on the 74 AIMs and subsets of top ranked 65, 55, 45, 35, 25 and 15 AIMs in order to assess the possibility of reducing number of markers. Correlation analysis was also employed to associate Awassi levels estimated by SNPs with estimated by farmers.

Proportion of polymorphism was highest for LA (96.2%) followed by the Ethiopian breeds (91.7 to 93.0%). Lowest proportion of polymorphic SNP was found in IA (84.3%). Expected heterozygosity was high (0.36) for LA followed by the local Ethiopian (LE) breeds (0.32) and then the IA (0.30). Highest genetic differentiation appeared between LE breeds and IA (F_{ST}~0.13) while the two Ethiopian breeds were closely related (F_{ST}=0.004). Surprisingly, LA sheep differentiated from IA at a higher level (F_{ST}=0.08) than it differentiated from W (F_{ST}=0.052) and from M (F_{ST}=0.065). Correlation coefficient (r) between individual Awassi level obtained from pedigree information and estimated from 74 SNP data was 0.98 for a small pedigreed research herd. All subsets of SNPs in this study provided consistent genotyping clusters and reproducible results and the top 45, 55, 65 SNPs yielded very similar estimates of ancestry proportions with the 74 SNPs. Mean and standard deviation of proportion of Awassi level in Chiro sheep flocks was 21.1±14.71 and 27.5±17.13% for ewes and lamb, respectively. Whereas, in Negassi-Amba the proportion of Awassi level was much lower with corresponding values of 11.0±10.53 and 9.0±7.36% for ewes and lambs, respectively. Higher within and between population variations were observed in crossbred populations found in the two villages. Best performing 13% cohort lambs at 8 months age had 5.9 and 10.4 Kg higher than the population mean in Negassi-Amba and Chiro, respectively. In general, lamb eight months weight was increased as the Awassi level increased. Lambing interval delayed as the Awassi level

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increased, however number of lambs weaned ewe^{-1} year^{-1} was not significantly different (P>0.05). In both locations productivity of ewe in production of eight months lamb weight year^{-1} was increased as the Awassi level increased to a certain level. Ear length and hair on leg score were appeared as primary predictors of Awassi level in Chiro (Wollo x Awassi) crossbred population (R^2=0.47, P>0.0001 for lambs and R^2=0.48, P>0.0001 for ewes). For Menz x Awassi ewes wither height appears as the main predictor which explained 32% of the total variation when alone and explained 42% of the variation when used with hair on leg score. In absence of pedigree and genome information, and when decision on admixture level is not too sensitive, farmers could be able to estimate the admixture level of individuals in their crossbred population.

This study provided insights regarding diversity and structure of local Ethiopian and Awassi breeds. Principal component analysis and model based structure analysis revealed clustering of populations according to their geographic location and breed development. Strong selection pressure on the Awassi sheep resulted in significant differentiation from LA. Low level of genetic variability and highest inbreeding level observed within the improved Awassi sheep breed should be noted and steps toward increasing diversity should take in to consideration. About 45 top ranked AIMs selected based on F_{ST} were good enough for accurate estimation of the level of ancestry in the crossbred sheep population. Considering the genetic variation created in the crossbred population, genetic improvement applying continuous selection within the crossbred population along with management improvement should be considered which progressively leads to the development of a composite population. Association analysis of Awassi levels with performances suggests crossbreeding of Wollo sheep with Awassi in Chiro and similar areas up to 50% Awassi level and strengthening the ongoing selective pure breeding programs of Menz sheep in Negasi-Amba village. Association analysis of Awassi levels with performances suggests crossbreeding of Wollo sheep with Awassi in Chiro and similar areas up to 50% Awassi level and strengthening the ongoing selective pure breeding programs of Menz sheep in Negasi-Amba village. The use of AIMs would inspire livestock breeding programs in developing countries by availing breed composition and pedigree information which persisted as marked constraint.

Key words: Admixture, Awassi, crossbreeding, Ethiopia, Menz, sheep, Wollo

Zusammenfassung

Das Ziel der vorliegenden Arbeit ist die Entwicklung eines kostengünstigen Set von Markern zur Schätzung der Admixture und die Identifizierung eines opimalen Admixture-levels von Awassi in einem bestehenden Kreuzungszuchtprogramms für Schafe im äthiopischen Hochland, das seit 1997 durchgeführt wird. Genetische Diversität, Populationsstruktur und Inzuchtniveau der unterschiedlichen Rassen, die für das Kreuzungszuchtprogramm verwendet werden, wurden analysiert. Verbesserte Awassi-schafe aus Israel wurden verwendet um die lokalen Rassen Menz (M) und Wollo (W) in den beiden Dörfern Negassi-Amba und Chiro zu verbessern. 50K SNP wurden über das gesamte Genom verteilt für M (n=34), W (n=18), lokale Awassi (LA) (n=24) und Improved Awassi (n=23) analysiert. Der Awass-Anteil wurde von 74 Individuen ermittelt, die die höchsten gereihten F_{ST} ancestry informative markers (AIMs) hatten. Diese Schätzer wurden mit der Leistung von Muttertieren und Lämmern in Verbindung gesetzt. In einem schrittweisen Verfahren wurden die AIMS auf 65, 55, 45, 35, 25 und 15 reduziert, um die Möglichkeit einer Reduktion von Markern zu evaluieren. Korrelationen und Regressionsanalyse wurden durchgeführt, um die Beziehung zwischen geschätzter Admixture, Pedigreeinformation, verschiedenen Körpermaßen, morphologischen Charakteristika und der Schätzung von Admixture nach Angaben von Bauern zu erheben. Polymorphismus war am höchsten bei LA (96.2%), gefolgt von den beiden äthiopischen Rassen (91.7 to 93.0 %), der niedrigste Wert (84.3 %) wurde bei IA erhoben. Die erwartete Heterozygosität ware hoch bei LA (0.36), gefolgt von beiden lokalen Rassen (0.32) und IA (0.30). Die größe genetische Differenzierung zeigte sich zwischen äthiopischen Rassen und IA (F_{ST}~0.13), die beiden äthiopischen Rassen waren eng verwandt (F_{ST}=0.004). LA zeigte größere Unterschiede zu IA (F_{ST}=0.08) als zu Menz (F_{ST}=0.065) und Wollo (F_{ST}=0.052). Korrelationskoeffizienten zwischen Awassi-level von Pedigreeinformation und von 74 SNP-set lag bei 0.98 für eine kleine Herde. Eine Reduktion auf 45, 55, 65 SNPs zeigte konsistent ähnliche Ergebnisse. Die Admixture-analyse zeigt, dass der Awassi-anteil in den unterschiedlichen Gebieten und auf verschiedenen Betrieben variiert. In Chiro war der Awassi-Anteil bei 21.1±14.71% für Muttertiere und 27.5±17.13 % für Lämmer. In Negassi-Amba der Awassi-Anteil war wesentlich geringer und lag für Muttertiere und Lämmer bei 11.0±10.53 und 9.0±7.36 %. Generell konnte beobachtet werden, dass mit zunehmenden Awassi-Anteil das Gewicht der Lämmer im Alter von 8 Monaten zunahm. Das Interval zwischen den Ablammungen stieg mit steigendem Awassi-anteil zwar an, aber die Anzahl an abgesetzten Lämmern pro Muttertier und Jahr war nicht signifikant unterschiedlich. Diese Studie gibt gute Einblicke in die Diversität und Struktur von lokalen äthiopischen Rassen und Awassi. Mit Hilfe der Principal component analyse konnten die unterschiedlichen Population geographisch

zugeordnet werden. Ein starker Selektionsdruck bei IA führte zu einer starken Differenzierung von LA, weniger in einer Variation innerhalb der Population. 45 AIMS zeigten gute Resultate bei der Schätzung der Admixture. Basierend auf dieser Studie kann für das Gebiet Chiro ein 50% Awassi-Anteil und für Negassi-Amba ein 25% Awassi-Anteil empfohlen werden. Zieht man die hohe genetische Variation der bestehenden Kreuzunspopulation in Betracht, kann darauf aufbauend eine Selektion innerhalb der Population zur Entwicklung einer Composite-Population durchgeführt werden.

Schlagwörter: Admixture, Äthiopien, Kreuzungszucht, Menz, Schafzucht, Wollo

1 Introduction

Small ruminant production is an important agricultural activity and has a substantial contribution to smallholder farmers in generating income and securing food in developing countries (Kosgey et al., 2006). Tropical developing countries rely on non-specialized multipurpose breeds, extensive production systems and poor control of breeding animals. Existing breeds are well known to adapt to the existing environmental situation which is characterized by feed scarcity and disease challenge (Baker et al., 1998; Haile et al., 2002; Gizaw et al., 2008a). Thus maintaining genetic diversity should be an important task in livestock breeding as it strengthens a population by increasing the likelihood that at least some individuals will be able to survive major disturbances and by making the population less susceptible to inherited disorders. However, local breeds may sometimes be unprofitable as they have limited genetic capacity to respond to an improved management even though they are well adapted to the existing environment. There is also a perception that the existing local breeds are less productive and unlikely to continue sustaining the fast growing demand for food that created by rapid human population growth, urbanization and income growth. Genetic diversity and understanding of population structure need to be understood to guide breed development programs to meet the current production need in various environments which allows sustained genetic improvement, to facilitate adaptation to the changing breeding objectives, and to device proper measures of utilization and conservation of livestock breeds (Notter, 1999; Dalvit et al., 2008).

Crossbreeding, which is the mating of individuals from different populations, is considered as one of the options and an attractive breed improvement method due to its quick benefit as the result of breed complementarity and heterosis effects (Hayes et al., 2009). Burrow (2012) also suggested combination of multiple breeds to achieve the optimum level of production. FAO (2007) documented the cross-border transfer of genetic material which increased dramatically in the recent decades. Sheep are among the most widely distributed species among all livestock. Consequently, the widely practiced breed combination resulted in about 443 composite sheep populations worldwide in 68 countries by 2005 (Shrestha, 2005). Remarkable results have been achieved in well-designed selective and crossbreeding schemes (Gootwine and Pollott, 2000; Waal and Combrinck, 2000; Pollott and Gootwine, 2004). The local Awassi managed by Bedouin farmers in the southern dry region of Israel also remain unprofitable and economic assessment showed that traditional extensive sheep farming in low input system based on the local Awassi breed was not positively contributing to family income and nutrition however still regarded as cultural benefit (Valle Zárate et al., 2009). The introduction of Improved Awassi (Afec-Awassi) to

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their flocks was successful and made the flocks profitable (Gootwine et al., 2009). Similarly, the introduction of different ram breeds in extensive semi-arid regions of Argentina improved carcass yield and conformation with varying performance among sires (Álvarez et al., 2010, 2013).

However in developing countries generally the adoption of livestock technologies has been low due to the environment and poor resource base of farmers (Iñiguez, 2011). Intensive programs have been favored in resourceful environments and well developed infrastructure and markets (Sölkner et al., 1998). The research and development efforts of sheep crossbreeding so far in Ethiopia also did not bring the anticipated benefit to the smallholder farmers. Proportion of exotic and crossbred sheep population in the country was only 0.2% in 2012/13 (CSA, 2013). However there is still a growing interest from the government and farmers side for sheep crossbreeding to satisfy the rising demand for meat. Institutional and infrastructural issues in multiplication and dissemination of genotypes from government farms to farmers as well as adaptation problems due to poor feed resource and health management are mentioned as reasons for the little success of crossbreeding (Hassen et al., 2002; Tibbo et al., 2005; Gizaw and Getachew, 2009). Determining the optimum combination of productivity and adaptability considering the prevailing environment is one of the key issues for the success of crossbreeding. Burrow (2012) suggested 25 to 75% adapted genes to be required for optimal production depending on the severity of the environment and the level of stress challenge, only exceptionally stressful environments require 100% adaptive genes.

The International Center for Agricultural Research in the Dry Areas (ICARDA), the International Livestock Research Institute (ILRI), University of Natural Resources and Life Sciences (BOKU) and the Ethiopian Agricultural Research System have been implemented a project on community based sheep breeding programs in eight communities of four districts (Afar, Bonga, Horro, Menz) in Ethiopia since 2007 (Haile et al., 2011). In all of the communities but one the sheep are pure local breeds. This community (Negasi-Amba village in Menz area) is also one of the three sheep crossbreeding village established by Debre Berhan Agricultural Research Center (DBARC) in 1997. In Negasi-Amba and another two villages (Serity and Chiro) Awassi x Menz crossbred rams have been distributed to increase the body size of the indigenous fat-tailed sheep breeds found in the areas (Gizaw and Getachew, 2008). It was extremely interesting to investigate how crosses (composites) of different blood levels perform under smallholder management. Setting the level of admixture for different locations based on the existing situation would be an essential part for the success of the on-going crossbreeding program as well as to design large scale crossbreeding breeding program in other similar areas. Routine recording of growth of lambs and fertility of

female animals has been part of the breeding program. Precise pedigree of the animals is compulsory to identify optimum level of admixture. However it has been challenging under smallholder farmer's situation due to uncontrolled mating and poor infrastructure. Pedigree records by small-scale dairy farmers in Kenya for example have been incomplete and contained a significant number of inaccuracies when compared with the genomic admixture level (Gorbach et al., 2010).

Use of SNP markers in assessing the current levels of genetic diversity, population structure, and detection signatures for natural and artificial selection has been studied in livestock (Kijas et al., 2012; Moradi et al., 2012; Periasamy et al., 2014). Before the advent of use of high density single nucleotides polymorphism (SNP) panels, microsatellites were extensively used in diversity studies. Microsatellites are highly polymorphic and very informative even though their genotyping and scoring are labor intensive (Ajmone-Marsan et al., 2014). Analyses of single nucleotide polymorphism (SNP) and microsatellite marker data resulted in similar conclusions with respect to population structure (Coates et al., 2009). Currently the use of the bi-allelic SNP has become very popular (VIgnal et al., 2002) due its abundance in the genome, widely distributed and amenable to cost effective and high throughput genotyping. Genome sequencing and the subsequent development of SNP chip data have also been extensively used to learn about population stratification and admixture in human populations (Bryc et al., 2010; Ding et al., 2011).

Use of genomic information may be expected to assist decision making in designing and implementing breed improvement program although its application in developing countries is limited mainly due to logistics and lack of knowledge and infrastructure. Even though more than 50K SNPS are available with varying level of information for the estimation of admixture level for sheep, only a small subset of highly informative markers need to be genotyped in order to accurately predict the admixture level with a minimal error rate and cost effective way. Use of sets of selected autosomal ancestry informative markers (AIMs) for accurate estimation of individual and population admixture levels has been reported (Benn-Torres et al, 2007; Negrini et al., 2009; Sölkner et al., 2010; Frkonja et al., 2012; Bray at. al, 2014).

Thus this study was focused on the use both high density 50KSNP and few AIMs to meet the following four objectives.

- To assess genetic diversity, population structure and levels of inbreeding of Ethiopian fat-tailed (Menz and Wollo) and Awassi breeds based on high density ovine 50KSNP markers.
- To identify a small and cost effective set ancestry informative markers for the estimation of admixture levels of Ethiopian fat-tailed and improved Awassi ancestors in the crossbred populations.
- To identify the optimal level of Awassi for different areas of small holder situation based on association of genomic estimated Awassi levels with lamb growth and ewe reproductive performances.
- To evaluate and associate the relationship of admixture levels estimated based on SNP data with different morphological measurements and characters, and admixture levels estimated by farmers.

2 Literature review

2.1 Sheep domestication and introduction to Africa

Sheep (*Ovis aries*) is among the first grazing livestock species domesticated 11,000 years ago from wild ancestor Asiatic mouflon (*O. orientalis*) in the Fertile Crescent (Zeder, 2008; Chessa et al., 2011). Then sheep differentiated and dispersed across Eurasia and Africa via separate migratory episodes. Environmental pressure shaped phenotypic variation and has left genetic footprints in the breeds raised in different agro-ecological zones (Lv et al., 2014). Molecular genetics (endogenous retrovirus and mitochondrial DNA) confirmed that the maritime trade and colonization had a major influence on sheep movement in the Mediterranean areas (Pedrosa et al., 2007; Chessa et al., 2011). Molecular genetics studies combined with the available archeological evidences confirmed that selection of domestic sheep with desired secondary characteristics common to the modern breeds occurred first in South-West Asia and then spread successfully in to Europe, Africa and the rest of Asia. A migratory episode involving sheep with improved production traits shaped the morphology, behavior and genetic structure of animals. Initially sheep were used for meat and later specialization for wool and milk commenced. Large number of local breeds (above 1,400) has been created worldwide due to the main evolutionary forces of mutation, selective breeding, adaptation, isolation and genetic drift caused by human intervention along with the environmental influence (FAO, 2007). Diversification of the breeds due to human and geographic influence is well studied. FAO (2007) documented the transfer of genetic material increased dramatically in the recent decades and sheep are among the widely distributed species among all livestock. Consequently, the widely practiced breed combination resulted in about 443 well documented composite sheep populations worldwide in 68 countries (Shrestha, 2005).

Compared to European and Asian sheep, the diversity of mitochondrial DNA (mtDNA) of African sheep has been less studied. Sheep population in Africa, Pakistan and China displayed a similarly homogenous retrotype pattern common to the population of South-West Asia, suggesting a direct migratory link of domestic sheep between these areas (Chessa et al., 2011). Muigai and Hanotte, (2013) also confirmed that the mtDNA data so far indicate that African sheep share a common maternal ancestry with European and Asian sheep, and that they likely originated from the same center(s) of domestication.

Indigenous African sheep genetic resources have been classified into two main groups, fat-tailed and thin-tailed sheep. The fat-tailed sheep are most widely distributed, being found in a large part of North Africa (from Egypt to Algeria) and in Eastern and Southern Africa (from Eritrea to South Africa). The thin-tailed sheep are present mainly in Morocco, Sudan and in West Africa. The first domesticated sheep is believed to have been thin-tailed as the ancestors of domestic sheep are thin tailed. Archaeological information supports separate introductions and dispersion histories for the African thin-tailed and fat-tailed sheep (Muigai and Hanotte, 2013). The first sheep entered Africa via the Isthmus of Suez and/or the southern Sinai Peninsula, between 7,500 and 7,000 BP. They were likely of the thin-tailed type. Fat-tailed sheep entered Africa through its northeastern part and the Horn of Africa. Mitochondrial DNA analysis supports a common maternal ancestral origin for all African sheep, while autosomal and Y chromosome DNA analysis indicates a distinct genetic history for African thin-tailed and sub-Saharan fat-tailed sheep.

Dispersal of two major haplotype groups was detected in modern sheep. A total of 57 haplotypes were observed which formed two distinct clades. Type A haplotypes were found in breeds from Asia (India, Indonesia, Mongolia, and Tibet), while type B haplotypes were observed at the highest frequency in breeds sourced from Europe (nine breeds from Austria, Finland, Spain, and northwestern Russia). A mixture of the two lineages was found in every breed except Suffolk and the Indian Garole, indicating introgression has played a major part during breed development and subsequent selection (Meadows et al., 2005). Parallel DNA and mtDNA studies of European, African and Asian domestic sheep suggest that there are three major and distinct lineages. These lineages are called Type A or Asian, Type B or European, and Type C, which has been identified in modern sheep from Turkey and China. All three types are believed to have been descended from different wild ancestor species of mouflon (*Ovis gmelini* spp), someplace in the Fertile Crescent.

2.2 Genetic diversity and population structure

Genetic diversity and understanding of population structure helps to guide breed development programs to meet the current production need in various environments which allows sustained genetic improvement, to facilitate adaptation to the changing breeding objectives, and to device measures of utilization and conservation of livestock breeds (Notter, 1999; Dalvit et al., 2008). Genetic variability is the clay of evolution, providing the base material on which adaptation and speciation depend. Genomic markers have been used to assess genetic variation among sheep breeds. Before the advent of use of high density SNP panels, microsatellites were extensively

used in diversity studies. Microsatellites are highly polymorphic and very informative even though their genotyping and scoring are labor intensive (Ajmone-Marsan et al., 2014). SNP markers are now quickly replacing microsatellites in genetic diversity studies due to their robustness, low cost and automatic allele calling. Previous results based on microsatellites and SNP markers shown that genetic diversity of sheep breeds associated with their area of origin in which populations that have been diverged more recently were generally closer together geographically (Peter et al., 2007; Gizaw et al., 2008a; Bozzi et al., 2009; Kijas et al., 2012). Isolation of populations and breed development history in the form of crossbreeding or selection had also affected the genetic diversity and population structure of livestock breeds (Epps et al., 2005; Dalvit et al., 2008; Ligda et al., 2009). Efforts to improve either production traits like milk, meat and wool or selection for morphological characters like coat color and horn resulted in differentiation of populations. Different selection priorities resulted in differentiation of polled Dorset from horned Dorset, improved Awassi from local Awassi (Gootwine, 2011) and clear differentiation of wool and meat type merino (Diez-Tascon et al., 2000). Small population size and intense selection to increase productivity resulted in low genetic diversity which might also be attributed to performance loss due to inbreeding (Barczak et al., 2009). Maintaining genetic diversity should be an important task in livestock breeding as it strengthens a population by increasing the likelihood that at least some individuals will be able to survive major disturbances and by making the population less susceptible to inherited disorders.

2.3 Classifying individuals in to populations

Classifying individuals in to populations is useful in population genetics. Usually it is defined subjectively based on linguistic, culture, physical characteristics and geographical location (Pritchard et al., 2000). The recent advent of genomic data allows using genomic information for assigning individuals to populations more precisely. Estimation of admixture proportion from genome data is based on the allele frequencies of the admixed population (Chain et al., 2001; Skotte et al., 2013). Allele frequencies of the admixed population are a linear combination of the contributing parental populations at the time when admixture occurs. One interest is to classify individuals of unknown origin in to pre-defined populations. The standard approach is to get DNA samples from a number of potential sources of population and estimate allele frequencies in each population at a series of unlinked loci. Using the estimated allele frequencies, it is then possible to compute the likelihood that a given genotype originated in each population. Individuals of unknown origin can be assigned to populations according to these likelihoods.

The other way is identify the actual subpopulations and assign individuals probabilistically to these populations. Bayesian clustering approach based on a model in which there are *K* populations (where *K* may be unknown), each of which is characterized by a set of allele frequencies at each locus (Rosenberg et al., 2003). The method attempts to assign individuals to populations on the basis of their genotypes, while simultaneously estimating population allele frequencies. The method can be applied in various types of markers. However it assumes that the markers are in linkage equilibrium with one another and in Hardey-Weinberg equilibrium within population. The primary interest of this model is the assignment of individuals to populations and also allows the presence of admixed individuals in the sample, whose genetic makeup is drawn from more than one of the *K* populations.

2.4 Genetic admixture

Genetic admixture occurs when individuals from two or more previously isolated populations begin interbreeding and form a new hybrid population and results in the introduction of new genetic linage in to the population (Shriver et al., 2003; Wang, 2003). Genetic admixture can be studied at population, individual or specific regions along the chromosome (locus level). Before the populations are mixed, every marker in the genome of an individual traces its ancestry to only one parental population. Consequently, ancestry for each individual at each marker, known as local ancestry, is constant for each individual across all loci. After one generation of random mating within the meta-population, an individual has inherited one chromosome from each parental population. Local ancestry is still uniform across all loci for a given individual. After a second generation of random mating and beyond, an individual's genome is a mosaic of chromosomal segments with ancestry switching from segment to segment among the parental populations. An ancestry switch refers to a change in ancestry in the interval between two markers and is the result of recombination during meiosis (Sölkner et al., 2010).

There are several characteristics of an admixed population. Admixed individuals not necessarily have the same proportion of ancestors from each parental population and all loci are not expected to share the same genealogical history. These two characteristics of admixed populations are sources of variance that must be accounted for when estimating local ancestry. At any given locus, allele frequencies can vary between the parental populations and the expected allele frequency in the admixed population is the linear combination of the allele frequencies in the parental populations with weights determined by the sample admixture proportion. Another important feature of admixed populations is that they can be more genetically diverse than the

parental populations if a locus is not polymorphic with the same alleles in all parental populations. The admixed population is expected to be polymorphic at both loci. Similar to the way in which allele frequencies at a locus may vary, the patterns of covariance between allele frequencies at linked loci, known as linkage disequilibrium, can also differ. As a result, the distribution of haplotype frequencies in the admixed population can be substantially different from the distributions of haplotype frequencies in the parental populations (Shriner et al., 2011).

Admixture mapping requires a genome-wide panel of markers that can differentiate genome regions in admixed individuals by their ancestral origins (Shriner et al., 2011). In human, recent mixture of African and European in African American population is of particular interest. Admixture mapping has been widely used in human in genetic association studies to study complex diseases (Tang et al., 2007; Via and Burchard 2009; Shriner et al., 2011), suggests the possibility of using it in livestock to identify genes associated with some kind of performances, morphological characters or disease associated with specific breeds.

2.5 Ancestry Informative Markers

Ancestry refers the proportion of genetic material descending from each founding population. Markers with frequencies that are highly differentiated among populations and are very homogenous within the population are particularly informative of ancestry and are called ancestry informative markers (Shriver et al., 2003). Ancestry informative markers (AIMs) are polymorphisms that exhibit large allele frequency differences between populations and can be used to infer ancestry at the level of population, sub-population and individual (Shriver et al., 2003). An ideal AIM should have one allele that is fixed (i.e., allele frequency of 1.0) in one ancestral population, and not present in the other. However most of the alleles are shared among different population. Their estimating ability highly depends on the informativeness to infer ancestry along the chromosome of the admixed individuals. Several measures of marker informativeness have been developed and tested in human and livestock to estimate the level of ancestry (Rosenberg et al., 2003; Shriver et al., 2003; Sölkner et al., 2010; Ding et al., 2011; Wilkinson et al., 2011; Frkonja et al., 2012; Galanter et al., 2012).

2.6 Number of markers required to classify individuals

Although many SNPs have been identified in the genome, most informative few markers provides sufficient power to discern and control population stratification with minimal error and in a cost effective way (Ding et al., 2011; Qin et al., 2013). The number of markers that are necessary to

estimate population admixture or individual ancestry depends on the informativeness of the markers and the required accuracy (Ding et al., 2011). The number of markers required is a function of genetic distance among populations. Ancestry proportion of individual from highly differentiated populations might be estimated from relatively lower markers compared to individuals from less differentiated populations (Ding et al., 2011). A panel of 150 validated SNPs that were highly informative in distinguishing northern Han (N-Han) from southern Han (S-Han) Chinese population (Qin et al., 2013). A panel of 93 AIMs is effective to ascertain population genetic structure in determining human continental origin (Nassir et al., 2009). Very small subset of highly informative markers in human has also been reported to predict ancestry with a minimal error. For instance Ding et al., (2011) reported that the top 20 ranked AIMs gave adequate classification of ancestral population. (Lao et al., 2006) also found as few as top 10 SNP markers reported to be enough to differentiate individuals from Africa, Europe, Asia and America and in their study no further gain in power of assignment was obtained by increasing the number of SNP markers. The authors also explained that the level of geographic structure estimated based on these 10 SNPs was similar to the value estimated based on the previously reported 377 or 40 microsatellites in the same set of samples (Rosenberg et al., 2003). A panel of 90 SNPs in cattle was as efficient as 19 to 23 microsatellites (Negrini et al., 2009). Negrini et al., (2009) also found that reducing the number to 35 top ranked SNPs with F_{ST} value of > 0.1 gave reasonable result in identifying the source of cattle breed of individuals of unknown origin with an acceptable loss in assignment rate.

2.7 Methods of ancestry estimation

Model-based ancestry estimation and principal component analysis (PCA) are two widely used approaches for the detection of population structure in admixed populations (Thornton et al., 2014). Both individual ancestry estimation method and PCA have been shown to give reliable inference for ancestry in admixed samples. Model-based approach models the probability of the observed genotype using ancestry proportions and population allele frequencies. It bases ancestry coefficient as a parameter of a statistic. STRUCTURE (Pritchard et al., 2000) and ADMIXTURE (Alexander et al., 2009) are commonly used software to estimate model based admixture. The programs compute probabilistic quantities called ancestry coefficients that represent the proportions of an individual genome that originate from multiple ancestral gene pools. Structure takes a Bayesian approach and relies on a Markov Chain Monte Carlo (MCMC) algorithm to sample the posterior distribution. ADMIXTURE also uses the same likelihood model but focuses on maximizing the likelihood rather than on sampling the posterior since high

dimensional optimization is much faster than high-dimensional MCMC. Ancestry coefficients can be estimated in both supervised and unsupervised methods.

Supervised method uses predefined source populations as ancestral populations based on least-squares regression of allele frequencies in hybrid and source populations. The total amount of intermixture estimated from the known gene frequencies in hybrid and total population (Roberts and Horons, 1965).

Unsupervised approaches estimate the ancestry proportion without additional information (Alexander et al, 2009). They attempt to infer ancestral gene pools from the data using likelihood methods. Ancestry estimation is challenging in case when ancestral populations are closely related.

2.7.1   Methods of selecting informative markers

Fewer markers might be obtained from low density BeadChip e.g. the Illumina GoldenGate Bovine3K Genotyping Beadchip was developed and made commercially available (Boichard et al., 2012) or markers might be selected from high density BeadChip in different ways; randomly selected across the genome (Frkonja et al., 2012), based on their informativeness (Ding et al., 2011; Frkonja et al., 2012) or based on specific regions or chromosomes (Sölkner et al., 2010). Selecting markers based on their informativeness allows estimation of ancestry levels at lower cost with reasonable accuracy. For example (Frkonja et al., 2012) suggested that 500 AIMs and about 5000 randomly selected SNPs resulted in comparable result with the full set of 40,492 SNPs. Further reduction of SNP markers to 98 and 48 top F_{ST} SNPs also gave pedigree and genome estimate correlation of 0.92 and 0.90, respectively.

Several measures of marker informativeness (ability of markers to differentiate between populations) have been developed to select the most AIMs from an ever-increasing wealth of genomic databases. However rationally choosing and deciding the number of markers required to meet the interest is compulsory. Rosenberg et al., (2003) developed a new parameter called informativeness for assignment (I_n), which is more closely related to F statistics index (F_{ST}) than absolute allele frequency differences (δ). Ding et al. (2011) compared different measures which F_{ST}, δ, Shannon information content (SIC), Fisher information content (FIC), and the Rosenberg's informativeness for assignment measure (I_n). Among the five measures used to select markers informativeness, absolute allele frequency differences, F_{ST} and I_n are likely to pick the same set of SNPs and F_{ST} and I_n have shown the highest Spearman correlation (Ding et al., 2011). Qin et. al.,

(2013) also found a strong correlation between F_{ST} and I_n. Four measurements; δ, Wahlund's f, Rosenberg's I_n and F_{ST} were compared to calculate marker information and found to have similar strong correlation among measures (Xu et al., 2008). Locus-specific branch length was also used to select informative and well distributed throughout the genome markers (Galanter et al., 2012). F_{ST} is a measurement that considers the difference in sample size. Ancestry informative markers generated by different methods are similar in estimating the ancestry proportion when the top AIMs are considered (Ding et al., 2011).

2.7.2   Importance of AIMs in admixture study

Admixture levels had estimated effectively based on available high density genomic markers (50k SNPs) in Bovine and Ovine population (Gorbach et al., 2010; Sölkner et al., 2010; Frkonja et al., 2012). When inferring genetic ancestries most of the alleles are common and only some markers are varying among populations. Though the price of genotyping with such a high density markers is getting down from time to time it is still expensive to genotype large numbers of animals. Thus, use of highly informative markers reduces the amount of genotyping required for ancestry inference. Use of fewer markers to estimate the proportion of ancestors in admixed populations with reasonable accuracy need to be the focus (Sölkner et al., 2010) and has been researched in Bovine and human (Negrini et al., 2009; Frkonja et al., 2012). Small set of markers has also been used to assess genetic structure in cattle (Edea et al., 2013).

With the availability of genetic ancestry estimates, admixed populations represent a valuable opportunity to study complex diseases and drug responses in human. Admixture studies widely practiced in human mainly in identifying genetic risk factors involved in complex traits or diseases showing prevalence differences between major continental groups (Freeman et al., 2004; Hoggart et al., 2004). The use of genetic ancestry estimations is a topic of growing importance in biomedical research (Via and Burchard 2009). Admixture between ethnic groups that differ in disease risk for genetic reasons provides an experiment of nature that can, in principle, be exploited to localize genes in the same manner as an experimental cross. Although advanced statistical methods are required to apply this approach, the underlying principle on which it relies to detect linkage is simple. Suppose, for instance, that risk alleles at a locus are differentially distributed between populations so as to generate a twofold higher risk of osteoporotic fractures in Europeans compared with West Africans. If we classify individuals of mixed European/West African descent according to whether they have 0, 1, or 2 gene copies of European ancestry at this locus, disease risk will be twofold higher in those with 2 copies than in those with 0 gene

copies of European ancestry. We do not have to compare disease risk among these three groups directly (which would require a cohort design). Instead we can study cases only, comparing at each locus on each gamete the observed and expected proportions of gene copies that have European ancestry.

2.8 Ethiopian sheep breeds

Origin and introduction of sheep in to Africa has not been well studied. Sheep population in Ethiopia estimated at 25.5 million heads (CSA, 2013) and 14 different types (Gizaw et al., 2007). Population structure of sheep in Ethiopia is associated with historical geographic isolation and ecological patterns. Ethiopian sheep breeds classified in to 4 breed major groups of sub-alpine short fat-tailed, highland long fat-tailed, lowland fat-rumped and low land thin-tailed based on their ecological, geographic location and tail type (Gizaw et al., 2008a). They were then further classified in to 6 breed groups namely short fat-tailed, Washera, thin-tailed, long fat-tailed, Bonga and Fat-rumped sheep (Gizaw et al., 2007).

Low global F_{ST} (0.046±0.004) but significant genetic differentiation was observed among all populations. F_{ST} values for all pairs of populations were significantly different from zero except between sub-alpine short fat-tailed groups (Menz, Wollo, Farta, Tikur, Semien and Sekota). Semien sheep was significantly differentiated from all other sub-alpine population. High level of within breed genetic diversity with observed heterozygosity in the range of 0.62 to 0.72 and expected heterozygosity in the range of 0.66 to 0.75 were observed based on 17 microsatellite genetic markers (Gizaw et al., 2007). Higher sheep flock size in marginal areas of the country (extreme highlands and lowlands) indicates that small ruminants played a higher role in maintaining the income as well as livelihood of small holder farmers and pastoralists as such areas that are not suitable for other agricultural activities (Gizaw, 2008; Getachew et al., 2010). Leta and Mesele, (2014) also reported the highest sheep density in the cool highlands of Ethiopia.

Sub-alpine short fat-tailed populations are characterized by relatively smaller body size than other groups, with mature weight in the range of 20.1±0.3 in Menz to 28.3±0.7 in Farta, low twinning rate 1.0±0.01 in Menz to 1.09±0.05 in Farta (Gizaw et al. 2008a). Size of the animal might be associated with the low feed resource base of the area and such breeds being adapted best to their environment. For example Menz sheep is smallest breed in the country, produce meat and coarse wool, are highly adapted to the cool Ethiopian highlands, and are tolerant of drought, seasonal variation in feed availability, and endo-parasite infection (Haile et al. 2002; Gizaw et al.

2008a; Getachew et al., 2015b). Higher coat color variability and genetic diversity observed in the sub-alpine short fat tailed population (Gizaw, 2008; Getachew et al., 2009). Darker coat color and coarse hair observed in Ethiopian short fat-tailed sheep breeds are in line with the morphological characteristics of sheep breeds identified as of ancient origin and considered as primitive breed (Chessa et al., 2011). Muigai and Hanotte (2013) also suggest the main ancestral population of southern African fat-tailed sheep likely originated in East Africa.

2.9 Breeding objectives and importance of sheep

Majority of sheep population in Ethiopia are indigenous unimproved breeds mainly created under the influence of natural selection and human intervention for morphological characteristics. In developing countries livestock in general are kept for subsistence and multipurpose role (Kosgey, 2004; Wurzinger et al., 2011). Sheep are mainly reared by small holder farmers and used mainly for income generation from the sale of live animals and food source (Duguma et al., 2010; Getachew et al., 2010; Liljestrand, 2012; Zonabend et al., 2014). Other intangible benefits like using sheep for insurance, means of saving and socio-cultural benefits are also well documented (Kosgey et al., 2004). Interest of farmers in the highland areas for coarse wool, coat color, tail type and presence and absence of horn is also documented (Gizaw, 2008; Getachew et al., 2010). Fast growing animals are most of the time sold to market as they provide for the immediate cash need. The role of sheep is more pronounced in the extreme highlands of the country as such areas are less suitable for crop production as well as larger animals like cattle due to environmental difficulties. Wollo, Menz, Tikur and Farta sheep breeds are among the short fat-tailed coarse-wool populations in the sub-alpine area has been targeted for Awassi crossbreeding. In developing countries, acceptance of new breeds by farmers is influenced not only by their productive performances, but also by non-production traits like beauty and appearance of the animal (Ndumu et al., 2008; Wurzinger et al., 2011). Traits like coat color, tail type, horn and ear size of sheep can also have significant influence on price in the predominant live animal marketing (Tadesse, 2009). At the beginning, exotic breed introduction targeted wool and meat production that overlooked the preference of farmers for appearance of sheep. Ignoring farmer preference led to low acceptance and resulted in low up-take rates.

2.10 Introduction of exotic sheep in to Ethiopia and crossbreeding efforts

The first introduction of exotic sheep breeds into Ethiopia dates back to 1944 when Merino sheep were introduced from Italy by an American aid organization and were maintained at Entoto

(located near Addis Ababa) sheep breeding station (DBHBMC, 2007). Romney, Corriedale, Hampshire and Rambouillet were introduced from Kenya in 1967 and were kept at Debre Berhan Sheep Breeding and Multiplication Center (DBSBMC) which was established in 1967 and located at Debre Berhan town, in North Shewa administrative zone of the Amhara region. Another state owned farm, Chilalo Agricultural Development Unit (CADU) was also established in the same year in the former province of Arsi. Both farms were engaged in crossbreeding of indigenous sheep with exotic breeds aiming to improve the surrounding highland sheep breeds in their respective areas. The detection of maedi-visna disease in the flock of CADU in 1988-89 prompted closure of the farm (BoA, 2000).

Wool sheep breeds like Merino, Romney, Corriedale, Hampshire and Rambouillet were targeted to cross with local sheep breeds aiming to supply wool for the Debre Berhan blanket factory established in 1967. These wool sheep breeds were not preferred by farmers in the highlands due to their physical characteristics like the face covered with hair, absence of horn in males and thin tail, and the suspected poor skin quality. Those breeds performed well in growth performance under station and farmer situation except Romney breed (DBHBMC, 2007). Documented letters found at Debre Berehan Sheep Breeding and Multiplication center (DBSBMC) sent from Ministry of Agriculture to request fattened sheep during the king regime showed that crossbreeding during that time was mainly limited to on-station to supply fattened sheep for the royals.

In 1980 Awassi sheep were introduced from Israel and kept at DBSBMC and Amed Guya Sheep Breeding and Multiplication Center (AGSBMC), the later was established in 1998. There were also continuous importations of purebred Awassi sheep totaling 45 (ram and ewe lambs). Introduction of Awassi sheep from Israel considered the preferred physical appearance. The breed is developed for milk and used as a triple purpose for meat, milk and wool (Pollott and Gootwine, 2004; Galal et al., 2008; Gürsoy, 2011). The improved Israeli Awassi is characterized by producing the highest amount of milk, having highest fertility and twinning rate, and heaviest body weight among all Awassi populations (Galal et al., 2008). The farms tried to maintain purebred Awassi flock and reached a total of 80 sheep of both sexes (67 at DBSBMC and 13 at AGSBMC) by the end of 2004. The small flock sizes, particularly at the AGSBMC, indicated that mating of related individuals was unavoidable, leading to inbreeding depression. The inbreeding rates per generation derived from the number of breeding males and females were 6.1% at Debre Berhan and 32.5% at Amed Guya (BoA, 2001). The two government farms have been engaged in multiplication and distribution of crossbred rams to farmers at a subsidized price. Ram dissemination was banned between the years 2001 and 2009 following the confirmed maedi-visna

disease in crossbreds and associated sheep flocks (DBHBMC, 2007). In 2011, about 170 pure Awassi sheep were imported from Israel and have been maintained at DBSBMC and AMSBMC farms.

Following the downfall of the monarchy (1974), the crossbreeding efforts were directed to produce and disseminate crossbred rams to individual smallholder farmers. DBSBMC and AMSBMC distributed more than 4,000 crossbred rams of different breeds (Awassi, Corriedale and Hampshire) to smallholder farmers at subsidized prices between the year 1974 to 2001 (DBHBMC, 2007). Hampshire and Corriedale breeds were initially used while these breeds were gradually replaced by Awassi following its introduction in 1980. Awassi breed is well accepted by Ethiopian farmers, especially in the highland areas, due to its preferred physical appearance similar to that of local breeds. In the first four years of ram distribution, individual smallholder farmers were targeted. Later, from 1979 to 1989, the focus was shifted to farmers organized in cooperatives. However, no performance evaluation was performed in the co-operatives, and animals were looted during the government change in 1991. Consequently, the co-operatives were abolished and dismantled with the government change (Emana, 2009) so that the focus was again changed to disseminate rams to individual smallholder farmers. The target has been on disseminating rams with 75% Awassi inheritance to farmers for crossbreeding with their local ewes aimed at replacing the local sheep breed through repeated backcrosses (DBSBMC, 2007).

2.11 Awassi crossbred ram production and dissemination

Until now introduction and maintaining of exotic breeds, as well as multiplication and dissemination of crossbreds are totally dependent on government farms. Efficient multiplication and dissemination of appropriate genotypes is one of the core elements in a breed improvement program. Sheep in government farms suffered from inbreeding due to small numbers of exotic animals and diseases (e.g. maedi-visna) associated with confinement. Low fertility with natural mating in the farms and lack of infrastructure and logistics (e.g. shortage of mating pens) limited the number of available Awassi crossbred rams for dissemination from the government farms. An informal survey in 1997 in South Wollo and North Shewa to evaluate the performance of ram dissemination in the Amhara region exposed that there were no any apparently crossbred sheep found even though a significant number of rams were disseminated to farmers all over the country (Tibbo, 2006; Gizaw and Getachew, 2009). Crossbred rams were castrated, disseminated to farmers having no or few breeding ewes or sold after castration (Gizaw and Getachew, 2009). The predominant practice of selling crossbred rams to individual farmers does not seem suitable for

smallholder situation. Firstly, farmers tempted to sell crossbred rams for their short term need and keeping and managing such a big animal might be difficult for a farmer. Secondly, rams were underutilized due to small flock size. Ram dissemination was not focused to specific areas and the effort of crossbreeding was diluted. Design of village based Awassi sheep crossbreeding schemes considering those limitations is feasible (Gizaw et al., 2014). Ahuya et al., (2005) also reported the previous government approach based on multiplication and dissemination of exotic bucks from government farms failed to bring the anticipated change.

2.12 Performance of crossbreds

At the end of 1980's and beginning of 1990's first research results on growth, reproductive performance and carcass performances from CADU farm (Osslon and Beyene, 1990) and Sheno (now Debre Berhan) Agricultural Research Center (Lemma et al., 1989; Demeke, 1993) were reported. An informal survey to evaluate the performance of crossbred rams disseminated to farmers was carried out in North Shewa and South Wollo districts of the Amhara Region by a team of researchers from DBARC and district agricultural extension experts in 1997 (Gizaw and Getachew, 2009). It was difficult to find either crossbred rams or offspring from disseminated rams in the surveyed areas. An on-farm evaluation of the performance of Awassi x local crossbred sheep under farmers' management was launched by DBARC in three villages in the highlands of Ethiopia in 1997. Details of the breeding program followed by DBARC are indicated (Gizaw and Getachew, 2009; Getachew et al., 2013). Similarly the Awassi x Tikur sheep crossbreeding started in two villages of North Wollo by Sirinka Agricultural Research Center in 2007. Better growth performance of Awassi crossbreds (Hassen et al., 2004; Gizaw and Getachew, 2009) and comparable reproductive performances of Awassi and Corriedale crossbreds (Demeke et al., 1995; Getachew et al., 2013) compared to their local counterparts were reported from the on-station and on-farm evaluation of sheep crossbreeding. Longer age at first lambing and lambing interval, and higher ewe post-partum weight of Awassi crossbred ewes were reported (Getachew et al., 2013; Lemma et al., 2014). Positive association of ewe postpartum weight with lamb birth weight has been reported (Olsson and Beyene 1990; Hassen et al. 2002) and was also resulted in better lamb growth and survival rate from crossbred ewes (Tibbo, 2006; Getachew et al., 2015b). The inferiority of Awassi crossbred ewes in age at first lambing and lambing interval are offset by their ability to raise their lambs to weaning age, which resulted in comparable (Getachew et al., 2013) and better Olsson and Beyene (1989) number of lambs weaned per ewe per year under farmers and on-station management, respectively. Higher price of Awassi crossbreds (about double) compared to locals under farmer management is a clear indication of this benefit. Use of

local breeds to produce crossbred lambs for sale is suggested to exploit the combined reproductive performance ability of local breeds and fast growing potential of crossbreds (Lemma et al., 2014), However this would require substantial organizational support for the straight breeding programs to continuously supply the parental populations.

The low performance levels of both local and crossbred sheep in Ethiopia and their response to supplementary feeding (Olsson and Beyene, 1990; Demeke et al., 1995) suggest that output from both local and crossbred can be increased by improving environmental conditions. In general, the performance levels of crossbred sheep in Ethiopia has been greatly influenced by many factors like location, management, breed composition level, market age and ewe breeds used. Thus variable research results on the performance of crossbreeding based on location, genotype and management suggested that the importance of differential recommendations for different locations. Determining the optimum level of exotic blood is lacking and should be worked out for different areas.

2.13 Association of admixture levels with phenotype

In human, admixture study has been widely used to study the association of disease gene specific to a certain population. If a certain phenotype like morphological characters has some genetic factors, and the frequency of an allele is higher in population 1 than population 2, then admixed population having that specific phenotype have more ancestry from population one than from population 2. If the markers are linked with the phenotype, this is because of linkage disequilibrium. Also interesting to know where the selected markers (SNP) found and whether they are associated with some identified genes.

There are some studies in human aiming to association of ancestral proportion with skin pigmentation and obesity related traits (Parra et al., 2004; Bonilla et al., 2004). Bonilla et al., (2004) found significant correlation between skin pigmentation and individual ancestry ($R^2=0.597$). A significant association was also found between bone mineral density and European admixture ($R^2=0.065$), but no such correlation was evident with body mass index or the remaining body composition measurements. Parra et. al., (2004) reported relationship of genetic ancestry level with skin color was found quite variable with moderately strong to weak correlation.

2.14 Application of admixture study for developing countries

The growing demand for livestock products mainly in developing countries required an urgent improvement of livestock productivity. However, breed utilization programs in general and particularly use of genomic tools for livestock breeding in developing countries are limited due to infrastructure and technical capacity. Crossbreeding has been believed to be one of the options of breed improvement due to breed complementarity and heterosis effect. FAO (2007) documented the transfer of genetic material increased dramatically in the recent decades and sheep are among the widely distributed species among all other livestock. Use of exotic sire for crossbreeding results in production of crossbreds with different levels of admixture have been created (Gizaw and Getachew, 2009; Gorbach et al., 2010; Peacock et al., 2011). Exotic breed introduction in tropical harsh environments should be focused on developing breeds which are more productive and resilient to the environment. Such breeding programs in developing countries are taking place with poor or no pedigree recording even though it is basic to match genotype with the environment (Gorbach et al., 2010; Philipsson et al., 2011). Among the benefits of genomic tools, parentage test, inferring genetic relationship and breed composition of animals in admixed population would be paramount in developing countries. Detecting the genome structures which are associated with adaptation to tropical environment has been investigated from genomic data (Moradi et al., 2012; Lv et al., 2014).

Usually breed composition estimated from the pedigree charts of animals, assuming strict halving of the contributions of individuals across generations of progeny. Human error in recording and fail to identify correct parents in the farm are common in farms which affects the accuracy of pedigree estimation. Recombination of the parental chromosomes during meiosis also leads to deviation from the pedigree estimate (Sölkner et al., 2010). Estimation of admixture level in livestock using genomic information are emerging following the advent of genome data and are interesting in many aspects (Freeman et al. 2004; Sölkner et al. 2010; Frkonja et al. 2011, Frkonja et al. 2012). The feasibility of estimation of crossbreeding levels using SNP data based on clustering algorithms in the absence of pedigree information were reported (Sölkner et al., 2010; Frkonja et al., 2012). This is particularly very important where pedigree recording is difficult and unreliable due to many factors as observed in most of the cases in developing countries. Even in the presence of complete pedigree in well-organized government farm in developing country had shown significant inaccuracy (Gorbach et al., 2010). Furthermore it did not tell about the true inbreeding level of each animal as the true base population allele frequency is not known. Detail of the extent of genome admixture and assessment of the level of inferred ancestral proportion

difference between animals has studied and able to detect the ancient Zebu and African taurine admixture and recent exotic European cattle introgression (Mbole-Kariuki et al., 2014). Ajmone-Marsan et al., (2014) also pointed out that tools of producing genomic information about livestock breed have been advanced faster than our capacity to process and understand the information.

3 Methodology

3.1 Study area

This study was superimposed on the ongoing Awassi x Local crossbreeding project which has been implemented in three villages by Debre Berehan Agricultural Research center (DBARC) since 1997. The three villages are Negasi-Amba, Chiro and Serity. Negasi-Amba and Serity are located in North Shewa whereas Chiro is in the South Wollo administrative zone of the Amhara regional state. Additional animals with pedigree were also collected from DBARC and Amed Guya sheep Breeding and Multiplication Center (AGSBMC). All study areas were found in the sub-alpine sheep-barley system of the Amhara Regional State in Ethiopia located with an altitude of about 3023 m. (Negasi-Amba and AGSBMC), 3224 m. (Chiro) and 2800 m. (Serity and DBARC). Lamb and ewe data obtained from Negasi-Amba and Chiro villages where with performance records and farmers breed composition estimate. Map of the study areas is indicated in Figure 1.

3.1.1 Characteristics of the production environment

Mixed crop-livestock dominated by sheep-barley is the principal production system in the study areas. Both areas are characterized by bi-modal rainfall with main rainy season (June to September) and erratic and unreliable short rainy season (February to March) and on average the areas receive about 900 mm rainfall annually. The main harvests in the areas are from the short rain because in most parts of the districts the rain during the main rainy season is too heavy and land is waterlogged. The areas are chronically food insecure because the unreliability of the short rainy season. In both areas sheep are considered a major income source among other agricultural activities. The sheep breeds are mainly reared for meat whereas coarse hair is also considered as minor income source for farmers. Sheep population of the areas is among the areas having highest sheep density in the country (Leta and Mesele, 2014).

Grazing on natural pasture and crop aftermaths are main feed sources for sheep in all areas. Among grasses Poa, *Festuca, Agrostis* and, to a lesser extent, *Andropogon* and among perennial legumes; the deep-rooted *Trifolium burchellianum* and *Trifolium acaule* are common in the areas (Mengistu, 2006). Supplementation of sheep is rarely practiced by some farmers using available feed sources like crop residues, local brewer by products, weeds, hay and cultivated forage, mainly oat and vetch species.
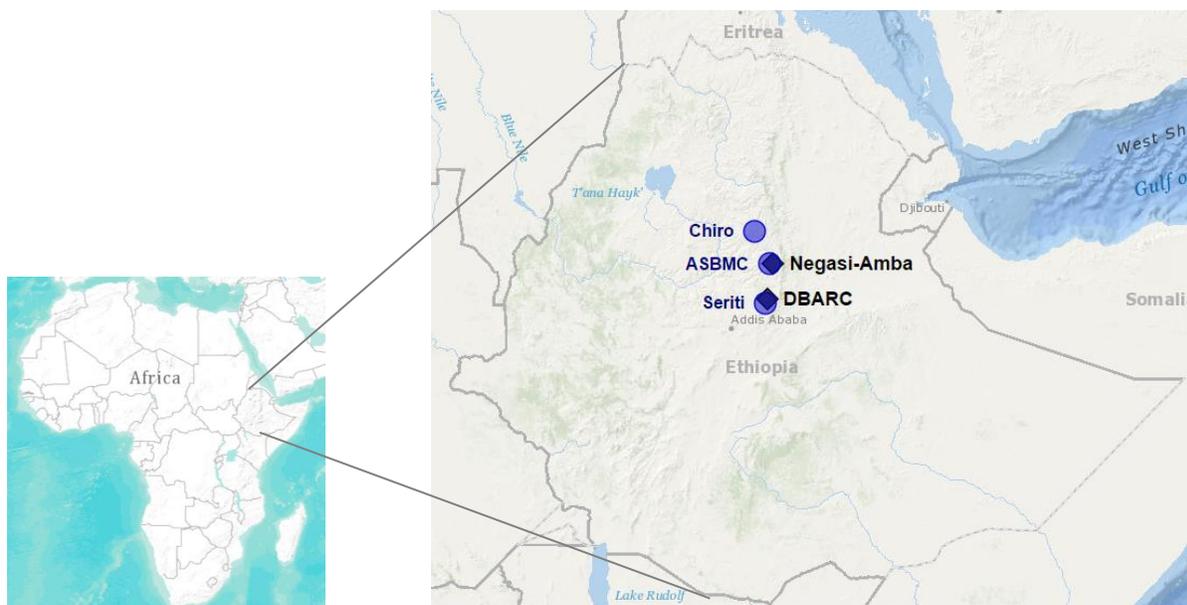
Figure 1. Map of the study areas. Top circle, middle circle, bottom circle, middle diamond and bottom diamond bullets represent Chiro, Amed Guya Sheep Breeding and Multiplication Center, Seriti, Negasi Amba and Debre Berhan Agricultural Research Center, respectively.

3.1.2 Description of the breeding program in the study areas

Details of the breeding program followed by DBARC were indicated in previous studies (Gizaw and Getachew, 2009; Getachew et al., 2013). In brief, the project was designed for disseminating high grade exotic crossbred (75% Awassi-25% Menz) rams to farmers for the purpose of upgrade the indigenous Ethiopian fat-tailed sheep breeds (Menz and Wollo) through continuous backcrossing. Indigenous sheep found in Negasi-Amba and Serity was Menz sheep breed where as the breed in Chiro village was Wollo. A ram was disseminated to a group of organized farmers based on their neighborhood and use of communal grazing so that communal ram use adopted within and among groups of farmers. The group farmers were responsible for use and care of the communal ram. A crossbred ram used for one year in a group was then transferred to another group to control inbreeding. During the process quite significant number of admixed population of Menz x Awassi and Wollo x Awassi with different admixture level has been produced in Negasi-Amba and Chiro villages, respectively. An enumerator was employed in each of the two sites for data collection.

3.2 Genetic and phenotypic data collection

A total of 754, purebred (n=111) and crossbred (n=643) sheep were used for the admixture analysis. Biological samples (ear tissue or blood) for DNA extraction and performance (growth and reproductive performance) were collected from the ongoing Awassi x Local crossbreeding project. Details about sampling are presented in Table 1. Ovine SNP50K data were obtained for three parental breeds, Menz, Wollo and Awassi. This data were used to assess the genetic diversity, population structure and linkage disequilibrium (LD) and runs of homozygosity (ROH) analysis of the parental breeds considering some other breeds as reference. Ovine SNP50K data from the ancestral breeds were also used to select ancestry informative markers (AIMs) differentiatiing Ethiopian fat-tailed and Awassi sheep breeds. Selected AIMs were used to genotype crossbred sheep population sampled from the three villages (Negasi-Amba, Chiro and serity) and two government farms (DBARC and AGSBMC).

Table 1. Detail summary of breed, location, sample type and number of observations.

Breed/ Population	Village	Sample type	Number of sample			
			UN_ID	Ewe	Lamb	Total
Ancestral breed						
Imp Awassi*	-	-	23	-	-	23
Imp Awassi	AGSBMC	FTA	18	-	-	18
Menz*	-	-	34	-	-	34
Menz	Dargegn	Ear tissue	-	18	-	18
Wollo	Chiro	Ear tissue	-	18	-	18
Admixed population						
MA	Negasi-Amba	Ear tissue	-	84	158	242
MA	Negasi-Amba	Blood using FTA	-	56	-	56
75% MA	Amed-Guya	Blood using FTA	16	-	-	16
WA	Chiro	Ear tissue	-	149	144	293
50% MA	DBARC	Ear tissue	16	-	-	16
MA	Serity	Ear tissue	-	20	-	20
Total			107	345	302	754

* = data obtained from the International Sheep Genome Consortium data base, Imp Awassi = improved Awassi, MA = Menz x Awassi crossbred population, WA = Wollo x Awassi crossbred, UN_ID = un-identified sex and class.

3.2.1 Genetic data collection

3.2.1.1 Descriptions of parental population

Three sheep breeds Menz, Wollo and Awassi were used as parental ancestors in order to estimate the admixture levels of Menz x Awassi and Wollo x Awassi crossbred population produced in Negasi-Amba and Chiro villages, respectively. Both Ethiopian Menz and Wollo sheep breeds are classified short fat-tailed breed have similar physical appearances and they are reared in sub-alpine and cold highland agro ecological zones of the country for meat (Gizaw et al., 2007) at an altitude of 2500 to 3600 m. The breeds are characterized by small size, having coarse wool and very low twinning rate of usually less than 3%. However Wollo sheep breed found to be slightly higher in body size and weight than Menz sheep breed (Gizaw et al., 2008a). These sheep breeds are mainly kept for income generation from the sale of live animals. However fiber, manure and skin are also products of sheep in the area (Gizaw et al., 2008a; Getachew et al., 2010). The Awassi sheep breed was introduced from Israel, well known for its adaptation to a wide range of environmental conditions and was widely accepted by many Asian and African countries (Galal et al., 2008; Gürsoy, 2011). Although the Awassi sheep imported from Israel is best known for its high milk production, the breed also used as a triple purpose (meat, milk and wool) in the middle East as well as other countries worldwide (Pollott and Gootwine, 2004; Galal et al., 2008).

3.2.1.2 Sampling DNA extraction, genotyping and quality control for ancestral breeds

Ovine SNP50K data from the two Ethiopian indigenous breeds (Menz and Wollo), exotic Awassi breed which have been used for crossbreeding of Menz and Wollo sheep were included in the study. Other breeds, African breeds (Kenyan Red Maasai, Egyptian Barki, South African Namaqua Afrikaner) South West Asian (Afshari, Moghany, Cypress fat-tailed, Sakiz), New Zealand Texel and Soay breed were also considered in some of the analyses. Ovine SNP50K data for Menz (n=34), Improved Awassi (n=24), local Awassi (n=23), Dorper (n=21), Red Maasai (n=45), Egyptian Barki (n=13), Namaqua Afrikaner (n=12), Afshari (n=37), Moghani (n=34), Cypress fat-tailed (n=30), Sakizl (n=22), NewZealand Texel (n=24), Soay (n=110) all available in ISGC database (ISGC, International Sheep Genomics Consortium, http://www.sheephapmap.org/) were used. The Awassi data were provided by Prof. Elisha Gootwine whereas the remaining data were provided by Prof. Johannes Lenstra.

Genomic DNA of the ear tissue samples collected from Wollo sheep (n=18) were extracted at Holeta Agricultural Research Center biotechnology laboratory, Holleta, Ethiopia. The samples

were genotyped using OvineSNP50 BeadChips manufactured by Illumina (Illumina Inc., San Diego, USA) containing 53862 SNPs by KOS GENETIC in Italy according to the manufacturer protocol. A total of 4494 SNPs were removed from Wollo SNP data having call rate of <0.99. One individual from Wollo breed was also removed due to poor genotyping rate (<95% SNP missing rate) and left with 49368 SNP and 18 individuals. A total of 49034 SNPs were available for Menz, Improved Awassi and local Awassi sheep from the ISGC. Quality control for Menz and Awassi sheep was indicated in Kijas et al., (2009, 2012) and Miller et al., (2011. In brief the quality control steps included the removal of markers with call rate <0.99, markers identified during clustering as having atypical X-clustering, SNP with minor allele frequency equal to zero and SNP with discordant genotypes. A total of 49368 SNPs for Wollo sheep and 49034 SNPs for Menz, local Awassi and Improved Awassi were used for minor allele frequency (MAF) and within breed diversity analysis. After removing SNP with unknown position and merging with common SNPs, a total of 99 individuals from the four breeds (Menz, Wollo, improved Awassi and local Awassi) sharing 47749 SNPs were ready for the remaining analysis. Data editing and quality check was carried out using PLINK (Purcell et al., 2007). Ovine SNP50K data from the two indigenous parental breeds (Menz and Wollo) and an exotic improved Awassi were used to identify informative markers which helped to estimate the proportion of breed in each of the Menz x Awassi and Wollo x Awassi composite populations.

3.2.1.3   Description of admixed population

For the admixed population, samples from Negasi-Amba (n=298), Chiro (n=293) and Serity (n=20) were obtained from farmers village. A total of 105 farmers, 54 in Negassi-Amba, 43 in Chiro and 8 in Serity were involved. Furthermore crossbreds with exotic inheritance of 50% Awassi (n=16) and 75% Awassi x 25% Menz (n=16) were collected from DBARC and ASBMC, respectively. Crossbred samples from Negasi-Amba and Chiro were from ewes with reproductive phenotypes and growing lamb with live weight at eight months age. Most of the samples were ear tissue collected using Allflex ear tissue sampler except 90 samples were blood sample collected using FTA cards. Ear tissue samples were collected between May and June 2012 whereas the FTA samples were collected in June 2009. Details of sample collection are presented in Table 1.

3.2.1.4   Ancestry Informative marker selection

SNPs which conceded the quality control criteria and common to the two indigenous and exotic breed (n=47749) were used to select AIMs. The two indigenous Ethiopian populations were merged as one breed as they are very close to each other having F_{ST} value of 0.004. AIMs that

showed large differentiation between local breeds and exotic Awassi were selected based on their F_{ST} value and fittingness in designing primers. The National Center for Biotechnology Information (NCBI) database http://www.ncbi.nlm.nih.gov/ and sheep genome browser v 3.1 http://www.livestockgenomics.csiro.au/sheep/oar3.1.php were used to identify 100 left and right nucleotide sequences for selected AIMs. A total of top F_{ST} ranked 105 SNP markers were selected from a list of 150 SNPs considering their compatibility in designing primers. Selected AIMs were be used to design SNP multiplex for Sequenom genotyping platform.

3.2.1.5 DNA extraction, and genotyping of admixed populations

Genomic DNA of the ear tissue samples collected from admixed crossbred populations was extracted at Holeta Agricultural Research Center biotechnology laboratory, Holleta, Ethiopia. Genome DNA extraction from FTA card was done at the Austrian Technology Institute (AIT) laboratory, Tulln, Austria. The protocols for DNA extraction from ear tissue sample and FTA cards are presented in Appendix table 1 and 2, respectively.

The AIM SNPs identified were analyzed using the Sequenom MassARRAY iPLEX system (Agena Biosciences (formerly Sequenom), USA) at Veterinary Medicine University laboratory, Tulln, Austria. In short, a section of DNA containing a SNP is amplified from each individual by PCR. This is followed by a high-fidelity single-base primer extension reaction over the SNP being assayed, using nucleotides of modified mass. The different alleles therefore produce oligonucleotides with mass differences that can be detected using highly accurate Matrix-Assisted Laser Desorption/Ionization Time-Of-Flight mass spectrometry (Buggs et al., 2010).

Three multiplex assays, each screening for 36, 36 and 33 SNPs, respectively, were designed using Assay Design Suite v1.0 software (Agena Biosciences (formerly Sequenom),USA) software. SNP genotyping was performed using the iPLEX® GOLD Complete Genotyping kit with SpectroCHIPs® II in the 384 format (Agena Biosciences, Germany), according to the manufacturer's protocol, with a single modification: To reduce unspecific primer extension, 5ng sheared salmon sperm DNA (Invitrogen, Austria) per reaction was added to the PCR mastermix. Results were analyzed with the Sequenom Typer 4.0 software (Agena Biosciences (formerly Sequenom), USA).

3.2.2 Phenotypic data collection

3.2.2.1 *Growth and reproductive performance*

Phenotypic data on growth and reproductive performances of crossbreed sheep having different Awassi levels were collected from flocks in Negasi-Amba and Chiro crossbreeding villages. Details on the number of animals and sampling locations are presented in Table 1. Data for lamb growth were collected between May and July, 2012. Lamb body weight measurement were recorded twice, one at ear tissue sampling date and the other ~2 month before the sampling date. Eight months weight was considered for the study because most of the lambs were at about 8 months of age at sampling time. This age is also close to the market age of lambs in those areas. Adjusted 8 months weight was calculated for each animal based on the regression coefficient from the two measurements as follows,

$$Adj8wt = b * (240 - daywt1) + wt1$$

Where $Adj8wt$ is adjusted 8 months weight, b is the regression coefficient of weight on days estimated as increase in weight between the two measurements divided by the number of days between the two measurements, *daywt1* is the age of lamb in days at the 1st weight measurement time and *wt1* is the weight of lamb in kg at the first measurement time.

Data on ewe lambing interval and number of lamb weaned ewe[-1] year^{-1} were obtained from database available at DBARC. Ewes with three lambing and above were considered for this study.

3.2.2.2 *Best and poor performing animals*

Ewes and lambs with best and poor performance were chosen within each of the two locations. Ewes were chosen based on lambing interval and number of lambs reached weaning age whereas lambs were chosen based on eight months weight performance. In addition to performance records farmer's observation about the performance of ewes and lambs were also considered in choosing best and worst sheep.

3.2.2.3 *Linear body measurements, morphological characters*

Linear body measurements and morphological characters were recorded from all sampled ewes and lambs in both locations. Linear body measurements were body length, chest girth, height at withers, tail length, tail width, ear length and leg length. Furthermore morphological characters like

hair smoothness score and hair on back leg score were observed and recorded for each sheep. Hair smoothness was scored from 1 to 5 using three selected farmers independently, where 1 was very coarse and 5 very smooth. Similarly the presence of hair on back leg was scored from 1 to 5, where 1 no hair on leg and 5 too much hair on leg. The the average of the three farmers were calculated and used for analysis. Details on body measurements and visual explanations are indicated in Appendix Table 3 and Appendix Figure 1, respectively.

3.3 Genetic and phenotypic data analysis

3.3.1 Genetic diversity analysis

A total of 49034 SNPs for Menz, local Awassi and Improved Awassi and 49368 SNPs for Wollo sheep were used for genetic diversity and MAF analysis. Genetic diversity measures were; proportion of polymorphic SNP (P_n), Polymorphism Information Content (PIC), Heterozygosity (Het), sometimes called observed heterozygosity which is the proportion of heterozygous individuals in the dataset allele diversity and allele diversity (Div), sometimes called expected heterozygosity which is the proportion of heterozygous individual in the data set when HWE holds. F statistics (F_{ST}) which is the proportion of total genetic variance contained in a sub population. F_{ST} is a pair-wise population measure of differentiation or relatedness based on genetic polymorphism data. F_{ST} between two populations at specific locus was calculated using SAS ALLELE procedure (SAS Institute Inc., 2008). Negative F_{ST} values that do not have biological interpretation were set to zero.

F_{ST} was calculated as:

$$F_{ST} = \frac{\sigma_p^2}{\bar{P}(1-\bar{P})}$$

Where, σ_p^2 is the variance in the frequency of an allele between sub-populations and $\bar{P}$ is the variance of the average frequency of the allele in the total population.

3.3.2 Principal component analysis

Principal component analysis (PCA) was performed on the 2 Ethiopian fat-tailed and 2 Awassi sheep breeds (set 1), Kenyan Red Maasai, Egyptian Barki and 2 South African breeds included on set 1 (set 2) and 4 Asian breeds included on set 2 (set 3) based on the 47749 SNP markers.

GenABEL R package (Aulchenko et al., 2007) was used for the PC analysis in R environment (R Development Core Team, 2013). To reduce the SNP ascertainment bias during SNP development, application of LD pruning on all 47749 SNP was performed using PLINK --indep (50 5 2) with window size of 50, the number of SNPs to shift the window at each step of 5 and variance inflation factor of 2. A total of 18381, 28961 and 36516 SNPs left for PCA for the data set 1, 2 and 3, respectively. However, PC plots were quite similar whether the SNPs are pruned or not so that the result shown are based on all 47749 SNPs.

3.3.3 Model based structure analysis for parental breeds

Genetic structure and admixture among breeds was analyzed using model-based clustering algorithm implemented in the software ADMIXTURE v. 1.2.3 (Alexander et al., 2009) for the same data sets used in PCA. Prior population information was ignored. The most probable number of population in the data set (K) was estimated using the default cross validation procedure by which prediction errors are obtained for each K values (Alexander and Lange, 2011). The K value that minimizes this estimated prediction error is then assumed to be the most suitable. Individual coefficient of ancestry proportion produced by ADMIXTURE software were graphically visualized using R software (R Development Core Team, 2013).

3.3.4 Linkage disequilibrium and ancestral effective population size

The numbers of autosomal SNPs used for LD were 36307, 43467, 40522 and 41409, for improved Awassi, local Awassi, Menz and Wollo, respectively after performing quality control (QC) of the genotypes with in each breed (Table 2). The QC excluded those markers having marker call rate of ≤0.95, minor allele frequency ≤0.05 and SNPs substantially deviating from Hardy-Weinberg equilibrium ($P<0.000001$). SNPs assigned to X or Y or 0 chromosomes were also excluded. The difference between total number of SNPs before QC and sum of SNPs excluded may not perfectly matched with total number of SNPs after QC. This is because of the possibility of same SNPs detected in more than one QC criterion.

LD as r^2 was calculated between pair-wise SNPs not more than 200 SNPs apart and up to 10 Mb apart on the same chromosome using the --r^2 --ld-window 200 --ld-window-Kb 10000 --ld-window --r^2 0 command in PLINK (Purcell et al., 2007).

The r^2 values were computed as:

$$r_{ij}^2 = \frac{(P_{ij} - P_i P_j)^2}{P_i(1 - P_i)P_j(1 - P_j)}$$

Where, P_{ij} is the frequency of the two marker pairs, P_i and P_j are the marginal allele frequencies in the ith and j^{th} SNP, respectively. The value of r^2 can vary from 0 to 1, where 0 means no correlation and 1 means perfect correlation between the SNP pairs.

Then the average r^2 value was stacked in to 5 to 15 Kb, 15 to 25 Kb, 25 to 35 Kb and so on to 9985 to 9995 Kb and the mean value calculated within each stack. For graphical presentation each bin was represented by the middle value, for example 10 Kb assigned for the first bin of 5 to 15 Kb and so on. Extent of LD across the genome was visualized on stacked r^2 values and plotted them as a function of inter-marker distance categories in the range of 0 to 1,000 Kb and 1,1000 Kb to 10,000 Kb.

Table 2. Quality measures and number of SNPs excluded from the linkage disequilibrium (LD) analysis.

Breed	Number of SNP before	Number of SNPs excluded due to				Number of SNPs after QC
		*Chr 0, 27, 28	Deviated from HWE	Genotype call rate < 0.95	MAF <0.05	
Improved Awassi	49034	1532	0	945	10376	36307
Local Awassi	49034	1532	0	467	3602	43467
Menz	49034	1311	2	75	7125	40522
Wollo	49368	1351	0	0	6608	41409

*= SNPs assigned to X or Y or 0 chromosomes, HWE= Hardy-Weinberg equilibrium, QC=quality control.

3.3.5 Runs of homozygosity

The genomic runs of homozygosity (ROH) are continuous stretches of homozygous genotypes without heterozygosity in the diploid. Autosomal markers from improved Awassi, local Awassi, Menz and Wollo sheep were used for ROH analysis. In addition Dorper sheep, Afshari, New Zealand Texel and Soay sheep were included as reference due to their unique features. Dorper was added as the breed have been used for crossbreeding in Ethiopia and many other countries, New Zealand Texel to represent breeds improved through selection and known for its growth and muscling, Afshari to represent sheep from sheep domestication area (Iran) and Soay represented highly inbred breed.

Stretches of consecutive homozygous genotypes were identified for each animal using PLINK v1.9 (Purcell et al., 2007). The previous PLINK v1.7 limited to detect segments beyond the specified window (Ferenčaković et al., 2013) however the new version PLINK v1.9 has revised considering this limitation. A ROH length of 1 Mb (to exclude very short and common ROH resulted from LD) and 15 consecutive homozygous SNP was set as minimum value to call ROH. One heterozygous genotype was permitted per window. SNP density of 1 SNP every 100 Kb was set as minimum to ensure low SNP density did not falsify ROH length. When two SNPs within a segment were far apart (>1000 Kb), that segment was split in two.

ROH length was categorized in to 8 groups based on their size. The groups were 1-5, 5-10, 10-15, 15-20, 20-25, 25-30, 30-35 and above 35 Mb. The mean sum of ROH with in each ROH length category was calculated by summing all ROH per animal in each ROH length category and averages this per breed population. The percentage of SNPs involved in ROH (locus autozygosity) was calculated at each locus. For each SNP, animals were scored as autozygous or non-autozygous based on the presence of a ROH encompassing the SNP. Then the locus autozygosity was calculated for each breed as the autozygosity score of individual divided by the total number of individual. Both results were visualized in R.

Genomic homozygosity was measured based on the percentage of individuals genome covered by ROH. Genomic homozygosity was estimated,

$$F_{ROH} = \frac{L_{ROH>1\,Mb}}{L_{AUTO}}$$

Where, $L_{ROH>1\,Mb}$ is the length of autosomal genome in ROH of above 1 Mb length per animal and L_{AUTO} is the total length of the autosomal genome covered by SNPs. The total length of autosomal genome covered by SNP was 2.78 Gega base (Gb).

3.3.6 Estimation of ancestry in admixed population

The ancestry contribution was estimated for the admixed population using 74 top ranked AIMs selected by their F_{ST} value. Admixture level in the crossbred population was estimated based on maximum likelihood-based clustering algorithm using the software ADMIXTURE v. 1.2.3 (Alexander et al., 2009) assuming two ($K=2$) parental population. Both unsupervised (prior population information was ignored) and supervised (with prior population information) were employed. Pearson's correlation was employed to compare the individual admixture estimates from supervised and unsupervised admixture analysis.

Validity of the 74 SNPs in estimating the level of Awassi was assessed by comparing the ancestry estimate based on the 74 AIMs with pedigree information. Ancestry contribution was also estimated based on top subsets of 65, 55, 45, 35, 25 and 15 SNPs in order to assess the possibility of reducing AIMs. Spearman's rank correlation coefficient was employed between the top ranked 74 and the subsets of top ranked SNPs. The accuracy and validity of ancestral estimates were also assessed using root mean square error (RMSE) which was used as a summary measure of precision in the estimate of individual ancestry proportion.

$$RMSE \;=\; \sqrt{\frac{1}{n}\sum(\hat{y}_i - y_i)^2}$$

Where y_i is the observed observation for the ith observation and $\hat{y}_i$ is the predicted value and n is the number of pairs of values of observed and predicted compared. Individual admixture estimate based on 74 SNPs was plotted against each subset of SNPs. R statistical software (R Development Core Team, 2013) was used for this statistical analysis and visualizing the results in graph.

3.3.7 Phenotypic data analysis

ROC UNIVARIATE (SAS Institute Inc., 2009) was employed within each location to check the quality of the phenotypic data. Calculated skewness values for residual adjusted 8 months weight were in the range of accepted value of -1 to 1 in both Negasi-Amba and Chiro villages. Observing the extreme values within location showed that all observations are in the range of mean±3 SD

suggested future analysis was valid for the given data set. Similar PROC UNIVARIATE also done for the ewe data set.

3.3.7.1 Growth and reproductive performance

For the analysis of growth and reproductive performance, lambs and ewes were classified in to six breed groups based on the admixture level estimated from the genotype. The breed groups were 0% Awassi (pure local breeds), <12.5% Awassi level, 12.5 to 25% Awassi level, 25 to 37.5% Awassi level, 37.5 to 50% and above 50% Awassi level. Growth, lambing interval (LI), number of lambs weaned ewe^{-1} year^{-1} (NLWEY) were analyzed using PROC MIXED of SAS/STAT (SAS Institute Inc., 2009). Lamb weight at 8 months was analyzed by fitting location to test the effect of location. In addition with in location analysis was implemented by fitting SNP estimated Awasssi level group and sex of lamb as fixed independent variables, owner of the lamb as random variable and 8 months weight as dependent variable.

Model to analyze lamb 8 month weight for each of Negasi-Amba and Chiro location was:

$$y_{ijkl} = \mu + b_i + s_j + f_k + \varepsilon_{ijkl}$$

Where, y_{ijkl} is lamb eight months weight, μ is the overall common constant for all values of y, b_i is the fixed effect of lamb Awassi level, s_j is the fixed effect of sex, f_k is the random effect of owner of lambs and ε_{ijkl} is the residual effect.

Lambing interval and NLWEY were analyzed by fitting breed group as fixed variables, owner of the ewe as random variable and LI or NLWEY as dependent variable.

Model to analyze lamb LI and NLWEY for each of Negasi-Amba and Chiro location was:

$$y_{ijk} = \mu + b_i + f_j + \varepsilon_{ijk}$$

Where, y_{ijk} is lambing interval or number of lambs weaned ewe^{-1} year^{-1}, μ is the overall common constant for all values of y, b_i is the fixed effect of ewe Awassi level, f_j is the random effect of owner of ewes and ε_{ijk} is the residual effect.

PROC GLM SAS/STAT (SAS Institute Inc., 2009) was used to analyze the association of extreme performances with Awassi level. Both lamb and ewe data were analyzed within location by fitting

performance level (poor, medium and worst) as fixed independent variable and Awassi level and performance of sheep as dependent variable.

The model was:

$$y_{ij} = \mu + p_i + \varepsilon_{ij}$$

Where, y_{ij} is either of the dependent variables in each location (Awassi level for lambs or ewes, 8 months weight and body condition score of lambs, lambing interval or number of lambs weaned ewe^{-1} year^{-1}), μ is the overall common constant for all values of y, p_i is the fixed effect of ewe or lamb performance level (poor, medium and top) and ε_{ij} is the residual effect.

3.3.7.2 Correlation and regression analysis of linear body measurements and admixture level estimates

Pearson's rank correlations were employed using PROC CORR (SAS Institute Inc., 2009) to assess the relationship between SNP estimated individual admixture level and 10 different linear body measurements and morphological characters (ear length, hair smoothness score, hair on leg score, tail length, tail width, back leg length upper, back leg length lower, body length, chest girth and wither height) for ewe and lambs. The analysis was done within each location. Scatter plot of SNP estimated Awassi level against each of body measurements or morphological characters was also visualized using R software for each pair. Multiple linear regressions were employed using PROC REG (SAS Institute Inc., 2009) to evaluate the prediction of Awassi level from linear body measurements and morphological characters. During the regression analysis individual Awassi level estimated based on the 74 SNP was fitted as dependent variable whereas the remaining 10 linear body measurements and morphological characters as predictor variables. All the 10 variables enter in to the model and stepwise procedure was used to select variables explaining more variation to predict the Awassi level. Coefficient of determination (R^2) and Mallows' Cp statistics were used to assess the fit of regression model.

3.3.7.3 Correlation between individual admixture level and farmers estimate

Pearson's rank correlations were employed using PROC CORR (SAS Institute Inc., 2009) to measure the relationship between farmer estimated individual Awassi level and SNP estimated Awassi level within each location for lambs and ewes separately. The relationship with their correlation values were visualized in scatter plot using R software. Three key farmers in each of

the two locations were asked to estimate the Awassi blood of each individual sheep based on their knowledge. Farmers were selected based on their knowledge and experience in sheep breeding.

4 Results and discussion

4.1 Genetic diversity and population structure

Genetic diversity measures for Menz, Wollo, local Awassi and improved Awassi sheep breeds are presented in Table 3. Proportion of polymorphism was highest for local Awassi (96.2%). The lowest proportion of polymorphic SNP was found for improved Awassi (84.26%). Ethiopian local breeds were intermediate when compared with the local and improved Awassi breeds. Local Awassi demonstrated the highest level of genetic diversity (Het=0.36) followed by the two Ethiopian (Het=0.32). While, improved Awassi had the lowest gene diversity (Het=0.30). Similar pattern was observed for PIC values. Minor allele frequencies in the four breeds based on different categories are presented in Figure 2. Proportion of monomorphic SNP was highest in improved Awassi and lowest in local Awassi, Ethiopian breeds were intermediate in proportion of monomorphic SNP.

Table 3. Genetic diversity measures for Menz (M), Wollo (W), local Awassi (LA) and Improved Awassi (IA) sheep breeds.

Breed	N	N_SNP	P_n	PIC	Het	Div	Pairwise F_{ST}		
							W	IA	LA
M	34	49034	93.00	0.255	0.316	0.320	0.00397	0.129	0.065
W	18	49368	91.71	0.258	0.330	0.324		0.123	0.052
IA	23	49034	84.26	0.236	0.310	0.296			0.087
LA	24	49034	96.22	0.283	0.356	0.357			

N=number of sheep, N_SNP=number of SNPs, P_n=proportion of polymorphic SNP, PIC=polymorphic information content, Het=Observed heterozygosity, Div=Gene diversity (expected heterozygosity).

Based on the pairwise estimate of F_{ST}, highest genetic differentiation appeared between local Ethiopian and improved Awassi sheep breeds (F_{ST}~0.13) while as expected, the two locally developed Ethiopian breeds were closely related (F_{ST}=0.004). Surprisingly, local Awassi sheep differentiated from improved Awassi at a higher level (F_{ST}=0.087) than the local Awassi differentiated from the two local Ethiopians, Wollo (F_{ST}=0.052) and Menz (F_{ST}=0.065) breeds (Table 3).
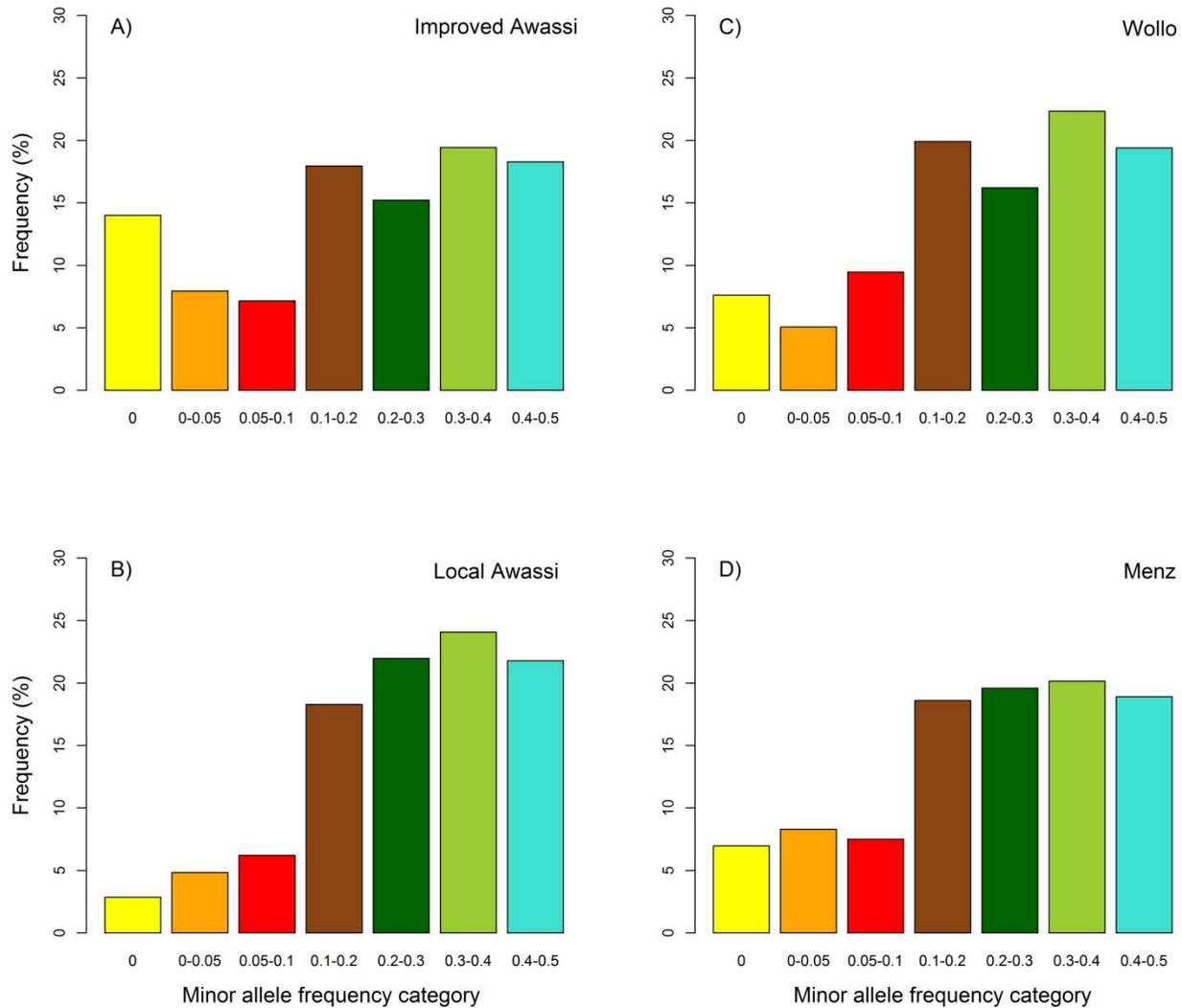
Figure 2. Minor allele frequency of Ethiopian and Awassi sheep breeds based on 47749 SNPs.

Genetic diversity measures for the two indigenous Ethiopian breeds (Menz and Wollo) and local Awassi were demonstrated higher level of genetic variability (Het = 0.32 - 0.36). Most of the world sheep breeds (Kijas et al., 2012), Suffolk, Rambouillet, Columbia, Polypay and Targhee (Zhang et al., 2013) and Italian sheep breeds (Ciani et al., 2014) had also shown higher genetic diversity in the range of 0.33 and 0.37. However, the value found for improved Awassi in this study was lower than those previous reports, which might be associated with the breed development history. Observed level of heterozygosity found in this result for the local Ethiopian, local Awassi and improved Awassi sheep breeds were higher than heterozygosity level of East African local cattle breeds reported in the range of 0.17 to 0.25 (Mbole-Kariuki et al., 2014).

The lower genetic diversity for Improved Awassi was also supported with excess of minor allele frequency (Figure 2). Relatively higher proportion monomorphic loci in local Ethiopian breeds compared to local Awassi might be hypothesized due to the effect of ascertainment bias in which the SNP discovery affects the level of polymorphism in a way breeds not considered in the SNP discovery tends to have less polymorphic loci (Clark et al., 2005). Ovine SNP50 BeadChip discovery was based on three separate experiments (Roche 454, Illumina GA SNP and Sanger sequencing). Among them 67.5% of SNP discovery was by Roche 454, primarily based on European breeds excluding African breeds (Kijas et al., 2012). However excess monomorphic loci in the improved Awassi might not be due to ascertainment bias as the Awassi breed was included in all the SNP discovery efforts. Rather might this be the result of strong selection to improve productivity of the breed. High level of monomorphic and loss of genetic variation due to random genetic drift and/or intense selection was also observed in cattle breeds (Zenger, et al., 2007; Bovine HapMap Consortium et al., 2009). To reduce the severity of the bias, more common subset of SNPs with MAF ≥0.1 (n=40734) and MAF ≥0.2 (n=32010) were used to assess level of polymorphism. Still the difference among breeds is consistently influenced (Table 4) in that local Awassi are more diverse than local Ethiopian breeds and Improved Awassi.

It is not surprising to assume that the local breeds maintained higher level of genetic variability than improved breeds as such local breeds are not under selection whereas intensive selection has been done on Improved Awassi breed to increase milk yield (Gootwine, 2011). This was supported with previous report on pig that local Chinese pigs showed higher level of genetic diversity than Western pigs (Ai et al., 2013). Higher gene diversity also observed for the Polpay and Targhee sheep breeds developed recently compared to the Suffolk and Rambouillet breeds which had longer improvement history (Zhang et al., 2013). Differentiation of polled Dorset from horned Dorset (Kijas et al., 2012) and significant variation among five different lines of Merino sheep were also reported (Diez-Tascon et al., 2000).

The observed reasonably high level of differentiation between local Ethiopian and improved Awassi supports the use of Awassi sheep to improve Ethiopian Menz and Wollo sheep breeds. Crossbreeding among highly differentiated populations possibly results in higher heterozygosity and increased productivity due to higher heterosis effect when heterozygosity results in a significantly greater effect or positive heterosis (Comings and MacMurray, 2000). However, looking for specific regions of the genome among breeds is suggested in order to design a crossbreeding breeding program to maintain important adaptive traits of the indigenous breed in the crossbreed population. Identification of chromosomal regions that contain signatures of

selection due to adaptation and other traits due to natural as well as artificial selection has been well documented (Kijas et al., 2012; Moradi et al., 2012; Fariello et al., 2014; Lv et al., 2014).

Table 4. Frequency of minor allele frequency (MAF) in different categories for the four breeds.

Breed	MAF = 0			MAF 0 to 0.05			MAF 0.05 to 0.1		
	Set 1	Set 2	Set 3	Set 1	Set 2	Set 3	Set 1	Set 2	Set 3
IAwassi	6681	2688	1093	3785	2682	1524	3416	2707	1642
LAwassi	1365	90	12	2309	532	96	2964	1376	418
Menz	3329	458	72	3956	1697	382	3577	2427	797
Wollo	3632	614	102	2422	975	228	4516	2893	945

IAwassi=improved Awassi, LAwassi=local Awassi, A total of 47749, 40434 and 32010 were used for set 1, 2 and 3, respectively. Set 1=based on all 47749 SNPs, set 2=contains 40434 SNPs after removing SNPs with MAF <0.1 and set 3=contains 32010 SNPs after removing SNPs with MAF <0.2 across all breeds.

4.1.1 Linkage disequilibrium

The r^2 value at very short distances as well as decline of it with genetic distance varied among breeds (Figure 3). Improved Awassi had the highest LD both at shorter and longer distances. Among the three local breeds (local Awassi, Menz and Wollo), Wollo sheep had the highest LD at shorter distance and consistently throughout the genome length. Lower LD was found in local Awassi and Menz sheep, in which local Awassi had the lowest LD in shorter distances of up to about 100 Kb while Menz sheep had the lowest LD in longer distances of after 200 Kb. Mean±standard deviation±SD r^2 for improved Awassi sheep breed was 0.43±0.36 in pairwise distance of 5 to 15 Kb apart which was higher compared to the values 0.3±0.32, 0.3±0.32, and 0.28±0.30 for Menz, Wollo and local Awassi, respectively. The r^2 value dropped to 0.28±0.28, 0.13±0.19, 0.14±0.19 and 0.12±0.17 at 55 to 75 Kb for improved Awassi, Menz, Wollo and local Awassi breeds, respectively. Decrease in LD measured by r^2 as a function of distance between marker pairs was consistent with many other findings in different sheep, cattle and pig populations (García-Gámez et al., 2012; Ai et al., 2013; Mbole-Kariuki et al., 2014; Pérez O'Brien et al., 2014). Observing the rate of decaying pattern, it was fast in a very short distant of up to 60 Kb for all the 4 sheep breeds. Then after, Menz sheep showed rapid decay than other local breeds whereas LD in improved Awassi extended being highest over distances up to 10,000 Kb.

The r^2 values obtained in this study for Ethiopian and Israeli local sheep breeds were in the range of 0.12 to 0.14 at marker pairs distance of 55 to 65 Kb which was in agreement with the r^2 value of 0.15 at 40 to 60 Kb reported for Churra sheep breed (García-Gámez et al., 2012). Compared to cattle, LD values were lower than the values reported 0.16 and 0.14 for taurine and indicine breeds at 100 Kb distance (Qanbari et al., 2010; Pérez O'Brien et al., 2014). Higher r^2 value of 0.2 was also reported in East African Zebu cattle at 55.4 Kb (Mbole-Kariuki et al., 2014). However LD for improved Awassi was highest at shorter distance (0.25 at 100 Kb and 0.28 at 40 to 60 Kb) and persistent throughout the genome distance indicated that the breed was selected from relatively small ancestral population and subjected to very narrow bottlenecks due to recent intense selection to improve milk yield, respectively. Isolated sheep breeds with less Ne (e.g. Soay and Wiltshire) and improved breeds like Dorset Horn and Texel breeds also showed higher LD compared to local breeds up to 1000 Kb (kijas et al., 2012). The influence of strong selection on LD by and recent reduction of effective population size was also reported in cattle (Lu et al., 2012; Pérez O'Brien et al., 2014). Looking the LD pattern among three local breeds, local Awassi had the lowest r^2 value at shorter distance implied the breed evolved from larger ancestral population. Whereas at longer distances Menz sheep showed the lowest LD value implied the breed have been subjected to a relatively less bottlenecks compared to local Awassi and Wollo sheep. Decaying of LD was very rapid for Soay breed which was started at the second highest at shortest distance and ends with the lowest r^2 value at the longest distance (10,000 Kb) which suggests that Soay breed was derived from a relatively small ancestral population and not subjected to very narrow bottlenecks (Appendix figure 1). Similar pattern was observed in N'Dama cattle breed (Bovine HapMap consortium et al., 2009).
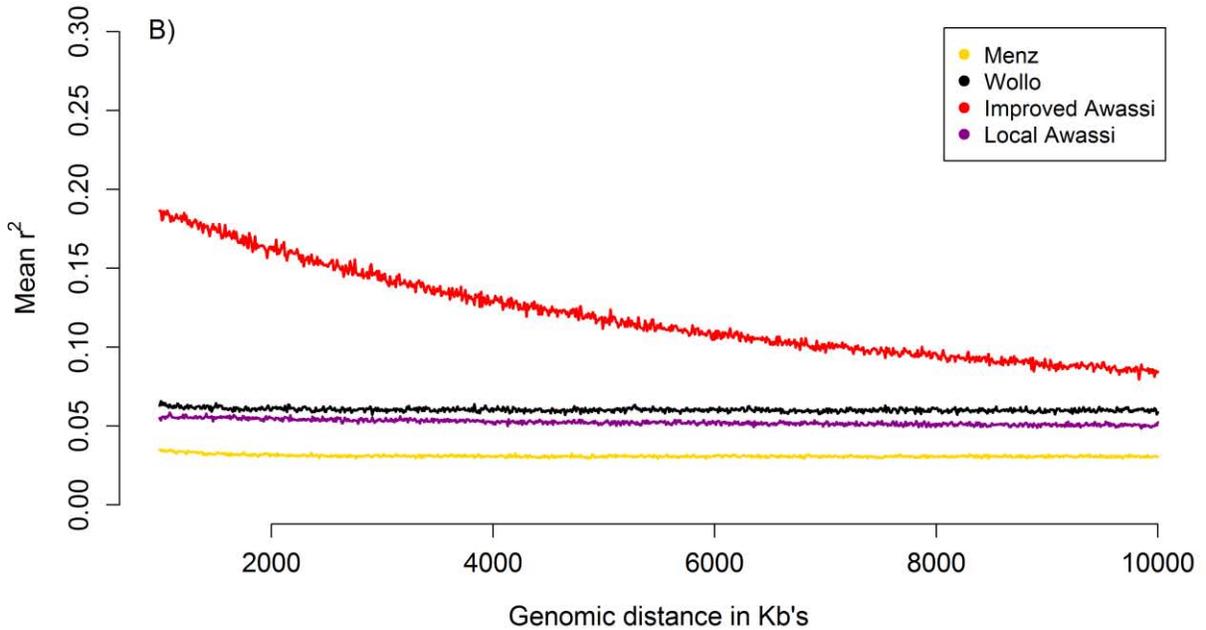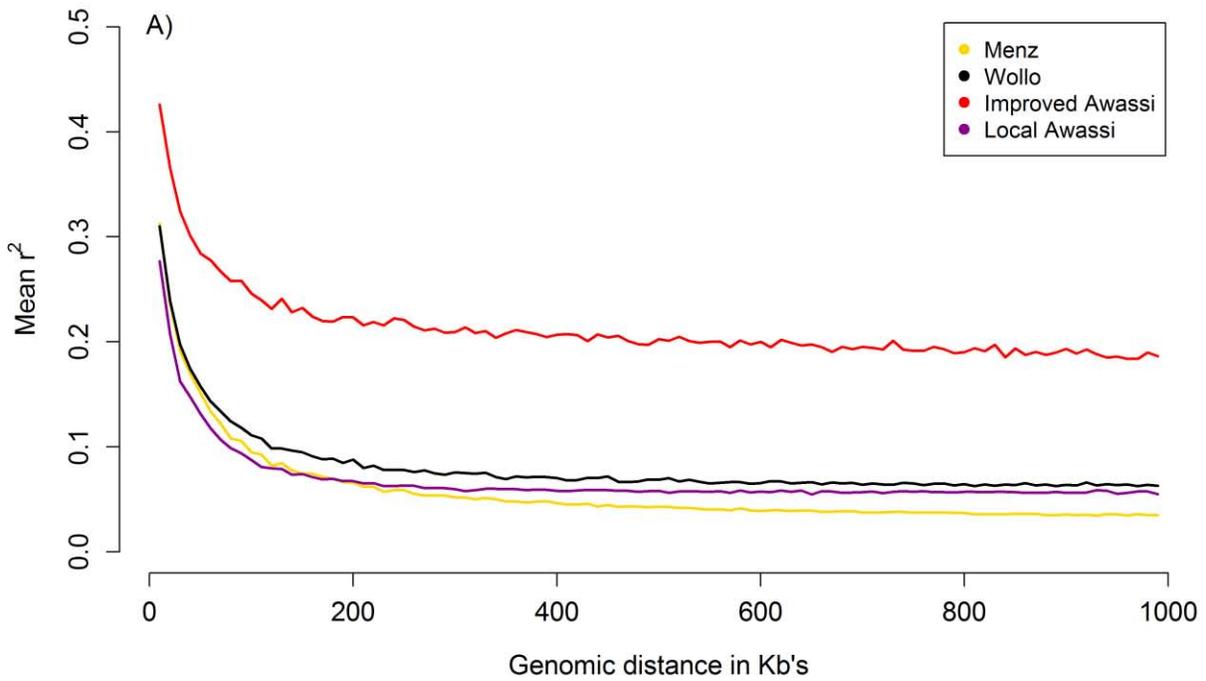
Figure 3. LD decay of average r^2 over genomic distance in kilo base's (Kb's). Average r^2 between SNPs in Menz, Wollo, Improved Awassi and local Awassi sheep breeds at varing distances in Kilobase pairs ranging from 0 to 1000 Kb (A) and 1000 Kb to 10,000 Kb (B).

4.1.2 Principal component analysis

Principal component analysis (PCA) explores the relationship among individuals and population groups. Principal component analysis for local Ethiopian and Awassi breeds (set 1) are presented in Figure 4. Individuals from the same population cluster together with small variation within the population of improved Awassi and Local Ethiopian population. Whereas the local Awassi breed showed a higher degree of variation. Both geographic location and breed development influenced breed differentiation. Based on the set 1 data, the first PC explains 16.28% of the total variation, associated with geographic location which separated Ethiopian and Israeli breeds. The second PC explains 5.5% of the total variation associated with breed development which separated the improved and local Awassi population (Figure 4).

When 4 another African breeds (African Dorper, Kenyan Red Massai, Egyptian Barki and Namaqua Afrikaner) were added on data set 1 (set 2), the first PC was attributed to geographic location in which the African breed separated from the Awassi and Egyptian breed with variation if 9.76% (Figure 5). In PC 1, Egyptian breed clustered with the Israeli breed corresponding to its geographic proximity to the Middle East. Namaqua Afrikaner and African Dorper were set apart from Ethiopian and Kenyan breeds and Improved Awassi set apart from the local Awassi. The second PC explained 5.6% of the variation and separated the Improved Awassi from the Local Awassi and Egyptian Barki.

When 2 Asian, (Afshari and Moghani) and 2 European (Cyprus Fat-Tailed and Sakiz) breeds were added to data set 2 (set 3), the first PC explained 8.08% of the variation and separated African from the rest of the breeds however the Egyptian Barki clustered to gether with the Asian and Awassi group (Figure 6). The second PC (4.39%) clearly separated Ethiopian breeds from South African breeds and the Red Maasai in between.

The variation explained in the first PCs found in this study was much lower than the variation explained by the first PC (65%) in cattle separating taurine and indicine breeds. This is consistent with the report suggesting sheep are weakly differentiated compared to cattle (Kijas et al., 2012). PC analysis showed that genetic SNP markers partitioned breeds according to their geographic location and breed development history observed in this study also in agreement with other studies in sheep (Kijas et al., 2012) and goat (Huson et al., 2014).
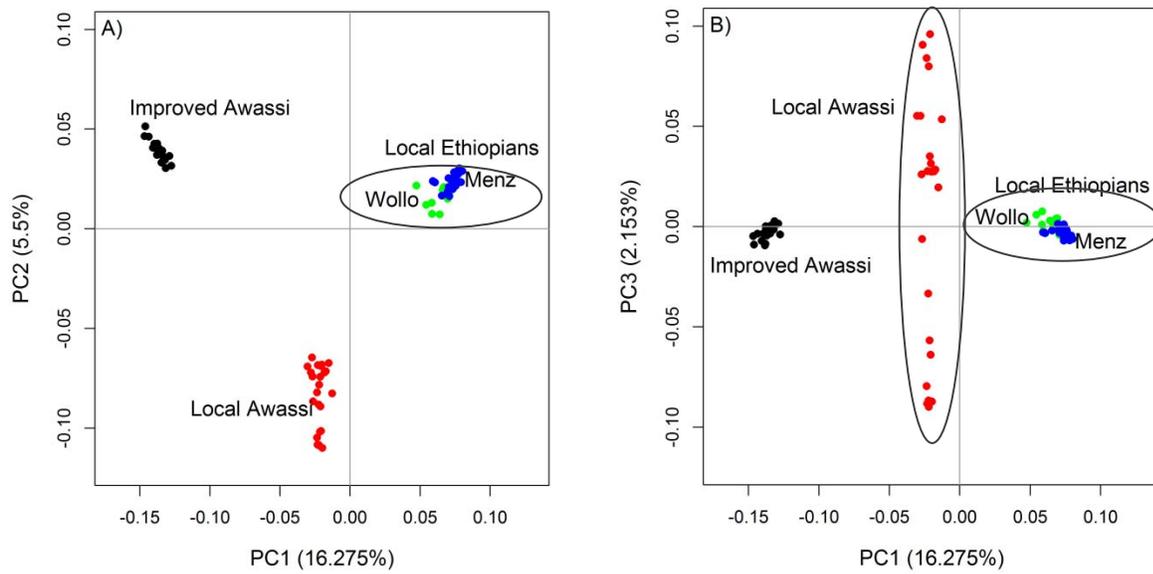
Figure 4. Principal componenet analysis, PC 1 and 2 (A) and PC 1 and 3 (B) of Menz, Wollo, local Awassi and improved Awassi sheep breeds.
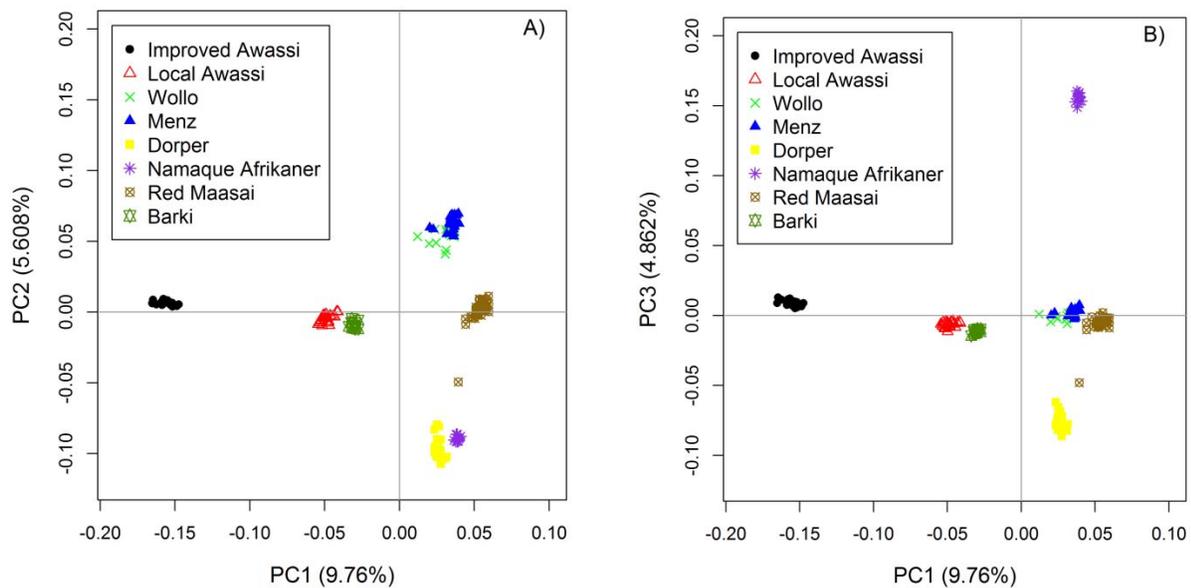
Figure 5. Principal componenet analysis, PC 1 and 2 (A) and PC 1 and 3 (B) of African and Awassi sheep breeds.
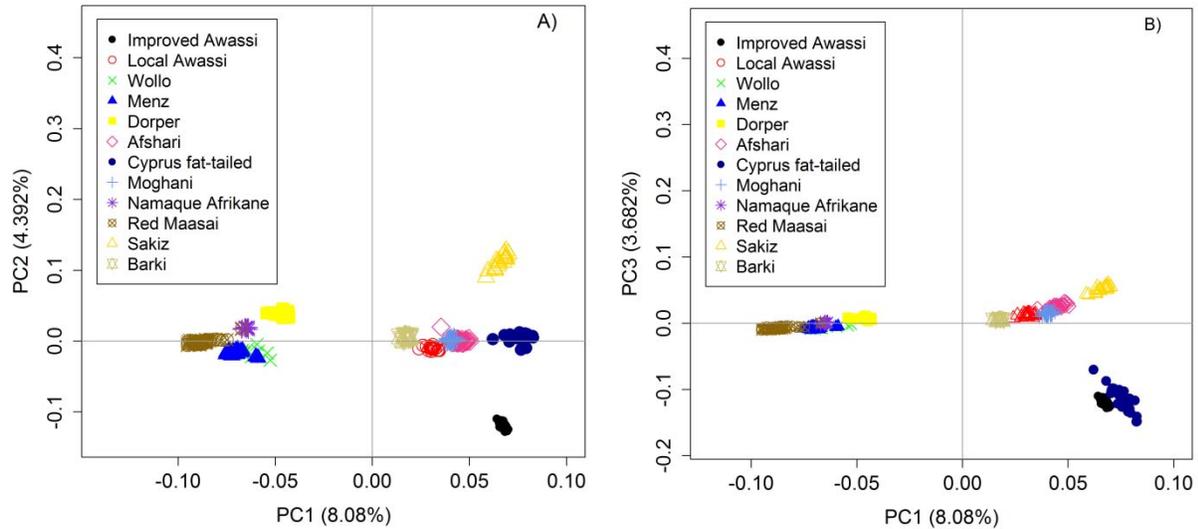
Figure 6. Principal component analysis, PC 1 and 2 (A) and PC 1 and 3 (B) for African, Asian, Awassi and European breeds.

4.1.3 Genetic structure analysis

Genotype frequency was analyzed using ADMIXTURE software to examine genetic structure of breeds. Varying the number of presumed ancestral population (K) revealed clusters consistent to known history of sheep breeds. ADMIXTURE software run from $K=2$ to $K=4$ using 47749 SNPs for the Ethiopian and Israeli breeds (set 1) are presented in Figure 7 and cross validation error from $K=1$ to 5 in Figure 8. Based on the cross validation (CV) error $K=3$ found as an optimal partitioning of breeds. At $K=2$ the improved Awassi separated completely from local Ethiopian breeds (Menz and Wollo) while the local Awassi showed as mixture of the improved Awassi and Ethiopian breeds. At $K=3$ local Awassi sheep separate from both the improved Awasssi and local Ethiopian breeds, while local Awassi still showed some admixture from both Improved Awassi and Ethiopian breed.

Consistent with the PC results, both man-made selection and geographic location were responsible for differentiation of sheep populations in admixture analysis. As clearly seen at $K=2$, local Ethiopian and improved Awassi breed were separated from each other. Local Awassi appeared as admixed population sharing from both populations. Surprisingly, consistent to the F_{ST} result found in this study, the model based admixture analysis also showed the mean contribution of Ethiopian population to local Awassi was greater (56.1%) than the contribution from Improved

Awassi (43.9%). This showed that man-made selection pressure to improve productivity of Awassi had much more influence than the geographic isolation between the two local Ethiopian and local Awassi breed. This higher relative genetic differentiation between improved Awassi and local Awassi compared to the differentiation between local Awassi and local Ethiopian breeds is also supported by the phenotypic performance variation. Talafha and Ababneh, (2011) summarized that local Awassi ewe produces in the range of 40 to 80 kg milk in 150 days of lactation under unimproved and improved management which is more close to local Ethiopian (produce no extra milk other than for the lamb to suck) compared to the improved Awassi (produces 500 L of milk over 240 days of lactation period).
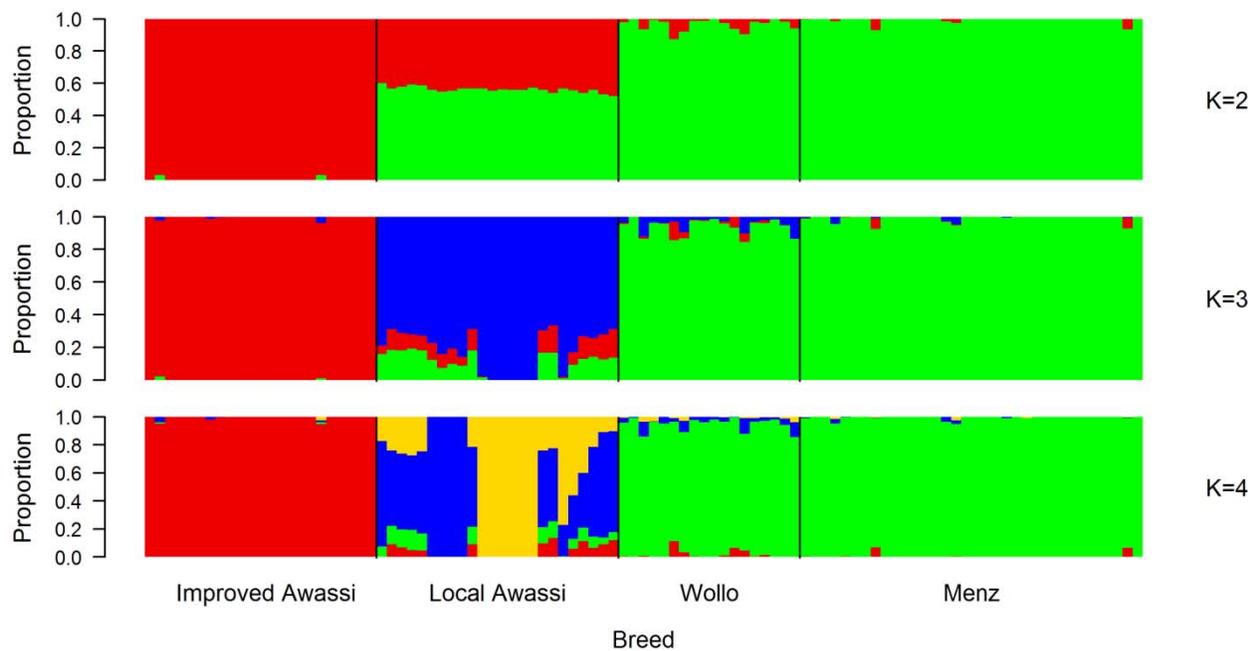
Figure 7. Model based admixture analysis of improved Awasssi, local Awassi, Wollo and Menz sheep breeds considering *K*=2 to 4 ancestral population. Breeds are separated by black lines. Each individual represented by a vertical bar and partitioned in to colored segments representing the proportion of each ancestors.
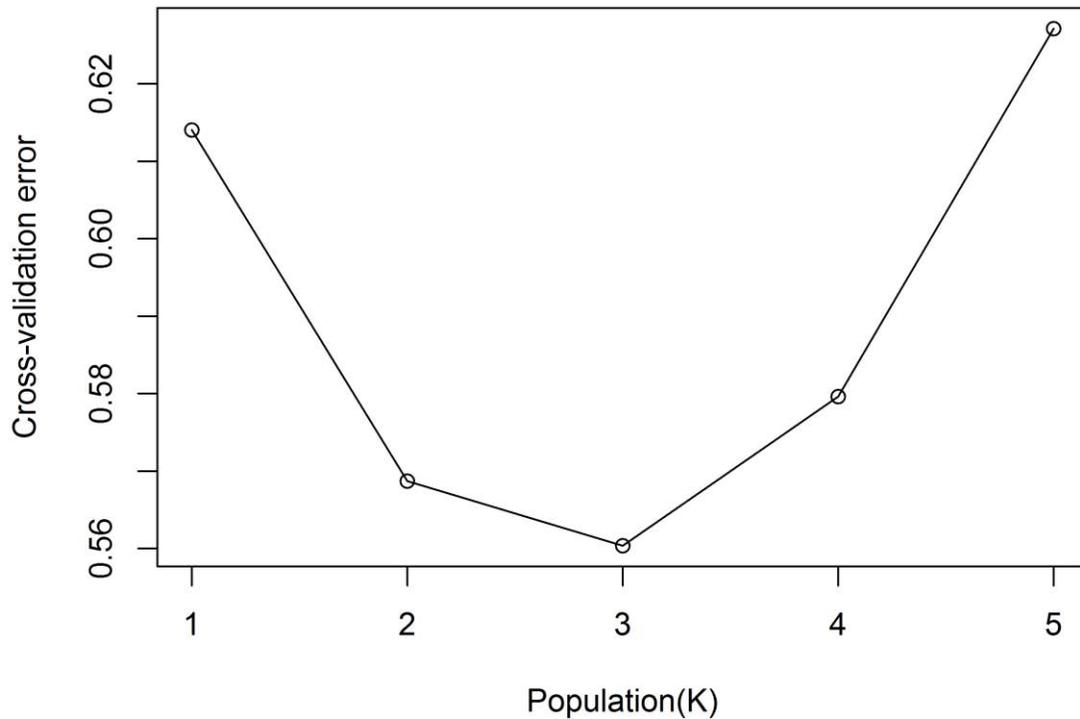
Figure 8. Cross validation error curve from $K=1$ to 5 for Ethiopian and Awassi breeds.

For the data set 2, $K=6$ found to be an optimal portioning and no other classification observed beyond this level (Figure 9 and 10). The six clusters correspond to Improved Awassi, Local Awassi and Egyptian Barki as one cluster, local Ethiopians, Dorper, Namaqua Afrikaner and Red Maasi populations. At $K=2$ improved Awassi separated from all other breeds however shared large portion with local Awassi and Egyptian Barki and little admixture with other African breeds. Namaqua Afrikaner, Dorper and Red Maasai were separated $K=3$, 4 and 5, separated at respectively. Local Awassi and Egyptian Barki showed similar genetic makeup up to $K=8$ with notable admixture from African breed and Improved Awassi until $K=5$.
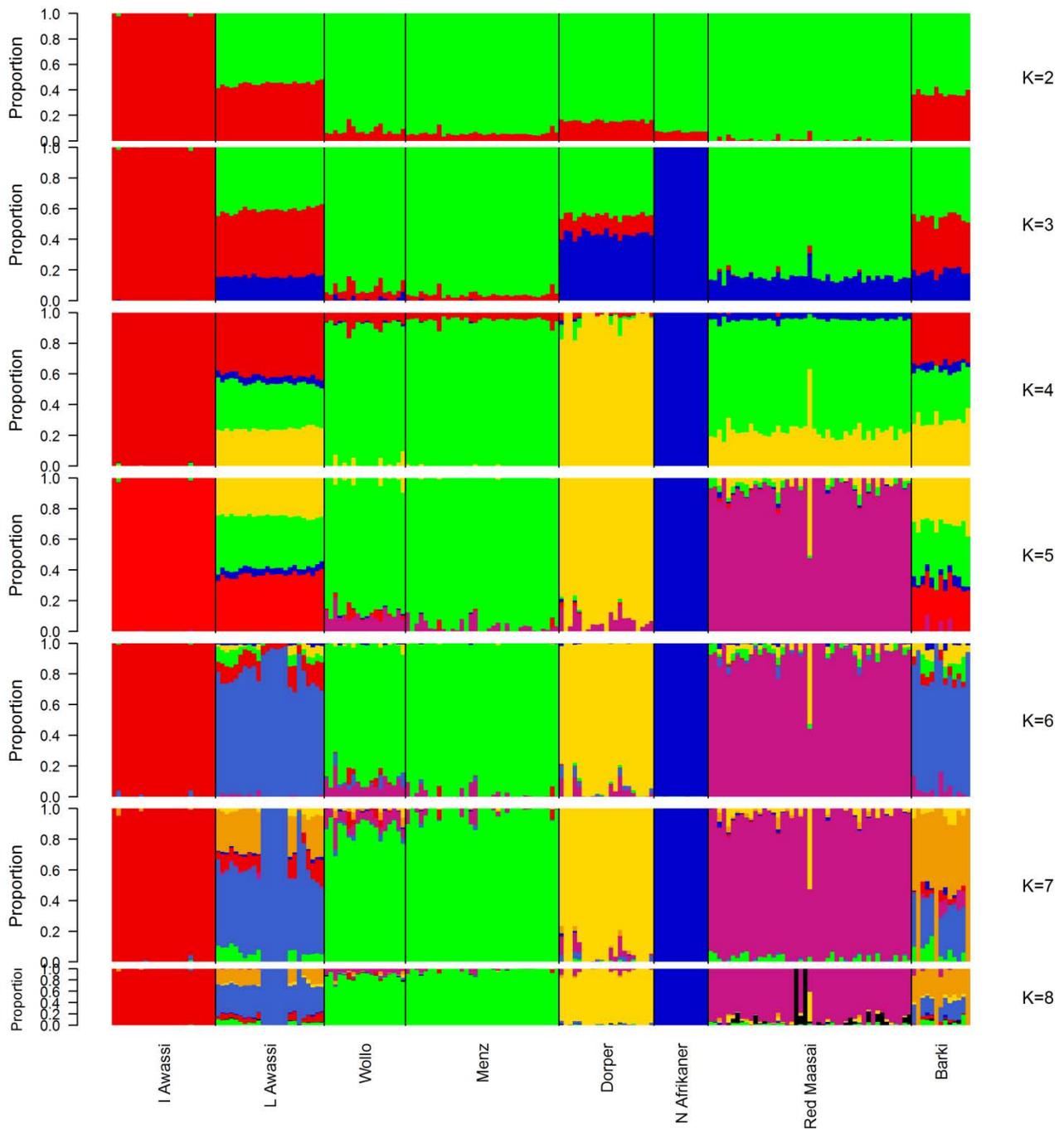
Figure 9. Model based admixture analysis of different African and Awassi sheep breeds considering *K*=2 to 8 ancestral population. Breeds are separated by black lines. Each individual represented by a vertical bar and partitioned in to colored segments representing the proportion of each ancestors. I Awassi=improved Awassi, L Awassi=local Awassi and N Afrikaner=Namaque Afrikaner.
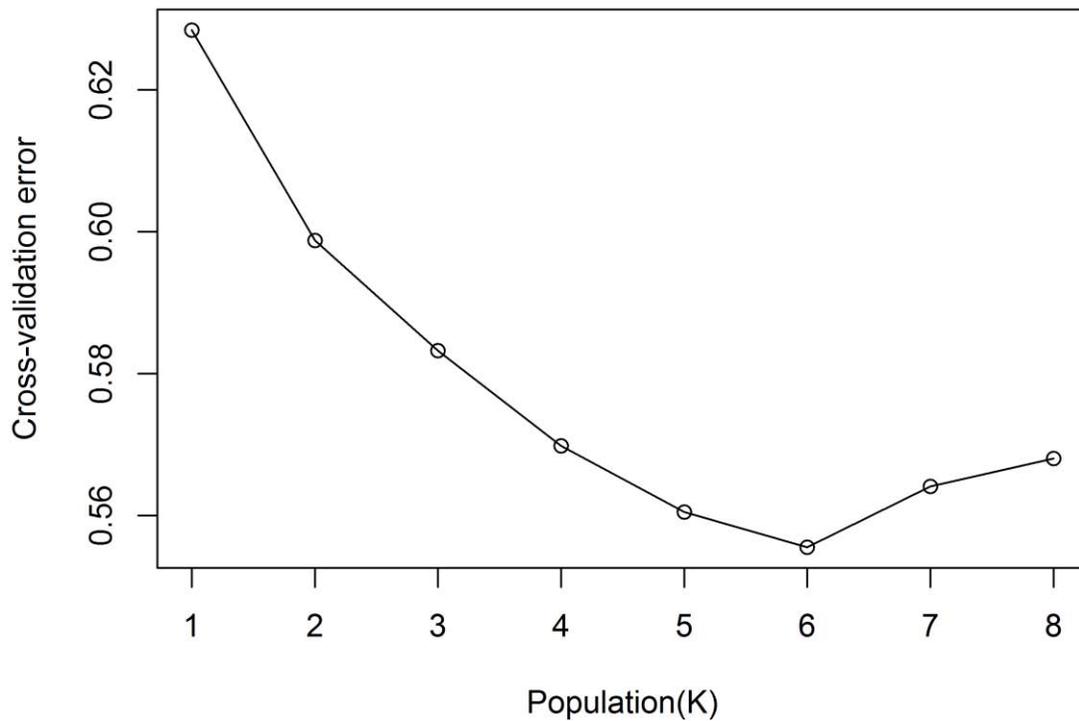
Figure 10. Cross validation error curve from *K*=1 to 8 for different African and Awassi sheep breeds.

For data set 3, *K*=9 was found as an optimal classification based on the cross validation error (Figure 12). Improved Awassi, Ethiopian fat-tailed, Dorper, Afshari, Cyprus Fat-Tailed, Namaqua Afrikaner, Sakiz and Red Maasai appeared as separate clusters (Figure 11). At *K*=2 African breeds were clustered together except Egyptian Barki which showed mixture of African and the rest of the breeds. Sakiz, Namaqua Afrikaner, Cyprus Fat-Tailed, Afshari and Dorper created their own cluster at *K*=2, 4, 5, 6 and 7, respectively.
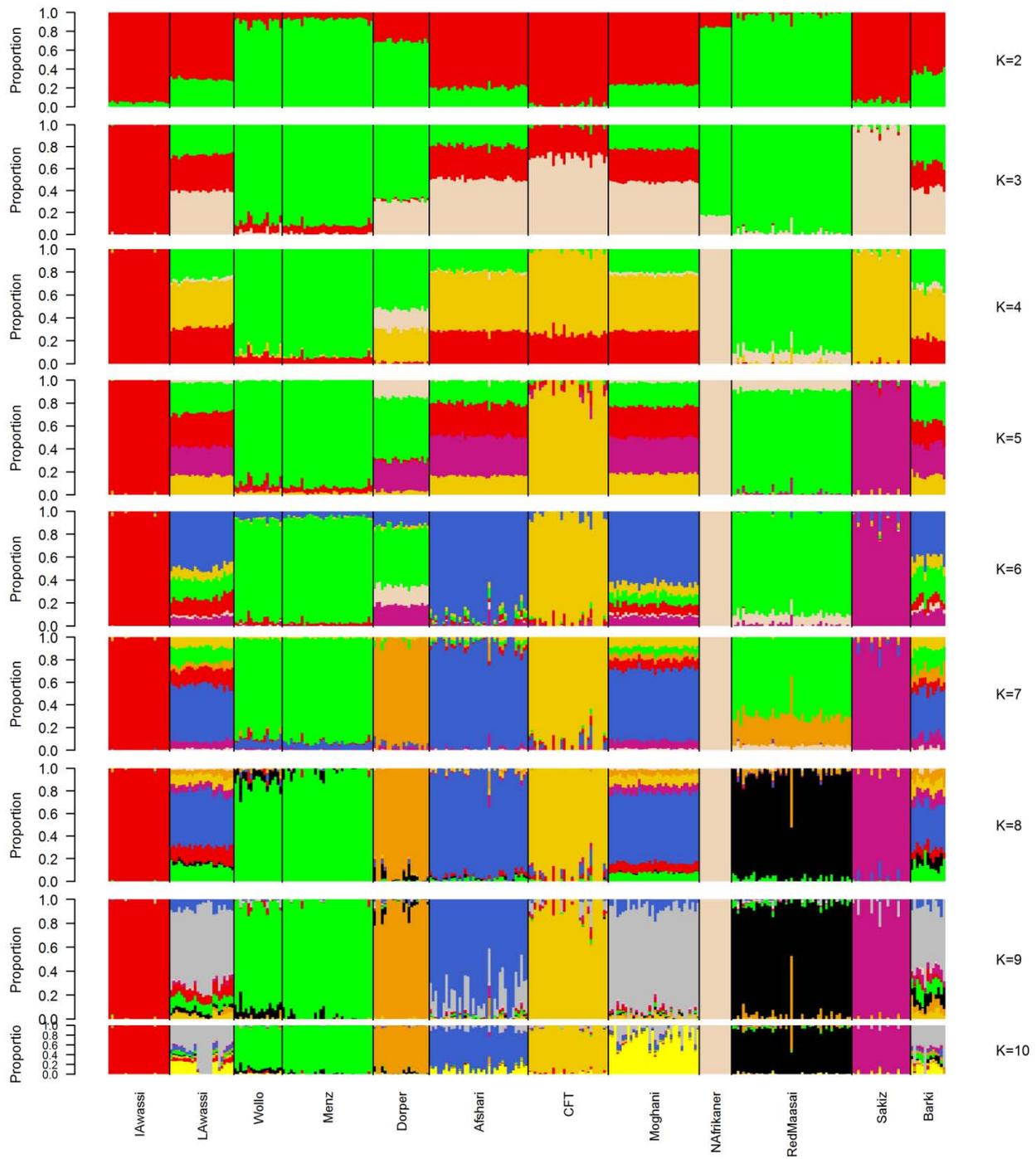
Figure 11. Model based admixture analysis of African, Awassi, Asian and European sheep breeds considering *K*=2 to 10 ancestral population. Breeds are separated by black lines. Each individual represented by a vertical bar and partitioned in to colored segments representing the proportion of each ancestors. IAwassi=improved Awassi, LAwassi=local Awassi and NAfrikaner=Namaque Afrikaner.
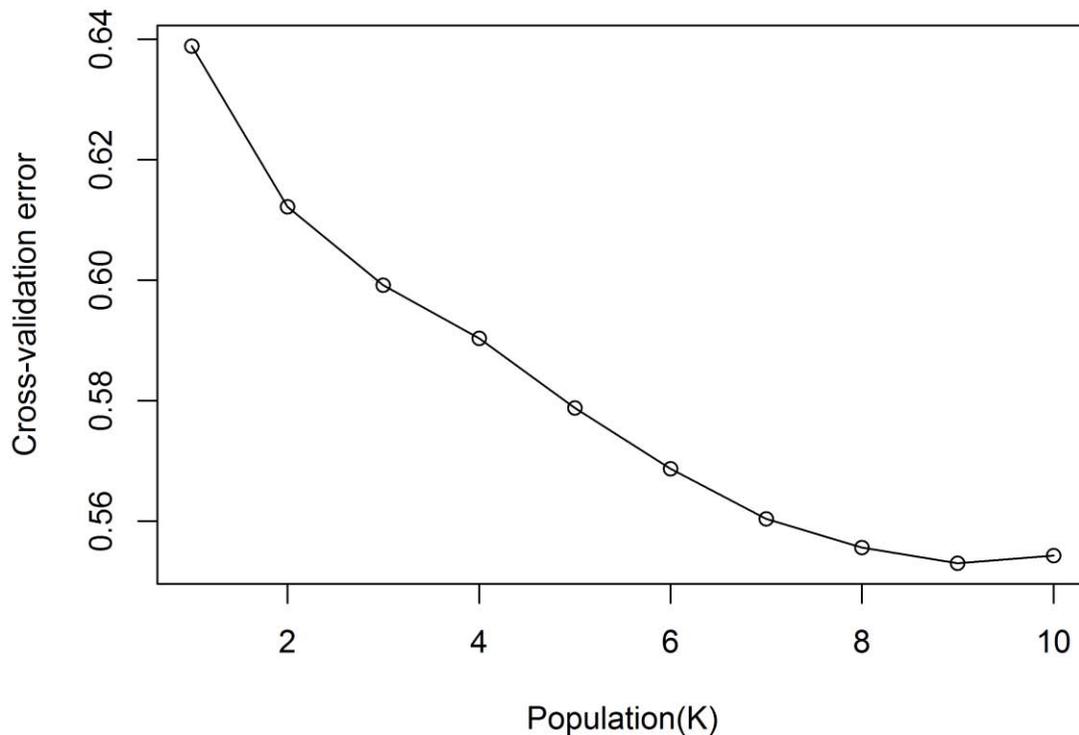
Figure 12. Cross validation error curve from *K*=1 to 10 of African, Awassi, Asian and European sheep breeds.

Considering Ethiopian breeds as reference in data set 3, Local Awassi, Barki and Moghani clustered in to a single group showed similar level of genetic admixture from other groups with larger proportion from Asian and lesser proportion from African and Improved Awassi until *K*=9. Ethiopian Menz and Wollo population are going together up to *K*=10 having low level of admixture from different breeds mainly from Red Maasai and Awassi. Comparing the two Ethiopian sheep breeds at *K*=8, level of admixture is relatively higher in Wollo (14%) compared to Menz (2.5%). The major proportion of admixture to the Ethiopian breeds was from the geographically nearest Red Maasai sheep with contribution of 8% and 1.7% for Wollo and Menz, respectively. Difference in level of admixture between the two closed and neighboring Ethiopian breed is interesting. More interestingly less admixture found in Menz sheep which is located in relatively closer distance to Red Maasai compared to that of Wollo. Awassi gene introgression was also found in lower proportion in Menz sheep (0.4%) compared to 4.2% in Egyptian Barki and 1.7% in Ethiopian Wollo sheep breed at *K*=9. The observed phenotypic performance, small sized and better adaptation in Menz sheep (Haile et al., 2002; Gizaw et al., 2007; Getachew et al.,

2015b) might be attributed to the breed has been evolved in a more harsher and less feed resource area. The role of selection on shaping the genome architecture of the current indigenous East African cattle also documented (Mbole-Kariuki et al., 2014). Both genetic and phenotypic uniqueness of the Menz breed compared to Wollo might indicate the Menz sheep has a potential to serve as source of valuable genes associated with adaptation traits. Strengthening the ongoing selective breeding and community based breed improvement program of Menz sheep (Gizaw et al., 2011, 2013; Haile et al., 2011), and further studies on finding aiming to find genes associated with adaptation is suggested.

4.1.4 Runs of homozygosity

Total ROH length for the 8 sheep breeds (improved Awassi, local Awassi, Menz, Wollo, Soay, New Zealand Texel, Dorper and Afshari) with different ROH length categories are presented in Figure 13. Among the two local Ethiopian and two Awassi breeds improved Awassi had the highest number and total length of ROH in all categories. Considering all the eight sheep breeds, improved Awassi and the highly inbred Soay showed comparable and highest total length of ROH. However, when ROH length are divided in to categories, Soay breed had the highest ROH length in categories of 1 to 10 Mb where as in the remaining length categories IA had the highest total length of ROH among the all sheep breeds considered in this study (Figure 13).
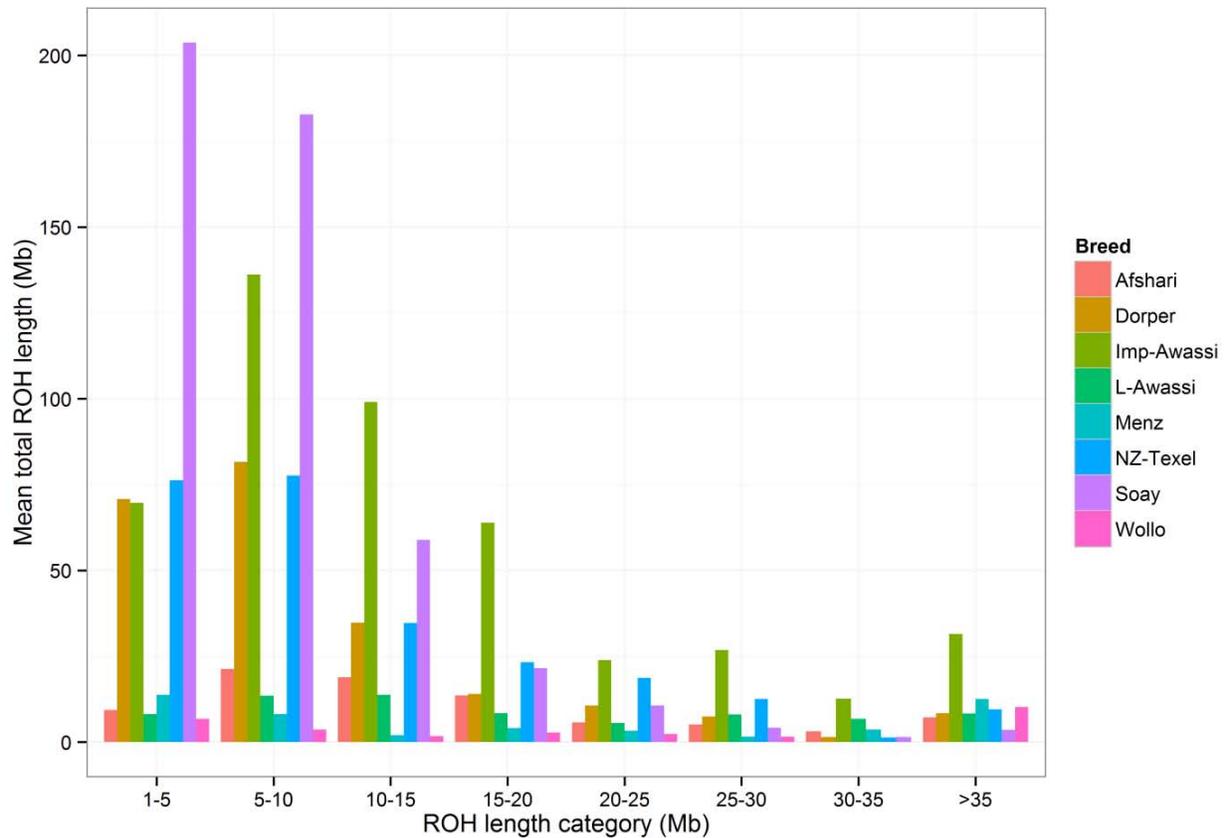
Figure 13. The mean sum of Run of Homozygosity (ROH) per breed in different ROH length category. The sum of ROH was calculated per animal, measured in megabases (Mb) within each ROH length category and averaged per breed.

ROH based on Ovine SNP50K data was used to estimate the level of inbreeding as it provides more accurate levels of inbreeding coefficient than estimating based on allele frequency (Ferenčaković et al., 2012; Curik et al., 2014). The mean, standard deviation (SD), minimum and maximum autosomal $F_{ROH>1\ Mb}$ for the breeds are presented in Table 5. Improved Awassi and Soay breed showed the highest level of inbreeding (total size of homozygous segment) value of 17.0 and 17.7%, encompassing 471.69 and 491.78 Mb of homozygous segment, respectively out of the total autosomal genome length covered by markers (2.78 Gb). Specialized breeds like Dorper and New Zealand Texel also showed quite higher level of inbreeding. As expected local breeds (Afshari, local Awassi, Menz and Wollo) had lower level of inbreeding in the range of 1.06% in Wollo and 3.67% in Menz. The level of inbreeding found for local sheep breeds was lower compared to values reported for cattle (Ferenčaković et al., 2012; Purfield et al., 2012; Curik et al., 2014) indicated higher gene flow among sheep breeds than cattle.

ROH length and number are varied among breeds. It is highly associated with the breed development history. Improvement of Awassi for milk yield resulted in larger number of ROH$_{>1\ Mb}$ (n=1234 with mean total length of 471.69 Mb) compared to local Awassi (n=167 with mean total length of 73.74 Mb). The percentage of SNPs involved in ROH across the genome showed some ROH-based selection signature in some of the breeds (Figure 14). Signature found on chromosome 2 in New Zealand Texel was highly prominent. Similarly, strong peak was observed on chromosome 2 when Texel breed was compared with other breeds using F_{ST} (Kijas et al., 2012).

Table 5. The mean, standard deviation, minimum and maximum autosomal $F_{ROH>1\ Mb}$.

Breed	N	$F_{ROH\ >1\ Mb}$ (%)			
		mean	SD	Minimum	Maximum
Afshari	31	3.51	3.23	0.00	10.71
Dorper	21	8.65	3.42	3.73	19.62
ImpAwassi	23	16.98	3.30	11.71	29.64
Lawassi	24	2.65	4.13	0.00	13.85
Menz	34	3.67	4.18	0.09	22.31
NTexel	24	9.23	3.38	4.91	20.87
Soay	110	17.70	1.93	13.30	23.94
Wollo	18	1.06	2.84	0.00	12.25

N=number of sheep, $F_{ROH>1\ Mb}$=percentage of autosomal genome covered by runs of homozygous, SD=standard deviation.

The highest levels of inbreeding and homozygous segments were observed in Soay and improved Awassi sheep. The highest ROH coverage observed in shorter and longer length category group are mostly indicative of historic and recent inbreeding, respectively. Soay sheep breed has the lowest length of ROH among all breads in longer length categories implied that the current level of inbreeding seems treated well. High amount of ROH in both short and long ROH length categories found in improved Awassi indicated that this breed experienced both recent and ancient inbreeding. Higher amount of ROH at 1 to 5 Mb was also observed in Angus and Herford breeds compared to other (e.g. Holstein) breed (Purfield et al., 2012). Pattern of most ROH coverage in the longer ROH categories was also observed in Holstein Friesian breed having less effective population size and inbreeding problem (Mc Parland et al., 2007). Soay breed also reported

previously as one of the isolated, small effective population size and highly inbred breed (Kijas et al., 2009, 2012).
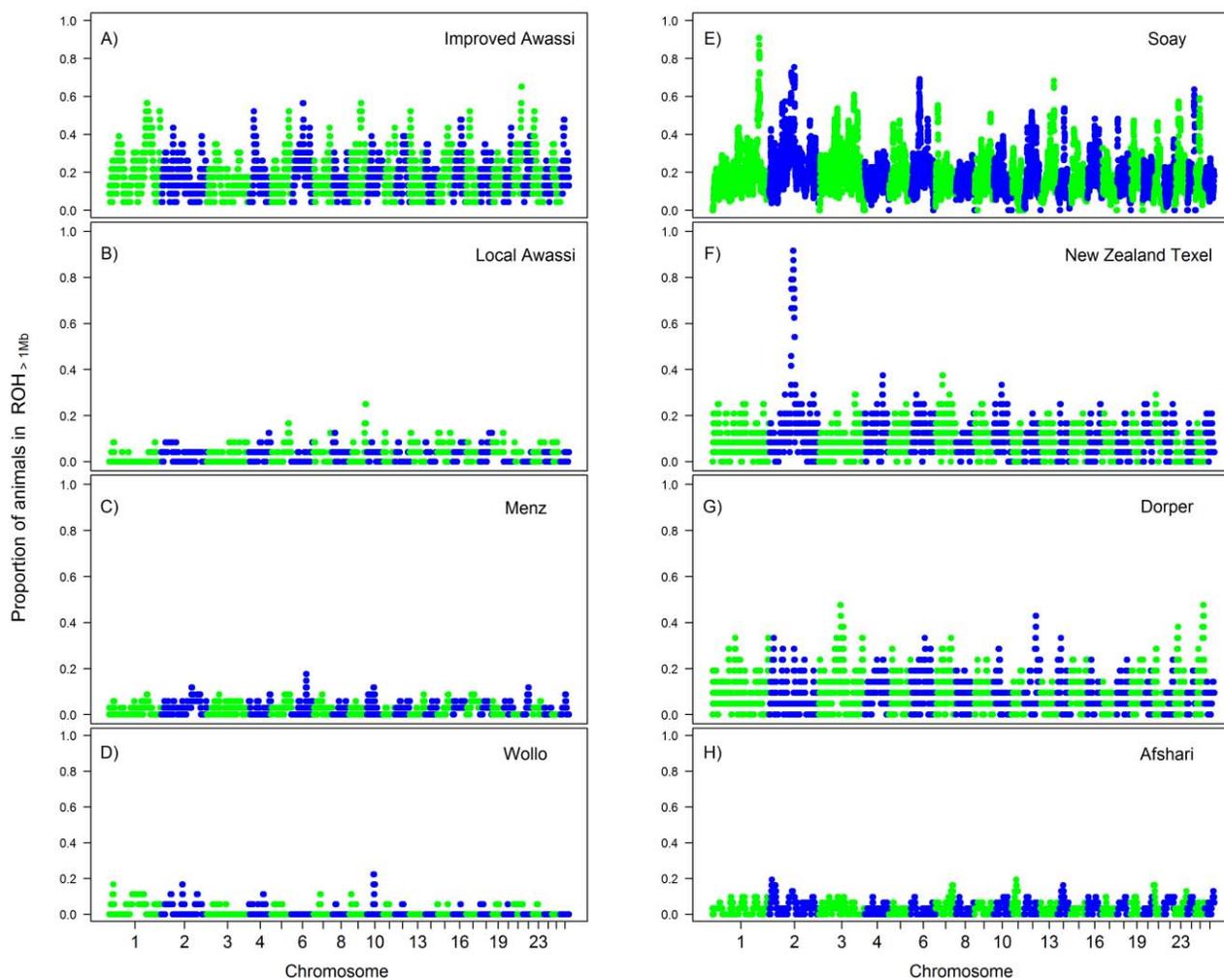
Figure 14. Manhattan plots of proportion of individuals in $F_{ROH>1\ Mb}$ for Improved Awassi (A), local Awassi (B), Menz (C), Wollo (D), Soay (E), New Zealand Texel (F), Dorper (G) and Afshari (H) sheep breeds.

4.2 Ancestry informative markers and estimated level of admixture

4.2.1 Characteristics of Selected markers

A total of 105 SNPs were selected from 50KSNP data based on their F_{ST} and were used to genotype admixed population. The top ranked 105 AIMs were distributed across the genome. The top ranked markers and their detail characteristics are presented in Table 6. A quality control in PLINK v1.9 (Purcell et al., 2007) was removed SNPs with genotype call rate of <0.9 and individuals with missing rate of <0.9. Finally a total of 74 SNPs (serial number 1 to 74) were left and used for the population and individual admixture analysis. Mean, minimum and maximum F_{ST} value of selected top 74 markers was, 0.76, 0.81 and 0.95 respectively.

Table 6. Selected ancestry informative markers. The marker name, chromosomal position, approximate location of the markers on the chromosome in megabases (Kb), two alleles, allele frequency of the first allele for each ancestral population, pairwise FST values and non-missing allele counts for each population are shown.

Ser No	SNP	Chr	SNP set	Position (Kb)	A1	A2	Allele frequency of A1		F_{ST}	N	
							IAW	Eth		IAW	Eth
1	OAR1_19651513.1	1	2	19652	C	A	0.00	0.84	0.81	46	104
2	OAR1_19750493.1	1	3	19750	A	G	0.15	0.92	0.76	46	104
3	OAR1_19817567.1	1	3	19818	G	A	0.02	0.91	0.88	46	104
4	s40404.1	1	2	34229	A	G	0.00	0.82	0.78	46	104
5	OAR1_119469795.1	1	1	119470	G	A	0.14	0.97	0.83	42	104
6	s37703.1	1	3	146952	C	A	0.16	0.97	0.83	44	104
7	OAR1_211426054.1	1	2	211426	G	A	0.09	0.90	0.80	46	104
8	OAR1_215302002.1	1	1	215302	A	G	0.24	0.99	0.78	46	104
9	OAR1_215364467.1	1	2	215364	G	A	0.24	1.00	0.80	46	104
10	OAR2_15631497.1	2	3	15631	A	G	0.11	0.94	0.83	38	104
11	OAR2_28358746.1	2	2	28359	A	G	0.04	0.99	0.95	46	104
12	OAR2_53589838.1	2	1	53590	A	G	0.00	0.85	0.82	46	104
13	s66123.1	2	1	53716	G	A	0.02	0.83	0.78	46	104
14	OAR2_146230561.1	2	1	146231	G	A	0.02	0.83	0.76	46	104
15	OAR2_154444578.1	2	1	154445	G	A	0.17	0.97	0.81	46	104
16	OAR2_154465026.1	2	2	154465	A	C	0.20	0.98	0.80	46	104
17	OAR2_167748075.1	2	3	167748	G	A	0.17	1.00	0.86	46	104
18	OAR2_219083907.1	2	3	219084	A	G	0.13	0.91	0.76	46	104

Ser No	SNP	Chr	SNP set	Position (Kb)	A1	A2	Allele frequency of A1		F_{ST}	N	
							IAW	Eth		IAW	Eth
19	OAR2_235562127.1	2	2	235562	A	G	0.07	0.89	0.81	46	104
20	s39898.1	3	2	14786	G	A	0.15	0.94	0.78	46	104
21	s54627.1	3	2	25815	A	G	0.00	0.83	0.79	46	104
22	OAR3_180522818.1	3	3	180523	A	G	0.09	0.90	0.79	46	104
23	OAR4_37110130.1	4	3	37110	A	G	0.13	0.97	0.84	46	104
24	OAR4_56106365.1	4	3	56106	A	G	0.20	0.99	0.82	46	104
25	s63232.1	5	3	16552	C	A	0.00	0.79	0.76	46	104
26	s04774.1	5	2	48865	A	G	0.07	0.89	0.81	46	104
27	OAR5_56291790.1	5	2	56292	A	G	0.00	0.84	0.80	46	104
28	OAR6_29577816.1	6	3	29578	A	G	0.15	0.95	0.80	46	104
29	OAR6_65652422.1	6	2	65652	G	A	0.24	0.99	0.78	46	104
30	OAR6_84531567.1	6	3	84532	G	A	0.20	0.96	0.77	46	104
31	OAR6_97185219.1	6	3	97185	G	A	0.00	0.83	0.79	46	104
32	OAR6_99879359.1	6	1	99879	A	T	0.07	0.90	0.82	46	104
33	s01880.1	7	3	30761	G	A	0.20	0.97	0.78	46	104
34	OAR7_89553402.1	7	3	89553	A	G	0.04	0.85	0.76	46	104
35	OAR9_20849838.1	9	3	20850	A	G	0.13	0.91	0.76	46	104
36	s61067.1	9	2	37903	A	G	0.13	0.91	0.76	46	104
37	OAR9_57111594.1	9	2	57112	G	A	0.15	1.00	0.87	46	104
38	OAR9_61775166.1	9	1	61775	A	G	0.09	0.96	0.87	46	104
39	OAR10_70905390.1	10	1	70905	A	C	0.17	0.94	0.76	46	104
40	OAR10_91533055.1	10	2	91533	G	A	0.07	0.87	0.76	46	104
41	OAR11_31531641.1	11	3	31532	A	G	0.22	0.99	0.80	46	104
42	s64521.1	11	1	58802	G	A	0.00	0.84	0.80	46	104
43	OAR11_62400735.1	11	3	62401	G	A	0.17	1.00	0.86	46	104
44	OAR12_76153608.1	12	2	76154	G	G	0.00	0.79	0.76	46	104
45	s48052.1	13	1	3579	C	A	0.00	0.81	0.77	46	104
46	OAR13_8946629.1	13	2	8947	G	A	0.11	0.89	0.76	46	104
47	OAR13_73857254.1	13	2	73857	G	A	0.17	0.94	0.76	46	104
48	s04113.1	13	3	80524	C	A	0.02	0.85	0.79	46	104
49	OAR13_81410207.1	13	2	81410	G	A	0.00	0.85	0.81	46	104
50	OAR14_1314896_X.1	14	3	1315	G	A	0.13	0.93	0.79	46	104
51	s24630.1	14	2	2723	A	G	0.02	0.92	0.88	46	104
52	s03339.1	14	3	7903	A	G	0.00	0.87	0.84	46	104
53	s37862.1	16	1	1386	G	A	0.09	0.90	0.80	46	104

Ser No	SNP	Chr	SNP set	Position (Kb)	A1	A2	Allele frequency of A1		F_{ST}	N	
							IAW	Eth		IAW	Eth
54	s13627.1	16	3	29101	A	G	0.00	0.86	0.82	46	104
55	OAR16_32866225.1	16	1	32866	A	G	0.13	0.93	0.79	46	104
56	OAR16_49974943.1	16	1	49975	G	A	0.07	0.88	0.79	46	104
57	s33640.1	16	2	67333	G	A	0.00	0.86	0.83	46	104
58	s08479.1	17	2	58921	A	G	0.00	0.93	0.92	46	104
59	OAR17_64750846.1	17	2	64751	A	G	0.00	0.85	0.82	46	104
60	s25024.1	17	3	66592	G	A	0.05	0.97	0.93	44	104
61	OAR18_62319441.1	18	2	62319	A	G	0.15	0.97	0.83	46	104
62	OAR20_7267196.1	20	1	7267	A	C	0.00	0.92	0.91	46	104
63	OAR21_6579878.1	21	3	6580	A	G	0.15	0.98	0.84	46	104
64	s52246.1	21	2	33311	A	G	0.15	0.93	0.77	46	104
65	OAR21_45614480.1	21	3	45614	A	G	0.16	0.96	0.80	44	104
66	OAR22_26079325.1	22	2	26079	C	A	0.04	0.90	0.84	46	104
67	OAR23_5044297.1	23	1	5044	G	A	0.00	0.86	0.83	46	104
68	s46432.1	23	1	52347	A	C	0.07	0.90	0.81	46	104
69	OAR24_8950654.1	24	2	8951	G	A	0.15	0.93	0.77	46	104
70	s29758.1	25	2	2868	C	A	0.02	0.91	0.87	46	104
71	OAR25_19228950.1	25	1	19229	A	G	0.15	0.94	0.78	46	104
72	s10741.1	25	1	44633	G	A	0.26	0.99	0.76	46	104
73	OAR26_27745939.1	26	3	27746	G	A	0.24	0.99	0.78	46	104
74	OAR26_33341119.1	26	1	33341	G	A	0.09	0.91	0.81	46	104
75	OAR1_78868061.1	1	1	78868	G	A	0.04	0.92	0.86	46	104
76	OAR1_204671262.1	1	1	204671	A	G	0.07	0.95	0.88	46	104
77	OAR1_204686791.1	1	3	204687	A	G	0.07	0.98	0.92	46	104
78	OAR2_39209186.1	2	1	39209	A	G	0.07	0.88	0.77	46	104
79	OAR2_141221105.1	2	2	141221	A	G	0.11	0.98	0.88	46	104
80	OAR2_146458539.1	2	1	146459	G	A	0.09	0.88	0.76	46	104
81	OAR3_66722206.1	3	2	66722	A	G	0.00	0.79	0.75	46	104
82	OAR3_142190582.1	3	1	142191	C	A	0.02	0.85	0.79	46	104
83	OAR3_183826897.1	3	1	183827	G	A	0.02	0.83	0.77	46	104
84	OAR4_53537262.1	4	2	53537	C	A	0.02	0.82	0.76	46	104
85	OAR5_113136843.1	5	3	113137	G	A	0.00	0.84	0.80	46	104
86	OAR6_19690807.1	6	1	19691	G	A	0.11	0.92	0.80	46	104
87	OAR7_83073383.1	7	1	83073	G	A	0.04	0.88	0.80	46	104
88	OAR7_95938778.1	7	2	95939	A	G	0.09	0.93	0.83	46	104

Ser No	SNP	Chr	SNP set	Position (Kb)	A1	A2	Allele frequency of A1		F_{ST}	N	
							IAW	Eth		IAW	Eth
89	OAR9_56685157.1	9	1	56685	G	A	0.15	0.95	0.80	46	104
90	s42079.1	9	1	58507	A	G	0.15	0.96	0.81	46	104
91	s31237.1	9	3	78129	A	G	0.09	0.96	0.87	46	104
92	OAR10_64481741.1	10	3	64482	A	G	0.15	0.97	0.83	46	104
93	s70144.1	11	1	8373	G	A	0.26	0.99	0.76	46	104
94	OAR11_48144420.1	11	1	48144	G	A	0.15	0.92	0.76	46	104
95	s75385.1	11	2	61193	A	G	0.24	1.00	0.80	46	104
96	s67736.1	12	2	31541	A	G	0.09	0.90	0.79	46	104
97	OAR12_83564723.1	12	3	83565	G	A	0.13	0.93	0.80	46	104
98	s69860.1	14	1	960	A	G	0.02	0.91	0.87	46	104
99	OAR14_4424829.1	14	2	4425	A	G	0.02	0.85	0.79	46	104
100	OAR14_50148641.1	14	2	50149	G	A	0.09	0.90	0.79	46	104
101	s05017.1	15	1	90028	A	G	0.00	0.94	0.93	46	104
102	OAR17_9906615.1	17	3	9907	G	A	0.11	0.94	0.82	46	104
103	OAR17_64771249.1	17	1	64771	A	C	0.00	0.85	0.82	46	104
104	OAR20_51262941.1	20	1	51263	G	A	0.09	0.88	0.76	46	104
105	DU481531_204.1	26	3	6467	A	G	0.04	0.97	0.92	46	104

Chr = chromosome, N = number of observations, IAW = improved Awassi, Eth = Ethiopian fat-tailed breed.

4.2.2 Correlation between pedigree information and genome estimated admixture levels

Mean, standard deviation (SD), minimum and maximum values of the Awassi level estimated based on 74 SNPs are presented for each category of Awassi level obtained from pedigree information (Table 7). The admixture estimate for pure Awassi was very close to the pedigree information compared to the estimate for 75% and 50% Awassi crossbreds. Individual admixture levels were estimated more accurately based on the genomic data using panels of pure reference animals than estimated based on pedigree alone (Sölkner et al., 2010). This is because pedigree information are calculated with the assumption of each offspring inherited half of the genes from each parent. However this might not be always true due to the effect of recombination of parental chromosomes in the process of crossbreeding (Sölkner et al., 2010). Furthermore man-made pedigree recording errors would create additional bias from the expected values both under small scale farmer and large scale ranches (Gorbach et al., 2010). When the selected markers are

informative, the observed highest precision in the pure Awassi population is expected as the effect of recombination and recording error is minimal for the pure population.

Correlation coefficient (r) between Awassi level based on pedigree information and admixture estimates from 74 SNP data was very high (r = 0.98). This value was slightly higher compared to the correlation value of 0.96 obtained from ~500 AIMs suggested to predict breed composition in cattle (Frkonja et al., 2012). This was also in agreement with the R^2 values in the range of 0.89 to 0.96 reported for different human population in prediction of admixture level using selected AIMs (Halder et al., 2008). This result confirmed that the selected 74 AIMs were excellent estimator of the admixture levels in the Awassi x local crossbred populations.

Table 7. Mean, standard deviation (SD), minimum and maximum values of the Awassi level estimated based on 74 SNPs for each category of pedigree admixture level.

Awassi level from Pedigree	N	Source	Estimated from 74 SNPs			
			Mean	SD	Min	Max
50%	18	DBARC	46.04	3.54	40.66	51.86
75%	17	AGSBMC	71.22	4.61	60.87	78.56
100%	17	AGSBMC	99.31	1.02	97.06	100.00

N=number of observations, SD=standard deviation, DBARC=Debre Berhan Agricultural Research Center, AGSBMC = Amed Guya Sheep Breeding and Multiplication Center.

4.2.3   Population and individual admixture levels of crossbreds

4.2.3.1   Effect of location

Frequency distribution of Awassi level in Negasi-Amba and Chiro villages are presented in Figure 15. Significant, however variable proportion of Awassi genotype has been produced in the two locations. The proportion as well as range of Awassi level was higher in Chiro village compared to the Negasi-Amba village. Distribution was also more skewed to the left (towards the local breeds) in Negasi-Amba compared to Chiro. The current mean±standard deviation (SD) proportion of Awassi level in Chiro sheep flocks was 21.1±14.71 and 27.5±17.13% for ewes and lamb, respectively. Whereas, in Negasi-Amba the proportion of Awassi level was much lower with corresponding values of 11.0±0.53 and 9.0±7.36% for ewes and lambs, respectively. Proportion of Awassi has been at increasing trend in Chiro site as was evident from the proportion of Awassi level being higher in replacement animals (lambs) than in ewes (Figure 16). However, in Negasi-

Amba the Awassi level looks static or in decreasing trend. The population MAFTA_ewe (NegAmb) in Negasi-Amba represents crossbred ewes before 3 years in that village (Figure 16). The current Awassi level in Negasi-Amba ewes was similar to that of the Awassi level before 3 years.
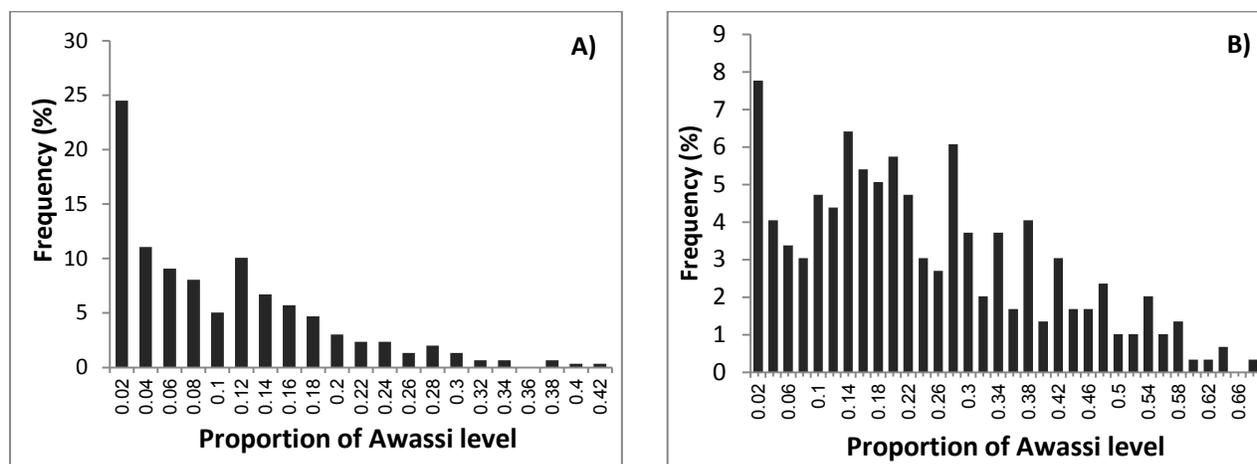
Figure 15. Frequency distribution of Awassi level in Negasi-Amba (A) Chiro (B) village.

Unsupervised and supervised admixture plot of crossbred populations for the crossbred and pure population from different location are presented in Figure 16A and B, respectively. Some level of Awassi introgression was observed in pure Ethiopian sheep population collected from three different locations. The Awassi introgression was higher in Wollo sheep breed (4.0%) than Menz sheep breed collected from two different locations in the range of 1.2-1.3%.This might result from the introduction of Awassi sheep at the beginning of 1980's or admixture analysis failing to separate the two ancestral breeds due to historic admixture of the breeds. The effect of this Awassi introgression on the individual admixture level estimate was tested by implementing supervised analysis. In supervised analysis ancestral populations are set to be completely divergent from each other where as in unsupervised analysis the ancestry proportion was estimated without any additional information (Alexander et al., 2009). Scatter plots of individual admixture levels estimated from supervised vs. unsupervised are presented in Figure 17. The correlation between the individual ancestry fraction estimated by supervised and unsupervised analysis was very high (r = 0.999). Fitted linear regression line of supervised on unsupervised (blue line) was perfectly matches with the diagonal line (red color). Observations deviated from the diagonal lines at lower and upper end was for Ethiopian local breeds set to proportion of 0 Awassi and Improved Awassi set to 1 Awassi level, respectively in supervised mode.
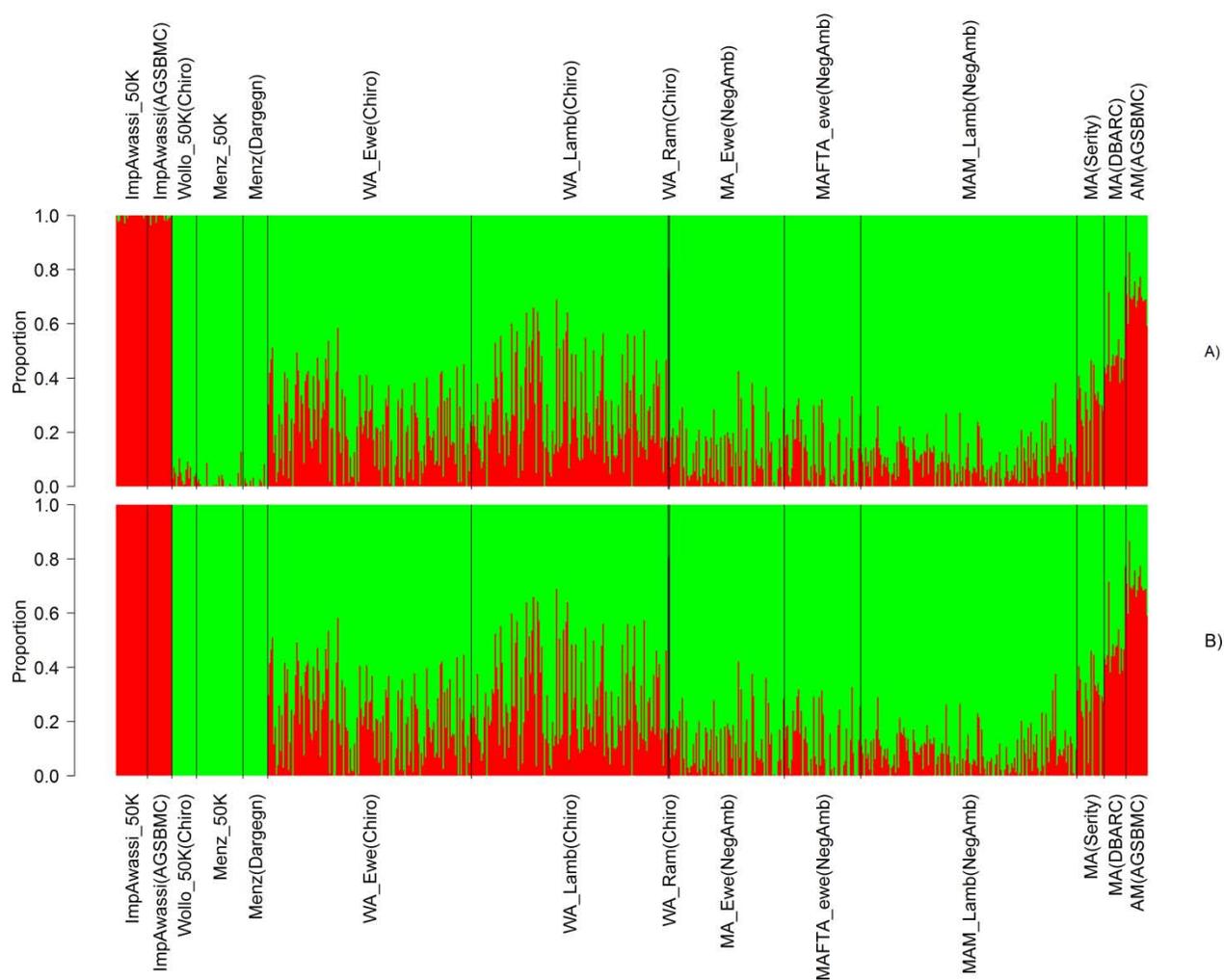
Figure 16. Unsupervised (A) and supervised (B) admixture plot of crossbred populations at *K*=2 estimated for each animal, individuals were represented by vertical line divided in to 2 colors, red color indicated the proportion of Awassi and green color for Ethiopian proportion. Each population was separated by black line and name of the populations are indicated at the bottom and top of the plot. Breed or population name and location in bracket were indicated. WA=Wollo x Awassi crossbred, MAFTA=Menz Awassi crossbred and the sample were collected 3 years before the others using FTA cards, MA=Wollo x Awassi crossbred population, DBARC=Debre Berhan Agricultural Research center, AGSBMC=Amed Guya Sheep Breeding and Multiplication Center.
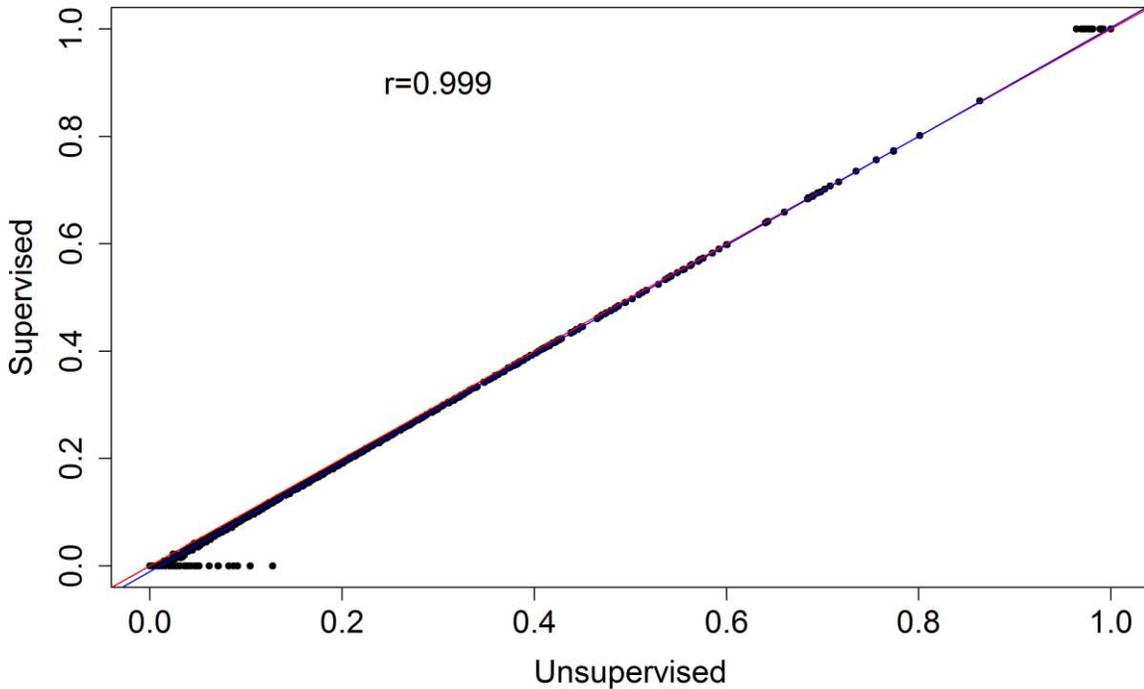
Figure 17. Scatter plots of individual admixture levels estimated from supervised vs. unsupervised. Blue line represents fitted linear regression line of supervised on unsupervised and red color represents the diagonal line when x=y.

4.2.3.2 *Effect of farmers*

Proportion of Awassi level and total number of sheep were highly varied among farmers in both Negasi-Amba and Chiro villages (Figure 18). A total of 37 (in Chiro) and 47 (in Negasi-Amba) farmers having at least 3 sheep were considered to describe proportion of Awassi level by farmer. Factors associated to variation among farmers need to be investigated however it might be associated with sheep flock size, resource of farmers to keep bigger crossbred animals, level of participation, interest of farmers, input access, variation in knowledge among farmers (Adugna, 2004; Basunathe et al., 2010; Mekonnen et al., 2010; Pequeños et al., 2013; Dehinenet et al., 2014). About 27% farmer in Chiro had Awassi level of above 30% in their flocks. Such farmers would have better potential to be upgraded towards breeding ram multiplier with little support from the government. This would share the load of government farms engaged in ram multiplication. Definitely this would be cost effective and more efficient than government farms as government farms has been suffered from budget constraint, lack of facilities and higher risk of disease outbreak associated with confinement. Ahuya et al., (2005) also reported the previous government

approach based on multiplication and dissemination of exotic bucks from government farms failed to bring anticipated change in Kenyan goat crossbreeding. Subsequently, the non-governmental organizations, German Development Cooperation (GIZ) and FARM-Africa initiated a community approach which was led by farmers and became more successful in significantly improving the livelihoods of resource poor families in Kenya (Peacock et al., 2011).
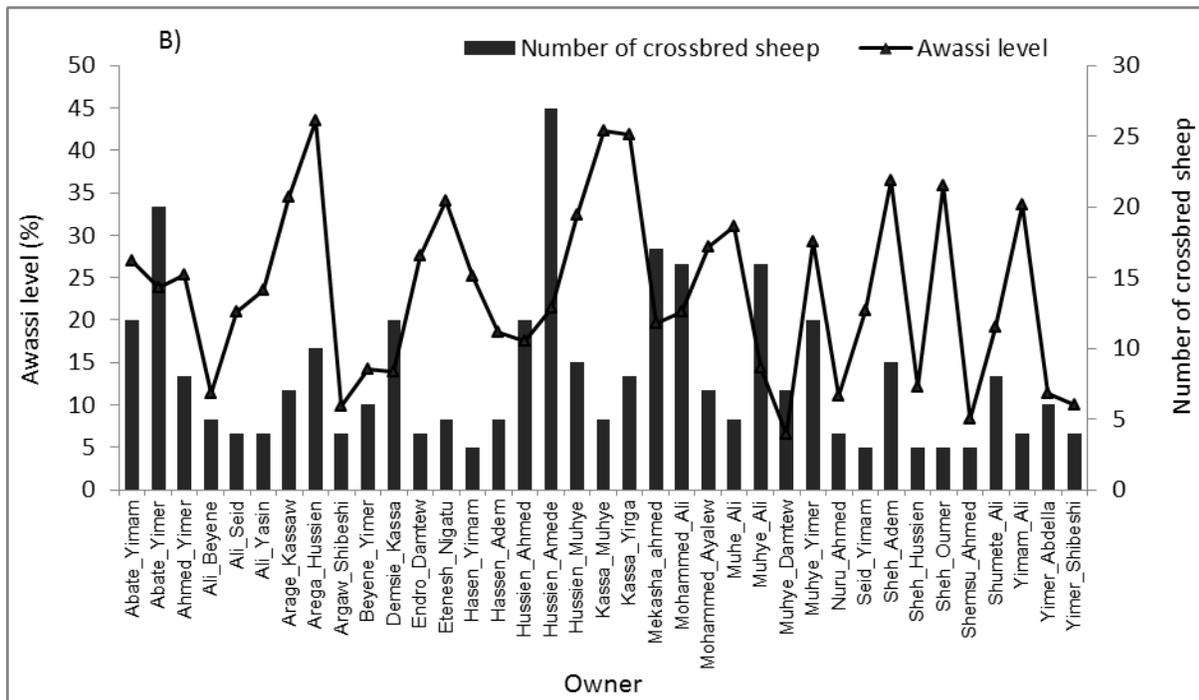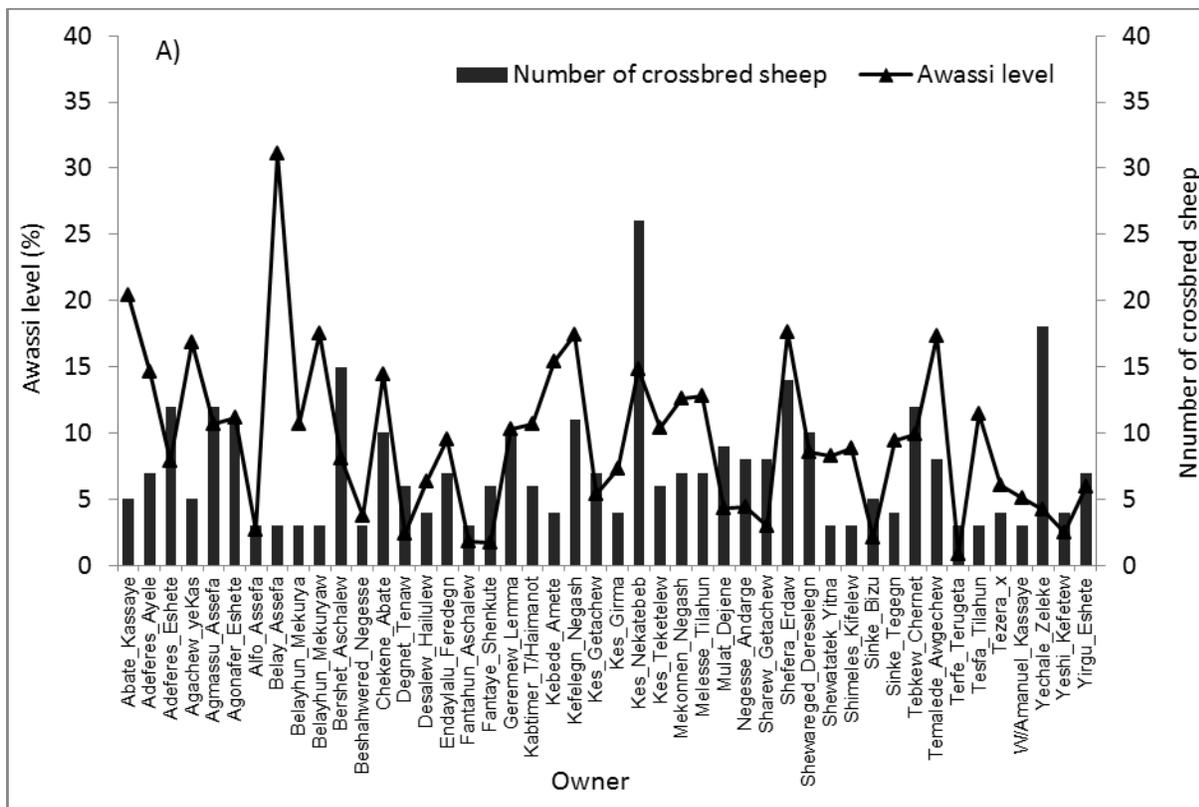
Figure 18. Mean Awassi level and total number of crossbred sheep produced by farmers in Negasi-Amba (A) and Chiro (B) villages. Bar plots are indicated the total number of crossbred sheep by an owner and line plots showed the average level of Awassi (%).

4.2.4 Estimation of ancestral contribution in admixed populations using different subsets of AIMs

Spearman's rank correlation between the Awassi levels estimated using top F_{ST} ranked 74 AIMs and subsets of top 65, 55, 45, 35, 25 and 15 AIMs are presented in Figure 19. Strong correlation coefficient (r) values were found in the range of 0.996 to 0.939. The values decreased as the number of AIMs in the subset decreased. Among the SNP subsets the lowest root mean square error (RMSE) was achieved with 65 SNPs (0.013) and increased to 0.020, 0.028, 0.037, 0.050 and 0.067 with top ranked 55, 45, 35, 25 and 15, respectively. Generally quite low RMSE were obtained in this study, which were lower than the values reported 0.09 and 0.182 when the ancestry estimate based on top 20 and 50 AIMs compared with the true ancestry, respectively (Ding et al., 2011), and comparable with RMSE=0.026 reported based on estimate from 105 AIMs and pedigree information (Halder et al., 2008) in human. All subsets of SNPs in this study provided consistent and reproducible results even though the top 45, 55, 65 SNPs yielded very similar estimates of ancestry compared to the top 74 AIMs as evidenced by strong correlations and lower RMSEs. The departure between the calculated and expected admixture proportion was also very minimal in the first 3 SNP subsets (Table 8). Each subset was compared with the top 74 SNPs having mean±SE value of 0.222±0.0092. All SNP subsets had produced similar estimate (P>0.05) with the mean value estimated based on the 74 SNPs. Thus about 45 top ranked AIMs selected based on F_{ST} were good enough for accurate estimation of the level of ancestry in the Awassi crossbred population. The number of markers required to estimate the level of admixture was low when compared with ~500 top F_{ST} ranked SNPs required to estimate the admixture level of Swiss Fleckvieh breed Frkonja et al., (2012).This was due to the Ethiopian sheep breed and Improved Awassi being genetically more divergent compared when to the divergence between the ancestral populations (Simmental and Red Holstein Friesian) of Swiss Fleckvieh breed as evident from their F_{ST} values. F_{ST} values were in the range of 0.81-0.95 for 105 SNPs in this study while F_{ST} values were much lower in the cattle ranging 0.623-0.783 for the top 98 SNPs. It was also reported that more AIMs were required to correctly differentiate individuals from less genetically divergent human populations than individuals from more genetically divergent population (Ding et al., 2011). Ding et al., (2011) suggested as few as top 20 ranked SNPs for accurate classification of ancestral population in human where the maximum F_{ST} value was 0.956. The number of markers required for population assignment depends on the population under consideration. In many developing countries, livestock crossbreeding has been implemented with poor or no pedigree recording. Thus this would provide a great opportunity to estimate the level of admixture in a cost effective way. Currently price per SNP is in the range of about €0.04 to 0.15; highly

dependent on the method and number of samples to be genotyped at a time. During the field visit during data collection many farmers showed interest to pay for the breed composition information. Such information particularly in Chiro site has been important to sell breeding rams to surrounding farmers as well as other customers coming from different parts of the country.

Table 8. Mean Awassi level estimated by different subset of top ranked SNPs and t-test results comparing each subset with the reference top 74 selected SNPs.

Subset of AIMs	N	Observed mean±SE	P-value
Top 65 SNPs	755	0.224±0.0092	0.891
Top 55 SNPs	755	0.222±0.0092	0.984
Top 45 SNPs	755	0.224±0.0092	0.906
Top 35 SNPs	755	0.235±0.0092	0.314
Top 25 SNPs	755	0.240±0.0094	0.222
Top 15 SNPs	755	0.239±0.0093	0.207

Each subset was compared with the top 74 SNPs having mean±SE value of 0.222±0.0092, P value of t-test indicate non-significant difference in mean, SE=standard error.
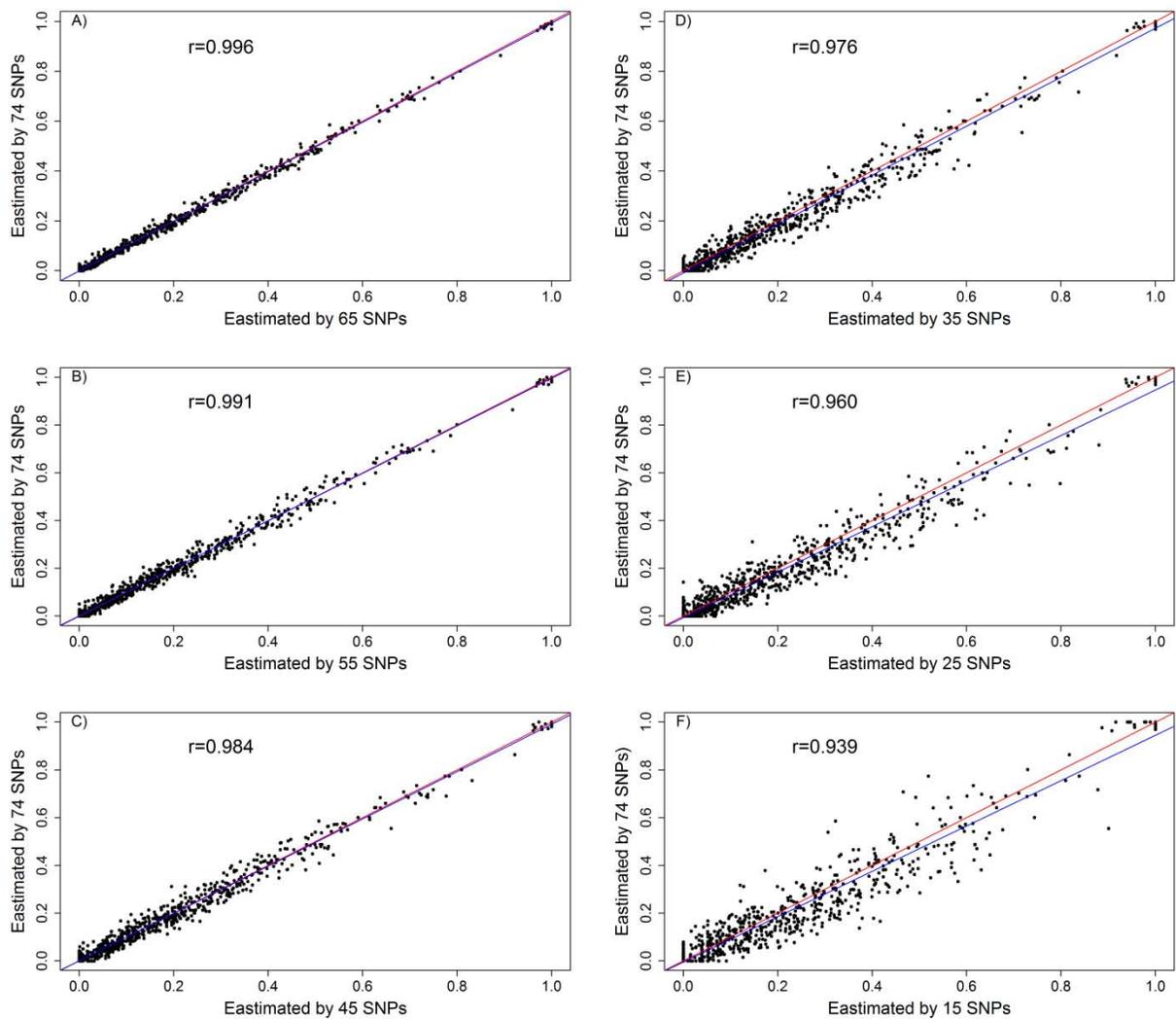
**Figure 19.** Correlation coefficient (r) values and scatter plot of individual Awassi level estimated by top 74 SNPs vs. individual Awassi level estimated by top 65, 55, 45, 35, 25, and 15 SNPs. In each plot the blue line is the linear regression line of individual Awassi level estimated by 74 SNPs on estimated by different subsets, and red line is the diagonal line with perfect linear relationship.

4.3 Performances of crossbreds

4.3.1 Lamb growth performance

The performances of Wollo-Awassi and Menz-Awassi composites having different Awassi blood levels were collected from the two crossbreeding villages (Negasi-Amba and Chiro). As indicated in the materials and methods, the Awassi levels were classified in to six groups; pure local (group 1) different Awassi admixture levels of <12.5% (group 2), 12.5 to 25% (group 3), 25 to 37.5% (group 4), 37.5 to 50% (group 5) and >50% (group 6). Fitting location by breed analysis was not appropriate as most of the lambs in Menz location were below 25% Awassi level. Thus regardless of Awassi level group, location and lamb sex fitted in the model and both significantly ($P<0.05$) affected lamb weight at eight months of age. Lambs in Chiro site were significantly heavier at eight months than lambs in Negasi-Amba and males were heavier than females.

Least square means±standard errors of crossbred populations with different Awassi levels and sex in the two locations are presented in Table 9. Awassi level significantly affected lamb eight months weight in both locations whereas the sex effect was significant in Chiro village only. The result showed that pure local lambs in both locations had similar live weight at eight months. In Negasi-Amba site, pure Menz sheep were lighter ($P<0.05$) at eight months weight compared to group 2 and 3 whereas similar ($P>0.05$) to higher level of Awassi (group 4) in that particular location. In Chiro site, lamb eight months weight was increased as the Awassi level increased up to 50%. Lambs with Awassi level of above 50% were not different ($P>0.05$) from the previous group (37.5 to 50% Awassi level). Male lambs were heavier ($P<0.05$) than females in Chiro site whereas the same ($P>0.05$) in Negasi-Amba. Similar effect of increased live weight with increasing the level of exotic gene was also reported (Hassen et al., 2002, 2004; Pollott and Gootwine, 2004; Gizaw and Getachew, 2009; Gizaw et al., 2012; Teklebrhan et al., 2014). Effect of location and interaction of breed by location on live weight of crossbred were also reported in a Kenyan Dorper with Red Maasai crossbreeding study (Zonabend et al., 2014).

Subjectively assessed body condition scores, determined on the live animal, can provide an acceptable and useful estimate of the proportion of fat in the live animal. Body condition scores for the crossbred population by Awassi level in Negasi-Amba and Chiro are presented in Figure 20. The results were consistent with eight months weight. The condition of lambs was not improved with increased Awassi level in Negasi-Amba whereas the reverse was true in Chiro village. In Menz, condition score slightly increased for the first crossbred group and then declined whereas in Wollo, lamb body condition was improved significantly as the Awassi level increased. The result

clearly showed the interaction of Awassi level by location. Pure Menz lambs had better body condition score than Wollo lambs at lower Awassi levels. Improvement of lamb growth and body condition in Chiro due to the increase in Awassi level might be associated with the combination both relatively better environment and farmers favored management for higher Awassi levels. Attractive price and increasing demand for higher Awassi level for breeding purpose particularly in Chiro site (Gizaw and Getachew, 2009; Teferra et al., 2014) also inspired farmers to provide better management for the crossbreds.

Table 9. Least square means±standard errors of crossbred population by different Awassi level groups and sex in Negasi-Amba and Chiro villages.

Class	Negasi-Amba		Chiro	
	N	LSM±SE	N	LSM±SE
Awassi level		*		***
0	62	15.2±0.42[a]	9	15.1±1.70 [a]
<12.5%	63	16.8±0.41[b]	22	17.8±1.08[a]
12.5 to 25%	35	17.1±0.55[b]	46	19.1±0.75[b]
25 to 37.5%	4	17.0±1.65[b,a]	30	21.7±0.93[c]
37.5 to 50%	-	-	18	24.8±1.20[d]
>50%	-	-	15	24.0±1.31[d]
Sex		ns		*
Male	86	16.9±0.55	66	21.6±0.66
Female	78	16.2±0.50	74	19.3±0.64

*** Significant at P=0.001, *significant at P=0.05, ns=non-significant at P=0.05, N=number of observations, LSM=least square means, SE=standard error.

The effect of Awassi crossbreeding on lamb growth was clearly observed in both locations attaining the overall crossbred population weights 16.8 and 20.7 Kg at eight months age in Negasi-Amba and Chiro, respectively (Table 10), which was better than the yearling weight of local Menz sheep reported 15.7 to 17.4 kg (Tibbo et al., 2005; Getachew et al., 2009; Gizaw et al., 2008a; Gizaw et al., 2008b). Advantage of Awassi crossbreeding was more noticeable in Chiro site. The eight months weight of 37.5 to 50% Awassi in Chiro (24.8 kg) was superior to the 75% Awassi reported 23.5 kg from station management at yearling age (Tibbo et al., 2005). It was also higher than that of indigenous breeds known as fast growing Washera sheep reported 21 kg at 9

months (Getachew et al., 2011; Mekuriaw et al., 2013), Horro sheep reported 17.8kg at 8 months (Abegaz et al., 2005).
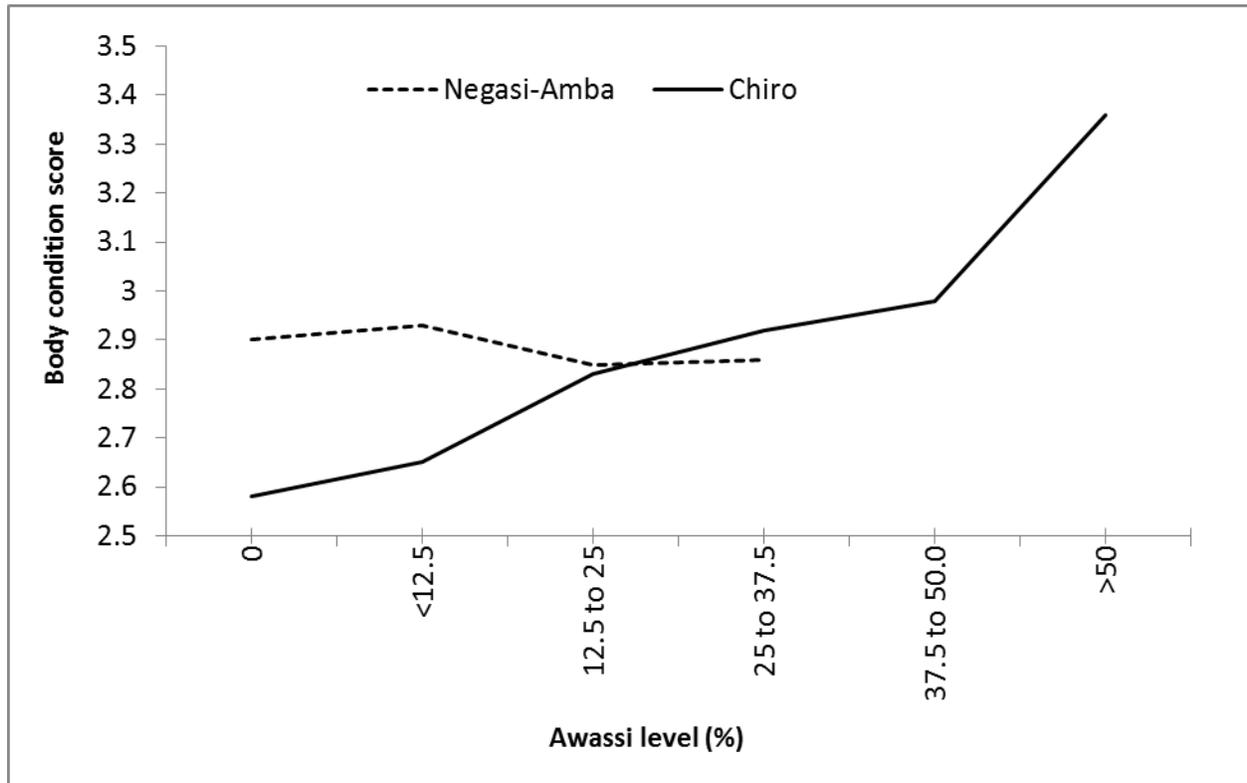
Figure 20. Least square means of body condition score for lambs having different Awassi level in Negasi Amba (Menz) and Chiro (Wollo) site

4.3.2 Lambing interval and number of lambs weaned

The combined analysis fitting location as fixed variable exhibited that location had no effect on lambing interval (LI) and number of lambs weaned ewe[-1] year^{-1} (result not presented here). Within location analysis for lambing interval, number of lambs weaned ewe[-1] year^{-1} (NLWEY) and ewe body condition score (BC) at time of measurement are presented in Table 10. In both locations, the effect of Awassi admixture on LI was significant ($P<0.05$) however not significant ($P>0.05$) for NLWEY and BC score. Lambing interval was longer as the Awassi level increased in both locations. Number of lambs weaned ewe[-1] year^{-1} also showed a decreasing trend with increased Awassi level however it was not significant. Similar to this study, a range of comparable to inferior performances were reported for age at first lambing, lambing interval and number of lambs born ewe[-1] year^{-1} for Awassi crossbred ewes (Olsson and Beyene 1990; Demeke et al. 1995; Getachew et al., 2013).

Table 10. Least square means±standard errors of lambing interval, number of lambs weaned ewe^{-1} year^{-1} and body condition score for the effect of Awassi level groups and sex in each of Negasi-Amba and Chiro sites.

Location/Awassi level	LI		NLWEY		BC	
	N	LSM±SE	N	LSM±SE	N	LSM±SE
Negasi-Amba		*		ns		ns
0	46	262±9.7[a]	34	1.25±0.06	28	2.5±0.12
<12.5%	28	290±12.5[a,b]	21	1.10±0.07	20	2.6±0.15
12.5 to 25%	42	298±10.2[b]	33	1.20±0.06	24	2.4±0.13
25 to 37.5%	10	303±20.9[a,b]	9	1.18±0.11	23	2.6±0.18
37.5 to 50%	-	-	-	-	-	-
Chiro		*		ns		ns
0	27	283±13.7[a]	27	1.24±0.07	14	2.9±0.15
<12.5%	20	280±16.0[a]	20	1.26±0.09	12	2.4±0.16
12.5 to 25%	38	297±11.6[a,b]	38	1.18±0.07	33	2.4±0.10
25 to 37.5%	36	305±11.9[a,b]	36	1.19±0.07	32	2.4±0.10
37.5 to 50%	18	334±16.8[b]	18	1.11±0.09	14	2.9±0.15

*significant at P=0.05, ns = non-significant at P=0.05, BC=body condition score, LI=lambing interval, NLWEY=number of lambs weaned ewe^{-1} year^{-1}, N=number of observations, LSM=least square means, SE=standard error.

4.3.3 Association of extreme performances of lamb growth and ewe reproduction on Awassi level

Higher within population variations in lamb growth and ewe reproductive performances were created due to crossbreeding in both locations. Weight of best performing 13% cohort lambs at 8 months of age was 5.9 and 10.4Kg higher than the population mean in Negasi-Amba and Chiro, respectively (Table 11). Similarly in ewes, the difference between best performing (15% Chiro and 23% in Negasi-Amba) and population average in lambing interval was about 2 to 3 months. In both locations best performance of lambs was associated with the level of Awassi however it was not significant (in the range of 6.8 to 10.1% Awassi level) in Negasi-Amba. However it was highly significant (P<0.001) in Chiro village in which best performing lambs had an average Awassi level of 37.1%, while the medium and worst performing lambs had 25.2% and 17.7% Awassi level,

respectively. Reproductive performances were not associated (*P*>0.05) with Awassi levels in both locations (Table 12).

The higher genetic variability of lamb growth in crossbred population particularly in Chiro village could be considered as an immense potential for improvement of productivity. Genetic improvement applying efficient selection within the crossbred population along with management improvement should be considered which progressively leads to the development of a composite population. Imposing selection on the parental breeds in the sheep breeding and multiplication centers based on the information from the pure lines and crossbreds might also be considered to maximize the benefit (Bijma and Arendonk, 1998).

Table 11. Least square means±standard errors of eight months weight, body condition score (BC) and Awassi level for top ranked and poor performing.

Performance level	Negasi-Amba				Chiro			
	N	8 months weight (kg)	BC	Awassi level (%)	N	8 months weight (kg)	BC	Awassi level (%)
		***	***	ns		***	***	***
Top	22	22.7 ± 0.37^a	3.0 ± 0.09^a	10.1 ± 1.50	19	30.6 ± 0.84^a	3.2 ± 0.11^a	37.1 ± 3.51^a
Medium	121	16.1 ± 0.16^b	2.9 ± 0.04^a	8.3 ± 0.63	98	19.8 ± 0.35^b	2.9 ± 0.05^b	25.2 ± 0.54^b
Poor	21	11.7 ± 0.37^c	2.6 ± 0.09^b	6.8 ± 1.47	25	13.9 ± 0.78^c	2.6 ± 0.10^c	17.7 ± 0.00^c
Overall	164	16.8 ± 0.18	2.8 ± 0.04	8.4 ± 0.73	142	20.7 ± 0.38	2.9 ± 0.05	26.7 ± 1.62

*** Significant at *P*=0.001, *significant at *P*=0.05, ns=non-significant at *P*=0.05, N=number of observations, LSM=least square means, SE=standard error.

Table 12. Least square mean±standand error of Awassi level and reproductive performances for top medium and worst performing ewes in Negasi-Amba and Chiro sites

Performance level	Negasi-Amba				Chiro			
	N	Awassi level (%)	LI	NLWEY	N	Awassi level (%)	LI	NLWEY
		ns	***	***		ns	***	***
Top	24	9.0±1.97	227±10.1[a]	1.61±0.03[a]	20	15.9±3.1	216±11.6[a]	1.89±0.041[a]
Medium	56	10.6±1.36	283±6.7[b]	1.18±0.02[b]	96	20.3±1.4	301±5.5[b]	1.19±0.020[b]
Poor	24	12.8±1.97	356±10.1[c]	0.77±0.03[c]	22	21.3±2.9	367±11.6[c]	0.69±0.041[c]
Overall	104	10.8±1.03	289±5.2	1.18±0.015	132	19.2±1.5	295±5.78	1.26±0.021

*** Significant at $P=0.001$, *significant at $P=0.05$, ns=non-significant at $P=0.05$, N=number of observations, LSM=least square means, LI=lambing interval, NLWEY=number of lambs weaned ewe^{-1} year^{-1}.

In most of the Awassi crossbreeding studies so far better growth and body weight and poor reproductive performance of Awassi crossbreds sheep has also been reported (Gizaw and Getachew, 2009; Getachew et al., 2013; Lemma et al., 2014). Combining the number of lambs weaned ewe^{-1} year^{-1} and lamb growth might be a useful measure to evaluate the overall productivity of crossbreeding. Combined data is lacking to capture the variability however point estimation based on the population mean might give rational indication. Eight months lamb weight ewe^{-1} year^{-1} was calculated by multiplying number of lambs weaned ewe^{-1} year^{-1} of a given Awassi level by 0.95 and then multiplied by the corresponding Awassi level of lamb eight months weight. The values were calculated considering two important assumptions; a ewe raised lamb/s of similar Awassi level to her Awassi level and survival rate of 95% between weaning and eight months for all Awassi levels. Plot of ewe^{-1} year^{-1} against the Awassi level group are indicated (Figure 21). The result showed that the inferiority of ewes in LI due to increased Awassi level was more than compensated by the fast growth of lambs. In both locations productivity of ewe in production of eight months lamb weight year^{-1} was increased as the Awassi level increased to a certain level and then started to decline. However, dropping point was variable in the two locations in that, in Chiro 37 to 50% and 12.5 to 25% in Negasi-Amba. Olsson and Beyene (1990) also reported total lamb weaning weights per ewe lambed were increased with increasing level of exotic genes up to 50% level. Lambing intervals obtained for the ewes with higher Awassi level well fit the annual mating system which might be suggested to improve the profitability of the farm by reducing higher lamb mortality occurred on lambs born during the dry seasons (December to May) (Getachew et al., 2015b).

Determining the optimum combination of productivity and adaptability considering the prevailing environment is paramount. Choosing the level of exotic ancestry should consider the existing environment and its potential for improved management. Burrow, (2012) suggested 25 to 75% adapted genes are required for optimal production depending on the severity of the environment and the level of stress challenge, only exceptionally stressful environments require 100% adaptive genes. Based on this result up to 50% Awassi level would be suggested for Chiro village and similar areas. However in Negasi-Amba Awassi the current situation could not support higher than 25% of Awassi blood. The Awassi level suggested to Chiro site in this study was slightly higher than Gizaw et. al., (2011) recommended 37.5% Awassi level under farmers management. Less number of lambs with higher Awassi level in the previous study (Gizaw et. al., 2011) and management change adopted through time might be probable reasons for this difference. Result found in Negasi-Amba supported by Demeke, (2013) who found that 37.5% Awassi was not different from 25% Awassi under station management near to Negasi-Amba village.
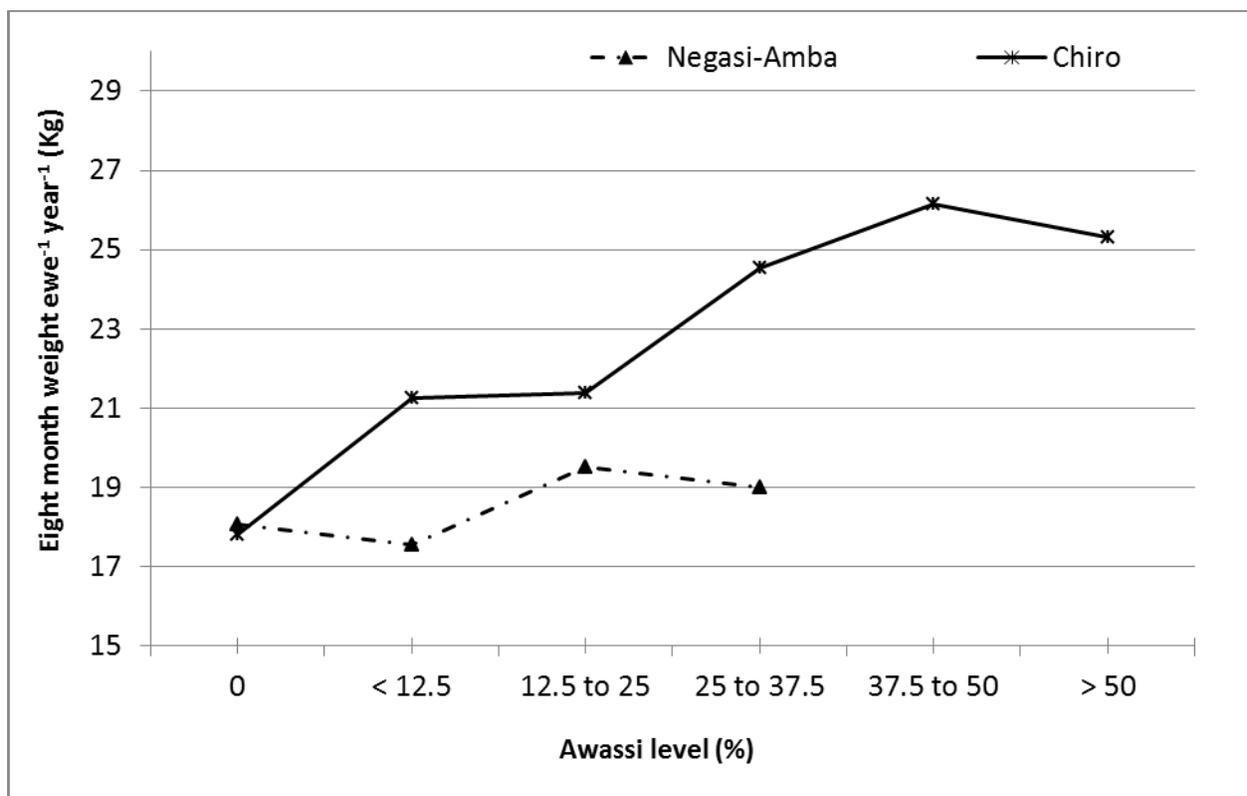

Figure 21. Mean eight months weight produced ewe^{-1} year^{-1} in Negasi-Amba and Chiro site.

4.3.4 Prediction of genomic admixture from morphological measurements

Correlation coefficients and plots of the Awassi levels estimated by SNP against different body measurement and morphological characters for lambs in Negasi-Amba, lambs in Chiro, ewes in Negasi-Amba and ewes in Chiro are presented in Figures 22, 23, 24 and 25 respectively. Generally correlation values were higher in Chiro flocks than in Negasi-Amba flocks. Only ear length and hair length had significant ($P<0.05$) correlation with the Awassi level for lambs in Negasi-Amba. In case of ewes in Negasi-Amba and both lambs and ewes in Chiro, all of the body measurements and morphological characters considered in this study were positively and significantly correlated with the genome estimated Awassi level with correlation coefficient (r) ranging from 0.16-0.63. Generally ear length, hair on leg and tail width in Chiro and withers height, hair on leg and ear length in Negasi-Amba had high correlation values in that order. Therefore ear length and hair on leg were chosen as the two important measurements to develop the prediction equations.

Prediction equations for Awassi level using different morphological characters and body measurements for ewes and lambs are presented in Table 13. Individual admixture levels were fairly predicted from morphological characters and body measurements. Ear length and hair on leg score appeared as primary predictors of Awassi level in Chiro (Wollo x Awassi) crossbred lambs and ewes (R^2 = 0.47, $P < 0.0001$, equation number (eq. no.) 14 for lambs and R^2 = 0.48, $P < 0.0001$, eq. no. 16 for ewes). These two variables were also relatively better predictors of Awassi level in Negasi-Amba (Menz x Awassi) crossbred lambs among other variables with R^2 = 0.18, $P < 0.001$ (eq. no. 2). Better prediction ability of ear length and hair on leg is attributed to the high phenotypic divergence in these traits between Ethiopian fat-tailed and improved Awassi breeds. For Menz x Awassi ewes however, wither height appears as the main predictor which explained 30% of the total variation when alone (eq. no. 5) and explained 42% of the variation when used with hair on leg score (eq. no. 6). The average Menz ewe has relatively small wither height (57.2 cm) compared to pure Wollo ewe 62.7 cm (Gizaw et al., 2007). This relatively larger variation between Menz sheep and improved Awassi compared to Wollo sheep and improved Awassi in wither height resulted in better prediction of Awassi level from wither height in Menz x Awassi crossbred population.

Similar to this study, traits with larger variation between populations like skin pigmentation in human had significant correlation between estimates of individual ancestry and skin pigmentation with R^2 = 0.16 to 0.21 (Shriver et al., 2003). Bonilla et al., (2004) also found significant correlation

between skin pigmentation and individual ancestry (R^2 = 0.597). This would provide good insight about the genes involved traits showing disease prevalence differences between major population groups like obesity, type 2 diabetes, prostate cancer and hypertension in that case (Shriver et al., 2003; Parra et al., 2004). Provided the large difference in ear length, abundance of hair on leg and wither height between populations, admixture mapping might also provide important insight in understanding the genetics of variation in these traits. Farmers in Menz area prefer to keep ewes with longer ear in their flock as they perceived such trait to be associated with productivity though not supported by research. Admixture mapping studies in order to identify associated production traits need to be investigated.
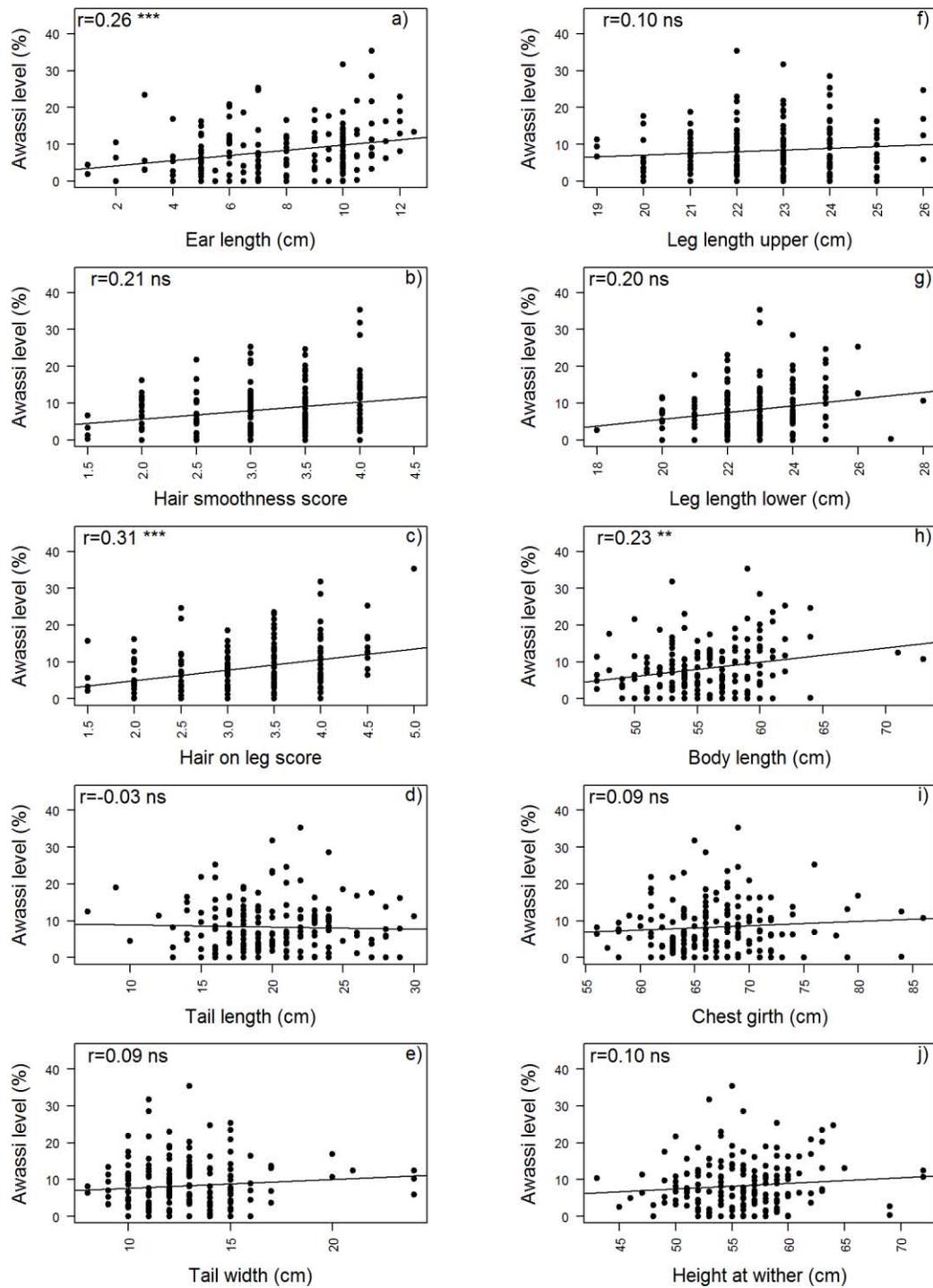
Figure 22. Scatter plot of genome estimated Awassi level vs. different morphological characters and body measurements; ear length (a), hair smoothness score (b), hair length score (c), tail length (d), tail width (e), leg length upper (f), leg length lower (g), body length (h), chest girth (i) and wither height (j) for crossbred lambs in Negasi-Amba site. *significant at P=0.05, ns=non-significant at P=0.05, r=Pearson's correlation coefficient.
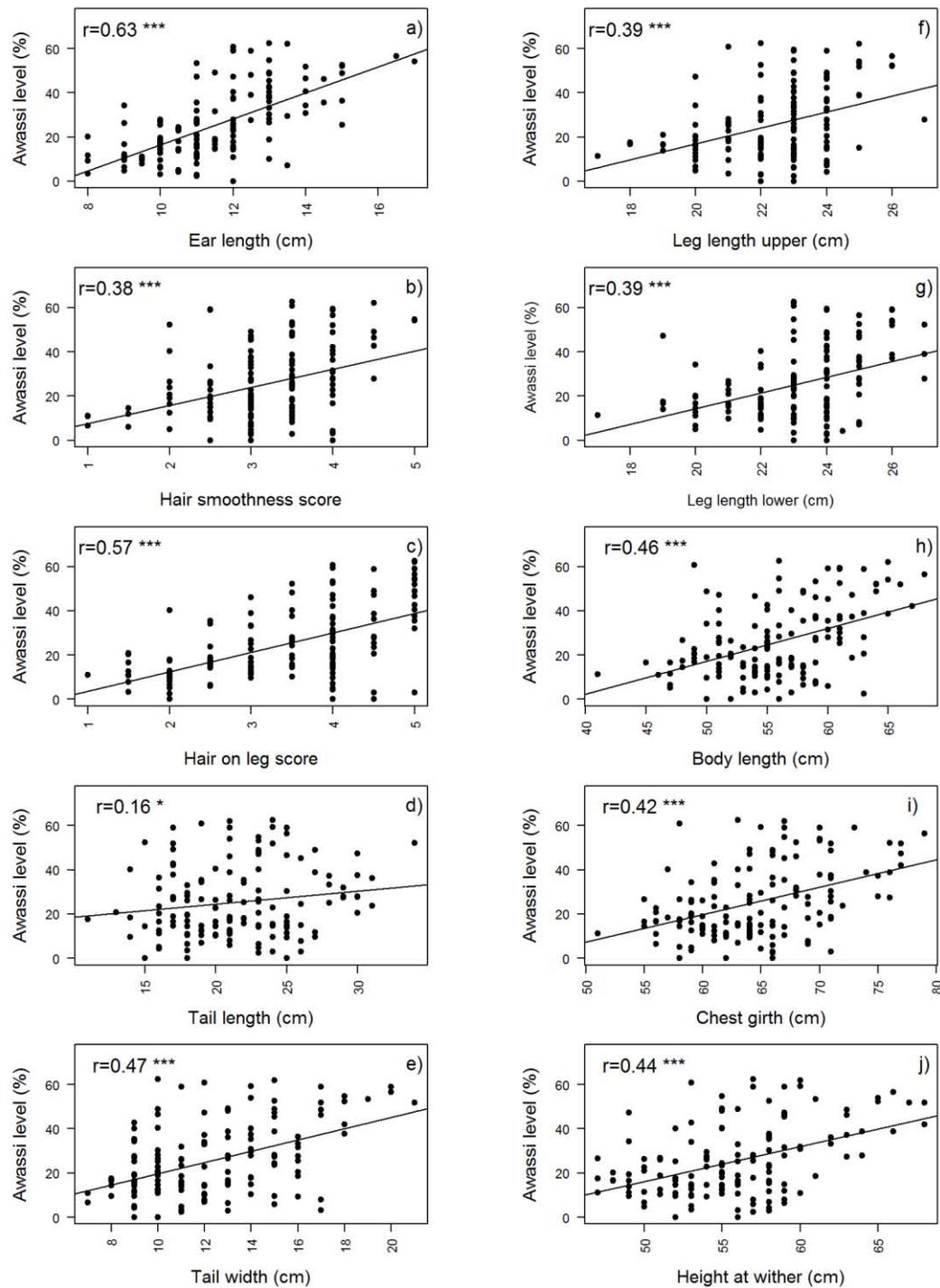
Figure 23. Scatter plot of genome estimated Awassi level vs. different morphological characters and body measurements; ear length (a), hair smoothness score (b), hair length score (c), tail length (d), tail width (e), leg length upper (f), leg length lower (g), body length (h), chest girth (i) and wither height (j) for crossbred lambs in Chiro site. *** Significant at *P*=0.001, *significant at *P*=0.05, r=Pearson's correlation coefficient.
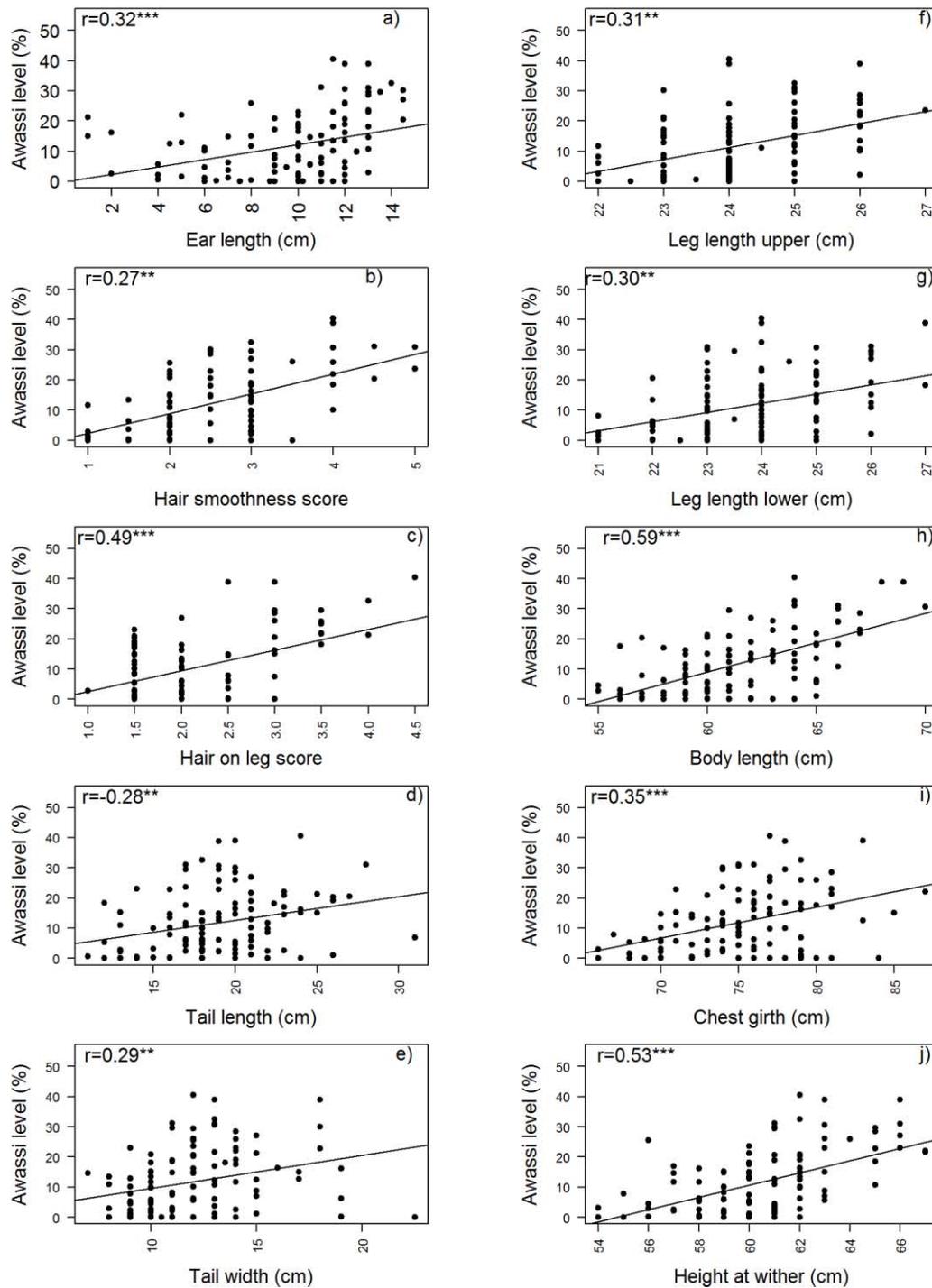
Figure 24. Scatter plot of genome estimated Awassi level vs. different morphological characters and body measurements; ear length (a), hair smoothness score (b), hair length score (c), tail length (d), tail width (e), leg length upper (f), leg length lower (g), body length (h), chest girth (i) and wither height (j) for crossbred ewes in Negasi-Amba site. ***Significant at P=0.001, **significant at P=0.01, r=Pearson's correlation coefficient.
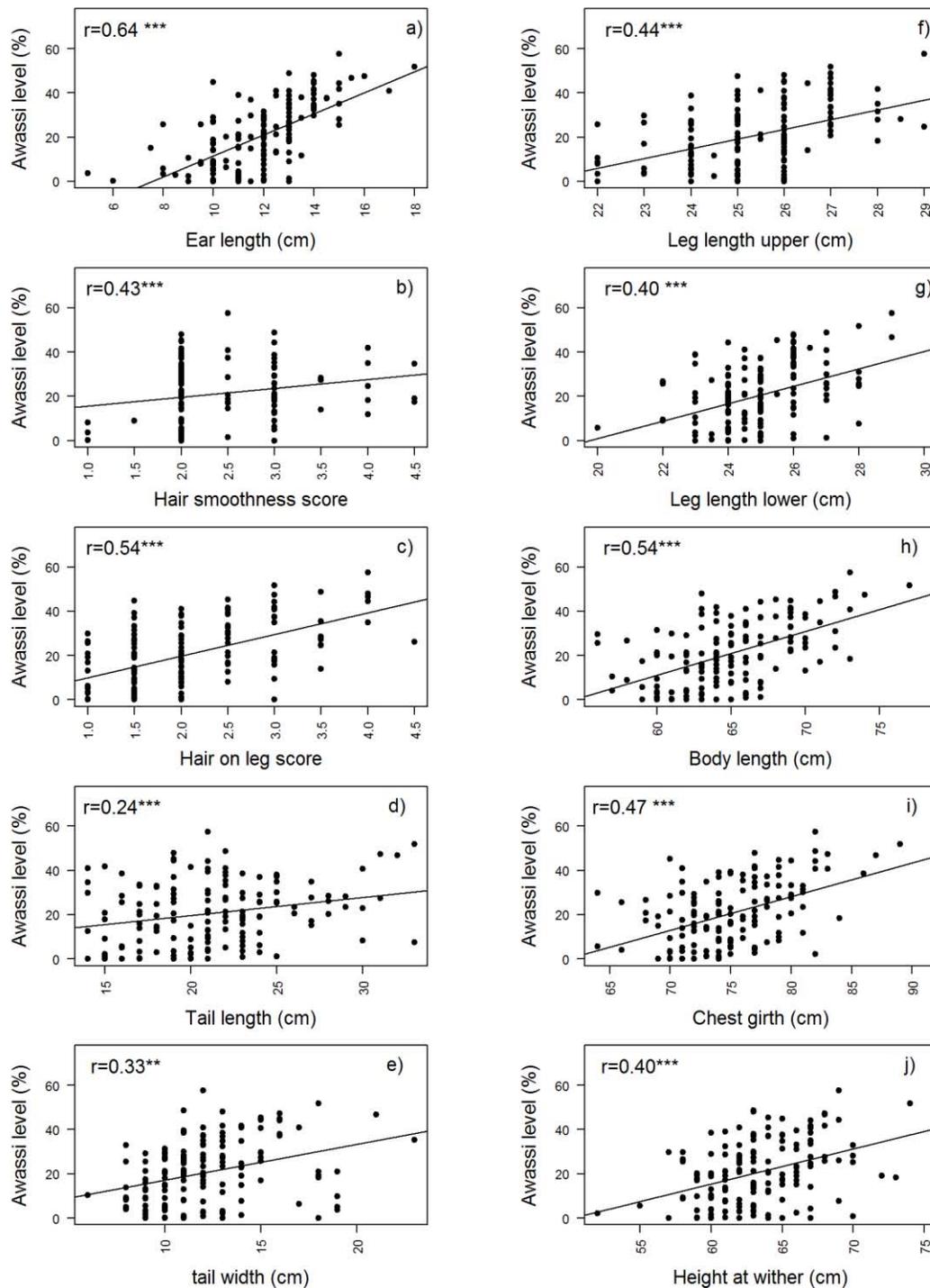
Figure 25. Scatter plot of genome estimated Awassi level vs. different morphological characters and body measurements; ear length (a), hair smoothness score (b), hair length score (c), tail length (d), tail width (e), leg length upper (f), leg length lower (g), body length (h), chest girth (i) and wither height (j) for crossbred ewes in Chiro site. *** Significant at *P*=0.001, **significant at *P*=0.01, r=Pearson's correlation coefficient.

Table 13. Prediction equation for Awassi level using different morphological characters and body measurements for ewes and lambs in Menz x Awassi and Wollo x Awassi crossbred populations.

Class/location	Equations for prediction of Awassi level (%)	R-square	Cp	Sig	Eq no
Negasi-Amba (Menz x Awassi crossbreds)					
Lamb	= 2.883 HOL − 0.913	0.10	32.03	***	1
	= 3.050 HOL + 0.759 EL − 7.407	0.18	16.21	***	2
	= 2.892 HOL + 0.747 EL + 0.327 BL − 25.033	0.22	9.94	**	3
	= 2.775 HOL + 0.783 EL + 0.714 BL -1.163 LLU − 20.027	0.25	5.02	**	4
Ewe	= 0.020 WH − 1.116	0.30	35.16	***	5
	= 0.017 WH + 0.049 HOL − 1.010	0.42	17.29	***	6
	= 0.016 WH + 0.043 HOL+ 0.006 TL − 1.058	0.45	12.66	***	7
	= 0.014 WH + 0.044 HOL+ 0.006 TL + 0.006 EL − 1.022	0.48	9.12	*	8
	= 0.013 WH + 0.040 HOL+ 0.005 TL + 0.007 EL + 0.008 TW − 1.001	0.52	5.66	*	9
	= 0.066 HOL + 0.009 EL − 0.118	**0.29**	**3.0**	*******	**10**
Chiro (Wollo x Awassi crossbreds)					
Lamb	= 5.770 EL − 40.082	0.37	53.30	***	11
	= 4.485 EL + 1.404 TW − 43.251	0.47	22.23	***	12
	= 3.081 EL + 1.196 TW + 5.085 HOL − 42.198	0.54	2.67	***	13
	= 3.877 EL + 6.036 HOL − 39.93	**0.47**	**2.78**	*******	**14**
Ewe	= 0.048 EL − 0.361	0.42	29.39	***	15
	= 0.037 EL + 0.051HOL − 0.336	0.48	14.31	***	16
	= 0.030 EL + 0.047 HOL + 0.007 CG − 0.764	0.51	6.09	***	17

Sig=level of significance for the added variable where, ***significant at $P=0.001$, ** significant at $P=0.01$, Cp=Mallows' Cp statistics used to assess the fit of regression model, HOL=*h*air on leg score, EL=*e*ar length, BL=body length, LLU=leg length upper, TW=tail width, TL=tail length, WH=wither height.

4.3.5   Accuracy of farmers estimate of Awassi level

On the ongoing crossbreeding program in Ethiopia farmers tried to estimate the breed composition of their animal by associating with their morphological changes of the crossbred. Pearson's correlation coefficient and scatter plot of Awassi level estimated by AIMs and estimated by farmers for lambs and ewes are presented in Figure 26 and 27, respectively. Farmers estimate in both location was significant ($P<0.05$) and highly consistent to the relationship between Awassi level and morphological characters observed in this study. Relationship between Awassi level and farmers estimate in Negasi-Amba lambs was relatively lower (r=0.29) compared to lambs in Chiro and ewes in both Negasi-Amba and Chiro found correlation coefficient in the range of 0.71 to 0.76. Level of precision in admixture level might depend on the desired stringency of assignment.

Empirical assignment of goats in to Sardinian, Maltese or crossbred group based on farm history and the farmer's estimate of average percentage of Sardian blood considering different phenotypic profiles was implemented and showed agreement with genetic approach assignment (Sechi et al., 2007). Indigenous knowledge of farmers in estimating heritability and genetic correlation among traits in sheep was also well documented (Haile et al., 2010). Estimating the level of admixture based on few markers is cost effective comparing to the use of large number of genotype. However still might not be possible for developing countries as the facilities, knowledge and logistics associated with sample collection, DNA extraction and genotyping. In absence of pedigree or genome information, and when decision on admixture level is not too sensitive, farmers could able to estimate the level of admixture in their crossbred population.
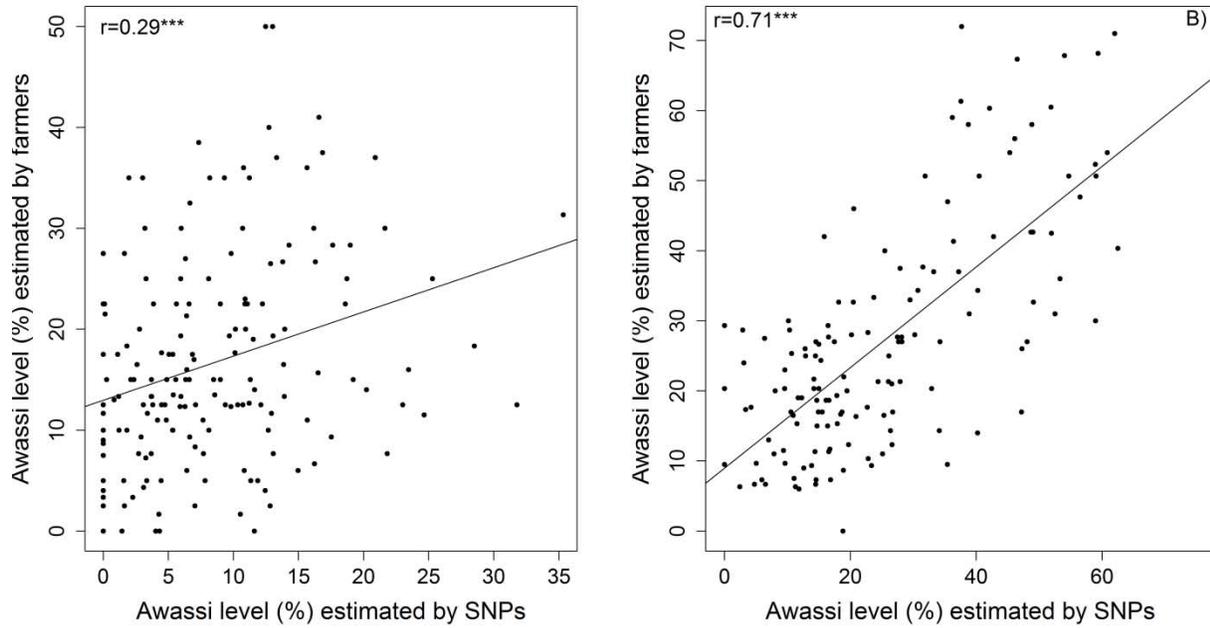
Figure 26. Scatter plot of Awassi level estimated by farmer vs. Awassi level estimated by SNP markers for Wollo x Awassi crossbred lambs in Negasi-Amba (A) and Chiro (B) villages.
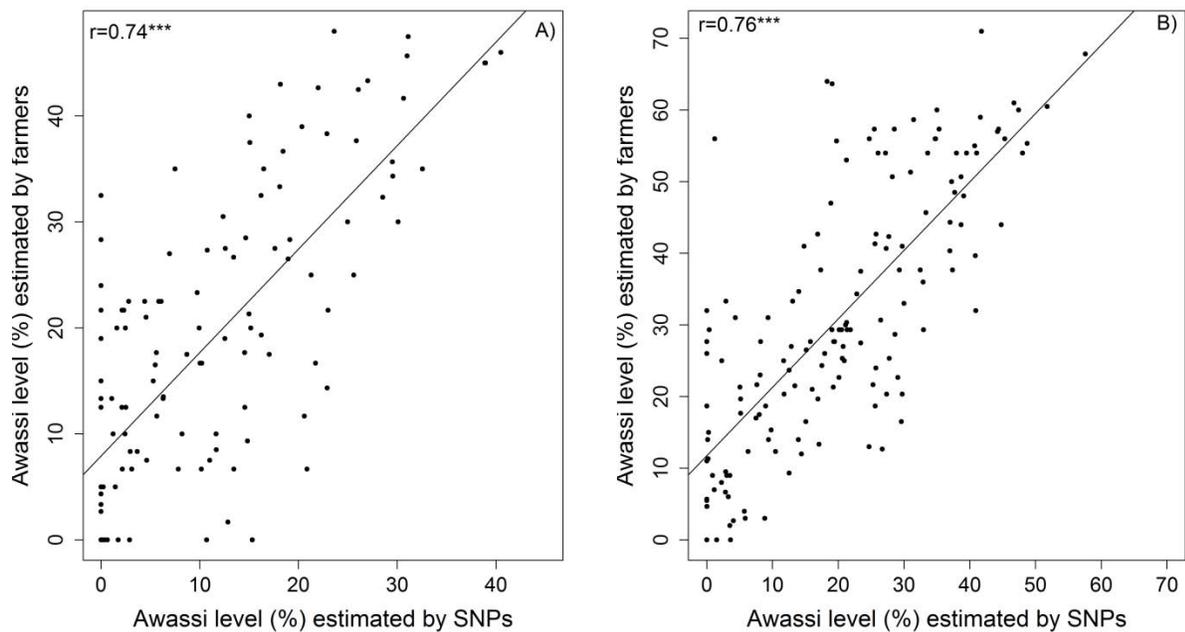

Figure 27. Scatter plot of Awassi level estimated by farmer vs. Awassi level estimated by SNP markers for Wollo x Awassi crossbred ewes in Negasi-Amba (A) and Chiro (B) villages.

5 Conclusions and recommendations

In this study ovine 50K SNP data distributed along the ovine genome were used to successfully assess genetic diversity, population structure and inbreeding levels of Ethiopian fat-tailed (Menz and Wollo), improved Awassi and local Awassi sheep breeds. Population structure, principal component analysis and distance measures consistently showed that both, man-made selection and geographic location, were responsible for differentiation of sheep populations. However, man-made selection pressure to improve productivity of Awassi for milk yield has more influence in separating improved Awassi from local Awassi than geographic isolation of the two local Ethiopian from local Awassi breed.

Genetic diversity, linkage disequilibrium (LD) and runs of homozygosity (ROH) analysis showed that local Ethiopian fat-tailed as well as local Awassi breeds have maintained higher levels of within breed genetic diversity and less inbreeding compared to improved Awassi. Observed higher levels of variability in local Ethiopian and local Awassi breeds imply that selection response for production and adaptation traits may be expected to continue. In addition, genetic distinctiveness observed in Ethiopian fat-tailed breeds, being small in body size and adapted to harsh areas (particularly for Menz sheep) indicates that the breed might carry genes responsible for adaptation to harsh low input environments. Proper utilization and conservations of such a breed may be valuable to cope with unpredictable future environments. However, lower LD in local sheep breeds compared to cattle and commercial pig breeds implies that more dense SNP panels are needed to implement genomic selection in sheep. Higher amount of ROH and LD in both short and long ROH length categories found in improved Awassi indicated that the breed derived from a small population and experienced recent inbreeding. This should be noted and steps toward increasing diversity should be taken into consideration. When costs are justifiable, looking for the extent of genomic structure induced by the current admixture of Ethiopian and Awassi based on high density SNP might be of future interest of study. This would help to identify if new LD that can extend to long distance has been created which then might help to reduce the number of markers needed for selection.

Ovine 50KSNP array also used as a powerful tool to identify AIMs for admixture and population structure studies. Individual admixture levels of the Menz x Awassi and Wollo x Awassi crossbred populations produced in two farmer villages (Negasi-Amba and Chiro) and government farms were estimated based on top 74 F_{ST} ranked ancestry informative markers (AIMs) selected from the Ethiopian fat-tailed and improved Awassi 50K SNP data. The top ranked ~45 AIMs selected

based on their F_{ST} value were found adequate to accurately estimate admixture levels in the Awassi x Ethiopian fat-tailed crossbred populations. Breed composition levels estimated based on AIMs were used to identify optimum Awassi levels for the on-going sheep crossbreeding programs implemented in different areas of Ethiopia. Farmers in Chiro village had already shown interest to pay for the accurate breed composition information at a meeting held with them during data collection for this study. Thus, information on breed composition using small sets of AIMs might be cost effective for smallholder farmers and would be easily integrated in the crossbreeding schemes to increase efficiency of selection and reliable exchange of breeding rams among farmers. Small sets of AIMs would also be worthwhile for many developing countries having admixed livestock populations in providing accurate breed composition in case of incomplete or absence of pedigree. The use of AIMs would inspire livestock breeding programs in by availing breed composition and pedigree information which persisted as marked constraint in many developing countries.

Association of individual admixture levels to lamb growth and ewe performance in this study showed that Awassi crossbreeding improved sheep productivity under farmer management in both locations. However, performance of crossbreds varied by locations which reinforces the importance of alternative breeding decisions for different areas regarding the optimal admixture level. Based on this result up to 50% Awassi level would be suggested for Chiro village and similar areas. However, Negasi-Amba village could not support higher than 25% of Awassi blood under the current environmental situation. Thus, promoting the Awassi crossbreeding on Wollo sheep breeds while strengthening the ongoing selective pure breeding programs of Menz sheep is suggested. Studies to identify genes under selection associated with adaptation traits could also be considered for Menz sheep.

Notable within genetic variability of lamb growth in the crossbred population particularly in Chiro village signifies the immense potential for improvement of productivity through selection. Thus, genetic improvement applying efficient selection within the crossbred population along with improved management should be considered, which progressively should lead to the development of a stabilized composite population. Accurate prediction of the extent of ancestry could also be a useful tool to assess the genetic structure of populations and to detect pure breeds for management of endangered populations.

Use of small sets of markers for admixture studies and identification of optimal Awassi level for different areas are the major findings of this study which are useful to enhance the current Awassi

sheep crossbreeding program. Future designing of a crossbreeding program should also rely on this finding considering the potential of the production areas and productivity level of crossbred populations. The potential of some farmers in maintaining higher levels of Awassi was notable in this study. Upgrading of such promising farmers to a multiplier level might be considered with support from the government and non-government organizations to ensure efficient ram dissemination among farmers.

Individual Awassi levels were predicted by farmers, morphological characters and body measurements with reasonable accuracies. In absence of pedigree and genome information, and when decision on admixture level is not too sensitive, farmers could be able to estimate the admixture levels of individuals in their crossbred population. Morphological characters like ear length, hair score on back legs of the sheep, and wither height were found to be the best predictors and could also be used to estimate the level of admixture. Farmer's experience and knowledge in identifying their animals should be encouraged through training.

Strengthening and optimizing the current Awassi crossbreeding program in a way to exploit the available large genetic variation and stabilizing the current crossbred population to the levels found to be best for a particular location is the main suggestion derived from the results of this thesis.

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7 Appendices

Appendix Table 1. Genome DNA Extraction protocol from ear punch

1) Open the Allflex tube via side cutter and transfer the tissue using tweezers to a 1,5ml extraction tube.
2) Apply 300µl Ear punch puffer
3) Apply 10µl 10% SDS
4) Apply 20µl Proteinase K
5) Apply 5µl RNAse
6) Close the lid of the tube and mix by flipping via fingers
7) Incubate the closed tube at 60°C over night
8) Cool down to room temperature (RT) on a shaker (1000 rpm)
9) Apply 400µl of cold Puffer III (4°C)
10) Close the lid and mix for one minute at 1000rpm
11) Incubate for 30min on crashed ice
12) Centrifuge at maximum speed at 4°C (or RT) for 10 minutes
13) Transfer the supernatant (containing the DNA) into a new tube – only use the clear liquid phase without any cell debris
14) For DNA precipitation apply 4°C cold 100% Ethanol (2 volumes) or Isopropanol (0,7 volumes) *)
15) Close the tube and mix by turning around several times (until the liquid phases are completely mixed)
16) Centrifuge at maximum speed at 4°C (or RT) for 5 minutes **)
17) A DNA pellet should be visible now on the bottom of the tube. The supernatant can be wasted by carefully decantation – the last liquid droplets can be removed using tissues.
18) Dry the pellet by incubating the opened tube at RT until the ethanol is completely evaporated (smelling test).
19) Apply 100µl Te 8 Puffer and incubate the closed tube on a shaker over night at room temperature. (DNA molecules need this time to dissolve completely)
20) Store the DNA at 4°C for short times and at -20°C for long periods. (Avoid freezing/thawing cycles because this will damage the DNA).

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*) The volume of Ethanol or Isopropanol depends on the volume of the transferred supernatant – for example: In case 200µl supernatant will be transferred to the new tube 400µl Ethanol or 140µl Isopropanol will be needed

**) In case there is no cooling centrifuge available incubate the tube at -20°C for 10minutes before centrifuging at RT.

Appendix Table 2. DNA isolation from FTA card

1. Three discs of 3 mm each taken from spotted blood on FTA card were put in 1.5 ml standard tube.
2. Add 360 µl of Whatman FTA Purification Reagent containing 60µg/ml Proteinase K were added.
3. Incubation has been made at room temperature (RT) overnight.
4. Use a pipette to mix the contents up and down without vortexing.
5. The used reagent was removed and discarded.
6. Add 360µl of Whatman FTA Purification Reagent without Proteinase K was put in the tube
7. Mix by pipetting
8. Discard the reagent used
9. Add 300 µl TE 8 solution
10. Incubate for an hour at 37 ^{0}C
11. Centrifuge for 1 minute at maximum speed
12. Transfer the supernatant in to another tube
13. Add 1000 µl absolute cold ethanol
14. Keep in refrigerator overnight
15. Centrifuge for 30 minute at maximum speed
16. Discard the supernatant
17. Wash with 400 µl 70% ethanol
18. Centrifuge for 10 minute at maximum speed
19. Discard the supernatant
20. Pellet dry
21. Add 50 µl TE

Appendix Table 3. Descriptions of body measurements

Measurements	Description
Body Length	Measured as the horizontal distance from the point of shoulder to the base of the tail
Chest Girth	The circumference of the body immediately behind the shoulder blades in a vertical plane perpendicular to the long axis of the body
Height at wither	the height of an animal from the bottom of the front foot to the highest point of the shoulder between the withers
Tail Length	Distance from the base to the tip of the tail on the outer side of the tail
Tail width	Width of the tail at the middle of the tail
Ear Length	The length of the ear of the external side from its root on the poll to the tip.
Hair smoothness score	Observed and scored from 1 to 5 where 1 was coarse and 5 very smooth
Hair on back leg	Observed and scored from 1 to 5 where 1 no hair on leg and 5 too much hair on leg
Leg length upper	Length of back leg from mid-point of the joint between upper and lower leg (hock) to stifle
Leg length lower	The length of back leg from tip of the hoof the middle of hock

All measurements were done while sheep was in standing position

Appendix Figure 1. Visual explanation for some body measurements and morphological characters. Top left: sheep with less hair on leg (left) and much hair on leg (right). Top middle: ear length example for local breed. Top right: ear length for crossbred. Middle left chest girth. Middle: leg length lower. Middle left: leg length upper. Bottom left: tail length, bottom middle and bottom right: body weight measurements.
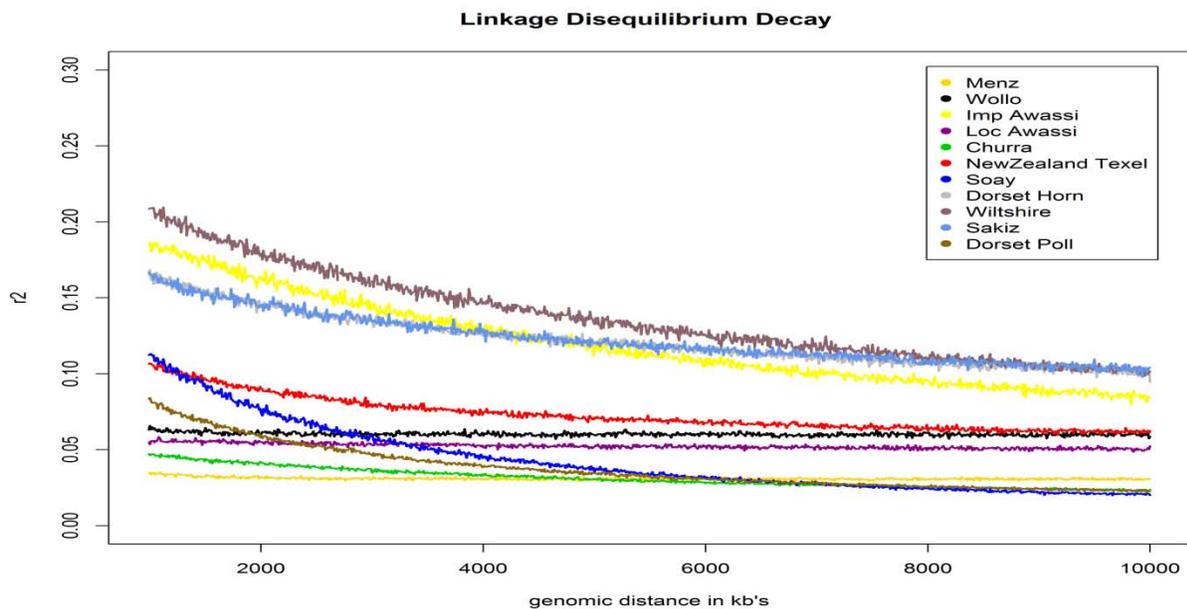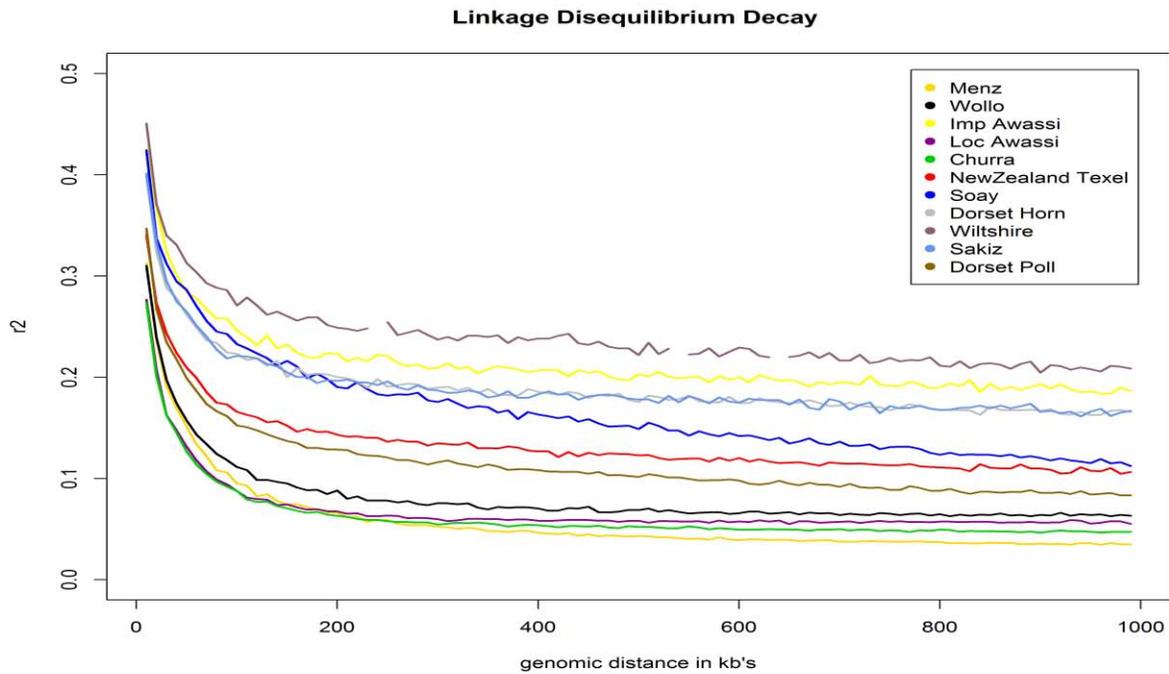
Appendix Figure 2. Linkage disequilibrium decay of average r^2 over distance. Average r^2 between SNPs in Ethiopian and other selected breeds from Middle Eastern, Asian and European breeds at varing distances in base pairs ranging from 0 to 1000 Kb (above) and 1000 Kb to 10 Mb (bottom).