Breeding program for indigenous chicken in Kenya

Analysis of diversity in indigenous chicken populations

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Thesis

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Abstract

The objective of this research was to generate knowledge required for the development of an indigenous chicken (IC) breeding program for enhanced productivity and improved human livelihood in Kenya. The initial step was to review five questions; what, why and how should we conserve IC in an effective and sustainable way, who are the stakeholders and what are their roles in the IC breeding program. The next step of the research focused on detecting distinctive IC ecotypes through morphological and genomic characterization. Indigenous chicken ecotypes were found to be populations with huge variability in the morphological features. Molecular characterization was carried out using microsatellite markers and whole genome re-sequenced data. The studied IC ecotypes are genetically distinct groups. The MHC-linked microsatellite markers divided the eight IC ecotypes studied into three mixed clusters, composing of individuals from the different ecotypes whereas non-MHC markers grouped ICs into two groups. Analysis revealed high genetic variation within the ecotype with highly diverse MHC-linked alleles which are known to be involved in disease resistance. Whole genome re-sequencing revealed genomic variability, regions affected by selection, candidate genes and mutations that can explain partially the phenotypic divergence between IC and commercial layers. Unlike commercial chickens, IC preserved a high genomic variability that may be important in addressing present and future challenges associated with environmental adaptation and farmers' breeding goals. Lastly, this study showed that there is an opportunity to improve IC through selection within the population. Genetic improvement utilizing within IC selection requires setting up a breeding program. The study described the systematic and logical steps in designing a breeding program by focusing on farmers' need, how to improve IC to fit the farming conditions, and management regimes.

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General introduction

Introduction

Relevance of indigenous chicken genetic resources in Africa

Globally, the poultry population is estimated at 19.46 billion, with Africa contributing 8.08% (FAOSTAT, 2012). Chickens are the most common and account for approximately 90% of the poultry population worldwide and 96.03% in Africa (FAOSTAT, 2012). Over 80% of the total chicken population in Sub-Saharan Africa are indigenous chicken (IC). In Kenya, over 75% of rural households keep IC (Magothe, 2012, Olwande et al., 2010). The popularity of IC among the resource poor rural households is attributed to their low production costs, better adaptability to poor quality feeds and harsh scavenging conditions, and higher tolerance to parasites and diseases (Ahlers et al., 2009, Besbes, 2009, Gondwe, 2004, Okeno, 2013). They are synonymous with resource poor rural households as a mitigation measure to overcome poverty and economic vulnerability (Okeno, 2013). Nutritionally, IC is a source of high quality protein, iron, zinc and vitamins (FAO, 2014, Ahlers et al., 2009). Their droppings are not only used as manure for crop production, but also as feeds (FAO, 2014), especially for dairy cattle and fish farming, as it is rich in nitrogen which is important for rumen microbial activities and accelerate the growth of planktons in fish ponds. Free roaming IC control pests and clean the environment by converting waste into nutritious products (Ahlers et al., 2009). They are also used for purposes such as cock fighting, treatment of illnesses, building of social relationships (Ngeno, 2011), as biological clock (Magothe et al., 2012), or execution of funeral rites and spiritual cleansing (Bett, 2012). Despite IC importance in wealth creation and mitigation of food insecurity, their potentials have not been fully realized because of their low productivity which is a key setback for their utilization.

Indigenous chicken genetic improvement in Africa

Various interventions enhancing IC productivity have been attempted in the past including breed substitution, crossbreeding/upgrading, and selection within population. Intensification of chicken production through substitution with exotic chicken breeds started in 1960s, with the most recently being the introduction of the Kuroiler chicken. The Kuroiler chicken from India has been introduced in African countries such as Uganda and Ethiopia. Comparative performance evaluation in Uganda demonstrated that Kuroiler chicken outperform IC under rural scavenging conditions with extra management intervention (feed supplementation and veterinary care) (Fotsa and Ngeno, 2011). The objective of substituting IC with exotic breeds was to have chickens with faster growth and higher egg production. However, adaptability of the introduced exotic chickens was a problem under the

prevailing conditions of production. Furthermore, substitution of local breeds with exotic breeds is opposed by the global move on conservation of indigenous genetic resources because it leads to the disappearance and displacement of the indigenous breeds (Kosgey et al., 2006; Hanotte et al., 2010). The only way to prevent breed substitution from happening would be to make the IC more valuable to farmers. This can be realised by genetic improvement of IC through within breed selection.

Crossbreeding or upgrading of IC with commercial exotic chickens through cockerels or pullets exchange was another genetic intervention implemented in the past in several African countries. The intervention started in 1950s in Nigeria where ICs were crossed with Rhode Island Red (RIR), Light Sussex and Black Australorp chicken (Oluyemi et al., 1979, Tiamiyu and 1999). Crossbreds demonstrated superiority in performance (Fayeye et al., 2005), but their survival rates were low and the intervention was categorized as unsuccessful (Magothe, 2012, Nyagah, 2007). A similar strategy was introduced in Malawi in the 1950s through the Smallholder Poultry Improvement Program (SPIP). The Black Australorp breed was used to upgrade IC, however, the program failed (Safalaoh, 2001, Gondwe, 2004). In Sudan, a crossbreeding program was initiated in 1956 and lasted for only three years because of poor distribution and adaptation of the exotic cocks (El-Zubeir, 1990, Musharaf, 1990). An upgrading program in Tanzania started in 1937 using RIR and Barred Plymouth Rocks genotypes imported from Europe and South Africa (Boki, 2000). The F1 crossbreds outperformed the IC in egg traits such as egg weight, length, breadth, volume and hatch weight (Malago and Baitilwake, 2009), but they were inferior in adaptability. Despite several years of funding, upgrading in Tanzania as in other countries has not been sustainable and end as soon as donors pull out (Boki, 2000). After the village crossbreeding or upgrading programs failed, a new model, where crossbreeding takes place on-station and the crossbreds are distributed to the smallholder farmers, was introduced by Danish International Development Agency (DANIDA) in 1999. This model was implemented in countries such as Benin, Burkina Faso, Eritrea, Kenya, Malawi, Mozambique, Senegal, and South Africa (FAO, 2010, Riise et al., 2005). As its predecessors, this model flopped in 2005 when DANIDA pulled out (Riise et al., 2005). Failures of these upgrading attempts have been attributed to poor planning which led to inefficient flow of inputs, lack of self-sustainability of the program, and adaptation challenges of the chickens (Magothe et al., 2012, Ndegwa et al., 2012, Okeno, 2013). This demonstrate that success of a crossbreeding or upgrading program requires proper planning, developed infrastructure, sustainable funding or financial independency and understanding the production environment.

Within breed selection was another strategy used in some African countries such as Egypt, Nigeria, and recently Ethiopia, to genetically improve IC. This strategy was successful in Egypt as it resulted in creation of Fayoumi breed which has a 60% higher egg production as compared to the IC (Hossary et al., 1995). Two pure lines of the Fayoumi breed were developed by selecting one for growth, and another one for egg production. In Ethiopia, selection within Horro IC was started in 2000 at the Debre Zeit Research Centre and has been successful in increasing egg production and body weight (Wondmeneh et al., 2014). Egg production in Horro chicken increased by 123.5% to 75 eggs at week 45, and age at first egg reduced to 148 from 203 days by generation five (Tadelle et al., 2013). The within breed selection, therefore, seems to be a promising strategy to improve productivity of IC compared to crossbreeding or upgrading. The baseline, however, is that, before initiating the breeding program for within breed selection, lessons learned from failures of crossbreeding or upgrading programs should be taken into account.

The Kenyan indigenous chicken and genetic improvement efforts

The Kenyan indigenous chickens are anticipated to have multiple origins of wild ancestors in South Asia and Island Southeast Asia (Lyimo et al., 2013, Mwacharo et al., 2013a). They are believed to have been introduced to the country through several entry points with the western (Magothe et al., 2012), and coastal region as the main entry points (Fuller et al., 2011). Archaeological and historic evidence indicate the presence of domestic chickens in the Shanga and Manda regions in coastal of Kenya by 800AD (Horton et al., 1993) and AD900-1400 (Chittick, 1984), respectively. Since the introduction, IC have spread through terrestrial routes (Mwacharo et al., 2013b) and are currently predominantly distributed in the rural areas in all the agro-ecological zones of Kenya. Currently, IC account for 77% (25 million) of the estimated 32.50 million chickens (FAOSTAT, 2013) and contributes 46.7% and 58.3% of the total egg and poultry meat produced annually (KNBS, 2010).

In Kenya, as in other African countries, several attempts were made to improve productivity of IC. These attempts have been made through both genetic and environmental interventions. Environmental interventions haves been mainly through the detection, prevention, and control of diseases. The task is carried out by the government extension agents (veterinarians and livestock production officers) (Nyagah, 2007). The genetic interventions began in the 1960's with importation of exotic breeds such as RIR, Light Sussex, New Hampshire Red, Black Australorp and White Leghorns (Permin and Pedersen, 2000; Nyagah, 2007). This was followed by formation of the National Poultry Development Project (NPDP) in

1976 to initiate commercialization of IC by improving the smallholder households' income and protein uptake (Nyagah, 2007, Wainaina, 1994). This was to be realised by improving the egg and meat productivity through crossbreeding or upgrading through cockerel or pullet exchange. Smallholder farmers were encouraged to exchange their IC cocks with commercial exotic cocks, while some farmers were given 10 to 15 pullets to mate with IC cocks. As in other African countries the program's impact was very minimal and was terminated in 1993 (Nyagah, 2007; Riise et al., 2005). The failures were similar to those experienced in other African countries, i.e. due to lack of a continuous supply of the exotic breeding stock, poor planning, and lack of understanding of production environment, among other reasons (Nyange, 1995).

Since the termination of the NPDP in 1993, no another attempt had been made to genetically improve IC until 2006 when the Smallholder Indigenous Chicken Improvement Programme (InCIP-www.incip.org) was initiated (Bett 2012; Magothe, 2013; Okeno 2013). Most of the InCIP activities were aimed to address the challenges that led to failure of the past productivity improvement interventions. Indigenous Chicken Improvement Programme activities involved the comprehensive mapping of the IC production value chain, including a situation analysis of the IC sub-sector, the characterization of marketing structures and production systems, the assessment of disease and parasite prevalence, the definition of breeding objectives, and the evaluation of different selection schemes. Most of these activities have been undertaken. The genetic diversity and population structure of IC, however, has not been investigated. Genetic diversity and population structure are important because they provide in-depth information which is vital for making informed decisions when setting up a breeding program for genetic improvement and conservation.

Rationale and objectives of the study

Indigenous chickens are important for food, nutrition, and income security among the poor rural households in many developing countries including Kenya. Their great potential for improving livelihoods has been recognised, as demonstrated by the increased demand for IC products. However, this potential has not been fully realised because the productivity of IC is still low, which is a key setback for its utilization. Furthermore, limited supplies of grains and vegetable proteins have led to competition between animals and humans for these products, thus limiting the intensification of chicken farming based on exotic birds in most developing countries. For grain deficient countries like Kenya, the solution to intensification of

chicken farming may lie with the genetic improvement of IC without altering their unique attributes and adaptation ability.

Indigenous chickens are widely distributed throughout Kenya under diversified geographical and agro-ecological conditions. Geographically isolated IC populations are subjected to local climatic conditions and each region is believed to host some unique types of chickens, hereafter called ecotypes. These ecotypes have been subjected to diversified ecological conditions, diets, parasites and diseases in their local habitats and along the dispersal routes. Such ecotypes are anticipated to exhibit high genetic diversity and possess unique combinations of alleles of genes that may confer adaptation to the local environment. However, insight into the underlying genetic diversity and adaptations, which has enable IC to adapt to varying conditions, is unknown. Studying of whole genomes by complete re-sequencing allows unravelling of mechanisms by which phenotypic diversity and adaptation to local environment are generated (Rubin et al. 2010).

Considering their importance and diversity, immediate steps must be taken to conserve and genetically improve these genetic resources for use by both the present and future generations. This can only be realized through genetic and phenotypic characterization of IC ecotypes and development of sustainable breeding programs utilizing IC that are adapted to the locally available feeds, disease challenges and harsh environmental conditions.

The objective of this research was to generate knowledge required for the development of IC breeding program for enhanced productivity and improved human livelihood in Kenya. The specific objectives of the study were: (i) to review five questions; what, why and how should we conserve IC in an effective and sustainable way, who are the stakeholders and what are their roles in conservation efforts, (ii) to characterize IC ecotypes morphologically, (iii) to investigate the genetic make-up of different ecotypes of IC in Kenya using both MHC-linked and non-MHC autosomal microsatellite markers, (iv) to identify genomic variation, genomic selection signatures and candidate mutations that may explain the phenotypic divergence between IC and high input commercial layers and (v) to propose a breeding program that can be implemented to enhanced IC productivity and improved human livelihood.

Outline of the thesis

This thesis consists of six chapters. Chapter 1 is the introduction. The chapter elaborates on the relevance of IC genetic resources. It gives an overview of the past IC genetic improvement attempts, and rationale of the study. Chapter 2 is a review focused on what, why and how should IC be conserved. This chapter also present

the stakeholders and their respective roles. Chapter 3 describes morphological features of IC ecotypes from five different regions in Kenya. The findings on the genetic diversity of eight IC ecotypes using two major histocompatibility complex (MHC), and ten non-MHC linked autosomal microsatellite markers, are described in Chapter 4. The chapter also present results from investigation on population sub-structure and allelic richness in the MHC and non-MHC regions. Chapter 5 presents the genomic variation, selection signatures, and mutations based on whole genome re-sequencing in IC and commercial layers. Chapter 6 elaborates on the practical relevance and utilization of the findings in chapters 2 to 5 in designing a breeding program for genetic improvement and conservation of IC.

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2

Indigenous chicken genetic resources in Kenya: their unique attributes and conservation options for improved use

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Abstract

The indigenous chicken (*Gallus gallus domesticus*) genetic resources (IC) comprise more than 80% of the overall poultry population in rural villages despite their low productivity. However, a holistic approach that increases productivity without increasing production costs or leading to loss of biodiversity is presently limited. Conversely, in most developing countries, there is almost no organizational structure for breeding programmes for improving and conserving IC. These locally adapted IC can only be conserved in the most rational and sustainable way by ensuring that they are functional part of different production systems. Their conservation should be through utilisation if they are to be of any benefit to the poor rural households. This discussion focuses on five very relevant questions that need to be answered if the conservation of IC is to be effective and sustainable: What, why and how should we conserve, who are the stakeholders and what are their roles?

Key words: indigenous chicken, ecotype, genotype, conservation

Introduction

The indigenous chicken *(Gallus gallus domesticus)* genetic resources (IC) comprise more than 80% of the overall poultry population in rural villages, despite their low productivity. Indigenous chicken are of importance for nutrition and income security among the poor rural households. They play a vital gender role for women, widows and orphaned children in terms of cash incomes and savings, food security, nutrition and socio-cultural activities (Kaudia and Kitalyi, 2002). They are highly adapted to the harsh scavenging conditions, poor nutrition and disease and parasite challenges. Their adaptation is attributed to hereditary characteristics that have resulted in differences in response to environmental stimulus. The reactions are closely linked with anatomy and physiological features, which have developed as a result of natural selection. According to Romanov et al. (1996), local adapted breeds possess genes and alleles that are pertinent to their adaptation to the local environments. In most developing countries, there is almost no organisational structure for IC breeding programmes. The current breeding strategies concentrate on specialized commercial chicken lines, derived by intense selection from a few breeds with a great genetic uniformity of traits under selection (Notter, 1999). Therefore, sustainable breeding strategies need to be developed which take into account their economically important and unique attributes. These locally adapted IC can only be conserved in the most rational and sustainable way by ensuring that they are a functional part of different production systems. Their conservation should be through utilisation if they are to be of any benefit to the poor rural households. This discussion will focus on five very relevant questions that need to be answered if the conservation of IC is to be effective and sustainable: What, why and how should we conserve, who are the stakeholders and what are their roles in conservation efforts?

What to conserve?
Origin of indigenous chickens

Chickens *(Gallus gallus domesticus)* are generally considered to have evolved from jungle fowl (Gallus gallus) inhabiting India, Indo-China, South China, Philippines and Indonesia (Moiseyeva et al., 2003). They are thought to have been domesticated in South-East Asia from where they were distributed to all parts of the world. Natural and, to some extent, human selection coupled with mutations and random drifts over time have resulted in the modifications and subsequent development of the various chicken genotypes presently available in various climatic regions.

Indigenous chicken genotypes in Kenya

Indigenous chicken have been characterized along genetic lines for feather (such as normal or frizzled feathered), body structure (such as naked neck, dwarf types) and colour variants (such as black, white, brown, mottled etc.). The phenotypes are a result of genes with major phenotypic effects, and hence are considered genotypes (Falconer, 1989). Some of the major genotypes available include the crested-head, frizzle, naked-neck, dwarf, tailless, bearded, normal feathered and feathered-shank (Figure 1) (Njenga, 2005). IC genotypes present in Kenya are shown in Figure 1. Performances also differ with presence of major genes. The dwarf gene has increased feed efficiency and egg mass production (Yeasmin et al., 2003). The dwarf genes are favoured by farmers due to better reproductive capacity. The bearded and feathered-shank types are adapted to cold environments (Bartels, 2003) and have been shown to have increased body weight and egg mass (Fayeye et al., 2006) for better egg and meat productivity in very cold environments. The crested-head genotype is considered to be a superior egg producer. These genotypes possess major genes known to significantly contribute to adaptability and fitness in the tropics (Horst, 1988) however, they have not been exploited, utilised nor conserved for present and future use.

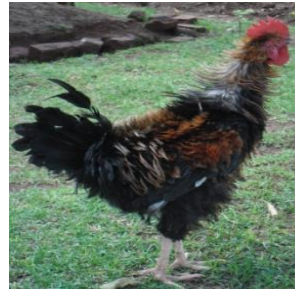|			
Game	Feathered shank	Bearded	Crested head
Naked-neck	Normal feathered	Frizzled feathered	Dwarf

Figure 1 Indigenous Chicken Genotypes in Kenya.

Indigenous chicken ecotypes in Kenya

An ecotype refers to chicken from one agro-ecological zone or area as distinguished from another. The names are derived from ecological zones and, in some areas, regional names have been used (Gondwe, 2005). The origin of an ecotype is a combination of separate adaptation, evolution, selection (natural and artificial), mutation and genetic drift. Natural selection pressure is imposed by climate change, endemic parasites and diseases as well as available nutrition. Directed selection, migration and mutation may have led to non-random or directional changes in the allele frequencies of the population. Thus each ecotype comprises a unique set of genes (a number of diverse adaptive and productive traits) with special utility in the tropics (Horst, 1989). Distinct ecotypes have been reported in Tanzania (Msoffe et al., 2001), Ethiopia (Tadelle and Ogle, 2001), Zimbabwe (Mcainsh et al., 2004), Botswana (Badubi et al., 2006) and Kenya (Ngeno, 2011). These ecotypes population presents a high between-and within-ecotype variation in body weight, egg weight, reproduction performance, plumage colour, comb type and skin colour. The annual egg production ranges from 20 to 100 eggs, mature live weight from 0.7 to 2.1 kg for females and from 1.2 to 3.2 kg for males (Tadelle et al., 2003; Ngeno, 2011). This large phenotypic variation points is important for selection, breeding and promotion of the most productive ecotype under specific management conditions.

Why conserve?

Unique attributes and properties of indigenous chickens: heat tolerance

Global temperature is predicted to increase globally (IPCC, 2007). The frequency and severity of droughts, which is associated with increased temperatures, is already high in Kenya and is expected to increase further in future (Ojwang et al., 2010), and such changes in temperature is expected to negatively impact levels of IC production. Direct effects involve heat exchanges between the birds and the surrounding environment that are related to radiation and temperature. High ambient temperature can compromise the ability of IC to dissipate heat and provoke heat stress. Heat tolerance is one of the adaptations which contribute to the performance of tropically derived breeds and their crosses in warm environments (Turner, 1984). Certain major genes have been found to be relevant to the indigenous breeds in their tropical production environment (Horst, 1989). There are a number of genes with major effects on the phenotype that seem to be of special interest for poultry keeping in smallholder systems in developing countries (FAO, 2010). The superior heat tolerance has been attributed to feather distribution gene, naked-neck (Na) and the feather structure gene, frizzle (F). These

genes cause a reduction in tropical heat stress by improving the IC ability to dissipate heat resulting in better performance.

Adaptation and nutrition

Indigenous chicken are known to be alert to predators, protective of their young, have high hatching ability, possess excellent foraging ability and long legs which are suitable for fast running. All these adaptation are necessary in a scavenging production system (Tadelle et al., 1999). Free-ranging chickens can fulfil their nutritional requirements for proteins, energy, vitamins and minerals by scavenging due to good foraging ability, and the ability to utilise high fibre diets. Indigenous chicken have anatomical and physiological adaptations to compensate for variations in the nutrient concentration of the diet. Kondra et al. (1974), in a study using strains of meat and egg-type chicken fed on high fibre, reported a significant increase in weight, size and number of various components of the digestive system. The study revealed that an addition of fibre to feed resulted in a relative increase in the weight of the alimentary canal, the crop, proventriculus, gizzard, length of the small and large intestines, caeca and total number and length of villi. Increase in size and number of various organs is considered to be an attempt to hold and process a relatively large volume of feed and extract the nutrients more efficiently (Kondra et al., 1974). The study concluded that chickens were capable of enlarging the length and weight of their digestive system, in accordance with the increased volume of feed of low nutrient density, so that required nutrients may be obtained. The anatomical and physiological adaptation to utilise diets of low and variable quality could be more advanced and complex in IC. Moreover, IC adaptability may be boosted by the possible adaption to local feeds found in the immediate environment.

Parasite tolerance

Most poultry kept in free-range scavenging systems are infected with various sorts of endo- and ecto-parasites. All rural scavenging chickens harbour one or more species of endo-parasitic worms. The most commonly encountered helminthes in scavenging systems are nematodes; *Ascaridia galli, Capillaria anatis, Capillaria contorta, Capillaria bursata, Capillaria obsignata, Cheilospirura hamulosa, Dispharynx nasuta, Gongylonema ingluvicola, Heterakis gallinarum, Strongyloides avium, Syngamus trachea, Tetrameres Americana* (Bagust, 1994). The common cestodes are *Amoebotaenia cuneata, Choanotaenia infundibulum, Hymenolepis cantaniana, Hymenolepis carioca, Raillietina echinobothrida, Raillietina tetragona and Raillietina cesticillus* (Bagust, 1994). In a study on the prevalence of IC gastro-

intestinal endoparasites in Kenya, Kaingu et al. (2010) used 710 adult free-ranging local chickens sampled from six districts, Kakamega (162), Bondo (81), Narok (81), Bomet (150), Turkana (70) and West Pokot (166). In that study, it was observed that 192 (27.04%) were infected with coccidial oocysts, 182 (25.63%) with *Ascaridia galli,* 10 (1.41%) with *Heterakis gallinarum*, 2 (0.3%) with *Syngamus trachea*, 37 (5.21%) with *Capillaria retunsa*, 8.45% with *Capillaria annulata*, 21 (2.96%) with *Raillietina tetragona*, 94 (13.24%), while 112 (15.8%) had no helminthes infestation. The most common ectoparasites included *Menacanthus stramineus, Menacanthus cornutus, Goniodes gigas, Lipeurus lawrensis tropicalis, Echidnophaga gallinacea, Menopon gallinae, Argus persicus, Cnemidocoptes mutans* and *Gonoicotes gallinae* (Njunga, 2003). It has been established that although they do not directly cause disease in the host, they weaken the immune system and can cause increased susceptibility to other more harmful disease agents (FAO, 2010). Most rural farmers are not aware of the presence of worms in their chickens, hence virtually no control measures are taken as long as the birds are still able to survive and reproduce. A study by Schou et al. (2007), revealed the existence of 24 major histocompatibility complex (MHC) haplotypes in IC, although only one exerted incomplete resistance to the helminth *Ascaridia galli*. Resistance to H*. beramporia, A. galli* and *T. mothedai* worms is linked to allele 276 whilst 251 and 264 are associated with increased susceptibility to *R. tetragona* (Schou et al., 2007). The MHC variability suggests that IC host different MHC genes which are associated with immune response, performance and life history strategies.

Disease resistance or tolerance

Indigenous chicken have a reputation for hardiness and resistance to diseases, and less susceptible ecotypes have been reported in different countries. In Egypt, Mandarah ecotype (Hassan et al., 2004), Poule De Benna ecotype in North and West Africa and Nkhuku ecotype in Southern Africa (FAO, 2007) have been reported to be able to endure Newcastle disease. Hassan et al. (2004) reported that the Mandarah ecotype in Egypt can withstand infectious bursal disease virus. Mdegela et al. (1998) and Msoffe et al. (2001) found that the Kuchi (game) ecotype was not easily affected by fowl typhoid, and Oluyemi et al. (1979) reported the Fayoumi breed to be less vulnerable to avian leucosis complex.

Among the genotypes, frizzled and naked-neck birds have been described as more disease resistant than other genotypes. Mahrous et al. (2008) revealed that these genotypes have a higher total antibody titre compared to their normal-feather counterparts. Using India ink, Hamal et al. (2006) demonstrated the phagocytic ability of naked-neck and frizzled birds to be more efficient compared to normally

feathered genotype. Moreover, the ability to efficiently dissipate heat can promote the immune system. From these studies it can be concluded that naked-neck and frizzled feathered can withstand some diseases.

Different alleles have been correlated either positively or negatively with certain traits. Allele 205 is positively associated with higher primary antibody responses against Newcastle disease (NCD), 307 is negatively correlated with elevated primary antibody responses against NCD and positively correlated with bodyweight (Lwelamira et al., 2008). Attention needs to be focused on breeding for resistance to reduce the levels of diseases, zoonotic transmission and economic losses. Although breeding for disease resistance is usually not straightforward, there could be negative correlations between disease resistant and production traits.

Kenya is already experiencing a number of problems due to climate change and variability (CVC) including more frequent droughts, prolonged dry spells, increased heat stress and disease outbreaks (IPCC, 2007). CVC can lead to a shift in the forage type available, their quality and quantity and, indirectly, affects IC performance. The quality and quantity of the forage materials is likely to be affected by impact of CVC due to changes in forage growth and dry matter (DM) yield, as increase in temperatures may increase lignification of plant tissues, reducing the digestibility and the rates of digestion. This may consequently lead to reduced nutrient availability for IC and ultimately to a reduction in performance. Conversely, CVC generally affect distribution and abundance of predators, competitors, disease vectors and parasites, which not only leads to the emergence of new diseases but also affects disease pattern. Therefore CVC effects on IC can limit their potential for providing food, nutrition, income and job securities to the human population. In the face of CVC challenges, adaptation of IC to tropical conditions (heat stress, poor quality, more frequent disease and parasite challenges) is imperative and has created a necessity for promoting the conservation of IC to maintain populations. Therefore, there is a need for sustainable breeding strategies to be developed which take into conserve IC as well as account for their economically important and unique attributes.

How can we conserve?
Conservation options for efficient egg and meat production
Options for in situ conservation (through utilization for egg and meat production) of IC are presented under the following four scenarios that ensure that the genotype is matched with the environment.

1. In the long term, under good management conditions, exotic chicken or their crossbreds with indigenous will most likely predominate (Option 1)

2. In systems where both eggs and meat are important and the management is suboptimal, there is a need for a dual-purpose chicken that is well adapted to these environments (Option 2).

3. In hot environments, exploitation of major genes that allow better heat dissipation may be suitable option of chicken production (Option 3).

4. In rural areas where the scavenging system is an integral part of the farming systems requiring low-inputs, low-output and periodic destruction of a large portion of the flock due to outbreaks of diseases, dependence on adapted indigenous chicken will exist for the foreseeable future (Option 4).

Option 1

Management conditions can be of two types, one is completely intensive (flock confinement under climate-controlled environment, use of commercial feeds and provision of health care) and the other with non-optimal conditions (housing not completely climate control environment). Two types of breeding program may be needed for these production environments, one which focuses on the use of purely exotic chicken and the other which uses hybrid birds. Exotic chickens are bred for industrial production because of their commercial efficiency. The system is run completely on a profit basis; therefore stocks are genetically selected for economically important traits of fast growth, high production and reproduction. When technical conditions are not optimum, and for farmers who want to keep exotic breeds and, at the same time, maintain the quality products and unique attributes of IC under this system may well need a breeding program which produces hybrid birds. Hybrid birds are created by crossing IC that has been selected for high performance with exotic breeds. Such hybrids benefit from directly transmitted gene effects of both the sire and dam but lack maternal effects, as defined as any influence of a dam on its progeny, excluding those from directly transmitted genes (Legates, 1972). Although maternal effects are apparent, dam-hens do not control chicks in this option, hence post hatch maternal effects are not expected.

Currently, some of the commercial breeders have included liveability in their breeding objectives which is an indirect way of increasing survival and disease resistance within the commercial flocks (Preisinger and Flock, 2000). Indigenous chickens with diverse alleles and MHC haplotypes are useful in crossbreeding as a means of promoting survivability and disease tolerance of commercial exotic birds. This option is most suitable for companies, organisations, financially stable individuals and employees in developing countries who want to invest in chicken farming, but is not practical for poor rural farmers.

OPTION 2

In this system, provision of inputs is low to medium. Water, supplementary feeds, night shelters, and a small amount of medication are provided. Birds are left to free range, and scavenged feed constitutes the substantial part of the total feed consumed, giving flocks that have low input costs with improved productivity and are highly adapted to the harsh scavenging conditions. Development of suitable synthetic breeds may be a better option for this scenario. A synthetic breed benefit from the unique attributes of the foundation breeds. Synthetic breeds, created through selection and crossbreeding, have improved growth traits, feed efficiency, sexual maturity and egg production abilities. Improved IC can be utilised as a foundation stock in the development of a synthetic breed. The ecotype lines selected are subjected to several generations of selection with emphasis on growth and egg production, until homogeneity is achieved. For example, the Fayoumi breed in Egypt and Desi breed in India were developed through several generations of selection and crossing. In Kenya, breeding programs may focus on IC ecotypes which are currently under evaluation to establish synthetic breeds. These ecotypes include Kakamega, Siaya (Bondo), Narok, Turkana, West Pokot, Taita Taveta, Lamu and Bomet. Organisational structures for breeding programmes have to be set-up which promote uptake of its products, as farmers are the clients of the breeding scheme. Farmers influence the breeding goal, as these depend on their customers' demands and preferences. To improve farmer's uptake of the program products, incentives suggested by FAO (2010) include free package of product and management training, frequent farm visit, health care provision (e.g. vaccinations), identification and rewarding best performing farmers are needed.

OPTION 3

In hot environments, where chicken are reared under scavenging systems, naked-neck and frizzle feathers IC genotypes may be suitable in the future, as they have a number of genes with major effects on the phenotype that are of special interest in controlling body temperatures in hot regions. These genes are known to restrict and affect the structure of the feathers which enable heat dissipation by convection. The advantage of these genes over their normally feathered counterparts in a hot, humid environment is evident in terms of feed intake, growth rate (Deeb and Cahaner, 1999) and weight gain (Yaccin et al., 1997). Naked neck and frizzle feather genotypes have increased growth rates, body weights, feed conversion, egg production and disease tolerance in tropical temperatures above 25°C (Mahrous et al., 2008). For example, the feather restriction or Naked Neck

gene results in 40% less feather coverage. This considerably reduces the need for protein input for feather production. Genetic improvement through selection within the flock could be a promising and sustainable strategy which could produce annual genetic changes of between 0.5-4%, in relation to the mean of the single or multiple traits that are of interest (FAO, 2010).

OPTION 4

In rural areas, the scavenging production system is predominant. The system is an integral part of farming where no feed is supplied at all and, instead, chicks and mature chickens are left to forage. Health care is not provided, although birds are sometimes provided with night shelters. Limited supplies of grains and vegetable proteins have led to competition between animals and man for these products, thus limiting the intensification of chicken farming. For grain-deficient countries, one solution may lie with the genetic improvement of the IC. The economic strength of IC lies in the low cost of production when compared to the value of the outputs. Such strategies have been used to develop IC in Egypt (Kosba et al., 2006), Iran (Kamali et al., 2007) and high yielding exotic hybrids (Siegel et al., 2006), and the birds described in option 2 and 3 could be used as breeding stock. Within a population kept under similar scavenging conditions, birds are first evaluated and best performing individuals (depending on the objective) are considered for subsequent improvement.

Who are the stakeholders and what are their roles?

Options for genetic improvement and in situ conservation of IC have been presented under the four scenarios described above, however the major challenge is determining who will implement these plans, who will convince the farmers, and what are their roles.

Stakeholders comprise nucleus flocks, farmers, collaborators (institutions involved in IC research activities), farmer's training centres and extension agents, cooperatives, consumers, networks, policy and planning developers. The stakeholders and their potential roles are presented in Figure 2.

Nucleus flocks, where genetic improvement is the major activity, need to be owned by a commercial breeding company (which is less dependent on external funding) or a research institution. They are responsible for spearheading the definition of breeding goals, implementation and evaluation of breeding schemes in collaboration with stakeholders in IC value chain.

Farmers are the clients of the breeding scheme and have responsibility for day-to-day management of the production population. They can influence the breeding goals depending on their customers' preferences for IC and IC products.

The main collaborators in the breeding program include national agricultural research systems (NARS), government and non-governmental organisations (NGOs). The NARS groups are responsible for the generation of new knowledge. Government and NGOs plays key roles in dissemination of the nucleus products.

Training centres have been set up for farmers to acquire and share knowledge, views and experiences. Extension agents are responsible for passing necessary knowledge and skills to farmers in all aspects of chicken breeding, feeding, diseases and parasites control and treatment and marketing. IC farmers need to be encouraged to form group enterprises, organised such that products from one enterprise will be an input in the next enterprise. Enterprises include farmers, processors, traders, co-operatives and extension services. To enhance efficient flow of products and services, capacity strengthening, micro-credit schemes, monitoring and evaluation systems need to be developed. Staff from government departments and NGO's will be needed to act as principal catalysts for the process of group formation, production and marketing. This way, farmers and traders will be able to intensify IC production and marketing, resulting in sustainable IC enterprises.

Consumers drive the programme in that they force the breeders and producers to focus breeding goals in ways that satisfy their (market) demands. Consumers influence the breeding traits through their preferences and purchasing power.
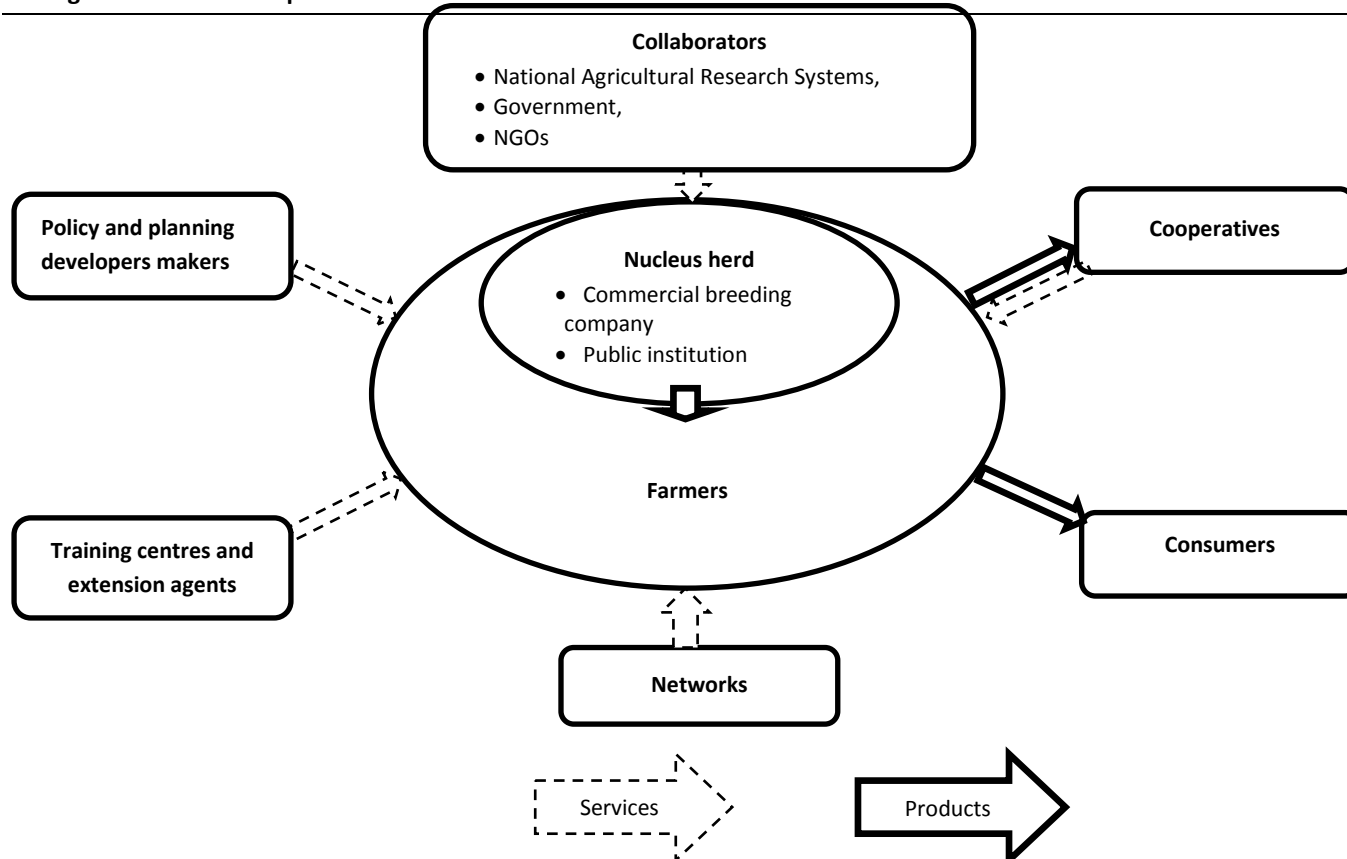
Figure 2 Organizational structures of the stakeholders in an Indigenous Chicken breeding programme.

Indigenous chicken improvement projects often fail because stakeholders' communication and involvement are inadequate. This paper proposes a novel method of including co-operating networks as stakeholder in IC breeding programs. Networks can bring together all other IC stakeholders. This can help IC stakeholders understand their networks and corresponding stakeholder involvement, allowing them to make more informed decisions. Networking could help researchers and policy makers to understand the problems encountered by IC farmers and how current policies and regulations affect the IC production value chain and which changes may be needed to improve the situation.

Conclusions

Indigenous chicken genetic resources dominate poultry flocks in rural villages, despite their low productivity, however they have great potential to contribute to improved rural livelihoods. However, a holistic approach that increases productivity without increasing production costs or leading to loss of biodiversity is currently not available. Conversely, in most developing countries, there is almost no organisational structure for breeding programmes for improving and conserving IC. Locally adapted IC can only be conserved in the most rational and sustainable way by ensuring that they are a functional part of different production systems. Their conservation should be through utilisation, if they are to be of any benefit to the poor rural households. This paper suggested options for *in-situ* conservation of IC via egg and meat production under four scenarios that ensure that the genotype is matched with the environment. Currently, options two and four are best suited for low to moderate income earners and poor rural farmers respectively, whereas option three suits to farmers in dry and warm regions. These suggested strategies could improve IC productivity leading to improved livelihood in rural households who are custodians of these indigenous genetic resources. Value chains generally have several players involved, and it is important that the role of various stakeholders in the IC production chain is clearly specified and their networks are well established.

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3

Morphological features of indigenous chicken ecotype populations of Kenya

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Abstract

This study characterized indigenous chicken (IC) ecotypes morphologically. Five IC ecotypes studied were Kakamega (KK), Siaya (BN), West Pokot (WP), Narok (NR) and Bomet (BM). Data on morphological features were collected from 1580 chickens and 151 for zoometric measurements. Descriptive statistics, non-parametric and F tests were used in analysis. A non-parametric Kruskal–Wallis, Binomial test and Mann–Whitney U test was used to evaluate whether the ecotype have effects on the qualitative morphological variables. Zoometric measurements was analysed with the PROC GLM of SAS. Results revealed that, black, black-white striped, brown and red body plumage colours were significantly different ($P < 0.05$) between the ecotypes. Feather morphology (%) was not significantly different ($P > 0.05$). Distribution of body feathers (%), comb types (%) and zoometric measurements were significantly different ($P < 0.05$). Eye colours varied significantly ($P < 0.001$) within the ecotypes unlike between the populations. In conclusion, IC ecotypes studied are heterogeneous population with huge variability in morphological features.

Key words: ecotype, indigenous chicken, morphological features

Introduction

Indigenous chicken (IC) genetic resources are a heterogeneous population which exhibit vast phenotypic variability (FAO, 2012) without standard phenotypic characteristics. They vary in body sizes, comb types, colours (plumage, eye, skin, shank and earlobe colours), outline and feather contours (Teketel, 1986; Ndirangu et al., 1991; Dana et al., 2010; Kingori, Wachira, A.M. & Tuitoek, 2010; Cabarles et al., 2012). Distinct phenotypic variations among IC in different regions (ecotypes) have been documented in some countries. In Uganda, Ssewanyana et al. (2003b) reported a wide phenotypic variability in plumage, shank, eye, earlobe, comb, skin, feathers, feather distribution, body size, comb type, wattle and earlobe sizes among IC population found in the Soroti, Mbale, Jinja, Masaka, Sembabule and Mbarara districts. Similarly, large variations in plumage colours, comb types, skin colours, shank colours, eye colours, earlobe colours and body positions among Ethiopian IC ecotypes (Tilili, Horro, Jarso, Tepi, Gelila, Debre-Elias, Melo-Hamusit, Gassay/Farta, Guangua and Mecha ecotypes) have been reported (Tadelle et al., 2003a; Halima, 2007; Bogale, 2008; Dana et al., 2010; Abera and Tegene, 2011). In Kenya, morphological variations of IC population have been reported by Ndirangu et al. (1991), Maina (2000), Njenga (2005) and Nyaga (2007). However, IC morphological characterization studies in Kenya were not based on the ecosystems and information of IC distributed in the specific regions of the country is presently limited. Each agro-ecological zone is anticipated to host chicken exhibiting different morphological characteristics. Therefore, there is a need to distinctively characterize morphologically IC populations in each agro-ecological zone.

The objective of this study was to characterize IC ecotypes morphologically. Information generated is crucial inputs to IC genetic improvement activities, future development of chicken breeds utilizing IC genetic resources and provide foundation for decision-making on conservation interventions needed.

Materials and methods

Study sites

The study was carried out in five administrative counties in Kenya; Bomet, Narok, Kakamega, Siaya and West Pokot counties. Counties were selected based on their geographical distances, ecological characteristics, coverage of the past chicken improvement programmes (distribution of exotic birds) and the socio-economic roles of IC (Okeno, 2012). In these counties, most rural households keep IC in rural households (MOLD, 2010; Okeno, 2012) and have wide variation in temperatures, annual rainfall and altitude.

Study population

Kakamega (KK), Siaya (BN), West Pokot (WP), Narok (NR) and Bomet (BM) ecotypes were studied. Indigenous chicken ecotypes were named according to the county of origin.

Sampling and data collection

Three divisions and three locations within each division in each county were randomly sampled. The households in the villages with highest number of IC in each location were recorded. Simple random sampling procedure was used to select households for interviews by randomly picking names of the households from the list. A pretested structured questionnaire was used to gather information. The main features in the questionnaire related to chicken morphological characteristics and flock size per morphological characteristics. Data were collected from free ranging IC through direct observations. Data on morphological features (qualitative data) collected included feather morphology, feather distribution (body and head), body plumage colours, skin colours, earlobe colours, comb types, eye colours, and shank colours. Zoometric measurements collected were body weight (BW) and shank length (SL). Measurements were taken in centimetres using a tape measure for SL and a digital weighing scale for BW. Only mature chicken (older than 8 months of age) were considered for morphological characterization.

Data were collected based on Cuesta (2008), Francesch et al. (2011), Cabarles et al. (2012) and FAO (2012). A total of 98, 122, 99, 96 and 87 IC farmers were interviewed in BN, KK, BM, NR and WP counties, respectively. Qualitative traits data were collected from a total of 1580 IC from BN (285), KK (415), BM (287), NR (282) and WP (311). Zoometric measurements data were collected from 151 IC from BN (31), KK (32), BM (29), NR (28) and WP (31).

Statistical analysis

Descriptive statistics were generated using frequency procedures and cross-tabulation using SPSS (SPSS, 2011). A non-parametric Kruskal–Wallis test was used to evaluate whether the ecotype have effects on the qualitative morphological variables (Dana et al., 2010; Cabarles et al., 2012). Variables with overall significant test based on Kruskal–Wallis test were followed-up with a Mann–Whitney U test to examine unique pairs. A Binomial test was used to analyse the significance of the differences in feather morphology (normal or frizzle), head feather distribution (crested or normal) and skin colour (white or yellow). The PROC GLM of SAS (SAS, 2008) was used for analysis of variance for BW and SL. A model that accounted for the fixed-effects of ecotype, sex and interaction between ecotype and sex was

fitted. The age of the bird was not included in the model because only adults, 8 months or older were sampled. Least-squares means were separated using least significant difference (LSD) option. The linear model fitted was:

$$Y_{ijk} = \mu + a_i + b_j + (ab)_{ij} + \varepsilon_{ijk}$$

where Y_{ijk} is the response expected in the dependent variable, µ is the mean of the population, ai is the effect of ecotype (i = KK, BN, BM, NR and WP), b_j is the effect of sex (j = Male or Female), $(ab)_{ij}$ is the effect of interaction between ecotype and sex and ε_{ijk} is the random error.

Results and discussion

Body plumage colours

Body plumage colours are presented in Table 1 and Figure 1. Among the body plumage colours, percentages of black, black-white striped, brown and red were significantly different (P < 0.05) between the ecotypes. BM, NR and WP ecotypes was dominated by black body plumaged chicken. BN ecotype had predominantly brown body plumage, whereas KK were dominated by black and white. Body plumage colouration play a role in survival success of IC raised under scavenging system characterized by frequent attack from predators. Indigenous chicken susceptibility levels to predators depend on the camouflaging ability to their numerous local habitats. Differences in camouflaging ability and reaction response to predator attack might have led to diverse frequencies in body plumage colouration. Farmers have colour preferences which influence body plumage colour frequencies among the ecotypes. In Ethiopia, plumage colours frequencies have been affected by farmer's preferences (Dana et al., 2010). Conversely, IC challenges under scavenging environment such as interaction with feather degradation bacteria, which degrade feather pigments might have contributed to the observed varied frequencies of the body plumage colours. According to Goldstein et al. (2004), adaption to microbial infections especially from bacteria which degrades feather pigments results in different feather colours. Additionally, different plumage colours may be due to the adaptive significance in the thermoregulation (Hill and McGraw, 2006; Protas and Patel, 2008) under tropical conditions.

Distribution of body and head feathers

Body feather distributions in the different body plumaged chickens within each ecotype are presented in Table 2 and Figure 2. Body feathers were distributed normally across the ecotypes (61–69 percent) except in KK population. Kakamega

population was composed of normal (31 percent), naked-neck (31 percent) and crested (35 percent) chicken.

Distribution (%) of head feathers (crested or normal) for the different body plumage colours within the populations are presented in Table 3. Distribution of head feathers were significantly different (P < 0.05) between the different body plumage colours within each population. Normal head-feathered-type chickens were dominant over crested in all the body plumage colours in BN, BM and WP populations.

Feather distribution is of vital importance in IC because of their physiological roles especially on thermoregulation. Different ecotypes might have evolved in feather arrangement to allow them to adapt and radiate in their respective habitats. In hot environments such as KK where IC are reared under the scavenging system, naked neck chicken may have been suitable because it allows better heat dissipation than other genotypes. Low percentages of the naked (Na) neck genotype in other counties except KK may be due to farmers' selection preferences and cultural issues.

Table 1 Body plumage of five ecotype populations (percentage of chickens within the population).

Body plumage colour	BM %)	NR (%)	BN (%)	KK (%)	WP (%)	N (%)
Black	22.9^c	23.8^{bc}	14.2^a	19.0^b	22.5^{ab}	321(20)*
Blacks-white striped	9.3^b	9.1^a	14.2^a	9.2^a	10.3^a	163(10)*
Blacks-White spotted	11.5	14.5	9.5	10.8	11.9	183(12)
Brown	13.6^a	8.4^{ab}	20.0^a	7.5b	11.9^b	187(12)*
Red	9.0^a	12.3^{ab}	5.5^a	15.2^b	10.0^b	171(11)*
Red-brown spotted	9	7.3	12.7	7.5	11.6	150(9)
Red-brown	11.1	6.9	6.2	5.5	5.5	110(7)
Grey	4.3	2.8	4.7	6.7	5.8	79(5)
White	8.2	14.5	12.7	18.6	9	206(13)
Other colours	1.1	0.4	0.3	0	1.6	10(1)
N	287	282	285	415	311	1580

BM, Bomet ecotype; KK, Kakamega ecotype; BN, Siaya ecotype; NR, Narok ecotype. N, figures within each column of body plumage; %, percent of total N within a population; N (%), figures and their percentage of each body plumage.

Note: Asterisks in last column indicate significant differences between ecotypes (columns) at the 5 percent (*) probability levels, based on the Kruskal–Wallis test.

[abc]Means in a row with one or more letter superscripts in common are not significantly different (P ≥ 0.05) based on the Mann–Whitney U test.

Black   Black-white striped   Black-white spotted

Brown   Red   Red-brown spotted

Red-brown   Grey   White

Figure 1 Body plumage colours of indigenous chicken ecotypes.

Feather morphology

Feather morphologies (normal or frizzle feathers) are presented in Table 2 and Figure 3. Two types of feather morphology were observed with normal feathered chickens being the majority. Statistical analysis revealed that feather morphology between the ecotypes was not significantly different (P > 0.05). Frizzle feathered chickens with the genes known to affect the structure of the feathers and enable heat dissipation constituted less than 10 percent. Frizzled chickens are crucial for cultural and traditional activities through their roles in rituals and sacrifices (Ojo, 2002) and may have contributed higher demand hence low frequency observed in

the study. For instance in KK county, frizzled IC are used for cultural purposes such as protection from witchcraft and payment of dowry. Conversely, frizzle feathering gene (F) is lethal when homozygous (Fayeye and Oketoyin, 2006) which may explain the low frequency in adult frizzle chicken in this study. Since homozygous F gene is lethal, farmers who may be aware of the condition may have done crossbreeding with other genotypes to conserve the allele.

Variations in shank and skin colours

Shank colours (white, yellow, green and black) with their respective percentages within each ecotype are presented in Table 2 and Figure 4. Results showed that majority of BM, NR, KK and WP population had yellow shank. White shanks contributed almost half (49 percent) of the BN population followed by yellow (39 percent). Skin colours (white or yellow) for the different ecotypes are presented in Table 2. Occurrences of white and yellow skin were significantly different (P < 0.001) within an ecotype but similar (P > 0.05) between the ecotypes. White skin was common in NR (52 percent), BN (62 percent) and WP (58 percent) population compared with yellow. Kakamega population recorded equal (50 percent) in both white and yellow skin colours.

Skin and shank colours are used as indicators of chicken health (nutritional and immune status), foraging efficiency and sexual attractiveness (Blount et al., 2003; Blas et al., 2006; Eriksson et al., 2008). Different skin colours observed in this study were in the range reported for Ethiopian IC where 52 and 48 percent had yellow and white skin, respectively (Dana et al., 2010). Colour diversity originates from the diet depending on the presence or absence of Oxycarotenoids (Seemann, 2000). Significant differences in shank and skin colours within the populations could be as a result of diet found in their local habitat, variation of genes from the ancestral lineages and the effect of consumer preference. Genetically, shank colour is controlled by three genes; dermal melanin (id+), inhibition of dermal melanin (Id), black extension factor (E) and autosomal white (W+) genes located in the Z sex chromosome (Smyth, 1990). White skin alleles are presumed to originate from red jungle fowl (*Gallus gallus*), whereas yellow skin is from hybridization of grey jungle fowl (*Gallus sonneratii*), Ceylon jungle fowl (*Gallus lafayettii*) and red jungle fowl (Eriksson et al., 2008; Cabarles et al., 2012) and might be the reasons for the different colours in IC population studied.

Table 2 Morphology and distribution of feathers, comb types and colours (skin, shank and eye) of five IC ecotypes.

		BM (%)	NR (%)	BN (%)	KK (%)	WP (%)	χ2
Feather morphology		**	**	**	**	**	5.6
	Normal	100	100	100	91	96	
	Frizzle	0	0	0	9	4	
Skin colour		**	**	**	**	**	6.4
	White	46	52	62	50	58	
	Yellow	54	48	38	50	42	
Body feather distribution		**	**	**	**	**	15.6**
	Normal	69	77	61	31	69	
	Naked neck	17	9	6	31	13	
	Feathered shank	7	1	22	3	11	
	Muff and bearded	0	4	0	0	0	
	Crest	7	9	11	35	7	
Shank colour		**	**	**	**	**	12.8*
	White	21	28	49	21	34	
	Yellow	75	38	39	53	39	
	Green	0	19	6	13	9	
	Black	4	15	6	13	18	
Comb type		**	**	**	**	**	9.8*
	Single	83	100	100	100	96	
	Pea	4	0	0	0	0	
	Rose	0	0	0	0	4	
	Cushion	13	0	0	0	0	
Eye colour		**	**	**	**	**	1.6
	Orange	87	63	89	92	76	
	Brown	9	16	0	0	16	
	Red	4	11	0	0	0	
	Pearl	0	11	11	8	8	
N		287	282	285	415	311	

BM, Bomet ecotype; KK, Kakamega ecotype; BN, Siaya ecotype; NR, Narok ecotype. N, figures within each column; %, percent of total N within a population. Note: Asterisks indicate significant differences between rows at the 5 percent (*) and 1 percent (**) probability levels, based on the Binomial test for feather morphology and skin colour and the Cochran test for distribution of body feathers, comb types, shank and ear lobe colours. χ2, the χ2 values in last column with "*" (5 percent) or "**" (1 percent) denote significant differences between columns (ecotypes) based on the Kruskal–Wallis test.

Normal	Naked neck	Feathered shank

Muff and bearded   Crested

Figure 2 Body feather distribution of indigenous chicken ecotypes.

Table 3 Distribution of head feathers (crested or normal) within ecotype populations (percentage of chickens within each population).

Body plumage colour	Head feathers	BN (%)	KK (%)	BM (%)	NR (%)	WP (%)	χ2
		**	**	**	**	**	
Black	Crested	1.1	5.9	1.4	0.4	1.1	7.7
	Normal	13.1	13.1	21.5	23.4	21.4	7.7
Black-white striped	Crested	2.1	3.9	0.1	0.6	0.9	21.5**
	Normal	12.1	5.3	9.2	8.5	9.4	21.5**
Black-white spotted	Crested	1.1	2.1	0.4	0.7	1.0	2.9
	Normal	8.4	8.7	11.1	13.8	10.9	2.9
Brown	Crested	3.4	3.9	0.2	4.2	1.1	16.1**
	Normal	16.6	3.6	13.4	4.2	10.8	16.1**
Red	Crested	0.9	5.6	0.5	0.4	0.3	6.3
	Normal	4.6	9.6	8.5	11.9	9.7	6.3
Grey	Crested	0.3	2.4	0.8	0.5	0.3	5.5
	Normal	4.4	4.3	3.5	2.3	5.5	5.5
White	Crested	1.7	5.5	0.1	0.7	0.8	3.3
	Normal	11	13.1	8.1	13.8	8.2	3.3
Red-brown spotted	Crested	0.3	3	0.3	0.6	0.5	10.6*
	Normal	12.4	4.5	8.7	6.7	11.1	10.6*
Red-brown	Crested	0.1	2.7	2.8	0.6	0.8	7.6
	Normal	6.1	2.8	8.3	6.3	4.7	7.6
Others	Crested	0.0	0.0	0.4	0.0	0.2	2.3
	Normal	0.3	0	0.7	0.4	1.3	2.3
N		285	415	287	282	311	

BM, Bomet ecotype; KK, Kakamega ecotype; BN, Siaya ecotype; NR, Narok ecotype.

N, figures within each column; %, percent of total N within a population.

Note: Asterisks indicate significant differences between rows at the 5 percent (*) and 1 percent (**) probability levels, based on the Binomial test for head feathers (crested or normal); χ2, the χ2 values in last column with "*" (5 percent) and 1 percent (**) denote significant differences between columns (ecotypes) based on the Kruskal–Wallis test.

| Normal | Frizzle |

Figure 3 Feather morphology of indigenous chicken ecotypes.

Comb types

Comb types (single, pea, rose and cushion) within and between the ecotypes with their respective percentages are presented in Table 2 and Figure 5. Comb types varied significantly (P < 0.001) within and between the ecotypes and could be associated with the variation in prevailing climatic conditions in their rearing environments and the effect of comb genes (Somes, 1990b; Duguma, 2006). All the ecotypes were dominated by single comb (above 83 percent) and in agreement with the reports by Egahi et al. (2010) and Apuno, Mbap and Ibrahim (2011) on Nigerian IC and contrary to those reported in Ethiopia by Dana et al. (2010). Comb is important in scavenging IC as it acts a cooling mechanism under hot tropical conditions because chickens cannot sweat. Single comb in IC helps in losing body heat up to 40 percent under prevailing environmental temperature of 80°F and below (Nesheim Austic and Card 1979).

| White | Yellow | Green | Black |

Figure 4 Shank colours of indigenous chicken ecotypes.

| Single | Pea | Cushion | Rose |

Figure 5 Comb types of indigenous chicken ecotypes.

Eye colours

Eye colours (orange, brown, red and pearl) for the different ecotypes are presented in Table 2. The widely distributed eye colour among the population was orange (>62 percent), whereas brown, red and pearl were below 17 percent. Eye colours within an ecotype varied significantly ($P < 0.001$) but not between ecotypes ($P > 0.05$). Within ecotypes diversities in eye colour could be attributed to genes of an individual, which affects blood supply and melanin levels, environmental effect in terms of availability of carotenoids and interaction of blood supply, melanin and carotenoids (Smyth, 1990; Stoddard and Prum, 2011; McCartney et al., 2014). Variation in colour is as a result of pigmentation of a number of structures within the eye (iris, retina, uveal tract and ciliary) due to sex-linked dermal melanin genes (id+ and idM) and its correlation with other genes expressing colours to other parts (Smyth, 1990; Cabarles et al., 2012) of the chicken body.

Table 4 Earlobe colours within different body plumage colours for each IC ecotype.

Body plumage colour	Earlobe colour	BN	KK	BM	NR	WP	χ2
		**	**	**	**	**	
Black	White	2.8	6.8	6.2	3.4	6.4	17.8**
	Red/Pink	5.3	8.9	12.7	10.0	12.4	
	Black	6.1	3.3	4.0	10.4	3.7	
Black-white stripes	White	3.9	3.8	2.8	3.2	3.3	4.1
	Red/Pink	7.6	4.7	6.5	4.3	6.0	
	Black	2.7	0.7	0.0	1.6	1.0	
Black-white spotted	White	2.3	3.3	2.9	6.1	3.3	1.6
	Red/Pink	6.2	6.6	7.9	4.5	7.6	
	Black	1.0	0.9	0.7	3.9	1.0	
Brown	White	4.4	2.8	3.6	2.2	1.8	11.4
	Red/Pink	11.2	4.7	7.6	4.2	10.0	
	Black	4.4	0.0	2.4	2.0	0.0	
Red	White	0.7	6.5	1.7	1.1	1.3	15.8**
	Red/Pink	2.9	7.5	6.9	10.7	8.5	
	Black	1.9	1.2	0.4	0.5	0.2	
Red-Brown spotted	White	2.2	2.6	2.7	2.3	2.9	1.7
	Red/Pink	9.4	4.4	6.2	4.2	8.4	
	Black	1.1	0.5	0.1	0.8	0.3	
Red-Brown	White	1.8	1.8	2.8	0.0	1.3	2.6
	Red/Pink	3.6	2.8	6.5	6.9	4.2	
	Black	0.8	0.9	1.8	0.0	0.0	
Grey	White	1.9	1.5	1.8	0.6	2.0	2.1
	Red/Pink	2.5	4.7	2.5	1.7	3.7	
	Black	0.3	0.5	0.0	0.5	0.1	
White	White	4.6	7.1	1.6	7.3	3.6	5.4
	Red/Pink	7.3	11.5	6.4	6.8	5.0	
	Black	0.8	0.0	0.2	0.4	0.4	
Other	White	0.3	0.0	0.0	0.0	0.0	1.3
	Red/Pink	0.0	0.0	1.1	0.0	1.3	
	Black	0.0	0.0	0.0	0.4	0.3	
N		285	415	287	282	311	

BM, Bomet ecotype; KK, Kakamega ecotype; BN, Siaya ecotype; NR, Narok ecotype. N, figures within each column; %, percent of total N within a population. Note: Asterisks indicate significant differences between rows at the 5 percent (*) and 1 percent (**) probability levels, based on the Cochran test.

χ2, the χ2 values in last column with "*" (5 percent) or "**" (1 percent) denote significant differences between columns (ecotypes) based on the Kruskal–Wallis test.
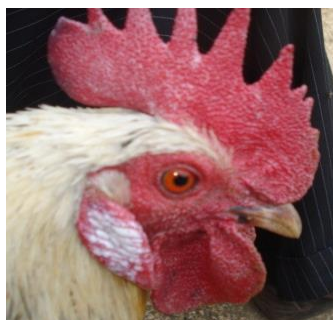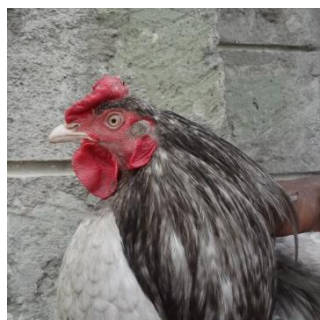
White earlobe   Red/pink earlobe

Figure 6 Earlobe colours of indigenous chicken ecotypes.

Earlobe colours

Table 4 and Figure 6 present the earlobe colours (white, red/pink and black). Generally, earlobe colours were significantly different (P < 0.05) within the populations. Red/pink earlobe was common in the populations. White earlobes were common in black-white spotted and white chicken. Earlobe pigmentation has a role in thermoregulation by absorbing or radiating solar radiation from chicken body (Stettenheim, 2000; Egahi et al., 2010). Differences in earlobe colours observed in this studied may be due to adaptability of IC ecotypes to their local habitats and differences in ancestral lineages. Ancestral lineages of white ear lobe are bankiva, murghi and gallus, and of red ear lobe are jabouillei and spadecius and their hybridization (Nishida et al., 2000; Ohta et al., 2000).

Shank length and Bodyweight

Variations in SL and BW for the different ecotypes are shown in Table 5. SL between ecotypes were significantly different (P < 0.05). Ecotypes with SL not significantly different (P > 0.05) were BM, KK, NR and WP as well as BN, KK and WP in another group. The longest shank was recorded in NR ecotype followed by BM, WP, KK and BN. SL can be used as a predictive live BW in IC particularly where weighing scales are not readily available, as is the case in most smallholder African rural farmers and meat markets (Mani, Abdullah and Von Kaufmann, 1991; Nesamvumi et al., 2000; Kabir et al., 2006). Shank lengths in chicken in this study were generally longer than Ethiopian (Dana et al., 2010) and Nigerian IC (Apuno, Mbap and Ibrahim, 2011) but in the range with some Tanzanian ecotypes (Msoffe

et al., 2001). Narok ecotypes with longer shanks are found in a relatively dry region with flat terrain and chicken may need to cover long distance in search of food.

Table 5 Least square means ± standard error of zoometric measurements of mature chicken under on-farm condition. Measurements were taken after one day of starving.

	BM	BN	KK	NK	WP
N	29	31	32	28	31
BW	1167.41± 69.56[b]	1367.45± 72.00[a]	1259.91± 58.65[ab]	1393.80± 76.96[a]	1348.81± 66.00[ab]
SL	10.92±0.32[a]	9.79 ± 0.35[b]	10.29± 0.31[ab]	11.08± 0.34[a]	10.43 ±0.32[ab]

BM, Bomet ecotype; KK, Kakamega ecotype; BN, Siaya ecotype; NR, Narok ecotype; N, number of chicken per ecotype; BW, Body weight; SL, shank length.
[abc]Means in a row with one or more letter superscripts in common are not significantly different (P ≥ 0.05) based on LSD.

Table 6 Least squares means ± standard error of bodyweight of mature female and male chicken under on-farm condition. Measurements were taken after one day of starving.

Ecotype	Female	Male
BM	1081.06 ±71.08[c]	1328.12 ±97.34[b]
BN	1321.66 ± 79.47[a]	1445.00± 104.06 [ab]
NR	1247.50 ±79.47[ab]	1643.57± 104.06[ab]
KK	1162.64 ± 66.77[bc]	1442.77 ± 91.77[ab]
WP	1108.12 ± 68.83[bc]	1776.66± 91.77[a]

BM, Bomet ecotype; KK, Kakamega ecotype; BN, Siaya ecotype; NR, Narok ecotype.
[abc]Means in a column with one or more letter superscripts in common are not significantly different (P ≥ 0.05) based on LSD.

Bodyweight were significantly different between the ecotypes (P < 0.05). On average, adults weighed 1 367, 1 259, 1 167, 1 393 and 1 348 g for BN, KK, BM, NR and WP, respectively (Table 5). BW for NR was not significantly different (P > 0.05) from BN, KK and WP. Significant difference (P < 0.05) between sexes with cocks being heavier than hens was recorded (Table 6). WP (heaviest) and BM (lightest) males were significantly different (P < 0.05). Females from BM and BN were the

lightest and heaviest, respectively. Narok, KK and WP females were not significantly different (P > 0.05), whereas BM hens were similar to KK and WP hens. The significant differences in average BW could be attributed to genetic make-up, feed availability and age of the birds (the precise age of birds were unknown due to lack records under on-farm).

Conclusion

Indigenous chicken ecotypes studied are heterogeneous population with large variability in morphological features. Black body plumage, normal feathers, single comb, white skin, orange eye colour and red/pink earlobes were the predominant morphological features.

Acknowledgments

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4

Genetic diversity of different indigenous chicken ecotypes using highly polymorphic MHC-linked and non-MHC microsatellite markers

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Abstract

The study investigated the genetic make-up of different ecotypes of indigenous chickens (ICs) in Kenya based on major histocompatibility complex (MHC)-linked and non-MHC microsatellite markers. Blood samples were collected from eight regions (48 birds per region) of Kenya: Kakamega (KK), Siaya (BN), West Pokot (WP), Turkana (TK), Bomet (BM), Narok (NR), Lamu (LM) and Taita-Taveta (TT) and genotyped using two MHC-linked and ten non-MHC markers. All MHC-linked and non-MHC markers were polymorphic with a total of 140 alleles, of which 56 were identified in MHC-linked markers. Mean number of alleles (Na and Ne), private alleles, heterozygosity and genetic distances were higher for MHC-linked markers compared with non-MHC markers. The *ad hoc* statistic ΔK detected the true numbers of clusters to be three for MHC-linked markers and two in non-MHC markers. In conclusion, ICs from Kenya belong into two to three genetically distinct groups. Different markers systems have different clustering system. MHC-linked markers divided ICs into three mixed clusters, composing of individuals from the different ecotypes whereas non-MHC markers grouped ICs into two groups. These IC ecotypes host many and highly diverse MHC-linked alleles. Higher allelic diversity indicated a huge amount of genetic variation in the MHC region of ICs and supported their reputation of being hardy and resistant to diseases.

Key words: ecotype, genetic diversity, indigenous chicken, MHC, population structure

Introduction

Indigenous chickens (ICs) (*Gallus gallus domesticus*) are widely distributed throughout Africa under diversified geographical and agro-ecological conditions. Geographically isolated IC populations are subjected to local climatic conditions and each region is thought to host some unique types of chickens, hereafter called ecotypes. Such ecotypes are anticipated to possess' unique combinations of alleles on genes that may confer adaptation to local environment (Mwacharo et al., 2007). These ecotypes may have evolved independently and genetically diverged as a result of natural selection. Some insight into Kenyan IC ecotypes (Kilifi, Taita, Muranga, Kisii, Kitui, Marsabit, Nandi, Meru, Homa Bay and Kakamega) have been achieved using microsatellite markers (Mwacharo et al., 2007, 2013). However, several ecotypes remain unknown. Furthermore, none of the studies described above did study the major histocompatibility complex (MHC) of ICs in different ecosystems.

Indigenous chicken populations are raised under scavenging conditions characterized by a high parasite and infectious disease agent load. In order to survive, these chickens have to display a large plasticity in their immune-related genes. The MHC is associated with immune response (Parmentier et al., 2004; Fulton et al., 2006; Nikbakht, Atefeh and Neda, 2013) and disease resistance (Lamont, 1989). Major histocompatibility complex can be used to study evolutionary process (Nikbakht, Atefeh and Neda, 2013). Microsatellite marker LEI0258 located within the MHC region has been used successfully in genetic diversity studies (Izadi, Ritland and Cheng, 2011; Chang et al., 2012).

The objective of this study was to investigate the genetic make-up of different ecotypes of ICs in Kenya using both MHC-linked and non-MHC autosomal microsatellite markers.

Materials and methods

Sampling

Blood samples were collected from different regions (counties) of Kenya following FAO guidelines (2011). The covered counties included: Kakamega (KK) and Siaya (BN) in the Western region; West Pokot (WP) and Turkana (TK) in North Rift; Bomet (BM) and Narok (NR) in South Rift; Lamu (LM) and Taita-Taveta (TT) in Coastal region (Supplementary Figure S1). Each county represents an ecotype. Two mature chickens per household located more than 0.5 km away from its neighbours were sampled, resulting in a total of 768 birds (i.e. 96 samples per ecotype). One bird per household was genotyped to reduce the probability of sampling genetically related birds (i.e. 48 per ecotype). All samples were collected from free ranging IC

populations. Blood samples (~2 ml in EDTA) were drawn from the wing vein of each bird.

DNA isolation, polymerase chain reaction (PCR) amplification and genotyping

Genomic DNA was obtained by standard phenol–chloroform extraction method. Individuals were genotyped with 12 microsatellite markers located on eight chromosomes (Supplementary Table S1). Two of these markers were MHC-linked markers (LEI0258 and MCW0371). The other 10 are non-MHC and are part of the 30 microsatellite markers recommended by ISAG/FAO (FAO, 2011) for chicken genetic diversity assessment. LEI0258 and MCW0371 were determined using PCR as described by McConnell et al. (1999) and Fulton et al. (2006).

Statistical analysis

MHC-linked markers LEI0258 and MCW0371 were examined together whereas ten non-MHC markers were analysed separately. Genetic diversity was assessed by calculating the number of alleles per marker and population (ecotype), observed (Ho) and expected (He) heterozygosity as well as fixation index (Fst) as a measure of genetic differentiation between populations. GenAlex software version 6.5b5 (Peakall and Smouse, 2012) was used to estimate observed mean (Na) and effective (Ne) number of alleles, Ho and He per population. Population software version 1.2.32 (Langella, 1999) was used for allele frequency and private allele identification. Population differentiation was estimated using Fst for each marker across ecotypes according to the variance-based method of Weir and Cockerham (1984) using FSTAT software Version 2.9.3.2 (Goudet, 2002). The significance of all the pair-wise Fst values was tested after permutations of multi-marker genotypes among samples and significance levels were reported after strict Bonferroni corrections to account for multiple comparisons (Rice, 1989). Genetic distances were calculated according to the Nei's standard genetic distance, Ds (Nei, 1972) and Dc (Cavalli-Sforza and Edwards, 1967) using Populations software version 1.2.32 (Langella, 1999). Analysis of molecular variance was performed to assess the percentage contribution of within and between population variations because of the geographic regions using the Arlequin 3.5 software (Excoffier and Lischer, 2011). The significance of the variance components was tested with 10 000 permutations. A Mantel test was used to investigate association between genetic differentiation (Rousset, 1997) and geographic distances (kilometres) between IC ecotypes using Isolation by Distance Web Service (IBDWS) version 3.23 (Jensen, Bohonak and Kelley, 2005). The significance level was calculated from 30 000 randomizations.

Structure software version 2.3.4 (Pritchard, Stephens and Donnerly, 2000) with the Bayesian model-based clustering method for inferring population structure using multi-locus genotypes was executed. The program was run 50 times for each assumed genetic cluster (K) value using the admixture and correlated allele frequencies model because IC ecotypes are likely to be similar due to common ancestry and frequent gene flow among nearly all the ecotypes within the terrestrial land of Kenya. Models with a burn-in period of 20 000 followed by 50 000 of Markov chain Monte Carlo iterations were implemented. Individuals were grouped into a predefined number of K clusters (K ranging from 1 to 8). Prior information on sampling locations was provided. Structure Harvester software version 0.6.93 (Earl and Vonholdt, 2011) was used to analyse the output of Structure program, to identify the optimal of clusters from K = 1 to 8. The *ad hoc* statistic ΔK, based on the rate of change in the log probability between successive K values, was used to detect the optimal numbers of clusters (Evanno, Regnaut and Goudet, 2005). Distruct software version 1.1 (Rosenberg, 2004) was used for graphical display. GSView software version 5.0 (Lang, 2012) was used to view Distruct postscript output. GenAlex software version 6.5b5 (Peakall and Smouse, 2012) was used for principal coordinate analysis (PCoA). The PCoA axes 1 (PC1) and 2 (PC2) were used to plot graphs.

Results
Genetic variability

All MHC-linked and non-MHC markers typed were polymorphic with a total of 140 alleles, of which 56 (40 percent) were identified in MCH-linked markers (Supplementary Table S1). Out of 56 alleles in MHC region, 46 alleles (194–550 bp) were observed in LEI0258 (Figure 1) and 10 for MCW0371 (198–207 bp). Observed number of alleles per locus ranged from 6 (ADL0268) to 46 (LEI0258). Twenty private alleles (14.3 percent of the total 140 alleles) were observed across eight IC populations (Table 1), of which 12 were detected in LEI0258 and 8 for non-MHC markers. Lamu ecotype had a higher number of private alleles compared with other ecotypes.

The Na and Ne per population were higher for MHC-linked markers compared with non-MHC markers (Table 1). Na per ecotype at MHC-linked markers ranged from 15.00 to 18.00 compared with 6.20 to 7.21 for non-MHC markers. Na for LEI0258 was higher than all other markers, ranging between 20 and 27 (Figure 1).
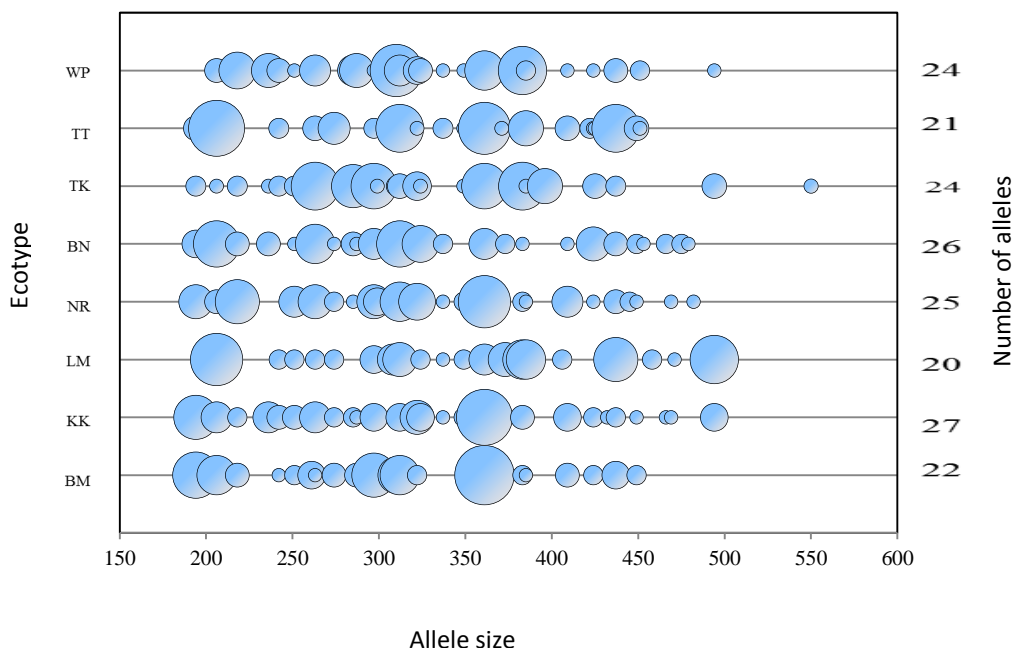
Figure 1 MHC-linked microsatellite marker LEI0258 allele frequencies in eight IC ecotypes. Alleles are identified by length in base pairs below and number of alleles per ecotype.

Among the ecotypes, KK had the highest but LM the lowest Na. MHC-linked markers had slightly higher levels of heterozygosity compared with non-MHC markers. Ho values per population ranged from 0.80 to 0.95 and from 0.67 to 0.71 for MHC-linked and non-MHC markers, respectively. He was higher for MHC-linked markers (0.84–0.88) than non-MHC loci (0.66–0.73). Values for He were slightly less than Ho in LM and TT at MHC-linked and non-MHC markers, respectively.

Table 1 Allelic patterns (Na, Ne, number of private alleles and He) in MHC-linked and non-MHC microsatellite markers across eight IC ecotypes in Kenya.

	Population	Na	Ne	Number of private alleles	Ho	He
MHC-linked microsatellite markers	BM	15.00	8.92	0	0.85	0.88
	KK	18.00	10.62	1	0.83	0.88
	LM	14.00	8.61	4	0.89	0.85
	NR	17.50	9.85	2	0.84	0.87
	BN	17.50	10.70	3	0.86	0.88
	TK	17.00	8.98	1	0.86	0.86
	TT	15.00	7.50	1	0.80	0.84
	WP	16.50	10.02	0	0.95	0.88
Non-MHC microsatellite markers	BM	6.30	3.47	1	0.69	0.70
	KK	6.70	3.72	1	0.71	0.72
	LM	5.70	3.24	0	0.67	0.68
	NR	6.50	3.42	1	0.69	0.70
	BN	7.10	3.82	1	0.70	0.73
	TK	6.40	3.23	2	0.67	0.68
	TT	6.20	3.06	1	0.67	0.66
	WP	6.70	2.72	1	0.68	0.71

BM, Bomet ecotype; KK, Kakamega ecotype; BN, Siaya ecotype; NR, Narok ecotype; TK, Turkana ecotype; TT, Taita-Taveta ecotype; WP, West Pokot ecotype.
Na, observed mean number of alleles; Ne, effective mean number of alleles; Ho, observed heterozygosity; He, expected heterozygosity.

Fst values varied between 0.01 and 0.03 for MHC-linked markers compared with 0.01–0.05 for non-MHC markers. Higher Fst values were observed for LM ecotype compared with the other populations. Ds and Dc between populations were higher in MHC-linked markers compared with non-MHC markers. Ds ranged from 0.02 to 0.33 and Dc from 0.13 to 0.46. Relatively long genetic distances were recorded between LM and the other ecotypes. Percentage of molecular variance for MHC-linked and non-MHC markers were high within individuals (>89 percent) compared among the ecotypes (<4 percent) and within individuals of an ecotype (<8 percent). A significant correlation between the genetic distances and geographic distances was observed. On average, MHC-linked markers produced a lower correlation coefficient (r) of 0.65 compared with 0.78 in non-MHC markers.

Population clustering

The *ad hoc* statistic ΔK detected the true numbers of clusters to be three for MHC-linked markers and two in non-MHC markers (Figure 2). PCoA-based principal coordinates 1 and 2 indicated three groups for MHC-linked markers and two in non-MHC markers (Figure 3). Although MHC-linked markers grouped ICs into three clusters, individuals from different ecotypes overlapped in principal coordinate space unlike distinct groups of LM (cluster one) and others (cluster two) for non-MHC markers.

Discussion

Genetic variability

All MHC-linked and non-MHC markers were highly polymorphic. Number of alleles including private alleles and their apparent difference in distribution across the ecotypes indicated the existence of genetic diversity between the populations. The genetic variations have been caused by differences in ancestral origin, migration routes, hybridization of origins, genetic drift, mutation and natural selection. ICs are anticipated to have multiple origins from wild ancestors in South Asia and Island Southeast Asia and have been introduced to Kenya through several entry points (Lyimo et al., 2013; Mwacharo et al., 2013a, 2013b). After introduction of ICs to Kenya, they spread separately via marine and multiple terrestrial routes (Mwacharo et al., 2013a, 2013b) and their variations could be due to adaptation to the local environmental conditions provided by the specific route. Ancestors of LM ecotype are anticipated to have originated from Asia and distributed to Kenya and Tanzania through common water body of Indian Ocean. Lamu ecotype has a local name as "Kuchi" which is similar in name to Kuchi ecotype in Tanzania. Kuchi chicken in Tanzania is similar to Shamo game birds from "Kõchi" Prefecture of Shikoku Island in Japan and its thought to be its origin (Lyimo et al., 2013). Genetic diversity of IC ecotypes might have been contributed by cross-breeding with chicken breeds from Asia and Europe. Cross-breeding started in 1950s in Kenya and was widely spread in the country through cockerel/pullet exchange programme initiated in 1976 (FAO, 2007).

MHC-linked microsatellite markers   Non-MHC microsatellite markers
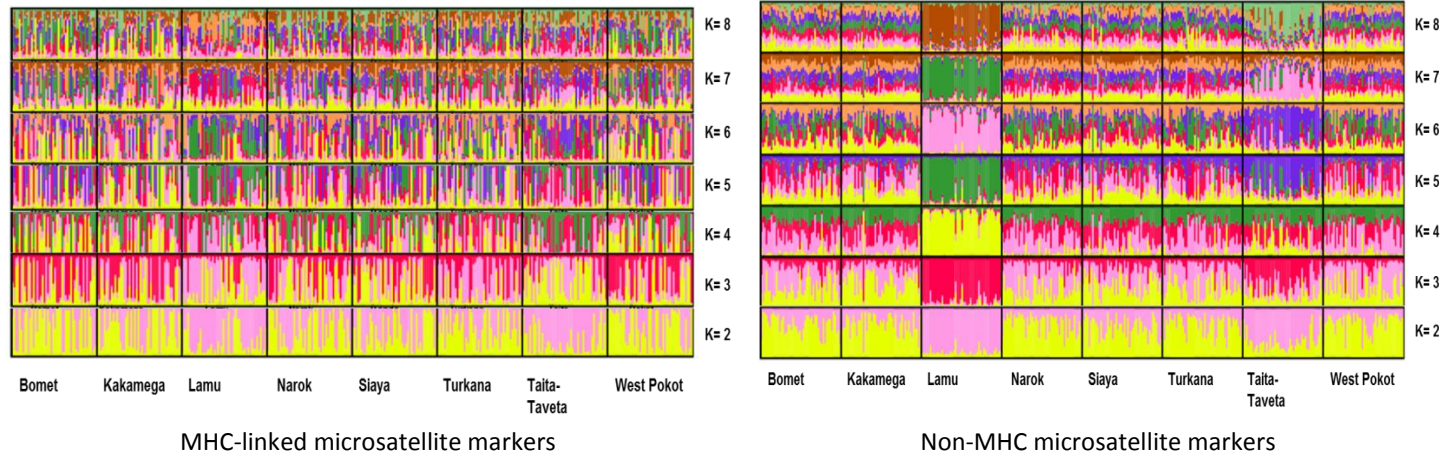
Figure 2 Proportions of admixtures observed in the eight IC ecotypes for MHC-linked and non-MHC microsatellite markers.

Number of alleles for LEI0258 per ecotype varied from 20 to 27 in the present study are in the same range as reported for Kuchi (22) and Medium (23) ecotypes in Tanzania (Lwelamira et al., 2008). Allelic diversity in MHC-linked markers is higher than non-MHC markers. The reason why the MHC region is so allele-rich and highly diversified is because survival is associated with additive allelic effects at the MHC locus (Eyto et al., 2007) and pathogen-driven selection (Nielsen, 2005). Such polymorphism of MHC region in ICs is maintained by pathogen-driven balancing selection because of varying pathogen resistance over space and time (Hughes and Nei, 1989; Hedrick, Lee and Garrigan, 2002). ICs studied are kept under harsh scavenging tropical conditions of varying heat stress and frequent disease outbreaks. A higher allelic diversity in the MHC region boosts IC's ability to tolerate disease challenges. However, this study did not prove the additive allelic effects of MHC locus.

Heterozygosity values for MHC-linked markers were higher than for non-MHC markers and in agreement with findings by Chazara et al. (2013) using wild species and unselected chicken populations. Ho were slightly lower than He in TT and LM ecotypes, suggesting selection against heterozygotes (Mogesse, 2007) and limited artificial selection (Dana, 2011) in these populations. Comparatively high Fst, Ds and Dc for LM population to the other ecotypes are as a result of restricted gene flow because of separation by Indian Ocean. Mantel test revealed positive correlation between genetic and geographic distances. Populations were sampled from eight different regions which are far apart (67–840 km) and the geographic distances had influence on the genetic structure of the farthest population (LM).

MHC-linked microsatellite markers   Non-MHC microsatellite markers
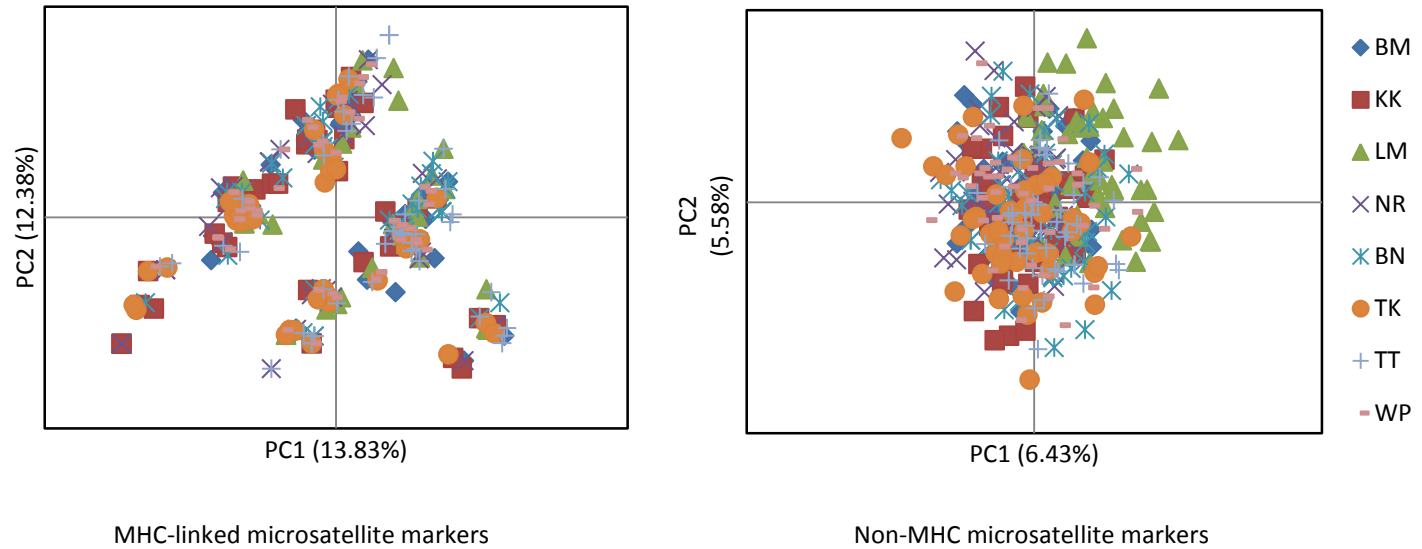
Figure 3 PCoA plot based on genetic distance for populations based on genetic distance for individuals for non-MHC and MHC-linked microsatellite markers.

Population clustering

Studying the genetic variability of ICs would be important in establishing whether the whole population may be treated as a single gene pool or whether it should be subdivided. Cluster analysis using MHC-linked markers indicated three genetically mixed clusters (i.e. each cluster is composed of individuals from all the ecotypes). Such clustering suggested similar directional selection in the MHC region of chicken clustered together. However, this study did not prove if the MHC is under selection. Non-MHC markers grouped ICs into two groups, LM (cluster one) and the other cluster composing of seven ecotypes (cluster two). Distinctness of LM from other IC populations indicated genetic uniqueness which could be as results of physical, geographical and socio-economic isolation of the populations. Main population barrier is Indian Ocean which isolated Lamu region from mainland. Population in cluster two are similar because of geographically proximity where, Western, North Rift valley and South Rift valley regions are very close, so it is easy for ICs to mix. Sharing of common routes from Nairobi to Turkana via Narok, Bomet, Siaya, Kakamega, and West Pokot might have made easier movement of ICs in these regions. Furthermore, residents of Western, North Rift valley and South Rift valley regions are sharing common working environment (huge tea plantations in Bomet, Kericho and Nandi counties). Interaction in such working place might have promoted the exchange of IC genetic material in form of gifts. Common cultures, living customs and intermarriages of tribes might have promoted frequent gene flow in the regions. Conversely, human interactions through trading between the studied regions as well as Kenya and other countries have led to mixing of chicken (Mwacharo et al., 2013).

Conclusion

Indigenous chicken from Kenya belong to two to three genetically distinct groups (LM and others). Different markers systems have different clustering system. MHC-linked markers divided ICs into three mixed clusters, composing of individuals from the different ecotypes whereas non-MHC markers grouped ICs into two groups (LM and others). These IC ecotypes host many and highly diverse MHC-linked alleles. Higher allelic diversity indicated a huge amount of genetic variation in the MHC region of IC and supported their reputation of being hardy and resistant to diseases. Natural balanced selection driven by pathogen has enabled ICs to maintain genetic diversity which is crucial for adaptation to harsh scavenging conditions and local disease challenges.

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Table S1 Characteristics of microsatellite markers used, including their chromosomal locations, fragment sizes, and number of alleles observed in overall samples.

Marker	Chromosome[1]	Expected Allele range (bp)[2]	Observe fragment size (bp)[3]	Observed number of alleles
ADL0268	1	102-116	101-112	6
MCW0111	1	96-120	91-118	10
MCW0183	7	296-326	288-322	10
MCW0206	2	221-249	225-249	7
MCW0222	3	220-226	300-318	7
MCW0034	2	212-246	217-246	12
MCW0037	3	154-160	175-187	8
MCW0067	10	176-186	175-187	7
MCW0069	26	158-176	158-177	10
MCW0081	5	112-135	114-135	7
LEI0258	16	-	194-550	46
MCW0371	16	-	198-207	10

[1] From the FAO (2011); [2] Expected allele size range from FAO (2011); [3] Detected allele size range (bp) in eight chicken populations.
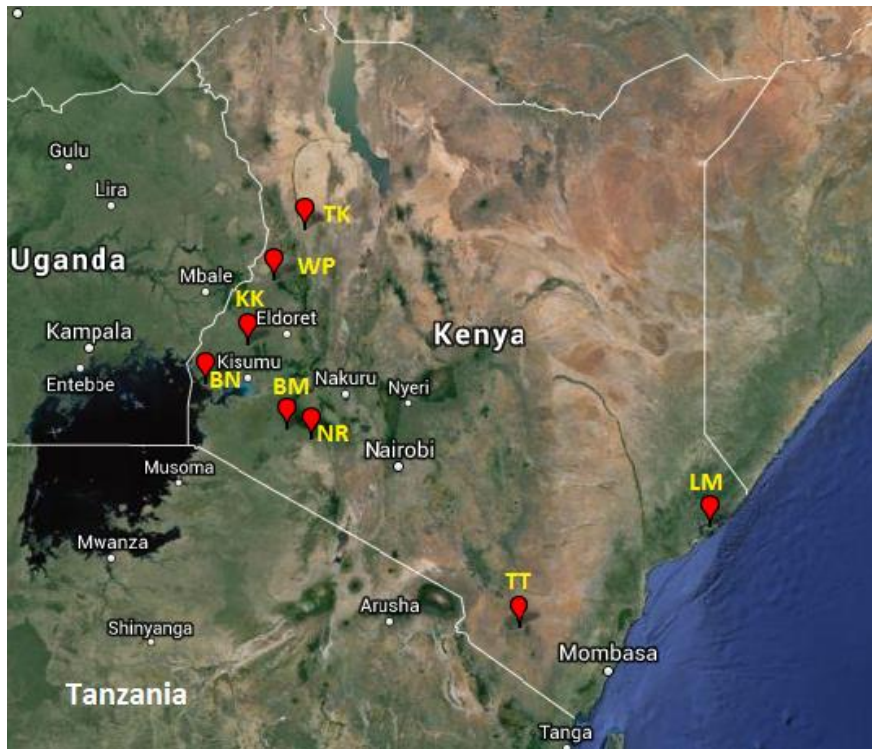
Figure S1 Map of Kenya showing locations where blood samples were collected; BM, Bomet ecotype; KK, Kakamega ecotype; BN, Siaya ecotype; NR, Narok ecotype; TT; Taita-Taveta; LM, Lamu.

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Genetic variation and signatures of selection in the genomes of Kenyan indigenous chicken and commercial layers

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Abstract

Breeding of indigenous chicken in developing countries is mainly done by the resource-poor rural households that raise birds in a scavenging system characterized by low inputs, adverse climatic conditions and high disease pressure. As a result, indigenous chicken may have developed specific genetic adaptations for such challenging environments. In this study, genomic variation of indigenous chicken kept under low input production systems was assessed using autosomal microsatellite markers and whole genome re-sequence data. Indigenous chickens were further compared to high input commercial layers to identify selection signatures and candidate mutations that may explain the phenotypic divergence between these populations. Commercial layers had much lower nucleotide diversity (0.31 - 0.36) than indigenous chicken (0.58 - 0.62). We also identified up to 59 genomic regions with high Fst values (0.44 - 0.85) between indigenous and commercial chickens, overlapping 16 genes. Five genes (S*LC26A8, BRPF3, MAPK13, PDIA4* and *MRPL32*) out of the 16 are associated with the missense variants that could explain partially the phenotypic divergence between these populations. Differently to commercial chickens, indigenous chickens preserved a high genomic variability that may be important, for addressing present and future challenges associated with adaptability to the environment and to cope with farmers breeding goals.

Key words: Genetic diversity, indigenous chicken, SNP, selection signatures, candidate mutations, genes

Introduction

Indigenous chicken (IC) populations are of importance for mitigation of income and food insecurity among the poor rural households in Africa. These domestic populations were introduced from South Asia and Island Southeast Asia (Lyimo et al., 2014; Wragg et al., 2012) and spread through several maritime and terrestrial routes (Mwacharo et al., 2013). During the dispersal process across the African continent, chickens have been subjected to diverse ecological conditions, diets, and diseases as well as reared in different sociocultural backgrounds. As a result, IC populations have a noticeable variety of phenotypes, displaying differences in body sizes, shank lengths, feather morphology, feather distribution (body and head), body plumage colours, skin colours, ear-lobe colours, comb types, eye colours and shank colours (Ngeno, 2011; Ngeno et al., 2014a; Ngeno et al., 2014b; Wragg et al., 2012). Nowadays, it is estimated that over 80% of the total chicken population in Sub-Saharan Africa are ICs, raised under extensive scavenging production system characterized by low inputs, harsh climatic conditions, poor nutrition and constant exposition to a wide variety of diseases (Ngeno, 2011; Okeno, 2013; Okeno et al., 2012). For instance, it has been observed that ICs kept under extensive production systems in Kenya, have typically high loads of gastro-intestinal parasites (Kaingu et al., 2010; Ondwassy et al., 2000).

Artificial selection driven by human needs has resulted in phenotypic, physiological and behavioural changes in chicken (Rubin et al., 2010). The selection pressure has been different in ICs from Africa compared to the international highly productive breeds. The first difference is the trait selection. In selection of IC, traits are more general, while in commercial populations the traits are more directed to the use of the bird either as broiler or layer. The second difference is the adaptation traits. Breeding of African chicken in challenging environments and production systems, explains their superior ability to withstand disease challenges associated with free-ranging and tropical heat stress (Magothe, 2012; Okeno, 2013). However, little is known of the underlying genetic basis that enables IC to adapt to these environments. Studying whole genomes by complete re-sequencing allows unravelling of mechanisms by which phenotypic diversity and adaptation to local environment are generated (Herrero-Medrano et al., 2014; Qanbari and Simianer 2014; Rubin et al., 2010; Wilkinson et al., 2013). In this study, genomic variation of IC kept under low input production system was assessed using autosomal microsatellite markers and whole genome re-sequenced data. Indigenous chickens were further compared to high input commercial layers to identify genomic selection signatures and candidate mutations that may explain the phenotypic divergence between these populations.

Methodology

Animals

A total of 384 samples from eight different regions in Kenya were genotyped with 12 autosomal microsatellite markers. The chickens selected belongs to the ecotypes Kakamega (KK) and Siaya (BN) in the Western region, West Pokot (WP) and Turkana (TK) in North Rift valley, Bomet (BM) and Narok (NR) in South Rift valley, Lamu (LM) and Taita-Taveta (TT) in the coastal region and are described in detail by Ngeno et al. (2014c).

One sample per ecotype was selected (Table S1) for whole genome re-sequencing based on the Principal Coordinate Analysis (PCoA) of the microsatellite data in order to find individuals representative for each ecotype. Analysis of the estimated (He) and observed heterozygosity (Ho) was performed within an ecotype using the 12 autosomal microsatellite markers. The representative individual per ecotype was selected for re-sequencing according to the fit of this sample in its ecotype based on the PCoA analysis and with a heterozygosity closed to the mean Ho of their respective ecotype. Sequences of two none related individuals (CL1 and CL2) of a commercial layer line were included in the study.

DNA isolation and genotyping

Genomic DNA was obtained by standard phenol–chloroform extraction. A total of 48 samples per ecotype were genotyped with twelve autosomal microsatellite markers as described by Ngeno et al. (2014c).

Sequence alignment and SNP discovery

Library preparation and 100bp paired-end sequencing were performed according to manufacturer's protocol (Illumina) on the selected DNA samples. Short reads were trimmed using Sickle Version 1.33 (Joshi and Fass, 2011). The resulting high-quality reads were aligned to the reference chicken genome (release: galGal4) using Burrows-Wheeler Aligner (BWA) v0.7.5a (Li and Durbin, 2009). SAMtools v0.1.19 (Li et al., 2009) was used to create archives in BAM format. The mpileup function of SAMtools was used to obtain the variant call format (VCF) files (Li et al., 2009). Variations were filtered for a minimum genotype SNP quality of 20 and for coverage in the range of 4x until twice the mean coverage of the genome studied using SAMtools v0.1.19 (Li et al., 2009). INDELs and variants on the sex chromosomes were removed for realistic comparison with autosomal microsatellite markers using VCFtools v0.1.12a (Danecek et al., 2011).

Data analysis

Autosomal microsatellite markers

Observed heterozygosity (Ho_micro) and expected heterozygosity (He_micro) were computed using twelve autosomal microsatellite markers for the selected samples with GenAlex 6.5b5 (Peakall and Smouse, 2012). The Nei's standard genetic distances (Ds) (Nei, 1972) for the 384 samples were calculated (Table S2) using Populations 1.2.32 (Langella, 1999) and used to perform a Neighbor Joining (NJ) hierarchical clustering among the ecotypes with Phylip v.3.6 (Felsenstein, 2004). Phylogenetic trees (Figure 1) were visualized with Dendroscope 3 (Huson and Scornavacca, 2012).

Whole genome re-sequenced data

Inbreeding coefficients (F_NGS) and nucleotide diversities (P_NGS) were computed for the entire genome using VCFtools v0.1.12a (Danecek et al., 2011). A phylogenetic tree was drawn with the IBS distance matrix (Table S2), estimated using Plink v1.07 (Purcell et al., 2007). The IBS distance matrix was used for NJ hierarchical clustering using Phylip.

Selection signature analyses

For the signature of selection analysis the eight IC were considered as one single population and the two CL chicken were considered as a second population. A three-step approach was used to identify regions affected by selection. Step one involved calculation of fixation index (Fst) values between IC and CL. The Fst values were estimated using VCFtools in bins of 40kb window sliding 20kb each time over the entire genome. Step two involved calculation of P_NGS within each 40Kb window for IC and CL using VCFtools. To reduce the number of false positives, windows with less than 10 variants were removed in both steps. Distribution of the Fst and P_NGS (Figure S1) were skewed and were normalized (ZFst, ZH_{IC} and ZH_{CL}) using Z-transformation (Rubin et al., 2010). Step three involved identification of regions affected by selection. Windows with transformed Fst (ZFst) above five standard deviations and/or nucleotide diversity below −1.8 for ZH_{IC} and ZH_{CL} were qualified as putative selection regions. The package ggplot2 in R environment (http://www.r-project.org/) was used to display the graphs.

Functional annotation of genomic variants and gene ontology

Functional annotations of genomic variants in the high ZFst regions were determined using Variant Effect Predictor VEP75 (McLaren et al., 2010). The option of SIFT predictions within VEP was used to predict if substitution of amino acid has

effect on protein function using the annotations found in Ensembl 75. BEDTools v2.17.0 (Quinlan and Hall, 2010) was used to intersect regions high in ZFst with the VEP file. Missense variants within the elevated Fst regions were extracted and their associated genes analysed for the Gene Ontology (GO) enrichment. BinGO v2.44 within Cytoscape v.2.8.3 (Maere et al., 2005) was used to check overrepresentation of GO terms enrichment for biological process using UniProt-GOA for chicken (Dimmer et al., 2012). Benjamini and Hochberg correction method designed for multiple comparisons was used to test significance at 0.05.

Results

Population structure and genetic diversity

The average coverage depth ranged between 20x and 27x for IC and 15x in CL. The number of genomic variants within autosomal chromosomes varied greatly among the chickens studied, ranging from 1.74 (KK) to 5.92 (BM) million (Table 1). The phylogenetic tree of IC ecotypes based on 12 autosomal microsatellite markers for the 384 samples and whole genome sequence (NGS) data were identical in their topology (Figure 1). For microsatellites markers, it was not possible to estimate the genetic distances. Ecotypes clustered according to their geographic origin. Thus, South Rift valley region (BM and NR), North Rift valley (TK and WP) and western (KK) region of the country formed separate groups. The only exception was the Siaya ecotype that grouped with the coastal cluster (LM and TT). Genetic diversity estimates computed from microsatellite markers and NGS data are presented in Table 2. Analysis of microsatellite markers in the selected samples of the IC ecotypes indicated close genetic diversity (He_micro = 0.69 - 0.75). Whole genome sequence analysis pointed similar trend of close genetic diversity among IC ecotypes (P_NGS = 0.56 - 0.62). Commercial layers had low P_NGS (0.31 to 0.36) and high F_NGS (>0.5) compared to IC P_NGS of 0.56 to 0.62 and F_NGS below 0.1.

Table 1 Number of variants using whole genome sequence data

Population	Variants			Exonic variants		Non-exonic variants	
	SNPs	INDEL'S	Total Variants	Missense	Synonymous	Intronic	Intergenic
BM	5,920,014	495,191	6,415,205	21,156	57,389	2,825,315	2,585,903
KK	1,743,918	139,963	1,883,881	5,693	16,133	872,747	788,694
LM	5,317,110	427,463	5,744,573	18,715	51,226	2,538,636	2,538,636
NR	4,493,840	369,504	4,863,344	15,141	40,669	2,156,496	2,013,255
BN	3,777,467	324,670	4,102,137	12,057	30,759	1,819,222	1,708,376
TK	2,994,608	239,131	3,233,739	8,866	24,544	1,483,557	1,377,739
TT	4,797,356	408,372	5,205,728	14,266	38,982	2,320,190	2,151,350
WP	4,279,520	383,797	4,663,317	13,638	35,458	2,073,505	1,945,635
CL1	4,715,983	413,196	5,129,179	15,407	41,871	2,064,578	2,418,618
CL2	4,749,606	411,101	5,160,707	15,857	43,605	2,268,562	2,090,118

BM, Bomet ecotype; KK, Kakamega ecotype; BN, Siaya ecotype; NR, Narok ecotype; TT; Taita-Taveta; LM, Lamu; CL1, Commercial layer 1; CL2, Commercial layer 2; SNP, Single nucleotide polymorphism; INDEL, insertion/deletion.

Table 2 Genetic diversity parameters using twelve autosomal microsatellite markers and whole genome re-sequence data

Population	Genetic diversity			Inbreeding
	Ho_micro	He_micro	P_NGS	F_NGS
BM	0.72	0.73	0.62	0.001
KK	0.73	0.74	0.59	0.018
LM	0.70	0.71	0.57	0.092
NR	0.71	0.73	0.60	0.009
BN	0.73	0.75	0.59	0.001
TK	0.70	0.71	0.56	0.024
TT	0.69	0.69	0.58	0.048
WP	0.73	0.74	0.60	0.001
CL1	-	-	0.31	0.545
CL2	-	-	0.36	0.531

BM, Bomet ecotype; KK, Kakamega ecotype; BN, Siaya ecotype; NR, Narok ecotype; TT; Taita-Taveta; LM, Lamu; CL1, Commercial layer 1; CL2, Commercial layer 2; Ho_micro, microsatellite markers observed heterozygozity; He_micro, microsatellite markers expected heterozygozity; P_NGS, entire genome nucleotide diversity using the NGS; F_NGS, entire genome inbreeding coefficient using the NGS.

a.   b.
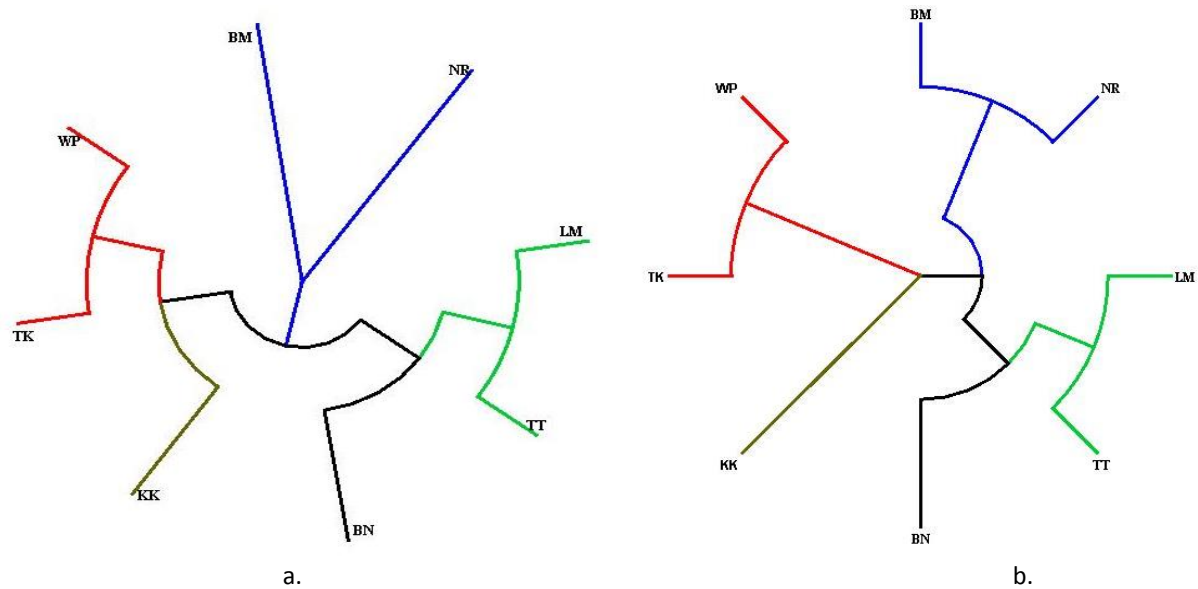
Figure 1 Dendrogram based on IBS distance between IC using SNP except SNPs from sex chromosome (a) and dendrogram based on Nei's standard genetic distance between IC for the 384 samples using 12 autosomal microsatellite markers (b).

Selection signature analysis

Analysis of signatures of selection was carried out by grouping the eight IC into one group and the two CL into another group. Putative selection signatures were identified by searching genome sites with significantly high Fst and low nucleotide diversity (Figure 2). A total of 59 regions with Fst values between 0.44 and 0.85 met the two criteria. These genomic regions overlapped with 16 genes, out of which five genes; *SLC26A8, BRPF3, MAPK13, PDIA4* and *MRPL32* were associated missense variants (26 missense variants). Gene Ontology analysis revealed 19 significant GO-terms (P<0.05) involving the genes *SLC26A8, BRPF3, MAPK13, PDIA4* and *MRPL32* (Table 3). The *SLC26A8* gene contained terms involved in transport whereas *BRPF3*, *MAPK13*, *PDIA4* and *MRPL32* genes had terms associated with metabolic process. *MAPK13* gene had two terms associated with regulation of interleukin-6 production. The *PDIA4* gene GO terms were related with response to endoplasmic reticulum stress and cell redox homeostasis. *BRPF3* was linked with protein amino acid acetylation. Three deleterious and 23 tolerated mutations were detected in the putative regions of selection. We observed a deleterious mutation in the gene *MAPK13* and tolerated in *MRPL32, PDIA4, BRPF3* and *SLC26A8* genes. The observed mutations overlapped with different amino acid change (Table 4).

Table 3 Different types of genes identified in the elevated Fst regions and their respective biological process GO-terms.

GO-ID	p-value	x	n	Description	Genes in test set
19532	0.00	1	45	Oxalate transport	*SLC26A8*
6835	0.00	1	125	Dicarboxylic acid transport	*SLC26A8*
44267	0.01	4	22405	Cellular protein metabolic process	*BRPF3|MAPK13|PDIA4|MRPL32*
32755	0.01	1	225	Positive regulation of interleukin-6 production	*MAPK13*
19538	0.01	4	26495	Protein metabolic process	*BRPF3|MAPK13|PDIA4|MRPL32*
6970	0.01	1	265	Response to osmotic stress	*MAPK13*
15711	0.01	1	315	Organic anion transport	*SLC26A8*
43966	0.02	1	405	Histone H3 acetylation	*BRPF3*
32675	0.02	1	425	Regulation of interleukin-6 production	*MAPK13*
34976	0.02	1	545	Response to endoplasmic reticulum stress	*PDIA4*
45454	0.02	1	565	Cell redox homeostasis	*PDIA4*
18105	0.03	1	635	Peptidyl-serine phosphorylation	*MAPK13*
18209	0.03	1	735	Peptidyl-serine modification	*MAPK13*
6820	0.03	1	745	Anion transport	*SLC26A8*
16573	0.03	1	775	Histone acetylation	*BRPF3*
44260	0.03	4	36715	Cellular macromolecule metabolic process	*BRPF3|MAPK13|PDIA4|MRPL32*
6473	0.04	1	875	Protein amino acid acetylation	*BRPF3*
46942	0.04	1	1095	Carboxylic acid transport	*SLC26A8*
15849	0.04	1	1095	Organic acid transport	*SLC26A8*

p-value, probability that x genes belong to a functional category C shared by n of the N genes in the reference set; x, test set genes; n, reference set genes.
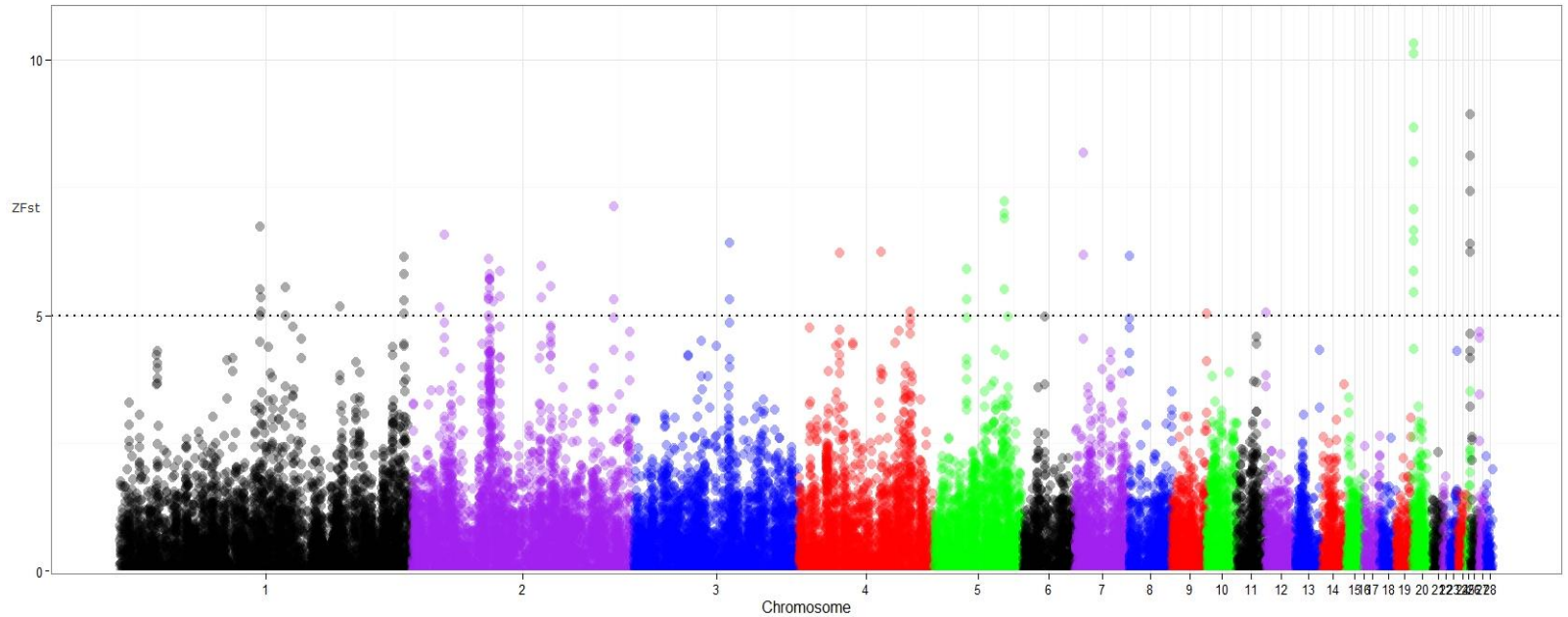
Figure 2 The ZFst distributions plotted along chicken autosomes 1–28. A dashed horizontal line indicates the cut-off (*Z*Fst > 5) used for extracting outliers.

Table 4 Different types of genes, amino acid change and nature of missense mutation in the elevated Fst regions.

Chromosome	Location	Number of variants	Mean Fst	Allele	Gene	Protein position	Amino acids	Codons	Gene symbol	Consequence
2	51752779	111	0.47	T	ENSGALG00000012337	8	M/L	Atg/Ttg	MRPL32	tolerated(0.22)
2	54490469	441	0.46	A	ENSGALG00000012415	31	E/D	gaA/gaT	PDIA4	tolerated(0.47)
2	54483729	441	0.46	G	ENSGALG00000012415	617	V/A	gTg/gCg	PDIA4	tolerated(1)
26	162632	116	0.74	T	ENSGALG00000000826	74	T/M	aCg/aTg	MAPK13	deleterious(0.04)
26	162632	100	0.68	T	ENSGALG00000000826	74	T/M	aCg/aTg	MAPK13	deleterious(0.04)
26	185048	100	0.68	T	ENSGALG00000000776	195	S/L	tCg/tTg	BRPF3	tolerated(0.11)
26	185048	71	0.53	T	ENSGALG00000000776	195	S/L	tCg/tTg	BRPF3	tolerated(0.11)
26	117838	96	0.55	G	ENSGALG00000000844	746	I/T	aTc/aCc	SLC26A8	tolerated(0.31)
26	184522	100	0.68	C	ENSGALG00000000776	20	A/P	Gcc/Ccc	BRPF3	tolerated(1)
26	184522	71	0.53	C	ENSGALG00000000776	20	A/P	Gcc/Ccc	BRPF3	tolerated(1)
26	129411	110	0.63	C	ENSGALG00000000844	136	M/V	Atg/Gtg	SLC26A8	tolerated(1)
26	129411	96	0.55	C	ENSGALG00000000844	136	M/V	Atg/Gtg	SLC26A8	tolerated(1)

Fst, fixation index

Discussion

Population structure and genetic diversity analysis

Quantification of genetic diversity and population substructure of chicken is useful to policy makers and other stakeholders in setting up genetic improvement and conservation programs. Complete information of breed stratification and their genetic diversity are vital aspects when deciding conservation actions with the goal of upholding adequate genetic variation within breeds. Characterization and subsequent identification of populations to be improved and conserved needs in-depth information on their genetic diversity (Herrero-Medrano et al., 2014). In this study, we investigated the level of genomic diversity of indigenous chicken and commercial layers with divergent selection and histories. Indigenous chicken from Kenya showed higher genomic diversity and low inbreeding than commercial layers. While commercial layers have been established from few chicken breeds (Elferink et al., 2012) subjected to intensive selection for egg production (Burt, 2005), indigenous chicken are kept under free-range scavenging production system characterized by limited human selection, free movement and random mating. Thus, the differences in the levels of artificial selection together with the higher effect of natural selection forces and free gene flow between the ICs can largely explain their higher genetic diversity.

Indigenous chicken from the same geographic area tent to cluster together in the phylogenetic tree, suggesting certain genetic structure of ICs, correlate with their geographic origin. On the other hand, we observed a closer relation of Siaya ecotype with the coastal group (represented by Lamu and Taita-Taveta population) despite the long geographical distances (753 to 790 kilometres apart). Lamu ecotype is known to be heavier, good in cock fighting due to their agility and parrot-type beak, aggressive in protecting young ones and thus fetches more market price compared other chicken types (Wesonga, 2013). For these reasons, Lamu ecotype also known as Kuchi has been introduced in other parts of Kenya such as Elgeyo/Marakwet County in western region by traders, developmental agencies and farmers, where Lamu ecotype was adopted as a gateway out of poverty (Wesonga 2013). Gene introgression from Lamu ecotype into Siaya ecotype is, therefore, a suitable reason for their closeness. The closeness in genetic estimates and topology of the phylogenetic trees obtained with microsatellite for the 384 samples and NGS data indicates that the set of microsatellite markers used in this study have a good reliability for population structure analysis. However, unlike the NGS, it was not possible to estimate the genetic distances using microsatellite markers for the selected samples (eight samples) due to small

sample size and thus indicates the reliability of NGS for genetic diversity studies over microsatellite markers when sample size is small. Data reliability is dependent on the sample size as well as the number of microsatellite markers (Dorji and Daugjinda, 2014) and the recommended minimum sample size per breed is 24 (Tadano et al., 2007; Osman et al., 2005) or 25 (Nassiri, 2009) for reliable allelic frequencies estimates.

Selective signatures

Identification of the regions of selective signatures in indigenous and commercial chicken offers the opportunity of understanding the forces of historical selection, genes and mutations underlying a phenotype differences between indigenous and commercial chicken. Detection of the genomics regions affected by selection is also crucial in understanding of the breeding goals (Elferink et al., 2012). Here, we calculated Fst between local populations from Kenya and commercial chickens for the detection of selection signatures. The same approach has been used in several species (Axelsson et al., 2013; Petersen et al., 2013; Ramey et al., 2013).

Natural and human induced selection pressures in chicken have likely left traceable signatures within their genome. Indigenous chicken is likely to have been shaped more by natural selection (Wragg et al., 2012) imposed by the local environmental conditions under scavenging production system, parasites, diets and diseases along the route of dispersal. Ecotypes of indigenous chicken studied originated from different agro-ecological zones ranging from arid to humid highlands with altitudes ranging from 0 to 2362 metre above sea level, 243 to 2000 mm annual rainfall, and 10 to 35^{0}C temperature (Table S1). The environmental conditions such as the frequency of droughts, dry spells duration, rainfall intensity, heat stress level and frequency of disease outbreaks in the agro-ecological zones may vary from those exposed to commercial layers. These climatic hazards impacts on the performance of indigenous chicken under free range scavenging system because it determine the amount of forage available to scavenge and the severity as well as the distribution of disease pathogens. Consequently, the response is likely to be different due to varied prevailing environmental conditions between indigenous and commercial chicken. Likewise, the impacts of human driven selection might have affected the indigenous chicken genome. The intensity of artificial selection on the genome may vary in the course, length and strength depending on the breeding goal. Specifically, breeding goals of farmers that rear indigenous chicken are different to the international breeding companies. While the former tend to focus on producing chickens with dual purpose for egg and meat production, better

mothering ability, fertility and adaptability, the later aim for traits involved in either egg or meat production.

Observed genomic regions with high Fst that overlapped with genes potentially favoured differently by selection in indigenous and commercial chicken. Concretely, the signatures of selection overlapped the genes *SLC26A8, BRPF3, MAPK13, PDIA4* and *MRPL32*. The *SLC26A8* gene is expressed in spermatocytes and developing spermatids and act as an anion transporter in the testis (Lohi et al., 2002). Mutation of *SLC26A8* gene in human males is associated with impairment of spermatogenesis and sterility in mice (Lohi et al., 2002; Rode et al., 2012). Deletion of *SLC26A8* in mice with homozygous alleles and low level in the individuals with the mutation causes sperm immobility and incapacitation resulting in infertility (Dirami et al., 2013). In the study, individuals with the disadvantageous mutations in this gene may have been strongly selected against due to infertility. Protein metabolic process, regulation of interleukin-6 production, serine phosphorylation and deleterious mutation were identified in the *MAPK13* gene. *MAPK* 13 which is part of the p38 *MAPK*, are vital signal components that are mainly activated by environmental stresses, inflammatory cytokines, physical stress, extracellular stress and less by growth stimuli factors (Cerezo-Guisado and Cuenda, 2010). In humans, *MAPK* respond to viral infection by impairing virus growth (Shi et al., 2013), play role in suppression of primary cutaneous melanoma proliferation (Gao et al., 2013), positive regulation of skin tumorogenesis (Schindler et al., 2009) and mediation of inflammatory responses (Kim et al., 2008). Divergence in the *MAPK13* gene was observed and this can explain the variation in performance, disease tolerance and ability to response to extreme temperatures and other environmental challenges. Selection in indigenous chicken may have favoured the different alleles in the *MAPK13* gene that could potentially result in better immunity and lower productivity. Indeed, indigenous chicken unlike commercial layers, are known for low productivity, better adaptability to poor quality feeds, high temperatures and more tolerant to diseases. On the other hand, intensively kept commercial layer is best performer in egg production but their susceptibility level to diseases and environmental challenges is upsurged. *PDIA4* gene is a member of Protein disulfide isomerases (PDIs) located in the endoplasmic reticulum. Although biological role of *PDIA4* gene is not well known, it has been noted that it is involved in the proteins synthesis, unfolding of protein response, calcium-binding, cells growth and proliferation (Chung, 2009, Raturi and Mutus, 2007). Inactivation of *PDIA4* promotes apoptosis in the mitochondrial pathway (Tufo et al., 2014). The *PDIA4* gene in this study had GO terms associated with response to endoplasmic reticulum stress and cell redox homeostasis. Intracellular changes such as calcium

or redox differences, fat and sugar rich diet, some drugs and state of micro-environment like hypoglycemia, hypoxia, and acidosis ignites endoplasmic stress (De Miguel and Pollock, 2013). Commercial layers are subjected mostly to drugs and feeds rich in energy unlike indigenous chicken, which might make them prone to endoplasmic stress, prompting the expression of *PDIA4* gene to counter the effect. *MRPL32* gene is a nuclear encoded gene and synthesizes protein in the mitochondrion. Lack of *MRPL32* causes assembling failure of ribosomes whereas partial processing causes decline in mitochondrial translation (Claypool and Koehler 2005). Consequently, malfunction of mitochondria affect organs (brain, muscle, and heart) that rely on high oxidative metabolism (Nolden et al., 2005). In human, *MRPL32* is involved in respiratory and respiratory chain complexes (Nolden et al., 2005). Biological Go-terms in this study, revealed the *MRPL32* gene to be associated with metabolic process suggesting variation in the metabolic response due to selection in the studied population. *BRPF3* gene is part of MOZ/MORF histone acetyltransferase (HAT) complex but its precise role remains unknown, although it has been associated with the regulation of transcriptional activation in MOZ/MORF, protein binding, blood coagulation and activation of platelets (Ullah et al., 2009). Commercial layers are kept under intensive system where health and housing management are provided unlike in indigenous chicken. Hence, the *BRPF3* gene might be expressed more in indigenous chicken as they are exposed more frequently to injuries under scavenging system.

The potential effects of missense SNP variants in the elevated Fst regions were predicted in this study. Missense variants in the high Fst regions were studied since they change the sequence of amino acid resulting in phenotypic differences (Strachan and Read, 1999). Mutation is the causative changes behind the variation and non-synonymous mutations detected within the genes could modify the amino acid sequence of the proteins resulting in a biological effect. The deleterious mutations found in the genes *MAPK13* and tolerated in *MRPL32, PDIA4, DIET1, BRPF3* and *SLC26A8* genes may reflect signatures of dissimilar selection pressures in indigenous and commercial chicken. The missense mutation which is the behind the single nucleotide changes in the genome which has resulted in different codons coding different amino acids may be advantageous to the chicken in terms of survival and performance. Majority of the identified mutations plays role in immunity responses. Change of codon to those coding for methionine, alanine, threonine, valine, leucine and proline amino acids promotes immune responses and antibodies (Li et al., 2007). Alanine amino acid promotes production of lymphocyte and antibody production. Amino acid coding for Leucine stimulates specific immunity and prevent inflammation (Li et al., 2007). Proline in *BRPF3* gene

destroys pathogens. Methionine play crucial role in immune protection in times of inflammation (Ruth and Field, 2013). The deleterious mutations in the *MAPK13* gene result in the change of Threonine amino acid to methionine. Methionine promotes performance (growth or egg production), removes fats from the body and contain sulphur which aid in production of huge amount of natural antioxidant and other sulphur amino acids (cysteine and taurine) which help in eradication of toxins in the body and promote development of strong and healthy tissues (Jacquie, 2013). Indigenous chicken are raised under free range condition, where they are exposed more to toxins from scavenged feeds and this might have led to high need for methionine to aid in toxin elimination. This can explain the reputation of IC being more tolerant to poor and rough quality scavenged diets and internal parasites.

Conclusion

Indigenous chickens have higher nucleotide diversity and lower inbreeding than commercial layers, representing a rich and diverse gene pool for addressing the present and future challenges associated with adaptability and farmers breeding goals. Furthermore, the analysis of signatures of selection in Kenyan chickens and commercial layers revealed genetic differences, probably because of selection driven by different breeding goals and production environments. Candidate mutations identified provide further understanding on the phenotypic divergence between indigenous chickens and commercial layers.

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Table S1 Numbers of samples, sampling regions and its Ecological characteristics

Region	Population	No. of samples	Microsatellite samples	Altitude, m asl (av.)	Rainfall, mm (av.)	Temperature, ºC (av.)	Description (Moisture index)
Western	KK	96	48	1942 (1774-2084)	1422 (1100-1500)	18 (13-24)	Cool highland
	BN	96	48	1503 (1338-1614)	1428 (1500-2000)	24 (17-30)	Hot and humid highland to lower midland (< -10)
North Rift valley	WP	96	48	6 (0-13)	950 (850-1100)	22.4 (20-25)	Coastal humid to dry sub-humid lowland (-10)
	TK	96	48	2064 (1755-2362)	699 (500-2000)	20 (11-28)	Arid and semiarid land lowland
South Rift valley	BM	96	48	1231 (1142-1323)	1427 (800-1600)	24 (17-30)	Inland humid to dry sub-humid lowland (-10)
	NR	96	48	815 (739-914)	243 (120-430)	30 (24-35)	Lowland
Coastal	LM	96	48	845 (528-1500)	603 (440-1900)	25 (18-31)	Coastal humid to dry sub-humid lowland (-10)
	TT	96	48	1692 (1281-2178)	1297 (400-1500)	20 (10-30)	Semi-arid to arid midland

BM, Bomet ecotype; KK, Kakamega ecotype; BN, Siaya ecotype; NR, Narok ecotype; TK, Turkana ecotype; TT, Taita-Taveta ecotype; WP, West Pokot ecotype; m asl, metres above sea level; av., average; Source; (Bett et al., 2011a; Singh et al., 2011; Fraga, 2002; Willam et al., 2008).

Table S2 Genetic distances between ecotypes based on Nei's standard genetic distance, Ds (above the diagonal) computed microsatellite markers and IBS distance using NGS (below diagonal).

BM		0.04	0.22	0.04	0.05	0.05	0.14	0.03
KK	0.25		0.26	0.06	0.05	0.05	0.16	0.04
LM	0.27	0.26		0.28	0.21	0.26	0.23	0.26
NR	0.25	0.25	0.27		0.06	0.07	0.15	0.05
BN	0.25	0.25	0.27	0.25		0.06	0.11	0.04
TK	0.25	0.25	0.27	0.25	0.25		0.14	0.04
TT	0.26	0.26	0.26	0.26	0.26	0.26		0.16
WP	0.25	0.25	0.27	0.25	0.25	0.25	0.26	

BM, Bomet ecotype; KK, Kakamega ecotype; BN, Siaya ecotype; NR, Narok ecotype; TK, Turkana ecotype; TT, Taita-Taveta ecotype; WP, West Pokot ecotype
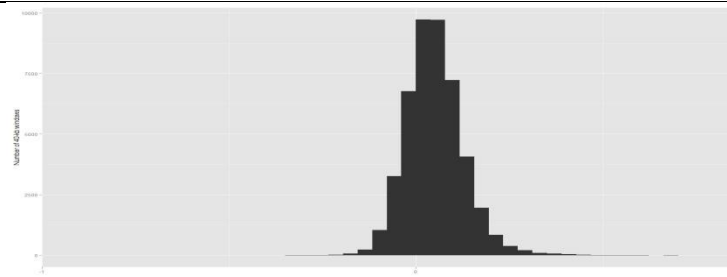
a. Distribution of un-transformed average pooled fixation index (Fst), for autosomal 40 kb (μ, mean= = 5.09E-02, σ, standard deviation = 7.76E-02)
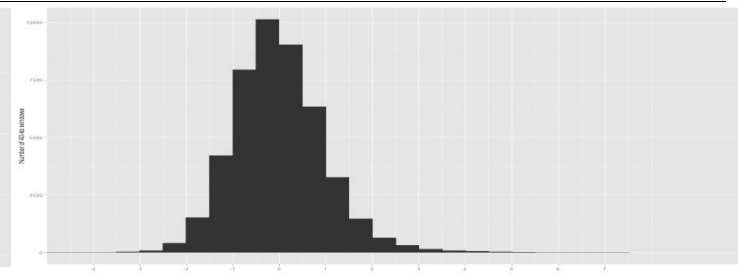
b. Distribution of Z-transformed average pooled fixation index (ZFst), for autosomal 40 kb (μ, mean= =0, σ, standard deviation =1).
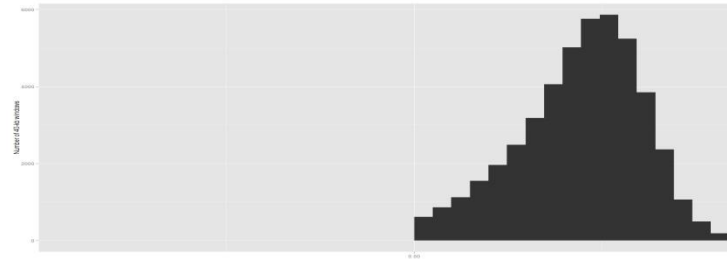
c. Distribution of un-transformed average pooled nucleotide diversity in IC (μ, mean= 4.44E-03; σ, standard deviation= 1.64E-03)
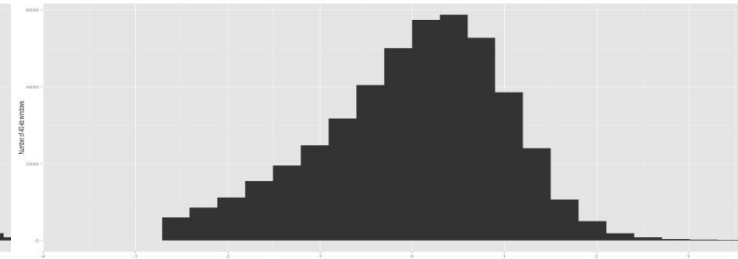
d. Distribution of Z-transformed average pooled nucleotide diversity in IC (ZH$_{IC}$) (μ, mean=0; σ, standard deviation=1)
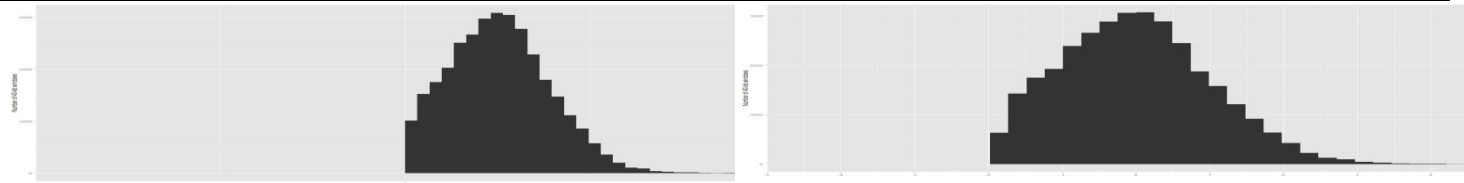
e. Distribution of un-transformed average pooled nucleotide diversity in CL (μ, mean= 2.56E-03; σ, standard deviation=1.34E-03)

f. Distribution of Z-transformed average pooled nucleotide diversity in IC (ZH$_{CL}$) (μ, mean=0; σ, standard deviation=1)

Figure S1 Distribution of un-transformed average pooled Fst (a), P_NGS for IC (b) and CL (c) and their Z-transformed distribution (ZFst, ZH$_{IC}$ and ZH$_{CL}$ respectively) for autosomal 40 kb windows sliding 20kb each time.

6

General discussion

Introduction

Indigenous chicken is an important genetic resource that needs to be better characterized, and strategies for improvement and conservation, need to be developed for the betterment of both, the present and future generations. Despite their importance, the potential of IC has remained undervalued for a long time and therefore, their potential to uplift the living standards of the farmers and bring about rural development, has not been fully realised. Indigenous chicken genetic potential for meat and eggs is still low compared to commercial exotic chicken, and cannot meet higher market demand for chicken products. There is, therefore, the need to genetically improve these IC by setting up a sustainable genetic improvement programs, to ensure that they are functional part of the production system. The aim of this study was to develop a breeding program for IC, for enhanced productivity and improved livelihood in Kenya. In this study, in-depth information was generated on what, why, how and who to improve and conserve IC (Chapter 2). It was revealed that IC can be improved and conserved under four *in-situ* scenarios that ensure they matched with the prevailing production system and climatic conditions. This chapter also present the stakeholders and their respective roles in the IC breeding program. In Chapter 3, genetic diversity of five IC ecotypes were characterized morphologically. Results indicated the studied IC to be heterogeneous population with huge variability in the morphological features. Chapter 4, presents an in-depth analysis of IC genetic diversity and population sub-structure carried out using two major histocompatibility complex (MHC), and ten non-MHC linked autosomal microsatellite markers. The eight IC ecotypes studied belong to 2-3 groups depending on the microsatellite marker system used. The MHC-linked markers generated 3 mixed clusters, while non-MHC markers grouped IC into two genetically distinct groups. The chapter also present results from investigation on population sub-structure and allelic richness in the MHC regions. The MHC-linked markers revealed highly allelic richness in the MHC region which is associated with adaptation to the local disease challenges. In Chapter 5, the genomic variation, selection signatures, and mutations based on whole genome re-sequencing in IC and commercial layers was investigated. Results revealed genomic variability, regions affected by selection, candidate genes and mutations that can explain partially the phenotypic divergence between IC and commercial layers. Unlike commercial chickens, IC preserved a high genomic variability.

The practical relevance and utilization of findings in Chapters 2-5 in designing a sustainable breeding program for genetic improvement and conservation of IC are synthesized and discussed in Chapter 6. The synthesis and discussion is focused on three areas; (a) is it possible to genetically improve IC? (b) should the breeding

program be aimed at pure or crossbreeding? and (c) how should the breeding population be managed to have a sustainable breeding population?

Is it possible to genetically improve indigenous chicken to fit the farming conditions?

Designing a breeding program is a process that involves decision making in a systematic and logical order. Analysis and description of production systems is the first step in the development of a successful breeding program. It helps in understanding the prevailing production environment, farmer's management practices as well as their associated factors. In designing a breeding program targeting the low input production system, it is important to take into account the low income level and therefore investment power of smallholder farmers. Production systems of IC can roughly be divided into three: the scavenging, semi-scavenging and intensive system (Besbes, 2009; Magothe et al., 2012). The focus of this discussion is placed on the scavenging production system where 95% of the IC in developing countries are raised (Tadelle et al., 2003a) and practiced by over 78% of households in Kenya (Okeno, 2013; Bett et al., 2011a; Okeno et al., 2012a).

The three different production systems (scavenging, semi-scavenging and intensive system) require use of different genotypes (commercial exotic, crossbred or IC) depending on their level of adaptation and input needed to exploit their genetic potential. Commercial exotic chicken breeds perform well as long as standard intensive management (full confinement, use of commercial feeds and provision of medication) are provided. Introduction of commercial exotic chicken, unavoidably need adjustment of input from low to high. In the low income and grain deficit countries like Kenya, the cost associated with intensive management especially commercial feeds are too high for majority of smallholder farmers (FAO, 2014). Such farmers prefer low-input-low output systems like the scavenging system. Scavenging systems, however, may not be favourable for exotic commercial chickens because they are not adapted to harsh scavenging as, feed resource may not meet the dietary requirement. When management conditions are not completely intensive and the commercially formulated diets are limited, crosses of commercial exotic breeds and IC may well suit this system (Chapter 2). Such crossbreds have moderate growth and egg productivity as well as adaptability (FAO, 2014). Although, the crossbreds can do better than exotic commercial breeds under scavenging conditions, their production and survival without extra care in terms of nutrition and health is below average. For instance, mortalities up to 50% or more were experienced in the past crossbreeding intervention in developing countries due to adaptation problems (FAO, 2004). In situation, where

supplementary feeds are not available or not affordable, use of IC is preferred (Chapter 2).

Indigenous chicken have essential characteristics that suit the scavenging production system; they are robust, highly adapted (Sonaiya and Swan, 2004), multi-coloured for easy camouflaging to the environment (Chapter 3), tolerant to local diseases and parasites condition (Singh et al., 2011; Kaingu et al., 2010). They are also known to have good foraging ability as they can walk longer distances and scavenging wider to meet dietary requirements, ability to utilize high fibre diets, in addition to being tolerant to extreme temperatures (Fraga, 2002). These IC have also wide variations in growth and egg production. Their annual egg production ranges from 20-100 eggs, mature live weight from 0.7- 3.1 kg for females and from 1.2-3.2 kg for males (Ndegwa and Kimani, 1996; GuÈye, 1998; Tadelle et al., 2013; Magothe et al., 2010; Dana et al., 2010a; Ngeno, 2011).

Previous studies have indicated that IC can be improved for meat, eggs or both due to their genetic potential (Okeno et al., 2013). Development of IC either for meat, egg or dual purpose is accompanied by challenges. Improving IC for meat production lowers egg production resulting in fewer eggs for hatching and subsequently fewer growers. In egg production, improving IC for egg numbers especially selecting less broody hens is essential because it would result in more eggs for market and hatching. However, when more emphasis is placed on egg production, the weight traits are negatively affected due to antagonistic relationship between these traits. Selection of IC either for meat or egg production is anticipated to compromise on the immune effectiveness and disease tolerance due to negative correlation between immune response with growth and egg traits. Reduction in fitness and disease immunity in turn increases the level of susceptibility and veterinary cost. Unlike meat or egg type IC, dual-purpose chicken may have lower meat and egg production; nonetheless they are superior in fitness and immunity (Okeno et al., 2013), which are important features needed for the scavenging system. In designing a breeding program targeting the scavenging production conditions, the most appropriate type of chicken to use is therefore the dual purpose IC.

Another logical step in setting up a breeding program after analysis of the production system is the definition of the breeding objective. Clear definition of breeding objective is a crucial step in addressing not only farmer's needs and expectations, but also consumer demands. Farmer and consumer requirements assist in specifying which traits should be improved, and in which direction. The breeding objective for IC in Kenya has been defined in consultation with the farmers and consumers. The breeding objective is to develop chicken with better

production (egg number, growth rate, and body size), reproduction (fertility and mothering ability) and adaptability (disease resistance) (Okeno et al., 2011a). Indigenous chicken breeding objective in Kenya is similar to the breeding objective for IC in Ethiopia (Dana et al., 2010b). Dual purpose breeding objective would produce IC that can serve the farmer with eggs and meat beside the ability to survive under scavenging conditions.

Genetic evaluation of potential parents is the next step in a breeding program. Knowledge on genetic parameters is paramount for genetic evaluation and the success of a breeding program. Heritability and genetic and phenotypic parameters are important for prediction of genetic gains and accuracy of genetic analysis. Genetic parameters can vary (for each trait) between IC population, and therefore, important that, estimates for each of the genetic cluster identified in Chapter 4 are estimated frequently. Genetic evaluation requires detailed and amount of pedigree and performance information, which are the key factors in determining the accuracy of the genetic evaluation. Comprehensive, consistent, and timely data collection under village conditions can be challenging. In most developing countries, pedigree and performance records are lacking. Lack of chicken identification, poor infrastructure and farmers are less knowledgeable has rendered data collection a challenging task under village set up (Gondwe, 2004; Besbes, 2009). Collection of some information is difficult for farmers, as it may need sophisticated equipment and technical expertise to carry out, such as disease resistance, which can include recording of disease symptoms and carrying out post-mortem analysis. Similarly, for reliability of data, pedigree and performance information for each individual bird, need to be collected. Hence, require infrastructures to be set up in every household which can be costly for the breeding program. Collection of information on chicken may require controlled mating, which is not the case in the scavenging production system, where chicken are left to mate freely (Dana et al., 2010c). To overcome the challenges faced under on farm situations, there would be a need to set-up a central data collection, processing, evaluation and monitoring point. It has been recommended that, the village challenges, can be addressed by setting up a centralized nucleus breeding schemes, which maintain the animals in a government farms or research centres (Kosgey et al., 2006b). Closed nucleus breeding schemes, which have been setup on-station in research institutions or government farms, such as the Horro IC breeding in Ethiopia (Wondmeneh et al., 2014) and Smallholder Indigenous Chicken Improvement Programme (InCIP-www.incip.org), have overcome the challenges faced under village situation. Ethiopian Horro breeding program has been effective and efficient in data recording and management (Tadelle et al., 2013). This study

therefore, adopts the Horro IC genetic improvement model of a two-tier (nucleus and commercial population) closed nucleus scheme, under on-station. Two-tier closed nucleus scheme, is preferred at initial stages for simplicity and effectiveness, while in the future, a three tier closed nucleus scheme (nucleus, multiplier and commercial units) proposed for IC in Kenya by Okeno et al. (2013) can be adopted. Although, closed nucleus scheme under on-station have been proposed in this study, the problems associated with the scheme such as lower genetic gains compared to open nucleus scheme (Abdel-Salam et al., 2010) are anticipated.

In conclusion, IC exhibit variation in egg and meat production and provides an opportunity to improve genetically through selection. These ICs can be improved genetically by setting up on-station closed nucleus breeding scheme, targeting to produce dual purpose birds.

Should the breeding program be aimed at purebred or crossbred?

There are different types of breeding strategies that can be applied to improve IC including introduction of exotic breeds, crossbreeding or selective breeding within IC (Kitalyi, 1998; FAO, 2004). Success and sustainability of each of the strategies may depend on farmers' breeding objective(s), resource availability, infrastructure, market demands, and compatibility of the genetic stock with the prevailing environmental conditions and cultural values of the society. The desire for the intensification of chicken production in Africa, through substitution with exotic chicken or crossbred has been found to increase productivity rapidly, within a short time but it has not been sustainable (Chapter 1). Use of breed substitution tends to lead to the predominance of a few highly specialized breeds, within which the breeding goals may be narrowly focused (Thornton, 2010). Narrowing focus, through the long-term process of one-sided intensive selection in exotic birds, is likely to have lowered their genetic diversity (Chapter 5), and poor resistance to diseases. Additionally, substitution of local breeds with exotic breeds might lead to the disappearance of the indigenous breeds (FAO, 2004) and it is opposed by the global move on the conservation of indigenous genetic resources (Kosgey et al., 2006a). Moreover, substitution strategy is capital intensive and infrastructure demanding, in order to suit the new breed to the prevailing condition. Substitution of IC with exotic chicken may be prevented, by making the IC valuable to farmers, and this would require genetic improvement.

An alternative strategy to breed substitution is crossbreeding and pure breed selection. Genetic improvement by means of crossbreeding IC with commercial exotic breeds has been considered a quick method. Farmers have been crossing IC with exotic commercial hybrid chicken, to have more eggs, faster growing chicken

for meat than parent IC, and moderately adapted chicken than exotic breed parent. Farmers in peri-urban and urban areas are already utilizing crosses of IC and commercial hybrids, under semi-scavenging production system (Okeno, 2013). Therefore, the use of crossbreds in developing countries is likely to remain for unforeseeable time or until IC has been improved to a level that their productivity is close or similar to their exotic counterparts, for fair competition. Crossbreeding increases IC productivity rapidly, but only under intensive or semi-scavenging management, of good nutrition, good health care and proper housing. Development of breeding program, targeting to produce crossbreds, which can be sustained only under intensive or semi-scavenging system, may not be feasible in most developing countries, due to high production cost and other complexities such cultural incompatibility of the new genotypes. Crossbreeding interventions have been implemented in the past (Nyagah, 2007, Riise et al., 2005). The interventions introduced different breeds of commercial exotic chicken. This resulted to unexpected shift in production system from low-to-high input which was beyond reach of most smallholder farmers (Nyagah, 2007). This could explain the backward shift, to low input system which could not support the crossbreds and subsequent termination of the program after the donors has pulled out. A well-organized crossbreeding scheme needs to maintain pure lines of IC and exotic breed as parent stocks, to breed the crossbred, and this is infrastructure and investment demanding. Conversely, crossbreeding can change the chicken morphological features, which can consequently result in rejection of the birds, by the farmers and consumers (Chapter 3).

Selection within indigenous breeds is an encouraging and viable approach for genetic improvement in the developing countries. Selection within an IC population, called Horro has been implemented successfully in Ethiopia, and has resulted in better egg production, compared to unimproved village chicken (Wondmeneh et al., 2014; Tadelle et al., 2013). Ethiopian Horro IC has increased egg production by 123.5% to 75 eggs by week 45 in five generations of selection for egg numbers (Tadelle et al., 2013). Although, purebred selection in IC population require more time to improve performance than crossbreeding and breed substitution, nevertheless, it can be tailored to fit the needs of local farmers and the prevailing environmental conditions. In conclusion, the breeding program should be aimed at purebred selection utilizing IC, as it is promising rather than breed substitution or crossbreeding. In Kenya, purebred selection of IC has been started under the Smallholder Indigenous Chicken Improvement Programme (InCIP), and is on-going.

Determination of the population size to recruit is another step in a breeding

program. Available space and market demands, can dictate the number of chickens that can be kept to produce commercial population. Currently, the carrying capacity available at InCIP breeding station (Egerton University) accommodates approximately 11,000 chickens. The nucleus size of the breeding scheme can be 500 cocks and 5,000 hens per generation (1 cock to 10 hens) to become nucleus breeding stock. The remainder space is utilized for other activities such as housing the selected population. The nucleus populations are multiplied to produce mature growers for the farmers. The number of mature growers per year per IC hen under on station is expected to be approximately 84 (Okeno et al., 2013) and this translates to 420,000 (5,000*84) mature growers of equal sex ratio. Breeding hens and cocks are selected from the mature growers. Approximately 0.24% (500 out of 210,000 cocks) best performing sires and 2.38% (5,000 out of 210,000 hens) dams are selected to constitute replacement stock. Sire selection intensity in this case is 3.117 and 2.353 for dams, which is in the range of 2.6 to 3.2 recommended for IC (Okeno et al., 2013) and 1.5 to 2.5 for commercial chicken (Lwelamira and Kifaro, 2010; Zerehdaran et al., 2009a). After selection, 414,500 (420,000-5,500) birds are left for market. Breeding stocks are culled after active productive life of 52 weeks for cocks, and 77 weeks for hens (Okeno et al., 2013). The carrying capacity at InCIP, cannot accommodate the anticipated 420,000 birds, hence they are produced in batches (hatch group) and sold in batches.

In Kenya, the total number of IC is approximately 77% (25 million) of the estimated 32.50 million chickens (FAOSTAT, 2013), and based on the mean flock size of 22.40 chickens per farmer (Okeno et al., 2012a), these IC are kept by an estimated 1.07 million farmers. A single farmer is expected to buy 22.40 growers per year, which translates to 18,504 farmers that can be served by the given current capacity. The available capacity cannot meet the demand of the 1.07 million IC farmers and therefore, the expansion of the breeding program including the addition of the multiplication unit, would be required. Addition of the multiplier level depends on the availability of resources.

Inbreeding can be a challenge in a breeding program. Inbreeding rate need to be monitored, and even predicted by carrying out frequent genetic evaluations, using SelAction (Rutten et al., 2002). To minimize reduction in the genetic diversity, and avoid deleterious effects in the long-term, due to selection in the IC breeding program, the tolerable inbreeding rate of 0.01 (FAO, 1998), should not be exceeded. To restrict the rate of inbreeding, the nucleus populations are mated with care and guided by the pedigree records. Inbreeding restriction can be achieved through selection of parents that minimize coefficient of coancestry (Meuwissen, 1997). Conversely, maintaining the effective population size within

the nucleus is another approach of restricting the rate of inbreeding. By adopting the suggested population size, would offer an effective population size of over 5,000, hence uphold IC diversity and possibly reduce or maintain their low level of inbreeding (Chapter 5).

Finally, the breeding program needs to be implemented (Chapter 2) and evaluated. The two main programs for evaluating the designed breeding program are ZPLAN (Willam et al., 2008) and SelAction (Rutten et al., 2002). Rate of inbreeding is one of the methods for evaluating breeding program and the lower the rate of inbreeding the better. In the IC breeding program, genetic and economic gains, inbreeding rate and reduction in genetic variance due to selection is predicted using SelAction (Zerehdaran et al., 2009b; Bijma et al., 2001).

Genetic progress is created in the nucleus, but the final aim is to improve the performance of the entire IC population. Thus, the genetic improvement generated in the nucleus has to be disseminated. Genetic material can be disseminated to the end users, through artificial insemination, fertilized eggs, chicks or growers. Farmers, individuals or private companies are encouraged to form enterprises, depending on their interests and objectives. Formation of enterprises, leads to specialization of production in the IC value chain, which can be more effective, unlike the customary poultry production, where each farmer is being involved in all the stages of production. Moreover, development of enterprises allows intensification of IC production and marketing, resulting in sustainable IC enterprises and subsequently the breeding program. Enterprises that can be formed are hatchery, chick, grower and mature chicken keepers as well as processors. Different enterprises are encouraged to form mutual contract agreements, so that output in one enterprise is input in another.

How should the breeding population be managed for sustainability?
Breeding objective has been identified for IC to be dual purpose, understanding of other factors that might affect the success of a breeding program, need consideration in the breeding objective. Chicken morphological characteristics like plumage colours, feather morphology and distribution, may affect the acceptability of chicken produced in the breeding program. Morphological features affect farmers and consumer's perception, preferences, adoption and the financial value of the chicken (Chapter 3). Farmers, depending on the region and culture inclinations, have different perceptions towards chicken plumage colours, and in their informal selection of parent stock, they consider chicken plumage colour (Okeno et al., 2011a). Breeding chicken with plumage colours like black, white, black-white striped, black-white spotted, red and brown, preferred by farmers

(Chapter 3), can promote the acceptability of chicken produce in the breeding program. However, black plumaged chickens are unacceptable in some regions of Kenya such as Eastern and Nyanza regions, due to association with traditional cultures like witchcraft and bad omen. Hence, different market niches require breeding for different plumage colours.

Development of dual purpose chicken for scavenging production system is a good idea (produce both eggs and meat and have ability to survive under scavenging environment). Nonetheless, it may be challenging due to antagonistic relationship between the different breeding objective traits. Increasing body size and egg production in the same bird is challenging, because negative correlation exist between egg traits (egg number) and growth traits (growth rate and live body weight). For example, in Ethiopian Horro IC, the genetic correlation between cumulative egg number at 24 weeks and bodyweight at 16 weeks is -0.12 (Wondmeneh et al., 2014). The genetic correlation between egg number and the antibody response against Newcastle disease is negative, but it is low, -0.04 (Okeno et al., 2011b), which would not pose serious challenges. Although the antagonistic relationship between the disease resistance and production traits is generally accepted, the actual genetic correlations for most of the diseases are unknown. Challenges associated with balancing between improving production, reproduction and adaptation (fitness and immunity), in a single bird can be addressed by a balanced selection approach (Lwelamira et al., 2009; Lwelamira, 2012). A balanced selection approach for dual purpose IC can be developed using selection index.

Genotype by environment interaction (GxE), which involves genotype re-ranking in different environments, and varying genetic variances (Falconer and Mackay, 1996; Lynch and Walsh, 1998) are factors that needs to be considered in a breeding program. The effect of GxE exist when the genetic correlation is lower than 0.60 (Mulder et al., 2006), hence problematic and re-ranking of genotypes needs to be considered in the breeding program (Mulder, 2007). Re-ranking of the genotypes can be problematic in genetic improvement, as it indicates that one genotype is not superior in all the production environments (Calus, 2006; Mulder, 2007). In IC, re-ranking of the genotypes can be challenging due to the differences between the on-station conditions (where breeding is carried out) and the village scavenging production environments. Management conditions provided by the village farmers can be different from the nucleus conditions. Challenges associated with the different production conditions, can be solved, by mimicking farmers' production conditions under on-station, for the improved IC to be adapted to the targeted scavenging environment. Scavenging conditions can be mimicked by ensuring the housing structures in the breeding station, are designed in such a manner that the

6 General discussion

chickens are able to freely move outside and scavenge for themselves. Such a mimicking production conditions within the nucleus unit can aid the young birds to adapt to the expected scavenging production environment in the rural villages, prior to distribution to the farmers. Conversely, the effect of GxE can be addressed by manipulating the environmental causes such as nutrition. For instance, under optimal management and environmental conditions, animals allocate more energy to growth unlike the suboptimal production conditions where most energy is re-allocated to fitness (Van der Waaij, 2004). For IC, GxE can be reduced by manipulating the scavenging production environment, by encouraging farmers to adopt the semi-scavenging production system, where feed supplementation is done. Supplementation is necessary, because the free range scavenged feeds can be scarce and seasonal, and therefore may not support high egg production as well as faster growth of the improved IC. Furthermore, GxE can be addressed by evaluating IC on-farm, as well as under diverse agro-ecological zones, to know their actual performance, and the estimated breeding values for the breeding objective traits be considered in selection. Contrary, GxE is not always problematic, depending on the level of genetic correlation between the different environments. In situation, where the genetic correlation between the different environments is greater than 0.8, GxE is not important (Robertson, 1959), and selection in the nucleus unit would results in similar genetic changes under farmer's village production conditions. Nevertheless, the genetic correlation of 0.8 has not been formally proofed, and it is suggested that any value below one can be substantial, if the economic significance of that particular trait is sufficiently high (Sae-Lim, 2013). In conclusion, the possible extent of GxE for IC in Kenya has not been studied and evaluation should be done after dissemination of improved chicken.

Intensive selection for production traits can have implication on the animal ability to cope up with the diverse and ever changing production environments. Animals subjected to selection for high productivity tend to re-distribute more resources to production traits and small portions to the biological processes such as exploratory behaviour (Lindqvist, 2008). For instance, the unselected red jungle fowl response actively by spending more time on foraging under food shortage, compared to commercial layers (Lindqvist, 2008). Intensive selection of the breeding objective traits and raising of IC chicks under intensive production conditions may affect their foraging behaviour. Introducing the improved IC, which has been subjected to selection and raised under high input intensive system, to low input scavenging system may require testing of the foraging ability. In case, the foraging ability is noted to be poor, chicks may need training so that they learn to cope with scavenging conditions of finding food and escaping from predators. Training can be

done prior to dissemination of chicks to the farmers, by allowing them to roam around the runs and scavenge feeds for themselves.

Diseases are a major setback in the success of chicken farming. Therefore, there is a need to enhance diseases resistance, since the genetic improvement achieved aggregate permanently and is transmittable to the progeny. Boosting disease resistance can replace the use of antibiotics and could lead to higher economic returns, because all the market niches such as organic market can be exploited. Genetic improvement for disease resistance is complicated, as it is influenced by genetics and environment especially nutritional factors as well as the interactions (Sivarajasingam, 1995). The challenge of incorporating disease resistance traits in IC breeding program may arise as a result of competition for resources with the production traits (egg and meat), and also due to low heritability, like in the case of liveability. Conversely, identification and collection of the appropriate phenotypes is another important reason why selection for disease resistance might be a challenge in IC breeding program. Although breeding for disease resistance is complicated and challenging, there is an opportunity to improve genetically via selection. Different breeds of chickens have variation in expressing the genes related to immunity (Redmond et al., 2009), providing an opportunity to genetically improve disease resistance, through selection of immune linked traits (Swaggerty et al., 2008). For instance, the heritability for antibody is low to moderate, and its consistent selection has been recorded to boost diseases resistance (Kaiser et al., 1998).

Analysis of IC revealed highly diverse MHC-linked alleles (Chapter 4) and several genes that play role in immunity (Chapter 5). These MHC alleles are correlated either positively or negatively with immunity. The MHC-LEI0258 allele 205 and 307 are correlated with primary antibody responses against Newcastle disease (Lwelamira et al., 2008). The LEI0258 allele 194 and 349 has been found by Fulton et al. (2006) to host MHC haplotype B11 which is known to confer resistance to Marek's disease virus (Wakenell et al., 1996) and similar alleles were detected in IC. Presently, disease resistance is being improved through selective modification of frequencies of the MHC alleles. The frequency of valuable MHC alleles can be increased or even fixed within the parents (Lamont, 1998). To boost disease resistance of IC, the MHC alleles and genes that are linked to immunological responses need to be incorporated in the breeding objective either directly or indirectly. To exploit the allelic diversity, the contribution of the identified MHC alleles to immunological response and their relation with other genes need to be ascertained to guide which alleles to select.

6 General discussion

Effective genetic improvement may not be achieved in isolation of other management interventions. Nutrition is an input critical for body functions especially for immune system. Nutritional augmentation improves immunity response, and has been used successfully during stress time in chicken (Mumma et al., 2006; Panda et al., 2008; Redmond et al., 2010) and if adopted as a daily routine in a feeding program, immunity can be enhanced. Likewise, nutrition (both quality and quantity) can be a major factor limiting the attainment of full productivity in IC. Chicken under scavenging environment forage freely and, although they get some feeds, they hardly meet the dietary requirement, because the quality and quantity of the scavenged diet are not only limited, but also there is high competition from other birds in the surrounding (Ahlers et al., 2009). The expression of genetic potential of improved IC may not be realised under village scavenging system where no supplemental feeds are provided. Furthermore, IC in Kenya exist in different genetic clusters (Chapter 4), that are anticipated to have varying levels of production (e.g. number of eggs) and physical characteristics (e.g. metabolic body size and average daily gain). For instance, IC in the different genetic clusters have different mature body weights and maturing rates (Table 1). These ICs in the different genetic clusters may have different nutritional requirements, but information on their nutrient requirements is presently limited. Thus, information on the nutrient requirements need to be determined both on-station and under village conditions to guide the formulation of appropriate diets.

Table 1 The least squares means of growth curve parameters for the Gompertz sigmoidal growth model by genetic cluster.

Growth curve parameters	Cluster 1	Cluster 2	Cluster 3
Final mature weight in grams (a)	2143.96	2442.13	2815.79
Scaling parameter (b)	1.28	1.30	2.14
Maturing index (c)	0.09	0.09	0.03

Cluster 1 (Bomet, Kakamega, Narok, Siaya, Turkana and West Pokot population), cluster 2 (Taita-Taveta population) and cluster 3 (Lamu population).

In conclusion, the introduction of genetic improvement, in combination with improvement in feeding, housing and health increases productivity e.g. egg production can improve by up to 50% (FAO, 2004). Therefore, the sustainability of IC needs a holistic management approach of genetic improvement (improving production, reproduction and disease resistance as well as evaluation of GxE), and

feeding (provision of feed supplements and genetic cluster based feeding) management.

Conclusion

This study showed that there is an opportunity to improve IC genetically. Understanding the genetic diversity is a prerequisite in setting up an effective breeding program and selection of population to use. The study showed the level of genetic diversity between and within IC population, which can be exploited for their genetic improvement. The study showed IC to be adapted to locally available feeds, disease challenges and local scavenging conditions as revealed by the presence of alleles and genes that boost immunity, hence suitable for the scavenging production system. Conversely, this study showed that there is an opportunity to improve IC through selection within the population. Genetic improvement utilizing within IC selection requires setting up a breeding program. Lastly, the study described the systematic and logical steps in designing a breeding program by focusing on farmers' need how to improve IC to fit the farming conditions, and management regimes.

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S

Summary

Summary

Indigenous chicken (IC) farming is associated with economic, environmental and societal benefits. They are synonymous with poor rural households as a mitigation measure to overcome poverty and food insecurity. Notwithstanding IC importance in enhancing livelihood, their potentials have not been fully realized because of their low productivity which is a key setback for its utilization. There is therefore the need to genetically improve productivity of these ICs. Genetic improvement can be realized through development of a sustainable breeding program.

In this study, development of IC breeding program was focused on and this was accomplished by analysing the diversity of IC populations. Chapter 1 of this thesis elaborates on the relevance of IC genetic resources and gives an overview of the past IC genetic improvement attempts. Chapter 2 is a review focused on what, why and how should IC be conserved. It was revealed that locally adapted IC can only be conserved in the most rational and sustainable way by ensuring that they are a functional part of different production systems. Their conservation should be through utilisation, if they are to be of any benefit to the poor rural households. This study suggested options for *in-situ* conservation of IC via egg and meat production under four scenarios that ensure that the genotype is matched with the prevailing production system and climatic conditions. This chapter also present the stakeholders and their respective roles.

Understanding genetic diversity is a prerequisite in setting up an effective breeding program and selection of population to use. Chapter 3 describes morphological features of IC ecotypes from five different regions in Kenya. This was exploratory study carried out on morphological data obtained from 1580 and 151 chickens for qualitative traits and zoometric measurements respectively. Results revealed that ecotypes are heterogeneous population with huge variability in morphological features.

Although morphological characterization was done in chapter 3 and found to be useful in describing different ecotypes of IC, it is not considered as ideal measure of genetic variation. Molecular markers are the current strategies for studying biodiversity. Among molecular markers, primary marker of choice for studying chicken biodiversity, have been microsatellites. The findings on the genetic diversity of eight IC ecotypes using two major histocompatibility complex (MHC), and ten non-MHC linked autosomal microsatellite markers, are described in Chapter 4. The chapter also present results from investigation on population sub-structure and allelic richness in the MHC regions. The MHC-linked microsatellite markers divided the eight IC ecotypes studied into three mixed clusters, composing of individuals from the different ecotypes whereas non-MHC markers grouped ICs

into two groups. Analysis revealed high genetic variation within the ecotype with highly diverse MHC-linked alleles which are known to be involved in disease resistance.

In chapter 5, genomic variation of IC was assessed using whole genome re-sequence data. Indigenous chickens were further compared to high input commercial layers to identify selection signatures and candidate mutations that may explain the phenotypic divergence between these populations. Whole genome re-sequencing revealed genomic variability, regions affected by selection, candidate genes and mutations that can explain partially the phenotypic divergence between IC and commercial layers. Unlike commercial chickens, IC preserved a high genomic variability that may be important in addressing present and future challenges associated with environmental adaptation and farmers' breeding goals.

Finally this study showed that there is an opportunity to improve IC through selection within the population. Genetic improvement utilizing within IC selection requires setting up a breeding program. The study described the systematic and logical steps in designing a breeding program by focusing on farmers' need, how to improve IC to fit the farming conditions, and management regimes.

S

Samenvatting

Samenvatting

Het houden van lokale kippen (LK) gaat gepaard met economische, omgevings-, en maatschappelijke voordelen. Ze brengen verlichting voor arme huishoudens om armoede te overwinnen en voedselzekerheid. Zonder af te doen aan het belang van de LK om de levensstandaard te verhogen, is hun potentieel nog niet volledig erkend vanwege hun lage productiviteit, wat het belangrijkste nadeel is voor hun gebruik. Het is daarom nodig om de productiviteit van deze LK genetisch te verbeteren. Genetische verbetering kan gerealiseerd worden via ontwikkeling van een duurzaam fokprogramma.

Deze studie is gespitst op de ontwikkeling van een fokprogramma voor LK en dit is bereikt door het bestuderen van de diversiteit van LK. Hoofdstuk 1 is gewijd aan het beland van LK genetische bronnen en geeft een overzicht van de eerdere pogingen om LK genetische te verbeteren. Hoofdstuk 2 is een review over welke, waarom, en hoe de LK zou moeten worden geconserveerd. Het is gebleken dat de lokaal aangepaste LK alleen behouden kunnen worden op een zeer rationele en duurzame manier, door te zorgen dat ze een functioneel onderdeel vormen van verschillende productiesystemen. Hun behoud moet worden bereikt door gebruik, als ze van enig nut zouden moeten zijn voor de lokale bevolking. Deze studie laat opties zien voor de in-situ instandhouding van LK via ei en vlees productie in vier scenario's die ervoor zorgen dat het genotype past bij de heersende productiesysteem en klimatologische omstandigheden. Dit hoofdstuk laat ook de belanghebbenden en hun rol hierin zien.

Begrip van genetische diversiteit is een voorwaarde voor het opzetten van een effectief fokprogramma en voor de selectie van een populatie hiervoor. Hoofdstuk 3 laat de uiterlijke kenmerken van LK ecotypes uit vijf regio's in Kenya zien. Dit was explorerend onderzoek op kwalitatieve data aan 1580 kippen en op zoömetrische data aan 151 kippen. De resultaten laten zien dat de ecotypes een heterogene populatie vormen met enorme variabiliteit in uiterlijke kenmerken.

Hoewel de morfologische karakterisering in hoofdstuk 3 is uitgevoerd en die nuttig bleek om de verschillende ecotypes LK te beschrijven, wordt het toch niet als de ideale manier gezien om genetische variatie te meten. Moleculaire merkers vormen de huidige strategie om biodiversiteit te bestuderen. Onder de moleculaire merkers werden de microsatelliet merkers als eerste keus gezien. De bevindingen op het gebied van genetische diversiteit van acht LK ecotypes voor twee major histocompatibility complex (MHC) en voor tien niet-MHC gerelateerde autosomale microsatelliet merkers, zijn beschreven in Hoofdstuk 4. Het hoofdstuk laat tevens resultaten zien van onderzoek aan populatie substructuur en allel-rijkdom in de MHC regio's. De MHC-gerelateerde microsatelliet merkers verdeelden de acht

ecotypes in drie gemixte clusters, die bestonden uit dieren van verschillende ecotypes, terwijl de niet-MHC gerelateerde merkers de LK in twee groepen verdeelden. Analyse liet een grote genetische variatie zien binnen het ecotype met veel diversiteit in MHC-gerelateerde allelen, waarvan bekend is dat ze zijn betrokken bij ziekte resistentie.

In Hoofdstuk 5 is de genomische variatie van LK onderzocht met totale genoom re-sequence data. Genomen van lokale kippen zijn vergeleken met die van hoog productieve commerciële leghennen om aanwijzingen van selectie en kandidaat mutaties te vinden die de fenotypische verschillen tussen deze populaties kunnen verklaren. Totale genoom re-sequencing liet genomische variabiliteit, regio's beïnvloed door selectie, kandidaat genen en mutaties zien die deels de fenotypische verschillen kunnen verklaren tussen LK en commerciële hennen. In tegenstelling tot commerciële kippen hebben de LK een hoge genomische variabiliteit behouden, welke belangrijk kan zijn in het aanpakken van huidige en toekomstige uitdagingen die gerelateerd zijn aan adaptatie aan de omgeving en de fokdoelen van boeren.

Tot slot heeft deze studie laten zien dat er een mogelijkheid is om LK te verbeteren door selectie binnen de populatie. Genetische verbetering door gebruik te maken van selectie in LK vraagt om het opzetten van een fokprogramma. Deze studie heeft de systematische en logische stappen beschreven die nodig zijn bij het ontwerpen van een fokprogramma door te focussen op de behoefte van de boeren, op hoe de LK verbeterd moeten worden om in de boeren omstandigheden te passen, en op het management stelsel.

P

Publications

Peer-reviewed publications

Ngeno, K., E.H. van der Waaij, A.K. Kahi and J.A.M. van Arendonk. 2014. Morphological features of indigenous chicken ecotype populations of Kenya. Animal Genetic Resources 55:115-124.

Ngeno, K., E.H. van der Waaij, H.J. Megens, A.K. Kahi, J.A.M. van Arendonk and R.P.M.A. Crooijmans. 2014. Genetic diversity of different indigenous chicken ecotypes using highly polymorphic MHC-linked and non-MHC microsatellite markers. Animal Genetic Resources: available online.

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Ngeno, K, T.M. Magothe, T.O. Okeno, B.O. Bebe and A.K. Kahi, 2013. Heritabilities and Correlations Between Body Weights and Growth Curve Parameters of Indigenous Chicken Populations Reared Intensively in Kenya. Research Journal of Poultry Sciences, 6: 43-52.

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Ngeno, K, B.O Bebe and A. K. Kahi. 2011. Estimation of Growth Parameters of Indigenous Chicken Populations Intensively Reared in Kenya. Egerton Journal of Science and Technology 11:13-28.

Publications under review or in preparation

Ngeno, K., J. M. Herrero-Medrano, R.P.M.A. Crooijmans, E.H. van der Waaij, H.J. Megens, A.K. Kahi and J.A.M. van Arendonk. Genetic variation and signatures of selection in the genomes of indigenous chicken and commercial layers. Animal Genetics.

Conference proceedings

Ngeno, K., J. M. Herrero-Medrano, E.H. van der Waaij, H.J. Megens, J.A.M. van Arendonk and R.P.M.A. Crooijmans. Genetic Variation and Signatures of Selection in the Genomes of Kenyan Indigenous Chicken and Commercial Layers. 9th Annual International Conference, Egerton University, 25th to 27th March, 2015.

Ngeno, K., E.H. van der Waaij, Hendrik-Jan Megens, A. K. Kahi, J. A.M. van Arendonk and R. P. M. A. Crooijmans. Major Histocompatibility Complex genetic diversity of Kenyan Indigenous Chicken populations based on microsatellite markers. 10th World Congress on Genetics Applied to Livestock Production (WCGALP) being held from 17 – 22 August 2014 at the Westin Bayshore Conference Center, Vancouver, British Columbia, Canada.

Ngeno, K, E. H. Vander Waaij and A. K. Kahi. Indigenous chicken genetic resources: Their unique attributes and conservation Options for improved use. Lillehammer, Norway 27th-29th of January 2014.

Ngeno, K., B. O. Bebe and A. K. Kahi. 2011. Genetic evaluation of growth traits in ecotypes of Kenyan indigenous chicken. Pan-African Conference, NAGRC&DB Headquarters, Entebbe, Uganda.

Ngeno, K., B. O. Bebe and A. K. Kahi. 2010. Heritability Estimates For Growth of Different Ecotypes of Indigenous Chicken Reared Intensively. 9th World Congress on Genetics Applied to Livestock Production Conference to be held 1st – 6th August 2010.

A

About the author

Kiplangat Ngeno was born in Narok County (Kenya) on 1st July 1983. He graduated with Bachelor of Science in Animal Production at Egerton University (Kenya) in 2007. Mr. Ngeno enrolled for Master of Science in Animal Production (Animal Breeding and Genetics option) in the same University in 2007. In 2009, he worked as an assistant lecturer, Department of Animal health, Mt Kenya University (Kenya). In 2010, he joined International Livestock Research Institute (ILRI) as a research technician before proceeding to Wageningen University in 2011 to pursue his PhD program in Animal Breeding and Genetics under the sponsorship of Wageningen University Sandwich Ph.D. Fellowship, Wageningen University ABG chair group, Egerton University and The Koepon Foundation. Currently, he is the secretary of Kenya Animal Breeding and Genomics Association (KABGA) and assistant lecturer in Animal Breeding and Genetics at the Department of Animal Science, Egerton University. The results of the Ph.D. project are shown in this thesis.

Training and Education

Education and Training

WIAS Introduction Course	2013
Course on philosophy of science and/or ethics	2014

Scientific Exposure   year

International conferences

Pan-African Conference, NAGRC&DB Headquarters, Entebbe, Uganda	2011
Conference on Genetic Resources for Food and Agriculture in a Changing Climate, Lillehammer, Norway	2014
Smallholder Indigenous Chicken Improvement Programme (InCIP) conference, LUANNAR University	2014
The 9th Egerton University International Conference	2015

Seminars and workshops

WIAS Science Day, Hotel de Nieuwe Wereld	2012
Smallholder Indigenous Chicken Improvement Programme (InCIP) Inception workshop, Egerton University	2012
Smallholder Indigenous Chicken Improvement Programme (InCIP) workshop, ARC Hotel, Egerton University	2013

Presentations

Pan-African Conference, Entebbe, Uganda, (oral)	2011
Conference on Genetic Resources for Food and Agriculture in a Changing Climate, Lillehammer, Norway (poster)	2014
LUANNAR, Malawi, (oral)	2014
The 9th Egerton University International Conference, Kenya,(oral)	2015

In-Depth Studies   year

Disciplinary and interdisciplinary courses

Genetic Improvement of Livestock, Wageningen	2011
Social genetic effects: Theory and genetic analysis	2013
Summer school EGS-ABG ILRI (Addis Ababa)	2013
Genomic selection in livestock	2014

Advanced statistics courses

WIAS Statistics for the Life Science	2013

Design of field experiments   2014

MSc level courses
Genetic Improvement of Livestock, Wageningen   2011

PhD students' discussion groups
PhD students' discussion group, ABG   2012

Professional Skills Support Courses   year
Research proposal writing   2011
Scientific Integrity   2013
Research proposal writing with focus on upgrading the value chain of
indigenous livestock   2014

Research Skills Training   year
Preparing own PhD research proposal   2011
Prepare report for International Network for Family Poultry Development
(INFPD) on the launch of the Kuroiler in Uganda   2011
Getting started with AS-Reml   2012
Artificial Insemination in chicken   2013

Didactic Skills Training   year
Lecturing
Chicken Artificial Insemination in Malawi (40 hours)   2014

Preparing course material
Chicken Artificial Insemination course   2014

Management Skills Training   year
Organisation of seminars and courses
Organisation of Chicken Artificial Insemination course in Kenya   2013

Education and Training Total   **45**

A

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C

Colophon

Colophon