

Breeding programs for indigenous chicken in Ethiopia
Analysis of diversity in production systems and chicken populations

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Thesis

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Abstract

The aim of this research was to generate information required to establish a sustainable breeding program for improving the productivity of locally adapted chickens to enhance the livelihood of rural farmers in Ethiopia. The first step was to characterize village poultry production environments and farmers' objectives for keeping chickens, and to identify factors affecting the choice of genetic stock used in villages. This was achieved by carrying out a questionnaire survey and a participatory group discussion with village farmers in different geographic regions of Ethiopia. The low input nature of village environments, the prevalence of disease and predators, and other factors such as the use of chickens both as sources of eggs and meat, and income determined the choice of chicken breed used by farmers, and thus, should be considered carefully before initiating new breeding programs. The highest importance attached to adaptation traits and the existence of particular preferences for chickens of certain plumage colours and comb shapes were also found to have effects on developing new breeds for village systems.

The next part of the thesis focused on identifying important and unique gene pools in local populations. This was achieved by characterizing the local chicken ecotypes both morphologically and molecular genetically. This way the genetic difference between the local populations and the level of genetic diversity within the populations was determined. Attributes important in breeding for tropical conditions such as the pea comb gene, and the naked neck gene have been identified. It was also revealed that the variability found within a single population could explain most of the genetic diversity (97%) in Ethiopian chicken populations. The result of this work is important both from conservation and utilization perspective and assists in maintaining indigenous genetic diversity for current and future generations.

Finally, the pedigreed Horro population that was kept on station was used for estimating genetic parameters for the production traits, monthly and cumulative part period egg numbers and growth to 16 weeks of age. Because the pedigreed population was established only recently, data of only 2 generations were available for estimating these genetic parameters. The results are promising but inaccurate due to insufficient amount of data. They would need to be re-estimated when more generations have been produced and thus more data has been generated.

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1

General introduction

Domestication of chicken

Chicken are the most popular poultry species worldwide in terms of their economic importance. Chicken are believed to have been domesticated from the jungle fowl. Four wild species of the jungle fowl exist: the red jungle fowl (*G. gallus*), the grey jungle fowl (*G. sonnerati*), the Ceylon jungle fowl (*G. lafayettei*) and the green jungle fowl (*G. varius*).

Whether chickens were domesticated from one or all of these species remains an open question. Taking into account the geographic range of the species (Crawford, 1990), archaeological discoveries (West and Zhou, 1988), protein polymorphisms and morphological characteristics (Moiseyeva *et al.*, 2003), these authors suggested that domestic chickens were derived from the red jungle fowl. However, questions still linger on whether only one or the 5 subspecies of red jungle fowl contributed to the genetics of domestic chickens. In a series of studies that analyzed 400 base pairs of the mtDNA D-loop region of four species of genus *Gallus* (*G. gallus*, *G. varius*, *G. lafayettei* and *G. sonnerati*), three subspecies of *G. gallus* (*G. g. gallus*, *G. g. spadiceus* and *G. g. bankiva*), nine domestic breeds of chicken from south Asia, south east Asia, Japan and Europe, Akishinomiya *et al.* (1994, 1996) presented evidence which suggested that domestic chickens are derived from a single continental population of *G. g. gallus*. However in a separate study, Liu *et al.* (2006) demonstrated that besides *G. g. gallus*, several other subspecies of the red jungle fowl were also involved in the genesis of modern chickens. Studies revealing that other wild species of jungle fowl have contributed to the genetics of modern chickens are abounding. A study by Nishibori *et al.* (2005) revealed genetic evidence for hybridization of species in the genus *Gallus* which suggests multiple species origins of domestic fowls. Erikson *et al.* (2008) by examining the origins of skin colour variations in domestic chickens, revealed that although the white skin allele in modern chickens is derived from the red jungle fowl the most likely origin of the yellow skin gene is the grey jungle fowl (*G. sonnerati*).

Another topic of discussion is whether the process of chicken domestication was a single event at a specific time or took place in several geographic locations and at different time periods. Crawford (1990) proposed that domestication of chickens took place in the Indus valley around 2500 - 2100 BC. On the contrary, archaeological discoveries in 16 Neolithic sites along the Huang He (Yellow River valley) in northeast China indicated that domestication of chickens may have taken place as early as 6000 BC (West and Zhou, 1988).

Based on the fact that the conditions around the 16 Chinese Neolithic sites are not typical of the natural environment for jungle fowls, West and Zhou (1988) proposed that domestication may have taken place in southeast Asia and the chicken were then moved to China by humans. In their study, Akishinomiya *et al.* (1994, 1996) also gave support to southeast Asia (Thailand and its neighbouring regions) as the cradle of domestic chickens. Liu *et al.* (2006) on the other hand found evidences implicating multiple maternal origins of chicken centered around south and southeast Asia.

Introduction of chicken to Africa

The introduction of the domesticated chicken to Africa is not well documented. The earliest known evidence was a drawing of the domestic cock, an ostrakon found in Egypt, depicting a red jungle fowl (Carter, 1923). This ostrakon was dated at c. 1425-1123 B.C. which was between the middle of the 18th dynasty and the period of the tomb of Ramesses IX of the 20th dynasty. According to Carter (1923), the ostrakon depicts fowls introduced to Egypt among tributes from a country between Syria and Babylonia. In the famous Annals of Tuthmosis III the fowls were then referred to as birds that "bear every day". However, chicken were not very common in Egypt until 332-330 BC (Clutton-Brock, 1992).

Little is known about the time and routes of introduction of chicken in Africa, except for Egypt. It is generally held that domesticated chicken spread into Europe and Africa after it appeared at Mohenjo-Daro in the Indus Valley by about 2000 B.C. Chicken were already present in most parts of Africa before the first European contact. Crawford (1990) indicated that chicken with black feathers, meat and bones were found in Mozambique in 1635, bearing the fibromelanosis mutant known at that time in India and not in Europe. According to this account, India is the most likely origin of chicken in Africa for two main reasons. The first reason was that trade between India and the east coast of Africa was well developed at an early date. Secondly, both eastern and western Africa share the same word for chicken that traced its root to India. However, it is not clear whether there were single or multiple routes and events of introduction. As was the case with many other livestock species, chicken could have been introduced into Africa through the Isthmus of Suez, the horn of Africa and through direct sea trading between Asiatic countries and coastal eastern Africa (Crawford, 1990; Clutton-Brock, 1992).

Archaeological evidences revealed a relatively recent presence of chickens in Africa, other than Egypt. A review by Clutton-Brock (1992) indicates that remains of chicken were recorded in southeast African iron age sites of Mozambique, Manekeni and Chibuene, dating to end of the first millennium, and the south African site of Ndongondwane, KwaZulu Natal, dating to 8th c. A.D. MacDonald (1992) found evidence of chicken remains from the west African Iron Age site of Jenne-jeno (Inland Niger Delta, Mali) dated to c. 450-850 A.D. and suggested that Asiatic chickens spread into west Africa before 850 A.D. The latest archaeological discovery was from Central Africa, in a tomb of the classic Kisalian Period at Sanga (Upemba), Zaire (10th -13th century A.D.) (Van Neer, 1990; cited by MacDonald, 1992). The primary reasons for domesticating animals in Africa were their cultural, ritual and social values. Their role as source of food came much later with the expansion of human population (Clutton-Brock, 1992). The conflict between archaeological findings to date on one hand and the apparently deep embedding of chicken in many African cultures, as well as the linguistic and ethnographic evidences on the other hand, suggest presence of chicken in Africa at much earlier dates (Williamson, 2000).

Hence, it is possible that chicken were present in Africa well before the earliest date yet attested by archaeological findings.

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Contribution of chickens to rural households in developing countries

Village chickens make substantial contributions to household food security throughout the developing world. Indigenous chicken serve as an investment and source of security for households in addition to their use as sources of meat and eggs for consumption and of income (Muchadeyi *et al.*, 2007). Chicken in general are a means of investment that is important to the welfare of women and children in traditional, low-input farming systems in the tropics. Besides rural households, these low-input, low-output poultry-husbandry systems are an integral component of the livelihoods of most of peri-urban, and some urban, households in most parts of the developing world. A review by Gueye (2000) indicated that an average family flock of five adult chickens (two males and three females) enables women in Central Tanzania to have an additional income equivalent to 10% of the average annual income. In the Niger Delta family poultry husbandry contributes 35% of the income of household women, which represents about 25% of Nigerian minimum wage and 50% of the per capita income (Alabi *et al.*, 2006). Experiences in many other developing countries have shown that village poultry can be used as an effective means of empowering women and as a tool for poverty alleviation (Kitalyi, 1998).

Indigenous poultry genetic resources

Dispersal of domestic chicken from its putative centres of domestication to different regions with diverse environmental conditions and people of different cultural orientations has contributed to the observed genetic differentiation of chicken populations across the world. Other factors that may have played a role in the genetic differentiation include founder effects and genetic drift.

Nearly 80% of the estimated 1.3 billion chickens in Africa comprise non-descript indigenous breeds raised by village farmers under extensive systems (Gueye, 1998). In Ethiopia the indigenous breeds contribute to more than 90% of the national chicken meat and egg output. Most indigenous birds of the developing countries, except those bred for cock fights, are non-specialized, and are known for their ability to survive on irregular supplies of feed and water, and with no to minimal health care. They form vital and integral parts of a “balanced” farming system

in terms of providing outputs matching available inputs. Studies on some of the indigenous birds from the tropics have shown that they are poor producers of eggs and meat production (Mathur *et al.*, 1989). Nevertheless, results from several productivity evaluation of indigenous chicken show that there are some highly productive indigenous bird populations exhibiting even higher performance levels compared to improved breeds under poor production circumstances (Mathur *et al.*, 1989).

Indigenous chicken have a number of adaptive traits and genes such as naked necks, minimum and frizzle feathers, black bones and meat, which have special utility in the hot and humid tropics (Horst, 1989). Indigenous chicken are known to be ideal mothers, good sitters, excellent foragers, hardy, and are believed to possess better natural immunity against common poultry diseases (Mathur *et al.*, 1989). A review by Islam and Nishibori (2009) indicated that in Bangladesh and many other developing countries, the meat and eggs of indigenous chicken is highly preferred for its taste and suitability for special dishes resulting in even higher market prices for these chickens than their exotic counterpart. Despite their importance indigenous breeds are under threat due to various factors such as changing production systems and indiscriminate cross-breeding (Besbes, 2009) and because of the low level of commercial interest on them. In general, their value remains underestimated and poorly documented compared to the specialized breeds in the western world.

Global databases on chicken genetic resources

At present there are three public domain electronic databases on animal genetic resources delivering information on the chicken in addition to other domestic animals. The first one is Domestic Animal Diversity Information System (DAD-IS) developed and managed by the Food and Agriculture Organization of the United Nations (FAO) global databank on animal genetic resources (AnGR); the second is the database of Oklahoma State University on Breeds of livestock of the World and the third one is the Domestic Animal Genetic Resources Information System (DAGRIS) which is developed and managed by the International Livestock Research Institute (ILRI).

DAD-IS (<http://www.fao.org/dad-is>) was initiated as a key communication and information tool for implementing the Global strategy for the management of farm AnGR, mainly to assist countries and country networks in their respective country programs (FAO, 1999). The DAGRIS virtual library (<http://dagris.ilri.cgiar.org>) has been developed to facilitate the compilation, organization and dissemination of information on the origin, distribution, diversity, present use and status of indigenous farm animal genetic resources from past and present research results. The current geographic scope of DAGRIS is Africa and selected Asian countries. In the future it aims to extend its coverage to other developing countries in Asia and Latin America and

the Caribbean (DAGRIS, 2007). The data base managed by Oklahoma State University does not provide much information on indigenous chicken genetic resources of developing countries.

Database for indigenous chicken of Ethiopia

The indigenous chicken of Ethiopia were referred to in various names and characterized on different grounds, as in many other parts of Africa. Teketel (1986) characterized them on the basis of plumage colour as, for example, 'Kei' (meaning red) or 'Tikur' (black). Tadelle (2003) referred to them as 'local chicken ecotypes' and Halima *et al.* (2007) as 'native chicken populations' both named on the basis of geographic region of sampling. Each 'local ecotype'/'native population' actually comprised chickens with wide range of morphologic or genetic diversity. In any case, thus far only 5 chicken 'types' of Ethiopia were listed in DAD-IS (FAO, 2008) and 10 in DAGRIS (DAGRIS, 2007) including those listed in DAD-IS. This small number represented in the databases indicates the shortage of data on chicken genetic resources of Ethiopia suggesting that much of the diversity that exists in the locally adapted populations still remains undocumented. Efforts to characterize and document both breed- and trait-level information for the indigenous chicken genetic resources should be given due consideration. Identification and characterization of animal genetic resources generally requires information on their population, adaptation to a specific environment, possession of traits of current or future value and socio-cultural importance, which are crucial inputs to decisions on conservation and utilization (Weigend and Romanov, 2001).

Genetic improvement of Ethiopian chickens

In the past many decades genetic improvement programs for increasing chicken productivity in Ethiopia mainly focused on use of imported temperate breeds. Many exotic breeds of chicken (White and brown Leghorns, Rhode Island Red, New Hampshire, Cornish, Australoup, Light Sussex etc.) were introduced over the years. The other approach to improve productivity of the village poultry production was based on use of crossbred animals. This involved crossing of local chicken to different levels of exotic blood. Evaluations of crossbred chicken at the Debre Zeit Agricultural Research Centre indicated that 62.5% white leghorn crosses showed superior performance to the locals as well as pure white leghorns in terms of egg production (DZARC, 1991). In a cross breeding program at Assela, Brannang and Persson (1990) also compared different York x local crosses. Their results indicated that egg production declined with increasing level of exotic inheritance (above 50%). Increasing the level of exotic blood also resulted in loss of broody behaviour, a trait of considerable economic value under village systems. Although the cross breeding programs produced successful results under experiment stations almost all of them were discontinued decades ago for various reasons. In the 1980s the Ministry of Agriculture initiated a cockerel distribution scheme. This involved importation and distribution of cockerels to

be used as breeding males in villages. This scheme again failed because farmers were unwilling to remove their local cocks and the exotic cocks failed to adapt in the village environments.

High yielding exotic breeds generally demand high input and thus promoting them is reasonable only if the farmer can verifiably benefit from the better commercialization potentials of exotic breeds. That is, easy accesses to markets, transport facilities, feed and veterinary products and timely availability of replacement stock etc. As long as the production condition causes stress (low nutrition, disease, high temperature, etc.) using high-yielding breeds can not be a sustainable option for improving village poultry. Instead they will create new dependencies and expose farmers to higher levels of risk. Therefore, breeding programs should be oriented in such a way as to address the underlying socioeconomic and production circumstances of village systems. This requires defining production environments and identifying the breeding practices, production objectives and trait choices of village farmers as inputs for developing appropriate breeding strategies (Solkner *et al.*, 1998).

Rationale and objectives of the study

The role of family poultry production as an affordable source of animal protein and income through out the developing world is well documented (Kitaliyi, 1998; Delgado *et al.*, 1999). In many African countries indigenous chickens kept under village systems are the major suppliers of poultry products (Gueye, 1998). In Ethiopia indigenous chickens are characterized by their small body size and poor production of meat and eggs (Mebratu, 1995). The average annual egg production of local genotypes ranges from 30 to 60 under free ranging village management which could be improved up to 100 eggs under improved management conditions (Dana and Ogle, 2000). The average egg weight is quite low, ranging from 38g to 46g (Teketel, 1986). Under research station management local birds were poor in feed efficiency (20 kg of feed required to produce 1 kg of eggs) and survival (Brannang and Persson, 1990) but demonstrated more sustained egg production at times of increased environmental temperatures and better fertility of eggs compared to their exotic counterpart (Teketel, 1986).

A recent study on evaluation of the growth performance of seven indigenous chicken populations indicated that average live weight to an age of 22 months ranged from 1045 to 1517 g for males and from 642 to 874 g for females, much lower compared to the average weights, 1736 and 1263 g, for the respective sexes of the Rhode Island Red breed kept under the same environment (Halima *et al.*, 2007). Local chicken attained 61 to 72 % of the weights of white leghorn chickens at 6 months of age with carcass weight of 559 g compared to 875 g for that of the white leghorn (Teketel, 1986).

Past attempts for increasing productivity of village poultry in Ethiopia pursued use of exotic breeds to replace indigenous chickens. This strategy failed to become a sustainable option mainly because it recurrently faced the problem of birds not being adopted widely by the rural farmers due to several socio-economic and environmental challenges (Teklewold *et al.*, 2006). One of the practical options to ensure conservation of genetic diversity is through utilization of indigenous genotypes by improving their competitiveness under the socioeconomic circumstances of their production environments. Recent studies showed that despite their low overall productivity indigenous chickens display wide range of variability in terms of morphological, production and genetic characteristics (Halima *et al.*, 2007) implying the potential for improvement through selective breeding. The extent of genetic diversity within and among the indigenous chicken populations, however, is not yet fully understood. The scope of the existing study on genetic diversity was limited particularly in detecting the extent of genetic differentiation among populations due to the small number of samples and microsatellite markers used compared to the number of markers recommended by FAO for chicken biodiversity studies, which was in the range of 20 to 30 (FAO, 2004). The extent of population differentiation could be detected more accurately by using larger number of loci because each locus will contain an independent history of the population depending on the amounts of random drift, mutation and migration that have occurred. Understanding the level of genetic diversity within and among chicken populations accurately is an important input in identifying populations for genetic improvement programs.

Developing appropriate animal breeding programs for village conditions requires defining the production environments and identifying the breeding practices, production objectives and trait choices of rural farmers (Solkner *et al.*, 1998). Furthermore, knowledge on genetic parameters for traits of economic importance to village poultry producers is critical. There is considerable mass of literature on genetic parameters of commercially important traits for industrial poultry populations (see reviews by Chambers, 1990; Fairfull and Gowe, 1990). However, these values may not be applicable to these indigenous chickens. So far no such estimates are available for Ethiopian chickens.

The overall objectives of this thesis were to characterize the production environments and diversity of chicken genotypes originating from different geographic regions of Ethiopia. This information will be used to set up a breeding scheme to improve productivity of Horro chicken, a common indigenous population in the highlands of Ethiopia.

The specific objectives of the study were: to initiate a breeding program for improving growth and egg production of indigenous chicken of Ethiopia, to describe the production environments in different geographic regions and identify the production objectives and trait preferences of village producers, to assess the morphological and genetic variations and describe the useful attributes of indigenous chicken populations, to estimate heritabilities and genetic and phenotypic correlations for growth and egg production traits in local chicken, to elucidate possible

southeast Asian contributions to the genetic variation of western commercial chicken breeds, and to propose schemes that can be implemented to stimulate the use of local chicken genetic resources for the production of eggs and meat

Thesis outline

This thesis is organized in 7 chapters which are described below.

Chapter 1 elaborates on the overall background and rationale of the study. It gives an overview of the domestication and introduction of chicken into Africa, significance of village poultry production, the genetic resource base and the needs for developing genetic improvement programs that enhance conservation of the existing diversity through improved competitiveness of indigenous breeds.

Chapter 2 describes certain morphological features of indigenous chicken of Ethiopia. It characterizes the different populations sampled from different geographic regions selected on agro-ecological basis. The peculiar morphological features of chicken from each region were presented in this chapter. The distribution and prevalence of certain genes, such as the Na and P genes, associated to the different qualitative traits and having relevance to breeding in tropical environments was presented.

Chapter 3 defines the socioeconomic characteristics of the village poultry production environments in different geographic regions, identifies the important functions of chickens, describes farmers' choice of chicken breeds and the underlying factors that determine the choice of genetic stock they use. The study included both questionnaire survey and a participatory group discussion. The questionnaire survey was used to collect data covering general information on village poultry production such as socio-management characteristics, production objectives, effective population size, farmers' breed choice and trait preferences, market preferences of specific traits, and traditional selection practices. The participatory group discussions were designed based on the different functions of chickens and 'traits' identified in the interviews in order to involve farmers in defining the breeding objective 'traits' and deriving their relative importance under the specific production environment.

Chapter 4 explores the genetic diversity of indigenous populations and the extent of population sub-structuring in Ethiopian chickens. It uses microsatellite data to infer the polymorphisms within and among the ecotypes and define the level of genetic differentiation among Ethiopian chicken populations using 20 microsatellite markers.

Chapter 5 is based on the breeding program initiated at Debre Zeit Agricultural Research Centre. It presents estimates on genetic parameters of growth and egg production in Horro chicken using a pedigree based on 26 sires and 260 dams. Estimates on body weights were based on measurements made every two weeks from hatch to 8 weeks and every 4 weeks afterwards until 16 weeks of age. Estimates on egg production traits were made for monthly egg numbers and

cumulative of monthly productions during the early part egg production period. The data were based on individual egg production records from start of laying to 44 weeks of age that were collected for 1 generation. Genetic parameters were estimated using animal model fitted with common environmental effects for growth traits and ignoring common environment for egg production traits.

Chapter 6 examines the possible Asian contributions to western commercial chicken and European traditional breeds from mitochondrial genetic diversity. A 365 bp fragment of the chicken mitochondrial DNA D-loop region of 160 commercial birds was sequenced, representing all important commercial types from multiple commercial companies that together represent more than 50% of worldwide commercial value. The same fragment for 16 Dutch fancy breeds (113 individuals) were also surveyed, comprising almost the entire breed diversity of The Netherlands.

Chapter 7 reflects on the outcome of the present research and discusses the findings in different perspectives. Importance of characterising indigenous chicken genetic resources, options for setting priorities for conservation of indigenous breeds and the roles of farmers and government were described. It examines the critical steps in developing breeding programs for improving village chickens and advises on implementation procedures. The lessons learned from the ongoing breeding program were also summarised. The final section of this chapter elaborates issues for consideration in setting up a sustainable genetic improvement program utilizing indigenous chickens.

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2

Production objectives and trait preferences of village poultry producers of Ethiopia: implications for designing breeding schemes utilizing indigenous chicken genetic resources

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Abstract

To generate information essential for the implementation of breeding schemes suitable for village poultry producers in Ethiopia, a survey was conducted aimed at defining the socioeconomic characteristics of the production environments in different geographic regions, understanding the important functions of chickens, identifying farmers' choice of chicken breeds and the underlying factors that determine the choice of genetic stock used. The survey included both questionnaire survey and a participatory group discussion. A total of 225 households (45 households from each of five *Woredas*) were interviewed. The questionnaire was designed to collect data covering general information on village poultry production such as socio-management characteristics, production objectives, population structure, breed choice and trait preferences, market preferences of specific traits, and farmers' selection practices. The participatory farmers' discussions were designed to involve stakeholders in defining the breeding objective 'traits' and deriving their relative importance in the production environment based on the different functions of chickens and 'traits' identified in the interviews. The results showed that production of eggs for consumption is the principal function of chickens in most regions followed by the use as source of income and meat for home consumption. The production system in all geographic regions studied revealed similar features generally characterized by extensive scavenging management, absence of immunization programs, increased risk of exposure of birds to disease and predators, and reproduction entirely based on uncontrolled natural mating and hatching of eggs using broody hens. Farmers' ratings of indigenous chickens with respect to modern breeds showed the highest significance of the adaptive traits in general, and the superior merits of indigenous chickens to high yielding exotic breeds in particular. Adaptation to the production environment was the most important attribute of chickens in all the study areas. The high significance attributed to reproduction traits indicates the need for maintaining broody behaviour and high level of hatchability while breeding for improved productivity of indigenous chickens for village conditions. The market price of chickens is primarily dictated by weight, but farmers rated growth (males) and number of eggs followed by growth (females) as the production traits they would like the most to be improved. Therefore, the ultimate breeding goal should be to develop a dual-purpose breed based on indigenous chicken genetic resources with any of the comb types other than single for all the regions studied having the most preferred white body plumage for farmers in the Amhara region and red body plumage for those in Oromia, Benshangul-Gumuz and Southern regions.

Key words: Indigenous chickens; breeding objectives; trait preference; Ethiopia

Introduction

Increased productivity of the poultry sub sector by using exotic breeds in Ethiopia failed to become a sustainable option mainly because this strategy recurrently faced the problem of birds not being adopted widely by the rural farmers due to several socio-economic and environmental challenges (Teklewold *et al.*, 2006). The management conditions under which the animals are produced vary along the existing production systems which were broadly classified into the village, small-scale commercial, and large-scale commercial systems based on flock size, production objectives and level of specialization and/or technology use (FAO, 2008). A review by Gueye (1998) indicated that nearly 80% of the estimated 1.3 billion chickens in Africa comprise indigenous breeds raised by village farmers under extensive systems. In Ethiopia the village system contributes to more than 90% of the national chicken meat and egg output. This system is generally characterized by small size of unimproved indigenous flock per household, birds maintained under scavenging regimens in the backyards with little or no supplemental feeding, no separate shelters except for night enclosures in the family house and lack of health care.

Despite their importance indigenous breeds are under threat due to various factors such as changing production systems and indiscriminate cross-breeding (Besbes, 2009). There are very few examples of breeding programmes for indigenous breeds in Africa and around the world. Recently a genetic improvement program has been initiated for increasing productivity of indigenous chickens of Ethiopia through selective breeding, as a means both to improve the livelihood of poor people as well as conserve the existing genetic diversity through utilization. Developing appropriate animal breeding programs for village conditions requires defining the production environments and identifying the breeding practices, production objectives and trait choices of rural farmers (Solkner *et al.*, 1998).

The traits traditionally considered as criteria for selecting breeding stock are important in describing the adaptive attributes and genetic merits of the indigenous chickens and in identifying farmers' choice of chicken breeds and the underlying factors that determine the choice of genetic stock used. The market preferences for specific traits identified in the current study could be used to compliment or stimulate further work on economic valuation of the traits (Scarpa, 1999). However, even in the absence of economic values, the results could be used to simulate alternative breeding schemes by using appropriate genetic parameters and deriving relative weights for the breeding objective traits using the desired-gain selection-index method as suggested by Solkner *et al.* (2008). Solomon (2008) found that farmers' ratings of trait categories they preferred to be improved in sheep in traditional systems were based on economic grounds and could be translated into economic weights that are comparable to economic values derived from profit equations. A similar approach could be adapted for developing breeding systems for indigenous poultry.

The objectives of this study were 1. to identify the socioeconomic characteristics of the production environments in different geographic regions; 2. to gain understanding of the traditional selection practices; and 3. to identify and prioritize the breeding objectives and trait preferences of village producers through a participatory approach.

Material and methods

In each of the study regions two types of data collection were applied. Firstly, individual farmers were interviewed and a list of detailed information was obtained. Secondly, and based on the results of the individual interviews, farmers were asked to discuss in groups on what they considered as most important regarding selection decisions and market value.

Description of study sites

The survey sites were selected considering agro-ecology, socio economic significance of chicken production and population of indigenous chickens based on the atlas published jointly by IFPRI and CSA (2006). Five *Woredas* (district) were covered in the study: Farta, Mandura, Horro, Konso and Sheka. The ecological and demographic features of the study areas were described in Tables 1 and 2.

Data collection and analysis

The interview was designed to collect two sets of data. The first set covered general information on household characteristics and poultry holdings. The second set included data on more specific aspects of village poultry production such as socio-management characteristics, production objectives, population structure, breed choice and trait preferences, market preferences of specific traits, and farmers' selection practices. A total of 225 households (45 households from each *Woreda*) were interviewed. The interview data were analyzed using descriptive statistics and the percentage of respondents was reported for each parameter.

The subsequent participatory farmers' discussions were designed to involve stakeholders in identifying the breeding objective 'traits' and deriving their relative importance in the different production environments. In total seven independent groups of farmers were formed in each region, where each group comprised of five to seven members. The groups consisted of neighbouring farmers following a transect walk in the villages. In order to address the variations in the opinions of farmers in different agro-ecological regions, the production system was classified into two 'sub-systems': low altitude and high altitude systems. Three regions were selected to represent the two 'sub-systems' (Mandura for the low altitude and, Farta and Horro for the high altitude production 'sub-system'). As point of departure for the discussions, the results of the individual interviews were summarized according to 1. identified overall objectives of keeping

Table 1 Ecological characteristics and human and chicken populations of sampling areas (Woreda*).

| | Farta | Mandura | Horro | Konso | Sheka |
|--|-----------------------------|-------------------------|----------------------------|-------------------------------|-------------------|
| Ecology | Cool to very cold sub-moist | Hot, sub-humid low land | Tepid to cool wet highland | Humid lowland to wet highland | Cool wet highland |
| Altitude (Range, m a.s.l., for Sampling Sites) | 2700-2870 | 1047-1426 | 2580-2810 | 1471-1898 | 2285 |
| Annual RF, mm | 1250-1599 | 900-1300 | 1200-1800 | 500-700 | 1400-2000 |
| Mean annual temp. (°C) | 9-25 | 25-32 | 22-26 | 24-37 | 13-25 |
| Human population | 256,513 | 31,000 | 84,596 | 206,607 | 47,955 |
| Av. family size | 7 | 5 | 6 | 5 | 7 |
| No of Chickens, total | 136,410 | 23,186 | 34,991 | 107,588 | 50,491 |
| No of indigenous chickens | 123,869 | 21,171 | 29,780 | 86071 | 46,456 |
| No of exotic Chickens** | 12,541 | 2,015 | 5,211 | 21518 | 4,035 |
| Av. flock size/ household | 3.7 | 3.7 | 2.5 | 2.6 | 7.4 |

* Woreda is an administrative domain at the 3rd level down a 'Region' and immediately below a Zone'

** Exotic chickens distributed by the office of Agric. since 2005 (this study was conducted in 2007)

chickens (egg or meat production, income generation, cultural/religious roles); 2. 'traits' affecting consumer preferences in purchasing and/or selling chickens (live weight, plumage colour, comb type); 3. 'traits' farmers desired to be considered in improving village chickens (adaptation, growth, egg production, plumage colour, 'qumena', comb type, reproduction). The 'traits' were defined in composite terms such as 'adaptation' (comprising disease and stress tolerance, flightiness/ability to escape predators, scavenging vigour), 'live weight/growth' (weight gain, live weight at market age/adulthood), 'egg production' (annual egg number, persistency of egg laying), 'reproduction' (broodiness, hatchability of eggs) and 'qumena' (conformation/erectness, visual attraction/colour, size). Farmers who had adopted exotic chickens (i.e. modern, genetically improved chickens, mainly Rhode Island Red) were asked to rate their opinions on the comparative production, reproduction and behavioural performance of indigenous chickens with respect to modern ones.

The discussions were aimed at coming to consensus regarding the ranking of the traits in the 3 categories, and in some cases on the preference for indigenous or exotic chickens. Per category, a list of the different functions of chickens and 'traits' identified in the interviews was prepared into separate flip charts and presented to each group for rating them according to their order of importance. The ratings were carried out by assigning different weights, ranging from 1 to 4 for the different functions of chickens and 'traits' affecting market preferences and, weights 1-5 and 1-7, respectively, to rate the relative importance of the 'traits' farmers desired to be improved in males and females (the highest weight = most important, the lowest weight = least important).

Table 2 Demographic characteristics of sample households (% of respondents).

| | Farta | Mandura | Horro | Konso | Sheka | Total |
|------------------------|-----------------|---|----------------|------------|--------------------------------------|-------|
| Male household head | 68.9 | 71.1 | 91.1 | 95.5 | 68.9 | 79.0 |
| female household head | 31.1 | 28.9 | 8.9 | 4.5 | 31.1 | 21.0 |
| Illiterate | 60.0 | 44.4 | 8.9 | 48.9 | 15.6 | 35.6 |
| Read & Write | 15.6 | 2.2 | 13.3 | 4.4 | 4.4 | 8.0 |
| Elementary + above | 24.4 | 53.3 | 77.8 | 46.7 | 80.0 | 56.4 |
| Muslim | 0.0 | 17.1 | 2.4 | 0.0 | 0.0 | 3.8 |
| Christian | 100.0 | 75.6 | 97.6 | 81.6 | 100.0 | 87.6 |
| Traditional | 0.0 | 7.3 | 0.0 | 5.3 | 0.0 | 2.4 |
| Major ethnic community | Amhara (100) | Amhara (44) Gumuz (28) Agew (19) Oromo (7) | Oromo (100) | Konso (96) | Shaka (84) Kaffa (7) Menja (5) | |

Each group discussed thoroughly and assigned relative weights, on consensus or majority vote otherwise, with the aid of a facilitator. Averages of the relative weights assigned by the groups in each region were finally ranked and compared using Wilcoxon signed ranks test.

To get an impression on the viability of the populations, the effective population size was determined (Falconer and MacKay, 1996):

$$N_e = (4 * N_m * N_f) / (N_m + N_f)$$

and the increase in inbreeding per generation as

$$\Delta F = 1 / (2N_e)$$

where; N_e is the effective population size, N_m the number of breeding males, N_f the number of breeding females and ΔF the inbreeding coefficient.

Results

Family and farm characteristics

The majority of the respondents were Christian males with at least elementary level of education (Table 1). Eight ethnic communities were comprised in the 5 survey sites. Except in Mandura, where the community was found to be a mixture of 3 communities other than the local Gumuz community (27.9%), all the other geographic regions were populated almost entirely by specific communities native to that area (Table 2).

Functions of chickens

Except in Mandura and Horro, where chickens are raised importantly as source of income, egg production (for home consumption) is the most important reason for keeping chickens in all regions studied. Meat production (for home consumption) is second in importance in Oromia

(Horro) and Southern regions. The function of chickens as source of cash income was rated to be as important as (Horro) or more important than egg and meat production (Mandura). It is second in importance to egg production in Farta. In Konso the principal purpose of raising chickens is for home consumption and their value as income source is third in importance. Only about 5% of the respondents in Farta and Konso included the cultural-religious role of chickens rating it fourth in importance whereas all the others did not state the significance of this function (Table 3).

Table 3 Farmers' rating^a of the relative importance of different functions of chickens.

| Functions of chicken | Farta | Mandura | Horro | Konso | Sheka |
|-------------------------|----------|----------|----------|----------|----------|
| Egg (home consumption) | 3.54 (1) | 3.47 (2) | 3.64 (1) | 3.90 (1) | 3.91 (1) |
| Meat (home consumption) | 1.24 (3) | 3.02 (3) | 2.76 (2) | 2.83 (2) | 3.54 (2) |
| Cultural/Religious | 0.19 (4) | 0.00 | 0.00 | 0.19 (4) | 0.00 |
| Source of income | 2.95 (2) | 4.00 (1) | 3.64 (1) | 0.49 (3) | 3.18 (3) |

Numbers in parenthesis indicate ranks based on Wilcoxon signed ranks test. Ranks of chicken functions within a column bearing different numbers are significantly different from each other (P<0.05)

^aThe importance of characters was rated based on weights attributed to each function of chickens by individual respondents; most important = 4, least important = 1

Socio-management factors

The major management factors describing chicken production in the different regions studied are presented in Tables 4 and 5. All of the households surveyed kept indigenous chickens managed extensively under traditional management regimens. Sixty two percent of the households in Konso and more than 75% of the households in Farta, Horro and Mandura practice supplementary feeding of scavenging chickens whereas confined management of chickens with commercial feeding is not known at all in any of the regions studied. Most of the farmers in the Amhara (Farta, 73%) and Oromia (Horro, 69%) regions sheltered chickens in the family house whereas almost equal proportion of those in Mandura and Sheka provided both separate shelter and sheltered in the family house. This is in contrast to Konso, where 80% of the farmers had separate shelters to house chickens.

Immunization services (Table 5) are almost non-existent (95%) for village chickens in all regions surveyed. However, unlike most of the farmers in the Amhara Region (Farta, 79%) where treatment of sick birds is not common, most households in Oromia region (Horro, 70%) and about 50% of the households in the Southern and Benshangul-Gumuz regions had awareness of, and access to curative medication.

There is no systematic mating in any of the regions studied. Thus, breeding of village chickens is completely uncontrolled and replacement stock is produced through natural incubation using broody hens. Whereas only 24% of the total number of respondents left broody hens to stop this behaviour naturally, the remaining majority practiced different methods to modify the broody behaviour, in times when incubation was not desired and the hens were required to resume laying faster. Some of the most popular methods reported were: hanging the hen up-side-down (59% in

Horro and 46% in Konso), moving the hen to neighbour houses (69% in Farta and 41% in Mandura). Together, these two are the most important methods commonly practiced by most of the farmers in the surveyed regions.

Changing the location of brooding nest is very popular in Konso (42%), little known in all other regions (3-9%).

Table 4 Housing and nutritional management of chickens under the village production system (% of respondents).

| Factor | Farta | Mandura | Horro | Konso | Sheka | Average |
|--|-------|---------|-------|-------|-------|---------|
| Housing | | | | | | |
| In the family house | 73 | 49 | 69 | 20 | 58 | 54 |
| Separate shelter | 22 | 51 | 31 | 80 | 42 | 45 |
| Separate house with other animals | 5 | 0 | 0 | 0 | 0 | 1 |
| Management system | | | | | | |
| Indigenous chicken, extensive management | 100 | 100 | 100 | 100 | 100 | 100 |
| Modern chicken, extensive management | 13 | 7 | 11 | 2 | 2 | 7 |
| Nutritional management | | | | | | |
| Scavenging | 22 | 16 | 2 | 38 | 7 | 17 |
| Scavenging + Supplement | 78 | 84 | 98 | 62 | 93 | 83 |
| Confined, complete ration | 0 | 0 | 0 | 0 | 0 | 0 |

Traits of adaptive and economic importance

In the discussion among farmers who had adopted modern chickens, the Rhode Island Red (RIR), the most popular and widely adopted chicken in the regions studied, was used as the reference breed. Only data from Farta, Horro and Sheka were considered because there a relatively larger number of farmers adopted (16% in Farta, 33% in Horro and 25% in Sheka). In terms of adaptive traits and consumption the indigenous chickens were considered favourable. Most of the respondents claimed that the modern breed is poor in disease and stress tolerance (86%) and in the ability to escape predators prevalent in their village conditions (96%). The modern breed generally required higher level of management (83%) often hard to afford and are poor scavengers (86%) compared to indigenous chickens. In addition, 77% of the farmers in Horro and 90% in Sheka claimed that hatchability of eggs obtained from the modern breed is inferior to eggs from indigenous chickens. Likewise, most of the respondents have the opinion that the eggs (90%) and meat (92%) obtained from modern breeds have poorer taste (Table 6). This was also confirmed by the lower market preference for eggs from exotic chickens. In the opinion of 98, 74 and 93% of the total respondents pooled over all regions RIR chickens were rated superior in egg production, meat yield and egg size, respectively, to the indigenous chickens (data not shown).

Plumage colour, live weight and comb type were important traits affecting market price of chickens (Table 7). Live weight is the most important attribute in all geographic markets followed

by plumage colour except in the Southern region where comb type affects market price more than plumage colour. The type of chicken breed does not have much influence on market preference. The market for eggs is not sensitive to the egg characteristics (egg size and shell colour) except that it attached higher preference for eggs of indigenous chickens to those from exotic breeds in all geographic regions (data not shown).

Table 5 Health and reproductive management of chickens under the village production system (% of respondents).

| Factor | Farta | Mandura | Horro | Konso | Sheka | Total |
|---------------------------------------|-------|---------|-------|-------|-------|-------|
| Vaccination & Immunization | | | | | | |
| No | 96 | 100 | 91 | 98 | 88 | 95 |
| Yes | 4 | 0 | 9 | 2 | 12 | 5 |
| Curative medication | | | | | | |
| No | 79 | 50 | 30 | 47 | 51 | 51 |
| Yes | 21 | 50 | 70 | 53 | 49 | 49 |
| Mating system | | | | | | |
| Uncontrolled, natural | 100 | 100 | 100 | 100 | 100 | 100 |
| Controlled | 0 | 0 | 0 | 0 | 0 | 0 |
| Incubation method | | | | | | |
| Natural (Broody hen) | 100 | 100 | 100 | 100 | 100 | 100 |
| Artificial incubation | 0 | 0 | 0 | 0 | 0 | 0 |
| Broody behaviour modification | | | | | | |
| Nothing | 12 | 36 | 14 | 13 | 43 | 24 |
| Hanging upside-down | 19 | 18 | 59 | 46 | 27 | 33 |
| Moving to neighbour houses | 69 | 41 | 16 | 0 | 24 | 33 |
| Submerge into water up to the breast | 0 | 2 | 2 | 0 | 0 | 1 |
| Change brooding place | 0 | 3 | 9 | 42 | 6 | 9 |

Farmers' selection practices

All farmers interviewed in the different regions practiced selection on breeding and replacement males and females based on four trait categories: plumage colour, live weight, comb type and 'qumena' (Table 8). Similar trait categories are used to select both males and females in all regions. Farmers in the Amhara (Farta) and Oromia (Horro) regions give the highest emphasis for plumage colour while in the Southern region (Konso and Sheka) live weight is used as the most important selection criteria. The emphasis given to each trait category is largely similar across the sexes except that, unlike for males, live weight is most important in Mandura (64%) and almost equally important to comb type in Farta for selecting breeding females.

Although each of these trait categories consisted of different component traits farmers described the specific trait components for only two of the four trait categories used as selection criteria, plumage colour and comb type (Table 9). White and red plumage colours were identified as the two important component traits used for selecting on the basis of body plumage. Red is the

most favoured plumage in the Benshangul-Gumuz (Mandura), Oromia (Horro) and Southern Regions (Konso and Sheka) whereas white is the body plumage colour more favoured by the Amhara community (Farta) irrespective of the sex of the birds. Farmers in the South, however, displayed a much stronger distaste for chickens having white plumage colour compared to the others. Similarly, farmers in all regions recognized only two types of combs for the trait category, comb type: ‘Netela’ meaning Single and, ‘Dirib’ that actually comprised all comb types other than ‘Single’ (i.e. rose, pea, walnut and duplex combs). ‘Dirib’ is a favoured comb type both for females (68%) and males (90%) suggesting that most of the farmers placed equally higher preference for any comb type other than single. No specific trait components were identified for the other trait categories, weight and ‘qumena’, except that all farmers stated that they selected birds that are ‘heavier’, in respect of their age mates, and those having attractive ‘qumena’ judging subjectively by hand ‘weighing’ and visual appraisal.

Table 6 Farmers’ rating of the characteristic attributes of indigenous chickens compared to a reference modern breed (MB)^a.

| Characteristics | Rating | Farta (%) | Horro (%) | Sheka (%) | Total (%) |
|------------------------------|----------------|-----------|-----------|-----------|-----------|
| Disease and stress tolerance | Superior to MB | 83 | 80 | 95 | 86 |
| | Equal | 0 | 0 | 5 | 2 |
| | Inferior to MB | 17 | 20 | 0 | 12 |
| Escape from predators | Superior to MB | 100 | 93 | 95 | 96 |
| | Equal | 0 | 0 | 0 | 0 |
| | Inferior to MB | 0 | 7 | 5 | 4 |
| Management level required | Higher | 0 | 36 | 16 | 17 |
| | Lower | 100 | 64 | 84 | 83 |
| | Equal | 0 | 0 | 0 | 0 |
| Scavenging behaviour | Superior to MB | 100 | 80 | 78 | 86 |
| | Equal | 0 | 7 | 11 | 6 |
| | Inferior to MB | 0 | 13 | 11 | 8 |
| Hatchability of eggs | Superior to MB | 33.3 | 77 | 90 | 67 |
| | Equal | 33.3 | 8 | 5 | 15 |
| | Inferior to MB | 33.3 | 15 | 5 | 18 |
| Taste of egg | Superior to MB | 83 | 93 | 95 | 90 |
| | Equal | 0 | 0 | 0 | 0 |
| | Inferior to MB | 17 | 7 | 5 | 10 |
| Taste of meat | Superior to MB | 83 | 93 | 100 | 92 |
| | Equal | 0 | 0 | 0 | 0 |
| | Inferior to MB | 17 | 7 | 0 | 8 |

^aRhode Island Red was the reference modern breed (MB)

Table 7 Farmers' rating^a of trait categories/factors most influencing price of live chickens marketed in different regions of Ethiopia.

| Trait category/ Factor | Farta | Mandura | Horro | Konso | Sheka |
|------------------------|----------|----------|----------|----------|----------|
| Plumage Colour | 2.04 (2) | 1.64 (2) | 2.24 (2) | 0.80 (3) | 0.96 (3) |
| Weight | 3.56 (1) | 3.84 (1) | 3.04 (1) | 3.72 (1) | 3.84 (1) |
| Comb Type | 1.44 (3) | 1.07 (3) | 1.60 (3) | 1.24 (2) | 1.08 (2) |
| Breed | 0.00 | 0.00 | 0.88 (4) | 0.64 (4) | 0.88 (4) |

Numbers in parenthesis indicate ranks based on Wilcoxon signed ranks test. Ranks of trait categories within a column bearing different numbers are significantly different from each other (P<0.05).

^aThe importance of characters was rated based on weights attributed to each character by individual respondents: most important = 4, least important = 1

Effective population size and inbreeding in village chickens

A considerable proportion, ranging from 31 to 55.6%, of the farmers interviewed in the different regions did not own breeding males. Most of them shared breeding males with neighbours. To get some impression on the effective population size and increase in inbreeding over generations, effective population size were calculated based on the flocks of farmers who possessed their own breeding males. The largest effective population size was recorded in Konso with the subsequent lowest inbreeding coefficient (Table 10).

Table 8 Trait categories used by farmers to select male and female breeding stock (% of farmers^a)

| Trait category | Farta | Mandura | Horro | Konso | Sheka | Total |
|----------------|-------|---------|-------|-------|-------|-------|
| Males | | | | | | |
| Plumage Colour | 81 | 57 | 82 | 15 | 35 | 57 |
| Weight | 33 | 55 | 52 | 70 | 54 | 52 |
| Comb Type | 40 | 21 | 30 | 36 | 30 | 31 |
| 'Qumena' | 12 | 33 | 39 | 24 | 38 | 29 |
| Female | | | | | | |
| Plumage Colour | 74 | 46 | 71 | 13 | 33 | 50 |
| Weight | 30 | 64 | 61 | 67 | 72 | 58 |
| Comb Type | 33 | 14 | 12 | 22 | 19 | 20 |
| 'Qumena' | 2 | 30 | 22 | 13 | 3 | 14 |

^aPercentages do not add up to 100% since respondents selected based on more than one trait category

Table 9 Farmers' preferences for specific traits in plumage colours and comb types of female (F) and male (M) chickens in different regions (percentage of farmers^a)

| Preferred Characteristics | Farta | | Mandura | | Horro | | Konso | | Sheka | | Total | |
|---------------------------|-------|----|---------|----|-------|----|-------|-----|-------|----|-------|----|
| | F | M | F | M | F | M | F | M | F | M | F | M |
| Plumage Colour | | | | | | | | | | | | |
| White | 68 | 76 | 46 | 33 | 19 | 29 | 20 | 6 | 5 | 17 | 32 | 32 |
| Red | 43 | 45 | 68 | 74 | 81 | 86 | 83 | 85 | 90 | 67 | 73 | 71 |
| Any Colour | 11 | 3 | 4 | 7 | 0 | 17 | 16 | 12 | 15 | 8 | 9 | 9 |
| Comb Type | | | | | | | | | | | | |
| Single (Netella) | 12 | 6 | 37 | 4 | 25 | 6 | 7 | 0 | 50 | 11 | 26 | 5 |
| 'Dirib' | 82 | 94 | 52 | 84 | 75 | 94 | 93 | 100 | 36 | 79 | 68 | 90 |
| Any Type | 6 | 0 | 11 | 12 | 0 | 0 | 0 | 0 | 14 | 11 | 6 | 5 |

^aPercentages do not add up to 100% since respondents selected based on more than one trait category

Rating trait categories for genetic improvement

Farmers' participatory rating of the importance of different trait categories is presented in Table 11. Adaptive traits (specifically disease and stress tolerance, flightiness and, scavenging vigour) in both males and females, growth in males and number of eggs in females, ranked 1st and equal in importance in low altitudes. In the highlands adaptation is second in importance to growth (males) and egg production (females). Plumage colour of birds (low altitude) and comb type (high altitude) were identified as the traits farmers would like the least to be improved in both classes of sex. Farmers in both altitude regimens attributed a comparable and high emphasis to traits related to reproduction in females, even more important than growth. 'Qumena' of birds is relatively more important to the farmers in low altitudes than those in the highlands.

Table 10 Possession of breeding males, effective population size and level of inbreeding of village chicken flock in the different regions.

| Study Area | Total No. of respondents (N) | Farmers not possessing breeding males | | Farmers rearing own breeding males (%) | Nm | Nf | Ne | ΔF |
|------------|------------------------------|---------------------------------------|------|--|------|------|------|------------|
| | | N | % | | | | | |
| Farta | 45 | 18 | 40 | 4.40 | 1.26 | 2.79 | 3.47 | 0.144 |
| Mandura | 45 | 25 | 55.6 | 31.1 | 1.75 | 2.58 | 4.17 | 0.120 |
| Horro | 45 | 14 | 31.1 | 24.4 | 1.84 | 3.76 | 4.94 | 0.101 |
| Konso | 45 | 20 | 44.4 | 22.2 | 1.96 | 3.9 | 5.22 | 0.096 |
| Sheka | 45 | 16 | 35.6 | 15.6 | 1.17 | 2.5 | 3.19 | 0.157 |

Nm = number of breeding males, Nf = number of breeding females, Ne = effective population size, ΔF = inbreeding coefficient

Table 11 Farmers' participatory rating of trait categories they would like the most to be improved for chickens in low (Mandura) & high (Farta & Horro) altitudes.

| Trait category for males | Low Altitude | High Altitude |
|---|--------------|---------------|
| Adaptation | 4.14 (1) | 3.93 (2) |
| Growth/Weight | 4.14 (1) | 4.21 (1) |
| Plumage Colour | 1.14 (4) | 3.07 (3) |
| Comb Type | 2.14 (3) | 1.86 (5) |
| 'Qumena' | 3.43 (2) | 1.93 (4) |
| Trait category for females | | |
| Adaptation | 5.14 (1) | 5.36 (2) |
| Number of eggs | 5.14 (1) | 6.00 (1) |
| Growth/Weight | 3.86 (4) | 3.86 (4) |
| Plumage Colour | 1.29 (6) | 3.57 (5) |
| Comb Type | 2.71 (5) | 1.64 (7) |
| Reproduction (broodiness, hatchability of eggs) | 5.00 (2) | 5.07 (3) |
| 'Qumena' | 4.86 (3) | 2.50 (6) |

Numbers in parenthesis indicate ranks based on Wilcoxon signed ranks test. Ranks of trait categories within a column bearing different numbers are significantly different from each other ($P < 0.05$).

Discussion

Functions of chickens

Like in any other village poultry systems in developing countries there is no specialized egg or meat chicken production in Ethiopia. Egg production is the principal function of chickens followed by the use as source of cash income and meat. Village chicken in other parts of Africa also played similar roles. In Zimbabwe chickens served as an investment and source of security for households in addition to their use as sources of meat and eggs for consumptions and of income (Muchadeyi *et al.*, 2007). Although previous studies in some parts of Africa (Gondwe, 2005; Muchadeyi *et al.*, 2007) indicated that the cultural/religious role of indigenous chicken types is important, the results of the present study did not support the significance of this function.

Socio-management characteristics and important attributes of indigenous chickens

The village poultry production environment in all geographic regions studied is generally characterized by extensive scavenging management, no immunization programs, increased risk of exposure of birds to disease and predators, and reproduction entirely based on uncontrolled natural mating and hatching of eggs using broody hens. Most of these features were also shared by many other African countries (Aboe *et al.*, 2006; Gondwe and Wollny, 2007; Harrison and Alders, 2010) although some countries such as Mozambique have started successful vaccination programs against one of the major killer diseases, Newcastle disease (Harrison and Alders, 2010). On average, 83% of the farmers in this study provided supplementary feeding. Recent studies in Ghana and Mozambique also showed that from 90 to 100% of farmers offered supplementary feeds to their chickens (Aboe *et al.*, 2006; Harrison and Alders, 2010). However, unlike farmers of Mozambique who mostly provided separate shelters and rarely housed their chickens in their homes (Harrison and Alders, 2010) more than 50% of the farmers in this study housed chickens in the family dwellings at night.

Farmers' ratings of indigenous chickens for various traits/ trait categories compared to a reference exotic breed revealed the important adaptive attributes of indigenous chickens. Adaptability of an animal is generally described in terms of traits enabling them to survive, reproduce and be productive in the limits of their production condition (Parayaga and Henshal, 2005). Indigenous chickens were rated to have superior merits with regard to traits such as disease tolerance, tolerance to cold and heat, ability to escape from predators, scavenging and broody behaviours and hatchability of eggs which are important in adaptation to the village environment; and those traits, such as taste of egg and meat, affecting consumption preference and consequently market value. A review by Islam and Nishibori (2009) indicated that in Bangladesh and many other developing countries, the meat and eggs of indigenous chickens is highly preferred for its

taste and suitability for special dishes resulting in even higher market prices for these chickens than their exotic counterpart. Earlier studies on adoption of poultry breeds in Ethiopia (Teklewold *et al.*, 2006) indicated that these trait categories were among the principal factors determining farmers' adoption of improved chicken breeds.

Morphologic traits such as plumage colour and comb type were also found to have significant economic values beside other quantitative traits related to growth and egg production. Like in other parts of the world (Jiang, 1999) there were specific choices for plumage colours affecting market preferences in the different geographic regions surveyed. The current result indicated that plumage colour followed by comb type is only second in importance to live weight in affecting market preference of chickens. In Northern Ethiopia both producer-sellers and intermediary traders attached the highest preference for plumage colour. For producer-sellers feather distribution, having either feathered or naked neck, is equally important as plumage colour followed by breed and comb type whereas for intermediaries comb type is second in importance (Aklilu *et al.*, 2007). The market preferences in this study were based on the opinions of producer-sellers and it was found that very little or no special preference was attributed to the type of breed marketed.

Farmers' selection practices

Farmers involved in virtually all forms of agricultural production practiced selection of varying scale and intensity for the traits they considered important under their production environment. Village farmers in this study traditionally attached greater selection emphasis to monogenic qualitative traits, plumage colour (white in the Amhara region and red in all the rest) and comb type, next to the only quantitative trait (growth). 'Qumena' as a composite trait category mainly deriving from general qualitative characteristics such as conformation is also given an important emphasis. This trait category was described similarly and attributed comparable level of importance in other species of livestock produced by village farmers (Solomon, 2008). Similarly, Muchadeyi *et al.* (2009) reported that poultry farmers in Zimbabwe traditionally selected compact and mature birds rather than angular and tallish ones as breeding stocks though they attached no emphasis to plumage colour.

There were almost no differences in the selection of male and female chickens in terms of both the selection criteria employed and emphasis given to the selection traits under the traditional selection practices. The selection practices were limited to trait categories which influenced market price differentials immediately and directly or observed and/or measured on the selection candidate itself. For instance, although egg production is the most important function of chickens in all households it was not considered as a selection criterion.

However, considering that the trait categories selected in both sexes were consistent to

those preferred by the local chicken market, it seems that market of chickens is the principal factor dictating farmers' selection practices in Ethiopia. Lack of information on egg production of the selection candidate was a less likely reason for farmers' not including this trait in their selection criteria because even in the absence of recording, it could have been possible to select the best female and male offspring for egg production at least by recalling the laying performance of their parents which should be simple due to the very small flock size owned per family.

Effective population size and inbreeding in village chickens

The effective population size ranged from 3.19 (Sheka) to 5.22 (Konso) and the number of breeding individuals is very small. The effective population size found in this study was too low compared, for instance, with the average size (15.4) reported for village chickens of Jordan (Abdelqader *et al.*, 2007). Subsequently the rate of inbreeding is quite high in all regions studied here particularly due to the small flock size characterizing this production system, an overall average of 3.4 chickens per household, which is extremely small compared to the average size of 42 reported for Jordan village chickens (Abdelqader *et al.*, 2007) and other African countries such as Ghana, Malawi, Zimbabwe and Mozambique where it ranged from 13 to 29 (Aboe *et al.*, 2006; Gondwe and Wollny, 2007; Muchadeyi *et al.*, 2007; Harrison and Alders, 2010). The extremely small flock size in this study confirms the drastic drop in the total population of chickens in Ethiopia since the past decade (Dana *et al.*, 2010). On the other hand, though the number of breeding individuals per household is low, the fact that market is also an important source of breeding males might contribute towards reducing further inbreeding. In any case, it should be noted that the estimates on the effective population size as well as rate of inbreeding in the village flocks are not very accurate due to the existing breeding system, which are entirely based on uncontrolled natural mating, and absence of breeding males in many households keeping chickens (see Table 8).

Selection traits and breeding objectives

Farmers' participatory definition and ratings of the trait categories they liked to be improved were different from those employed traditionally as selection criteria. They included additional economically important traits related to adaptation, egg production and reproduction. For instance, farmers both in the low altitude (Mandura) and high altitude regions (Farta and Horro) traditionally exerted the highest emphasis on body plumage colour next to body weight for selecting males and females. However, following the participatory rating of trait categories it was one of the traits farmers would like the least to be considered in improving both classes of sex. Adaptation to the production environment was the most important attribute of chickens both in the lowland and highlands in males as well as females (except in the highlands where it is considered

2nd in importance following egg number). Almost similar order of ranking was reported by Muchadeyi *et al.* (2009) for village chickens in Zimbabwe where farmers across all ecological regions attributed the highest importance to reproductive performance, growth and survival in the production environment rating plumage colour as the least important. In Jordan, village farmers considered egg production as the most important criterion, followed by mothering ability and body weight, for selecting their breeding stock (Abdelqader *et al.*, 2007). Thus, it is advisable to incorporate these traits in the selection schemes while setting up breeding programs targeting village poultry producers in different regions of Ethiopia.

Conclusion

There is a clear need to base genetic improvement programs for village poultry producers on indigenous chicken genetic resources. This is emphasized by the fact that the adaptive traits in general, and the superior merits of indigenous chickens to high yielding exotic breeds in particular, were rated of the highest significance by the local farmers. Egg production is the principal function of chickens followed in respective order by their use as source of cash income and meat. The market price of chickens is primarily dictated by weight, but farmers rated growth (males) and number of eggs followed by growth (females) as the traits they would like the most to be improved. Therefore, the ultimate breeding goal should be to develop a productive dual- purpose breed that can survive and reproduce under the production environment of village farmers.

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3

Morphological features of indigenous chicken populations of Ethiopia

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Summary

This study describes the variations in the physical features and the useful attributes of different populations of indigenous chickens. Five populations of chickens in different regions of Ethiopia were studied based on 13 qualitative traits recorded on a total of 1,125 chickens. Additional measurements on quantitative traits, shank length and body weight, were also included. Descriptive statistics, non parametric and F-tests were used to analyze the data. Each population studied possessed multiple variants of plumage colours and other physical features. However, white body plumage is one of the prominent features of the Farta chickens whereas red is predominant in the other populations. Pea comb is the dominant comb type in all regions. Most of the chickens in the high altitude regions have yellow skins. The geographic distribution and frequency of the naked neck chickens is generally small and the available small proportion is found mainly in the low altitude regions. Males in all populations are heavier and taller than the females. Body weights ranged from 1411 (Konso) to 1700 g/bird (Horro) in adult males and from 1011 (Konso) to 1517 g/bird (Sheka) in females. Most of the morphological traits studied showed very low level of associations with each other.

Key words: Indigenous chickens, morphological characters, Ethiopia

Introduction

The indigenous chickens of Ethiopia were referred to in various names and characterized on different grounds, as in many other parts of Africa. Teketel (1986) characterized them on the basis of plumage colour as, for example, 'Kei' (meaning red) or 'Tikur' (black). Tadelle (2003) referred to them as 'local chicken ecotypes' and Halima *et al.* (2007b) as 'native chicken populations' both named on the basis of geographic region of sampling. Each 'local ecotype'/'native population' actually comprised chickens with wide range of morphologic or genetic diversity. In any case, thus far only 5 chicken 'types' of Ethiopia were listed in the DAD-IS of FAO (derived from FAO, 2008) and 10 in the DAGRIS of ILRI (derived from DAGRIS, 2008) including those listed in DAD-IS. This small number represented in the databases indicates the shortage of data on chicken genetic resources of Ethiopia suggesting that much of the diversity that exists in the locally adapted populations still remains undocumented.

Identification and characterization of the chicken genetic resources generally requires information on their population, adaptation to a specific environment, possession of traits of current or future value and socio-cultural importance, which are crucial inputs to decisions on conservation and utilization (Weigend and Romanov, 2001). Indigenous chickens of the tropics are important reservoirs of useful genes and possess a number of adaptive traits (Horst, 1989).

Genetic variations in chickens could be described, among other approaches, using monogenic traits based on pigmentation differences and comb types. Pigmentation differences, occurring due to melanin, produce a variety of plumage colours in the chickens. The presence and level of melanin pigments such as trichochrome is related to feather colour and is considered to be indicative of genetic differences among certain plumage colours (Smyth, 1990). Similarly, the presence or absence of the carotenoid pigments, primarily xanthophylls, in the feed is responsible for the diversity in skin colour of chickens. The genetic basis of this variation was described by Eriksson *et al.* (2008).

Besides their significance in describing genetic variations and adaptive attributes, qualitative morphological traits have important economic value in chickens. There are specific choices for plumage and skin colours affecting preferences of different geographic markets around the world (Smyth, 1990; Jiang, 1999). In Ethiopia, though there is no specific choice for skin colour, plumage colour was only second in importance to live weight in affecting market preference of chickens (Nigussie *et al.*, unpublished) and in certain communities of Ethiopia (Leulseged, 1998) and other parts of Africa (Gueye, 1998) it has cultural-religious functions as well. In Northern Ethiopia both producer-sellers and intermediary traders of chickens attached the highest market preference to plumage colour and feather distribution followed by comb type (Aklilu, 2007). This clearly suggests that qualitative traits with specific characters must be

carefully identified and considered in developing breeding strategies.

Objectives of this study were to describe the physical features of different populations of indigenous chickens and assess the morphological variations among the populations in order to depict the useful attributes of indigenous chickens. This work will also contribute to the existing scarce information on the indigenous chicken genetic resources of Ethiopia.

Materials and methods

A list of physical descriptors was prepared to record both qualitative morphological characters and certain quantitative traits. In each of the study regions individual households only keeping local chickens were selected. Moreover, each of the selected farmers was interviewed to describe the family history of the flock and only unrelated adult birds were sampled for the recording. Neighbouring households were skipped to avoid the risk of sampling chickens sharing the same cock.

Naming of indigenous chickens

There are certain discrepancies in nomenclature of indigenous chickens of Ethiopia that forfeited retrieval, utilization and comparison of results, published or unpublished thus far. To avoid such discrepancies and limit further variations we adopted the naming referred to by Halima *et al.* (2007b) using the term 'indigenous' instead of 'native', in the context of the classification proposed by Tixier-Boichard *et al.* cited in Weigend and Romanov (2001) for chickens comprising domesticated but unselected populations.

Description of study areas

The study areas were selected considering agro-ecology, socio economic significance of chicken production and population of indigenous chickens based on the atlas published jointly by IFPRI and CSA (2006). Five *woredas** were covered in the study: Farta (Amhara Region), Mandura (Benshangul Gumuz Region), Horro (Oromia Region), and, Konso and Sheka (Southern Region). The location of the study areas was shown in Fig. 1 and their ecological and demographic features were described in Table 1.

Data collection and analysis

Morphological variations were studied based on feather distribution (presence or absence of feathers on the neck), feather morphology, colours of the body plumage, neck, breast and back feathers, shank colour, skin colour, earlobe colour, comb type, and head and body shapes. Data were recorded on a total of 1,125 indigenous chickens of both sexes: 225 chickens (of

approximately 8 months or older) in each of Farta, Mandura, Horro, Konso and Sheka *Woredas*, following the FAO descriptors for chicken genetic resources (FAO, 1986). Descriptions on comb types were based on illustrations presented by Somes (1990). The morphologic variables were recorded in different character states (see Appendix 1). Each character state was recorded as a binomial variable (1 if present and 0 if not). Measuring tapes and spring balance, respectively, were used to measure shank length and body weight of individual chickens in the field.

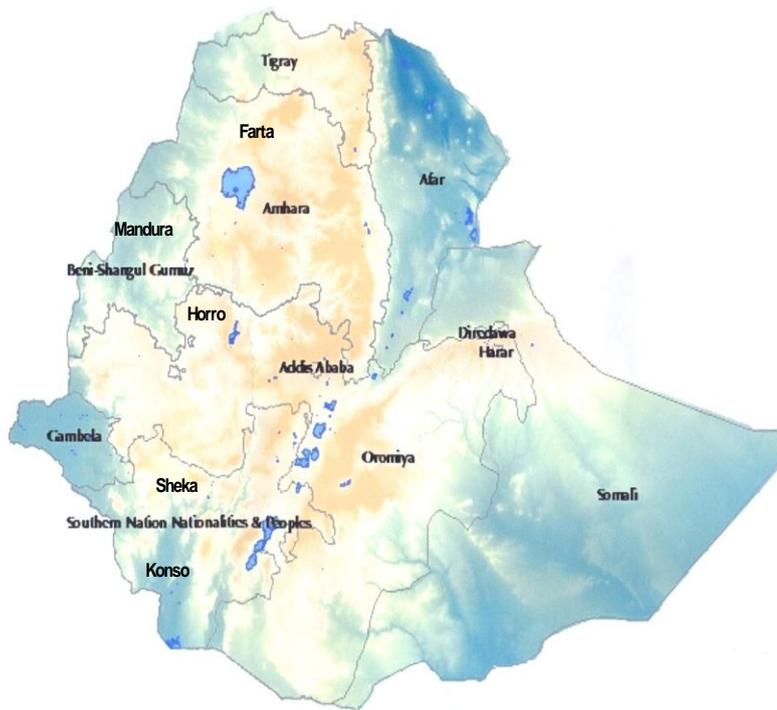


Fig. 1. Map of Ethiopia showing location of sampled populations of indigenous chickens, Farta, Mandura, Horro, Konso and Sheka. (Pink areas denote high altitude regions and sky blue sheds represent low altitude regions. See Table 1 for detailed descriptions of sampling sites)

Data were analyzed using the SPSS 12.0 statistical package (SPSS, 2003). Binomial variables from records on qualitative morphologic characters were reported as percentages. The qualitative data were analyzed for descriptive statistics using frequency procedures and cross-tabulation of SPSS. Kruskal-Wallis test was applied to test the effects of populations/ regions of sampling/ on each of the qualitative morphological variables. Binomial test was used to analyze the significance of the differences within population in feather morphology, feather distribution and skin colour whereas Cochran's test was applied to test the differences in shank and ear lobe

colours, comb type and head and body shapes.

The GLM procedure of SPSS was used to analyze the quantitative data, fitting live weight and shank length as independent variables and region of sampling (the populations) and sex of the chickens as fixed factors. Age of the chickens was not included in the model because only adults, 8 months or older, were sampled.

Table 1 Ecological and demographic characteristics of sampling areas.

| <i>Woreda</i> * | Farta | Mandura | Horro | Konso | Sheka |
|--|-----------------------------|----------------------------|----------------------------|-------------------------------|---------------------|
| Ecology | Cool to very cold sub-moist | Hot, sub-humid lowland | Tepid to cool wet highland | Humid lowland to wet highland | Cool wet highland |
| Altitude (range, m asl., for sampling sites) | 2700-2870 | 1047-1426 | 2580-2810 | 1471-1898 | 2285 |
| Annual RF, mm | 1250-1599 | 900-1300 | 1200-1800 | 500-700 | 1400-2000 |
| Mean annual temp., °C | 9-25 | 25-32 | 22-26 | 24-37 | 13-25 |
| Human population | 256,513 | 31,000 | 84,596 | 206,607 | 47,955 |
| Av. family size | 7 | 5 | 6 | 5 | 7 |
| No. of chickens, total | 136,410 | 23,186 | 34,991 | 107,588 | 50,491 |
| No. of local chickens | 123,869 | 21,171 | 29,780 | 86071 | 46,456 |
| No. of exotic chickens** | 12,541 | 2,015 | 5,211 | 21518 | 4,035 |
| Major ethnic community | Amhara | Amhara, Gumuz, Agew, Oromo | Oromo | Konso | Sheka, Kaffa, Menja |

* *Woreda* is an administrative domain at the 3rd level down a 'Region' and immediately below a 'Zone'

** Exotic chickens distributed by the office of Agric. since 2005 (this study was conducted in 2007)

Results and Discussion

Description of the populations

The morphological characteristics of the different populations of indigenous chickens in this study were shown in Tables 2, 3, 4, 5, 6 and 7. The specific features of each population are elaborated in the following sections. The data disaggregated by sex was only presented for morphological traits showing some interesting variations between the sexes to limit the size of the text.

Farta chickens. The Farta chickens are found in the Amhara regional state, North Ethiopia at altitudes ranging from 2700-2870 m asl in a cool to very cold, sub-moist ecological zone (Fig. 1). The population of these chickens is about 123,800 and they are kept by the Amhara community (Table 1). They are maintained under scavenging regimens with occasional supplementation and sheltered in the family house (Dana *et al.*, unpublished). The chickens have predominantly white body plumage colour occurring at similar frequency in both sexes. Red (25%) and 'Gabsima'

(wheaten strips on a black background) are the typical plumage colours in males but are not observed in females (Fig.2; Table 6). The other peculiar feature in males is the black breast (locally referred to as ‘Libe Tikur’) which is almost absent in females (Table 2). Naked neck chickens were not found in the population. About 55% of the birds have yellow skin colour, 65% of which are males (Table 4). The population is mainly pea combed (54%) followed by duplex combs (26%). Crest head (locally referred to as ‘Gutya’) and blocky body shape are the predominant features in both sexes (Table 5). The average shank length of adult males is 8.2 cm and that of adult females is 6.6 cm. Adult males weigh about 1630 g and females 1054 g per bird (Tables 7).

Table 2 Description of body plumage and breast feather colours of indigenous populations of chickens sampled from different regions (percentage of chickens within population, number of chickens sampled per population =225, N = 1125).

| Feather colour | White | Black | Red | Geb sim a | Teter im a | Brow n | Kokim a | Grey | Zigrim a | Golde n | Multipl e |
|---------------------|------------------|------------------|------------------|----------------|-----------------|-----------------|----------------|-----------------|-----------------|---------|----------------|
| Body plumage N (%) | 184 (16) | 81 (7) | 227 (20) | 82 (7) | 66 (6) | 217 (19) | 31 (3) | 66 (6) | 131 (12) | 6 (1) | 34 (3) |
| | ** | | | | ** | ** | ** | ** | ** | | ** |
| Farta | 33 ^a | 5 | 15 ^a | 8 | 11 ^a | 12 ^a | 3 | 2 | 5 | 0 | 4 |
| Mandur | 17 ^b | 8 | 19 ^b | 7 | 5 ^b | 20 | 1 | 8 | 10 | 0 | 1 |
| Horro | 14 ^{bc} | 5 | 22 ^{bc} | 5 | 2 ^b | 16 | 6 ^a | 9 | 18 ^a | 0 | 2 |
| Konso | 11 ^{bc} | 9 | 21 ^{bc} | 10 | 6 ^c | 18 | 3 | 5 | 11 | 0 | 7 ^a |
| Sheka | 7 ^c | 9 | 23 ^c | 7 | 4 ^b | 30 ^b | 1 | 6 | 11 | 0 | 1 |
| Breast colour N (%) | 193 (17) | 174 (16) | 23 (2) | 24 (2) | 99 (9) | 372 (33) | 15 (1) | 163 (15) | 37 (3) | 3 (0) | 22 (2) |
| | ** | * | ** | ** | ** | ** | | ** | ** | | |
| Farta | 33 ^a | 19 ^{ab} | 0 | 0 | 16 ^a | 21 ^a | 1 | 7 | 0 | 0 | 3 |
| Mandur | 16 | 13 ^b | 0 | 1 | 9 ^b | 32 ^b | 0 | 22 ^a | 4 | 0 | 3 |
| Horro | 13 | 20 ^a | 1 | 1 | 2 ^c | 29 ^c | 3 | 25 ^a | 2 | 0 | 3 |
| Konso | 14 | 12 ^b | 7 ^a | 6 ^a | 6 ^b | 34 ^b | 2 | 9 | 7 ^a | 0 | 3 |
| Sheka | 10 | 13 ^b | 2 | 3 | 9 ^b | 49 ^d | 1 | 9 | 3 | 0 | 1 |

Note: different superscript letters within each column indicate significant differences between the populations of regions, based on Kruskal-Wallis test (*=P<0.05, ** = P<0.01). **Geb sima**, wheaten strips on black background; **Teterima**, black or red speckles on white background; **Kokima**, white or greyish strips on brown or reddish background; **Zigrima**, black and white spotted feather; *N (%)*, figures within each row of body plumage and breast colours denote the number of individuals having the specific feather colour out of the total number of chickens (1125) sampled in all populations, and the numbers in parenthesis show their respective proportions.

Mandura chickens. The population is found in the Benshangul Gumuz regional state, North West Ethiopia at an altitude ranging from 1047-1426 m asl in a hot, sub-humid lowland ecological zone (Fig. 1). They are reared by mixed communities of Amhara, Gumuz and Agaw. The population of these chickens is relatively small, estimated to be only 21, 200 (Table 1). Most of the households keeping these chickens provided separate shelters for housing during the night, while they spend the day scavenging in the backyards supplemented with grains and food leftover (Nigussie *et al.*, unpublished). Brown is the most predominant plumage in the population (Fig. 3) followed by red,

white, and 'kokima' (red/yellowish strips on grey or whitish background) (Tables 2). Complete red is typical of males (38% of male plumage) and absent in females. Hens have all variants of colours including 'zigrima' (24%), the most predominant, which is almost absent in males (Table 6). The majority of males (about 40%) possess shining red back feathers which are entirely absent in females. Almost all chickens have normal feather distribution except a small proportion (3%) of naked neck chickens (Table 4). The majority of the birds have white skin colour, regardless of sex and most of the chickens are pea combed (55%). The average shank length of adult males is 8.4 cm and that of females is 7.1 cm. Adult males weigh about 1652 g and females 1426 g per bird (Table 7).

Table 3 Description of neck and back feather colours of indigenous populations of chickens sampled from different regions (percentage of chickens within population, number of chickens sampled per population =225, N = 1125).

| Feather colour | White | Black | Red | Geb-sim | Teterim | Brown | Kokim | Grey | Zigrim | Golde | Multiple |
|----------------|-----------------|----------------|------------------|----------------|-----------------|------------------|----------------|----------------|-----------------|-----------------|-----------------|
| Neck colour | 203 | 77 | 173 | 71 | 63 | 176 | 22 | 14 | 103 | 172 | 51 |
| N (%) | (18) | (7) | (15) | (6) | (6) | (16) | (2) | (1) | (9) | (15) | (5) |
| | ** | * | ** | | ** | ** | ** | | ** | ** | ** |
| Farta | 35 ^a | 6 | 10 ^a | 7 | 10 ^a | 8 ^a | 1 | 0 | 4 | 19 | 4 |
| Mandura | 21 ^b | 8 | 13 ^{ab} | 4 | 6 ^b | 15 ^b | 1 | 1 | 9 | 18 | 4 |
| Horro | 14 | 2 ^a | 14 ^{bc} | 7 | 1 ^c | 14 ^b | 5 ^a | 2 | 18 ^a | 20 | 3 |
| Konso | 13 | 9 | 19 ^c | 8 | 6 ^b | 18 ^{bc} | 2 | 1 | 8 | 8 ^a | 8 ^a |
| Sheka | 8 | 9 | 22 ^c | 5 | 5 ^b | 23 ^c | 0 | 1 | 7 | 10 ^a | 10 ^a |
| Back colour | 181 | 95 | 242 | 66 | 71 | 216 | 32 | 56 | 132 | 9 | 25 |
| N (%) | (16) | (8) | (22) | (6) | (6) | (19) | (3) | (5) | (12) | (1) | (2) |
| | ** | | | | * | ** | ** | ** | ** | | * |
| Farta | 33 ^a | 7 | 20 ^a | 7 | 10 | 12 | 2 | 1 | 5 | 0 | 3 |
| Mandura | 16 | 9 | 19 ^a | 6 | 7 | 20 | 1 | 7 ^a | 12 | 2 | 1 |
| Horro | 14 | 6 | 21 ^{ab} | 3 ^a | 2 ^a | 16 | 7 ^a | 9 ^a | 20 ^a | 1 | 1 |
| Konso | 12 | 2 ^a | 22 ^{ab} | 7 | 7 | 19 | 3 | 4 | 11 | 0 | 4 |
| Sheka | 7 ^b | 9 | 26 ^b | 6 | 7 | 28 ^a | 1 | 4 | 11 | 2 | 3 |

Note: different superscript letters within each column indicate significant differences between the populations of regions, based on Kruskal-Wallis test (*=P<0.05, ** = P<0.01).

Geb-sima, wheaten strips on black background; **Teterima**, black or red speckles on white background; **Kokima**, white or greyish strips on brown or reddish background; **Zigrima**, black and white spotted feather; *N (%)*, figures within each row of body plumage and breast colours denote the number of individuals having the specific feather colour out of the total number of chickens (1125) sampled in all populations, and the numbers in parenthesis show their respective proportions.

Horro chickens. Found in Oromia regional state, Western Ethiopia at an altitude ranging from 2580 to 2810 m asl in a tepid to cool wet highland ecological zone (Fig. 1). The size of the population is estimated at about 29,800 and the ethnic community keeping this population is the

Oromo (Table 1). The Horro chickens are reared under scavenging management with supplemental feeding and in most cases the birds are sheltered in the family house during the night

Table 4 Variations in morphology and distribution of feathers and colours of skin, shank and earlobe of indigenous populations of chickens (percentage of chickens within population).

| | Farta (%) | Mandura (%) | Horro (%) | Konso (%) | Sheka (%) | Total (%) | Chi-Sq. [#] |
|----------------------|-----------|-------------|-----------|-----------------|-----------|-----------|----------------------|
| Feather morphology | | | ** | ** | | | |
| Normal | 53 | 52 | 66 | 64 | 54 | 58 | 16.5 |
| Silky | 47 | 48 | 34 | 36 | 46 | 42 | 16.5 |
| Feather distribution | ** | ** | ** | ** | ** | | |
| Normal | 100 | 97 | 100 | 97 | 96 | 98 | 14.9 |
| Naked neck | 0 | 3 | 0 | 3 | 4 | 2 | 14.9 |
| Skin colour | | ** | ** | * | ** | | |
| White | 45 | 68 | 35 | 58 | 34 | 48 | 76.5 |
| Yellow | 55 | 32 | 65 | 42 | 66 | 52 | 76.5 |
| Shank colour | ** | ** | ** | ** | ** | | |
| White | 13 | 16 | 31 | 31 | 28 | 28 | 34.4 |
| Black | 5 | 21 | 12 | 15 | 12 | 12 | 25.0 |
| Yellow | 81 | 63 | 57 | 54 ^a | 60 | 60 | 43.4 |
| Ear lobe colour | ** | ** | ** | ** | ** | | |
| White | 26 | 56 | 52 | 43 | 24 | 40 | 79.1 |
| Red | 62 | 41 | 45 | 44 | 68 | 52 | 53.4 |
| Yellow | 12 | 3 | 3 | 13 | 8 | 8 | 28.3 |

Note: Asterisks indicate significant differences between rows at 5% (*) and 1% (**) probability levels, based on Binomial test for feather morphology, feather distribution and skin colour, and Cochran test for shank and ear lobe colours, Chi-Sq., the Chi-Square values within a row denote significant differences between populations or regions (P<0.01), based on Kruskal-Wallis Test.

Table 5 Variations in comb type and head and body shapes of indigenous populations of chickens (percentage of chickens within the population).

| | Farta (%) | Mandura (%) | Horro (%) | Konso (%) | Sheka (%) | Total (%) | Chi-Sq. |
|------------|-----------|-------------|-----------|-----------|-----------|-----------|---------|
| Comb type | ** | ** | ** | ** | ** | | |
| Single | 6 | 13 | 13 | 14 | 20 | 13 | 18.8 |
| Rose | 14 | 15 | 14 | 22 | 12 | 16 | 9.9 |
| Pea | 52 | 55 | 56 | 49 | 54 | 53 | |
| Walnut | 1 | 3 | 4 | 10 | 9 | 6 | 25.5 |
| Duplex | 27 | 14 | 13 | 4 | 4 | 13 | 68.2 |
| Head shape | ** | ** | ** | ** | ** | | |
| Snake head | 6 | 15 | 23 | 35 | 21 | 20 | 61.8 |
| Crest | 75 | 51 | 33 | 1 | 8 | 34 | 371.4 |
| Flat | 19 | 34 | 44 | 64 | 71 | 46 | 165.7 |
| Body shape | ** | ** | ** | ** | ** | | |
| Blocky | 88 | 84 | 90 | 82 | 88 | 87 | |
| Triangular | 4 | 8 | 8 | 15 | 11 | 9 | 18.7 |
| Wedge | 8 | 8 | 2 | 3 | 1 | 4 | 23.8 |

Note: Asterisks indicate significant differences between rows at the 1% probability level according to Cochran's test. Chi-Sq., the Chi-Square values within a row denote significant differences between populations or regions (P<0.01), based on Kruskal-Wallis Test.

(Nigussie *et al.*, unpublished). The single most important plumage of males is dark red (60%) (Fig. 4). Only 3% of the females possessed red colour, the most frequent being 'zigrima' which is totally absent in males. All chickens have feathered necks. Yellow is the dominant skin colour in both sexes (Table 6). The predominant body shape is blocky (Table 5). However, quite a large

proportion of cocks (22%) have a triangular body shape. The average shank length of adult males is 8.8 cm and that of females is about 6.8 cm. Adult males weigh about 1700 g and females 1372 g per bird (Table 7).

Konso chickens. Found in Southern Nations, Nationalities and Peoples regional state, South Ethiopia at an altitude ranging from 1471 to 1898 m asl in a humid lowland to wet highland ecological zone (Fig. 1). The population of the population is estimated at about 107,600 and the major ethnic community keeping this population is the Konso (Table 1). The Konso chickens are reared under scavenging management. The proportion of households practicing supplementary feeding is the smallest compared to farmers in other regions, although still about 62% supplemented their chickens (Nigussie *et al.*, unpublished). Unlike in other regions, most of the farmers (82%) here provided separate housing for their chickens. Most of the cocks (43%) have red body plumage whereas brown (28%), 'zigrima' (17%) and black (15%) are the prominent plumage colours in hens. About 4% of the cocks and less than 2% of the hens have naked necks (Fig. 5). Both white (54%) and yellow (46%) skin colours are available (Table 4). However, 56% of the cocks have yellow skin colour (Table 6). The birds are mainly pea combed (49%) followed by a relatively large proportion of rose comb (22%). The shape of the head is mainly flat (45%) and most of the chickens have blocky body shapes (Table 5) although about 17% of the cocks and 13% of the hens have triangular body shape, respectively. The average shank length of adult males is 10.1 cm and that of females is 7.1 cm. Adult males weigh about 1411 g and females 1011 g per bird (Tables 6).

Sheka chickens. The population is found in the Southern Nations, Nationalities and Peoples regional state, South Ethiopia at an altitude 2285 m asl in a cool wet highland ecological zone (Fig. 1). They are reared mainly by the Sheka and other very small populations of Kaffa and Menja communities. The population of the Sheka population is about 46,450 (Table 1). The proportion of households providing separate housing and sheltering the chickens in the family house is almost equal. Most households practice supplementary feeding (Nigussie *et al.*, unpublished). Brown is the predominant plumage followed by red, 'zigrima' and black (Fig. 6; Table 2). Cocks (42%) have chiefly red plumage (Table 6). Brown breast is typical of both sexes. But black is the second largest type of breast colour in cocks (22%), locally referred to as 'Libe Tikur'. Cocks are chiefly red or golden on the neck while hens are mainly brown necked. Six percent of the hens and 3% of the cocks have naked necks. The majority of the chickens in the population have white skin, yellow shank and red earlobe colours (Table 4). The population is mainly pea combed (54%) with 20% single combs. The average shank length of adult males is 9.4 cm and that of females is about 7.8 cm. Adult males weigh about 1697 g and females 1517 g per bird (Table 7).

Table 6 Variations in certain morphological characters between sexes of indigenous chicken populations (% of chickens within sex).

| Trait category | Character | Farta | | Mandura | | Horro | | Konso | | Sheka | | Total (%) | |
|----------------------------------|------------|-------|-------|---------|-------|-------|-------|-------|-------|-------|-------|-----------|-------|
| | | M (%) | F (%) | M (%) | F (%) | M (%) | F (%) | M (%) | F (%) | M (%) | F (%) | M (%) | F (%) |
| Body plumage colour ¹ | White | 34.0 | 32.1 | 19.6 | 14.4 | 12.3 | 14.5 | 13.3 | 8.7 | 6.5 | 7.7 | 18.7 | 14.2 |
| | Black | 2.7 | 9.0 | 3.7 | 11.0 | 2.7 | 6.6 | 2.0 | 15.0 | 9.3 | 8.5 | 4.1 | 10.0 |
| | Red | 24.5 | 1.3 | 38.3 | 0.8 | 60.3 | 3.3 | 42.9 | 3.9 | 41.7 | 6.0 | 39.3 | 3.2 |
| | Gebsuma | 12.9 | 0.0 | 12.1 | 2.5 | 9.6 | 1.3 | 19.4 | 2.4 | 13.9 | 0.9 | 13.6 | 1.5 |
| | Brown | 8.2 | 20.5 | 16.8 | 22.0 | 4.1 | 22.4 | 5.1 | 28.3 | 22.2 | 36.8 | 11.6 | 26.1 |
| | Kokima | 0.0 | 6.4 | 0.0 | 2.5 | 1.4 | 8.6 | 0.0 | 4.7 | 0.0 | 2.6 | 0.2 | 5.1 |
| Feather morphology | Zigrima | 0.0 | 15.4 | 0.9 | 23.7 | 0.0 | 27.6 | 3.1 | 16.5 | 0.0 | 20.5 | 0.7 | 21.4 |
| | Normal | 39.5 | 79.5 | 27.1 | 75.4 | 28.8 | 84.2 | 35.7 | 86.6 | 26.9 | 78.6 | 32.3 | 81.4 |
| Skin colour | Silky | 60.5 | 20.5 | 72.9 | 24.6 | 71.2 | 15.8 | 64.3 | 13.4 | 73.1 | 21.4 | 67.7 | 18.6 |
| | White | 35.4 | 64.1 | 69.2 | 66.1 | 45.2 | 30.3 | 43.9 | 69.3 | 37.0 | 31.6 | 44.7 | 48.1 |
| | Yellow | 65.6 | 35.9 | 30.8 | 33.9 | 54.8 | 69.7 | 56.1 | 30.7 | 63.0 | 68.4 | 55.3 | 51.9 |
| Comb type | Single | 6.8 | 5.1 | 9.3 | 16.1 | 9.6 | 14.5 | 14.3 | 14.2 | 13.9 | 25.6 | 10.7 | 15.6 |
| | Rose | 2.0 | 35.9 | 2.8 | 26.3 | 6.8 | 18.4 | 16.3 | 26.8 | 8.3 | 16.2 | 6.7 | 23.7 |
| | Pea | 48.3 | 59.0 | 52.3 | 57.6 | 34.2 | 65.1 | 48.0 | 49.6 | 56.5 | 52.1 | 48.8 | 56.8 |
| | Walnut | 2.0 | 0.0 | 6.5 | 0.0 | 12.3 | 0.0 | 15.3 | 6.3 | 13.9 | 4.3 | 9.3 | 2.2 |
| | Duplex | 40.8 | 0.0 | 29.0 | 0.0 | 37.1 | 2.0 | 6.1 | 3.1 | 7.4 | 1.7 | 24.7 | 1.5 |
| Body shape | Blocky | 82.3 | 98.7 | 71.0 | 95.8 | 71.2 | 99.3 | 76.5 | 86.6 | 77.8 | 97.4 | 76.4 | 95.4 |
| | Triangular | 4.8 | 1.3 | 13.1 | 4.2 | 21.9 | 0.7 | 17.3 | 12.6 | 20.4 | 2.6 | 14.4 | 4.4 |
| | Wedge | 12.9 | 0.0 | 15.9 | 0.0 | 6.8 | 0.0 | 6.1 | 0.8 | 1.9 | 0 | 9.2 | 0.2 |

¹The percentage values for plumage colours do not add up to 100 because only the major colour variants were considered here.

Note: **Gebsuma**, wheaten strips on black background, ; **Kokima**, white or greyish strips on brown or reddish background; **Zigrima**, black and white spotted feather.

Table 7 Adult live body weight and shank length of the different populations of indigenous chickens.

| | | Farta | Mandura | Horro | Konso | Sheka | Total |
|---------------------------------|--------|----------------------------|----------------------------|----------------------------|----------------------------|----------------------------|--------------|
| Live weight (gm/bird, \pm SD) | Male | 1630 ^a (685) | 1652 ^b (504) | 1700 ^b (437) | 1411 ^a (281) | 1697 ^b (497) | 1612** (458) |
| | Female | 1054 ^a (298) | 1426 ^b (349) | 1372 ^b (344) | 1011 ^a (223) | 1517 ^b (355) | 1266** (373) |
| Shank length (cm, \pm SD) | Male | 8.2 ^a (1.2) | 8.4 ^b (1.3) | 8.8 ^b (1.0) | 10.1 ^c (0.6) | 9.4 ^c (0.9) | 9.1** (1.1) |
| | Female | 6.6 ^a (0.5) | 7.1 ^a (0.7) | 6.8 ^a (0.6) | 7.1 ^a (0.6) | 7.8 ^b (0.6) | 7.0** (0.7) |

Note: Means in a row with different superscript letters denote significant differences between populations or sampling regions ($P < 0.05$) and asterisks within a column indicate significant differences between males and females for each parameter at the 1% (**) level of probability.

Chicken populations

The chicken population of Ethiopia seems to be consistently declining in the last few years; According to FAO (2000), it was estimated that there are 65 million heads of chicken, more than 95% of which comprised indigenous chickens. Estimates on the population of indigenous chickens were 42.9 million in 2003 (CACC, 2003) which declined to about 30 million in 2005 (CSA, 2005). The average flock size of indigenous chickens kept per rural small holder family varied from 6 to 10 (Alemu, 1995; Halima *et al.*, 2007a). The average estimated size of indigenous flock per household was quite small in the current study, only about 3.5 (ranging from 2.1 in Konso to 6.5 in Sheka).



Figure 2. A single combed ‘gebsima’ male, and a female chicken of the white plumage that predominantly characterize the Farta population

Morphological Variations

Tables 2 and 3 show the proportions of the different body plumage, breast, neck and back feather colours in the chicken populations of the different regions. In line with the results reported from other regions of Ethiopia (Halima *et al.*, 2007b), each population in this study possessed multiple variants of feather colours although there were highly significant differences between the different populations in the proportion of specific feather colours characterizing them. White, red, brown and ‘zigrima’ are the dominant colours describing most of the populations, except for the breast colour which comprises large proportions of black and grey instead of red and ‘zigrima’ feathers (Table 3). Presence of multiple variants of feather colours within a population is also a typical feature characterizing indigenous fowls in other parts of Africa (Gueye, 1998; Badubi *et al.*, 2006) and Asia (Bhuiyan *et al.*, 2005).

The Farta population comprised the largest proportion (33-35%) of chickens with white body plumage, breast, neck and back feather colour. Similarly, Halima *et al.* (2007b) also reported the white feather colour of Farta chicken population as one of its prominent features. Conversely, the populations in the southern (Konso and Sheka), Benshangul-Gumuz (Mandura) and Oromia regions (Horro) constituted larger number of chickens with red body plumage compared to the population in the north (Farta). Interestingly, this pattern is compatible with the farmers' stated preferences for plumage colour in the respective regions reported by Nigussie *et al.* (unpublished) as a separate part of this study. The fact that farmers consider plumage colour as one of the important selection criteria in the traditional breeding practices appeared to have affected the frequencies of the most preferred white and red plumage colours favourably.

Golden colour is a characteristic peculiar to the neck feather (Table 3). The proportion of chickens having the golden neck feather is significantly smaller in the southern populations of Konso and Sheka (8-10%) compared to all the others (19-20%).



Figure 3. A Mandura chicken with a silky feather morphology prevalent in the population And predominantly characterizing the males

The populations in the high altitudes (Farta, Horro and Sheka) constituted larger proportions of yellow skinned chickens relative to the others and, except in Farta, there were significant differences between the proportions of yellow and white skinned chickens in all

regions (Table 4). However, in spite of the ecological region, the proportion of males having yellow skins was larger than that of females. This is probably because the scavenging feed resource base is relatively better in the high altitude regions compared to low altitude areas and that the foraging behaviour of cocks is stronger than the hens. Yellow skin colour is the result of the expression of carotenoid pigments in the skins of birds (Smyth, 1990) and according to Eriksson *et al.* (2008), it is generally considered to be associated with the individual's adaptive fitness reflecting its nutritional status or health which, in turn, is indicative of its foraging efficiency and immune status.



Figure 4. Male and female chickens of Horro. Males are predominantly of deep red body plumage colour.

The naked neck (*Na*) gene was described as one of the major genes in local chickens of the tropics with desirable effects on heat tolerance and adult fitness (Horst, 1989). However, the number of chickens expressing this gene was quite small (23 out of a total of 1125, i.e. <2%) in the populations we studied (Table 4). As would be expected, these are found mainly among the populations in low altitude regions having warm climates (Mandura in the west and Konso in the southern region). The exact size and geographic distribution of naked neck chickens in Ethiopia was not clearly established and only a very limited number of works was reported so far (Teketel, 1986). The total frequency of chickens carrying the *Na* gene in the populations we studied was smaller than the proportion (6%) reported in Nigeria (Gueye, 1998)

and 3.6% in Botswana (Badubi *et al.*, 2006). Important reason is that farmers did not prefer the naked neck chickens (Aklilu, 2007) ultimately favouring selection against this valuable gene. Thus, it appears that the future of the *Na* gene is at stake unless measures are taken towards its conservation.



Figure 5. The Konso chicken scavenging in the family backyard. Some of the naked neck chicken recorded in this study were found in the Konso population.

The overall pattern of the variation in comb types is similar to that reported by Halima *et al.* (2007b). The highest proportion of single, rose and walnut combs were found in the southern populations (rose and walnut in Konso, and single and walnut in Sheka) whereas the Farta population constituted significantly larger proportion (27%) of chickens with duplex comb (Table 5). On the other hand, the major proportion of indigenous chickens in all regions studied carried the pea comb (from 49 to 56%). The pea comb gene (*P*) is known to be related to an important effect in breeding for tropical conditions in terms of reduced frequency of breast blisters and improved late juvenile growth (Horst, 1989). Although the effect of the *P* gene on growth might be indirect, the reduced frequency of breast blisters directly results from the presence of a ridge of thickened skin in pea combed birds that runs the length of the keel over the breast bone (Somes, 1990). However, the high frequency of pea comb and the contrary very low frequency of walnut in the current populations probably needs further verification. Somes (1990) indicated the possibility of classification errors with regard to comb types and suggested that it is useful to examine the

breast ridge in distinguishing between birds with pea and single combs, and between those with rose and walnut combs. The breast ridge is a well established manifestation of the *P* gene which is also characteristic of walnut-combed birds. In the present study breast ridge was not investigated.

Most of the chickens in the northern population (75% in Farta) identified with crest head whereas flat head was found to be a characteristic feature of those in the south (64% in Konso and 71% in Sheka). The populations were significantly different from each other in terms of head shape characteristics except that comparable proportions of flat head chickens were found in the south (Table 5). This probably suggests that head shape could be considered as one of the most important morphological characteristics to discriminate between different populations of indigenous chickens.

Average body weight of adult males and females varied significantly among the populations. Females in Mandura, Horro and Sheka populations were significantly heavier than those in Farta and Konso populations (Table 6). The weight ranges for males, 1.4 (Konso) to 1.7 kg (Horro), and females, 1.0 (Konso) to 1.5 kg (Sheka), in the current study were within the ranges reported earlier by Mebratu (1997) for different 'plumage colour types' of indigenous chickens of Ethiopia reared under confined management regimens (1.3 to 1.7 kg for males and 1.0 to 1.2 kg for females). However, the ranges in this study were much higher than those reported by



Figure 6. A Sheka male showing a triangular body shape found at a much higher proportion compared to males in all other populations, except the Horro. However, it is a characteristic feature of males in all populations.

Halima *et al.* (2007b) for 7 indigenous populations of chickens in north Ethiopia kept under intensive management conditions (1.0-1.5 kg for males and 0.64-0.87 kg for females at 22 weeks of age).

The discrepancies could either be due to the variation in the age of the birds (there were no records of the exact age of birds in this study) or were simply indicative of the negative effects of confined management on the performance of local chickens. Studies in Ethiopia showed that indigenous chickens have very poor adaptation to confined environments and suffered huge mortality (up to 90%) and morbidity resulting in poor performance (Brannang and Pearson, 1990).

Males were significantly heavier and particularly so in the Farta, Horro, and Konso populations by 36, 20 and 28%, respectively, compared to the females. Cocks in the Konso population and the hens of Sheka have significantly longer shanks compared to their counterparts in other populations. Males in all populations have significantly longer shanks, about 17 (Farta and Sheka) to 30% (Konso) longer compared to the females. The chickens in this study were generally shorter relative to their Tanzanian counterparts (Msoffe *et al.*, 2001). However, the ranges in shank length were almost similar to those reported by Badubi *et al.* (2006) for the indigenous chickens of Botswana and close to the figures reported by Halima *et al.* (2007b), especially for the 'Mecha' chickens of Ethiopia.

Variations in morphological characteristics between males and females

Most of the morphological characteristics varied between the male and female sexes. Interesting variations were observed in the body plumage colour, feather morphology and comb type. Males in most regions were largely found to be identified with silky, bright red plumage and higher proportions of duplex combs where as females had peculiarly mixed plumage colours (zigrima and kokima) with larger proportion of rose combs.

Absence of feathers on the neck (naked neck), recorded at a very low frequency (23 birds out of a total of 1125), characterized both sexes. About 70% of the naked neck chickens had brown, white or red body plumage colours, which is simply because about 56% of the chickens in the entire populations carried these colours.

Generally, silky feather morphology, red body plumage and back feather colour and black breast were the prominent features observed at high frequencies in males probably suggesting that a considerable proportion of cocks in the regions studied carried at least some of the physical features ascribed to the red jungle fowl. Crawford (1990) described that the feather colour of red jungle fowl is retained almost exactly in the black-breasted red phenotype of domestic fowl and in males the colours are enhanced by modifications in feather morphology.

Conclusion

Though generally not considered as ideal measures of genetic variability, morphological traits were found to be useful in describing different populations of indigenous chickens. The populations in this study carried multiple variants of plumage colours and other physical features. However, there were certain features characterizing each population such as the distinctly predominant white plumage colour and crest head of the Farta chickens in the north and the prominent red body plumage and flat head in the southern populations. Likewise, the populations in the high altitude regions were predominantly (55% in Farta, 65% in Horro and, 66% in Sheka) characterized by yellow skin colour, a trait reflecting the adaptive fitness of birds under foraging environments. Other attributes, important in breeding for tropical conditions, have also been identified such as the pea comb gene, in populations of all regions, and the naked neck gene, particularly in those of low altitude areas. However, the limited geographic distribution and the very small frequency of the naked neck chickens we found in this study suggests that the future of the *Na* gene associated to this trait is at stake unless measures are taken towards its conservation.

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Appendix 1. Definition of variables used to describe morphological characters.

| Morphological character | Variable (dummy) ¹ | Description ² |
|-------------------------|-------------------------------|--|
| Feather distribution | 1 | Normal/feathered neck |
| | 2 | Naked neck |
| Feather morphology | 1 | Normal feathers |
| | 2 | Silky feathers |
| Plumage colours: | 1 | Complete white |
| Body plumage | 2 | Complete black |
| Neck feather | 3 | Complete red |
| Breast feather | 4 | Gebsima |
| Back feather | 5 | Teterima |
| | 6 | Brown |
| | 7 | Kokima |
| | 8 | Grey |
| | 9 | Zigrima |
| | 10 | Golden |
| | 11 | Multiple mixed colours |
| Shank colour | 1 | Has white shanks |
| | 2 | Has black shanks |
| | 3 | Has yellow shanks |
| Skin colour | 1 | Has white skin |
| | 2 | Has yellow skin |
| Earlobe colour | 1 | Has white earlobes |
| | 2 | Has red earlobes |
| | 3 | Has yellow earlobes |
| Comb type ³ | 1 | Is single combed |
| | 2 | Is rose combed |
| | 3 | Has pea comb type |
| | 4 | Is walnut combed |
| | 5 | Has duplex comb type |
| Head shape | 1 | Looks like 'snake head' |
| | 2 | Looks like 'snake head' but also has hair/ is crest head |
| | 3 | Head shape is flat |
| Body shape ⁴ | 1 | Blocky shaped |
| | 2 | Triangular |
| | 3 | Wedge shaped |

¹Each character state was recorded as a binomial variable (1 if present and 0, if not).

²Colour descriptors are local feather colour identifications used by farmers: Gebsima, wheaten strips on black background; Teterima, black or red speckles on white background; Kokima, white or greyish strips on brown or reddish background; Zigrima, black and white spotted feather.

³Descriptions on comb types were based on illustrations presented by *Somes (1990)*.

⁴Blocky body shape is meant to represent a horizontal, oblong body resembling the distinct characteristic shape of the Rhode Island Red and the Rhode Island White. Wedge shape represents almost the opposite feature, oblong but vertical.

4

Microsatellite DNA analysis of genetic diversity and population structuring in Ethiopian chickens

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Abstract

The aim of this study was to analyze the genetic diversity and population structure of indigenous chickens kept under village systems. The study was done using 20 microsatellite markers for genotyping a total of 252 chickens representing 5 ecotype populations sampled from different geographical regions of Ethiopia. The populations studied were: Konso and Sheka from south and southwest, Horro from central west, and Farta and Mandura from north Ethiopia, respectively. In total, 171 alleles were observed overall the populations. Forty one private alleles were identified in all populations occurring at very low frequencies (1%). The number of alleles per locus over all the populations ranged from 4 to 16 and the mean number of alleles per locus for each population ranged from 5.4 (Konso) to 6.4 (Farta). The observed heterozygosity varied from 5.1 (Farta) to 5.7 (Mandura) and the expected heterozygosity from 0.55 (Farta) to 0.61 (Mandura). The total number of loci showing significant deviations from Hardy-Weinberg equilibrium (HWE) in each population ranged from 1 to 7; 1 each in Sheka (MCW14) and Horro (MCW81), 3 in Konso, 5 in Mandura and 7 in Farta populations. All populations deviated significantly from HWE except Horro. The overall population heterozygote deficiency (F_{IT}) was 0.095. Most of the F_{IT} was accounted for by the within-population heterozygote deficiency ($F_{IS} = 0.064$), the heterozygote deficiency due to population subdivision (F_{ST}) only being 0.033. The modified Cavalli-Sforza genetic distance (D_A) ranged from 0.041 between Horro and Mandura to 0.80 between Konso and Farta populations. The standard distance (D_S) ranged from 0.036 (between Sheka and Horro; and between Horro and Mandura) to 0.98 (between Konso and Horro). Pairwise F_{ST} distances between populations generated similar results to D_S , values ranging from 0.016 to 0.056. The results showed that the genetic differentiation among chicken ecotypes of Ethiopia is weak ($F_{ST} < 0.1$). AMOVA analysis also showed that the between-populations variation accounted for less than 4% of the diversity in overall the populations. There was no evidence of population structuring along agro ecological regions of sampling. Generally, the results indicated that almost all of the differences in the populations of Ethiopian chickens could be explained by the genetic variability of individuals within-population. Most of the microsatellite loci showed high level of polymorphism suggesting that they can be used in future efforts to assess genetic diversity of indigenous chickens.

Key words: indigenous chicken ecotypes, Ethiopia, genetic diversity, microsatellite loci, population structure

Introduction

Village chickens make substantial contributions to household food security throughout the developing world. Indigenous chicken serve as an investment and source of security for poor households in addition to their use as sources of meat and eggs for consumption and as income source (Kitaliyi, 1998).

Dispersal of domestic chicken from its putative centres of domestication to different regions with diverse environmental conditions and people of different cultural orientations has contributed to the observed genetic differentiation of chicken populations across the world. Assessing the genetic diversity of different locally adapted chicken populations kept in the village systems is useful for understanding the parameters for breed differentiation and for genetic conservation of these populations. There are many reasons for conserving genetic variation in indigenous chickens. They have a number of adaptive traits and genes such as naked necks, minimum and frizzle feathers, black bones and meat, which have special utility in the hot and humid tropics (Horst, 1989). Indigenous chicken are known to be ideal mothers, good sitters, excellent foragers, hardy, and are believed to possess better natural immunity against common poultry diseases (Mathur *et al.*, 1989). They could be a source of unique alleles and can contribute to the search for genes associated with health and quality traits (Muchadeyi *et al.*, 2007).

Ethiopia is situated at a strategic location at the horn of Africa which made it one of the potential routes of entry for different livestock species to Africa (Hanotte *et al.*, 2002). This country is also known for its agro-climatic and geographic divergence. As a consequence, Ethiopia is expected to be one of the core centres of diversity in farm animal genetic resources. More than 95% of the total chicken populations of Ethiopia are comprised of the indigenous genotypes varying in feather and shank colour, comb type, and other morphologic and production characteristics (Dana *et al.*, 2010a). However, the extent and level of genetic variability within and among the populations is poorly understood. Recent studies based on 10 (Tadelle, 2003) and 7 (Halima *et al.*, 2009) microsatellite markers revealed the presence of large variability within-populations of indigenous chickens sampled from different geographic regions of Ethiopia. Although these findings give a useful insight into the genetic diversity of indigenous chickens their scope was limited particularly in detecting the extent of population structuring among ecotypes due to the small number of samples and microsatellite markers used. Genetic differentiation of populations could be detected more accurately by using larger number of loci because each locus will contain an independent history of the population depending on the amounts of random drift, mutation and migration that have occurred. In 2004, a joint FAO-ISAG working group recommended 30 microsatellite markers for biodiversity study of chicken (<http://dad.fao.org/en/refer/library/guideline/marker.pdf>). The present study was done using 20 of

these markers for genotyping five indigenous chicken ecotypes sampled from different geographical regions of Ethiopia. The aim of the study was to determine the genetic diversity and population structuring of indigenous chickens kept under village systems. Information on the diversity of indigenous genetic resources is an essential tool for designing utilization and conservation programs.

Materials and methods

Sampling

The chicken populations were sampled from 5 geographic regions: Konso and Sheka from the south and southwest, Horro from the central west, and Farta and Mandura from the northern regions of Ethiopia, respectively. Ecological features of the sampling locations and information on sample size are described in Table 1. All samples were collected from non selected indigenous populations. A total of 252 animals representing five populations were analyzed. Blood samples were collected from the wing vein of each chicken onto Whatman FTA filter cards (Whatman International Ltd). One chicken was sampled per household and neighbouring households where the flocks could have shared cocks were avoided to prevent sampling of directly related animals.

Table 1 Information on samples and sampling locations of chicken populations.

| Population | n | n | Administrative Region | Geographic location of sampling site | Altitude (m asl) | Ecological description |
|------------|----|----|------------------------|--------------------------------------|------------------|-----------------------------|
| | M | F | | | | |
| Konso | 20 | 29 | Southern State | South | 1471 | Humid lowland |
| Sheka | 23 | 27 | Southern State | South West | 2285 | Cool wet highland |
| Horro | 16 | 34 | Oromia State | West | 2580 | Tepid to cool wet highland |
| Mandura | 17 | 35 | Benshangul-Gumuz State | North West | 1047 | Hot, sub-humid lowland |
| Farta | 5 | 46 | Amhara State | North | 2700 | Cool to very cold sub-moist |

DNA polymorphisms

Twenty microsatellite markers were analyzed. These are part of the 30 marker loci recommended by FAO (<http://dad.fao.org/en/refer/library/guideline/marker.pdf>) MoDAD project for surveying chicken biodiversity. Primer sequences, additional references on the markers and details on the amplification protocol and primer annealing temperatures can be found in the web site of the AVIANDIV project on genetic diversity of European chicken breeds (<http://w3.tzv.fal.de/aviandiv>). Multiplex PCR was carried out according to FAO

recommendations (<http://dad.fao.org/en/refer/library/guidelin/marker.pdf>).

Analysis of genetic diversity

Genetic diversity within populations was measured as the mean number of alleles per locus, the observed (H_O) and expected (H_E) heterozygosities, deviations from Hardy-Weinberg equilibrium and within-population inbreeding coefficient (F_{IS}). Exact tests for deviations from Hardy-Weinberg equilibrium were performed using the GENEPOP package version 4.0.10 (Raymond and Rousset, 1995). A probability test was performed using a Markov chain method (dememorization 1,000, batches 500, and iterations per batch 5,000). Significance levels were calculated per locus, per population, and over all loci and populations combined.

Population differentiation was measured as the number of private alleles (alleles found in only one population) and coefficient of inbreeding between populations (F_{ST}). Genetic differentiation between populations was also estimated using the pairwise F_{ST} . The difference in the pairwise F_{ST} values was tested by permuting multilocus genotypes among samples and significance levels were reported after strict Bonferroni corrections to account for multiple comparisons. Values of F_{IT} (inbreeding coefficients over-all the population), F_{ST} and F_{IS} were estimated based on Weir and Cockerham (1984). FSTAT program version 2.9.3 (Goudet, 2001) (<http://www.unil.ch/izea/software/fstat.html>) was used to compute the fixation indices.

Population relatedness was estimated according to the standard genetic distance, D_S (Nei, 1972) and the modified Cavalli-Sforza distance, D_A (Nei *et al.*, 1983) based on allele frequencies using POPULATIONS version 1.2.30 (<http://bioinformatics.org/~tryphon/populations/>). Analysis of Molecular Variance (AMOVA) was carried out to assess the percentage contribution of the within and between population variations to the overall variability observed in Ethiopian chickens using ARLEQUIN version 3.5 (Excoffer *et al.*, 2006).

A Mantel test was performed using IBDWS version 3.16 (<http://ibdws.sdsu.edu>) to test for correlations between genetic and geographical distances (isolation by distance). The centre of geographic origin of each ecotype was used as the geographical localization of the breeds and the genetic differentiation [$F_{ST}/(1 - F_{ST})$] (Raymond and Rousset, 1995) was used to fit the genetic distance. The significance level was calculated from 10000 randomizations.

Results

Polymorphism of microsatellite markers

The characteristics and the variations for each microsatellite marker used in this study are presented in Table 2. In total, 171 alleles were observed from the 20 loci surveyed in 252 birds.

The number of alleles per locus over all the populations ranged from 4 (MCW 222, MCW248, LEI166) to 16 (LEI234) with a mean of 8.6 (171/20). Markers LEI94, MCW34, LEI234, MCW183 and MCW330, with more than 10 alleles each, were more highly polymorphic in terms of the number of alleles. The expected heterozygosity (H_E) per locus ranged between 0.34 (MCW248) and 0.86 (LEI234) and the genetic variation between the populations (F_{ST}) per locus was between 0.01 (MCW14) and 0.12 (MCW81). Loci LEI194, MCW34, MCW295 and LEI234 showed higher values of H_E compared to others where as only MCW81 showed high value for F_{ST} (>10%).

Table 3 presents the frequencies of private alleles found in the chicken populations studied. Private alleles were detected in 18 of the 20 microsatellite loci. The highest number of unique alleles was detected in MCW330, which had 6 unique alleles across the populations. A total of 41 private alleles (of 171 alleles, 24%) were detected over all the loci, ranging from 5 (Konso and Horro) to 11 (Sheka and Farta). However, the frequency of private alleles in the populations was very low. Thirty eight of the 41 private alleles (95%) had a frequency of less than 3%. Twenty eight of these only occurred at a frequency of 1%. The highest frequency of unique alleles was 5%, which were detected in 2 loci (MCW206 and MCW330) specifically in the Horro population.

Results of HWE test giving exact P-values per locus and per population estimated by the Markov chain method are given in (Table S1). The total number of loci showing significant ($P < 0.05$) deviation from HWE in each population ranged from 1 to 7, 1 each in Sheka (MCW14) and Horro (MCW81), 3 in Konso, 5 in Mandura and 7 in Farta populations.

Some of the microsatellite loci that deviated from HWE were specific to certain populations. Three loci (LEI 94, LEI166 and MCW183) deviated from HWE in only the Farta population. Deviations of loci MCW37 and MCW34 were specific to Konso and Mandura populations, respectively.

Three loci had significant HW disequilibrium across 2 populations; ADL278 and MCW330 in Mandura and Farta, and MCW206 in Konso and Farta. Two loci significantly deviated from HWE across 3 populations, MCW81 in Konso, Horro and Mandura and MCW14 in Sheka, Mandura and Farta.. Fisher's chi square test showed that except for the Horro population the genotype frequencies in each of the populations significantly ($P < 0.05$ in Konso and Sheka; $P < 0.001$ in Mandura and Farta) deviated from HWE (Table S2).

Genetic variability

The mean number of alleles (MNA) per breed varied from 5.35 in the Konso ecotype to 6.10 in the Farta ecotype. The observed heterozygosity (H_O) varied from 5.1 (Farta) to 5.7 (Mandura) and the expected heterozygosity (H_E) ranged from 0.55 (Farta) to 0.61 (Mandura). The average

heterozygosity overall loci and populations in our study was 0.545 (Table 4).

Genetic relationships and population structuring

The modified Cavalli-Sforza distance (D_A) and Nei's standard genetic distance (D_S) generated for each pair of breeds were given in Table 5. The D_A genetic distance ranged from 0.041 between Horro and Mandura to 0.80 between Konso and Farta. The D_S distance ranged from 0.036 (between Sheka and Horro, and between Horro and Mandura) to 0.98 between Konso and Horro. The Konso was the most divergent ecotype in terms of both genetic distance measures.

Table 2 Characteristics of microsatellite loci used, chromosomal location, fragment size, and number of alleles observed for each locus.

| Locus | Chromosome ¹ | Expected fragment size (bp) ² | Observed Fragment size (bp) ³ | Observed n of alleles ⁴ | H_E ⁵ | F_{ST} ⁶ |
|--------|-------------------------|--|--|------------------------------------|--------------------|-----------------------|
| LEI94 | 4 | 247-287 | 245-285 | 14 | 0.79 | 0.022 |
| ADL268 | 1 | 102-116 | 103-117 | 7 | 0.64 | 0.044 |
| MCW216 | 13 | 139-149 | 141-147 | 5 | 0.56 | 0.070 |
| MCW248 | W29 | 205-225 | 213-221 | 4 | 0.34 | 0.032 |
| LEI166 | 3 | 354-370 | 345-355 | 4 | 0.48 | 0.048 |
| MCW34 | 2 | 212-246 | 221-243 | 12 | 0.71 | 0.060 |
| MCW69 | E60C04W23 | 158-176 | 156-174 | 9 | 0.57 | 0.076 |
| ADL278 | 8 | 114-126 | 112-122 | 9 | 0.62 | 0.048 |
| MCW295 | 4 | 88-106 | 86-100 | 8 | 0.70 | 0.056 |
| MCW37 | 3 | 154-160 | 151-156 | 6 | 0.66 | 0.033 |
| LEI234 | 2 | 216-364 | 211-315 | 16 | 0.86 | 0.024 |
| MCW222 | 3 | 220-226 | 216-222 | 4 | 0.58 | 0.042 |
| MCW16 | 3 | 162-206 | 136-154 | 8 | 0.68 | 0.055 |
| MCW81 | 5 | 112-135 | 112-134 | 9 | 0.38 | 0.118 |
| MCW111 | 1 | 96-120 | 99-113 | 7 | 0.64 | 0.056 |
| MCW206 | 2 | 221-249 | 217-243 | 10 | 0.56 | 0.030 |
| MCW14 | 6 | 164-182 | 164-186 | 10 | 0.38 | 0.010 |
| MCW183 | 7 | 296-326 | 293-325 | 12 | 0.61 | 0.047 |
| MCW67 | 10 | 176-186 | 175-183 | 5 | 0.54 | 0.017 |
| MCW330 | 17 | 256-300 | 248-294 | 12 | 0.62 | 0.088 |
| Mean | | | | 8.6 | 0.60 | 0.048 |

¹ From the AVIANDIV project (<http://w3.tzv.fal.de/aviandiv>).

² Expected allele size range from FAO (<http://dad.fao.org/en/refer/library/guideline/marker.pdf>)

³ Detected allele size range (bp) 5 chicken populations.

⁴ Number of alleles across 5 chicken populations.

⁵ Unbiased expected heterozygosity (Nei, 1987).

⁶ F_{ST} = fixation index (Weir and Cockerham, 1984).

Table 6 shows pairwise F_{ST} estimates for population differentiation based on allele frequency variations. The least differentiated populations were Sheka and Horro (pairwise F_{ST} = 0.0162) and

Horro and Mandura (pairwise $F_{ST} = 0.0155$). Konso and Horro were the most differentiated pair of populations with pairwise F_{ST} of 0.0558 followed by Konso and Farta (pairwise $F_{ST} = 0.0486$). Generally, the pairwise F_{ST} distances produced similar results as the D_S distances.

Table 3 Private alleles in base pairs (frequencies in parenthesis) observed for the 5 chicken ecotypes of Ethiopia.

| Locus | Konso | Sheka | Horro | Mandura | Farta |
|--------|-------------|---|----------------------------|----------------------------|----------------------------|
| LEI94 | | 285 (0.010) | | | 267 (0.010) |
| ADL268 | | | | 107 (0.010) 117 (0.010) | |
| MCW216 | - | - | - | - | - |
| MCW248 | 219 (0.010) | | | | |
| LEI166 | | | | | 347 (0.020) |
| MCW34 | | | | 237 (0.010) | |
| MCW69 | | 158 (0.010) | | 174 (0.029) | |
| ADL278 | | 117 (0.010) | | | 114 (0.010) 120 (0.029) |
| MCW295 | | | | | 94 (0.010) |
| MCW37 | 156 (0.010) | | | | |
| LEI234 | | 267 (0.010) 271 (0.020) | | | 211 (0.023) |
| MCW222 | - | - | - | - | - |
| MCW16 | | | | 136 (0.010) 154 (0.010) | |
| MCW81 | 130 (0.010) | 120 (0.010) | | | 132 (0.020) |
| MCW111 | | | 113 (0.010) | | |
| MCW206 | | | 217 (0.020) 227 (0.051) | 223 (0.029) | 219 (0.020) |
| MCW14 | 176 (0.010) | 166 (0.020) 172 (0.010) 186 (0.010) | | | |
| MCW183 | | | 313 (0.010) | 325 (0.010) | 323 (0.010) |
| MCW67 | 179 (0.010) | | | | |
| MCW330 | | 248 (0.011) 272 (0.011) | 276 (0.051) | 268 (0.010) | 284 (0.020) 294 (0.010) |

Table 4 Mean number of alleles, observed (H_O) and expected (H_E) heterozygosities and inbreeding coefficients (F_{IS}) for the chicken populations.

| Population | n | Alleles/locus (SD) | H_O (SD) | H_E (SD) | F_{IS} |
|------------|----|--------------------|---------------|---------------|---------------------|
| Konso | 49 | 5.35 (2.01) | 0.546 (0.129) | 0.592 (0.139) | 0.079* |
| Sheka | 50 | 6.10 (2.66) | 0.551 (0.155) | 0.592 (0.142) | 0.069* |
| Horro | 50 | 5.65 (2.24) | 0.552 (0.166) | 0.565 (0.157) | 0.021 ^{NS} |
| Mandura | 52 | 6.10 (2.27) | 0.567 (0.166) | 0.613 (0.151) | 0.079* |
| Farta | 51 | 6.35 (2.55) | 0.510 (0.158) | 0.546 (0.174) | 0.071* |

* Significantly different from 0 at $P < 0.05$.

Table 5 Genetic distances between ecotypes based on Nei's (Nei, 1972) unbiased standard distance, D_S (above the diagonal) and the modified Cavalli-Sforza distance, D_A (below diagonal) computed using allele frequencies of 20 microsatellite loci.

| Ecotype | Konso | Sheka | Horro | Mandura | Farta |
|---------|-------|-------|-------|---------|-------|
| Konso | - | 0.087 | 0.098 | 0.091 | 0.083 |
| Sheka | 0.077 | - | 0.036 | 0.056 | 0.073 |
| Horro | 0.077 | 0.042 | - | 0.036 | 0.040 |
| Mandura | 0.075 | 0.044 | 0.041 | - | 0.041 |
| Farta | 0.080 | 0.073 | 0.054 | 0.046 | - |

Table 6 Proportion of genetic variability due to population substructuring (pairwise F_{ST}) among Ethiopian ecotype populations (values given below diagonal).

| | Konso | Sheka | Horro | Mandura | Farta |
|---------|--------|--------|--------|---------|-------|
| Konso | | * | * | * | * |
| Sheka | 0.0449 | | * | * | * |
| Horro | 0.0558 | 0.0162 | | * | * |
| Mandura | 0.0456 | 0.0252 | 0.0155 | | * |
| Farta | 0.0486 | 0.0424 | 0.021 | 0.0204 | |

* $P < 0.05$ (above diagonal); corresponding to pairwise significance after standard Bonferroni corrections.

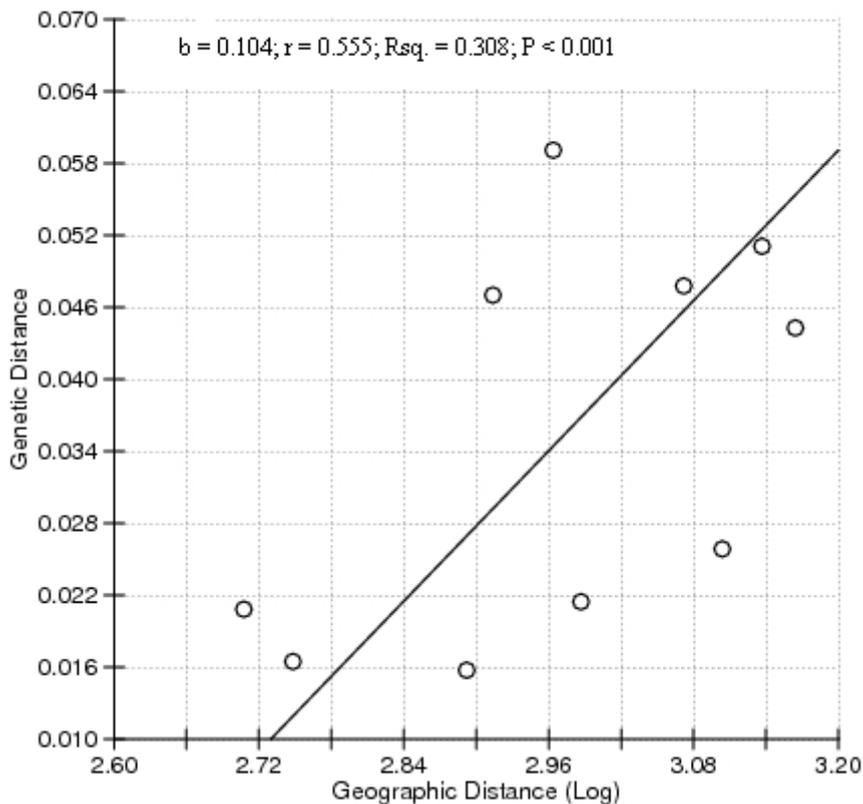


Figure 1 MANTEL test for the correlation between genetic distance ($F_{ST}/(1 - F_{ST})$) and $\log(\text{geographic distance})$ among the 5 indigenous chicken populations of Ethiopia (b = regression coefficient; r = correlation coefficient; $R_{sq.}$ = coefficient of determination).

The estimate of the mean inbreeding coefficient overall the populations (F_{IT}) was 0.095 (99% CI = 0.077-0.133). The mean inbreeding coefficient between-populations (F_{ST}) was 0.033 (99% CI = 0.024-0.045) and that found within-populations (F_{IS}) was 0.064 (99% CI = 0.043-0.084). This shows that the within-population heterozygote deficiency was much larger than the between-population heterozygote deficiency and accounted for most of the F_{IT} overall the populations. A similar pattern was observed using AMOVA analysis which indicated that more than 96% of the genetic variation was found among individuals within-populations and the difference among the ecotypes represented a very small proportion of the total variability (Table 7). Mantel test revealed a significant correlation between genetic and geographical distance (isolation by distance) among the populations ($r = 0.56$, $P = 0.001$) (Figure 1).

Table 7 AMOVA analysis of Ethiopian indigenous chicken populations based on microsatellite DNA variation.

| Source of variation | Sum of squares | Variance components | Percentage variation |
|---------------------|----------------|---------------------|----------------------|
| Among ecotypes | 104.833 | 0.20497 | 3.40259 |
| Within ecotypes | 2874.181 | 5.81896 | 96.59741 |
| Total | 2979.014 | 6.02393 | |

Fixation index overall loci (F_{ST}) = 0.03403 ($P = 0.0000$, after 1023 permutations).

Discussion

This study revealed the existence of high allelic and genetic variability within chicken populations. Moreover, most of the markers used in this study were polymorphic. Four of the markers, LEI94, MCW34, MCW295 and LEI234, showed a combination of high H_E (0.71-0.86) and many alleles. These markers would be more effective for studies on diversity of local chickens. On the contrary, MCW0248 and LEI166, which had low H_E values and relatively a smaller number of alleles at the same time appear to be less suitable for this kind of study.

However, with regard to the number of alleles, all of the markers were polymorphic, detecting at least four alleles per locus in any single population tested. The range in the number of alleles per locus observed in this study (4 to 16) was close to that reported by van Marle-Koster *et al.* (2000) for locally adapted South African chickens where it varied from 4 to 12 across the 18 markers they used, which included 12 of the 20 markers used in our study. The mean number of alleles (MNA) per locus in each population was quite high indicating the existence of high within-population genetic variability. The values of the different populations were close to each other. The ranges (5.4 to 6.4) were higher than those reported by Tadelle (2003) for 5 Ethiopian populations (4.2 to 5.3) using 10 microsatellite markers but comparable to the values (4.85 to 6.29) reported by Halima *et al.* (2009) for 7 populations from northwest Ethiopia using 7 microsatellite markers. However, whereas we included 7 of the 10 loci used by Tadelle, none of the loci used by

Halima *et al.* were incorporated in our analysis. The values found here were also higher than those reported by Hillel *et al.* (2003) for 52 chicken populations based on 22 microsatellite markers, 15 of which were included in our study. They found that average number of alleles per locus in unselected indigenous populations, morphologically selected European breeds, layers and broilers were 4.1, 3.5, 3.4 and 3.6, respectively.

The existence of strong within-population diversity was also confirmed by the high values of the observed and expected heterozygosities in each population. Comparable level of diversity seems to exist within each population as observed from the close heterozygosity values of each populations. Similarly, Tadelle (2003) reported heterozygosity values ranging from 0.55 to 0.63 in 5 indigenous chicken ecotypes of Ethiopia. Although direct comparison of diversity values reported by different workers has major limitations owing to the different genetic background, sample size, and number and type of marker employed our results indicated the presence of considerable polymorphism within Ethiopian chicken populations relative to indigenous chicken populations in other regions of the world. The mean heterozygosity across all loci and populations in our study (0.582) was comparable to the mean value (0.516) reported for Iranian native chicken populations typed for 5 microsatellite markers (Shabazi *et al.*, 2007). Our results also agree with those reported by Muchadeyi *et al.* (2007) who found large heterozygosity values in Zimbabwean village chicken populations, much larger compared to pure bred layer and broiler lines using 30 microsatellite loci. All the 20 loci we used in this study were incorporated in their work. These authors suggested that migration of birds within village flocks resulting in continuous gene flow between flocks could be responsible for conserving the heterozygosity found in village populations. The same argument could explain the heterozygosity in Ethiopian populations since the village poultry system in both countries shared similar characteristic features (Muchadeyi *et al.*, 2009; Dana *et al.*, 2010b).

All of the loci in the present study have also been used previously to study indigenous and pure bred populations by Muchadeyi *et al.* (2007). Contrary to our results indicating substantial number of loci deviating from Hardy-Weinberg proportions, they reported no loci that deviated from Hardy-Weinberg proportions. However, the overall population heterozygote deficiency for Ethiopian ecotypes ($F_{IT} = 0.095$) was still comparable to the value reported for Zimbabwean chickens by these authors ($F_{IT} = 0.084$).

In domestic species, heterozygote deficiencies can be explained by several factors such as the presence of unamplified alleles ("null" alleles), selection, population subdivision (Wahlund's effects), or inbreeding. Null alleles were not detected in the current study. The level of heterozygosity in the populations was quite high suggesting that the degree of selection imposed by humans on the populations is limited. Likewise, the observed low range (0.02-0.08) in the F_{IS} values of the populations does not present strong evidence of inbreeding. During inbreeding or a

bottleneck period allele numbers usually reduced faster than the heterozygosity (Nei *et al.*, 1975). In fact, the Ethiopian populations exhibited large number of alleles. The deviation from the Hardy-Weinberg equilibrium might be attributed to the so called *Wahlund's effect* where the frequency of homozygotes tends to be higher than the Hardy-Weinberg proportion when a population is subdivided into many breeding units (Nei, 1987).

The between-population genetic diversity was quite low. Although private alleles were observed in each population their frequency was very low, not more than 1%. The pairwise F_{ST} distances (0.016-0.056) revealed the poor divergence of the Ethiopian populations. Using all 30 microsatellite markers recommended by FAO (FAO, 2004), Mwacharo *et al.* (2007) reported comparable values (pairwise $F_{ST} = 0.003$ -0.040) for Kenyan chickens, indicating the absence of structuring among the ecotype populations.

We used the modified Cavalli-Sforza's distance (D_A) which is used for closely related populations and the D_S distance to compare the current results with literature values. The D_A genetic distance was slightly lower (0.041-0.080) than the standard genetic distance (D_S) (0.036-0.098). One interesting difference, however, was that Konso and Farta were the most separated populations in respect of the D_A distance ($D_A = 0.080$) whereas Konso and Horro were the most distant populations when measured using the standard genetic distance ($D_S = 0.098$). However, as with F_{ST} values the genetic distances between the Ethiopian populations were also low. The mean D_S (0.064) was only slightly higher than the corresponding value found between Zimbabwean ecotypes (0.04) (Muchadeyi *et al.*, 2007).

Generally, the Ethiopian chicken populations showed high level of within- but low level of between-population genetic variability. Close to 70% of the total inbreeding (F_{IT}) over all populations was due to the within-population inbreeding (F_{IS}). This result was also confirmed by the AMOVA analysis, which showed that each ecotype population could explain 97% of the genetic diversity found in Ethiopian chickens. A similar pattern was reported by Shabatzi *et al.* (2007) for native Iranian populations, where about 92% of the genetic variation was found among individuals within-populations, while only 8% existed among populations. According to Tixier-Boichard *et al.* (2009), the variety of motivations of village farmers for keeping chickens, including product quality, adaptation to environment and cultural uses shows that within population diversity is a major objective of keeping village chickens. Despite the fact that the chicken populations in the present study were sampled across wide geographic range (510-1460 km) they were not clearly structured along geographical regions. Although the Mantel test for the correlation between genetic differentiation and geographic distances revealed that the populations exhibited isolation by distance, the coefficient of determination of the regression model was quite low ($R_{sq} = 0.312$).

Conclusion

The Ethiopian chicken populations generally showed weak genetic differentiation ($F_{ST} < 0.1$). There is no evidence of population substructuring along agro ecological regions of sampling. Almost all of the differences in the populations could be explained by the genetic variability of individuals within-population. The high polymorphism of microsatellite loci observed in this study suggested that these markers can be utilized in future efforts to assess genetic diversity of indigenous chickens.

Acknowledgement

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Table S1 Exact P-Values of the Hardy-Weinberg probability test for each of 20 microsatellite loci in 5 Ethiopian chicken populations.

| Locus | Konso | Sheka | Horro | Mandura | Farta |
|--------|---------------|---------------|--------------|---------------|---------------|
| LEI94 | 0.6406 | 0.5876 | 0.5085 | 0.2249 | 0.0004 |
| ADL268 | 0.9743 | 0.2946 | 0.2432 | 0.1051 | 0.4174 |
| MCW216 | 0.2374 | 0.5028 | 0.5366 | 0.677 | 0.4821 |
| MCW248 | 0.7299 | 0.0837 | 0.7605 | 0.6946 | 0.6433 |
| LEI166 | 1.0000 | 0.8797 | 0.0715 | 0.1264 | 0.0094 |
| MCW34 | 0.1331 | 0.1737 | 0.1891 | 0.0458 | 0.4196 |
| MCW69 | 0.1018 | 0.2004 | 0.9871 | 0.0933 | 0.7741 |
| ADL278 | 0.3439 | 0.6136 | 0.3087 | 0.0121 | 0.0007 |
| MCW295 | 0.1643 | 0.0843 | 0.7179 | 0.805 | 0.1751 |
| MCW37 | 0.0076 | 0.569 | 0.1637 | 0.6549 | 0.2422 |
| LEI234 | 0.6619 | 0.1683 | 0.0505 | 0.3859 | 0.3453 |
| MCW222 | 0.6953 | 0.0504 | 0.9734 | 0.1596 | 0.0897 |
| MCW16 | 0.539 | 0.0907 | 0.332 | 0.3475 | 0.1631 |
| MCW81 | 0.0361 | 0.2654 | 0.012 | 0.0012 | 1.0000 |
| MCW111 | 0.0613 | 0.4145 | 0.9085 | 0.2591 | 0.8394 |
| MCW206 | 0.0193 | 0.156 | 0.3643 | 0.0741 | 0.0099 |
| MCW14 | 1.0000 | 0.0051 | 0.6803 | 0.0002 | 0.0460 |
| MCW183 | 0.3798 | 0.2186 | 0.0721 | 0.0822 | 0.0021 |
| MCW67 | 0.2274 | 1.0000 | 0.8676 | 0.5177 | 0.2748 |
| MCW330 | 0.1841 | 0.2226 | 0.6128 | 0.0413 | 0.0459 |

Figures in bold face indicate P values of significant deviations from HWE

Table S2 Fisher's Chi-square test for Hardy-Weinberg Equilibrium overall loci in 5 Ethiopian chicken populations.

| Population | Chi2 | DF | P |
|------------|---------|----|----------------------|
| Konso | 59.7446 | 40 | 0.023 |
| Sheka | 62.9379 | 40 | 0.0118 |
| Horro | 47.817 | 40 | 0.1851 ^{NS} |
| Mandura | 92.5535 | 40 | 0.000 |
| Farta | 99.3663 | 40 | 0.000 |

NS: Non Significant

5

Genetic and phenotypic parameter estimates for body weights and egg production in Horro chicken of Ethiopia

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Abstract

A breeding program has been established in 2008 to improve productivity of Horro chicken, an indigenous population in the western highlands of Ethiopia. The pedigree descended from 26 sires and 260 dams. Body weights were measured every two weeks from hatch to 8 weeks then every 4 weeks for the next 8 weeks. Egg production was recorded to 44 weeks of age for 1 generation. Genetic parameters were estimated using animal model fitted with common environmental effects for growth traits and ignoring common environment for egg production traits. Direct heritabilities ranged from low (0.15 ± 0.08), for body weight at 6 weeks, to moderate (0.40 ± 0.23), for hatch weight. Heritabilities of common environmental effects on growth were high at hatch (0.39 ± 0.10) and remained low afterwards. Age at first egg showed a very low heritability (0.06 ± 0.15). Heritabilities of egg numbers in the first, second, third and fourth months of laying were $0.32 (\pm 0.13)$, $0.20 (\pm 0.16)$, $0.56 (\pm 0.15)$ and $0.25 (\pm 0.14)$, respectively. Heritabilities of cumulative of monthly records of egg numbers were from 0.24 ± 0.16 (for the first two months, EP12) to 0.35 ± 0.16 (over the 6 months, EP16). Body weight at 16 weeks of age (BW16) has a strong genetic correlation with the cumulative of monthly records: 0.92 (with EP12), 0.69 (with EP36) and 0.73 (with EP16). Besides their strong association, BW16 and EP16 showed higher heritability, relative to their respective trait categories. These two traits seemed to have common genes and utilizing them as selection traits would be expected to improve both egg production and growth performance of local chicken. However, the standard errors of estimates in this study were mostly high indicating that the estimates have low precision. Parameter estimations based on more data are needed before applying the current results in breeding programmes.

Key Words: indigenous chicken, growth, egg production, heritability, correlations

Introduction

Indigenous chickens comprise about 80% of the national flocks in Africa and Asia. Compared to their modern counterparts indigenous chickens are generally poor producers of eggs and meat. Consequently they are being replaced by commercial strains in many developing countries. In some countries this strategy was pursued for decades to increase productivity under village systems but failed to bring sustainable improvement (Teklewold et al. 2006). In fact, it posed a serious threat to the existing genetic diversity of indigenous chickens (Besbes 2009).

Despite their low growth rates and egg production, indigenous chickens are generally better in disease resistance and could maintain higher level of performance under poor nutrition and high environmental temperatures compared to commercial strains under village systems (Horst 1989). This is clear evidence of the positive attributes of indigenous chickens. Studies on biodiversity of indigenous chickens in many parts of Africa revealed the presence of high genetic variability within ecotype populations (Muchadeyi et al. 2007; Mwacharo et al. 2007; Halima et al. 2009) indicating the potential for genetic improvement of these chickens through selective breeding. The present work is based on a selection scheme initiated in 2008 to improve growth and egg production of Horro chickens.

Horro is an indigenous chicken type named after the geographic region of origin located in the western part of Ethiopia near the Blue Nile gorge. There are about 30,000 chickens restricted to this original environment (Dana et al. 2010). The population has a wide range of morphologic and genetic diversity. The program aims to make Horro chickens more profitable for the poor people in these regions and conserve the existing genetic diversity. If this program is successful then it will be used as a benchmark for improving other indigenous chicken genetic resources.

Knowledge on genetic parameters is essential for any genetic improvement program. There is a lot of literature on genetic parameters for growth and egg production of commercial poultry populations (see reviews by Chambers 1990; Fairfull and Gowe 1990); however, these values may not be applicable to these indigenous chickens. There are some estimates for growth (Norris and Nigambi 2006; Gondwe 2005) and egg production (Francesch et al. 1997; Sang et al. 2006; Kamali et al. 2007; Lwelamira et al. 2009) traits in unselected indigenous chickens of Africa and other countries but there are no estimates for Ethiopian chickens.

The aim of this study was to estimate heritabilities and genetic and phenotypic correlations for growth and egg production traits to understand which traits should be included in breeding programmes for Horro chickens.

Materials and methods

Experimental population and traits measured

The study was done at the Ethiopian Institute of Agricultural Research, Debre Zeit Agricultural Research Centre (DZARC). The population was established from 3000 eggs purchased from two village market sheds in Horro. The pedigree descended from 26 sires and 260 dams and were hatched and raised at the poultry research farm of DZARC. The offspring were hatched in 3 batches between January and February 2008. Birds in all age classes were provided *ad libitum* access to feed and water. Starting chick feed (20%CP and 2,950-3,000 kcal/kg) for the first 3 weeks and grower ration (18%CP and 2,850-2,900 kcal/kg) from 3 to 10 weeks. Between 10 to 16, 16 to 18 and from 18 weeks onward the birds were provided with pullet ration (16%CP and 2,700-2,750 kcal/kg), pullet/layer blend and layer ration (17-18%CP and 2,700-2,750 kcal/kg), respectively. The chickens were reared in a single deep litter house until 18 weeks of age under a standard housing space, with natural lightning after 8 weeks of age. After 18 weeks of age a total of 240 females and 24 males were picked randomly and transferred to layer houses and reared in floor cages with 1 male and 10 females in each pen. Each pen had a trap nest for individual recording of egg production and pedigree. The remaining animals were sold due to limitations in housing space. All chickens were vaccinated against Newcastle and Marek's diseases at one day old, Gumboro at 1 week and fowl pox at 10 weeks.

Live weight growth was measured every 2 weeks for the first 8 weeks then every 4 weeks for the next 8 weeks. Traits recorded were: body weights at hatch (BW0) and body weights in weeks 2 (BW2), 6 (BW6), 8 (BW8), 12 (BW12) and 16 (BW16). Age at first egg (AFE) was recorded for each hen. Early part egg production record, defined as the number of eggs produced from housing to about 44 weeks of age, was used to study egg production traits. Egg production was recorded for six 4-week periods: 21 to 24, 25 to 28, 29 to 32, 33 to 36, 37 to 40, and 41 to 44 weeks of age. Each of these 4 week intervals comprised the monthly records of egg numbers; M1, M2, M3, M4, M5 and M6, respectively. The cumulative of monthly egg production records were used for analyzing part period production. The number of eggs produced in periods 1 (EP12), 2 (EP36) and 3 (EP16) were the cumulative number of eggs produced from months 1 to 2, 3 to 6 and 1 to 6, respectively. Box-Cox transformation was used to achieve normality in egg production data (Besbes et al. 1993).

Statistical analyses

Descriptive statistics of growth and egg production data were carried out in the SAS package (SAS 2001) using all available records. Only records from hens which survived to 44 weeks of age were included in the genetic analysis of egg traits. Parameter estimates for both growth and egg traits

were obtained by univariate animal model using ASREML (Gilmour et al. 2006). Heritabilities of growth traits were estimated including a common environment effect. The following linear model was used:

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

Where, \mathbf{Y} = vector of observations; \mathbf{b} = vector of fixed effects of sex and hatch number; \mathbf{a} = vector of random direct genetic effects; \mathbf{c} = vector of random common environmental effects; e = vector of residual effects; and \mathbf{X} , \mathbf{Za} and \mathbf{Zc} are incidence matrices relating records to fixed, direct genetic and common environmental effects, respectively. Maternal genetic effects could not be estimated due to the small data size. The common environmental effect did not exist for body weights in weeks 12 and 16 and was, thus, excluded from the model. A similar procedure was used for analysing egg production traits but ignoring common environment from the model and using hatch number, house and pen as the fixed effects. Correlations were estimated using a bivariate analysis. Because convergence could not be achieved when the common environmental effect was included in the model, correlations were estimated with animal as the only random effect.

Results

Basic statistics

Table 1 shows means of body weights for Horro chicken to 16 weeks of age. Means of body weights from hatch to 16 weeks of age ranged from 25 to 701 g in males and from 25 to 573 g in females. Overall, the mean hatch weight of Horro chicken was about 25 g which increased to 621 g at 16 weeks of age.

The average number of eggs produced monthly and cumulative of part records for the laying period were shown in Table 2. Hens attained sexual maturity at an average of 190 days. About 16% of the hens started laying between 21-24 weeks of age. Most of the hens housed did not lay during this period and only started laying after 25 weeks of age. Mean monthly egg numbers ranged from 0.7 at the beginning of laying to 9 in the fourth month. Peak egg production was achieved at the fourth month of laying which started to drop in the following months. Averages of cumulative of monthly records in the first 2 months (EP12), from month 3 to 6 (EP36) and the total over 6 months (EP16) were around 5, 32 and 34, respectively. Hen-day rate of egg production, defined as the number of egg produced by the hens housed divided by the product of the number of days in production and the number of hens alive, increased from 2.5% in the first

month to 32% in the fourth month of laying and declined afterwards. Mortality in the laying house increased from 8 in the first month (21-24 weeks) to 11% in the second month but steadily decreased and remained low in the following periods. The total rate of mortality during the laying period was 29%, slightly lower compared to the total mortality from hatch to 16 weeks of age (32%) most of which occurred after 6 weeks of age (see table 1 for the number of animals that survived at different ages).

Table 1 Means of body weights^a of Horro chicken by sex and for both sexes combined.

| Male | n ^b | Mean, g (±SE) | Female | n ^b | Mean, g (±SE) |
|---------------------|----------------|---------------|--------|----------------|---------------|
| BW0 | 642 | 24.9 (0.13) | BW0 | 872 | 24.6 (0.10) |
| BW2 | 642 | 59.6 (0.46) | BW2 | 871 | 52.2 (0.37) |
| BW4 | 641 | 113.9 (1.10) | BW4 | 871 | 93.4 (0.87) |
| BW6 | 640 | 181.6 (1.58) | BW6 | 870 | 146.0 (1.31) |
| BW8 | 606 | 277.8 (2.60) | BW8 | 849 | 216.0 (2.19) |
| BW12 | 528 | 485.5 (5.97) | BW12 | 764 | 388.3 (4.51) |
| BW16 | 388 | 701.1 (12.13) | BW16 | 646 | 572.7 (7.27) |
| Both sexes combined | n ^b | Mean, g (±SE) | | | |
| BW0 | 1514 | 24.7 (0.08) | | | |
| BW2 | 1513 | 55.4 (0.30) | | | |
| BW4 | 1512 | 102.1 (0.73) | | | |
| BW6 | 1510 | 161.1 (1.10) | | | |
| BW8 | 1455 | 241.8 (1.86) | | | |
| BW12 | 1292 | 428.0 (3.85) | | | |
| BW16 | 1034 | 620.9 (6.71) | | | |

^aBW0, hatch weight; BW2, BW4, BW6, BW8, BW12, BW16, body weights at 2, 4, 6, 8, 12 and 16 weeks of age, respectively

^bNumber of animals

Table 2 Mean monthly^a and cumulative^b number of eggs, hen-day (HDP) and hen-housed (HHP) rates of egg production and mortality during the early part laying period, to 44 weeks of age, in Horro chicken.

| Trait | Period (week) | Hens housed, n | Mean, n (±SE) | HDP (%) | HHP (%) | Mortality (%) |
|-------|---------------|----------------|---------------|---------|---------|---------------|
| M1 | 21-24 | 328 | 0.71 (0.13) | 2.53 | 2.34 | 7.6 |
| M2 | 25-28 | 303 | 4.06 (0.33) | 14.50 | 12.87 | 11.2 |
| M3 | 29-32 | 269 | 7.82 (0.41) | 27.93 | 26.69 | 4.5 |
| M4 | 33-36 | 257 | 8.98 (0.44) | 32.07 | 30.82 | 4.0 |
| M5 | 37-40 | 247 | 8.25 (0.44) | 29.47 | 28.51 | 3.2 |
| M6 | 41-44 | 239 | 7.34 (0.39) | 26.23 | 25.57 | 2.5 |
| EP12 | 21-28 | 328 | 4.78 (0.41) | 8.68 | 7.12 | 18.0 |
| EP36 | 29-44 | 269 | 31.77 (1.31) | 30.31 | 26.26 | 13.4 |
| EP16 | 21-44 | 328 | 33.64 (1.56) | 23.55 | 16.73 | 29.0 |
| AFE | | 203 | 190.00 (1.77) | | | |

AFE, age at first egg (in days)

^aM1, M2, M3, M4, M5, M6, egg numbers in the first, second, third, fourth, fifth and sixth months, respectively

^bEP12, EP36 and EP16, cumulative number of eggs produced from months 1 to 2, 3 to 6 and 1 to 6, respectively

Fig. 1 shows the distribution of the number of eggs produced in relation to the number of hens that survived to 44 weeks of age. More than 13% of the hens did not lay at all through out this period. Relatively the largest proportion of hens (37 of 203, 18.2%) laid between 31-40 eggs. The top 10% hens produced between 71 to a little more than 90 eggs. However, the proportion of hens that laid more than 80 eggs was less than 5%. The associations between the phenotypic performance of body weight growth and total egg production at 44 weeks of age were shown in Fig. 2. The top 10 % of hens with superior egg production had higher body weight at 16 weeks of age compared both to the hens that laid from 31 to 40 eggs and non-layers. On average the body weight of non-layers remained unchanged from 12 to 16 weeks of age.

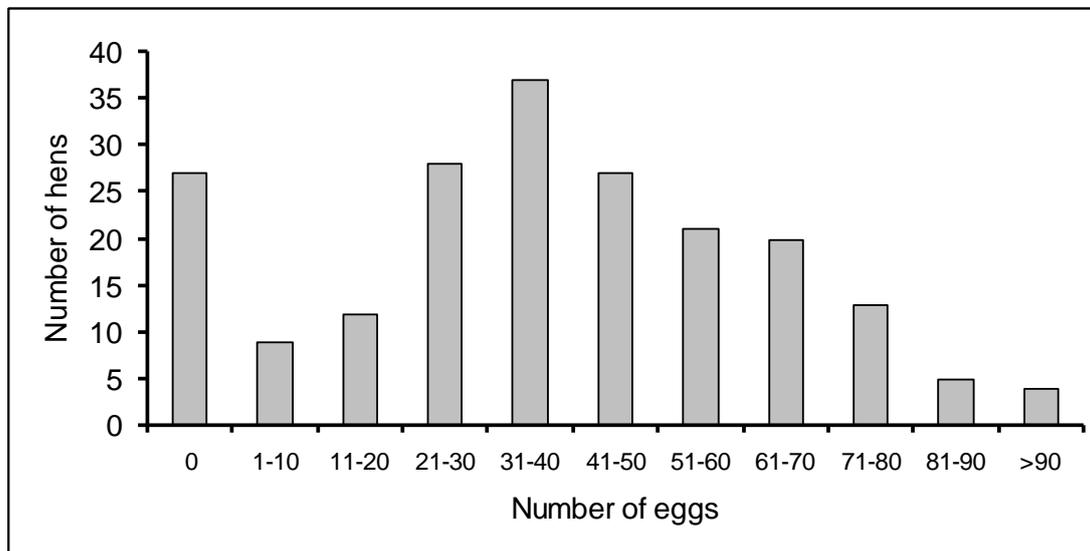


Fig. 1 Frequency distribution of the number of eggs produced by hens that survived through out the recording period (44 weeks of age).

Heritabilities of growth and egg production

Tables 3 and 4 present the variance components and heritabilities for growth and egg production traits. Estimates on additive genetic variances for growth traits ranged from 3.9 for body weight at hatch to 9673 for body weight at 16 weeks of age. Environmental variances also showed similar trends, generally increasing from hatch to 16 weeks of age. Common environmental variances were observed for body weights at hatch and those at weeks 2, 6 and 8 while they were not detected for body weights in weeks 12 and 16 due to lack of convergence.

Estimates of direct heritability of growth traits ranged from 0.15 (BW6) to 0.40 (BW0). The values were moderate for body weight at 16 weeks of age (0.23) and that of hatch weight but remained low for the rest of the traits. Common environmental effect was moderate for hatch weight (0.39) but almost non existent for the remaining traits. Age at first egg showed a very low

heritability (0.06). Heritabilities of monthly egg numbers ranged from 0.20 to 0.32, except for M3 for which heritability was 0.56. Heritabilities of cumulative part record egg numbers were from 0.24 (EP12) to 0.35 (EP16).

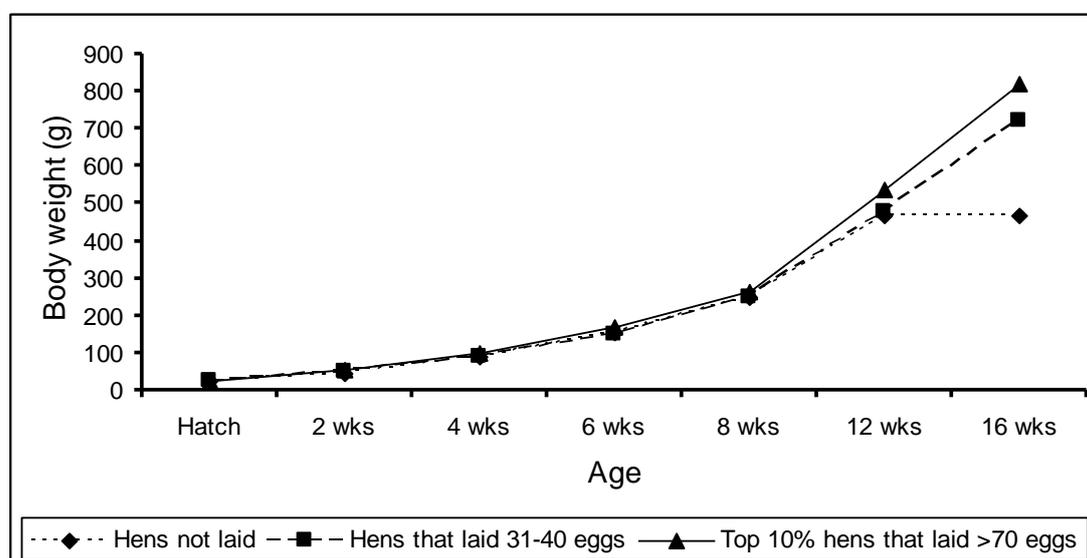


Fig. 2 The trends in growth performance of hens to 16 weeks in relation to their total egg production at 44 weeks of age.

Table 3 Variance components^a and heritabilities^b of body weights^c in Horro chickens (for hens survived to 44 weeks of age).

| Trait | Anim-als, n | Reco-rds, n | Sires, n | Dams, n | σ^2_a | σ^2_c | σ^2_e | h^2 (\pm SE.) | c^2 (\pm SE.) |
|-------|-------------|-------------|----------|---------|--------------|--------------|--------------|--------------------|--------------------|
| BW0 | 1456 | 1307 | 25 | 143 | 3.9 | 3.7 | 2.0 | 0.40 (0.23) | 0.39 (0.10) |
| BW2 | 1434 | 1306 | 25 | 142 | 19.1 | 11.4 | 71.7 | 0.19 (0.11) | 0.11 (0.05) |
| BW6 | 1330 | 1303 | 25 | 141 | 197.4 | 43.8 | 1073.3 | 0.15 (0.08) | 0.03 (0.03) |
| BW8 | 1262 | 1248 | 25 | 138 | 516.9 | 36.6 | 2643.2 | 0.16 (0.08) | 0.01 (0.03) |
| BW12 | 1092 | 1090 | 25 | 136 | 2399.0 | | 12410.0 | 0.16 (0.05) | - |
| BW16 | 845 | 845 | 25 | 132 | 9673.0 | | 33220.0 | 0.23 (0.06) | - |

^a σ^2_a , σ^2_c and σ^2_e , additive genetic, common environmental and residual variances, respectively

^b h^2 and c^2 , heritabilities of direct genetic and common environmental effects, respectively

^c BW0, hatch weight; BW2, BW6, BW8, BW12, BW16, body weights at 2, 6, 8, 12 and 16 weeks of age, respectively

Correlations within and among growth and egg production traits

Table 5 shows the relationships within and among body weights and cumulative number of eggs produced between 1-2 (EP12), 3-6 (EP36) and 1-6 (EP16) months of laying. The correlations between hatch weight and most other traits were generally low. Among other growth traits,

genetic correlations ranged from 0.51 (BW2 with BW16) to 0.99 (BW12 with BW16) and phenotypic correlations from 0.27 (BW2 with BW16) to 0.85 (BW6 with BW8). Genetic correlations among part record egg numbers ranged from 0.79 (EP12 with EP36) to 0.98 (EP36 with EP16).

Table 4 Variance components^a and heritabilities^b of monthly^c and cumulative^d egg numbers during early part laying period in Horro chickens (for hens survived to 44 weeks of age).

| Trait | Period (weeks) | Animals, n | Records, n | Sires, n | Dams, n | σ^2_a | σ^2_e | h^2 (\pm SE.) |
|-------|----------------|------------|------------|----------|---------|--------------|--------------|--------------------|
| M1 | 21-24 | 203 | 176 | 23 | 69 | 0.1 | 0.2 | 0.32 (0.13) |
| M2 | 25-28 | 203 | 176 | 23 | 69 | 2.6 | 10.2 | 0.20 (0.16) |
| M3 | 29-32 | 203 | 176 | 23 | 69 | 15.5 | 12.2 | 0.56 (0.15) |
| M4 | 33-36 | 203 | 176 | 23 | 69 | 7.1 | 21.8 | 0.25 (0.14) |
| EP12 | 21-28 | 203 | 176 | 23 | 69 | 3.8 | 11.9 | 0.24 (0.16) |
| EP36 | 29-44 | 203 | 176 | 23 | 69 | 67.6 | 174.3 | 0.28 (0.15) |
| EP16 | 21-44 | 203 | 176 | 23 | 69 | 115.9 | 216.5 | 0.35 (0.16) |
| AFE | | 203 | 176 | 23 | 69 | 31.5 | 458.5 | 0.06 (0.15) |

AFE, age at first egg (in days)

^a σ^2_a , σ^2_c and σ^2_e , additive genetic, common environmental and residual variances, respectively

^b h^2 , heritability

^c M1, M2, M3, M4, egg numbers in the first, second, third and fourth months, respectively

^d EP12, EP36 and EP16, cumulative number of eggs produced from months 1 to 2, 3 to 6 and 1 to 6, respectively

The correlations between body weights and part record egg numbers did not converge for growth traits in weeks 6, 8 and 12 with egg traits. Correlations of the egg traits with body weight at hatch and weight in week 2 were generally low (Table 5). Interesting genetic correlations were observed for body weight at 16 weeks with part record egg numbers. Body weight at this age was strongly and positively correlated with EP12 (0.92), EP36 (0.69) and EP16 (0.73). Negative genetic correlation existed between BW6 and EP12 (-0.54). The phenotypic correlations between body weight and part record egg numbers generally appeared to be low, ranging from 0.06 (BW0 with EP12) to 0.38 (BW16 with EP16). However, the standard errors of all estimates between growth and egg production traits were quite high reflecting the small sample size. Table 6 presents the correlations between monthly and cumulative part record egg numbers. The highest correlations were found between the number of eggs recorded in the third month (M3) and cumulative part record of the first 2 months (EP12) ($r_g = 0.83$, $r_p = 0.39$) while the other part records, EP36 and EP16, were strongly correlated with M4 ($r_g = 0.74$ to 0.81 , $r_p = 0.68$ to 0.73).

Table 5 Genetic (below diagonal) and phenotypic (above diagonal) correlations between body weights^a and cumulative early part period egg numbers^b.

| Trait | BW0 | BW2 | BW6 | BW8 | BW12 | BW16 | EP12 | EP36 | EP16 |
|-------|----------------|-----------------|-----------------|----------------|----------------|----------------|----------------|----------------|----------------|
| BW0 | | 0.45 (0.03) | 0.22 (0.03) | 0.15 (0.03) | 0.09 (0.03) | 0.10 (0.03) | 0.06 (0.08) | 0.19 (0.07) | 0.19 (0.08) |
| BW2 | 0.71 (0.08) | | 0.64 (0.02) | 0.53 (0.02) | 0.37 (0.03) | 0.27 (0.03) | 0.16 (0.07) | 0.07 (0.08) | 0.13 (0.08) |
| BW6 | 0.46 (0.10) | 0.85 (0.06) | | 0.85 (0.01) | 0.59 (0.01) | 0.40 (0.03) | 0.25 (0.07) | 0.16 (0.07) | 0.21 (0.07) |
| BW8 | 0.37 (0.11) | 0.77 (0.08) | 0.97 (0.02) | | 0.74 (0.01) | 0.56 (0.02) | 0.19 (0.07) | 0.11 (0.08) | 0.15 (0.07) |
| BW12 | 0.25 (0.13) | 0.54 (0.13) | 0.68 (0.11) | 0.86 (0.06) | | 0.82 (0.01) | - | - | - |
| BW16 | 0.30 (0.12) | 0.51 (0.13) | 0.67 (0.12) | 0.82 (0.08) | 0.99 (0.03) | | 0.35 (0.07) | 0.31 (0.07) | 0.38 (0.06) |
| EP12 | 0.30 (0.38) | 0.22 (0.48) | -0.54 (0.93) | - | - | 0.92 (0.35) | | 0.39 (0.06) | 0.59 (0.05) |
| EP36 | 0.40 (0.36) | -0.16 (0.56) | - | - | - | 0.69 (0.43) | 0.80 (0.32) | | 0.96 (0.01) |
| EP16 | 0.42 (0.31) | 0.02 (0.46) | - | 0.15 (0.07) | - | 0.73 (0.32) | 0.88 (0.21) | 0.98 (0.02) | |

^aBW0, hatch weight; BW2, BW6, BW8, BW12, BW16, body weights at weeks 2, 6, 8, 12 & 16, respectively

^bEP12, EP36 and EP16, cumulative number of eggs produced from months 1 to 2, 3 to 6 and 1 to 6, respectively

Table 6 Genetic (below diagonal) and phenotypic (above diagonal) correlations between monthly^a and cumulative^b number of eggs produced during the early part laying period.

| Trait | M1 | M2 | M3 | M4 | Ep12 | Ep36 | Ep16 |
|-------|--------------|-------------|-------------|-------------|-------------|-------------|-------------|
| M1 | | 0.38 (0.06) | 0.10 (0.07) | 0.18 (0.07) | 0.48 (0.05) | 0.22 (0.07) | 0.38 (0.06) |
| M2 | - | | 0.40 (0.06) | 0.16 (0.07) | - | 0.39 (0.06) | 0.59 (0.04) |
| M3 | 0.29 (0.37) | 0.94 (0.32) | | 0.25 (0.07) | 0.39 (0.06) | 0.58 (0.04) | 0.59 (0.04) |
| M4 | -0.15 (0.47) | 0.71 (0.52) | 0.97 (0.39) | | 0.16 (0.07) | 0.73 (0.03) | 0.68 (0.04) |
| EP12 | - | - | 0.83 (0.26) | 0.46 (0.45) | | 0.39 (0.06) | 0.59 (0.05) |
| EP36 | 0.16 (0.46) | - | - | 0.81 (0.19) | 0.80 (0.32) | | 0.96 (0.01) |
| EP16 | 0.38 (0.37) | - | - | 0.74 (0.21) | 0.88 (0.21) | 0.98 (0.02) | |

^aM1, M2, M3, M4, egg numbers in the first, second, third and fourth months, respectively

^bEP12, EP36 and EP16, cumulative number of eggs produced from months 1 to 2, 3 to 6 and 1 to 6, respectively

Discussion

The mean body weights of Horro chicken were generally within the ranges reported for unselected indigenous populations in north western Ethiopia (Halima et al. 2007) and many other countries of Africa (Gueye 1998). The average number of eggs as well as the rate of lay to 44 weeks of age was quite low. Comparative data on early part period egg production of other Ethiopian local chickens is not available. The peak production was attained in the fourth month of lay on the level of 32% (9 eggs/hen). The figures generally confirm previous reports showing that indigenous chickens of Ethiopia and of many other African countries are poor egg layers (Gueye 1998; Dana and Ogle 2002).

Body weights to 16 weeks of age were used to characterize the growth of chicken in this study. Selection for rapid early growth at a market age (40-50 days) has been the most common approach in broiler chicken breeding programmes (Emmerson 2003). Our results showed that body weight at 16 weeks of age has a positive correlation to growth from 2 to 12 weeks of age. The correlations were particularly strong with certain growth traits ($r_g = 0.82$ with BW8, and 0.99 with BW12). Body weight at 16 weeks was also relatively the most heritable among the other growth traits measured. Therefore, since chickens in Ethiopia are kept for both meat and egg production attaining mature body size at earlier ages is not the target of the production system, and thus, selection at 16 weeks of age could be the most suitable approach to improve growth.

Heritabilities of monthly egg productions decreased from 0.32 in month 1 to 0.25 at peak egg production in month 4, except for month 3 which was exceptionally high ($h^2 = 0.56$). A comparable pattern of heritability changes in monthly egg numbers has also been reported by Anang et al. (2002) and Wolc and Szwaczkowski (2009). Heritabilities of cumulative part period egg numbers (0.24-0.35) were within the range reported by Sang et al. (2006) who found moderate values (0.24-0.37) in five Korean native chicken strains for total egg numbers from start to 270 days of lay and the figures (0.31-0.32) reported by Lwelamira et al. (2009) for cumulative number of eggs produced in the first 90 days of laying in indigenous Tanzanian chickens. Sabri et al. (1999) also reported heritabilities of 0.27, 0.19 and 0.30 for egg numbers produced between 26-30, 50-54 and 26-54 weeks period, respectively, for White Leghorn hens in a subtropical environment. Higher values were reported by Anang et al. (2000) for cumulative egg production of the first 5 months in White Leghorn chickens ($h^2 = 0.46$) and by Kamali et al. (2007) for the first 12 weeks of egg production ($h^2 = 0.49$) in Iranian indigenous fowls compared to our results.

Part period egg numbers were relatively more heritable and consistent than monthly egg productions. Most of the monthly egg production traits were poorly related with each other and with cumulative egg production while the correlations among the latter traits remained quite high. Particularly, the total number of eggs produced to 44 weeks of age (EP3) was found to be the most heritable trait ($h^2 = 0.35$) having a strong positive correlation with BW16 ($r_g = 0.73$). These two traits seemed to have common genes and utilizing them as selection traits would be expected to improve both egg production and growth performance of local chicken. The standard errors of estimates in this study were mostly high indicating that the estimates have low precision and parameter estimations based on more data are needed before applying the current results in breeding programmes.

However, the trends drawn from the phenotypic performances of growth and total egg production to 44 weeks of age showed that hens heavier at 16 weeks of age laid higher number of eggs where as the non-layers weighed less suggesting that body weight at 16 weeks of age could

be a good indicator for egg production, which is in agreement with the high genetic correlation (0.69-0.92). Hens with the highest egg production (> 70 eggs at 44 weeks of age) comprised about 10% of the flock, and thus, might be considered as potential candidates for selection based on phenotypic performance (see Fig. 1 and 2). This can form the basis for selection instead of random picking for the following generation.

Selection based on early period part records, up to 40 weeks of age could result in increased egg production of chickens (Fairfull and Gowe 1990; Poggenpoel et al. 1996). Estimates for part records can be used as selection criteria to improve both part and annual egg production and any loss in accuracy is compensated by the reduction in generation interval, thus maximizing genetic gain per unit of time (Ayyagari et al. 1980). Hicks et al. (1998) also showed that selection based on partial records of the individual and all available ancestral records resulted in the shortest generation interval and was the most efficient strategy for maximizing egg production in laying hens compared to other strategies using full records. Various models have been proposed to predict annual egg production from early part record egg production (McMillan et al. 1986; Grossman and Koops 2001).

Conclusion

Growth and egg production are economically the most important traits in small holder poultry production systems. An earlier study in Ethiopia showed that farmers across all geographic regions rated them as the traits they wanted to be improved the most (Dana et al. 2010). Since chickens under rural production systems are kept both for meat and egg production selection for genetic improvement of local chickens should seek to improve the two traits simultaneously. This study revealed that body weight at 16 weeks of age has a strong genetic correlation with the total number of eggs recorded from housing to 44 weeks of age. These two traits also showed higher level of heritability, relative to their respective trait categories. However, the precision of estimates particularly on egg production traits is low due to the small number of records used. Therefore, further work is recommended to confirm the current results using larger number of records.

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6

East Asian contributions to Dutch traditional and western commercial chickens inferred from mtDNA analysis

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Summary

Understanding the complex origin of domesticated populations is of vital importance for understanding, preserving, and exploiting breed genetic diversity. Here we aim to assess Asian contributions to European traditional breeds and western commercial chicken from mitochondrial genetic diversity. To this end, a 365 bp fragment of the chicken mtDNA D-loop region of 16 Dutch fancy breeds (113 individuals) was surveyed, comprising almost the entire breed diversity of The Netherlands. We also sequenced the same fragment for 160 commercial birds representing all important commercial types from multiple commercial companies that together represent more than 50% of worldwide commercial value. We identified 20 different haplotypes. The haplotypes clustered into five clades. The commonest clade (E-clade) supposedly originates from the Indian subcontinent. In addition, both in commercial chicken and Dutch fancy breeds many haplotypes were found with a clear East-Asian origin. However, the erratic occurrence of many different East-Asian mitochondrial clades indicates there were many independent instances where breeders used imported exotic chickens for enhancing local breeds. Nucleotide diversity and haplotype diversity analyses showed the influence of the introgression of East-Asian chicken on genetic diversity. All populations that had haplotypes of multiple origin displayed high inferred diversity as opposed to most populations that had only a single mitochondrial haplotype signature. Most fancy breeds were found to have a much lower within population diversity compared to broilers and layers though this is not the case for mitochondrial estimates in fancy breeds that have multiple origin haplotypes.

Keywords mtDNA D-loop, diversity, Dutch fancy breeds, broilers, layers

Introduction

The hypothesis that the chicken has been domesticated from the red jungle fowl is widely accepted (West & Zhou 1988, Crawford 1990, Moiseyeva *et al.* 2003) although evidence exists (Nishibori *et al.* 2005, Erikson *et al.* 2008) that other wild species of jungle fowl contributed genetic material to modern chickens. In addition, different opinions prevailed regarding the geographic region of domestication. Crawford (1990) proposed that domestication of chickens took place in the Indus valley around 2500 – 2100 BC. However, archaeological and genetic evidences suggested that Southeast Asia is the origin of chicken domestication (West & Zhou 1988, Akishinonomiya *et al.* 1994, 1996). Liu *et al.* (2006) on the other hand found evidences implicating multiple maternal origins of chicken centred around South and Southeast Asia, reconciling these earlier finding by providing genetic evidence for domestication of chickens to have taken place in multiple geographic locations.

Many routes have been proposed to explain the migration of domestic chickens to Europe. A review by Shahbazi *et al.* (2007) indicates that chickens were introduced to Iran from the Indus valley around 2500-2000 BC from where they spread to Europe, to be introduced to Greece and Italy across the Aegean Sea or directly to central Europe through Scythia and Southern Russia. Other possible routes include dispersion through Iran to the Mediterranean and, through China and Russia to Europe (Crawford 1995). West & Zhou (1988) suggested that North European chickens were introduced from China through Russia, and not from the Indus Valley. Based on the fact that chickens in the Mediterranean were morphologically different from those found in Northern Europe, West & Zhou (1988) shared the opinion that Mediterranean chickens could have been introduced through Iran.

Dispersal of domestic chickens from their putative centres of domestication to different regions with diverse environmental conditions and people of different cultural orientations has contributed to the observed genetic differentiation of chicken populations. Other factors that may have played a role in the genetic differentiation include founder effects, migration, mutation, natural and artificial selection. Most of indigenous pure breeds and varieties and the multitude of fancy breeds found today in Europe and North America were developed during the late 19th century by breeding for exhibition traits using local and imported stocks (Crawford 1995). It was also during the last centuries that chicken from South and Southeast Asia were imported to Europe and were documented to have played a role in the formation of these European breeds.

For Dutch fancy breeds, early paintings and existing literature showed that formation of many breeds was influenced by Asiatic (and to lesser extent by east European and Mediterranean) chickens. For instance, one or more of the Asiatic chicken classes such as Malays, Japanese

bantams, and Sumatras were involved in the formation of Friesian fowls, Dutch bantams, Breda fowls, Booted bantams, Barnevelders, Kraienkoppes and Hamburgs. Polish bearded and Polish non-bearded are thought to be influenced by chicken from east Europe. The Lakenvelders, Assendelft fowls, Drente fowls, Holland fowls and Groninger Mews are country fowls with no recorded history of genetic influence from Asiatic chickens (see supplementary information, Text S1).

From the 1950s, some of the most productive breeds and varieties that were developed by fanciers were subjected to intensive selection for quantitative traits giving rise to the breeds currently being used in the broiler and layer industry (Muir *et al.* 2008). Broilers descend from Plymouth Rock females that have been selected for reproductive traits while the sire lines, selected for growth traits, are based on the White Cornish breed. The Plymouth Rock has a documented origin that includes Asian breeds such as Java Fowl, Brahma and Cochin, while the Cornish are thought to be partially descended from Asian fighting cocks. Commercial egg layers consist mainly of White Leghorns or their crosses which are bred for the production of white shelled eggs. The brown-egg layers on the other hand are derived from the Rhode Island Red breed (Crawford 1995), that originated in the USA and documented to include several Chinese chicken breeds in its heritage.

Therefore, both European fancy and Western commercial breeds of chicken are thought to carry genes from Asian populations of domestic chickens. However, the sources of variations and genetic relationships among fancy breeds of the Netherlands and commercial chicken populations with their potential progenitors and the probable influence of other continental populations are not clear. Up to now, studies investigating the origin of European traditional and Western commercial breeds, such as by way of mitochondrial haplotype analysis, have been limited in scope.

This study aims to elucidate possible Southeast Asian contributions to the genetic variation of European traditional breeds, and western commercial chicken by ascertaining mitochondrial genetic diversity. We do this by systematically investigating the majority of chicken breeds of one European country, breeds that are known to be highly diverse in origin and encompass breeds of known Asian origin as well as country fowls with no known non-European contributions. In addition, we systematically investigate Western commercial populations by including all major types (white egg layers, brown egg layers, dam broiler and sire broiler lines) representing more than 50% of commercially sold chicken in the world. We furthermore compare the level of genetic diversity within and between the Dutch fancy and commercial breeds.

Materials and methods

Samples

In this study, 273 individuals were selected from populations and breeds included commercial chickens (n = 160) selected for economically important traits, and fancy breeds (n = 113) from the Netherlands. The name and sample size for each breed is shown in Table 1.

Genomic DNA was extracted from whole blood of each chicken using the Genra Kit (<http://www1.qiagen.com/Products/GenomicDnaStabilizationPurification/GenraPuregeneBloodKit.aspx>).

MtDNA D-loop sequencing

In total 635 bp of the chicken mtDNA D-loop was amplified for 273 individuals with the primers L16750 (5'-AGGACTGCTTGAAAAGC-3') and CR1b (5'-CCATACACGCAAACCGTCTC-3'). PCR was performed under standard conditions (annealing temperature of 50°C). Sequencing reactions were carried out using the Big Dye terminator cycle sequencing kit version 3.1 and analyzed on an ABI 3100 automated DNA analyzer. In this study we used 365 base pairs covering the D-loop from base 30 to 394 of the reference mtDNA sequence of *G. g. domesticus* (X52392, Desjardins & Morais 1990).

Sequence analysis

Phylogenetic relationships and network analysis

All of the 273 mtDNA D-loop sequences were manually edited and aligned with the aid of the CLUSTALX program (Thompson *et al.* 1997). A rooted neighbour-joining (NJ) tree was reconstructed to identify phylogenetic clades employing the Kimura 2-parameter model. The CLUSTALX package was used to draw the tree applying 1000 bootstrap replications to test reliability of the branching order. The red jungle fowls (*G. g. bankiva*, AB007718 and *G. g. gallus*, AB007720) were used as out groups to root the tree using NJPLOT95. Median-joining (MJ) networks (Bandelt *et al.* 1999) were constructed using NETWORK version 4.5.6 (<http://fluxusengineering.com>) to determine the genetic relationship among the clades that were observed from the NJ tree.

To explore the genetic relationships of the haplotypes generated in this study and those of other chicken populations from different geographic regions and from areas close to the possible centres of chicken domestication, a total of 830 sequences were retrieved from the Genbank. The sources and accession numbers of these sequences and their geographic regions were described by Liu *et al.* (2006).

Table 1 Sample information and haplotype frequency of Dutch fancy and commercial chicken populations analyzed in this study.

| Breed | Abbreviation | Sample source | n | No. of haplotypes | Haplotype (frequency) ¹ |
|---------------------------------------|--------------|---------------|-----|-------------------|---|
| Fancy | | | | | |
| Groninger Mew | GrM | Netherlands | 7 | 3 | E1 (1), E3 (1), E7 (5), |
| Lakenvelder | Lak | Netherlands | 6 | 2 | C1 (2), E1 (4) |
| Drente fowl | DrF | Netherlands | 7 | 1 | E1 (7) |
| Assendelft fowl | AsF | Netherlands | 8 | 1 | E1 (8), |
| Frisian fowl | FrF | Netherlands | 5 | 2 | A3 (2), E1 (3) |
| Hamburgh | Ham | Netherlands | 6 | 2 | E2 (2), E7 (4) |
| Polish bearded | PoB | Netherlands | 6 | 1 | E1 (6) |
| Dutch owl-bearded | DoB | Netherlands | 6 | 1 | E1 (6) |
| Polish non-bearded | PnB | Netherlands | 7 | 1 | E1 (7) |
| Breda fowl | BrF | Netherlands | 8 | 3 | A2 (2), D1(3), E4 (3) |
| Brabanter | Bra | Netherlands | 8 | 3 | E1 (5), E3 (1), E5 (2) |
| Dutch Bantam | DuB | Netherlands | 7 | 3 | A1 (1), E1 (5), E11 (1) |
| Booted bantam | BoB | Netherlands | 8 | 2 | C1 (4),E1 (4) |
| Barnevelder | Bar | Netherlands | 8 | 1 | E1 (8) |
| Holland fowl | HoF | Netherlands | 8 | 1 | E1 (8) |
| Kraienkoppe | Kra | Netherlands | 8 | 1 | E1 (8) |
| All fancy | | | 113 | 12 | A1 (1), A2 (2), A3 (2), C1 (6), D1 (3), E1 (80), E2 (2), E3 (2), E4 (3), E5 (2), E7 (9), E11 (1) |
| Commercial | | | | | |
| Broiler Male line 1 | BM1 | Company A | 15 | 4 | B1 (2), E1 (1), E5 (11), E9 (1) |
| Broiler Female line 2 | BF2 | Company A | 15 | 4 | B1 (1), E1 (1), E5 (12), E6 (1) |
| Broiler Male line 3 | BM3 | Company B | 21 | 4 | A4 (4), B1 (7), E1 (2), E5 (8) |
| Broiler Female line 4 | BF4 | Company B | 42 | 4 | A4 (2), B1 (5), E1 (2), E5 (33) |
| Brown-Egg Layer | BE | | 51 | 7 | A1 (5), A2 (7), A5 (1), E1 (9), E3 (5), E8 (4), E10 (20) |
| White-Egg Layer | WE | | 16 | 4 | A2 (1), E7 (7), E8 (1), E12 (7) |
| Broilers line 1 and 2 combined | B1-2 | | 30 | 5 | B1 (3), E1 (2), E5 (23), E6 (1), E9 (1) |
| Broilers line 3 and 4 combined | B3-4 | | 63 | 4 | A4 (6), B1 (12), E1 (4), E5 (41) |
| Combined broiler Male lines (1 & 3) | BM1-3 | | 36 | 5 | A4 (4), B1 (9), E1 (3), E5 (19), E9 (1) |
| Combined Broiler Female lines (2 & 4) | BF2-4 | | 57 | 5 | A4 (2), B1 (6), E1 (3), E5 (45), E6 (1) |
| All Broiler | B | | 93 | 6 | A4 (6), B1 (15), E1 (6), E5 (64), E6 (1), E9 (1) |
| All Layer | L | | 67 | 9 | A1 (5), A2 (8), A5 (1), E1 (9), E3 (5), E7 (7), E8 (5), E10 (20), E12 (7) |
| All commercial | Com | | 160 | 14 | A1 (5), A2 (8), A4 (6), A5 (1), B1 (15), E1 (15), E3 (5), E5 (64), E6 (1), E7 (7), E8 (5), E9 (1), E10 (20), E12 (7) |
| Fancy plus commercial | Fancy-Com | | 273 | 20 | A1 (6), A2 (10), A3 (2), A4 (6), A5 (1), B1 (15), C1 (6), D1 (3), E1 (95), E2 (2), E3 (7), E4 (3), E5 (66), E6 (1), E7 (16), E8 (5), E9 (1), E10 (20), E11 (1), E12 (7) |

¹Figures in parenthesis indicate the frequency of that particular haplotype

Genetic diversity and population structuring

Haplotype and nucleotide diversities were calculated using ARLEQUIN version 3.1 (Excoffier *et al.* 2006). All 113 sequences of the Dutch breeds were only considered in 1 group according to

Table 2 Diversity of Dutch fancy and commercial populations of chicken and distribution of individuals in the clades

| Breed ¹ | Haplotype diversity (± SE) | Nucleotide diversity (± SE) | Distribution of individuals in each clade, n (%) ² | | | | |
|--------------------|-------------------------------|--------------------------------|---|---------|--------|--------|----------|
| | | | A | B | C | D | E |
| Fancy | | | | | | | |
| GrM | 0.524 (0.209) | 0.0021 (0.002) | | | | | 8 (100) |
| Lak | 0.533 (0.172) | 0.0101 (0.007) | | | 2 (33) | | 4 (67) |
| DrF | 0.000 | 0.000 | | | | | 8 (100) |
| AsF | 0.000 | 0.000 | | | | | 8 (100) |
| FrF | 0.600 (0.175) | 0.0098 (0.007) | 2 (40) | | | | 3 (60) |
| Ham | 0.533 (0.172) | 0.0029 (0.003) | | | | | 8 (100) |
| PoB | 0.000 | 0.000 | | | | | 8 (100) |
| DoB | 0.000 | 0.000 | | | | | 8 (100) |
| PnB | 0.000 | 0.000 | | | | | 8 (100) |
| BrF | 0.750 (0.097) | 0.0198 (0.012) | 2 (25) | | | 3 (38) | 3 (38) |
| Bra | 0.607 (0.164) | 0.0018 (0.002) | | | | | 8 (100) |
| DuB | 0.524 (0.209) | 0.0067 (0.005) | 1 (14) | | | | 6 (86) |
| BoB | 0.571 (0.095) | 0.0109 (0.007) | | | 4 (50) | | 4 (50) |
| Bar | 0.000 | 0.000 | | | | | 8 (100) |
| HoF | 0.000 | 0.000 | | | | | 8 (100) |
| Kra | 0.000 | 0.000 | | | | | 8 (100) |
| All Fancy | 0.490 (0.057) | 0.0063 (0.003) | 5 (4) | | 6 (5) | 3 (3) | 99 (88) |
| Commercial | | | | | | | |
| BM1 | 0.467 (0.148) | 0.0067 (0.004) | | 2 (13) | | | 13 (87) |
| BF2 | 0.371 (0.153) | 0.0042 (0.003) | | 1 (7) | | | 14 (93) |
| BM3 | 0.733 (0.052) | 0.0149 (0.008) | 4 (19) | 7 (33) | | | 10 (48) |
| BF4 | 0.373 (0.089) | 0.0073 (0.004) | 2 (5) | 5 (12) | | | 35 (83) |
| BE | 0.786 (0.039) | 0.0113 (0.006) | 13 (25) | | | | 38 (75) |
| WE | 0.650 (0.075) | 0.0059 (0.003) | 1 (6) | | | | 15 (94) |
| B1- 2 | 0.409 (0.108) | 0.0053 (0.003) | | 3 (10) | | | 27 (90) |
| B3-4 | 0.536 (0.062) | 0.0110 (0.006) | 6 (10) | 12 (19) | | | 45 (69) |
| BM1-3 | 0.657 (0.063) | 0.0134 (0.007) | 4 (11) | 9 (25) | | | 23 (64) |
| BF2-4 | 0.368 (0.077) | 0.0061 (0.004) | 2 (3) | 6 (11) | | | 49 (86) |
| B | 0.497 (0.056) | 0.0102 (0.005) | 6 (7) | 15 (16) | | | 72 (77) |
| L | 0.853 (0.024) | 0.0113 (0.006) | 14 (21) | | | | 53 (79) |
| Com | 0.801 (0.026) | 0.0112 (0.006) | 20 (13) | 15 (9) | | | 125 (78) |
| Fancy-Com | 0.807 (0.016) | 0.0092 (0.005) | 25 (9) | 15 (6) | 6 (2) | 3 (1) | 224 (82) |

¹Breed: Abbreviations for breeds are given in Table 1.

² n (%): Number of individuals in the clade, figures in parenthesis indicate percentage of individuals in the clade out of the total number of samples for the breed.

their original populations where as the 160 sequences of commercial chickens were grouped into different sub-populations to study the extent of genetic structuring in the populations. Analysis of molecular variance (AMOVA) was computed and variance components were estimated among and within the population groups.

Results

Sequence variation and haplotype sharing

A total of 20 haplotypes (HM015602-21) were identified in this study (Supplemental Files Table S1). Twelve haplotypes were identified in the fancy breeds, while 14 haplotypes were identified in the commercial chickens. In total 31 substitutions (2 of which were transversions) were detected. Information on the samples and the distribution of haplotypes are shown in Table 1. Six dominant haplotypes (A2, B1, E1, E5, E7 and E10) were present in 222 chickens (of 273). Except haplotypes E1 and E7, less than 3 individuals of fancy chickens shared haplotypes with commercial chickens. Six haplotypes found in 16 samples (of 113), were specific to fancy chickens, while 8 haplotypes, found in 56 chickens (of 160), were unique to commercial samples (Figure 1).

Table 3 AMOVA analysis of Dutch fancy and commercial populations of chicken based on mtDNA-control region variation

| Group (G) | Source of variation (%) | | |
|--|-------------------------|---------------------------------|--------------------|
| | Among groups | Among populations within groups | Within populations |
| Fancy ¹ - no grouping | 27.47 (0.000) | | 72.53 (0.000) |
| Commercial ² - no grouping | 19.95 (0.000) | | 80.05 (0.000) |
| Fancy versus commercial | 5.81 (0.065) | 18.07 (0.000) | 76.11 (0.000) |
| Broiler versus layer (G1 = BM1-3, BF2-4; G2 = BE, WE) | 11.30 (0.329) | 11.48 (0.000) | 77.19 (0.000) |
| Broiler males versus broiler females (G1 = BM1, BM3; G2 = BF2, BF4) | 2.35 (0.319) | 11.15 (0.022) | 86.50 (0.003) |
| Broilers from company A versus broilers from company B (G1 = BM1, BF2; G2 = BM3, BF4) | -2.94 (0.647) | 14.85 (0.006) | 88.09 (0.004) |

¹The sub-populations were formed of the 16 fancy breeds (see Table 1).

²The sub-populations were formed of BM1-3, BF2-4, BM1, BF2, BM3, BF4, BE, WE (see Table 1 for abbreviations).

Note: Significance tests were based on 1023 permutations, P values are indicated in parentheses.

bantam (A1) and 2 Breda fowl animals (A2). Haplotypes A4 and A5 are uniquely found in this study. The broiler haplotype (B1) and haplotype C1, containing Lakenvelder and Booted bantam animals are the same as haplotype B1 and C6 described by Liu *et al.* (2006). Haplotype E1, the major E-clade haplotype was also described by Liu *et al.* (2006). All the other haplotypes in the clade were distributed around it with one mutation difference, except for E9, E10 and E11 which were separated by 2 differences from E1 (Figure 3). The haplotypes E3, E5, E7, E8, E10 and E12 correspond to haplotypes E3, E6, E8, E9, E11 and E15 described by Liu *et al.* (2006).

Eight of the haplotypes identified in this study were not found by Liu *et al.* (2006). Four haplotypes out of these 8 belonged to 3 fancy breeds (D1 and E4 found in 6 Breda fowls, E2 found in 2 Hamburg fowls, and E11 found in 1 Dutch bantam). The other four novel haplotypes were

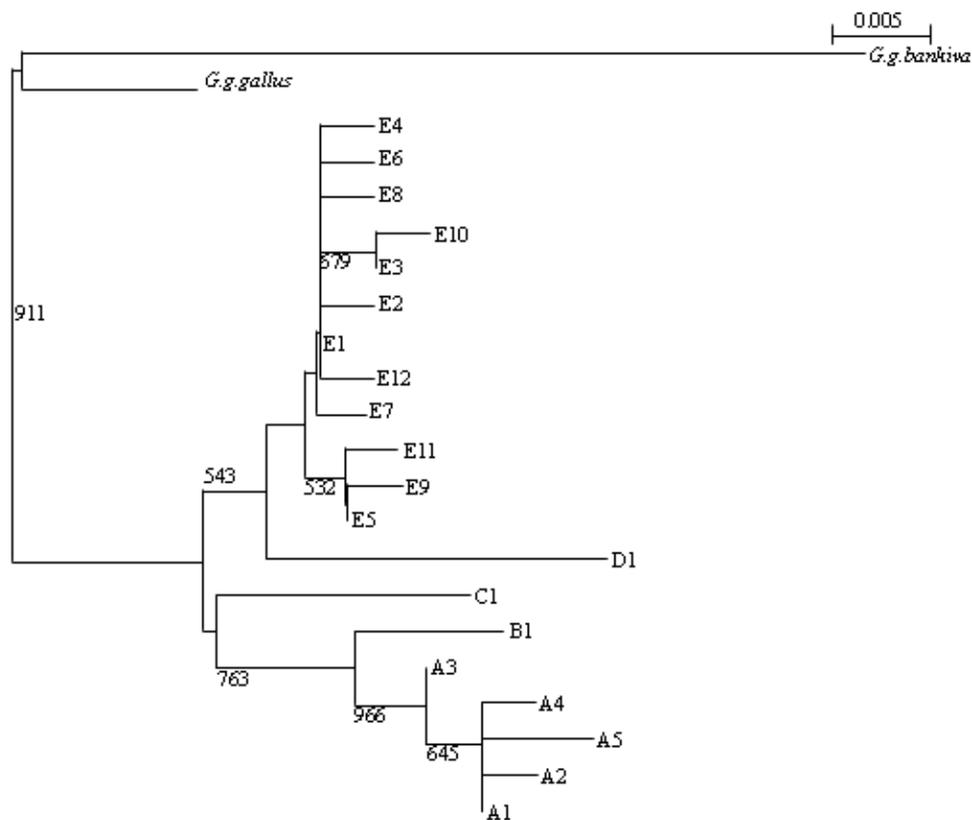


Figure 2 A neighbour-joining tree for the 20 haplotypes of mtDNA control-region sequences of 113 Dutch fancy and 160 commercial chickens. The CLUSTALX package was used to draw the tree applying 1000 bootstrap replications to test reliability of the branching order. The red jungle fowls (*G.g.bankiva*, AB007718 and *G.g.gallus*, AB007720) were used as out groups to root the tree using NJPLOT95. Only bootstrap values greater than 50% are presented.

found in commercial breeds (A4 in four individuals from broiler male line 3, and two individuals from broiler female line 4; A5 found in 1 brown-egg layer; E6 and E9 found in 1 individual each from broiler female line 2 and broiler male line 1).

Genetic diversity

Eight (of 16) fancy breeds belonged to a single haplotype, E1. Equal proportions of the remaining 8 breeds have 2 and 3 haplotypes. The haplotype diversity in these breeds ranged from 0.52 (Groninger Mew and Dutch bantam) to 0.75 (Breda fowl) while the nucleotide diversity values ranged between 0.0018 (Brabanter) and 0.0198 (Breda fowl). Each of the commercial breeds belonged to 4 haplotypes, except the brown-egg layers which belonged to 7 (Table 1). The haplotype and nucleotide diversities of commercial breeds ranged, respectively, from 0.37-0.78 and 0.0042-0.0149. All fancy chickens considered together showed haplotype diversity of 0.49 and nucleotide diversity of 0.0063; the respective figures for the commercial populations were 0.80 and 0.0112 (Table 2).

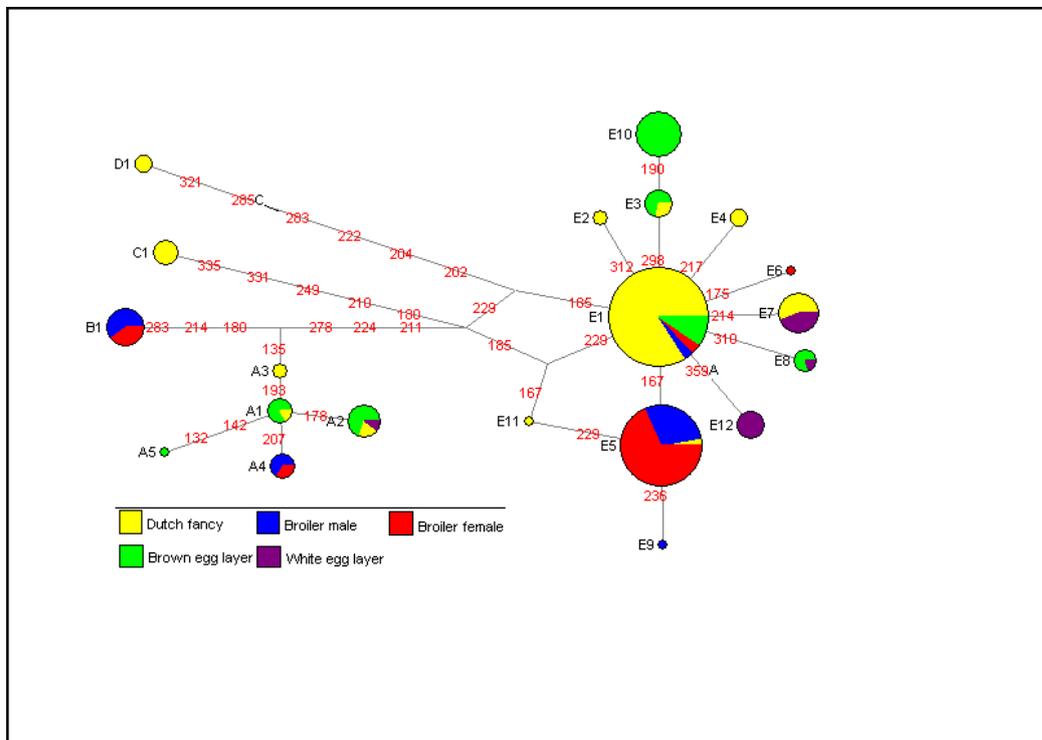


Figure 3 Median-joining network among 20 mtDNA haplotypes of Dutch fancy and commercial breeds of chicken based on 365 bp of control-region sequences, regions 30-394 of the reference sequence, *G. g. domesticus*, X52392 (Desjardins & Morais 1990). Suffixes A and C to the nucleotide positions 359 and 285, respectively, designate transversions. Circle areas are proportional to haplotype frequencies.

Population structure

Estimates of a global AMOVA for Dutch fancy chickens, treating all 16 breeds as one group, indicated that 72.5% ($P < 0.0001$) of the genetic variance was due to variation within populations. At the same time, the diversity among breeds (27.5%) was also found to be highly significant ($P <$

0.0001). Likewise, significant genetic variation was observed both within (80%, $P < 0.0001$) and among (20%, $P < 0.0001$) the breeds in commercial populations, when all breeds were treated as a single group. The variation among the fancy and commercial populations (5.8%) was non significant ($P > 0.05$). When commercial populations were considered separately and grouped as: broilers versus layers and broiler males versus broiler females, only 11.3% ($P > 0.05$) of the variation among broilers and layers; and 2.4% ($P > 0.05$) of the variation among broiler males and broiler females could be attributed to the genetic differences among the groups. Further grouping of the broilers by the source of the samples, as broilers from company A versus those from B, indicated that genetic differences due to company groups were confounded by large error of estimations (Table 3). In general, the diversity among breeds was larger for fancy populations compared to commercial populations. However, there exists higher level of diversity within commercial breeds than in fancy breeds.

Discussion

This study presents a systematic survey of mitochondrial haplotype diversity of a nearly all recognized traditional breeds of chicken of The Netherlands. The breeds studied vary in background. Some are thought to originate from country fowl, while others have a known or putative history of contributions of chicken from other parts of Europe or even from other continents. Some fancy breeds such as the Breda fowl, Booted bantam, Dutch owl-bearded, Polish bearded, Polish non-bearded and Hamburg are thought to be relatively old, featuring in 16th and 17th century paintings, while other Dutch breeds were created in the 20th century to meet new demands for commercial egg and meat production (Supplemental Files Text S1). Despite the diverse backgrounds of Dutch breeds, the common mitochondrial theme is the E-clade, and specifically the E1 haplotype; all breeds have E-clade haplotypes, and only the Breda fowl and the Hamburg do not have the E1 haplotype. This clade is the same clade (clade E) described by Liu *et al.* (2006) that mainly contained chickens from Europe, the Middle East and India. Considering the distribution patterns of the clade and the high proportion of unique haplotypes in India, Liu *et al.* (2006) suggested that the origin of this clade might be the Indian subcontinent. The consistent occurrence of this clade in the entire traditional chicken diversity of a Western European country suggests that European chicken are originally mostly if not exclusively derived from the Indian subcontinent.

Apart from the consistent occurrence of the E-clade in Dutch fancy breeds, there is a sporadic occurrence of a few other clades, with only a few of the occurrences being shared between breeds. Three breeds had clade A haplotypes, with none of the haplotypes overlapping between breeds.

Haplotypes in clade A were found before among large numbers of Chinese (109) and Japanese (16), and a small number of European (2), and Iranian (2) chickens (Liu *et al.* 2006). Two Dutch breeds (Lakenvelder and Booted bantam) shared the same clade-C (C1) haplotype, a group of haplotypes found so far only in Chinese and Japanese chickens. Clade D, found only in Breda Fowl, was clustered in Liu *et al.* clade F which exclusively contained samples from Yunnan, South China in that study. The erratic occurrence of East-Asian haplotypes in Western European breeds suggests that there is no ancient occurrence of these haplotypes that would have likely created a more uniform distribution. Rather, the irregular distribution of these haplotypes appears much more the product of the happenstance availability of exotic chicken in combination with the whims and fancies of chicken breeders in past centuries.

As with the Dutch fancy breeds, the dominant haplotypes of the commercial chicken are in the E-clade. All commercial breeds originated in Europe or North America, and particularly the broilers were thought to have a very high proportion of Asian contributions to their origin. However, from the mitochondrial signatures, even the broilers appear to be mostly descending from European chicken. Nevertheless, the pattern of E-clade diversity in commercial birds is much more complex; the broilers have mostly E5, in the brown egg layers E10 is dominant, and in the white egg layers E7 and E12 make up virtually all the variation. The E-clade is nowadays occurring throughout the globe, no doubt largely due to recent translocations by European traders. However, there may be a more ancient dispersal of the E-clade for instance into Asia that needs further investigation, and that makes a precise origin of the commercial chicken breeds currently difficult to ascertain.

Like in the Dutch fancy breeds, East-Asian haplotypes can be found in the commercial breeds. But unlike in the Dutch fancy breeds, the pattern of occurrence of these haplotypes is much more consistent. The A1 and A2 haplotypes are found mostly in the brown egg layers (and a single occurrence in a white egg layer), and are shared with a few Dutch breeds. The occurrence of these A-clade haplotypes, which are found at high frequency in Chinese chicken, is in line with the historical records that suggest a contribution of Cochins and Langshans to brown layers. A unique A-clade haplotype, A4, occurs only in broilers, and interestingly only in broilers of one company, both in dam and sire lines, suggesting crossbreeding of what are considered to be distinct breeds, which is supported by SNP markers (Megens *et al.* 2009), during the history of these commercial lines. The A4 haplotype appears therefore to be a rare occurrence in the broilers, and in these commercial chickens the B-clade, specifically the B1 haplotype that is occurring at high frequency also in China and Japan, appears to be the common theme. This indicates introgression of East-Asian chicken of a very different source compared to the brown egg layers

and Rhode Island Red, despite the historical records indicating a common origin from similar dual purpose breeds.

The occurrence of mitochondrial haplotypes that are mostly East-Asian in distribution in Western European traditional breeds and commercial broilers and layers confirms historical records that indicated such influences. The occurrence of many different clades indicates that many different Asian breeds may have been used. Although East Asian haplotypes constitute a minority in most populations surveyed here, the per cent contribution of East Asian chicken to current Dutch and commercial chicken populations is difficult to establish from mtDNA alone.

The influence of the introgression on genetic diversity becomes apparent from the nucleotide diversity and haplotype diversity analyses. All populations that had haplotypes of multiple origin displayed high inferred diversity as opposed to most populations that had only a single origin mitochondrial signature (mostly the E1 haplotype). Interestingly, most fancy breeds have been shown to have a much lower within population diversity compared to broilers and layers based on nuclear markers (Eding *et al.* 2006), but this is not the case for mitochondrial estimates in fancy breeds that have multiple origin haplotypes. The results of AMOVA showed that there was genetic differentiation among Dutch fancy breeds and also among commercial breeds. The ragged mismatch distribution curve of fancy and commercial populations also supports the presence of population subdivision and the heterogeneity between populations (Supplemental Files Table S2, Figure S2).

Understanding the complex origin of chicken populations is of vital importance for understanding, preserving, and exploiting chicken genetic diversity. A complex origin, in this study shown to occur in broilers, brown egg layers, and several Dutch traditional fancy breeds, may influence nuclear haplotype diversity, linkage disequilibrium, and hence affects all mapping and association analyses including genomic selection.

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Text S1 History of Dutch fancy breeds used in this study

Groninger Mew (gold pencilled)

This is pencilled breed that existed for centuries in north-western Europe. At the same time, this type of breed was also common in the Groninger areas of northern Netherlands. The breed existed in north-western Groninger areas and in the adjoining German territory. They exhibited no apparent difference with a breed known as East Friesian Mew that existed across the German border. The hens lay white shelled eggs that weighed 55-60 g. Their body weight ranged from 1600 to 2100 g.

Lakenvelder

The earliest history of the Lakenvelder documented in 1727 shows that this breed was kept around the hamlets of Lakervelt for eggs and meat. The birds have a white sheet draped over a black body, having a colour pattern that also occurs in cattle, pigs and goats. In 1835 the Lakenvelders are found in Westfalen, Germany. They have a clear white body between the deep velvet-shining black hackle and tail. Lakenvelders produce white eggs that weigh on average 55 g. Their body weight ranged from 1400 to 2000 g.

Drente fowl (partridge; pile)

The Drente Fowl is present in the Drente province in the north-east of the Netherlands, since the 17th century. But it was close to extinction by the beginning of the 20th century. Later they increased in number and were used by a few commercial poultry farms after the Second World War. These fowls lay white shelled eggs weighting about 55 g. Their body weight ranged from 1300 to 1900 g.

Assendelft fowl (yellow pencilled)

The Assendelft Fowl is a rare breed which has been used for eggs in the province of Noord Holland, in the northwest part of the Netherlands. In the middle of the 20th century Assendelft fowls were transported to England and used for breeding Hamburgs. Morphologically it is highly similar with the Friesian Fowl. Assendelft birds weigh from 1200 to 1700 g.

Friesian fowl (silver pencilled)

Friesian fowls have small size and their feather pattern varied between silver pencilled, red mottled and yellow-white pencilled. The silver pencilled fowls are very common in Friesland (north Netherlands) as well as all over the northwest Europe. In the 18th century cockfighting was

popular in northern Netherlands. Thus, the 'biting-cocks' emerged later by crossing Friesian fowls with Orloffs or Malays. They lay white-shelled eggs weighing between 50 and 55 g. Their weight ranged from 1200 to 1600 g.

Hamburgh (gold pencilled; silver spangled)

This breed consists of three strains with three colourings of different origin in the Netherlands, Germany and England. The pencilled colourings might have originated in the Netherlands. Old Dutch paintings showed that the pencilled Hamburgs existed in the Netherlands by about 1625. The silver spangled Hamburgs were the result of latter breeding efforts and the different colour varieties that followed carried blood of Minorcas and Sumatras. They weigh from 1200 to 2500 g and are ideal as show birds.

Polish bearded (buff-laced; frizzled silver)

These are fowls with beards and crest. The first Polish arrived in England in 1835. In England, Australia and USA they are called Polish and a distinction is made between fowl with and without beards. Descriptions of the fowls with a crest and beard existed as early as 1600, known as Patavonians. Crested fowls with and without beards were found in many of the old paintings since 1600s by Albert Cuyp (1620-1691), Malchior d'Hondecoeter (1636-1695) and Jan Steen (1626-1679). The frizzled silver fowls lay white shelled eggs and weighed from 1500 to 2500 g.

Dutch owl-bearded (black bearded white)

This is one of the ancient breeds depicted in the 16th century Dutch paintings. It is black bearded with white feathers. The breed was close to extinction around 1900. It was crossed with the bearded Thuringian fowl to increase its population size although it still remained small in number. These fowls lay white shelled eggs weighing about 55 g and their body weight ranged from 1600 to 2500 g.

Polish non-bearded (white crested black; black crested white)

This breed appears both as white crested black and black crested white though the latter are less popular. The source of Dutch crested fowls is believed to be southeast Russia. They were introduced to the Netherlands probably in the 16th century when the Dutch traded with Russia. Crested fowls with or without beard were depicted in the paintings of many famous Dutch painters in the 17th century.

Breda fowl (cuckoo)

The Bredas are one of the ancient fowls of the Netherlands. They are large birds found in the town of Breda, in the southern part of the Netherlands, and surroundings. Portraits from 350 years ago displayed birds that resembled the present day Bredas. The Bredas originated from crested fowls and latter crossed with Chinese Langshans and Cuckoo Malines to produce more meat. They lay white-shelled eggs which weigh 55-60 g. Their weight ranges from 1750-3000 g.

Brabanter (black bearded-buff)

The ancestors of this breed were said to have been brought from Persia by Dutch sailors. They have beards in three parts and a very small closed tuft of feathers. Like the other crested fowls the Brabanter has notably large nostrils. Brabanters lay white shelled eggs and their body weights ranged from 1500 to 2500 g.

Dutch bantam (partridge; females- light and dark partridge; golden duck wings)

The Dutch Bantams, formerly known as Partridge Bantams, are the ancestors of Dutch and German Bantams found in areas of Netherlands adjoining Germany. Part of their ancestry is said to come from Japanese and English Game Bantams. For a long time the Dutch Bantams were kept by rural fanciers only for fun and later considered as a standard breed in 1906. They are one of the smallest bantams laying near white shelled eggs weighing about 30-35g.

Booted bantam (non bearded mille fleur; bearded citron mille fleur)

This is a very old ornamental and true bantam breed originating in East Asia. It descends from a small group of Asiatic bantam breeds that produced the clean legged Japanese bantams and other varieties with feathered legs. Paintings by Adrian van Utrecht show that the booted bantam was already known in the Netherlands as early as the 16th century. There are also other rare varieties of booted bantam with a beard. Like the beardless varieties their legs are heavily feathered and have vulture hocks. Booted bantams only weighed from 600 to 800 g.

Barnevelder (dark)

This breed is named after the town Barneveld in the province of Gelderland in the central eastern part of the Netherlands. The Barnevelder is at present a popular exhibition breed with wide and deep shape and beautiful colours. Since 1850 farm chickens from Barneveld and the surroundings were crossed with Cochins for dark brown eggs. Around 1855 these crossings were mated with partridge Brahma's where as latter around 1900 Langshans were also used for crossing. The Langshan, a much older pure breed, is particularly believed to have large influence on the

Barnevelder. They are large fowls weighing 2500 to 3500 g and laying dark brown eggs.

Kraienkoppe (silver partridge)

The Kraienkoppe was originated in the middle of the 19th century on both sides of the Dutch-German border, around Enscheda, eastern Netherlands. The ancestors of this breed include Malays, silver partridge Leghorns and country fowls. They were first exhibited in Enscheda in 1892 as ‘Twente Grays’ having a silver partridge colour, though at present they also exist in partridge colour and renamed as ‘Twente Fowls’. They lay large eggs with yellow-brown colour and weigh from 2000 to 2500 g.

Reference

Palthe A.W. van Wulfften & Simons P.C.M. (1992) C.S.van Gink’s Poultry Paintings 1890-1968. pp. 1- 136. Published by Dutch Branch of the World’s Poultry Association, Beekbergen, The Netherlands.

Table S1 Names and accession numbers of haplotypes defined by Liu *et al.*

(2006) that were used to compare with the haplotypes observed in this study

| Haplotype name | Accession number | Reference | Corresponding haplotype found in this study |
|----------------|------------------|--------------------------|---|
| A1-A5 | HM015602-06 | This study | |
| B1 | HM015607 | This study | |
| C1 | HM015608 | This study | |
| D1 | HM015609 | This study | |
| E1-E12 | HM015610-21 | This study | |
| LiuA1 | AY392372 | Liu <i>et al.</i> (2006) | A1 |
| LiuA2 | AF512321 | Liu <i>et al.</i> (2006) | A2 |
| LiuA7 | AF512201 | Liu <i>et al.</i> (2006) | A3 |
| LiuB1 | AF512088 | Liu <i>et al.</i> (2006) | B1 |
| LiuC6 | AB009427 | Liu <i>et al.</i> (2006) | C1 |
| LiuD1 | AY588636 | Liu <i>et al.</i> (2006) | - |
| LiuE1 | AY392269 | Liu <i>et al.</i> (2006) | E1 |
| LiuE3 | AF128318 | Liu <i>et al.</i> (2006) | E3 |
| LiuE6 | AY392310 | Liu <i>et al.</i> (2006) | E5 |
| LiuE8 | AY704696 | Liu <i>et al.</i> (2006) | E7 |
| LiuE9 | AY392336 | Liu <i>et al.</i> (2006) | E8 |
| LiuE11 | AY645015 | Liu <i>et al.</i> (2006) | E10 |
| LiuE15 | AY704712 | Liu <i>et al.</i> (2006) | E12 |
| LiuF1 | AF512285 | Liu <i>et al.</i> (2006) | - |
| LiuG1 | AF512288 | Liu <i>et al.</i> (2006) | - |

Reference

Liu Yi-Ping, Wu G-S., Yao Y-G., Miao Y-W., Luikart G., Baig M., Beja-Pereira A., Ding Z-L., Palanichamy M. G. & Zhang Y-P. (2006) Multiple maternal origins of chickens: out of the Asian jungles. *Molecular Phylogenetics and Evolution* **38**, 12-19.

Table S2 Summary statistics of Dutch fancy and commercial populations of chicken, broilers and layers showing raggedness and Fu's F_s values

| Population | Raggedness, r (95% CI) ¹ | P (r) | F_s (95% CI) ² | P (F_s) |
|------------|---------------------------------------|-----------|-----------------------------|-------------|
| Fancy | 0.093 (0.02-0.29) | 0.000 | 0.365 (-3.35-5.13) | 0.448 |
| Commercial | 0.098 (0.02-0.28) | 0.000 | 0.441 (-3.47-5.30) | 0.424 |
| Broiler | 0.089 (0.02-0.27) | 0.000 | 0.383 (-3.68-5.10) | 0.447 |
| Layer | 0.093 (0.02-0.30) | 0.000 | 0.368 (-3.39-4.90) | 0.429 |

¹Raggedness, r (95% CI): raggedness statistic (Rogers & Harpending 1992).

² F_s (95% CI): Fu's F_s statistic (Fu 1997).

The confidence intervals (CI) and significance levels (P) for the two statistics (r and F_s) were computed using coalescent simulations provided in DNASP version 5 (Librado & Rozas 2009).

References

Fu X-Y. (1997) Statistical tests of neutrality of mutations against population growth, hitchhiking and background selection. *Genetics* **147**, 915-25.

Librado P. & Rozas J. (2009) *DNASP* version 5: A software for comprehensive analysis of DNA polymorphism data. Available at: <http://www2.ub.es/dnasp> (last accessed on 18 December 2009).

Rogers A.R. & Harpending H. (1992) Population growth curves in the distribution of pairwise genetic differences. *Molecular Biology and Evolution* **9**, 552-69.

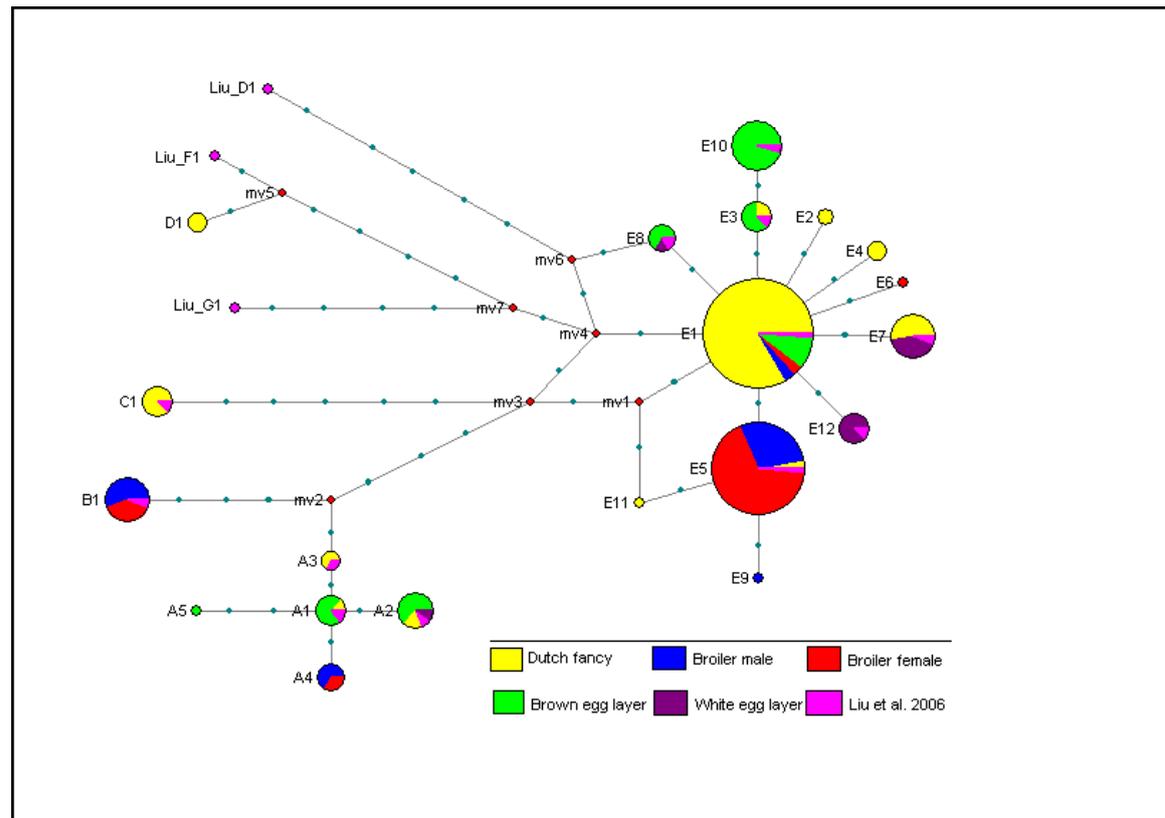


Figure S1 Median-Joining network of haplotypes observed in this study and haplotypes of this study observed in domestic chicken haplotypes of Liu *et al.* (2006). Circle areas are proportional to haplotype frequencies. Dots on the lines denote the number of mutations separating the haplotypes.

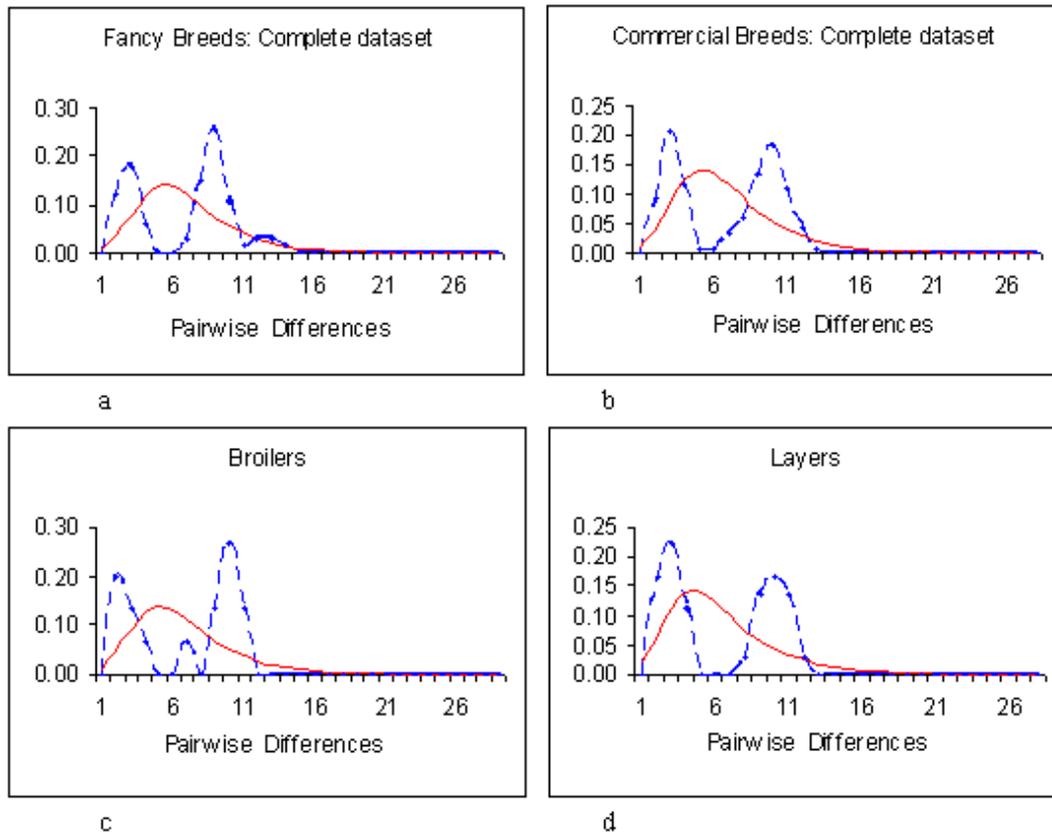


Figure S2 Mismatch distribution for mtDNA types of Dutch fancy (a) and commercial (b) populations and broiler (c) and layer (d) breeds. The red solid lines in each figure denote the expected values for population expansion.

7

General discussion

Introduction

The first sections of this final chapter present the roles of chicken production in Ethiopia and the needs and methods for characterizing indigenous chicken genetic resources. Procedures for setting priorities for conservation of indigenous breeds and the roles of farmers and government were described. It examines the critical steps in developing breeding programs for improving village chickens and advises on implementation procedures. The lessons learned from the ongoing breeding program were also incorporated. The final section of this chapter elaborates issues to be considered in setting up a sustainable genetic improvement program utilizing indigenous chickens.

Role of village chicken in Ethiopia

The role of local chickens to households of rural communities in developing countries is well recognized. This is evident from the fact that indigenous chicken comprise from 70-95% of the national chicken flocks in Africa and Asia. In Ethiopia they comprise about 98% of the national poultry flock (CSA, 2005). Rearing chickens is a simple means of earning cash income and chickens are often the only assets of rural women. They serve as cheap sources of protein in many developing countries. The contribution of local flocks to overall chicken meat and egg production in Ethiopia reaches up to 90% (Alemu, 1995).

Genetic resources: characterization and conservation

Indigenous chickens of the tropics are important reservoirs of useful genes and possess a number of adaptive traits (Horst, 1989). However, they are generally poor producers of meat and eggs. As a result they are losing their functions in the villages. Introduction of exotic genetic material continues to be seen as a solution to low productivity of local breeds even in areas where the exotic genotypes are poorly adapted. Consequently local breeds have often been diluted by indiscriminate cross breeding with imported stock (FAO, 2007) and in many countries are even replaced with modern breeds (Besbes, 2009). The genetic erosion of these local breeds may lead to the loss of valuable genetic variability in specific characteristics. This reflects a need to conserving indigenous breeds having unique genetic features and values for sustainable utilization.

Characterization

Identification and characterization of the existing genetic diversity is an important step for making decisions on conservation and sustainable utilization of the resources. Different phenotypic and genetic techniques can be used to characterize the biodiversity of chickens: morphological traits, phenotypic performance, protein polymorphisms, immunogenetic markers and molecular markers. Current strategies for assessing farm animal biodiversity are based on molecular markers. Molecular markers are useful to assess genetic and evolutionary relationships among populations

and to explore parentage within populations. Although there are different classes of molecular markers microsatellites are the primary marker class of choice for chicken biodiversity studies (Hanotte and Jianlin, 2005; Simianer, 2007). Their high mutation rate and co-dominant nature permit the estimation of within and between breed genetic diversity, and genetic admixture among breeds even if they are closely related. In the current study microsatellite markers were used to assess the diversity in Ethiopian chickens, the results indicating that most (97%) of the genetic variation in the populations was ascribed to the with-in breed/population diversity (Chapter 4) which has been shaped by subtle combinations of human and natural selection. The problem with microsatellite markers, however, is that they only indicate neutral diversity and do not provide information on functional trait diversity. Single nucleotide polymorphism is a new molecular marker system which offers opportunities to assess the genetic diversity in farm animal species. They could be the marker of choice for diversity studies in the future because they can be used in assessing either neutral or functional variation (FAO, 2007).

Documentation of existing genetic resources is a very crucial component of breed conservation. Documentation should not be limited to molecular information. It should include the description of the population sizes and phenotypic characteristics of breeds, their economic performance, any special traits they may have, and their cultural/historical importance. Characterizing populations also in these terms (Chapters 2, 3 and 5) is quite important both from utilization and conservation perspectives.

Conservation priorities

It is unrealistic and unnecessary to consider all populations for conservation. To establish preservation measures of chicken genetic resources, priorities need to be defined. A working hypothesis to estimate the value of a breed or population is that potentially important genetic resources are those which are characterized by unique genetic features (Weigend and Romanov, 2001). The more genetically distant a breed or population is the more likely it is to carry unique genetic features. The degree of genetic uniqueness is determined based on measurements of between population distances using genetic markers. Gibson, *et al.* cited in Hanotte and Jianlin (2005) suggested two criteria to select priority breeds for conservation using molecular information: breeds with the largest within-breed diversity and/or the ones which contribute to maximize the conservation of between-breed diversity.

However, estimating the value of a breed or population entirely based on its genetic uniqueness does not give the full picture of the real value the breeds/populations have at different levels (livestock keeper, community, national, global) and the different functions of livestock. Evaluation of breed should also include its total economic value which comprises direct use values (food, fertilizer, hide, socio-cultural), indirect use values (benefits deriving from ecosystem

functions), option and quasi-option values (insurance, future use), bequest value (benefit accruing to any individual from the knowledge that others might benefit from a resource in the future), and existence value (the satisfaction of knowing that a particular asset exists) (Hiemstra *et al.*, 2006). In the current study we found that chickens have dominantly direct use value, chiefly as sources of eggs and meat for consumption, and to a very little extent an option value, in all geographic regions and farming communities (Chapter 2).

Therefore, decisions on conservation priorities should not be made only based on molecular information as it does not take in to account the economic value of a breed. Molecular markers can serve as an important initial guide to evaluate breeds as genetic resources based on their genetic uniqueness. Decisions on selecting breeds for conservation have to consider a range of other criteria including the degree of endangerment, adaptation to a specific environment, possession of traits of current or future economic importance or scientific interest, and the cultural or historical value of the breed (Ruane, 1999). Among these criteria the degree of endangerment is probably the most important factor in conservation decisions. Regardless of their genetic uniqueness, breeds of large, stable population size should not be prioritized for conservation action (Ruane, 1999). Our study based on microsatellite markers indicated absence of genetic uniqueness between the Ethiopian chicken populations (Chapter 4). However, this information should be integrated with the criteria described above and with the information on morphological traits (Chapter 3) and productive performances of the populations to guide decision makers on prioritizing breeds for conservation. We only analyzed production performance of one population (Chapter 5). Certainly, it will be much useful to collect phenotypic information on more populations to assess the variation in performance characteristics across breeds/populations. Since direct use value (as food) is the chief economic value of village chickens of Ethiopia local breeds that produce more meat and eggs could have a better chance of being in the priority list relative to the less productive ones, given that they stand favorably with regards to the other criteria.

Methods of conservation

Theoretically, three types of conservation measures can be implemented: *in situ* conservation, *ex situ in vivo* conservation and *ex situ in vitro* conservation. There is often inconsistent use of these terminologies in different reports. The definitions used in the FAO report (FAO, 2007) are adopted here. Accordingly, *in situ* conservation refers to conservation of livestock through continued use by livestock keepers in the production environment in which the livestock evolved or are now normally found and bred. *Ex situ in vivo* conservation refers to conservation through maintenance of live animal populations not kept under normal management conditions (e.g. zoological parks and in some cases governmental farms) and/or outside of the area in which they evolved or are now normally found. *Ex situ in vitro* conservation refers to conservation external to

the living animal in an artificial environment, under cryogenic conditions including the cryoconservation of embryos, semen, oocytes, somatic cells or tissues having the potential to reconstitute live animals (including animals for gene introgression and synthetic breeds) at a later date.

Government institutes and research centers are the most important players of conservation activities in Ethiopia. Well-established genebanks are present for *ex situ in vitro* conservation of plant and forest germplasms which has been initiated by the government several decades ago under the Institute of Biodiversity Conservation (IBD). Although the livestock component has been included recently it is not very active at the moment particularly in conserving local chicken populations.

In Ethiopia the government is also involved in poultry breeding activities through its research centers. Research centers own nucleus farms and sell breeding stock to small farmers. Not all types of conservation strategies are capable of safeguarding all values of a breed or population described above (Hiemstra *et al.*, 2006). Neither the IBD nor research centers are capable of undertaking all types of conservation schemes. IBD does not have the facility and manpower to maintain animals and thus should focus on a strategy that can not generate direct use value, viz. *ex situ in vitro* conservation. On the contrary, research centers which have no germplasm storage facilities but capable to rear animals. As a result they are appropriate for *ex situ in vivo* conservation, which can safeguard the direct use value of chickens.

The ideal method of conservation is *in situ* or on-farm conservation by the farmers. However, this can not be sustained unless it brings adequate economic benefits to the livelihoods of the farming communities. Hiemstra *et al.* (2006) summarized the needs for integrating different approaches to support conservation of animal genetic resources: ‘Given the constant evolution of agricultural production and marketing, *in situ* or on-farm management of genetic resources should include the genetic improvement of the animal genetic resource so as to ensure its competitiveness as a future livelihood option. Given the uncertainties about *in situ* and on-farm conservation, it is prudent to give serious consideration to other options of conservation (*ex situ in vivo* or *in vitro*) as complementary approaches’. The current breeding program described in this thesis is initiated recognizing the need to improve local breed in order to conserve it as well as improving its egg and meat production level. The ultimate goal is to develop a genetic improvement scheme using a locally adapted population of chickens (Chapter 5). This will increase competitiveness of local breeds in the rural settings and at the same time will serve as an *ex situ in vivo* conservation scheme complementing other conservation strategies. In the next paragraph various aspects of such a scheme are discussed.

Design of breeding program for village chickens

Animal breeding is one of the formal instruments for a country's animal genetic resources development and genetic improvement. The major tasks of a breeding program are genetic improvement and dissemination of improved material to end users. The important starting points for developing successful breeding programs in developing countries include: describing the production system, identifying the production objectives and breeding goals of village farmers (FAO, 2010). Our study on poultry production systems indicated that village production environments in Ethiopia are low input systems with high disease and predation risk (Chapter 2). Chickens are raised importantly as sources of eggs and meat (for home consumption) and income. Farmers defined the breeding goal that reflected the village circumstances and their production objectives: to develop chicken breeds that can adapt well to the local environment, heavy and produce more eggs primarily for home consumption and sale in the local markets.

Defining the breeding goal includes giving each goal trait a value, the weighted summation of which could be used to derive the aggregate genotype to be improved. The values used for weighing traits are generally called economic values or economic weights, or simply goal values (Groen, 2003). Valuation of traits of indigenous animal genetic resources depends on the context of the production system and must consider economic and non-market attributes as well (Romano, 1999). Here we used the approach presented by Solomon *et al.* (2009) for weighing traits in the breeding goal based on farmers' preferences. In this approach relative weights for breeding goal traits were derived from farmers' ratings of traits they desired to be improved. Although the current study is only limited to identification and weighting of trait categories these values can be used to develop a desired-gain-selection index to achieve a desired amount of genetic gain in selected traits (Solomon *et al.*, 2009). Similarly, simple mathematical models are available (see ICAR/FAO, 2000a; ICAR/FAO, 2000b) that can be used to directly derive the goal values from the weights assigned to each trait.

The other important steps in developing a successful breeding program include developing data recording and collection system, breeding value estimation, selecting parents for the next generation and developing a system for mating selected individuals, and designing a structure to disseminate the genetic progress created in the breeding population into the production population (Bijma *et al.*, 2005).

The structure of data recording system in breeding programs depends on the species and the traits in the breeding goal. Collection of phenotypic information on animals and their relatives at village level is not feasible for most of the traits in chickens reared under free-range systems. Measurements on disease resistance (which involves daily follow-up and recording of clinical observations on disease signs and symptoms observed in sick birds, macroscopic postmortem examinations, histopathology etc.) require special equipment and expertise and as a result can not

be handled under village circumstances. Likewise, collection of data on pedigree information and egg production, and reproduction traits such as hatchability requires specific farms for individual recording. Small flock size, uncontrolled mating, absence of housing and recording are characteristic features of village poultry production systems in Ethiopia (Chapter 2). Under such circumstances it is suggested that genetic improvement programs should be centrally organized in a population maintained in government farms or research centers under nucleus breeding schemes (Kosgey *et al.*, 2006) although this requires long term commitment. A closed nucleus breeding scheme could be a more appropriate strategy considering the problem of obtaining reliable pedigree information outside nucleus farms which is required for running open nucleus schemes. Village-based selection schemes where the breeding activities are carried out by the smallholder communities are being used for genetic improvement of local sheep breeds (Solomon *et al.*, 2009; Tadelle *et al.*, 2010). However, like with open nucleus schemes this approach will also not be easy to implement in chicken breeding because of the difficulty to establish a reliable data recording system. Due to these reasons the genetic improvement program initiated in this study is a closed nucleus scheme utilizing one of the locally adapted breeds.

The breeding methods normally considered for tropical conditions include purebred selection for improving local breeds, substitution of local breeds by other local breeds or exotic breeds and using cross breeding systems. The classical decision diagram that provides the favorable breeding method based on breed differences and expected heterosis effects is not suitable for making decisions in village conditions. According to Solkner *et al.* (1998), the major reasons for this are that the decision diagram does not include the definition of a breeding objective and the comparative evaluation of different genotypes under village environments does not exist and as a result, the main input parameters for the decision diagram are missing. Under Ethiopian circumstances, both selection and cross breeding could be more appropriate over breed substitution. Improving local breeds through selection may even be advantageous over cross breeding. Selection within local breeds will allow continuous genetic improvement. However, the genetic progress of selective breeding is slow and may not be compatible with the fast growing demand for food. Progress is generally obtained within a shorter time period using cross breeding. A cross breeding scheme could be designed utilizing selectively bred pure local breeds with exotic strains. This may be used to achieve heterosis for fitness traits and complementarity for other traits. However, evaluating the potential of different breeds under village conditions in relation to the overall breeding objectives is important for deciding on the type of breeding scheme.

Maintenance of genetic variation is a condition for continuous genetic improvement. In the absence of selection genetic variation is lost by genetic drift and gained by mutation, and thus the minimum population size to maintain genetic variation is a function of the mutation rate (Hill, 2000). However, in populations undergoing selection there is a reduction in genetic variance due

to selection (Bulmer effect). To avoid long-term loss of genetic diversity under selection schemes an upper limit shall be set to the level of inbreeding since the loss of genetic variation within a breed is related to the rate of inbreeding (ΔF). The acceptable level of inbreeding to prevent serious deleterious effects is about 0.01 (van Arendonk and Bijma, 2003). Woolliams *et al.* (1999) and Woolliams and Bijma (2000) developed a general theory to predict rates of inbreeding in populations undergoing selection. This approach facilitates a deterministic optimization of short and long-term response in breeding schemes.

Currently, a software (SelAction) is available (Ruten *et al.*, 2002), which can be used to predict the rate of inbreeding by deterministic simulation of selection schemes based on the long term genetic contribution theory of Bijma *et al.* (2001). The predicted rate of inbreeding can be used to calculate the effective population size, N_e . Constraining inbreeding is an important element of breeding schemes. Meuwissen (1997) developed a dynamic selection tool which maximizes genetic gain while restricting the rate of inbreeding. The method allows the selection of a group of parents from a given set of selection candidates, in which the genetic merit is maximized while the average coefficient of coancestry is constrained. Alternative breeding schemes can be judged by comparing their selection response at the same rate of inbreeding. The scheme with the highest selection response at the same rate of inbreeding is the best scheme (Dekkers *et al.*, 2004).

Genetic progress created in the breeding program can be disseminated to village farms in the form of day old chicks, or fertile hatching eggs. Multiplication of improved breeds is an important prerequisite for dissemination of genetic superiority in the breeding population to end users. In industrial poultry breeding multipliers constitute a separate tier in the breeding structure operating on profit basis and selling their products as hybrid offspring for table egg or broiler producers. In Ethiopia, multiplication of poultry breeds (exclusively modern breeds) was being carried out by government farms established in many regions across the country under the Ministry of Agriculture. The multiplication centers sold either fertile eggs directly for the surrounding farmers for hatching using broody hens or 2-3 months old chickens to the extension services and NGOs for large scale distribution. However, this system was generally found to be inefficient and demonstrated poor impact in village poultry development. As a result during the past few years these farms were privatized to operate commercial farming. Hence, it is vital to devise appropriate mechanisms for multiplying and disseminating improved breeds.

Currently the two well recognized models to improve village poultry production are the Bangladesh and Kuroiler models. The Bangladesh poultry development model is a micro-credit based model emphasizing on the entire supply chain. The beneficiaries are specialized in different activities ranging from vaccination and medication supply, rearing of (crossbred) chicks of

different age classes, fertile egg production, hatching, or feed supply. Some of the reasons for the success of the program are that the different activities of the beneficiaries are tightly integrated and inter-dependent and the actors in all levels profit from their activity. Constraint of the program in Bangladesh was that there are insufficient numbers of high quality chickens produced that are able to withstand the local environments (Dolberg *et al.*, 2002; Fakhru Islam and Jabbar, 2005).

The Kuroiler is a dual purpose breed for village production developed by Kegg farm, a commercial company in India. The Kuroiler model gives a novel approach for integrating breeding programs to rural households and could serve as an example for developing similar programs in Ethiopia. Perhaps the most important starting point for the success of the Kuroiler breeding program was that it was based on adequate understanding of the village production environment and the needs of farmers. This was reflected by the fact that the Kuroiler breed has retained many of the desirable features of indigenous chickens such as hardiness, feather colors, ability to escape predators and disease resistance. As a result the breed could easily fit into the village environment and was readily adopted by smallholder farmers. The strategy used to disseminate the breed was also the other key reason for the success of the breeding program over rural households. This was done by developing effective organizational structures reaching out to the end users, supplying complimentary inputs along with the breed, providing technical back stopping and designing viable financial arrangements (Ahuja *et al.*, 2008). The program was able to maintain the involvement of rural farmers by involving them in the supply and sales chain.

Implementation of the breeding program

Genetic improvement must bring positive benefits to the village farmers both in the short and long term. However, it is only one component of village poultry improvement. Other aspects such as improving housing, feeding, health care, supply links, marketing of products and training of farmers are necessary and require the involvement of multiple stakeholders. In Ethiopia breeding schemes for improving village chickens are part of the government's initiative for improving food security. The government has been involved in designing the breeding program. As a result, it makes important decisions in the genetic improvement scheme including use of superior stock. Therefore, the first key step before implementing the breeding scheme is to communicate the plan to the responsible government bodies and make it an integral part of the national livestock development plan. Developing viable forms of cooperation between the village community, advisory service staff and decision makers at different levels is very important for the success of the breeding program (Solkner *et al.*, 1998).

Effective management of the breeding program is the other important issue for the

success of the scheme. This includes adequate planning of infrastructure and manpower for recording, data management and processing. It should be planned with due consideration of available resources and facilities. Appropriate facilities should be available for accurate data recording. In the current study data on egg production of individual animals were recorded from birds housed in groups on deep litter floor equipped with trap nests (Chapter 5). Battery cage equipped with automatic egg weighing and counting devices for individual performance recording has been the commonly used system in industrial breeding programs. This system is no longer acceptable mainly due to concerns over animal well being. Moreover, recent studies showed that individual performance in breeding farms is not a good indicator for group performance of hens in production farms because the social interactions within birds in a group effect individual variations in egg laying performance (Burel *et al.*, 2002). The method of housing used in this study is advantageous in these regards. However, its major drawback is that it has high labor requirement since each hen in the nest has to be removed manually. Besides increased labor cost, manual operation of trap nests has an additional drawback. In this study we observed that laying hens in the trap nests are often overlooked by farm attendants and stay locked inside for a long period without feed and water, causing considerable stress on the hens and affecting their performance negatively. Using an automatic registration system can overcome this problem. Burel *et al.* (2002) presented a system that automatically registers and recognizes individual egg laying performance of hens housed in groups. Other methods such as the Funnel Nest Box (FNB) and Electronic Pope Hole (EPH) have also been developed for automatic recording of individual performance in group housing system. Thurner *et al.* (2006) evaluated the FNB method and found it to be a reliable system for the recording of individual performance of hens in floor management.

However, these technologies are generally very expensive and complicated even for commercial breeding companies and difficult to implement under the circumstances in developing countries. More importantly, selection of individuals in nucleus farms based on individually recorded traits might give rise to G-E interaction because under normal production circumstances chickens are reared in pens where traits such as egg production and feed intake are measured at group level. Recently, Biscarini *et al.* (2008, 2010) have presented a novel approach that might help to overcome this problem. They developed a method to estimate breeding values of individuals based on phenotypic information pooled over hens housed in groups. This work showed that estimates based on pooled records can be used effectively. This opens ways to include information collected at group level under village conditions. This approach is cost effective and simple to adopt, and has a promising application in the nucleus breeding schemes.

The other important issue is that models used for analyzing phenotypic information of hens housed in groups should take into account effects due to competition within groups. Interaction among individuals makes a significant contribution to heritable variation (Bijma *et al.*,

2007a). Bijma *et al.* (2007b) presented statistical methodology that enables the design of selection programs to effectively reduce competitive interactions. Disregarding the associative effects in the model could lead to biased genetic parameters and reductions of genetic progress. The associative genetic effects could not be estimated in the present breeding experiment due to the limited data.

Finally, a breeding program has to be implemented in a cost effective way. It is necessary to cut unnecessary costs at all levels of implementing the scheme. For instance, removing excess male animals from the breeding population as early as possible contributes to cost reduction. This can be done by sexing day old chicks. Although there are simple methods for sexing day old chicks of most commercial cross breeds by using plumage color this is not applicable for local chickens. However, the traditional vent sexing technique can be employed to sex day old local chickens by giving proper training to the technicians working in the breeding program. Lack of expertise in vent sexing in the current work resulted in the need for keeping all chickens including large number of unwanted males until sexual differentiation is physically visible. This takes at least 8 weeks causing unnecessary strains of measurements and increased labor and feed cost as well as housing space. Traits to be selected for should represent the breeding goal defined by village farmers. This is very important to achieve genetic improvement in the desired direction. In addition, recording involves cost and measurements on traits that are not relevant to the breeding goal will impose unnecessary cost on the breeding program.

Challenges to the breeding program

One of the potential problems of nucleus breeding schemes could be genotype-environment interaction since the selection and breeding activity is carried out under a production system that is different from village environment. Although it is difficult to emulate the village production system it is important to consider the possibility of modifying the rearing environment in the nucleus farm. For instance, the housing system could be modified to provide outdoor runs where the birds are allowed to maintain their scavenging and flighty behaviors, which are important to survival in village environments. Adjusting the nutritional level under which the chickens are selected in the nucleus farm to a certain standard that could reflect the level afforded under village conditions might also be considered. However, the challenge here is that the scavenging resource base in the village environments is highly seasonal, fluctuating both in terms of quality and quantity and can not support increased egg production without supplementary feeding (Kitalyi, 1998). This means any breeding scheme will require improved feeding in the villages (not for the data recording, but in the ultimate production birds); otherwise there will not be any improvement under village circumstances. If feed intake remains to be the limiting factor in villages, as it is

currently, then the only improvement in egg number that can be made is due to increased feed efficiency.

In any case, the presence of genotype-environment interaction should be tested by evaluating the breeds in village farms. If strong genotype-environment interactions occur performance testing for breeding value estimation under village situations will be favored relative to testing under on-station conditions (Bijma *et al.*, 2005). The opportunities to record performance in villages should be explored anticipating the problem.

The other challenge with a closed nucleus scheme is that it does not allow introduction of new animals in to the nucleus population. The only source of new variations in such populations is by mutations. Mutational variations are, however, small (about 0.1 to 0.5% of the environmental variance) and are mostly detrimental with respect to fitness (Falconer and MacKay, 1996). To overcome loss of variation in the breeding population the founder population in the nucleus scheme should begin with an adequate sized sample of animals that should ideally be unrelated, non-inbred and fertile. It is necessary to maintain a sufficient effective population size by ensuring that as many animals as possible contribute to the next generation. Moreover, the contribution of mutation as a source of new variation in closed populations is not irrelevant in the long term as long as the effective population size is large enough (larger than ~50) (Piter Bijma, *Personal Communication*).

From the breeding experiment in this study (Chapter 5) we observed that ensuring larger sized sample of animals in the founder population may not be straight forward in local chickens and needs extra caution. Many practical aspects should be considered in determining the number of eggs required to hatch and establish founder animals. The standards for calculating hatchability percentage, proportion of quality chicks hatched, and rates of mortality at different ages for local chickens are outside the ranges recognized for commercial breeds. Decisions on the number of eggs to be purchased should account for poor quality eggs collected from villages that are not suitable for incubation (about 10%), low hatchability of eggs (about 50%), and relatively large proportion of poor quality chicks discarded after hatching (3-4%). Although under standard circumstances hatchability of eggs from indigenous chickens could be quite high (79%, Lemlem and Tesfay, 2010) it should be noted that eggs purchased in large numbers from village markets are diverse in quality mainly due to the varying storage conditions and duration of storage. Farmers particularly with small number of laying hens need to collect eggs over a long period of time, often more than 2 or 3 weeks (the recommended practice for hatching eggs being storage under cold environment for a maximum of 7 to 12 days), to get sufficient number for selling. Finally, the potential number of animals that could survive to sexual maturity and contribute offspring should be calculated. This study showed that about 32% of the animals housed at the starting phase died during the first 16 weeks and 29% of those in the layer house died during the

first 44 weeks of laying period (Chapter 5). And yet not all hens that survived contributed offspring to the next generation. Ignoring hens that only laid between 1 and 10 eggs, which accounted for about 4%, more than 13% of hens that survived did not lay at all during the 44 weeks of laying period (Chapter 5).

In addition to the number of eggs sampled the method of sampling animals to establish the breeding population is very important. Eggs should be purchased with considerable care so as to capture the actual diversity in the local breed chosen for genetic improvement. It could be difficult to assure this because retailers and small traders often bring eggs from many different sources to village market sheds. Therefore, the eggs should be purchased from primary producer-sellers in the villages by making a brief interview on the history of the farmer's flock. However, the most preferred approach is to buy from individual households at the farm gate making first hand assessment on the breed composition of the farmers' flock.

Sustainability of village chicken breeding programs

In developing countries, genetic interventions have commonly not been either effective or sustained. A symposium conducted jointly by the 7th World Congress on Genetics Applied to Livestock Production and FAO developed specific recommendations for establishing sustainable animal breeding programs. In the recommendations it was noted that livestock development interventions that disregard requirements of animal genetic resources used in the system are inconsistent with sustainable production systems and livelihoods; and the broad range in production systems within and across countries and regions requires unique rather than globally-uniform genetic material to sustain human livelihoods (INRA and CIRAD, 2002). Past experiences in poultry breeding in Ethiopia provide clear evidence supporting these opinions. Poultry breeding had been carried out in Ethiopia for the last many decades starting 1950's using exotic breeds to improve village chickens (Alemu, 1995). The technical reasons for failure of these efforts were that the decisions on breed choice were not based on the needs of the farmers and did not adequately consider the circumstances of village production environments (Teklewold *et al.*, 2006). Moreover, improving village poultry productivity can not be achieved by transferring a single technological innovation. Improving the production conditions gives the basic environment for utilization of an improved genetic stock to its genetic potential. Production levels could be increased only when the genetic superiority of the new breed is expressed. Extra benefit obtained from increased production levels increases profitability and encourages farmers to keep and manage the new breeds better, there by contributing to sustaining the breeding program.

Successful establishment of genetic improvement action depends particularly on availability of human resource and institutional capacity and the involvement of stakeholders (INRA and CIRAD, 2002). Both the government and donors play important role in establishing a

breeding program for improving village chickens. However, to be sustainable in the long term, a breeding program should be financially independent. Financial sustainability of the breeding program could be attained by ensuring participation of local people in the supply and sales sector (Ahuja *et al.*, 2008). Failure to ensure financial sustainability is probably the most dangerous risk to the breeding program. In genetic improvement schemes where breeding animals are reared in nucleus farms dependence over external funding would result in total loss of the genetic gain over a short period of time whenever funding is disrupted.

Finally, it is necessary to encourage involvement of private sectors in breeding chickens for village systems. The Kegg farm described in the previous section provides an outstanding example on the potential role of private sectors in promoting rural poultry production (Ahuja *et al.*, 2008).

Conclusion

Characterizing livestock breeds should consider various aspects. Molecular markers do not provide adequate information for breed characterization although they can serve as an important initial guide to evaluate breeds as genetic resources based on their genetic uniqueness. A range of other criteria should be considered such as description of the population sizes and phenotypic information on the breeds, their economic performance, any special traits they may have, and their cultural/historical importance. It should also include clear definition of the production environment under which the breed has evolved and produced. Although different types of conservation measures can be implemented conservation through utilization is the most appropriate strategy to maintain the genetic diversity in indigenous chicken genetic resources. This can be realized by developing nucleus schemes for genetic improvement of locally adapted breeds. Technical aspects of designing and implementing these schemes have been discussed. To be successful the breeding schemes should be based on the needs and production objectives of the farmers. Nucleus breeding schemes require long term commitment and should be financial independent to be sustainable. Maintenance of the sustainability and involvement of farmers in the breeding schemes are key to success. By developing appropriate breeding schemes it is possible to generate genetic progress in locally adapted breeds, which provides promising opportunities to support resource poor livestock keepers.

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Summary

Chicken rearing is one of the most suitable activities to improve the livelihood of the poor. It requires small investment and it is relatively easy to improve productivity in the rural setting. Earnings from the sale of eggs and chickens are often the only sources of cash income for rural women in Ethiopia.

The indigenous chickens of Ethiopia, kept under village management systems, contribute to more than 90% of the total output. They are generally considered to have poor genetic potential for egg and meat production. However, there are populations of indigenous chickens that, unlike commercial breeds, are productive under harsh village conditions. Identifying productive populations and assessing genetic diversity within and between populations for determining breeding strategies for improving the indigenous chickens of Ethiopia were the main aims of this project.

Before being able to set up a selective breeding scheme it is essential to find out what to select for. This was achieved by characterizing village poultry production systems, production environments, and farmers' objectives for keeping chickens, and to identify factors affecting the breed choice of farmers (Chapter 2). This work included both a questionnaire survey and a participatory group discussion involving a total of 225 households in 5 geographic regions of Ethiopia. Once this was achieved, a second important factor is to characterize the local chicken ecotypes both morphologically and molecular genetically. This way the genetic differences between the local populations could be determined, and also the level of genetic diversity within the local chicken populations. This allows recognition of important and unique gene pools in local populations, important both from conservation and utilization perspective and assists in maintaining indigenous genetic diversity for current and future generations. In this project a total of 1,125 chickens from five populations of chickens, originating from different regions of Ethiopia, were studied based on qualitative and quantitative traits. This allowed describing morphological variation among the populations (Chapter 3). Blood samples from these populations were collected and genotyped using 20 microsatellite markers to determine the within- and between-population genetic diversity (Chapter 4). Partly simultaneous to this describing of genetic and phenotypic variation, eggs from a large population of chickens, the Horro, were collected and brooded to start a selection experiment. The experiment had 3 main aims: 1. to characterize the Horro chickens under controlled circumstances; 2. to create a pedigreed population that allowed first estimations of genetic parameters for some production traits that were considered of economic importance by the farmers; 3. to start selective breeding to improve these production traits of the Horro breed. This work was done on station at the poultry research farm of the

Ethiopian Institute of Agricultural Research (Chapter 5).

The assessment of farmers' objectives showed that production of eggs for consumption is the principal function of chickens in most regions, followed by the use as source of income, and meat for home consumption. The production system in all 5 geographic regions studied could be characterized as extensive scavenging management, absence of immunization programs, increased risk of exposure of birds to disease and predators, and reproduction entirely based on uncontrolled natural mating and hatching of eggs using broody hens (Chapter 2). The farmers rated the adaptive traits of indigenous chickens, in particular the superior merits of indigenous chickens to high yielding exotic breeds, as most important. Reproduction traits, such as broody behaviour and high level of hatchability, were considered very important. These are thus traits that need to be maintained while breeding for improved productivity of indigenous chickens for village conditions. The market price of chickens is primarily dictated by weight, but farmers rated growth (males) and number of eggs followed by growth (females) as the production traits they would like the most to be improved (Chapter 2).

The populations in this study carried multiple variants of plumage colours and other physical features. However, some populations had specific features. The Farta chickens in the north, for example, have a predominantly white plumage colour and crest head, and the chickens in the south and west have a prominently red body plumage and flat head. Likewise, the populations in the high altitude regions were predominantly (55% in Farta, 65% in Horro and, 66% in Sheka) characterized by yellow skin colour, a trait reflecting the adaptive fitness of birds under foraging environments (Chapter 3). Also other attributes that are important in breeding for tropical conditions have been identified such as the pea comb gene, in populations of all regions, and the naked neck gene, particularly in those of low altitude areas.

The pedigreed Horro population that was kept on station was used for estimating genetic parameters for the production traits, monthly and cumulative part period egg numbers and growth to 16 weeks of age (Chapter 5). Heritabilities of egg numbers in the first, second, third and fourth months of laying were 0.32 (± 0.13), 0.20 (± 0.16), 0.56 (± 0.15) and 0.25 (± 0.14), respectively. Heritabilities of cumulative of monthly records of egg numbers were from 0.24 ± 0.16 (for the first two months, EP12) to 0.35 ± 0.16 (over the first 6 months, EP16). Body weight at 16 weeks of age (BW16) had a genetic correlation with the cumulative of monthly records of: 0.92 (with EP12), 0.69 (with EP36) and 0.73 (with EP16). Because BW16 and EP16 were fairly strongly correlated, it could be hypothesized that chicks that are able to grow well to 16 weeks of age also will be able to lay eggs relatively well. In addition it is likely that chicks that are relatively heavy at 16 weeks of age will also be relatively heavy at adult age. Because BW16 is easy to measure and the heritability is fairly moderate (0.23 ± 0.06), it seems a good trait to consider as selection criterion as it is expected to improve both adult weight and egg production. Because the pedigreed population

was established only recently, data of only 2 generations were available for estimating these genetic parameters. The results are promising but inaccurate due to insufficient amount of data. They would need to be re-estimated when more generations have been produced and thus more data has been generated.

Results of genetic diversity analysis indicated that the variability found within a single population could explain most of the genetic diversity (97%) in Ethiopian chicken populations (Chapter 4). This suggests that not much selective breeding has been performed thus far and that there is considerable potential for genetic improvement of local chickens through selective breeding. From socio-economic assessment of village poultry producers we recommend that the breeding program should aim to develop a dual-purpose breed based on indigenous chicken genetic resources with any of the comb types other than single for all the regions studied having the most preferred white body plumage for farmers in the Amhara region and red body plumage for those in Oromia, Benshangul-Gumuz and Southern regions.

This research provides a bench mark for further improvement of the ongoing breeding program. A follow up research building on the ongoing program is recommended in order to develop pure lines of Horro chickens selected for traits identified as the most important by rural farmers which could be followed by cross breeding of improved lines of Horro chickens with exotic strains. The ultimate goal of the breeding program should be to develop a blue print for improving village poultry production by integrating breeding, marketing, and training of farmers.

Samenvatting

Pluimveehouderij is een van meest toepasselijke activiteiten om het leven van arme mensen te verbeteren. Het vereist weinig investeringen en het is relatief eenvoudig om de productiviteit te verhogen in een landelijke omgeving. Verdiensten uit de verkoop van eieren en kippen zijn vaak de enige bron van inkomsten voor plattelandsvrouwen in Ethiopië.

De oorspronkelijke kippen van Ethiopië die onder dorpsouderij omstandigheden worden gehouden, dragen meer dan 90% van de totale productie bij. Over het algemeen worden ze beschouwd als slecht genetisch potentieel voor ei en vleesproductie. Echter, er zijn populaties lokale kippen die, anders dan commerciële exotisch kippen, productief zijn onder moeilijke lokale dorpsomstandigheden. De voornaamste doelen van dit project waren het identificeren van productieve populaties, het bepalen van de genetische diversiteit binnen en tussen deze populaties en vervolgens het ontwikkelen van fokkerijstrategieën om de productiviteit van de lokale kippen van Ethiopië te verbeteren.

Voordat een fokprogramma kan worden opgezet is het essentieel om te bepalen waarop geselecteerd moet worden. Dit is bewerkstelligd door het karakteriseren van dorpskippenproductiesystemen, productieomgeving, het doel waarvoor boeren de kippen houden en wat hun keus van kippenras bepaald (Hoofdstuk 2). Dit werk omvatte zowel een enquête als een groepsdiscussie waarbij 225 huishoudens in 5 geografische gebieden van Ethiopië waren betrokken. Vervolgens is een tweede belangrijk punt het karakteriseren van de lokale kippenrassen, zowel morfologisch als moleculair genetisch. Hierdoor konden genetische verschillen tussen lokale kippenpopulaties worden vastgesteld, alsook de hoeveelheid genetische variatie binnen de populaties. Hierdoor werd het mogelijk om unieke genenpoelen te identificeren in lokale populaties, wat belangrijk is vanuit het oogpunt van zowel conservering als toepassing en bijdraagt aan het behoud van lokale rassen voor de huidige en toekomstige generaties. In dit project zijn 1.125 kippen van 5 populaties kippen uit verschillende regio's uit Ethiopië bestudeerd op kwalitatieve en kwantitatieve kenmerken. Hierdoor kon morfologische variatie tussen de populaties worden beschreven (Hoofdstuk 3). Bloedmonsters van deze populaties zijn verzameld en getypeerd voor 20 microsateliet merkers om binnen en tussen populatie genetische diversiteit te bepalen (Hoofdstuk 4). Deels tegelijkertijd met deze genetische en fenotypische beschrijving van de populaties zijn eieren van een grote populatie kippen, de Horro, verzameld en uitgedroed als start van een selectieexperiment. Het experiment had 3 doelen: 1. Het beschrijven van de Horro onder gecontroleerde omstandigheden; 2. Het creëren van een populatie met afstammingsgegevens waardoor een eerste schatting van genetische parameters mogelijk was voor productiekenmerken die van economische betekenis werden geacht door de boeren; 3. Het starten van selectie om productie van de Horro te verbeteren. Dit werk is gedaan op het pluimveeonderzoeksbedrijf van

het Ethiopian Institute of Agricultural Research (Hoofdstuk 5).

De bepaling van de doelen van de boeren liet zien dat de productie van eieren voor consumptie in de meeste regio's de meest belangrijke functie is van de kippen, gevolgd door een bron van inkomsten en vlees voor thuisconsumptie. Het productiesysteem in alle 5 geografische regio's kon worden gekarakteriseerd als extensief scharrelmanagement: afwezigheid van immunisatieprogramma's, groter risico om kippen bloot te stellen aan ziekte en roofdieren, en de reproductie volledig gebaseerd op ongecontroleerde natuurlijke paring en uitkomst van de eieren door gebruik te maken van broedse hennen (Hoofdstuk 2). De boeren scoorden het adaptief vermogen van de lokale kippen, in het bijzonder de voordelen van de lokale ten opzichte van de commerciële rassen, als belangrijkste reden om lokale kippen te houden. Reproductiekenmerken zoals broedsheid en uitkomstpercentage werden als heel belangrijk ervaren. Dit zijn daarom kenmerken die moeten worden behouden bij het verhogen van de productiviteit van deze dieren door fokkerij. De marktprijs van kippen wordt voornamelijk bepaald door gewicht, maar boeren vinden groei (hanen) en daarnaast ook aantal eieren (hennen) kenmerken die ze het liefst verbeterd zouden zien (Hoofdstuk 2).

De populaties in dit onderzoek hadden diverse verenkleuren en andere fysieke kenmerken. Sommige populaties hadden speciale kenmerken. De Farta kippen in het noorden, bijvoorbeeld, zijn overwegend wit met een puntige kop, terwijl de kippen in het zuiden en westen overwegend rode veren hadden en een platte kop. Populaties uit de hoog gelegen gebieden hadden overwegend een gele huid (55% in Farta, 65% in Horro en 66% in Sheka), een adaptief kenmerk van kippen in een scharrel omgeving (Hoofdstuk 3). Er zijn ook andere kenmerken geïdentificeerd die belangrijk zijn voor fokkerij onder tropische omstandigheden, zoals de erwtenkam in alle regio's en de naakte nek vooral in de laaglandgebieden.

De Horro populatie met afstammingsgegevens die op het proefbedrijf wordt gehouden is gebruikt voor de schatting van genetische parameters voor de productiekenmerken, maandelijkse en cumulatieve deelproductie van eieren en groei tot 16 weken (Hoofdstuk 5). Erfelijkheidsgraden voor eiproductie in de eerste, tweede, derde en vierde maand van leg waren respectievelijk 0.32 (± 0.13), 0.20 (± 0.16), 0.56 (± 0.15) en 0.25 (± 0.14). Erfelijkheidsgraden van cumulatieve of maandproductie waren 0.24 ± 0.16 (voor de eerste 2 maanden, EP12) tot 0.35 ± 0.16 (over de eerste 6 maanden, EP16). Lichaamsgewicht op 16 weken (BW16) had een genetische correlatie met cumulatieve maandproductie van 0.92 (met EP12), 0.69 (met EP36) en 0.73 (met EP16). Omdat BW16 en EP16 vrij sterk gecorreleerd waren kon gehypothetiseerd worden dat dieren die goed kunnen groeien tot 16 weken ook vrij goed in staat zijn tot eiproductie. Daarnaast is het waarschijnlijk dat de kuikens die relatief zwaar zijn op 16 weken ook relatief zwaar zullen zijn op volwassen leeftijd. Omdat BW16 makkelijk te meten is en omdat de erfelijkheidsgraad redelijk is (0.23 ± 0.06), lijkt het een goed kenmerk om als selectiecrterium te

gebruiken omdat de verwachting is dat daardoor zowel eiproductie als volwassen gewicht zullen verbeteren. Omdat de populatie met afstammingsgegevens nog maar kort geleden tot stand is gekomen konden data van maar 2 generaties worden gebruikt bij het schatten van de genetische parameters. De resultaten zijn veelbelovend maar onnauwkeurig door onvoldoende data. Ze zullen opnieuw geschat moeten worden wanneer data van meerdere generaties beschikbaar zijn.

Resultaten van de genetische diversiteitsstudie geven aan dat de variatie binnen een populatie de meeste genetische variatie verklaard (97%) in Ethiopische kippen populaties (Hoofdstuk 4). Dit suggereert dat er niet veel selectie heeft plaatsgevonden en dat er daarmee veel potentieel is voor genetische verbetering door selectie. Resultaten van de sociaal-economische analyse van lokale kippenproductie suggereren dat het fokprogramma zich zal moeten richten op de ontwikkeling van een dubbeldoel kip, gebaseerd op lokale genetische bronnen en met alle kamtypes behalve een enkele kam. Met betrekking tot verenkleur zou die wit moeten zijn voor boeren in de Amhara region en rood voor boeren in de Oromia, Benshangul-Gumuz en zuidelijke regio's.

Dit onderzoek levert een uitgangspunt voor verdere verbetering van het lopende fokprogramma. Een vervolgonderzoek voortbouwend op het lopende programma wordt aangeraden zodat de zuivere lijn Horro kippen verder kan worden geselecteerd voor kenmerken die als belangrijk worden aangemerkt door lokale boeren. Het fokprogramma kan uitgebreid worden met kruisingen met commerciële exotische lijnen. Het uiteindelijke doel van het fokprogramma is om een richtlijn te ontwikkelen om lokale kippenproductie te verbeteren door fokkerij, marketing en training van boeren te integreren.

About the author

Nigussie Dana was born in Sidamo, Ethiopia. He completed his Bsc in animal science at Alemaya University of Agriculture (now Haramaya University) in 1987. He began his career as a graduate assistant at Debre Zeit Agricultural Research Center (DZARC) in 1992. He holds his Msc in biology with specialization in livestock systems from the Swedish University of Agricultural Sciences in 1999. He joined back DZARC, which was then transferred to the Ethiopian Institute of Agricultural Research. Nigussie served at various positions and capacities in the animal science department and poultry research program of the institute. He started his PhD study in 2006 with a particular research interest to develop a breeding program for indigenous chicken genetic resources of Ethiopia.

List of Publications (2006-2010)

Journals

- Dana, N., Hendrik-Jan Megens, H. J., Crooijmans, R.P.M.A., Hanotte, O., Mwacharo, J., Groenen, M.A.M. and van Arendonk, J.A.M., 2010. East Asian contributions to Dutch traditional and western commercial chickens inferred from mtDNA analysis, *Animal Genetics (in press)*.
- Dana, N., vander Waaij, L. H., Dessie, T. and van Arendonk, J.A.M., 2010. Production objectives and trait preferences of village poultry producers of Ethiopia: implications for designing breeding schemes utilizing indigenous chicken genetic resources, *Tropical Animal Health and Production*, 42, 1519-1529.
- Dana, N., Dessie, T., vander Waaij, L.H. and van Arendonk, J.A.M., 2010. Morphological features of indigenous chicken populations of Ethiopia, *Animal Genetic Resources*, 46, 11-23
- Dana, N., vander Waaij, E.H. and van Arendonk, J.A.M., 2010. Genetic and phenotypic parameter estimates for body weights and egg production in Horro chicken of Ethiopia, *Tropical Animal Health and Production*, DOI: 10.1007/s11250-010-9649-4
- Duguma, R., Dana, N. and Yami, A., 2006. Marek's disease vaccination opened the door to rear indigenous chickens of Ethiopia under confined management, *International Journal of Applied Research in Veterinary Medicine*, 4(2), 121-127
- Dana, N., Duguma, R., Teklewold, H. and Aliye, S., 2006. Transforming village poultry systems into small agro-business ventures: a partnership model for the transfer of livestock technologies in Ethiopia. *Livestock Research for Rural Development*, 18(169), <http://www.lrrd.org/lrrd18/12/dana18169.htm>
- Teklewold, H., Dadi, L., Yami, A. and Dana, N., 2006. Determinants of adoption of poultry technology: a double hurdle approach, *Livestock Research for Rural Development*, 18(3), (<http://www.cipav.org.co/lrrd/lrrd18/3/tek118040.htm>)

Conference Proceedings

- Dana, N., vander Waaij, E.H. and van Arendonk, J.A.M., 2010. Genetic parameter estimates for body weights in Horro chicken of Ethiopia, Poster presented in the *proceedings of the 9th World Congress for Genetics Applied to Livestock Production*, August 6-11, 2010, Leipzig, Germany
- Dana, N., vander Waaij, E.H. and van Arendonk, J.A.M., 2010. Heritabilities and genetic and phenotypic correlations for egg production traits in Horro chicken of Ethiopia, Poster to be presented in the *proceedings of the 5th Conference of the All African Society of Animal Production*, October 25-28, 2010, Addis Ababa, Ethiopia

Dana, N., Duguma, R., Teklewold, H., vander Waaij, E.H. and van Arendonk, J.A.M., 2008. Use of value chain approaches for transforming village poultry systems in Ethiopia, *Proceedings of an International Conference for improved competitiveness in poultry production for Africa*, 5-9 May 2008, Sally, Senegal.

Dana, N., de Haan, N., Minae, S., GuerneBleich, E. and Dessie, T., 2008. Mapping of poultry value chains in Ethiopia. In: *Proceedings of the 16th annual conference of the Ethiopian Society of Animal Production*, October 8-10, 2008. A CD-ROM.

Research Reports

Teklewold, H., Dadi, L., Yami, A. and Dana, N., 2006. Adopting Poultry breeds in the highlands of Ethiopia. Ethiopian Institute of Agricultural Research. *Research Report 65*. Addis Ababa, Ethiopia, 26 pp

Duguma, R., Yami, A. and Dana, N., 2006. Health Management of local chickens under confinement. Ethiopian Institute of Agricultural Research. *Research Report 69*. Addis Ababa, Ethiopia

Submitted

Dessie, T., Dana, N., Ayalew, W. and Hanotte, O., 2010. Current state of knowledge on indigenous chicken genetic resources of the tropics: domestication, distribution and documentation of information on the genetic resources. *World's Poultry Science Journal*.

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| Training and Supervision Plan | | Graduate School WIAS |
|--------------------------------------|---|-----------------------------|
| Name PhD student | Nigussie Dana Mullu | |
| Project title | Quantitative genetic analysis of production traits to support breeding programs utilizing local chicken | |
| Group | Animal Breeding and Genomics Centre | |
| Daily supervisor(s) | Dr. E.H. van der Waaij | |
| Supervisor(s) | Prof. Dr. Johan A..M. van Arendonk | |
| Project term | From September 2006 | Until Sep. 2010 |
| Submitted | September 20, 2010 | final |



| | |
|--|-----------|
| The Basic Package (3 credits) | credits * |
| WIAS Introduction Course | 1.5 |
| Philosophy of science and/or ethics | 1.5 |
| Subtotal Basic Package | 3 |
| Scientific Exposure (12 credits) | credits |
| International conferences | |
| 5 th Conference of All African Society of Animal Production, 25-28 Oct, 2010, Ethiopia | 1.0 |
| 9th World Congress for Genetics Applies to Livestock Production, 6-11 Aug, 2010, Germany | 1.5 |
| International conference for improved competitiveness of poultry value chains in Africa, 5-9 May, 2008, Sally, Senegal | 1.5 |
| FAO regional workshop on socioeconomic support to livelihood of smallholder farmers by strengthening avian influenza controlling strategies, 18-20 June, 2008, Kenya | 1.0 |
| Seminars and workshops | |
| Conservation Genetics of Animal Populations, Wageningen, 21 Nov 2006, Netherlands | 0.3 |
| 15th Annual conference of Ethiopian Society of animal Production (ESAP), 4-6 Oct, 2007, Ethiopia | 1.0 |
| FAO workshop on mapping poultry value chains of Ethiopia, 11 Oct, 2007, Ethiopia | 0.3 |
| 16th annual conference of ESAP, 8-10 Oct. 2008, Ethiopia | 1.0 |
| Presentations | |
| Mapping poultry value chains of Ethiopia, ESAP, 10 Oct 2008, Ethiopia (Oral) | 1.0 |
| Use of poultry value chains for transforming village poultry systems in Ethiopia, 6 May 2008, Senegal (Oral) | 1.0 |
| Genetic parameter estimates for body weights of Horro chicken of Ethiopia, 6-11 Aug 2010, Germany (Poster) | 1.0 |
| Heritabilities and genetic and phenotypic correlations for egg production traits of Horro chicken of Ethiopia, 25-28 Oct 2010, Ethiopia (Poster) | 1.0 |
| Subtotal Scientific Exposure | 12 |

| Training and Supervision Plan | | Graduate School WIAS |
|--------------------------------------|---|-----------------------------|
| Name PhD student | Nigussie Dana Mullu | |
| Project title | Quantitative genetic analysis of production traits to support breeding programs utilizing local chicken | |
| Group | Animal Breeding and Genomics Centre | |
| Daily supervisor(s) | Dr. Liesbeth vander Waaij | |
| Supervisor(s) | Prof. Dr. Johan A..M. van Arendonk | |
| Project term | From September 2006 | Until Sep. 2010 |
| Submitted | September 20, 2010 | final |



| In-Depth Studies (23 Credits) | credits |
|---|-----------|
| <i>Disciplinary and interdisciplinary courses</i> | |
| Design of Animal Experiment | 1.0 |
| Mathematical modelling in biology | 1.5 |
| Introduction to R statistical package | 1 |
| Quantitative Genetics with a focus on selection theory, 7-11 June 2010, Netherlands | 1.5 |
| Statistics for life sciences, 1-4 June 2010, Netherlands | 1 |
| Integrated crop and livestock production, 5-23 June 2005, ICARDA, Syria | 4.5 |
| <i>MSc level courses</i> | |
| Animal Breeding and Genetics, Sept. - Oct. 2006, Wageningen, NL | 6.0 |
| Genetic Improvement of Livestock, Nov.- Dec. 2006, Wageningen, NL | 6.0 |
| Subtotal In-Depth Studies | 23 |
| Professional Skills Support Courses (4 Credits) | |
| Training workshop on research proposal writing, 28 Apr-2 May, 2005, Kenya | 2 |
| Design and Analysis of field experiments, 21-26 May 2001, Ethiopia | 1.8 |
| Subtotal Professional Skills Support Courses | 4 |
| Research Skills Training (8 Credits) | |
| Preparing own PhD research proposal (maximum 6 credits), in 2005/06 | 6.0 |
| External training period (one month or more is 2 credits) | |
| Special research assignments (apart from PhD project): grant project preparation in 2009, Ethiopia | 1.5 |
| Subtotal Research Skills Training | 8 |
| Education and Training Total | 48 |

* one ECTS credit equals a study load of approximately 28 hours

Colophon

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