1st CHAPTER

General introduction

Background

Vietnam is an agricultural country with 70% of the population living in rural areas. More than 80% of the total agricultural households keep chickens (Vang, 2003 and Burgos *et al*., 2008). In 2006, the chicken population in Vietnam was estimated about 152.7 millions. The distribution ranges from 2.9 million in the Northwest to 40.6 million birds in the Red River Delta. Local chickens make up more than 70% of the country's total chicken population (Desvaux *et al*., 2008). They are mainly kept in the traditional extensive backyard/household production, representing about 94% of all poultry producers (Hong Hanh *et al*., 2007).

Chicken is the country's second most important meat source after pork (Burgos *et al*., 2008) and plays an integral role in the smallholder farming systems. They are used to meet the multiple social, economic and cultural needs of households (Epprecht, 2005 and Burgos *et al*., 2008). Vietnamese local chicken breeds are specific for particular regions and they are assumed showing specific adaptation to climate, disease, local low input and low output production system (Vang, 2003). Hence they may represent a large natural gene pool as reservoir for future breeding to meet specific objectives.

Vietnamese local chickens consist of different phenotypes kept in distinct agro-ecological zones of Vietnam, which stretches 1 650 km from North to South. Geographical isolation of the populations could result in sub-structuring through drift, mutation and divergent selective forces. However, it is not known to what degree Vietnamese local chicken populations differ. An assessment of genetic diversity using molecular markers may serve as a initial guide to identify unique and valuable genetic resources. Recently, several studies to assess genetic structure of chicken populations using molecular tools such as microsatellite markers (Hillel *et al*., 2007; Muchadeyi *et al*., 2007; Chen *et al*., 2008; Berthouly *et al.,* 2008; Granevitze *et al*., 2007; 2009 and Bodzsar *et al*., 2009) and mitochondrial DNA (Liu *et al*., 2004; Liu *et al*., 2006; Oka *et al*., 2007; Muchadeyi *et al*., 2008 and Silva *et al*., 2008) were published. An assessment of the genetic structure employing these molecular tools provides different insights into diversity within and between indigenous chicken populations. These two marker types have a different mode of inheritance. Microsatellites are autosomal markers while mitochondrial DNA is maternally inherited.

The genetic potential of indigenous chickens as a reservoir of genomic variation and major genes with relevance to improve adaptability has already been reported by several reports (Horst and Mathur, 1992; Horst, 1989; Garces and Casey, 2003 and FAO, 2007a). Understanding about the existing variation that already exists and how it can be conserved and accessed effectively needs to be gained. Romanov *et al*. (1996) suggested that local chickens might contain genes and alleles pertinent to their adaptation to particular environmental conditions and local breeding goals. Therefore, maintaining local breeds is needed to permit genetic adaptation of populations to unforeseen breeding requirements in the future and as a source of research material (Horst, 1989; Besbes, 2009 and Tixier-Boichard *et al*., 2009). The erosion of local chicken populations may be linked to the loss of valuable genetic variability and unique characteristics. The convention on biological diversity (http://www.biodiv.org) has put the need to conserve farm animal genetic diversity on the agenda. In farm animal diversity conservation, a unified approach accounting for two main roads to conservation has been established. This includes prevention of breed extinction and management of genetic diversity (Simianer, 2005).

There is a growing recognition that preservation of local chicken breeds is not only important to ensure the livelihoods of poor farmers who depend on these breeds, but their conservation is regarded as a national policy, as locally adapted chicken genetic resources could become future assets in breeding programs. Decision making in conservation requires specification of model parameters such as diversity, breed values, extinction probabilities and conservation potentials (Simianer, 2005). Beside phenotypic characterization, assessment of genetic characterization of local breeds is a prerequisite for this purpose (Wollny, 2003). Efforts should be made to preserve the important and unique characteristics that Vietnamese local chicken genetic resources possess. Genotypic characterization and conservation priorities in Vietnamese local chicken populations therefore need urgent attention.

Role of chickens in smallholder faming

In developing countries nearly all families at the village level, even the poor and landless, own poultry (Mack *et al*., 2005). Major initiatives have been undertaken to develop

poultry as a tool for rural development. Poultry constitutes an important contribution to rural household's food security and income generation (Kitalyi and Mayer, 1998; Coplan and Alders, 2005; Alders and Pym, 2010). They are used to address gender inequalities. Women have more control and decision making powers on chickens than men (Kitalyi and Mayer, 1998; Guèye, 2000). In addition, village chickens are required for special festivals and essential for many traditional ceremonies (Coplan and Alder, 2005; Alders and Pym, 2010). Furthermore, chickens show the greatest variability of population types and make an important contribution to biodiversity (Tixier-Boichard *et al*., 2009).

In Vietnam, chicken accounts for 70% of total poultry population. In 2006, the total poultry meat production (slaughtered poultry) was estimated to be 344.4 thousand tons and the number of eggs produced was 3.97 billions (GSO, 2007). Tung and Rasmussen (2005) showed that 31.7% and 17.8% of the total output of poultry production was consumed by semi-subsistence and semi-commercial poultry keepers, respectively. Poultry used as a source of protein to improve the nutrition for Vietnamese was reported by Epprecht (2005). The most important livestock-based source of income for the poorest income quintile is derived from poultry. Epprecht (2005) and Epprecht *et al*. (2007) reported that poultry accounts for about one quarter of the total household's income from livestock and further indicated that poultry serves as a 'sell-for-cash' tool for poor households.

Cuc *et al*. (2006) suggested a shift in gender ratio in chicken production due to the ownership of Vietnamese H'mong chickens by women. A similar observation was found by Burgos *et al*. (2008) who recognized the importance of Vietnamese poultry for children and women. The Vietnamese local chicken breeds also are an essential part of cultural and social activities (Vang, 2003 and FAO, 2009), for example Ho chicken are used for entertainment in religious celebrations and Ac and H'mong chicken are used for traditional medical purposes.

Chicken genetic diversity and assessment of genetic diversity

In developing countries, it had been widely assumed that local chickens have adapted to their local production systems which often are characterised by a limited supply of

resources and a lack of proper management program. They may present a diverse gene pool that could comprise unique genetic features. Some information on the genetic make-up of local chickens was reported (Horst, 1989). Thereby, their major genes with important effects on tropical oriented breeding already proved for their special utility in the tropics, such as dwarf (Dw), naked neck (Na), frizzle (E), silky (H), non-inhibitor (Id), fibro-melanosis (Fm), pea comb (P), blue shell (O) and slow feathering (K).

A wide diversity of indigenous chicken breeds in the tropics could form the basis for genetic improvement and diversification to produce more productive breeds adapted to specific environments and requirements (Horst, 1989). Therefore, the estimation of genetic diversity of the local chickens should be carried out to support conservation strategies and utilisations of their performance values.

Microsatellites and mitochondrial DNA (mtDNA) sequences have already proved to be useful for assessing genetic variability, while single nucleotide polymorphisms (SNPs) are becoming more and more popular due to their very high density and availability of high throughput genotyping techniques. Microsatellites are tandem repeats in the genomic DNA with very short (1-5bp) simple sequence motifs, and hence they are autosomally inherited (Tautz, 1989). Major advantages of these highly polymorphic markers are their locus specificity, abundance and random distribution over the genome, co-dominant inheritance, ease and speed of their application and suitability for semi-automated analysis (Weigend and Romanov, 2001). Unlike microsatellite markers, mtDNA is maternally inherited. The mtDNA is a circular molecule of 16 785 bp in size (Desjardins and Morais, 1990). The displacement loop (D-loop) region of the mtDNA contains elements that control the replication of the molecule and is highly polymorphic. MtDNA is used to infer regions of domestication and to identify the number of maternal lineages and their geographic origins (FAO, 2007b).

A combination of these two markers is a complementary approach that combines the highly polymorphic microsatellites whose high mutation rates allow for small-scale resolution of more recent demographic events with mtDNA which shed light on phylogeographic events dating further back in time (Feulner *et al*., 2004). An assessment of genetic structure based on these two markers with different modes of inheritance provides more insights into the evolutionary forces shaping genetic diversity.

Total genetic diversity includes within and between breed diversity. Genetic diversity within a breed can be estimated by the number of alleles, the expected heterozygosity (Frankham *et al*., 2002) and marker estimated kinships within a breed (Eding and Meuwissen, 2001). Genetic diversity between breeds can be assessed by various measures. A parameter for assessing diversity between breeds is the genetic differentiation or fixation indices which reveal the partitioning of genetic diversity (Wright, 1969). A wide range of studies for the assessment of genetic diversity were conducted using genetic distances (Nei, 1972 and Reynolds *et al*., 1983). A unified approach to assess genetic diversity within and between populations is based on marker estimated kinships (Eding and Meuwissen, 2001). Bayesian clustering approaches have been suggested for admixture analysis of different populations (Pritchard *et al*., 2000). This approach has already been proven to be useful to study the structure of populations of various farm animals (Rosenberg *et al*., 2001; Fabuel *et al*., 2004; Granevitz *et al*., 2009; Leroy *et al.,* 2009; Li and Kantanen, 2009).

Recently, assessments of genetic diversity of chicken populations using the same set of microsatellite markers suggested by FAO (2004) have been published in several studies (Cuc *et al*., 2006; Hillel *et al.,* 2007; Muchadeyi *et al*., 2007; Chen *et al*., 2008; Granevitze *et al*., 2007; 2009 and Bodzsar *et al*., 2009). Using the same markers in these studies allows unbiased comparisons. In a large scale study including 64 chicken populations from various continents and management systems, Granevitze *et al*. (2007) found considerable variation of within breed diversity (i.e., numbers of alleles/population and heterozygosity values). This variation reflected differences in population history and management. The average number of alleles and expected heterozygosity (3.6 ± 0.87 alleles and 0.51 ± 0.07, respectively) was rather lower than that observed in human (Ayub *et al*., 2003), cattle (Sodhi *et al*., 2005) and pig (Behl *et al*., 2006). Highest levels of within-population diversity were found in non-managed local populations, in some standardised breeds kept with a large population size and in some commercial broiler lines. A wide variation of within breed diversity was found for European fancy breeds. White-egg layer lines revealed the lowest level of diversity of all commercial lines. Therefore, white-egg layer lines might be considered to be in a more critical situation than other commercial lines concerning their future development, while some local breeds do represent an important reservoir of genetic diversity.

In the analysis of the population structure of a wide range of chicken breeds and lines from various continents and management systems, Granevitze *et al.* (2009) found six main clusters which were related to their geographical origins and histories. These six main clusters were formed by 1) brown egg layers, 2) predominantly broilers, 3) native Chinese breeds or breeds with recent Asian origin, 4) predominantly breeds of European derivation, 5) populations with no known history, and 6) the other populations shared their genome with some clusters defined as "Multi-clusters". Within a country, different observations on population structure were revealed. Chen *et al.* (2008) and Bodzsar *et al.* (2009) found sub-structuring in Chinese and Hungarian chicken breeds, respectively, while Zimbabwean chicken populations do not exhibit a typical breed structure (Muchadeyi *et al.*, 2007). This implies a higher level of gene flow among agro-ecological zones or populations in the African country (i.e., Zimbabwe) than Asian and European countries (i.e., Vietnam and Hungary, respectively).

Some studies on Vietnamese chicken populations employing microsatellite data were published (Cuc *et al.,* 2006 and Berthouly *et al.,* 2009). These studies were carried out in a single province (i.e., Mai Son district, Son La province and Ha Giang province, respectively). The populations in these studies showed high diversity and no substructure. In addition, the highest genetic diversity was found in a H'mong population when comparing it to a wide range of globally collected chicken breeds (Granevitze *et al.*, 2007).

In the analysis of mtDNA sequences, haplotype network analysis clusters individuals based on haplotypes they possess and indicates how different these haplotypes are from those in other individual. The median networks of haplotypes were generated by partitioning the groups of haplotypes to portray mtDNA relationships and infer about population expansion and domestication events (Bandelt *et al.*, 1995). The ancient haplotypes can be distinguished from young ones due to their higher frequencies and central positions surrounded by derived haplotypes in a star like topology (MacHugh and Bradley, 2001). Bandelt *et al.* (1995) showed that the median networks of haplotypes provide a much more useful and informative mitochondrial portrait of the populations concerned than can be obtained from other traditional tree building approaches such as maximum parsimony, maximum likelihood and distance methods.