Strategy to assess genetic diversity and conserve Vietnamese animal genetic resources based on molecular parameters

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Abstract

Vietnam is one of the countries, which is richest in biodiversity in the world. There are 50 local breeds known. They show specific adaptation to climate or disease and the local low input - low output production system. Despite their importance, their population sizes have been decreasing, some breeds are dangerous and threatened of extinction. The erosion of local breeds could be linked with the loss of valuable genetic variability and unique characteristics.

Moreover, identification of the breeds is mainly based on: their original areas, names called by local people and phenotypic characteristics. According to definition for breed of FAO, there might be more breeds to be discovered in next years. Therefore, identification of population to conserve need to be an attention. Microsatellite was assessed to be very useful to identify breeds for conservation.

Microsatellites developed by the European Commission – funded project of Development of Strategy and Application of Molecular Tools to Assess Biodiversity in Chicken Genetic Resources (AVIANDIV), Institute of Animal Breeding and Biotechnogy at Hohenheim University - Germany and the Project of Animal Biodiversity Assessment in Vietnam (BIODIVA) funded by France as well as Food and Agriculture Organization of United Nations (2004) - Secondary guidelines for development of national farm animal genetic resources management plans: measurement of domestic animal genetic diversity (MoDAD): Recommended microsatellite markers should be used to assess genetic diversity of chickens, pigs and ruminant, respectively.

The result will identify breeds for efficient conservation measures and to monitor genetic variation within conservation flocks. It enables to contribute objective information on the global assessment and evaluation of the state of the world animal genetic resources.

Keyword: Genetic Animal Resource Conservation, Genetic Diversity, and Vietnam

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1 Introduction

Vietnam is one of the countries, which is richest in biodiversity in the world. There are 50 local breeds known (). They show specific adaptation to climate or disease and the local low input - low output production system. Despite their importance, their population sizes have been decreasing, some breeds are dangerous and threatened of extinction. The erosion of local breeds could be linked with the loss of valuable genetic variability and unique characteristics.

Moreover, identification of the breeds is mainly based on: their original areas, names called by local people and phenotypic characteristics. According to definition for breed of FAO, there might be more breeds to be discovered in next years. Therefore, identification of population to conserve need to be an attention.

The conservation of natural genetic variation is important not only for ethical and aesthetic reasons but also to ensure that the earth's living resources may be used even more efficiently and sustainably in agriculture, forestry, food production and other industries (Karp *et al.*, 1998). In addition to producing food, animal genetic resources serve as a "storehouse" for the wide range of desirable production traits (FAO, 1995). The genetic potential of indigenous breeds as a reservoir of genomic variation and major genes with relevance to improve adaptability has been recognized (Horst, 1989). More needs to be known about the variation that already exists and how it can be conserved and accessed effectively (Karp *et al*., 1998). Romanov *et al*. (1996) suggested that local chickens might contain the genes and alleles pertinent to their adaptation to particular environment and local breeding goals. Such local breeds are needed to maintain

genetic resources permitting adaptation to unforeseen breeding requirements in the future and a source of research material.

The definition of breeds is subjective and in many cases oriented towards few phenotypic traits whereas large parts of the genomic make-up might be common between breeds. The presence of various breeds still gives a question of whether we have genetically distinct populations. Therefore, to set up efficient conservation measures, reliable information about genetic difference between individual, populations and breeds are required. In the process of genetic evaluation to develop conservation measures, it is of special interest to assess genetic variation between suggested subpopulations by utilizing modern molecular tools. Advances in molecular technology have enabled the assessment of genetic variability at the DNA level. Microsatellites are tandem repeat with very short (1-5bp) simple sequence motifs and considered to be evenly distributed in the genome (Tautz, 1989). The basic units of the simple tandem repeats consist of small numbers of base pairs (i.e., CAC, GATA, GACA etc.). Repeat units consist of $(A)_n$, $(TG)_n$, $(CA)_n$ or $(AAT)_n$ repeat (Tautz, 1989; Smeets *et al*., 1989). These repeats can be amplified by using PCR since they are well distributed in animal genomes and are multi-allelic in nature (Tautz, 1989). The number of repeat units that an individual has at a given locus can be resolved using poly acrlyamide gels. From the gels, two genetic markers for most individual can be seen; each individual inherits one length of nucleotide repeats from the mother and the other from the father (individuals with one band received the same band from both their mother and their father).

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 automated analysis (Weigend and Romanov, 2001; Crooijmans *et al*., 1996). It is found that genetic distances for use with microsatellite loci are preferable for phylogenetic reconstruction of taxa that are sufficiently diverged (Roy *et al*., 1994; Goldstein *et al*., 1995). Microsatellite markers are available from databases for chicken such as: ttp:w3.tzv.fal.de/aviandiv/index.html; ttp:w3.tzv.fal.de/aviandiv/index.html; http:/www.thearkdb.org/browse or have been reported by many authors. For example Crooijmans *et al*. (1994; 1996) reported 101 microsatellite markers mapped. Microsatellites have been used by FAO as first priority molecular tool for the MoDAD project to study biodiversity. Currently, microsatellites provide a powerful tool for quantitative trait locus research and for studying genetic diversity within and between populations of humans and all livestock species (Maudet *et al*., 2002; Stephen *et al*., 2002; Han *et al*., 2002; Groenen *et al*., 1995; Garcia de leon *et al*., 1995, Vanhala *et al*. 1998; Zhou and Lamont, 1999; Wimmers *et al*., 2000; Tadelle, 2003; Zhang *et al*., 2002).

The conservation of animal genetic resources is not simply of national importance but also an international issue (Le Viet Ly, 1994). The primary goal for conservation of biodiversity should focus on the diversity between and within indigenous populations of farm animals. The genetic characterization of local breeds is a prerequisite for this purpose. Effort should be made to preserve the important and unique characteristics animal genetic resources possess.

The objectives of the study is to assess genetic diversity and to identify population of Vietnamese local breeds.

2.0 Material and method

2.1 The study area and experimental populations

This work will be carried out in the provinces of Viet nam where local breeds are located. Thirty blood samples per each breed which is selected randomly will be collected. A drop of venous blood will be collected from ulnar vein of each individual onto FTA Micro card (Whatman Co). The filter paper will be allowed to dry, sealed in aluminum bags and kept at room temperature, awaiting DNA isolation.

2.2 Laboratory procedures

DNA will be isolated. The DNA will be amplified using polymerase chain reaction (PCR). Multiplex reactions that included two to five pairs of microsatellite primers will be run in one PCR reaction. Microsatellites developed by the European Commission – funded project of Development of Strategy and Application of Molecular Tools to Assess Biodiversity in Chicken

Genetic Resources (AVIANDIV), Institute of Animal Breeding and Biotechnogy at Hohenheim University - Germany and the Project of Animal Biodiversity Assessment in Vietnam (BIODIVA) funded by France should be used to assess genetic diversity of chickens, pigs and ruminant, respectively. PCR product will be scored

2.3 Statistical analysis

The microsatellite data set will be formatted using the microsatellite toolkit for MS Excel 97 or 2000 (Park, 1999).

Measurement of genetic variation within populations

The observed number of alleles at each locus in each sample and the respective allele frequency will be counted using the FSTAT version 2.9.3 (Goudet, 2001).

Mean observed heterozygosity and expected (under the assumption that the population is in Hardy-Weinberg equilibrium) heterozygosity (Nei, 1987) estimates for each population and overall will be calculated with microsatellite toolkit and FSTAT version 2.9.3 package software. Observed heterozygosity at a locus is given by direct counting of the number of heterozygotes in the sample divided by the number of individuals typed at the locus. An unbiased estimate of expected heterozygosity will be given (Nei, 1987).

$$H = \left[\frac{2n}{2n-1}\right]\left[1-\sum_{i=1}^{k}\left(x_i^2\right)\right]$$

Where: n is the number of individuals x is allele frequency at locus 1, k is number of alleles at locus 1

F statistics goes back to Wright, but FSTAT is based on the method of Weir and Cockerham (1984). Fis value (inbreeding coefficient) per each locus and sample and Fit, Fis will be estimated using FSTAT versions 2.9.3 and Genepop versions 3.4 (Raymond and Rousset, 2004). A chi–square test to test for Hardy–Weinberg equilibrium estimated for each locus and overall will be done using Genepop version 3.4.

Measurement of genetic variation between populations

Genetic relationships among populations and feather colour variants will be determined by multilocus estimator of Fst (Proportion of genetic variability due to population differences) between all pairs of samples (Weir and Cockerham, 1984) and pair wise tests of differentiation for each pair of samples were performed using FSTAT 2.9.3. For each pairs of sample, multi – locus genotypes will be randomised between two samples and permutation performed 3000.

Based on microsatellite genotyping, three different methods of genetic distances calculated will be used Nei's standard genetic (Nei, 1972), Cavalli – Sforza's chord measure (Cavalli – Sforza and Edward, 1967) and Reynolds's genetic distance (Reynolds *et al*., 1983) using Phylip computer package version 3.5 (Felsenstein, 1993). The three genetic distance measures used have somewhat different assumptions. Nei's distance is assumed that the genetic variability in the population is at equilibrium between mutation and genetic drift. The two others genetic distances are assumed that there is no mutation, and that all gene frequency changes are by genetic drift alone. Nei's (1972) standard genetic distance was used to draw the tree.

Standard genetic distance D, according to Nei (1972) is described as:

$$D = -\ln\left[\frac{\sum_m\sum_i p_{lmi}\,p_{2mi}}{\left[\sum_m\sum_i {p_{lmi}}^2\right]^{1/2}\left[\sum_m\sum_i {p_{2mi}}^2\right]^{1/2}}\right]$$

Where: *m* is summed over loci, *i* over alleles at the m-th locus,

P_{1mi} is frequency of the i-th allele at the m-th locus in population 1,

P_{2mi} is frequency of the i-th allele at the m-th locus in population 2.

Cavalli-Storza's chord genetic distance (Cavalli-Sforza and Edwards, 1967) is calculated as:

$$D^2 = 4\sum_m \left[\frac{1 - \sum_i p_{1mi}^{1/2} p_{2mi}^{1/2}}{\sum_m (a_m - 1)} \right]$$

Where: *m* indexes the loci, *i* is summed over the alleles at the m-th locus, *a* is the number of alleles at the m-th locus, P_{1mi} is frequency of the i-th allele at the m-th locus in population 1 and P_{2mi} is frequency of the i-th allele at the m-th locus in population 1.

Reynolds's genetic distance (Reynolds *et al*., 1983) is illustrated as:

$$D^2 = \frac{\sum_m \sum_i [p_{1mi} - p_{2mi}]^2}{2\sum_m \left[1 - \sum_i p_{1mi} p_{2mi} \right]}$$

Where: *m* indexes the loci, *i* is summed over the alleles at the m-th locus, *a* is the number of alleles at the m-th locus, P_{1mi} is frequency of the i-th allele at the m-th locus in population 1 and P_{2mi} is frequency of the i-th allele at the m-th locus in population 1.
Nei's standard genetic distance (Nei, 1972) is widely used for populations (Maudet, 2002; Eding and Laval, 1998). Trees constructed with this genetic distance are expected to draw a reliable phylogeny of species (Eding and Laval, 1998). Neighbour Joining (NJ) also gives higher bootstrap values in most cases and use of the NJ method is advisable (Eding and Laval, 1998; Nei, 1987). Based on Nei's (1972) standard genetic distance, phylogenetic trees will be reconstructed using the Neighbor – joining method (Saitou and Nei, 1987) and Phylip computer package version 3.5. Bootstrapping will be performed 1000 re-samplings to test the robustness of the tree.

3. Expected result

Microsatellite markes allele distribution for each breed and total will be indicated. Specific allele for each breed will be presented. Genetic diversity and Hardy–Weinberg testing will be calculated. Population differentiation and relationship will be tested and phylogenetic tree will be reconstructed

The result will identify breeds for efficient conservation measures and to monitor genetic variation within conservation flocks. It enables to contribute objective information on the global assessment and evaluation of the state of the world animal genetic resources.

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