Analysis of Bhutanese native chickens genetic diversity by microsatellite markers

Nedup Dorji¹, Monchai Daungjinda* and Yupin Phasuk¹

Abstract: Four Bhutanese native chickens and commercial lines were genotyped at 18 microsatellite loci to investigate genetic diversity. A total of 160 alleles were scored with the mean value of 8.87 ± 0.55 across populations. Substantial genetic variations were examined for all populations with Khuilay and Yuebjha Narp represented the highest and lowest genetic variability among Bhutanese native chickens. The genetic differentiation was moderate among six chicken populations. A Neighbour-joining tree revealed a distinct separation of Bhutanese native chickens from commercial chickens. It is concluded that Bhutanese native chicken represented unique population and valuable genetic resources for effective conservation and utilization.

Keywords: genetic variability, microsatellites, native chickens

Introduction

Native chickens are reservoir for global chicken genetic resources. However, an increase demand for poultry products has attributed to genetic loss as focused for superior performance. Similarly, pullets and roosters of commercial lines were distributed to Bhutanese farmers in early 1970s to boost the rural chicken production. The existence of native chickens is because of socio-cultural purposes and scavenging behaviour (require low inputs). For example, preference of local chickens for sacrificing, slaughtering to entertain the guests or visitors, and believe in consuming fresh meat to retrieve the health of women during pregnancy and after birth.

Many native chicken strains are present in Bhutan with poor documentation and conserving for chicken genetic resources proves to be the main concern especially when populations remained uncharacterized. Thus, four familiar strains were included (Table 1) based on their socioeconomic roles: Seim is the most common chicken resembling to Red Junglefowl, Yuebjha Narp (Black) is thought to have medicinal values, Khuilay (Naked Neck) are well known for heat tolerance suitable to hot and humid environment and Phulom (Frizzle) are specific only for few Bhutanese tribes purposed for meat. Thus, the objective of this paper was to investigate the genetic variation among Bhutanese chicken populations.

Recently, the population variations involved with multiallele detectable markers particularly microsatellites because the highest polymorphism was observed with microsatellite than allozyme and random amplified polymorphic DNA (Zhang et al., 2002). In addition, main characteristics of microsatellite loci such as, distributed over the genome, codominant, high mutational rate, and high reproducibility (Dodgson et al., 1997) are

¹Faculty of Agriculture, Khon Kaen University, Thailand
* Correspondence e-mail: monchai@kku.ac.th
fundamental criteria for marker in the field of population genetics. Many studies were investigated and consequently reported the reliability of microsatellites in determining and differentiating among different fowl populations genetically (Nassiri et al., 2007; Davila et al., 2009; Nedup et al., 2010; Osei-Amponash et al., 2010).

Material and methods

Experimental population sizes

The chicken blood were sampled from four Bhutanese native chicken and two commercial lines; Seim (SM = 30), Yuebjha Narp (YN = 24), Khuilay (KL = 25), Phulom (PL = 26), Broiler (BR = 30) and White Leghorn (WH = 30). Sample sizes were based on Tadano et al. (2007) suggestions and will be referred as population, for the sake of convenience. Bhutanese chickens were sampled from backyard and were unrelated. DNA was isolation as described in Goodwin et al. (2007).

Microsatellite genotyping

A set of 18 microsatellite primers were based on FAO/ISAG recommendation and were also used in AVINDA project (Hillel et al., 2003) and Mazandaran native chickens (Nassiri et al., 2007). Microsatellite loci amplification was performed by polymerase chain reaction (PCR). The PCR undergoes three successive phases; Initial denaturation at 94°C for 5 mins followed by 30 cycles that includes denaturation at 94°C for 30

<table>
<thead>
<tr>
<th>Population</th>
<th>Distribution</th>
<th>Comb type</th>
<th>Plumage</th>
<th>Shank and beak</th>
</tr>
</thead>
<tbody>
<tr>
<td>Seim</td>
<td>Throughout</td>
<td>Rose,</td>
<td>Male are golden brown, sometimes reddish brown saddle.</td>
<td>Black, Yellowish</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Pea, Single</td>
<td>Female are brownish red with dark-greenish strip following at each feather</td>
<td></td>
</tr>
<tr>
<td>Yuebjha Narp</td>
<td>Southwest</td>
<td>Rose,</td>
<td>Both sexes are entirely black.</td>
<td>Black</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Pea</td>
<td>Name derived from morphology.</td>
<td></td>
</tr>
<tr>
<td>Khuilay (Naked neck)</td>
<td>South, Southwest</td>
<td>Rose, Pea</td>
<td>Featherless skin at neck is bright red.</td>
<td>Yellowish, Whitish</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Single</td>
<td>Generally soft-feather red however, diverse plumage colour present (such white, partridge)</td>
<td></td>
</tr>
<tr>
<td>Phulom</td>
<td>South, Southwest</td>
<td>Rose, Pea</td>
<td>Feathers faced outwards (various colour such as Seim, black).</td>
<td>Yellowish, Black</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Single</td>
<td>Like frizzle chicken breed.</td>
<td></td>
</tr>
</tbody>
</table>
secs, annealing temperature Tm C for 45 secs, and extension 72\degree C for 45 secs. The final phase is the extension at 72\degree C for 2 mins. SYNGENE™ Gel documentary system (Sygene, Inc., UK) were employ for scoring the bands.

Statistical analysis approach

The band(s) scored referred as alleles hereafter, were analyzed mean number alleles (MNA), observed (H_o) and expected (H_e) heterozygosity because they are important parameters for assessing the population variations (Nassiri et al., 2007). Chi-square test \( \chi^2 \) was tested if the population was in Hardy Weinberg equilibrium. The subpopulation division was determined by Wright’s fixation index \( F_{ST} \) by GENEPOP 4.0. A Neighbour-joining (NJ) method of NTSYSPC Ver 2.10 was used to construct a phylogenetic tree based on Nei’s unbiased genetic distance.

Results and discussion

Microsatellite allele distribution and population diversity

In the present study, the genetic variability both within and among six chicken populations were analyzed based on MNA, H_o and H_e as summarized in Table 2. Since estimated H_o value depends on the sample sizes H_e is used to infer the population diversity calculated from allele frequency. A total number of 160 alleles were scored with the MNA of 8.87 \( \pm \) 0.55 (160 / 18). The allele numbers ranged from 4 to 13 at MCW111 and LEI94, respectively indicating the selected loci were reliable and informative because standard error is minimized while estimating genetic distance (Nassiri et al., 2007).

Among populations considering parameters, the lowest diversity was examined for Yuebjha Narp (MNA = 7.94 \( \pm \) 0.40, H_o = 0.442 \( \pm \) 0.054, and H_e = 0.94 \( \pm \) 0.016) moreover, lower than commercial chickens. While Khuilay exhibited the greatest diversity (MNA = 9.50 \( \pm \) 0.68, H_o = 0.489 \( \pm \) 0.051, and H_e = 0.833 \( \pm \) 0.015). Khuilay has highly diversified plumage colour (including soft-red, white, black, partridge, and speckled) and possibly gene flow from India. Overall, the derived results were slightly lower than Thai native chicken populations (Nedup et al., 2010) however, greater than Mazandaran native chickens (Nassiri et al., 2007) using a same set of markers. Thus, the Bhutanese chicken still contains substantial genetic variations for effective genetic utilization. On contrary, low genetic diversity was examined for commercial lines as they were developed from few breeds followed by intensive selection whereby some genes or alleles might be lost. Similarly, the maximum deviation from HWE was observed for broiler and White Leghorn as they were selected decades for production. The genetic diversity between six populations was determined by F_{ST} and the mean value of 0.079 reflects the populations are moderately differentiated.

Genetic relationships

The NJ topology clearly distinguished between Bhutanese native chickens from commercial chickens which may infer that Bhutanese native chickens is not yet diluted since introductions of exotic breeds to improve rural poultry production. If focused to improve, Khuilay
(Naked Neck) represented genetically similar with commercial broilers, thus we may assume Khulay to improve for meat type. Moreover, the Khulay carcass is easy to un-pluck. Generally, chickens are raised in Bhutan for egg purposes where in Khulay and White Leghorn examined for low genetic distance hence, such population might be developed for egg type as well as meat type however, further study using functional genes might be essential to confirm the result.

**Conclusions and recommendation**

The present study revealed substantial genetic diversity exists because of strong Buddhist believes the cultural practices. Moreover, Bhutanese
chickens may tentatively consider as unique populations which might be conserved to meet unpredictable breeding requirements. Nevertheless, the sampling was from few villages thus, the result might not actually refer the exact genetic variations within and among Bhutanese chickens. It might be thus, be recommended to sample as many villages as possible for further investigation of Bhutanese native chickens genetic diversity.

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References


