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308 Lasani Town, Sargodha Road, Faisalabad - Pakistan
Mob: +92 300 3008585, Fax: +92 41 8815544
E-mail: editorijps@gmail.com

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Genetic Differentiation in the *Oh-Shamo* (Japanese Large Game) Breed of Chickens Assessed by Microsatellite DNA Polymorphisms

Takao Oka[1,2] and Masaoki Tsudzuki[1,2]
[1]Graduate School of Biosphere Science, [2]Japanese Avian Bioresource Project Research Center, Hiroshima University, Higashi-Hiroshima, Hiroshima 739-8528, Japan

Abstract: The *Oh-Shamo* is a native Japanese chicken breed that has been improved for cock fighting purpose for approximately 400 years. Two types of *Oh-Shamo* have been created: one type concentrates on offence and the other type is specialized for defense. In addition to these *Oh-Shamo* types for cock fighting, commercial stocks have been established to produce brand meat in Japan. In this article, we revealed the genetic differentiation among *Oh-Shamo* populations based on the difference in the use and the manner of fighting, as well as genetic diversity in these populations. MNA, AR, H_O and H_E were 2.55-4.03, 2.52-3.75, 0.420-0.577 and 0.422-0.559, respectively, throughout both fighting- and meat-purpose populations. There was a tendency for fighting-type *Oh-Shamo* populations to show higher genetic diversity. Inbreeding was not observed in both fighting-purpose and meat-purpose *Oh-Shamo* populations. Neighbor-joining tree topology based on D_{PS} genetic distance clearly separated fighting-purpose *Oh-Shamo* from meat-purpose *Oh-Shamo*. Thus, commercial *Oh-Shamo* for meat production is genetically distinct from the original *Oh-Shamo* used for cock fighting. On the other hand, no conspicuous genetic differentiation was observed between offence- and defense-types of fighting-purpose *Oh-Shamo*.

Key words: Genetic differentiation, genetic diversity, Japanese Large Game, *Oh-Shamo*, microsatellite, native Japanese chickens

INTRODUCTION

In Japan, there are many kinds of ornamental breeds of chickens (Tsudzuki, 2003). Among them, the *Oh-Shamo* is a breed for cock fighting that has a large and erect body and which has been designated as a "National Natural Treasure" of Japan. In addition to its cock-fighting purpose, a part of the *Oh-Shamo* are raised for their statuesque figures and beautiful plumage colors. Also, the meat of the *Oh-Shamo* is so delicious that commercial *Oh-Shamo* stocks used for meat production purpose exclusively have been established recently in Japan. Commercial meat-type *Oh-Shamo* are used to produce brand meat by crossing them to foreign breeds (*e.g.*, White Plymouth Rock, Barred Plymouth Rock and Rhode Island Red).

The *Oh-Shamo* used for cock fighting can be classified into two types depending on their fighting behaviour and body size. One is the type that concentrates on offence by kicking and pecking, while the other is the type that concentrates on defense with sparse or no kicking and pecking. The former weighs more than 4 kg while the latter is less than 4 kg.

In spite of these differences in their utility (cock fighting and meat production) and the manner of fighting behaviour, genetic relationships have not been investigated among such kinds of birds. In the present study, we examined genetic diversity and differentiation in *Oh-Shamo* populations kept for cock-fighting and meat-production purposes based on microsatellite DNA polymorphisms.

MATERIALS AND METHODS

We collected blood samples from four populations of the *Oh-Shamo*; namely a population of offence-type birds (O population), a population of defense-type birds (D population) and two populations of commercial birds for meat production (tentatively named M and L populations). The number of blood samples (birds) examined was 30, 18, 24 and 24 in O, D, M and L populations, respectively.

Using genomic DNA extracted from blood with a standard phenol-chloroform method (Sambrook and Russell, 2001), we amplified ISAG/FAO recommended 30 microsatellite DNA markers with the exception of *MCW0284*. Amplification of the marker DNA was performed with a thermal cycler (GenAmp 9700 PCR System; Applied Biosystems, Foster City, CA, USA) according to the same conditions described by Oka *et al*. (2010). Subsequently, we genotyped the 29 microsatellites using an automated DNA sequencer (ABI PRISM 310 Genetic Analyzer; Applied Biosystems) and a GeneMapper software 3.7 (Applied Biosystems).

Corresponding Author: Masaoki Tsudzuki, Graduate School of Biosphere Science, Hiroshima University, Higashi-Hiroshima, Hiroshima 739-8528, Japan

Table 1: Genetic diversity indices estimated in each population of *Oh-Shamo* chickens[(1)]

Populations[(2)]	No. of birds	MNA	AR	H_O	H_E	F_{IS}
O	30	4.03 (1.97)^{b}	3.75 (1.67)^{b}	0.538 (0.017)^{b}	0.559 (0.028)^{b}	0.038^{ns}
D	18	3.48 (1.27)^{b}	3.48 (1.27)^{b}	0.577 (0.022)^{b}	0.553 (0.028)^{b}	-0.043^{ns}
M	24	2.55 (0.78)^{a}	2.52 (0.75)^{a}	0.420 (0.019)^{a}	0.422 (0.031)^{a}	0.006^{ns}
L	24	2.97 (0.78)^{ab}	2.91 (0.75)^{ab}	0.496 (0.019)^{ab}	0.483 (0.031)^{ab}	-0.026^{ns}

[(1)] Microsatellites recommended by ISAG/FAO (FAO, 2004) were analyzed (excluding *MCW0284)*

MNA: mean number of alleles

AR: allelic richness (corrected value of number of alleles calculated with the correction based on a minimum population size of population D (n = 18))

H_O and H_E: observed and expected heterozygosity

F_{IS}: inbreeding coefficient. Numerals in parentheses show standard deviations

MNA, AR, H_O and H_E were compared among populations using the Kruskal-Wallis test with the Scheffe test for pairwise comparison (values with different superscripts show statistical difference, P<0.05). The superscript ns shows no statistical significance (P>0.05)

[(2)]O and D are the populations of traditional *Oh-Shamos* for cock fighting, while M and L are the populations of new-type commercial *Oh-Shamos* to be used in meat production

With the genotyping data and FSTAT 2.9.3 program (Goudet, 1995), we estimated the corrected number of alleles (allelic richness: AR), expected heterozygosity (H_E) and inbreeding coefficients (F_{IS}) in each population, along with the mean number of alleles (MNA) and observed heterozygosity (H_O) and compared the genetic diversity among populations. Furthermore, we constructed a neighbor-joining (NJ) phylogenetic tree (Saitou and Nei, 1987) based on the D_{PS} genetic distance (Bowcock *et al.*, 1994) and revealed the genetic relationship among individuals.

RESULTS

Genetic diversity indices (MNA, AR, H_O, H_E and F_{IS}) estimated for *Oh-Shamo* populations are presented in Table 1. MNA and AR varied from 2.55 (population M) to 4.03 (O) and from 2.52 (M) to 3.75 (O), respectively. H_O and H_E ranged from 0.402 (M) to 0.577 (D) and from 0.422 (M) to 0.559 (O), respectively. Population M showed the lowest values (P<0.05) of genetic diversity indices (MNA, AR, H_O and H_E) in the Kruskal-Wallis test with Scheffe's multiple comparison, while there were no differences in the indices among populations O, D and L. The F_{IS} values estimated were not significant in all populations (P>0.05).

In the D_{PS}-NJ tree (Fig. 1), the birds for traditional cock fighting were clearly separated from commercial birds for meat production. Within the commercial birds, individuals in populations M and L were also clearly separated from each other and formed different clusters. On the other hand, the individuals from populations O and D were included into the same cluster.

DISCUSSION

Osman *et al.* (2006) reported that two *Oh-Shamo* populations for cock-fighting purpose showed MNA of 3.90 and 4.35 and H_E of 0.62. Oka *et al.* (2010) also reported that *Oh-Shamo* populations for commercial meat production showed MNA of 2.61-3.50 and H_E of 0.46-0.53. The genetic diversity of the four *Oh-Shamo* populations, which included both cock-fighting and commercial meat purposes, estimated in the present study (Table 1) was similar to that reported in those previous studies. The relatively low diversity in the M and L populations is thought to reflect the relatively small number of founder birds that contributed to the establishment of these closed colonies. The F_{IS} value presents a degree of non-random mating; in other words, a deviation from the Hardy-Weinberg equilibrium (Weir and Cockerham, 1984). In the present study, all four populations showed no significance in the F_{IS} value, which indicates that no inbreeding had occurred in all the four populations. It is thought in the case of commercial meat purpose *Oh-Shamo* that intentional and schematic matings had been performed based on scientific breeding theory. On the other hand, breeders of the fighting-purpose *Oh-Shamo* seem not to know about such theory. However, they are avoiding inbreeding by exchanging birds from all over Japan. The *Oh-Shamo* for cock-fighting must be healthy and strong. According to interviews with breeders, they all knew that inbreeding must be avoided to produce healthy and strong birds.

In the D_{PS}-NJ tree, offence-type and defense-type *Oh-Shamo* for cock fighting were included within the same cluster and apparently separated from the M and L *Oh-Shamo* populations for meat production. Thus, the offence-type and defense-type of the *Oh-Shamo* for cock fighting are recognized as the same breed, irrespective of their differences in body size and the manner of fighting. The body shape of males in the L population is not as erect as that of the fighting-type *Oh-Shamo*. Furthermore, there is an indication that this population was established by crossing with another breed to increase meat amount. The tree topology is thought to reflect this information well. There is no information that the M population has been established by introducing genes from other breeds. However, the length and amount of their feathers are apparently longer and richer, respectively, than those of fighting-type *Oh-Shamo*, although the body shape is generally similar to each other between the M-stock and the fighting-type *Oh-*

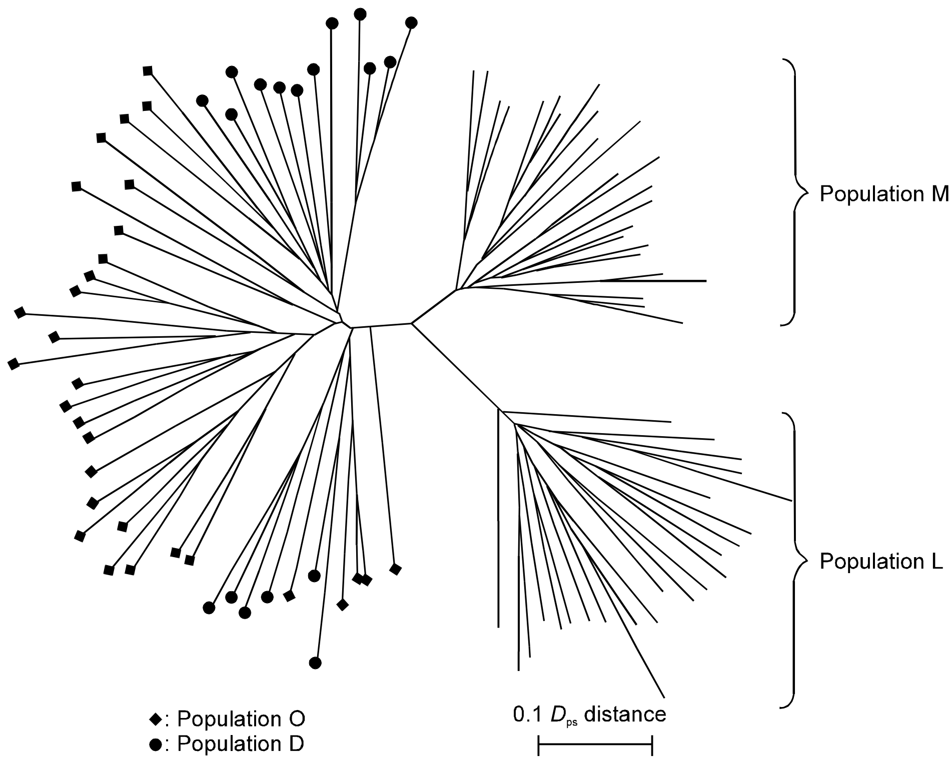

Fig. 1: Unrooted neighbour-joining tree representing the relationship among 96 *Oh-Shamo* individuals. The tree was constructed based on D_{PS} genetic distance calculated from polymorphisms of 29 microsatellites. Populations O and D consist of traditional birds for cock fighting. Birds in population O are offence-type fighters and those in population D are defense-type fighters. Populations M and L are composed of new-type commercial birds for meat production

Shamo. Judging from the tree topology, there is a possibility that the M stock was established by crossing with other breeds in the process of breeding. Summarizing these, the fighting-type *Oh-Shamo* should be maintained by avoiding the cross with the meat-type *Oh-Shamo*, because apparent genetic discrimination was observed between them. Although offence-type and defense-type *Oh-Shamo* were recognized as one breed, it would be interesting to maintain the two types separately into the future because of the large difference in their manner of fighting.

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