# Genetic Variability and Relationships of Native Japanese Chickens Assessed by Microsatellite DNA Profiling - Focusing on the Breeds Established in Kochi Prefecture, Japan -

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**ABSTRACT :** Blood samples were collected from eight native Japanese breeds of chickens (Miyadi-dori, Ohiki, Onaga-dori, Shoukoku, Tosa-Jidori, Tosa-Kukin, Toutenkou and Uzurao) and two foreign breeds of chickens (White Leghorn and Rhode Island Red) to examine the genetic variability and relationships among the breeds by using a microsatellite DNA technique. Except for the Shoukoku breed, the other Japanese chicken breeds all originate from Kochi Prefecture. Ohiki, Onaga-dori, Tosa-Jidori, Toutenkou and Uzurao are fancy fowl, and Miyadi-dori and Tosa-Kukin are utility fowl. Among the fancy fowl, Ohiki, Onaga-dori, and Toutenkou males have thick and long feathers in the saddle and tail. Genetic variabilities of the 20 microsatellites examined, varied depending on the breed: the mean number of alleles per locus ranged from 2.05 (Miyadi-dori) to 3.90 (Rhode Island Red); proportion of polymorphic loci ranged from 0.75 (Miyadi-dori) to 1.00 (Rhode Island Red, Shoukoku and Uzurao); and mean expected heterozygosity ranged from 0.330 (Miyadi-dori) to 0.607 (Rhode Island Red). Unique microsatellite alleles were detected in each breed. Using the neighbour-joining method, phylogenetic trees were constructed based on the genetic distances of $D_A$ and $D_{ST}$. Among the breeds originating from Kochi Prefecture, fancy and utility breeds belonged to different clusters. Among the fancy breeds, those having thick and long feathers in the tail and saddle showed a close genetic relationship to the Shoukoku breed, which also has thick and long feathers in the tail and saddle.
***(Asian-Aust. J. Anim. Sci. 2005. Vol 18, No. 6 : 755-761)***

**Key Words :** Genetic Relationship, Genetic Variability, Japanese Native Japanese Chicken, Microsatellite, Kochi Prefecture

## INTRODUCTION

In Japan, there are approximately 50 breeds of native chickens (Tsudzuki, 2003). Most of them were bred by the end of the Edo Era (1603 to 1867) for their special plumage, crowing ability, and cockfighting ability. Others were bred to produce eggs and/or meat through the Meiji Era (1868 to1912) to the Taishou Era (1912 to1926).

Among the Japanese breeds, seven originated from Kochi Prefecture; they are Tosa-Jidori, Onaga-dori (Tosa-no-Onaga-dori), Ohiki (Minohiki Chabo), Toutenkou, Uzurao (Uzura Chabo), Tosa-Kukin, and Miyadi-dori. This is the largest number of breeds that were established in any one prefecture. The former five are fancy (ornamental) breeds that were established by the end of the Edo Era, and the latter two are utility breeds developed during and after the Meiji Era (Oana, 1951; Tsudzuki, 2003). Kochi Prefecture is located on the Pacific Ocean side of Japan's Shikoku Island (http://www.jetro.go.jp/investjapan/).

The earliest study of genetic relationships among native Japanese chickens was performed based on external characteristics and old literature (Oana, 1951). Later, osteometrical and somatometrical studies were carried out (Nishida et al., 1985 a,b). In addition, several phylogenetic and variability studies based on blood groups and/or blood protein polymorphisms have been performed (Hashiguchi et al., 1981; Okabayashi et al., 1998; Okada et al., 1980, 1984, 1989; Tanabe and Mizutani, 1980; Tanabe et al., 1991). However, these investigations detected only a limited number of polymorphic loci and alleles per locus. In addition, the genetic relationships of native Japanese chicken breeds reported in these studies were not always in accordance with those from the morphological and literature studies. As a result, the genetic relationships among native Japanese chicken breeds remain unclear.

Recently, microsatellites have become the preferred type of genetic markers for several reasons, including their abundance, random distribution, codominant inheritance, high variability, and possibility of automated detection. Additionally, microsatellites have the ability to show genetic polymorphisms even in species in which classical genetic markers have been proven to be unsuccessful (Goldstein and Pollock, 1997; Petit et al., 1997). Accordingly, microsatellite markers have proven useful in assessing genetic variation and diversity in livestock (Buchanan et al., 1994; MacHugh et al., 1994; Martinez et al., 2000; Pandey et al., 2002).

A large number of chicken microsatellite markers have been determined (Groenen et al., 2000) and have been

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Received July 12, 2004; Accepted December 27, 2004

**Table 1.** List of chicken breeds used in this study

| Breeds | Breed abbreviations | No. of individuals examined | Source of samples |
|---|---|---|---|
| Miyadi-dori | MYA | 24 | Kochi Prefecture (fancier) |
| Ohiki | OHK | 28 | Kochi Prefecture (fanciers) |
| Onaga-dori | ONA | 27 | Kochi Prefecture (fanciers) |
| Rhode Island Red | RIR | 27 | HS-NLBC[1] |
| Shoukoku | SHO | 24 | Mie Prefecture (fancier) |
| Tosa-Jidori | TJI | 24 | KPLES[2] |
| Tosa-Kukin | TKU | 26 | Kochi Prefecture (fanciers) |
| Toutenkou | TOT | 37 | Kochi Prefecture (fancier) |
| Uzurao | UZU | 23 | Kochi Prefecture (fanciers) |
| White Leghorn | WL | 24 | OS-NLBC[3] |

[1] Hyogo Station, National Livestock Breeding Center.
[2] Kochi Prefectural Livestock Experiment Station.
[3] Okazaki Station, National Livestock Breeding Center.

employed to evaluate the genetic diversity of chicken populations (Groen et al., 1994; Crooijmans et al., 1996a; Ponsuksili et al., 1996; Vanhala et al., 1998; van Marle-Köster and Nel, 2000; Emara et al., 2002). Recently, two studies revealed the genetic relationships of native Japanese chickens based on microsatellite DNA polymorphisms (Takahashi et al., 1998; Osman et al., 2004). However, these studies did not focus on the breeds originating from Kochi Prefecture, which are the main breeds in Japan.

This paper describes the genetic variability and relationships of native chickens originating from Kochi Prefecture, Japan. The results will be useful in order to support decisions regarding conservation and further use of the breeds.

## MATERIALS AND METHODS

### Chicken breeds

Table 1 shows the breeds and sampling sites of blood samples used in this study. We assessed eight native Japanese breeds [Miyadi-dori (MYA), Ohiki (OHK), Onaga-dori (ONA), Shoukoku (SHO), Tosa-Jidori (TJI), Tosa-Kukin (TKU), Toutenkou (TOT), and Uzurao (UZU)] and two foreign breeds [White Leghorn (WL) and Rhode Island Red (RIR)]. Details of breed features are described elsewhere (Mitsui, 1979; Hawksworth, 1982; Kuroda et al., 1987; Tsudzuki, 2003). The following is a brief description of the native Japanese breeds screened in this study. TJI has a small body size (600 to 675 g), and its ancestor is thought to have been introduced to Japan more than 2,000 years ago. The overall appearance of this breed is similar to that of the Red Jungle Fowl. UZU is similar to TJI in its body size, but this breed shows a sloping back and has no tail feathers. ONA is an especially distinguished-looking breed. Some of the tail feathers and all of the saddle hackles in the males show no molting during their lives, continue to extend, and sometimes exceed 10 m in length. OHK has a small body size, but is somewhat larger than TJI. In spite of this small body size, the tail feathers and saddle hackles of males are considerably thick and long. TOT is characterized by prolonged crowing (15 sec in average). Excepting the plumage color, TOT has great similarity to SHO in the body shape. SHO is one of the oldest breeds in Japan. It is believed to have an ancestor of Chinese origin. Tail feathers and saddle hackles of males are thick and long. TKU is a utility breed having a large (3,300 to 4,500 g), Cochin-type body. MYA is also a utility fowl with black plumage and short legs the color of lead. This breed has a single comb and white ear lobes. The whole body shape is similar to that of the Creeper in Germany (van Wulfften Palthe, 1992).

### DNA extraction and microsatellite amplification

The blood samples were collected from the ulnar vein. Genomic DNA was extracted from the whole blood by using a DNeasy Tissue Kit (QIAGEN, Tokyo). Prior to the PCR amplification, DNA concentration of individual samples was adjusted to 20 ng/μl.

A number of chicken microsatellite markers are available at the Web Site of the Chicken Genome Project (http://poultry.mph.msu.edu/). In this study, we used 20 microsatellite primer pairs, taking the genome coverage of the loci into consideration. Details of the microsatellite markers used are described in Table 2. Polymerase chain reactions (PCR) were carried out following the protocols suggested by Cheng and Crittenden (1994) and Crooijmans et al. (1996b), with some modifications. The protocol is as follows. PCR amplifications were carried out in a 20 μl reaction mixture containing 20 ng DNA, 1.5 mM $MgCl_2$, 50 mM KCl, 10 mM Tris-HCl (pH 8.3), 0.001% gelatin, 100 μM dNTPs, 0.5U of AmpliTaq Gold (Applied Biosystems, Foster City, USA) and 10 pM of each primer. Each reaction was performed according to the following conditions: first there were an initial denaturation at 95°C for 9 min and 43 cycles of denaturation at 95°C for 1 min. Next, there was an annealing for 1 min at an appropriate temperature (50 to

**Table 2.** Details of 20 microsatellites screened in this study

| Loci[1] | Chro. No. | Repeat motif (5'-3') | Forward primer (5'-3') | Reverse primer (5'-3') | Annealing (°C) |
|---|---|---|---|---|---|
| ADL 262 | 23 | $(TG)_9$ | GTGCAGACACAGAGGGAAAG | TCACATGCACACAGAGATGC | 55 |
| LEI 092 | 6 | $(TG)_9$ | GATCTACATTTGTGCAGTGTC | TCCTTGGTCTGACTCTCCATG | 50 |
| LEI 096 | 2 | $(TG)_9$ | GATCAGATTGGTTCCCTGTG | TGGGTGAAGTTTCCTCGTAG | 55 |
| LEI 099 | 12 | $(TG)_9$ | GATCTGGCAGAACAGAAACAG | ATATTTCACACCTGACCTGCG | 55 |
| LEI 135 | 28 | $(TG)_9$ | CACAATGAAGGATGAATAGTGC | AATTCACAGTTACACCTGAGG | 55 |
| LEI 209 | 1 | $(AC)_{17}AT(AC)_4(GC)_4$ | AATTTGGTGTCATACCTCTCC | GACTTTCCAGTGTCTCGTTTAG | 55 |
| MCW 067 | 10 | $(TG)_9$ | GCACTACTGTGTGCTGCAGTTT | GAGATGTAGTGCCACATTCCGAC | 55 |
| MCW 145 | 1 | $(TG)_{10}$ | ACTTT ATTCT CCAAA TTTGG CT | AAACA CAATG GCAAC GGAAA C | 55 |
| MCW 183 | 7 | $(TG)_{10}$ | ATCCCAGTGTCGAGTATCCGA | TGAGATTTACTGGAGCCTGCC | 55 |
| MCW 193 | 5 | $(TG)_{10}$ | TATTCAATAGAGTTACGCTGTC | ATTACGTCTGCACCAGTACAG | 50 |
| MCW 214 | 5 | $(TG)_{10}$ | CAACAGTAACCATACATCTGC | TACCTGGATTCTTTCATCAGG | 50 |
| MCW 217 | 18 | $(TG)_{10}$ | GATCTTTCTGGAACAGATTTC | CTGCACTTGGTTCAGGTTCTG | 55 |
| MCW 222 | 3 | $(TG)_{10}$ | GCAGTTACATTGAAATGATTCC | TTCTCAAAACACCTAGAAGAC | 55 |
| MCW 233 | 27 | $(TG)_{10}$ | TCCAGCAGTAAGTATAGCTGC | TGTTAGCTGCAGGGTATTAGC | 55 |
| MCW 240 | 4 | $(TG)_{10}$ | CAAAACCGGTGTCACCTACTG | GGTTATTTCTTCAGTGACTTCC | 50 |
| MCW 252 | 3 | $(TG)_{10}$ | CTGCTCAAGCCCATCAAATGG | CGATAACATCTGACACTGCC | 55 |
| MCW 295 | 4 | $(TG)_{10}$ | ATCACTACAGAACACCCTCTC | TATGTATGCACGCAGATATCC | 55 |
| MCW 301 | 24 | $(TG)_{10}$ | GGAGAGGAGACAACTGTATTC | AGGGTGAGAGGTAACAAGTGC | 55 |
| MCW 322 | 13 | $(TG)_{10}$ | GATCTCCCTAGCTACAAACC | CTTCCGCCTTCTTGAGAGTC | 55 |
| MCW 330 | 17 | $(AC)_{17}AT(AC)_4(GC)_4$ | TGGACCTCATCAGTCTGACAG | AATGTTCTCATAGAGTTCCTGC | 55 |

[1] The Chicken Genome Project Web Site (http://poultry.mph.msu.edu/) provides PCR primer information to amplify each locus.

55°C) for each marker. This was followed by an extension at 72°C for 1 min, using a Perkin-Elmer Cetus 9600 thermalcycler (Applied Biosystems). Finally, there was a final extension at 72°C for 10 min.

The PCR fragments were electrophoresed in a 5% polyacrylamide gel using an automated ABI 377 DNA sequencer with the internal size standard Genescan 350-TAMRA (Applied Biosystems). Data were collected with the ABI PRISM 377 (version 2.5) software (Applied Biosystems). Microsatellites were sized and genotyped using the GENESCAN (version 3.1) and GENOTYPER (version 2.5) software programs (Applied Biosystems).

### Statistical analysis

Allele frequency of each locus and breed was directly calculated from the observed genotypes. Genetic variability of each breed was evaluated by calculating the mean number of alleles per locus (*MNA*), proportion of polymorphic loci ($P_{poly}$: Lewontin and Hubby, 1966), and unbiased expected heterozygosity ($H_e$: Nei, 1978).

Two genetic distances ($D_A$: Nei et al., 1983 and $D_{ST}$: Nei, 1972) were calculated between all pairs of breeds, and two phylogenetic trees were constructed based on the neighbor-joining (NJ) method (Saitou and Nei, 1987). The reliability of the tree obtained was examined by a bootstrap test with 1,000 replicate re-sampling of loci with replacement. These procedures were conducted by using the POPTREE program (http://www.bio.psu.edu/People/Faculty/Nei/software.htm), and the tree was visualized on the TREEVIEW program (http://taxonomy.zoology.gla.ac.uk/rod).

## RESULTS

### Genetic variability

Genetic variabilities estimated for each breed are summarized in Table 3. A total of 155 alleles were observed at the 20 loci in 264 individuals from 10 chicken breeds. The number of alleles per locus across breeds ranged from 3 (ADL 262) to 15 (LEI 209) with the *MNA* of 7.75. The *MNA* in each breed ranged from 2.05 (MYA) to 3.90 (RIR). The lowest value of the $P_{poly}$ (0.75) was found in the MYA breed, while three breeds (RIR, SHO and UZU) showed polymorphisms in all the 20 microsatellites (i.e. $P_{poly} = 1.00$). The $H_e$ ranged from 0.330 (MYA) to 0.607 (RIR). In other words, the MYA had the lowest genetic variability and the RIR showed the highest variability.

All breeds had two or more private alleles (Table 4). There were seven private alleles in WL, five in ONA and SHO, four in TKU and RIR, three in UZU, and two in each of MYA, OHK, TJI and TOT.

### Genetic distances

Matrices of genetic distances ($D_A$ and $D_{ST}$) between every pair of breeds were calculated from the allele frequencies at the 20 loci and are presented in Table 5. Two dendrograms drawn from the genetic distance matrices are shown in Figure 1A and B.

*$D_A$ distance* : The average genetic distance among all breeds was 0.485±0.095. The lowest distance (0.275) was observed between the ONA and SHO breeds, and the highest distance (0.645) between the UZU and WL breeds. The $D_A$ values measured between the WL and Japanese

**Table 3.** Statistics of microsatellite variability in terms of the number of different alleles at each locus, the mean number of alleles per locus (*MNA*), proportion of polymorphic loci ($P_{poly}$), and mean expected heterozygosity (*He*), estimated for 10 chicken breeds

| Loci | Breeds[2] | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | All[1] | MYA | OHK | ONA | RIR | SHO | TJI | TKU | TOT | UZU | WL |
| ADL 262 | 3 | 1 | 3 | 1 | 3 | 2 | 2 | 2 | 2 | 3 | 3 |
| LEI 092 | 12 | 3 | 4 | 7 | 3 | 2 | 1 | 3 | 2 | 4 | 3 |
| LEI 096 | 7 | 2 | 4 | 3 | 4 | 2 | 3 | 3 | 2 | 5 | 6 |
| LEI 099 | 7 | 1 | 1 | 2 | 4 | 3 | 1 | 3 | 1 | 3 | 4 |
| LEI 135 | 8 | 4 | 3 | 5 | 4 | 3 | 3 | 4 | 3 | 5 | 1 |
| LEI 209 | 15 | 2 | 3 | 6 | 7 | 6 | 4 | 6 | 2 | 5 | 5 |
| MCW 067 | 4 | 1 | 3 | 3 | 3 | 3 | 2 | 1 | 3 | 4 | 3 |
| MCW 145 | 7 | 1 | 3 | 3 | 5 | 4 | 2 | 2 | 1 | 3 | 3 |
| MCW 183 | 9 | 1 | 3 | 2 | 4 | 2 | 2 | 6 | 2 | 4 | 3 |
| MCW 193 | 9 | 3 | 4 | 2 | 2 | 3 | 3 | 2 | 5 | 2 | 5 |
| MCW 214 | 11 | 3 | 2 | 5 | 2 | 4 | 3 | 4 | 3 | 5 | 4 |
| MCW 217 | 7 | 2 | 3 | 3 | 5 | 5 | 3 | 4 | 2 | 3 | 1 |
| MCW 222 | 5 | 2 | 3 | 3 | 3 | 2 | 2 | 2 | 2 | 2 | 2 |
| MCW 233 | 5 | 2 | 5 | 3 | 4 | 3 | 3 | 3 | 3 | 4 | 1 |
| MCW 240 | 11 | 2 | 5 | 6 | 5 | 5 | 3 | 4 | 4 | 5 | 2 |
| MCW 252 | 8 | 2 | 5 | 4 | 4 | 4 | 3 | 2 | 4 | 3 | 3 |
| MCW 295 | 7 | 2 | 2 | 2 | 4 | 4 | 4 | 3 | 2 | 3 | 3 |
| MCW 301 | 11 | 2 | 3 | 4 | 5 | 4 | 3 | 4 | 2 | 4 | 3 |
| MCW 322 | 3 | 3 | 3 | 3 | 3 | 3 | 3 | 3 | 2 | 2 | 2 |
| MCW 330 | 6 | 2 | 2 | 2 | 4 | 2 | 4 | 2 | 3 | 4 | 1 |
| *MNA* | 7.75 | 2.05 | 3.20 | 3.45 | 3.90 | 3.30 | 2.70 | 3.15 | 2.50 | 3.65 | 2.90 |
| *SE of MNA* | | 0.19 | 0.24 | 0.36 | 0.26 | 0.26 | 0.19 | 0.29 | 0.22 | 0.23 | 0.32 |
| $P_{poly}$ | | 0.75 | 0.95 | 0.95 | 1.00 | 1.00 | 0.90 | 0.95 | 0.90 | 1.00 | 0.80 |
| $H_e$ | | 0.330 | 0.486 | 0.403 | 0.607 | 0.461 | 0.362 | 0.473 | 0.351 | 0.566 | 0.459 |
| *SE of He* | | 0.055 | 0.045 | 0.045 | 0.027 | 0.045 | 0.049 | 0.047 | 0.052 | 0.031 | 0.056 |

[1] Number of different alleles per locus across all breeds.
[2] See Table 1 for the abbreviation.

**Table 4.** Microsatellite alleles specific to each breed

| Breeds[1] | No. of alleles | Locus name and allele sizes (bp) |
|---|---|---|
| MYA | 2 | LEI 135: (140), MCW 217: (147) |
| OHK | 2 | MCW 183: (298), MCW 301: (285) |
| ONA | 5 | LEI 092: (265, 273, 281), LEI 209: (166), MCW 240: (175) |
| RIR | 4 | LEI 099: (123), MCW 145: (204), MCW 183: (306), MCW 240: (197) |
| SHO | 5 | LEI 099: (117), LEI 092: (243), MCW 217: (163), MCW 295: (93), MCW 301: (267) |
| TJI | 2 | MCW 193: (311), MCW 330: (258) |
| TKU | 4 | MCW 183: (296), MCW 222: (224), MCW 240: (187), MCW 295: (99) |
| TOT | 2 | LEI 135: (152), LEI 209: (138) |
| UZU | 3 | LEI 099: (135), MCW 301: (305), MCW 330: (266) |
| WL | 7 | LEI 096: (242), LEI 209: (164, 172), MCW 183: (310), MCW 214: (277, 289), MCW 252: (297) |

[1] See Table 1 for the abbreviation.

breeds were generally high (0.531 to 0.645), and constantly greater than the average value (0.485) of 45 breed pairs. While the $D_A$ values measured between RIR and Japanese breeds ranged from 0.393 to 0.538, the mean value (0.466) is less than the average value (0.485) of 45 breed pairs.

*$D_{ST}$ distance* : The average genetic distance among all breeds was 0.847±0.248. The lowest distance (0.403) was observed between the ONA and SHO breeds, and the highest distance (1.413) between the UZU and WL breeds. The $D_{ST}$ values determined between the WL and Japanese breeds were generally high (1.024 to 1.413), and constantly greater than the average value (0.847) of 45 breed pairs. While the $D_{ST}$ values determined between RIR and Japanese breeds ranged from 0.602 to 1.009, the mean value (0.801) is less than the average value (0.847) of 45 breed pairs.

**NJ tree**

Figure 1 A illustrates the genetic relationships among breeds as the NJ tree was reconstructed based on the $D_A$ distance matrix. Chicken breeds were divided into three major groups. The first group consisted of TOT, ONA, OHK, SHO and UZU; the second group comprised WL, RIR, TKU, and MYA; and the third group included only one breed, TJI. Figure 1B illustrates the genetic

**Table 5.** Matrix of $D_A$ (below diagonal) and $D_{ST}$ (above diagonal) genetic disrtances estimated between 10 chicken breeds based on 20 microsatellites[1]

| | MYA | OHK | ONA | RIR | SHO | TJI | TKU | TOT | UZU | WL |
|---|---|---|---|---|---|---|---|---|---|---|
| MYA | 0 | 0.959 | 0.717 | 0.610 | 0.835 | 0.916 | 1.025 | 0.781 | 0.708 | 1.024 |
| OHK | 0.594 | 0 | 0.476 | 0.977 | 0.413 | 0.672 | 1.080 | 0.505 | 0.549 | 1.257 |
| ONA | 0.463 | 0.319 | 0 | 0.809 | 0.403 | 0.868 | 1.062 | 0.607 | 0.714 | 1.362 |
| RIR | 0.425 | 0.518 | 0.468 | 0 | 0.851 | 0.766 | 0.602 | 1.009 | 0.846 | 0.739 |
| SHO | 0.474 | 0.300 | 0.275 | 0.473 | 0 | 0.733 | 0.839 | 0.585 | 0.520 | 1.063 |
| TJI | 0.542 | 0.447 | 0.491 | 0.418 | 0.423 | 0 | 1.026 | 1.050 | 0.740 | 1.234 |
| TKU | 0.580 | 0.594 | 0.571 | 0.393 | 0.498 | 0.566 | 0 | 1.094 | 0.841 | 1.056 |
| TOT | 0.519 | 0.379 | 0.356 | 0.538 | 0.411 | 0.547 | 0.582 | 0 | 0.613 | 1.152 |
| UZU | 0.516 | 0.352 | 0.407 | 0.442 | 0.353 | 0.445 | 0.504 | 0.474 | 0 | 1.413 |
| WL | 0.547 | 0.603 | 0.638 | 0.418 | 0.560 | 0.623 | 0.531 | 0.614 | 0.645 | 0 |

[1] Calculation of genetic distances $D_A$ and $D_{ST}$ was performed based on Nei et al. (1983) and Nei (1972), respectively.
See Table 1 for the abbreviation of breed names.

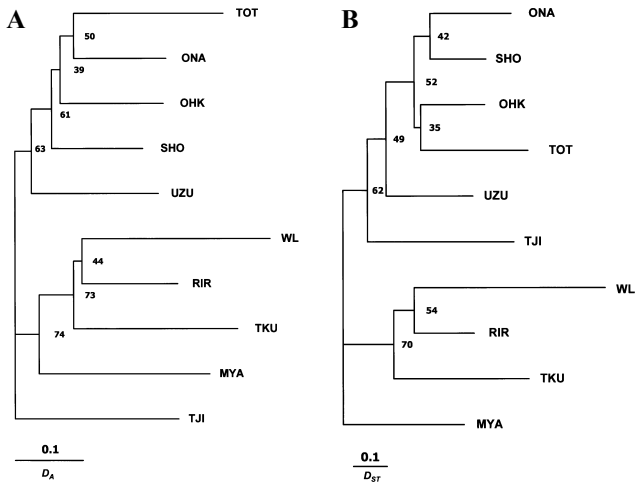


**Figure 1.** Neighbor-joining trees showing the genetic relationships among eight breeds of native Japanese chickens and two foreign breeds (White Leghorn and Rhode Island Red) using $D_A$ (A) and $D_{ST}$ (B) genetic distances calculated from 20 microsatellites. The numbers at the node indicate bootstrap values in percentage (1,000 replicates). MYA: Miyadi-dori, OHK: Ohiki, ONA: Onaga-dori, SHO: Shoukoku, TJI: Tosa-Jidori, TKU: Tosa-Kukin, TOT: Toutenkou, UZU: Uzurao, RIR: Rhode Island Red, WL: White Leghorn.

relationships between breeds as the NJ tree was reconstructed based on the $D_{ST}$ distance matrix. Breeds were clustered into three major groups. The first group consisted of ONA, SHO, OHK, TOT, UZU and TJI; the second group was composed of WL, RIR and TKU; and the third group included only one breed, MYA.

## DISCUSSION

### Genetic variability

Genotyping at various microsatellite loci across the genome indicated that the chicken breeds examined were genetically different from each other. The microsatellite allelic composition and frequencies differed among the 10 breeds. While several alleles were shared among the 10 breeds, some alleles were observed to be breed-specific. Recently in Japan, native Japanese chickens are being mated to foreign breeds to produce better tasting, high-quality meat (Japan Chicken Association, 2003). Breed-specific alleles may be a diagnostic marker to trace the origin of meat, when ordinary chicken meat has been attempted to be passed off as high-quality meat.

In our study, the number of alleles per locus across all breeds examined was three to 15 (Table 3). This result is similar to those of Crooijmans et al. (1993, 1996a), Cheng et al. (1995), and Ponsuksili et al. (1996). As well, the *MNA* in each breed varied from 2.05 to 3.90. The Kochi breeds varied from 2.05 to 3.65. This result, also, is similar to that of van Marle-Köster and Nel (2000), in which the *MNA* varied from 2.3 to 4.3 in five chicken lines from South Africa (Koekoek, New Hampshire Red, Naked-Neck, Lebowa-Venda, and Ovambo) and two chicken populations each from Mozambique and Botswana. Furthermore, Groen et al. (1994) and Emara et al. (2002) also observed similar values of *MNA* in their studies.

In the present study, the $H_e$ value ranged from 0.330 to 0.607 with the values of 0.330 to 0.566 in the Kochi breeds. This result is generally similar to that of van Marle-Köster and Nel (2000) mentioned above, in which the $H_e$ values ranged from 0.314 to 0.612. Also, using microsatellite markers, Vanhala et al. (1998) reported similar $H_e$ values ranging from 0.290 to 0.670 in eight chicken lines (three White Leghorn hybrids, three Finish Landrace lines, a Rhode Island Red line, and a broiler hybrid line). Judging from the values of *MNA* and $H_e$, the genetic variability displayed by the Kochi Prefecture chicken breeds is generally similar to that of chickens reared elsewhere in the world.

The $P_{poly}$ and $H_e$ values of native Japanese breeds in the present study were 0.75 to 1.00 and 0.330 to 0.566, respectively (Table 3). These values are higher than those reported by Hashiguchi et al. (1981) and Okada et al. (1989). These discrepancies are thought to result from the

differences of genetic markers used. We used microsatellite DNA polymorphisms, while both Hashiguchi et al. (1981) and Okada et al. (1989) reported on the $P_{poly}$ and $H_e$ based on blood groups and/or protein polymorphisms.

**Genetic relationships**

The present study demonstrated for the first time the relationships between chicken breeds originating from Kochi Prefecture using microsatellite DNA polymorphisms. According to the NJ trees (Figure 1A and B), the utility breeds (MYA and TKU) and the fancy breeds (OHK, ONA, TJI, TOT, and UZU) originating from Kochi Prefecture, belonged to different clusters. This result is thought to accurately reflect their breeding history. The breeding of the MYA started late in the Meiji Era (1868 to 1912) based on the mating of a Black Minorca male and a Kamochi-dori female (Kamimoto, 1951). The Kamochi-dori seems to have been a small chicken with a bearded face and feathered short shanks, native to Kochi Prefecture (Sawada, 1978). However, this chicken has become extinct, and details are unknown; for example, it is unknown whether it was a pure breed or a hybrid. The TKU breed was established during and after the Meiji Era by mating some Japanese chicken with the Cochin breed, which has Chinese origin and was brought to Japan via England (Mitsui, 1979). Whereas, OHK, ONA, TJI, TOT, and UZU had already been in Kochi Prefecture by the end of the Edo Era (1603 to 1867) many years earlier (Oana, 1951). It is noteworthy that among the Kochi fancy breeds, those having thick and long tail feathers and saddle hackles (OHK, ONA, and TOT) showed a close relationship to the SHO breed, which is one of the oldest Japanese breeds and also has thick and long feathers in the tail and saddle.

There are few reports that dealt with genetic relationships between the chicken breeds originating from Kochi Prefecture. Okada et al. (1984) and Okabayashi et al. (1998) reported on the basis of blood groups and/or blood protein polymorphisms that ONA is genetically close to TOT and apparently far from SHO. This contrasts with our result in which ONA showed a closer genetic distance to SHO than to TOT (Table 5). Oana (1951) purported, based on morphology and old literature, that ONA directly descended from SHO. Although our study revealed that ONA is genetically closer to SHO than to TOT, there is no evidence from the present study to show SHO is a direct ancestor of ONA. The present study only showed that the genetic distance between ONA and SHO is slightly smaller than that between ONA and TOT.

According to Oana (1951), UZU was thought to be directly derived from TJI by a single mutation, because the two breeds are very similar in body shape and size, except for tail morphology. UZU looks like a type of TJI, lacking a rump. In the NJ tree, based on $D_A$ (Figure 1A), TJI was located far from UZU, differing from the hypothesis of Oana (1951). However, in the NJ tree based on $D_{ST}$ (Figure 1B), TJI and UZU showed a close relationship, supporting the hypothesis. Further studies are necessary to reveal the genetic relationship between the TJI and UZU breeds.

## ACKNOWLEDGMENTS

This study was supported in part by a grant-in-aid from Nankoku City, Kochi Prefecture, Japan, and that from the Integrated Research Program for Functionality and Safety of Food Toward the Establishment of a Healthy Diet, The Ministry of Agriculture, Forestry and Fisheries of Japan. We are grateful to the members of Kochi Prefectural Livestock Experimental Station, and Okazaki and Hyogo Stations, National Livestock Breeding Center, for their kind help in collecting blood samples. We are also grateful to fanciers M. Tajima, T. Ikemoto, Y. Sawada, H. Nakahira, Y. Hamada, K. Nishiguchi, T. Matsuoka, and M. Komatsu (Kochi Prefecture) and S. Nishikawa and T. Nakanishi (Mie Prefecture) for their kind help in the collection of blood samples. We wish to thank Dr. H. H. Cheng, USDA, for his kind supply of primer sets of microsatellites.

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