The Genetic Variability and Relationships of Japanese and Foreign Chickens Assessed by Microsatellite DNA Profiling

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ABSTRACT : This is the first study in which genetic variability and relationships of a large number of Japanese chicken breeds were revealed along with those of several foreign breeds by using microsatellite DNA polymorphisms. Twenty-eight breeds (34 populations) of native Japanese chickens and seven foreign breeds or varieties were analyzed. The mean number of alleles per locus, the proportion of the polymorphic loci, and the expected average heterozygosity ranged from 1.75 to 4.70, from 0.55 to 1.00, and from 0.21 to 0.67, respectively. Microsatellite alleles being unique to a particular population were detected in some populations. The D_A genetic distance between populations was obtained from allele frequency for every pair of the populations to construct a neighbor-joining tree. According to the phylogenetic tree, excluding a few exceptions, native Japanese chicken breeds and foreign breeds were clearly separated from each other. Furthermore, the tree topology divided native Japanese chickens into four main classes, which was almost in accordance with the classification based on body morphology; that is, (1) Cochin type, (2) Malay type, (3) layer type, and (4) intermediate type between Malay and layer types. This is the first finding for native Japanese chickens. (**Key Words :** Genetic Relationship, Genetic Variability, Native Japanese Chickens, Microsatellite)

INTRODUCTION

In Japan, there are approximately 50 breeds of native chickens (Tsudzuki, 2003). Most of today's Japanese chicken breeds are thought to have been mainly established from three original breeds, Jidori, Shoukoku, and Oh-Shamo during Japan's period of isolation (1635-1854) (Oana, 1951). Exactly speaking, Jidori is not a word to specify a breed, but a generic name used for various breeds whose ancestors inhabited Japan more than 2,000 years ago. It is thought that in ancient Japan there were many kinds of

Jidoris throughout the country, although there are only three or a bit more breeds of Jidoris in recent Japan. The Shoukoku is thought to have been introduced into Japan from China between the 8th and 12th centuries. The Oh-Shamo is thought to have been derived from a Malay-type chicken introduced into Japan from Thailand in the early 17th century. Most Japanese chicken breeds were developed for special plumage, body shape, crowing, and cockfighting. In addition to these ornamental breeds established during Japan's isolation period, some breeds were established to produce eggs and/or meat during the late stages of the 19th century to the early stages of the 20th century.

Oana (1951) first presumed the origin and lineage of native Japanese chickens based on external appearance and old literature. Thereafter, his hypotheses have become a kind of "Bible" for fanciers and researchers of native Japanese chickens. Later, to confirm the hypotheses of Oana (1951), osteometrical and somatometrical studies were carried out (Nishida et al., 1985a, b). In addition, on the basis of blood groups and/or blood protein polymorphisms, several phylogenetic and variability studies were performed (Hashiguchi et al., 1981; Okabayashi et al., 1998; Okada et al., 1980, 1984, 1989; Tanabe and Mizutani,

Table 1. List of chicken breeds/populations used in this study

Breeds	Breed/population abbreviations	No. of individuals examined	Source of samples
Aidu-Jidori	ADU	24	FAHE[1]
Barred plymouth rock	BPR	24	HS-NLBC[2]
Chabo	CHA	23	ESAH-APAFRC[3], IPLRC[4], LRD-MSTPC[5]
Ehime-Jidori	EHM	24	Kagoshima University
Gifu-Jidori	GJI-G	19	GPLRI[6]
	GJI-T	24	Tokyo Agricultural University
Hinai-dori	HIN	24	APLES[7]
Jitokko	JTK	22	IPLRC, KPLES[8], MLES[9]
Kinpa	KIN	13	ESAH-APAFRC
Koeyoshi	KOE	26	Aomori Prefecture (fanciers)
Kuro-Kashiwa	KRK	24	YPLRI[10]
Ko-Shamo	KSM-H	24	Hiroshima Prefecture (fancier)
	KSM-K	24	Kochi Prefecture (fanciers)
Kumamoto	KUM	16	HS-NLBC
Kawachi-Yakko	KWA	23	Mie Prefecture (fanciers)
Minohiki-dori	MIN	28	IPLRC, SPLES[11]
Mie-Jidori	MJI	30	Mie Prefecture (fanciers)
Miyadi-dori	MYA	24	Kochi Prefecture (fancier)
Nagoya	NAG	24	HS-NLBC
New hampshire red	NHR	24	HS-NLBC
Ohiki	OHK	28	Kochi Prefecture (fanciers)
Onaga-dori	ONA	27	Kochi Prefecture (fanciers)
Oh-Shamo	OSM-H	24	Hiroshima Prefecture (fancier)
	OSM-K	24	Kochi Prefecture (fancier)
Red cornish	RC	24	HS-NLBC
Rhode island rRed	RIR	27	HS-NLBC
Satsuma-dori	SAT	24	Kagoshima Prefecture (fanciers)
Shoukoku	SHO-A	24	IPLRC, LRD-MSTPC
	SHO-B	24	Mie Prefecture (fancier)
Tosa-Jidori	TJI	24	KOPLES[12]
Tosa-Kukin	TKU	26	Kochi Prefecture (fanciers)
Toumaru	TMA	22	NARILRC[13]
Toutenkou	TOT	37	Kochi Prefecture (fancier)
Ukokkei (black)	UKO-B	20	Hiroshima Prefecture (fancier)
Ukokkei (white)	UKO-W	24	Hiroshima Prefecture (fancier)
Uzurao	UZU	23	Kochi Prefecture (fanciers)
White cornish	WC	24	HS-NLBC
White leghorn	WL	24	OS-NLBC[14]
White plymouth rock	WPR	24	HS-NLBC
Yakido	YKD-A	24	LRD-MSTPC
	YKD-B	24	Mie Prefecture (fanciers)

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[7] Akita Prefectural Livestock Experiment Station.

[8] Kagoshima Prefectural Livestock Experiment Station.

[9] Miyazaki Livestock Experiment Station.

[10] Yamaguchi Prefectural Livestock Research Institute.

[11] Shizuoka Prefectural Livestock Experiment Station.

[12] Kochi Prefectural Livestock Experiment Station.

[13] Niigata Agriculture Research Institute Livestock Research Center.

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1980; Tanabe et al., 1991). However, the genetic relationships of native Japanese chicken breeds resulted from these studies were not always in concordance with those from the morphological and literature studies. So, the genetic relationships among native Japanese chicken breeds are still unclear.

Microsatellites show simple repeat sequence polymorphisms (Tautz, 1989). Microsatellites alleles differ in the number of repetitive di-, tri-, or tetra-nucleotide units, and this length variation is detected with polymerase chain reaction (PCR) by using pairs of primers. Recently, microsatellites have become the preferred type of genetic

markers because of their abundance, ease of identification, random distribution, codominant inheritance, high variability, and possibility of automated detection (Milligan et al., 1994; Goldstein and Pollock, 1997; Petit et al., 1997; Paszek et al., 1998). Microsatellite markers have been proven to be useful for assessing genetic variation and diversity in livestock (Buchanan et al., 1994; MacHugh et al., 1994; Martinez et al., 2000; Pandey et al., 2002; Dorji et al., 2003). A large number of chicken microsatellite markers are currently being developed (Groenen et al., 2000; Schmid et al., 2000) and have been employed to evaluate genetic diversity of chicken populations (Crooijmans et al., 1996; Ponsuksili et al., 1996; Vanhala et al., 1998; van Marle-Köster and Nel, 2000; Emara et al., 2002; Hilell et al., 2003). Recently, we studied genetic relationships of native Japanese chickens based on microsatellite DNA polymorphisms (Osman et al., 2004, 2005, 2006). However, these studies were conducted on a small number of breeds in each work.

The aim of this study is to synthetically investigate genetic relationships between a large number of native Japanese breeds of chickens, along with other imported commercial breeds present in Japan.

MATERIALS AND METHODS

Chicken breeds

To make a synthetic analysis, we combined new and earlier (Osman et al., 2004, 2005, 2006) data in this study. Table 1 shows the breeds and number of individuals examined. We investigated 28 breeds of native Japanese chickens (34 populations) (Aidu-Jidori (ADU, Chabo (CHA), Ehime-Jidori (EHM), Gifu-Jidori (GJJ-G and GJI-T), Hinai-dori (HIN), Jitokko (JTK), Kinpa (KIN), Koeyoshi (KOE), Kuro-Kashiwa (KRK), Ko-Shamo (KSM-H and KSM-K), Kumamoto (KUM), Kawachi-Yakko (KWA), Minohiki-dori (MIN), Mie-Jidori (MJI), Miyadi-dori (MYA), Nagoya (NAG), Ohiki (OHK), Onaga-dori (ONA), Oh-Shamo (OSM-H and OSM-K), Satsuma-dori (SAT), Shoukoku (SHO-A and SHO-B), Tosa-Jidori (TJI), Tosa-Kukin (TKU), Toumaru (TMA), Toutenkou (TOT), Ukokkei (UKO-B and UKO-W), Uzurao (UZU), Yakido (YKD-A and YKD-B)), and seven foreign breeds (Barred Plymouth Rock (BPR), New Hampshire Red (NHR), Red Cornish (RC), Rhode Island Red (RIR), White Cornish (WC), White Leghorn (WL), and White Plymouth Rock (WPR)). The data of ADU, BPR, EHM, KUM, NAG, NHR, RC, WC, and WPR were newly obtained, and the others were from our earlier studies (Osman et al., 2004, 2005, 2006). Breed features are described elsewhere (Mitsui, 1979; Kuroda and Yamaguchi, 1987; Okada, 1991; Roberts, 1997; Tsudzuki, 2003).

Based on chicken morphology and old literature, Oana (1951) discussed the origin and lineage of native Japanese chicken breeds in his book. Among the Japanese breeds used in this study, GJI, MJI, and TJI belong to the Jidori group. As described in the introductory section, the ancestors of SHO and OSM are thought to have been imported from China and Thailand, respectively. In addition to these, the ancestors of CHA and UKO are thought to have been imported from Vietnam and China (or India), respectively, around the early stages of the 17th century (Oana, 1951). KUM, MYA, NAG, and TKU are utility breeds for eggs and/or meat production that were bred, through the late stages of the 19th century to the early stages of the 20th century, by mating Japanese indigenous chickens and newly imported foreign breeds (e.g. Cochin, Black Minorca) (Mitsui, 1979). Although the historical events of ADU and EHM are unclear, other Japanese breeds (HIN, JTK, KIN, KOE, KRK, KSM, KWA, MIN, OHK, ONA, SAT, TMA, TOT, UZU, and YKD) are thought to have been established by the middle stage of the 19th century based on various kinds of crossbreeding with SHO, OSM, and various types of Jidoris, and subsequent selective propagation (Oana, 1951).

DNA extraction and microsatellite amplification

Using genomic DNA extracted from the whole blood collected from the ulnar vein, 20 microsatellites, selected by taking the genome coverage of the loci into consideration, were amplified by PCR. The microsatellites used were ADL0262 (Chromosome 23), LEI0092 (Chr. 6), LEI0096 (Chr. 2), LEI0099 (Chr. 12), LEI0135 (Chr. 28), LEI0209 (Chr. 1), MCW0067 (Chr. 10), MCW0145 (Chr. 1), MCW0183 (Chr. 7), MCW0193 (Chr. 5), MCW0214 (Chr. 5), MCW0217 (Chr. 18), MCW0222 (Chr. 3), MCW0233 (Chr. 27), MCW0240 (Chr. 4), MCW0252 (Chr. 3), MCW0295 (Chr. 4), MCW0301 (Chr. 24), MCW0322 (Chr. 13), and MCW0330 (Chr. 17). The PCR fragments were sized and genotyped using an automated ABI 377 sequencer (Applied Biosystems, Foster City, CA). Details of the microsatellites and the experimental methods are described in our earlier papers (Osman et al., 2004, 2005).

Statistical analysis

The genetic variability of each population was assessed 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).

Genetic divergence between the populations was calculated according to D_A genetic distance (Nei et al., 1983) using DISPAN computer program (Ota, internet source), based on the allele frequency of each locus and population directly calculated from the observed genotypes using the program Excel Microsatellite Toolkit (Park,

Table 2-i). 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 (*H_e*), estimated for 41 chicken breeds

Loci	Range (bp)	All[2]	Breeds/populations[1]																			
			ADU	BPR	CHA	EHM	GJI-G	GJI-T	HIN	JTK	KIN	KOE	KRK	KSM-H	KSM-K	KUM	KWA	MIN	MJI	MYA	NAG	NHR
ADL 262	105-107	3	3	2	3	3	3	3	2	3	1	1	1	1	1	2	1	2	3	1	2	2
LEI 092	233-281	16	4	4	3	6	4	3	4	8	3	2	3	6	3	1	1	5	5	3	2	4
LEI 096	216-246	10	2	3	3	5	4	3	5	4	2	1	2	3	3	3	2	4	3	2	3	3
LEI 099	113-135	9	3	3	3	4	3	2	3	3	3	1	1	4	3	2	4	5	2	1	2	4
LEI 135	130-152	9	3	5	3	5	3	3	4	6	1	2	2	5	5	3	6	3	3	4	3	4
LEI 209	138-188	18	7	3	4	6	4	4	3	2	4	2	1	5	5	4	4	4	3	2	4	3
MCW 067	171-183	7	3	3	2	5	2	2	3	3	3	1	2	3	3	2	3	2	4	1	2	3
MCW 145	180-212	9	3	5	5	4	2	3	4	5	2	1	3	4	2	4	3	4	2	1	3	4
MCW 183	294-322	13	3	3	2	3	2	2	2	3	1	3	3	5	3	3	2	1	3	1	1	6
MCW 193	297-321	12	2	3	4	7	3	4	6	6	2	2	2	3	6	2	3	3	5	3	4	4
MCW 214	269-305	17	2	3	3	6	4	3	3	5	4	1	2	3	1	3	3	3	6	3	2	3
MCW 217	147-173	8	5	4	2	5	5	3	2	4	2	3	2	4	2	1	1	4	4	2	2	4
MCW 222	214-224	6	2	2	5	3	3	4	3	4	2	2	3	2	3	2	3	3	3	2	2	3
MCW 233	205-217	7	2	3	2	4	3	3	4	2	2	2	3	3	3	2	4	2	2	2	2	3
MCW 240	171-203	14	2	3	3	10	5	4	4	5	3	1	1	4	5	2	2	2	1	2	1	4
MCW 252	277-307	14	3	2	4	6	4	2	4	6	4	2	2	3	3	2	4	7	2	2	2	5
MCW 295	87-105	9	3	2	4	6	4	2	5	4	2	3	2	4	4	1	1	3	3	2	3	3
MCW 301	263-305	18	2	3	6	8	3	2	3	8	3	3	3	2	5	3	4	3	2	4	5	5
MCW 322	251-261	6	3	3	2	3	3	1	2	2	2	1	2	3	2	3	5	3	3	3	2	3
MCW 330	256-288	12	1	3	2	3	4	1	3	4	1	1	2	2	2	2	2	2	3	2	3	3
		217																				
MNA		10.85	2.90	3.10	3.25	5.10	3.40	2.70	3.45	4.35	2.35	1.75	2.10	3.45	3.20	2.35	2.85	3.30	3.15	2.05	2.45	3.65
P_poly			0.95	1.00	1.00	1.00	1.00	0.90	1.00	1.00	0.80	0.55	0.80	0.95	0.90	0.85	0.80	0.95	0.95	0.75	0.90	1.00
H_e			0.42	0.47	0.47	0.63	0.52	0.43	0.51	0.60	0.35	0.21	0.28	0.47	0.45	0.34	0.37	0.51	0.37	0.33	0.29	0.60

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

Table 2-ii). 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 (*H_e*), estimated for 41 chicken breeds

Loci	Range (bp)	All[2]	Breeds/populations[1]																				
			OHK	ONA	OSM-H	OSM-K	RC	RIR	SAT	SHO-A	SHO-B	TJI	TKU	TMA	TOT	UKO-B	UKO-W	UZU	WC	WL	WPR	YKD-A	YKD-B
ADL 262	105-107	3	3	1	2	2	3	3	2	2	3	2	2	2	2	2	3	1	3	3	2	2	1
LEI 092	233-281	16	4	7	6	3	5	3	5	2	4	1	3	2	2	1	2	4	2	3	3	5	5
LEI 096	216-246	10	4	3	4	5	5	4	6	2	4	3	3	2	2	4	4	5	2	6	3	1	2
LEI 099	113-135	9	1	2	5	3	4	4	2	3	2	1	3	1	1	1	2	3	3	4	5	4	4
LEI 135	130-152	9	3	5	6	4	4	4	6	3	3	3	4	3	3	3	4	5	3	1	4	4	3
LEI 209	138-188	18	3	6	11	8	5	7	6	6	4	4	6	1	2	4	5	5	2	5	5	3	5
MCW 067	171-183	7	3	3	3	4	3	3	3	3	4	2	1	2	3	2	3	4	2	3	3	1	4
MCW 145	180-212	9	3	3	4	2	6	5	4	4	7	2	2	1	3	4	3	2	3	6	3	3	3
MCW 183	294-322	13	3	2	3	6	3	4	5	2	2	2	6	3	2	2	5	4	2	3	4	2	2
MCW 193	297-321	12	4	2	4	4	4	2	6	3	4	3	2	2	5	3	4	2	2	5	4	2	2
MCW 214	269-305	17	2	5	5	3	5	2	6	4	6	3	4	4	3	4	6	5	3	4	3	1	2
MCW 217	147-173	8	3	3	6	5	6	5	6	4	3	4	3	2	4	3	2	1	4	2	3	2	3
MCW 222	214-224	6	3	3	2	3	3	3	4	2	3	2	2	2	2	2	3	2	2	2	3	3	3
MCW 233	205-217	7	5	3	4	3	4	4	4	3	5	3	3	4	3	4	4	4	3	1	3	2	3
MCW 240	171-203	14	5	6	4	5	5	5	4	5	5	3	4	1	4	3	5	3	2	5	5	5	3
MCW 252	277-307	14	5	4	3	5	5	4	6	4	7	3	2	2	4	3	3	3	2	3	2	2	3
MCW 295	87-105	9	2	2	3	4	5	4	3	4	5	4	3	2	2	3	5	3	3	3	3	3	3
MCW 301	263-305	18	3	4	6	5	5	5	7	4	5	3	4	2	2	5	4	4	3	3	5	4	4
MCW 322	251-261	6	3	3	2	2	3	3	5	3	3	3	2	2	3	2	2	2	2	2	2	2	4
MCW 330	256-288	12	2	2	4	2	3	4	4	2	5	4	2	2	3	4	4	2	1	5	1	1	1
		217																					
MNA		10.85	3.20	3.45	4.35	3.90	4.30	3.90	4.70	3.30	4.25	2.70	3.15	2.20	2.50	2.95	3.75	3.60	2.30	2.90	3.75	2.60	3.00
P_poly			0.95	0.95	1.00	1.00	1.00	1.00	1.00	1.00	1.00	0.90	0.95	0.85	0.90	0.90	1.00	1.00	0.95	0.80	1.00	0.80	0.90
H_e			0.49	0.40	0.62	0.62	0.66	0.61	0.67	0.46	0.54	0.36	0.47	0.30	0.35	0.40	0.56	0.57	0.37	0.46	0.58	0.38	0.40

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

internet source). Takezaki and Nei (1996) studied the efficiencies of many methods for calculating genetic distances from microsatellite DNA data, for example, D_A (Nei et al., 1983), D_c (Cavalli-Sforza and Edwards, 1967), D_{sw} (Shriver et al., 1995), and $(\delta\mu)^2$ (Goldstein et al., 1995) distances. Their results indicated that the D_A and D_c distances are most efficient in obtaining the correct tree topology. Since the D_A distance is the modified D_c distance, we selected the D_A distance in our study.

The Phylogenetic tree was constructed using neighbour-joining (NJ) clustering method (Saitou and Nei, 1987) based on the D_A distance. Bootstrap resampling (n = 1,000) was performed to test the robustness of the dendrogram topologies. The tree was visualized using the TREEVIEW program (Page, internet source).

RESULTS

Genetic variability

The allele and genotype frequencies of 20 microsatellite loci were determined in 34 populations of 28 breeds of native Japanese chickens and seven foreign chicken populations. Genetic variability estimated for each population is summarized in Table 2. The allele size range

Table 3. Microsatellite alleles specific to each breed/population

Breeds/ populations[1]	No. of alleles	Locus and allele sizes (bp)
ADU	0	
BPR	0	
CHA	2	MCW0222: (214), MCW0301: (301)
EHM	2	MCW0067: (183), MCW0301: (303)
GJI-G	2	MCW0330: (260, 272)
GJI-T	0	
HIN	0	
JTK	3	MCW0252: (277), MCW0301: (289), MCW0183: (322)
KIN	0	0
KOE	0	0
KRK	2	LEI0092: (263), LEI0096: (244)
KSM-H	0	0
KSM-K	1	MCW0193: (297)
KUM	0	0
KWA	3	MCW0233: (209, 211), MCW0322: (259)
MIN	2	MCW0214: (305), MCW0252: (307)
MJI	2	MCW0067: (171), MCW0330: (278)
MYA	1	MCW0217: (147)
NAG	2	MCW0193: (321), MCW0214: (301)
NHR	1	MCW0301: (295)
OHK	0	
ONA	1	LEI0092: (265)
OSM-H	1	LEI0209: (160)
OSM-K	0	
RC	1	MCW0214: (297)
RIR	0	0
SAT	1	MCW0322: (261)
SHO-A	1	LEI0099: (117)
SHO-B	4	LEI0092: (233), MCW0214: (299), MCW0252: (285), MCW0330: (274)
TJI	0	0
TKU	0	0
TMA	1	MCW0214: (291)
TOT	0	0
UKO-B	1	MCW0252: (305)
UKO-W	1	MCW0240: (203)
UZU	0	0
WC	0	0
WL	2	LEI0209: (164, 172)
WPR	0	0
YKD-A	0	0
YKD-B	1	MCW0067: (173)

[1] See Table 1 for the abbreviation.

of polymorphic loci was from 2 bp difference (105 to 107 bp) at locus ADL0262 to 50 bp difference (138 to 188 bp) at locus LEI0209 and showed a large variation across the loci. A total of 217 alleles were observed at the 20 loci in 986 individuals from 41 chicken populations. The number of alleles per locus across all breeds/populations ranged from 3 (ADL0262) to 18 (LEI0209 and MCW0301) with the *MNA* of 10.85. The *MNA* in each population ranged from 1.75 (KOE) to 4.70 (SAT). The lowest value of the P_{poly} (0.55) was estimated in the KOE population, while 17 populations

(BPR, CHA, EHM, GJI-G, HIN, JTK, NHR, OSM-H, OSM-K, RC, RIR, SAT, SHO-A, SHO-B, UKO-W, UZU, and WPR) showed polymorphisms in all of the 20 microsatellite markers (i.e. $P_{poly} = 1.00$). The H_e varied from 0.21 (KOE) to 0.67 (SAT).

Twenty-three populations had one or more private alleles, that is, alleles unique to a single population (Table 3). There were four private alleles in the SHO-B; three in the JTK and KWA, two in the CHA, EHM, GJI-G, KRK, MIN, MJI, NAG, and WL, and one in the KSM-K, MYA, NHR, ONA OSM-H, RC, SAT, SHO-A, UKO-B, UKO-W, TMA, and YKD-B. There were no private alleles in 18 populations (ADU, BPR, GJI-T, HNI, KIN, KOE, KSM-H, KUM, OHK, OSM-K, RIR, TJI, TKU, TOT, UZU, WC, WPR, and YKD-A).

Genetic distance

The D_A genetic distance matrix estimated between every pair of populations is presented in Table 4. There were 820 possible population pairs, of which the D_A values ranged from 0.103 to 0.716 with the mean value (±SD) of 0.463±0.095. The lowest (0.103) and highest (0.716) distances were estimated between OSM-H and OSM-K and between KOE and NAG populations, respectively. The D_A values estimated between Japanese breed populations and each of the BPR, NHR, RC, RIR, WC, WL, and WPR ranged from 0.373 to 0.621, from 0.236 to 0.550, from 0.309 to 0.576, from 0.271 to 0.570, from 0.378 to 0.674, from 0.427 to 0.648, and from 0.271 to 0.577, respectively, with the mean values (±SD) of 0.508±0.056, 0.432±0.071, 0.447±0.062, 0.431±0.071, 0.548±0.078, 0.552±0.057, and 0.453±0.071, respectively.

Phylogenetic relationships

Figure 1 visualizes the genetic relationships among breeds/populations as the NJ tree reconstructed based on the D_A distance matrix (Table 4). According to the NJ tree, excluding a few exceptions, native Japanese chicken breeds and foreign breeds were clearly separated from each other (Clusters A and B). As for exceptional cases, four Japanese breeds (KRK, TMA, KUM, and TKU) were combined with six foreign breeds (WL, BRB, WPR, RIR, NHR, and RC). In addition, an American breed WC was clustered with three Japanese breed populations (HIN, UKO-B, and UKO-W).

Within native Japanese breeds, there were two major clusters: one was composed of HIN, UKO-B, and UKO-W (Cluster C), and a second consisted of 22 breeds (27 populations) (Cluster D). In Cluster C, both HIN and UKO breeds have a Cochin-type (meat-type) body, although their body size is not so large. Cluster D was divided into two subclusters: one included KOE, MJI, OSM-H, OSM-K, YKD-A, YKD-B, KIN, KSM-H, and KSM-M (Cluster E),

Table 4. i) Pairwise genetic distance (D_A) estimated between 41 chicken breeds/populations

Breeds/populations[1]	ADU	BPR	CHA	EHM	GJI-G	GJI-T	HIN	JTK	KIN	KOE	KRK	KSM-H	KSM-K	KUM	KWA	MIN	MJI	MYA	NAG	NHR	OHK
ADU	0																				
BPR	0.544	0																			
CHA	0.490	0.543	0																		
EHM	0.409	0.437	0.264	0																	
GJI-G	0.465	0.466	0.469	0.339	0																
GJI-T	0.459	0.565	0.462	0.335	0.300	0															
HIN	0.524	0.431	0.425	0.338	0.381	0.434	0														
JTK	0.441	0.428	0.396	0.320	0.426	0.429	0.282	0													
KIN	0.557	0.595	0.490	0.368	0.568	0.615	0.437	0.401	0												
KOE	0.469	0.536	0.564	0.405	0.546	0.566	0.506	0.495	0.403	0											
KRK	0.498	0.531	0.481	0.409	0.536	0.525	0.429	0.461	0.468	0.573	0										
KSA-H	0.482	0.498	0.374	0.351	0.436	0.488	0.405	0.338	0.307	0.439	0.545	0									
KSA-K	0.485	0.591	0.352	0.346	0.433	0.444	0.410	0.368	0.308	0.484	0.584	0.140	0								
KUM	0.635	0.488	0.578	0.509	0.549	0.635	0.478	0.577	0.656	0.686	0.557	0.614	0.628	0							
KWA	0.579	0.539	0.437	0.445	0.485	0.484	0.425	0.451	0.504	0.642	0.673	0.400	0.398	0.638	0						
MIN	0.575	0.555	0.496	0.459	0.500	0.547	0.452	0.412	0.554	0.611	0.617	0.433	0.465	0.610	0.506	0					
MJI	0.654	0.515	0.532	0.429	0.573	0.498	0.438	0.446	0.493	0.463	0.511	0.475	0.533	0.504	0.526	0.549	0				
MYA	0.595	0.472	0.597	0.457	0.563	0.579	0.483	0.482	0.538	0.547	0.585	0.434	0.484	0.570	0.472	0.589	0.602	0			
NAG	0.574	0.446	0.491	0.479	0.492	0.555	0.408	0.393	0.573	0.716	0.594	0.462	0.500	0.577	0.516	0.509	0.510	0.575	0		
NHR	0.476	0.304	0.446	0.355	0.386	0.507	0.236	0.375	0.493	0.491	0.413	0.453	0.486	0.356	0.490	0.495	0.467	0.484	0.368	0	
OHK	0.460	0.589	0.447	0.324	0.437	0.446	0.491	0.470	0.525	0.656	0.538	0.506	0.427	0.664	0.541	0.530	0.649	0.594	0.499	0.538	0
ONA	0.411	0.562	0.490	0.344	0.416	0.411	0.500	0.542	0.597	0.561	0.575	0.488	0.473	0.635	0.529	0.552	0.633	0.470	0.563	0.550	0.314
OSM-H	0.458	0.527	0.359	0.306	0.454	0.399	0.378	0.334	0.422	0.441	0.459	0.242	0.266	0.610	0.446	0.384	0.425	0.517	0.437	0.390	0.431
OSM-K	0.461	0.503	0.408	0.309	0.404	0.413	0.356	0.335	0.381	0.426	0.485	0.225	0.250	0.532	0.423	0.365	0.373	0.492	0.441	0.409	0.421
RC	0.462	0.296	0.491	0.346	0.392	0.481	0.334	0.387	0.529	0.466	0.489	0.446	0.485	0.548	0.512	0.445	0.460	0.424	0.143	0.551	
RIR	0.488	0.325	0.453	0.324	0.393	0.458	0.271	0.394	0.570	0.504	0.481	0.475	0.495	0.366	0.525	0.519	0.415	0.423	0.399	0.165	0.517
SAT	0.390	0.373	0.397	0.249	0.317	0.326	0.289	0.202	0.419	0.465	0.412	0.290	0.342	0.434	0.430	0.380	0.404	0.435	0.337	0.286	0.404
SHO-A	0.338	0.527	0.396	0.275	0.376	0.411	0.439	0.419	0.465	0.459	0.513	0.371	0.376	0.544	0.469	0.409	0.574	0.478	0.556	0.483	0.298
SHO-B	0.348	0.454	0.392	0.264	0.403	0.367	0.360	0.336	0.396	0.488	0.418	0.374	0.395	0.566	0.477	0.463	0.511	0.496	0.539	0.417	0.324
TJI	0.514	0.553	0.432	0.333	0.486	0.394	0.374	0.426	0.492	0.559	0.466	0.430	0.399	0.640	0.437	0.518	0.563	0.500	0.484	0.430	0.436
TKU	0.529	0.512	0.476	0.409	0.490	0.493	0.493	0.455	0.609	0.597	0.501	0.540	0.570	0.400	0.476	0.549	0.454	0.580	0.587	0.386	0.594
TMA	0.476	0.467	0.468	0.345	0.505	0.586	0.393	0.429	0.370	0.531	0.362	0.499	0.501	0.566	0.538	0.594	0.551	0.538	0.569	0.422	0.538
TOT	0.518	0.621	0.564	0.438	0.497	0.534	0.546	0.535	0.539	0.624	0.574	0.480	0.490	0.659	0.561	0.582	0.632	0.519	0.490	0.532	0.379
UKO-B	0.527	0.486	0.403	0.354	0.494	0.517	0.449	0.438	0.541	0.482	0.580	0.515	0.525	0.575	0.566	0.607	0.571	0.527	0.532	0.459	0.505
UKO-W	0.383	0.446	0.404	0.315	0.402	0.429	0.290	0.313	0.443	0.434	0.426	0.403	0.408	0.551	0.507	0.440	0.514	0.497	0.402	0.314	0.380
UZU	0.523	0.514	0.367	0.286	0.420	0.461	0.418	0.422	0.421	0.546	0.469	0.355	0.395	0.580	0.521	0.431	0.541	0.517	0.536	0.430	0.352
WC	0.586	0.537	0.539	0.439	0.469	0.499	0.416	0.466	0.674	0.596	0.566	0.609	0.602	0.480	0.655	0.641	0.550	0.535	0.531	0.401	0.634
WL	0.532	0.497	0.590	0.427	0.511	0.552	0.443	0.479	0.587	0.556	0.550	0.537	0.607	0.648	0.534	0.588	0.546	0.547	0.564	0.371	0.603
WPR	0.516	0.333	0.495	0.364	0.387	0.518	0.271	0.375	0.474	0.491	0.442	0.414	0.479	0.425	0.562	0.481	0.401	0.577	0.391	0.174	0.522
YKD-A	0.643	0.478	0.538	0.419	0.523	0.588	0.401	0.452	0.465	0.491	0.396	0.398	0.477	0.637	0.567	0.545	0.468	0.436	0.546	0.450	0.611
YKD-B	0.607	0.490	0.484	0.379	0.534	0.574	0.400	0.383	0.339	0.442	0.459	0.259	0.358	0.613	0.459	0.481	0.425	0.451	0.515	0.425	0.590

and the other was comprised of the remaining 16 breeds (18 populations) (Cluster F). All breeds in Cluster E, excluding MJI, have an erect body (Malay-type body) and a pea comb, and belong to the Shamo or Shamo-related group. Cluster F was divided into two further subclusters: one consisted of MIN, NAG, JTK, and SAT (Cluster G), and the other was composed of the remaining 12 breeds (14 populations) (Cluster H). The breeds in Cluster G, except NAG, have a somewhat erect body (intermediate-type body between the Malay and layer types), a pea comb, and thick tail feathers. On the other hand, all the breeds in Cluster H have a layer-type body, although UZU and CHA show mutation in tail morphology (rumplessness in UZU and erect tail feathers in CHA). OHK, ONA, TOT, and SHO breeds were combined as one group (Cluster I). These four breeds have a larger number of tail feathers and saddle hackles, and these feathers are quite long and tend to drag on the ground, even though they have the usual layer-type body shape.

DISCUSSION

Genetic variability

Genotyping at various microsatellite loci across the genome indicated that the chicken populations examined were genetically different. The microsatellite allelic composition and frequencies differed among the 41 chicken populations, and some alleles were observed as breed/population-private alleles. Nowadays, native Japanese chickens are mated to imported breeds to produce specialized delicious meat (Japan Chicken Association, 2003), because native Japanese chicken meat is high in quality. Breed/population-private alleles may be used as a diagnostic marker to trace the origin of meat when bland-chicken meat has been fraudulently transacted.

In the present study, the number of alleles per locus across all populations studied were three to 18 (Table 2). This result is generally similar to those of Crooijmans et al. (1993, 1996), Cheng et al. (1995), Ponsuksili et al. (1996), and Hilell et al. (2003). While, the *MNA* in each breed varied from 1.75 to 4.70. This result also resembles that of van Marle-Köster and Nel (2000), in which the *MNA* varied from 2.30 to 4.30 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, Emara et al. (2002) and Hilell et al. (2003) also observed similar values of *MNA* in their studies.

In our study, the H_e value ranged from 0.21 to 0.67. This result generally resembles that of van Marle-Köster and Nel (2000) mentioned above, in which the H_e values ranged from 0.31 to 0.61. Also, using microsatellite markers, Vanhala et al. (1998) reported similar H_e values ranging

Table 4. ii) Pairwise genetic distance (D_A) estimated between 41 chicken breeds/populations

Breeds/populations[1]	ONA	OSM-H	OSM-K	RC	RIR	SAT	SHO-A	SHO-B	TJI	TKU	TMA	TOT	UKO-B	UKO-W	UZU	WC	WL	WPR	YKD-A	YKD-B
ADU																				
BPR																				
CHA																				
EHM																				
GJI-G																				
GJI-T																				
HIN																				
JTK																				
KIN																				
KOE																				
KRK																				
KSA-H																				
KSA-K																				
KUM																				
KWA																				
MIN																				
MJI																				
MYA																				
NAG																				
NHR																				
OHK																				
ONA	0																			
OSM-H	0.464	0																		
OSM-K	0.488	0.103	0																	
RC	0.501	0.414	0.424	0																
RIR	0.468	0.407	0.406	0.165	0															
SAT	0.415	0.255	0.220	0.309	0.273	0														
SHO-A	0.275	0.328	0.319	0.468	0.473	0.351	0													
SHO-B	0.308	0.364	0.369	0.419	0.400	0.323	0.184	0												
TJI	0.447	0.351	0.364	0.431	0.418	0.365	0.413	0.367	0											
TKU	0.578	0.510	0.485	0.410	0.395	0.384	0.499	0.426	0.570	0										
TMA	0.586	0.536	0.518	0.445	0.425	0.404	0.485	0.310	0.447	0.491	0									
TOT	0.359	0.414	0.454	0.576	0.535	0.424	0.412	0.412	0.505	0.582	0.559	0								
UKO-B	0.435	0.495	0.512	0.403	0.325	0.383	0.462	0.454	0.498	0.522	0.472	0.536	0							
UKO-W	0.440	0.332	0.328	0.354	0.361	0.234	0.389	0.366	0.387	0.475	0.426	0.471	0.275	0						
UZU	0.409	0.360	0.363	0.478	0.441	0.353	0.355	0.333	0.438	0.505	0.466	0.475	0.503	0.438	0					
WC	0.569	0.568	0.557	0.364	0.286	0.430	0.616	0.519	0.558	0.576	0.531	0.667	0.378	0.389	0.588	0				
WL	0.634	0.575	0.568	0.388	0.420	0.461	0.558	0.489	0.648	0.531	0.535	0.614	0.577	0.454	0.645	0.480	0			
WPR	0.575	0.410	0.367	0.217	0.230	0.373	0.514	0.434	0.509	0.434	0.441	0.547	0.510	0.370	0.385	0.441	0.387	0		
YKD-A	0.550	0.368	0.368	0.453	0.417	0.410	0.468	0.388	0.444	0.529	0.435	0.552	0.487	0.440	0.540	0.594	0.544	0.483	0	
YKD-B	0.561	0.294	0.282	0.475	0.425	0.373	0.442	0.355	0.425	0.546	0.428	0.550	0.501	0.445	0.487	0.595	0.524	0.457	0.138	0

[1] See Table 1 for the abbreviation.

from 0.29 to 0.67 in eight chicken lines (three White Leghorn hybrids, three Finish Landrace lines, a Rhode Island Red line, and a broiler hybrid line). Furthermore, Hilell et al. (2003) found similar values in the study with 52 populations of European and commercial chickens. Native Chinese chicken breeds analysed by Cheng et al. (2004) also showed approximately similar values of H_e. Judging from the values of MNA and H_e, the genetic variability possessed by native Japanese chicken breeds is generally similar to that of chickens reared in the world.

Genetic relationships among breeds/populations

The phylogenetic dendrogram (Figure 1) well reflected origins and body morphology of chicken breeds. Japanese breeds and foreign commercial breeds were clearly separated (Clusters A and B), although some exceptions were recognized. In cluster A, Japanese breeds KUM and TKU were combined with foreign commercial breeds. However, this seems to be a reasonable result, because the KUM and TKU are utility breeds that were created from the Cochin breed. The Cochin breed also has contributed to the establishment of most of foreign commercial breeds (Roberts, 1997). In cluster A, Japanese breeds KRK and TMA were also combined with foreign commercial breeds far from almost all Japanese breeds. This result is well

consistent with the report of Okabayashi et al. (1998), in which they analyzed genetic relationships of chicken breeds based on blood protein polymorphisms. In the report, they suggested the possibility that the TMA would receive the gene flow from some Chinese breed. Oana (1951) also assumed that TMA was derived from Oh-Toumaru, which was a large breed imported from China in the early stage of the 17th century. Although Oh-Toumaru has been extinct and details of this breed are unknown, there is a possibility that Oh-Toumaru was a breed genetically similar to Cochin. In this case, the phenomenon might occur that TMA is genetically close to foreign commercial breeds, which were also genetically influenced by the Cochin. However, both KRK and TMA do not have cochin-type body, but have rather layer-type body.

Oana (1951) presumed that KRK and TMA were genetically close to SHO. However, from the present study, we could not obtain the evidence that SHO is genetically close to KRK and TMA. Further studies will be necessary to confirm the origin of KRK and TMA and genetic relationship between these two breeds and other Japanese breeds.

In Japanese chickens (Cluster B), UKO and HIN comprised of one cluster (Cluster C). So far, there is no report or hypothesis that UKO and HIN are genetically
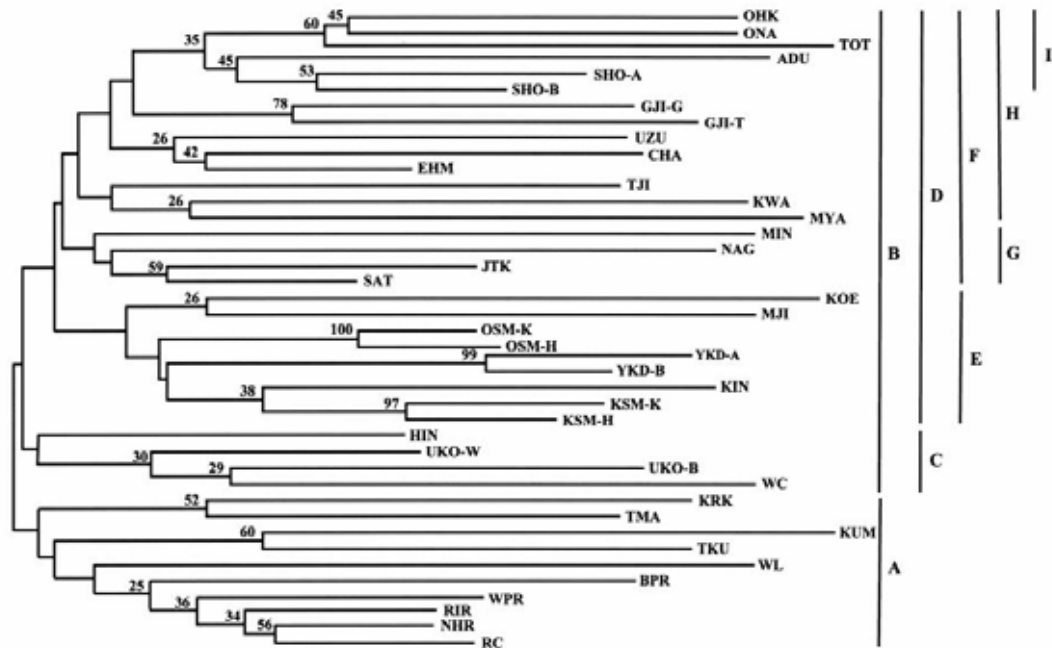
Figure 1. Neighbour-joining tree showing the genetic relationships among 28 breeds (34 populations) of native Japanese chickens and seven foreign breeds or varieties, using D_A genetic distance calculated from 20 microsatellite loci. Bootstrap values less than 25% are not shown. See Table 1 for the abbreviations of breed/population names.

close. However, both of these breeds show Cochin-type body shape, which is rarely seen in Japanese fancy breeds. Thus, there might be some close breeding history between them. Additional studies will be necessary to confirm it.

The ancestor of UKO is thought to have been introduced into Japan at the early stages of the 17th century (Oana, 1951). There is no literature or assumption that, from the introduction time to the present day, crossbreeding has been frequently done between the UKO and other breeds. The tree topology of Figure 1 seems to well reflect this history, because Cluster C, to which UKO belonged, was clearly distinguished from Cluster D in which many Japanese breeds were included.

Cluster E of Figure 1 was composed of so-called Shamo-group and Shamo-related breeds. Based on the external morphology, OSM, YKD, KIN, and KSM are classified as Shamo-group breeds having Malay-type body shape (Oana, 1951). KOE has a similar body shape to OSM, and is thought to be a Shamo-related breed (Oana, 1951). Oana (1951) presumed that one more ancestral breed of KOE is TMA. However, it is difficult from this study to think that TMA is an ancestral breed of KOE, because TMA showed a far distance from KOE in Figure 1. As an exceptional case, MJI was combined in Cluster E. MJI is one of Jidoris and has a layer-type body shape, greatly differing from the morphology of Shamo-group and Shamo-related breeds. It is likely that random gene drift and/or bottleneck effect may lead to this result. According to

anonymous fanciers, the number of MJI is very small in recent Japan.

MIN, NAG, JTK, and SAT were combined together in Cluster G. This result supported Oana's hypothesis based on morphological observation that MIN, JTK, and SAT are genetically close (Oana, 1951). Having a pea comb, thick tail feathers, and a somewhat erect body shape are common in these breeds. On the other hand, NAG has a single comb and a Cochin-type body, greatly differing from these three breeds in external appearance. NAG was established in Aich Prefecture by crossbreeding of Cochin and some native Japanese breed (Oana, 1951). However, the name of the Japanese breed is unknown. The origin of MIN is also in Aich Prefecture. Thus, there is a possibility that the MIN breed was used to create the NAG.

In Cluster H, TJI, KWA, and MYA showed a close relationship. According to the assumption of Oana (1951), TJI contributed to the establishment of KWA, which was supported by the present study. MYA was a breed established in Kochi Prefecture by crossbreeding Black Minorca and Kamochi-dori (Sawada, 1978). The Kamochi-dori has been extinct and details are unknown about this breed. However, it is clear that the origin of the Kamochi-dori is in Kochi Prefecture. TJI has its origin also in Kochi Prefecture. Accordingly, there is a possibility that the Kamochi-dori has close genetic relationship to TJI, which might result in the somewhat close relationship between TJI and MYA.

In Cluster H, CHA was combined with EHM. According to anonymous fanciers, EHM is not a true Jidori, but a descendant of crossbreds between CHA and some other breeds, although EHM has the word Jidori in its breed name, Ehime-Jidori. Our result supported the fanciers' view.

OHK, ONA, TOT, ADU, and SHO were combined in Cluster I as seen in Figure 1. OHK, ONA, TOT, and SHO are grace breeds whose males have a large amount of flowing tail feathers and saddle hackles. These feathers are long and frequently drag on the ground. Oana (1951) assumed based on the external appearance of these breeds that OHK, ONA, TOT, and SHO are genetically close. This has been supported by the present study at a DNA level.

According to anonymous fanciers, ADU is not a true Jidori, but a descendant of hybrids between SHO and some other breed, although ADU has the word Jidori in its breed name, Aidu-Jidori. Our result supported the fanciers' opinion because ADU showed a close relationship to SHO.

GJI showed a somewhat close relationship to the breeds, OHK, ONA, TOT, and SHO, having thick and long tail feathers. The GJI shows usual morphology in tail feathers and saddle hackles as typically seen in the Leghorn breeds. So far, there is no report or assumption that GJI is genetically close to these long tailed breeds. Further studies will be necessary to confirm the genetic relationship between the GJI and long tailed breeds.

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