Pascoe PL, Patton SJ, Critcher R, and Dixon DR, 1996. Robertsonian polymorphism in the marine gastropod, *Nucella lapillus*: advances in karyology using rDNA loci and NORs. Chromosoma 104:455–460.

Phillips RB, Pleyte KA, and Hartleys E, 1988. Stock-specific differences in the number of chromosome positions of the nucleolar organizer regions in arctic char (*Salvelinus alpinus*). Cytogenet Cell Genet 48:9–12.

Sabelli B and Spada G, 1981. Guida illustrata all'identificazione delle conchiglie del Mediterraneo. Boll Malac (Suppl) XVII(3–4) G.I. 18

Sabelli B and Spada G, 1986. Guida illustrata all'identificazione delle conchiglie del Mediterraneo. Boll Malac. (Suppl) XXII(1–4) G.I. 22.

Schmid M and Guttenbach M, 1988. Evolutionary diversity of reverse (R) fluorescent chromosome bands in vertebrates. Chromosoma 97:101–114.

Schmid M, Feichtinger W, Weimer R, Mais C, Bolaños F, and Leon P, 1995. Chromosome banding in Amphibia. XXI. Inversion polymorphism and multiple nucleolus organizer regions in *Agalychnis callidryas* (Anura, Hylidae). Cytogenet Cell Genet 69:18–26.

Schmid M, Haaf T, Geile B, and Sims S, 1983. Chromosome banding in Amphibia. VII. an unusual XY/XX sex chromosome system in *Gastrotheca riobambae* (Anura, Hylidae). Chromosoma 88:69–82.

Sella G, Vitturi R, Ramella L, Colomba MS. 1995. Chromosomal nucleolar organizer region (NOR) phenotypes in nine species of the genus *Ophryotrocha* (Polychaeta, Dorvilleidae). Mar Biol 124:425–433.

Singh L, Purdom IF, and Jones KW, 1980. Sex chromosomes associated satellite DNA: evolution and conservation. Chromosoma 79:137–157.

Sola L, Monaco PJ, and Rasch EM, 1990. Cytogenetics of bisexual/unisexual species of *Poecilia.* 1. C-bands, Ag-NOR polymorphism, and sex chromosomes in three populations of *Poecilia latipinna.* Cytogenet Cell Genet 53:148–154.

Staiger H, 1954. Der chromosomendimorphismus beim Prosobranchier *Purpura lapillus* in Beziehung zur ökologie der Art. Chromosoma 6:419–478.

Sumner AT, 1972. A simple technique for demonstrating centromeric heterochromatin. Exp Cell Res 101:235–243.

Sysoev AV and Kantor YI, 1987. Deep-sea gastropods of the genus Aforia (Turridae) of the Pacific: species composition, systematics, and functional morphology of the digestive system. Veliger 30:105–126.

Taylor JD and Morris NJ, 1988. Relationships of Neogastropoda. Malacol Rev 4(suppl):167–179.

Thiriot-Quievreux C and Ayraud N, 1982. Les karyotypes de quelques espèces de bivalves et de gastèropodes marins. Mar Biol 70:165–172.

Vitturi R, 1992. Conventionally stained chromosomes, constitutive heterochromatin and nucleolus organizer regions in *Milax nigricans* (Gastropoda, Pulmonata). Chromatin 1:147–155.

Vitturi R and Catalano E, 1988. A male XO sex-determining mechanism in *Theodoxus meridionalis* (Neritidae) (Prosobranchia, Archaeogastropoda). Cytologia 53:131–138.

Vitturi R and Catalano E, 1990. Spermatocyte chromosome banding studies in *Buccinulum corneum* (Prosobranchia: Neogastropoda): variation in silver-NOR banding pattern. Mar Biol 104:259–263.

Vitturi R, Catalano E, Colombera D, Avila AL, and Fucà A, 1993. Multiple sex-chromosome system of *Pterotrachea hippocampus* (Mollusca: Mesogastropoda). Mar Biol 115:581–585.

Vitturi R, Catalano E, Macaluso M, and Maiorca A, 1987. Spermatocyte chromosomes in six species of Neogastropoda (Mollusca, Prosobranchia). Biol Zent bl 106: 81–88.

Vitturi R, Catalano E, Macaluso M, and Zava B, 1988. The karyology of *Littorina neritoides* (Linnaeus, 1758) (Mollusca, Prosobranchia). Malacologia 29:319–324.

Vitturi R, Colombera D, Catalano E, and Amico FP, 1991. Spermatocyte chromosome analysis of *Helicella virgata* (Pulmonata: Helicidae): silver-stained and C-banded chromosomes. J Hered 82:339–343.

Vitturi R, Libertini A, Mazzola A, Colomba MS, Sarà G, 1996. Characterization of mitotic chromosomes of four species of the genus *Diplodus* Rafinesque, 1810 (Pisces, Sparidae): karyotypes and chromosomal nucleolar organizer region (NOR) phenotypes. J Fish Biol 49:1128–1137.

Vitturi R, Libertini A, Panozzo M, and Mezzapelle G. 1995. Karyotype analysis and genome size in three Mediterranean species of periwinkles (Prosobranchia: Mesogastropoda). Malacologia 37:123–132.

Volpi EV, Pelliccia F, Lanza V, Di Castro M, and Rocchi A, 1992. Morphological differentiation of a sex chromosome and ribosomal genes in *Asellus aquaticus* (Crust. Isop.). Heredity 69:478–482.

White MJD, 1973. Animal cytology and evolution, 3rd ed. Cambridge: Cambridge University Press.

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Genetic Relationships Among Japanese Native Breeds of Chicken Based on Microsatellite DNA Polymorphisms

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Genetic relationships among Japanese native breeds of chickens were studied on the basis of microsatellite DNA polymorphisms. DNA samples from 10 Japanese native breeds (Iwate-Jidori, Aizu-Jidori, Sadohige-Jidori, Siba-Tori, Onaga-Dori, Echigonankin, Hinai, Kinpa, Koeyoshi, and Tomaru) and one imported breed (White Leghorn) were analyzed using eight microsatellite markers that were isolated from a microsatellite DNA-enriched library of chickens (Takahashi et al. 1996). The PCR primers to detect $(CA)_n$ repeat length polymorphisms were synthesized based on the sequences of clones, and these markers were typed by PCR amplification and electrophoresis using a DNA sequencer. Since all eight microsatellite markers were polymorphic, genetic distance between the breeds could be calculated based on the frequencies of alleles of the microsatellites and phylogenetic relationships between the breeds could be estimated. Most Japanese native chickens were grouped into three groups that correspond to the origin breeds, Jidori, Shokoku, and Shamo. The results suggest that microsatellite DNA markers are a useful tool for studying the genetic relationships among chicken breeds.

Japan was essentially isolated from the outside world from 1635 to 1854. In that period many unique breeds were developed for special plumage, crowing, and cockfighting. There are more than 30 distinctive breeds. Seventeen of them have been designated as national treasures of Japan. Since most Japanese chicken breeds have low egg production and meat yield, many of these breeds are in danger of disappearing. They are valuable as genetic resources and are being conserved within the Ministry of Agriculture, Forestry and Fisheries (MAFF). The National Institute of Agrobiological Resources of MAFF has been collecting semen of various Japanese breeds and preserves samples in a frozen state. To date 14 breeds (3 varieties) are conserved.

Genetic relationships among Japanese native breeds of chickens have been studied based on blood protein polymorphisms (Hashiguchi et al. 1981; Okada et al. 1980; Tanabe and Mizutani 1980). However, these reports indicated a limited number of polymorphic loci and alleles per loci, so consensus about the genetic relationships among breeds of Japanese native chickens has not been established. In addition, the results of earlier reports do not agree with the morphological characteristics of breeds.

Microsatellite repeat sequences, for example, $(CA)_n$ repeats, are well dispersed in the genome, highly polymorphic, and have been shown to be powerful tools in genome mapping of chickens (Cheng et al. 1995). The application of the microsatellites to characterize chicken breeds is relatively recent. Recently we reported an efficient method for cloning microsatellites in chickens (Takahashi et al. 1996). The purpose of this study is to define the genetic relationships among Japanese native breeds of chickens on the basis of microsatellite DNA polymorphisms.

Materials and Methods

Samples

We studied unrelated chickens belonging to 10 breeds (11 populations) of Japanese native chickens: Iwate-Jidori (19 individuals), Aizu-Jidori (20), Sadohige-Jidori (22), Siba-Tori (16), Onaga-Dori [sampled in Kochi Prefecture (22) and Fukushima Prefecture (15)], Echigonankin (12), Hinai (15), Kinpa (22), Koeyoshi (24), Tomaru (15), and one imported breed—White Leghorn

Table 1. Polymerase chain reaction (PCR) primers for microsatellite markers

Clone	GenBank[a]	Repeat	Forward primer	Reverse primer	Alleles	Range[b]
1	U60782	$(CA)_{12}$	5′–TTTCACACGCAGCCTTTCTCCCG–3′	5′–GTCATTCCTGCCTCCCCTTGAC–3′	6	120–130
2	U60783	$(CA)_{9}$	5′–GTGCAGCTCAGTTGGACACACGC–3′	5′–CAGCGGGTAACGGCGGCGGGACA–3′	10	72–100
3	U60786	$(CA)_{11}$	5′–CAACTTCACTGCCTTCCCATTTG–3′	5′–AACAGAGGAGAAATGGGAATAGTG–3′	5	114–126
4	U60787	$(CA)_{8}$	5′–GATGCCCTCAGCCACCAGCCCT–3′	5′–CACCCAGCAAACAGGAGCCCAC–3′	2	144–146
5	U60791	$(CA)_{9}$	5′–TTAGCAAGGATAGGGGTGGAACA–3′	5′–AACAGAGAACACACTACGCAGCCT–3′	6	93–103
6	U60792	$(CA)_{12}$	5′–GTCCTTTCTCTGTCCTTCCCACT–3′	5′–GTCTTGCTTCTAGGAGTCAGGCT–3′	7	144–158
7	U60793	$(CA)_{8}$	5′–AGAGGTGGGCAGGTGGGCATGAG–3′	5′–CAGCATCCTTAATAGCAGTTTTCC–3′	4	174–180
8	AF012928	$(CA)_{11}$	5′–GTTGTGGTGGGCTCGTTTGTCTG–3′	5′–GTGGGGAAACCGAAAGCACCG–3′	5	110–120

[a] GenBank accession number.

[b] Allele size ranges and means are in DNA base pairs.

[a strong egg shell line (24) and a weak egg shell line (24)]. The two lines of White Leghorn were developed by two-way selection for egg shell strength with nondestructive deformation (Nirasawa et al. 1995).

Detection of Chicken Microsatellite DNA Polymorphisms

From a $(CA)_n$-enriched library (Takahashi et al. 1996), eight clones were randomly selected and the nucleotide sequences were determined. The sequences have been registered in GenBank with accession numbers U60782, U60783, U60786, U60787, U60791, U60792, U60793, and AF012928. The primer sequences for PCR are shown in Table 1. The PCR primer pairs of the clones to detect $(CA)_n$ repeat

Table 2. Gene frequencies at the microsatellite DNA loci in each population

Marker	Size (bp)	Iwate-Jidori	Aizu-Jidori	Sadohige-Jidori	Siba-Tori	Onaga-Dori		Echigo-nankin	Hinai	Kinpa	Koeyoshi	Tomaru	White Leghorn	
						Kochi	Fukushima						Strong Egg	Weak Egg
U60782	120	0.0000	1.0000	0.0455	0.0000	0.0909	0.1333	0.5000	0.0333	0.0000	0.5208	0.4000	0.2500	0.1042
	122	0.0000	0.0000	0.0909	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000
	124	0.1316	0.0000	0.2727	0.0000	0.0000	0.0000	0.2917	0.0000	0.0455	0.0000	0.0000	0.3333	0.1458
	126	0.0526	0.0000	0.5909	0.4063	0.0682	0.3000	0.0000	0.9667	0.0000	0.0000	0.0000	0.0000	0.0000
	128	0.8158	0.0000	0.0000	0.4375	0.8409	0.5667	0.2083	0.0000	0.9545	0.4792	0.0000	0.0000	0.0000
	130	0.0000	0.0000	0.0000	0.1563	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.6000	0.4167	0.7500
U60783	72	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0833	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000
	74	0.0000	0.0000	0.0909	0.0000	0.0000	0.0000	0.0000	0.0000	0.4545	0.0000	0.0000	0.0000	0.0000
	76	0.0000	0.5500	0.0000	0.3438	0.0000	0.0333	0.1667	0.0333	0.0000	0.0208	0.0000	0.0000	0.0000
	80	0.0000	0.0000	0.1136	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000
	82	0.0000	0.4500	0.0227	0.0000	0.0000	0.1000	0.0000	0.6667	0.0000	0.0000	0.1333	0.3958	0.4583
	84	1.0000	0.0000	0.0000	0.0313	0.4773	0.7333	0.7500	0.0000	0.3636	0.5625	0.0000	0.0000	0.0000
	86	0.0000	0.0000	0.5227	0.0000	0.3182	0.1333	0.0000	0.2333	0.1818	0.1875	0.0000	0.0000	0.0000
	88	0.0000	0.0000	0.0000	0.4063	0.1818	0.0000	0.0000	0.0667	0.0000	0.2292	0.8667	0.6042	0.5417
	98	0.0000	0.0000	0.2500	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000
	100	0.0000	0.0000	0.0000	0.2188	0.0227	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000
U60786	114	0.2105	0.3000	0.1818	0.0000	0.0455	0.0667	0.0000	0.0000	0.0909	1.0000	1.0000	0.0000	0.0000
	120	0.7895	0.1500	0.2500	0.3438	0.9091	0.8667	0.4583	0.7333	0.5909	0.0000	0.0000	0.8333	0.5833
	122	0.0000	0.0000	0.4773	0.0625	0.0227	0.0000	0.5417	0.1333	0.3182	0.0000	0.0000	0.1667	0.0000
	124	0.0000	0.0000	0.0909	0.5938	0.0000	0.0000	0.0000	0.0667	0.0000	0.0000	0.0000	0.0000	0.4167
	126	0.0000	0.5500	0.0000	0.0000	0.0227	0.0667	0.0000	0.0667	0.0000	0.0000	0.0000	0.0000	0.0000
U60787	144	0.6053	0.3250	0.8409	0.6563	0.3182	0.3000	0.0000	0.0333	0.0000	0.0000	0.0000	0.9583	0.9583
	145	0.3947	0.6750	0.1591	0.3438	0.6818	0.7000	1.0000	0.9667	1.0000	1.0000	1.0000	0.0417	0.0417
U60791	93	0.2368	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000
	95	0.6842	0.5500	0.8636	0.9688	0.8409	0.8333	0.7500	0.7333	1.0000	0.9792	0.7333	0.0000	0.0000
	97	0.0000	0.2250	0.0000	0.0313	0.0909	0.1333	0.0000	0.2333	0.0000	0.0208	0.0667	1.0000	1.0000
	99	0.0789	0.2250	0.1364	0.0000	0.0682	0.0333	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000
	101	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0333	0.0000	0.0000	0.2000	0.0000	0.0000
	103	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.2500	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000
U60792	144	0.0000	0.0000	0.9091	0.1250	0.1136	0.0000	0.2500	0.2333	0.0000	0.4375	0.0000	0.0000	0.0000
	146	0.1842	0.0000	0.0000	0.0313	0.0000	0.0000	0.5000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000
	148	0.0000	0.0000	0.0000	0.0000	0.0227	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000
	150	0.2368	1.0000	0.0000	0.5625	0.2273	0.2000	0.0833	0.1667	0.0000	0.5625	0.0000	0.0000	0.2500
	152	0.3684	0.0000	0.0000	0.0000	0.6136	0.8000	0.0000	0.5667	0.2955	0.0000	0.3667	0.0000	0.0000
	154	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.1000	0.0000	0.0000
	158	0.2105	0.0000	0.0909	0.2813	0.0227	0.0000	0.1667	0.0333	0.7045	0.0000	0.5333	1.0000	0.7500
U60793	174	0.1053	0.0000	0.0455	0.9063	0.2727	0.5333	0.0000	1.0000	0.0000	0.5000	0.0000	0.0000	0.0000
	176	0.0000	0.0000	0.6136	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000
	178	0.8947	1.0000	0.0000	0.0938	0.7273	0.4667	1.0000	0.0000	1.0000	0.5000	1.0000	1.0000	1.0000
	180	0.0000	0.0000	0.3409	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000
AF012928	110	0.2895	0.1250	0.1136	0.5938	0.0682	0.1000	0.0000	0.1667	0.1591	0.0000	0.9000	0.0000	0.0000
	114	0.0000	0.0000	0.5909	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	1.0000
	116	0.0000	0.4250	0.2045	0.0938	0.4318	0.2333	0.8750	0.7667	0.4091	0.5833	0.0000	0.6667	0.0000
	118	0.0789	0.0000	0.0455	0.2813	0.0455	0.0000	0.0833	0.0333	0.4318	0.4167	0.1000	0.0000	0.0000
	120	0.6316	0.4500	0.0455	0.0313	0.4545	0.6667	0.0417	0.0333	0.0000	0.0000	0.0000	0.3333	0.0000

length polymorphisms were synthesized and 5′ ends of CA strand primers (forward primers) were fluorescently labeled.

Chicken genomic DNA used as a template for the PCR reaction was isolated from blood using a DNA isolation kit (SepaGene, Sanko Jyunyaku, Japan). PCR was performed: 9 min (94°C) and 35 cycles of 30 s (94°C), 30 s (62°C), and 1 min (72°C). A reaction value of 20 μl contained 50 ng genomic DNA, 1.5 mM $MgCl_2$, 50 mM KCl, 10 mM Tris-HCl (pH 8.3), 0.001% gelatin, 100 μM dNTP, 0.025 U of AmpliTaq Gold (Perkin Elmer) and 25 pmols of each primer. PCR products were separated on 6% Long Ranger™ gel (AT Biochem) containing 8 M urea. Analyses of fragments were performed using an automated DNA sequencer (A.L.F.II, Pharmacia, Sweden) and a computer program (Fragment Manager, Pharmacia, Sweden).

Statistics

Alleles were designated according to PCR product size and allelic frequencies were estimated by directly counting. Genetic differences among populations were studied by calculating the D_A distance (Nei 1983). Takezaki and Nei (1996) studied the efficiencies of several genetic distance measures, 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), when they are applied to microsatellite DNA loci. Their computer simulation showed that D_A and D_C distance are most efficient in obtaining the correct tree topology. Since the D_A distance is the modified D_C distance, we chose the D_A distance in this study. From the distances, a majority-rule consensus tree based on 1000 bootstrap replicates was constructed by the neighbor-joining (NJ) method (Saitou and Nei 1987) incorporated into the "njbafd" and "treeview" programs (provided by Dr. N. Takezaki, National Institute of Genetics, Mishima, Japan).

Results and Discussion

All eight microsatellites examined were polymorphic. A total of 45 alleles were detected and the average number of alleles per locus was 5.6 (Table 1). Gene frequencies at the microsatellite DNA loci in each population are shown in Table 2. Excepting Aizu-Jidori and Tomaru, Japanese chicken breeds and White Leghorn were clearly separated from each other (Figure 1). Within Japanese native breeds, three groups could be differentiated: (1) Sadohige-Jidori, Hinai and Siba-Tori; (2) Iwate-Jidori and Onaga-Dori; and (3) Echigonankin and Kinpa. Koeyoshi was close to the second and third groups.

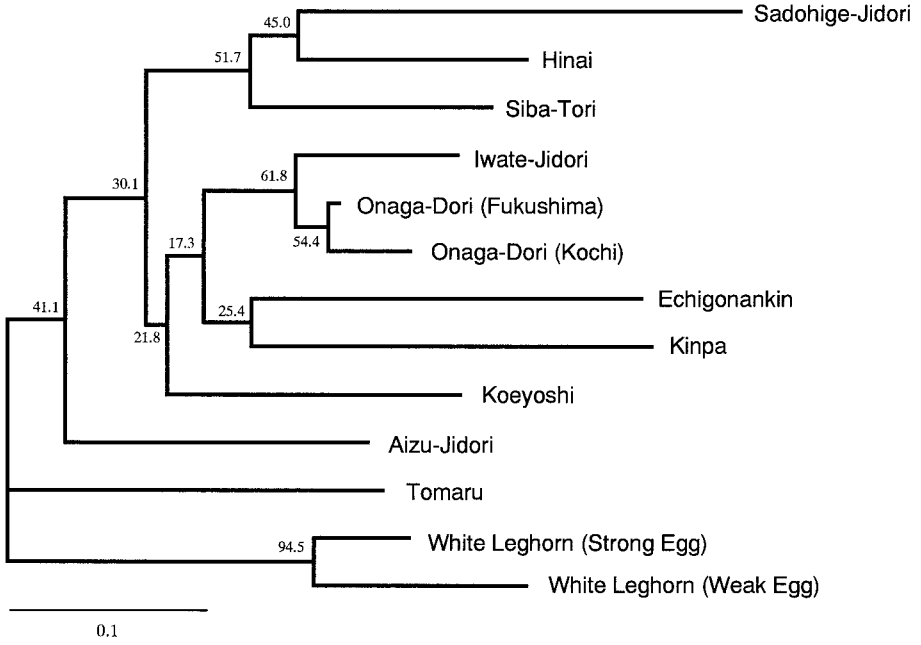

Figure 1. NJ dendrograms of Japanese native chickens and White Leghorn based on D_A (Nei 1983). Numbers on the nodes are percentage bootstrap values from 1,000 replications of resampled loci.

There are no indigenous chickens in Japan in the true sense of the word. Chicken breeds in Japan were introduced into Japan at various times. Most of today's Japanese chicken breeds were established from three original breeds, Jidori, Shokoku, and Shamo (Koana 1951). Jidori means *indigenous chicken* and retains primitive chicken characteristics. Jidori is thought to have been introduced into Japan from China about 2,000 years ago. Shokoku, which have long hackle and saddle feathers, is thought to have been introduced into Japan from China between the 8th and 12th centuries. Some varieties of Shokoku were exported from Japan to other countries in the 19th century and their offspring are known as Phoenix and Yokohama (Hawksworth 1994; Stromberg 1996). Shamo is thought to be derived from a Malay-type chicken introduced into Japan from Thailand in the 16th or 17th century for cockfighting. In addition, other types of breeds such as Oh-Tomaru, Chabo, and Silky were introduced into Japan from China. During Japan's period of isolation (1635–1854), breeds were crossed to improve plumage, crowing, or cockfighting ability, resulting in more than 30 breeds of chicken being recognized. Since most Japanese chicken breeds are crossbreeds of imported breeds and closely related to each other, it is difficult to identify the origin of each Japanese chicken breed. There are some reports concerning genetic relationships among Japanese chicken breeds based on blood protein polymorphisms (Hashiguchi et al. 1981; Okada et al. 1980; Tanabe and Mizutani 1980). Although some breeds were common to all of these studies, the results were inconsistent. Our study demonstrates for the first time that the main breeds of Japanese chickens can be distinguished using microsatellite DNA polymorphisms. Compared to morphological characteristics of breeds (Koana 1951), the three groups of Japanese chickens based on microsatellite markers correspond to the original breed: the first group (Sadohige-Jidori, Hinai, and Siba-Tori) corresponds to Jidori, the second group (Iwate-Jidori and Onaga-Dori) corresponds to Shokoku, and the third group (Kinpa and Echigonankin) corresponds to Shamo.

Siba-Tori is a variety of Jidori that originated in Niigata Prefecture. Sadohige-Jidori is thought to be an isolated breed of Siba-Tori on Sado island. Beard (*hige* in Japanese) is a special characteristic of Sadohige-Jidori. Hinai is a meat type breed established in Akita Prefecture, which is near Niigata Prefecture. This breed was thought to be established by crossing Jidori with Shamo. Our results do not contradict the presumed histories of the three breeds.

Onaga-Dori (*long tail fowl*) is the most famous Japanese breed because males have very long tail feathers. The main tail

feathers of males do not molt and can grow by 90 cm each year. The tail feathers sometimes grow longer than 8 m, and the record is 12 m. This breed is thought to have originated by mutation from Shokoku and was established in Kochi Prefecture in the 18th century (Koana 1951). Iwate-Jidori was found in Iwate Prefecture in 1975, and was believed to be a variety of Jidori and was honored as a poultry treasure of Japan in 1984. However, in this study, the genetic distance between Iwate-Jidori and Onaga-Dori was found to be relatively close. This suggests that Iwate-Jidori may be a variety of Shokoku or a crossbreed between Shokoku and other breeds.

Kinpa and Echigonankin are small varieties of Shamo. Kinpa can be found in Akita Prefecture and Echigonankin can be found in Niigata Prefecture. Our results agree with the morphological characteristics of the breeds. Koeyoshi, found in Akita and Aomori Prefectures, is famous for the prolonged crowing ability of males. Although the history of Koeyoshi is unclear, this breed appears to be a crossbreed between Shamo, Shokoku, and Tomaru. Our results suggest that Koeyoshi may be a crossbreed between Shamo and Shokoku.

Aizu-Jidori, found in Fukushima Prefecture, is thought to be a variety of Jidori. However, Aizu-Jidori did not belong to the group of Jidori in this study. The relationships between Aizu-Jidori and the other Japanese breeds could not be elucidated, however, Aizu-Jidori may be a crossbreed of Jidori, Shokoku, and Shamo. Tomaru is found in Niigata Prefecture and is famous for the prolonged crowing ability of males. The cocks can crow for up to 18 s. Tomaru is a large breed; the adult male body weight is about 3.5 kg. This breed is thought to be derived from a breed of Oh-Tomaru (large Tomaru) imported from China in the 16th or 17th century. In this study, the genetic distances between Tomaru and the other Japanese breeds were relatively far. Our results do not contradict the presumed history of Tomaru.

In conclusion, microsatellite markers are a useful tool for studying the genetic relationships among closely related breeds of chickens. Since the markers in this study are highly polymorphic, they can be also applied for linkage mapping of chickens.

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References

Cavalli-Sforza LL and Edwards AW, 1967. Phylogenetic analysis: models and estimation procedures. Am J Hum Genet 19:233–257.

Cheng HH, Levin I, Vallejo R, Khatib H, Dodgson JB, Crittenden LB, and Hillel J, 1995. Development of a genetic map of the chicken with markers of high utility. Poult Sci 74:1855–1874.

Goldstein DB, Ruiz Linares A, Cavalli-Sforza LL, and Feldman MW, 1995. Genetic absolute dating based on microsatellites and the origin of modern humans. Proc Natl Acad Sci USA 92:6723–6727.

Hashiguchi T, Tsuneyoshi M, Nishida T, Higashiuwatoko H, and Hiraoka E, 1981. Phylogenetic relationships determined by the blood protein types of fowls (in Japanese). Jpn J Zootech Sci 52:713–729.

Hawksworth D, 1994. British poultry standards. Oxford: Blackwell Science.

Koana H, 1951. A history of Japanese chickens (in Japanese). Tokyo: Nihonkei-Kenkyusha.

Nei M, 1983. Genetic polymorphism and the role of mutation in evolution. In: Evolution of genes and proteins (Nei M and Koehn R, eds). Sunderland, Massachusetts: Sinauer; 165–190.

Nirasawa K, Naito M, Oishi T, and Komiyama T, 1995. Two-way selection for egg shell strength with non-destructive deformation as selection criterion in chickens (in Japanese). Jpn Poult Sci 32:128–136.

Okada I, Toyokawa K, and Takayasu I, 1980. Genetic relationships of some native chicken breeds in the northern Tohoku district of Japan (in Japanese). Jpn Poult Sci 17:337–343.

Saitou N and Nei M, 1987. The neighbor-joining method: a new method for reconstructing phylogenetic trees. Mol Biol Evol 4:406–425.

Shriver M, Jin L, Boerwinkle E, Deka R, and Ferrell RE, 1995. A novel measure of genetic distance for highly polymorphic tandem repeat loci. Mol Biol Evol 12:914–920.

Stromberg L, 1996. Poultry of the world. Ontario: Silvio Mattacchione & Co.

Takahashi H, Nirasawa K, and Furukawa T, 1996. An efficient method to clone chicken microsatellite repeat sequences. Jpn Poult Sci 33:292–299.

Takezaki N and Nei M, 1996. Genetic distances and reconstruction of phylogenetic trees from microsatellite DNA. Genetics 144:389–399.

Tanabe Y and Mizutani M, 1980. Studies of the phylogenetic relationships of the Japanese native fowl breeds (in Japanese). Jpn Poult Sci 17:116–121.

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A New Allelic Series for the Underwhite Gene on Mouse Chromosome 15

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A new allelic series at the underwhite gene is described. Three of the alleles in the series—*uw, uw*^{d}, and *Uw*^{dbr}—arose as spontaneous mutations on different genetic backgrounds at The Jackson Laboratory. We report here the visible phenotypes and dominance hierarchy of these alleles, all of which are defined by a reduction of pigmentation in both eye and coat color. Electron microscopic analysis of retinal epithelium suggests that the primary defect is in the melanosome. The degree of severity of melanosome anomalies in the retina correlates with the degree of hypopigmentation in the coat. The perturbed gene and its gene product are unknown. We show that the *uw* locus is genetically distinct from *Myo10,* a suggested candidate gene for this mutation.

The first mutant allele to reveal the underwhite gene, *uw,* arose as a spontaneous mutation in the C57BL/6J inbred strain and was originally described by Dickie (1964). This allele produces the lightest coat color phenotype. The homozygote is characterized by lack of eye pigmentation at birth, dark reddish eyes as adults, and a light buff with white underfur coat color. The *uw* gene was mapped to proximal Chr 15 (Davisson et al. 1990; Eicher and Green 1972). For many years thereafter it was the phenotypic marker of choice for locating genes on Chr 15.

Two new spontaneous mutations causing coat color lightening have occurred at The Jackson Laboratory and have been shown by direct tests for allelism to be additional mutant alleles of the *uw* gene. MacPike and Mobraaten (1984) described a new semidominant mutation in the B10.PL(73NS)/Sn congenic strain that was provisionally called dominant brown (gene symbol *Dbr*). Cook and Davisson (1993) reported *Dbr* to be a semidominant allele of *uw*; the gene symbol for dominant brown became *Uw*^{dbr}. *Uw*^{dbr} is dominant to the wild-type allele for full color and to all recessive alleles at the underwhite gene. On the nonagouti (*a*/*a*) background, homozygotes, *Uw*^{dbr}/*Uw*^{dbr}, are light beige; heterozygotes, *Uw*^{dbr}/+, appear dark brown (Cook and Davisson 1993). Eye pig-