A Study on Genetic Differentiation and Phylogenetic Relationships among East Asian Titmice (Family Paridae) and Relatives

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By means of protein electrophoresis on 23 loci (15 enzymes), genetic variation and differentiation among eight East Asian Titmice (family Paridae, genus *Parus*), two long-tailed tits (family Aegithalidae, genus *Aegithalos*), a nuthatch (family Sittidae, *Sitta*) and Bearded Tit (family Paradoxornithidae, genus *Panurus*) were analyzed. A genealogic dendrogram is constructed by the UPGMA method, using genetic distances among the species. The results of the present study suggests that the East Asian *Parus* species investigated can be divided into four groups, i.e., groups consisting of (1) *Parus montanus*, (2) *P. ater*, *P. venustulus* and *P. varius*, (3) *P. major* and *P. monticolus*, and (4) *P. spilonotus* and *P. holsti*. *P. spilonotus* seems to have a close relationship to *P. holsti*, but distant relationships to *P. major* and *P. monticolus*. *Aegithalos*, *Sitta* and *Panurus* are relatively closely related to each other, whereas the *Parus* species are shown to have a family level genetic distance in the Passeriformes.

In this study, we could not get enough samples of some species (especially *P. montanus* and *P. spilonotus*). And since we obtained samples from dealers, there are some possibility that samples were collected from only one population of each species. The data of other samples from several populations and that of other species are needed to determine the phylogeny of the East Asian *Parus* species.

Key Words: East Asian Titmice, Isozyme, Paridae, *Parus*, Phylogeny

Titmice (family Paridae) are small birds which inhabit the forests of Africa, Europe, Asia and North America. They are very acrobatic in clinging to branches by their feet and have relatively short beaks and stout feet. Snow (1967) classified 46 species of the Paridae into three genera without recognizing subgenera: *Parus* (44 species), *Melanochlora* (1 species), and *Sylviparus* (1 species). His classification has been accepted widely at the present, whereas Thielcke (1968) classified 43 species of *Parus* into 10 subgenera. We follow Thielcke in recognition of subgenera of *Parus*. Fourteen *Parus* species that belong to 8 subgenera are distributed in East Asia (Mongolia, China, the Korean Peninsula, Taiwan and Japan).

In earlier studies, Vaurie (1950, 1957) discussed the taxonomic problems and relationships of Palearctic species of the Paridae using morphological characters, but the phylogenetic relationships of Asian titmice are still poorly known.

Recently there have been systematic studies of the Paridae in Europe and North America based on isozymes and DNA analysis (Gill *et al.* 1989, Sheldon *et al.* 1992, Kvist *et al.* 1996, Slikas *et al.* 1996). However, there are no comparable studies on Asian species, which makes a study of Asian titmice desirable.

The Aegithalidae, Sittidae and Paradoxornithidae (*Panurus*) are considered to be closely related to the Paridae. *Aegithalos* and a few related genera have been treated as a separate family, the Aegithalidae (Snow, 1967), but they are often considered as genera or a subfamily of the Paridae (e.g., Hellmayr 1903, Mayr & Amandon 1951). *Panurus* has been classified in the subfamily Panurinae (=Paradoxornithinae) of the family Muscicapidae (s. lat.) (Deignan 1964). In addition, the Sittidae have sometimes been considered as a subfamily of the Paridae (Vaurie 1959).

This study attempts to reveal the phylogenetic relationships among the little studied members of the Paridae of East Asia and their relationships to *Aegithalos*, *Sitta* and *Panurus*. These species were examined by electrophoretic analysis on 23 loci (15 enzymes) with tissues (liver, muscle and heart) taken from 59 individual birds.

MATERIALS AND METHODS

We analyzed 59 individual birds belonging to 6 subgenera and 8 species of the Paridae, one species of the Paradoxornithinae, two species of the Aegithalidae, and one species of the Sittidae. They were obtained from pet dealers between March 1986 and May 1997, so detailed locality and population number of the samples are not known. The species, sample sizes and localities of the samples used in this study are given in Table 1. The liver, muscles and heart were removed from half-

Table 1. Studied species, sample size and locality.

Family	Species	English name	Sample size	Locality
Paradoxornithidae	<i>Panurus biarmicus</i>	Bearded Tit	2	China
Aegithalidae	<i>Aegithalos caudatus</i>	Long-tailed Tit	3	China
	<i>Aegithalos concinnus</i>	Red-headed Tit	2	Taiwan
Paridae	<i>Parus (Poecile) montanus</i>	Willow Tit	1	China
	<i>Parus (Periparus) ater</i>	Coal Tit	7	Taiwan
	<i>Parus (Pardaliparus) venustulus</i>	Yellow-bellied Tit	12	China
	<i>Parus (Parus) major</i>	Great Tit	7	China
	<i>Parus (Parus) monticolus</i>	Green-backed Tit	11	Taiwan
	<i>Parus (Parus) spilonotus</i>	Chinese Yellow Tit	2	China
	<i>Parus (Machlolophus) holsti</i>	Formosan Yellow Tit	6	Taiwan
	<i>Parus (Sittiparus) varius</i>	Varied Tit	5	Taiwan
Sittidae	<i>Sitta europaea</i>	Nuthatch	1	China
Total	12 species		51 individuals	

Order of the species follow Snow (1967), Deignan (1964) and Thielcke (1968). The subgenera of *Parus* after Thielcke (1968) are given in parenthesis.

defrosted birds, homogenized, and centrifuged at $15,000\times g$ for 20 minutes at 2°C . The supernatant was then divided into aliquots and stored in capillary glass tubes at -80°C . The extracts of the tissues were analyzed by starch gel electrophoresis to determine the allelic composition of each protein locus. The combinations of tissues and buffer systems for enzyme detection are shown in Table 2. The staining

Table 2. Combinations of tissues and buffer systems for detection of enzymes.

Protein	Abbr.	Tissues	Buffers*
Creatine kinase	CK	Muscle, Heart	Tris-HCl
Diaphorase	DIA	Liver	Tris-HCl
Esterase-D	ESD	Liver	Tris-citrate
Glutamate oxaloacetate transaminase	GOT	Liver	Tris-citrate
α -Glycerophosphate dehydrogenase	α -GPDH	Muscle	Tris-citrate
Glucosephosphate isomerase	GPI	Muscle	Tris-citrate
Isocitrate dehydrogenase	IDH	Liver	Tris-citrate
Lactate dehydrogenase	LDH	Muscle, Heart	Tris-citrate
Malate dehydrogenase	MDH	Liver	Tris-citrate
Peptidase	PEP	Liver	Poulik
6-Phosphoglucose dehydrogenase	6PGD	Liver	Tris-citrate
Phosphoglucosemutase	PGM	Liver	Tris-citrate
General protein	PT	Muscle	Tris-citrate
Sorbitol dehydrogenase	SDH	Liver	Tris-citrate
Superoxide dismutase	SOD	Liver	Tris-citrate

*Tris-HCl, Poulik: Selander *et al.* (1971), Tris-citrate: Numachi *et al.* (1983).

method for enzymes were essentially the same as those described by Bell *et al.* (1982), Shaw & Prasad (1970), Selander *et al.* (1971), Hopkinson *et al.* (1973) and Numachi *et al.* (1979). These enzymes and proteins are assumed to be coded by 23 gene loci. Allozymes representing alleles for individual loci are designated alphabetically in decreasing order of mobility. Isozyme systems under the control of distinct loci were identified numerically, counting from the anodal side.

Genetic differentiations in populations, both the proportion of polymorphic loci and heterozygosities, were calculated as follows. The proportion of polymorphic loci were identified as the proportion of genetic loci that showed variations. Heterozygosities were calculated in two ways: observed heterozygosities were calculated by averaging the proportion of individual heterozygous birds observed per locus, and expected heterozygosities were calculated as the proportion of heterozygosities expected from allele frequencies, assuming Hardy-Weinberg equilibria. Genetic identities and genetic distances between species were calculated using Nei's (1972) formula, and the values of genetic distances were clustered into a dendrogram using the unweighted pair-group method of analysis with arithmetic means (UPGMA) (Sneath & Sokal 1973).

Table 3. Alleles and their frequencies from the variable loci of *Panurus*, *Aegithalos*, *Parus* and *Sitta*. Alleles are shown as a, b and c.

Species	CK-1	CK-2	DIA	ESD-1	ESD-2	GOT-1	GOT-2	α -GPDH	GPI	IDH-1	IDH-2
<i>Panurus biarmicus</i>	b	b	a	a	b	b	b	b	e	c	a
<i>Aegithalos caudatus</i>	b	b	c	b	a	b	a	a	b	a	b
<i>Aegithalos concinnus</i>	b	b	c	b	a	b	b	a	a	c	b
<i>Parus montanus</i>	b	b	b	b	a	b	a	c	d	c	a
<i>Parus ater</i>	b	b	a	b=0.2857 c=0.7143	a	b	b=0.2857 c=0.7143	c	c	c	a
<i>Parus venustus</i>	b	b	a=0.8750 b=0.0417 c=0.0833	b	a=0.0833 b=0.9167	b	a	c	c	a=0.4167 c=0.5833	a
<i>Parus major</i>	b	b	a	b	c	b	a	c	c	c	a
<i>Parus monticolus</i>	a=0.0909 b=0.9091	a=0.0455 b=0.9545	a	b	c	b	a	c	c	c	a
<i>Parus spilonotus</i>	b	b	a	b	a	b	a	d	c	b=0.5000 c=0.5000	a
<i>Parus holsti</i>	b	b	a	b	a	a=0.5000 b=0.5000	a	d	c	c	a
<i>Parus varius</i>	b	b=0.9000 c=0.1000	a=0.8000 b=0.2000	b	a=0.2000 b=0.8000	a=0.2000 b=0.8000	a	c	c	c	a
<i>Sitta europaea</i>	b	c	d	b	a	c	b	c	e	d	c

Table 3. Continued.

Species	LDH-1	LDH-2	MDH-1	MDH-2	PEP	6PGD	PGM-1	PGM-2	PT-1	PT-2	SDH	SOD
<i>Parus biarmicus</i>	c	c	a	b	b	g	a	c	a	a	c	c=0.5000 d=0.5000
<i>Aegithalos caudatus</i>	c	b	a	b	c	h	a	d	a	a	a	b=0.5000 d=0.5000
<i>Aegithalos concinnus</i>	c	b	a	b	d	g	a	c	a	a	a	d
<i>Parus montanus</i>	b	a	a	b	e	b	b	c	b	a	c	a
<i>Parus ater</i>	b	b	a	b	d	c=0.0714 e=0.9286	a=0.1429 b=0.8571	c	b	a	c	b
<i>Parus venustulus</i>	b	b	a	b	c=0.1667 d=0.8333	c=0.1250 e=0.8750	b	e	b	a	c	b
<i>Parus major</i>	a	b	a	a=0.1429 b=0.8571	d	c=0.8751 e=0.1429	b=0.9286 c=0.0714	c	b	a	d	a=0.9286 c=0.0714
<i>Parus monticolus</i>	b	b	a	b	d	d	b	c	b	a	d	a=0.9545 d=0.0455
<i>Parus spilonotus</i>	b	a	a	b	c	f	b	c	b	a	e	b
<i>Parus holsti</i>	b	b	a	b	c	a	b	c	b	a	b	b
<i>Parus varius</i>	b	b	a	b	d	b	b	b=0.9000 c=0.1000	b	a	c	b
<i>Sitta europaea</i>	c	b	a	a	a	g	a	a=0.5000 c=0.5000	a	a	a	e

RESULTS

1) Genetic variation

Alleles detected at each locus and the frequency of allele occurrences are shown in Table 3. The number of birds investigated for each species ranged from one to twelve, with intraspecific genetic variations found in all species of *Parus* studied except *P. montanus*, of which there was only one specimen. No intraspecific genetic variation was detected in both species of *Aegithalos* and *Panurus* tested (*Sitta* was represented by one specimen).

The species with the highest genetic variation was *P. venustulus* and *P. varius*, which showed variations at 5 loci. Fourteen loci showed genetic variations within a species: SOD (variation found in four species), 6PGD (three species), CK-2, DIA, ESD-2, GOT-1, IDH-1, PGM-1 and PGM-2 (two species), CK-1, ESD-1, GOT-2, MDH-2, and PEP (one species). The largest number of alleles found at any one locus was eight at 6PGD.

The proportions of polymorphic loci and average heterozygosities (observed and expected) of the species studied are shown in Table 4 and those data for other avian groups are shown in Table 5. The mean proportion of polymorphic loci of the 8 species of *Parus* was 0.125 (range 0 - 0.217), which is relatively lower than

Table 4. Proportion of polymorphic loci and heterozygosities (observed and expected) of each species.

Species	Proportion of polymorphic loci	Average heterozygosity	
		Observed	Expected
Paradoxornithidae			
<i>Panurus biarmicus</i>	0.043	0.043	0.022
Aegithalidae			
<i>Aegithalos caudatus</i>	0.043	0.043	0.022
<i>Aegithalos concinnus</i>	0	0	0
Aegithalidae Mean	0.022	0.022	0.011
Paridae			
<i>Parus montanus</i>	0	0	0
<i>Parus ater</i>	0.174	0.031	0.052
<i>Parus venustulus</i>	0.217	0.018	0.059
<i>Parus major</i>	0.174	0.025	0.033
<i>Parus monticolus</i>	0.130	0.008	0.015
<i>Parus spilonotus</i>	0.043	0	0.022
<i>Parus holsti</i>	0.043	0.029	0.022
<i>Parus varius</i>	0.217	0.035	0.057
Paridae Mean	0.125	0.018	0.033
Sittidae			
<i>Sitta europaea</i>	0.043	0.043	0.022

Table 5. Proportion of polymorphic loci and expected heterozygosities in various groups of birds.

Taxon	Loci	Proportion of polymorphic loci	Expected Average heterozygosity	Source
Non-Passeriformes				
Phasianidae (<i>Gallus</i>)	32	0.125	0.046	Ohta <i>et al.</i> (in press)
Phasianidae	23	0.184	0.045	Gutiérrez <i>et al.</i> (1983)
Procellariiformes	23	—	0.003	Kuroda <i>et al.</i> (1990)
Anatidae	10	0.019	0.003	Numachi <i>et al.</i> (1983)
Alcidae	24	0.045	0.017	Watada <i>et al.</i> (1987)
Mean		0.093	0.023	
Passeriformes				
Mimidae	23	0.009	0.015	Avise <i>et al.</i> (1982)
Muscicapidae	25~27	0.167	0.043	Avise <i>et al.</i> (1980a)
Paridae	23	0.125	0.033	Present study
Emberizidae	20~21	0.151	0.039	Avise <i>et al.</i> (1980b)
Emberizidae	20	0.140	0.042	Watada <i>et al.</i> (1995)
Parulidae	16~22	0.392	0.134	Barrowclough & Corbin (1978)
Vireonidae	23	0.115	0.036	Avise <i>et al.</i> (1982)
Estrildidae	26	0.061	0.017	Kakizawa & Watada (1985)
Mean		0.145	0.045	

for the Passeriformes (0.145).

Of the 23 loci tested, inter-specific variations were found in 19 loci (CK-1, CK-2, DIA, ESD-1, ESD-2, GOT-1, GOT-2, α -GPDH, GPI, IDH-1, LDH-1, LDH-2, MDH-2, PEP, 6PGD, PGM-1, PGM-2, SDH and SOD) in the 8 species of *Parus*. LDH-1 showed a single allele except in *P. major*.

Inter-specific variations of two *Aegithalos* species were found in 7 loci (GOT-2, GPI, IDH-1, PEP, 6PGD, PGM-2 and SOD).

Genetic variations between *Parus*, *Aegithalos*, *Sitta* and *Panurus* were found in 21 loci (CK-1, CK-2, DIA, ESD-1, ESD-2, GOT-1, GOT-2, α -GPDH, GPI, IDH-1, IDH-2, LDH-1, LDH-2, MDH-2, PEP, 6PGD, PGM-1, PGM-2, PT-1, SDH and SOD). IDH-2 showed a single allele in *Parus* and *Panurus* and PT-1 showed a single allele in *Aegithalos*, *Sitta* and *Panurus*.

2) Genetic differentiation

The genetic identity and distances were calculated from the data given in Table 6 according to Nei's (1972) formula. The results obtained from the analysis of the 23 loci showed that genetic variation was found among all the species of *Parus*, *Aegithalos*, *Sitta* and *Panurus* examined. The lowest genetic distance was found between *P. major* and *P. monticolus* (0.0892), and the second lowest one between *P. venustulus* and *P. varius* (0.1012). These values probably indicate relatively low

Table 6. Matrix of genetic identity (above diagonal) and genetic distance (below diagonal) between in the species studied.

	<i>Panurus</i> <i>biarmicus</i>	<i>Aegithalos</i> <i>caudatus</i>	<i>Aegithalos</i> <i>concinus</i>	<i>Parus</i> <i>montanus</i>	<i>Parus</i> <i>ater</i>	<i>Parus</i> <i>venustus</i>	<i>Parus</i> <i>major</i>	<i>Parus</i> <i>monticolus</i>	<i>Parus</i> <i>spilonotus</i>	<i>Parus</i> <i>holsti</i>	<i>Parus</i> <i>varius</i>	<i>Sitta</i> <i>europaea</i>
<i>Panurus biarmicus</i>	—	0.4111	0.5934	0.4396	0.5160	0.4702	0.4422	0.4378	0.4222	0.4222	0.4709	0.4222
<i>Aegithalos caudatus</i>	0.8889	—	0.7253	0.3956	0.4031	0.4645	0.3959	0.3935	0.4667	0.4889	0.4256	0.4444
<i>Aegithalos concinnus</i>	0.5218	0.3211	—	0.4348	0.5231	0.4296	0.4800	0.4778	0.4176	0.4616	0.4478	0.5495
<i>Parus montanus</i>	0.8219	0.9273	0.8329	—	0.6762	0.6593	0.6505	0.6929	0.6814	0.6374	0.7255	0.2857
<i>Parus ater</i>	0.6617	0.9086	0.6480	0.3913	—	0.8443	0.7325	0.7650	0.7062	0.7514	0.8252	0.3257
<i>Parus venustus</i>	0.7546	0.7667	0.8450	0.4166	0.1693	—	0.7434	0.7747	0.6987	0.7346	0.9038	0.2757
<i>Parus major</i>	0.8160	0.9266	0.7340	0.4300	0.3113	0.2966	—	0.9146	0.6386	0.6832	0.7461	0.2969
<i>Parus monticolus</i>	0.8259	0.9326	0.7385	0.3669	0.2678	0.2553	0.0892	—	0.6804	0.7247	0.7881	0.2838
<i>Parus spilonotus</i>	0.8622	0.7621	0.8732	0.3837	0.3478	0.3586	0.4486	0.3851	—	0.8444	0.6927	0.2875
<i>Parus holsti</i>	0.8622	0.7156	0.7731	0.4504	0.2859	0.3085	0.3809	0.3220	0.1691	—	0.7471	0.2444
<i>Parus varius</i>	0.7531	0.8542	0.8034	0.3209	0.1921	0.1012	0.2928	0.2381	0.3671	0.2916	—	0.2889
<i>Sitta europaea</i>	0.8622	0.8109	0.5988	1.2527	1.1218	1.2885	1.2143	1.2594	1.4088	1.2417	1.2465	—

genetic differentiation between the two species, respectively. The highest genetic distance (1.4088) was found between *P. spilonotus* and *Sitta europaea*. Among the *Parus* studied, the highest genetic distance (0.4504) occurred between *P. montanus* and *P. holsti*. The mean genetic distances of avian taxa at various levels of taxonomic divergency are shown in Table 7. This shows an average genetic distance at

Table 7. Mean genetic distances in avian taxa at various levels of taxonomic divergence.

Taxon	Subspecies	Species	Genus	Family	Source
Non-Passeriformes					
Phasianidae	—	0.092	—	—	Ohta <i>et al.</i> (in press)
Procellariiformes	—	0.137	0.350	0.554	Kuroda <i>et al.</i> (1990)
Procellariiformes	—	—	0.435	0.683	Barrowclough <i>et al.</i> (1981)
Anatidae	0.000	0.016	0.285	0.674	Numachi <i>et al.</i> (1983)
Alcidae	—	0.151	0.442	—	Watada <i>et al.</i> (1987)
Non-Passeriformes Mean	0.000	0.099	0.378	0.637	
Passeriformes					
Mimidae	—	0.084	0.223	—	Avise <i>et al.</i> (1982)
Muscicapidae and Mimidae	—	0.024	0.344	0.780	Avise <i>et al.</i> (1980a)
Emberizidae and Fringillidae	0.009	0.123	0.253	0.592	Avise <i>et al.</i> (1980b)
Emberizidae	—	0.331	0.617	—	Watada <i>et al.</i> (1995)
Paridae	—	0.309	—	0.955	Present study
Parulidae	—	0.100	0.179	—	Barrowclough & Corbin (1978)
Parulidae	—	0.060	0.175	0.998	Avise <i>et al.</i> (1980c)
Vireonidae	—	0.360	—	—	Avise <i>et al.</i> (1982)
Icteridae	—	0.011	0.248	—	Smith <i>et al.</i> (1976)
Estrildidae	0.001	0.193	0.397	—	Kakizawa & Watada (1985)
Sturnidae	0.007	0.035	0.397	—	Corbin <i>et al.</i> (1974)
Passeriformes Mean	0.006	0.148	0.315	0.831	

species level is 0.148 for the Passeriformes and 0.099 for the non-Passeriformes. The mean genetic distance of the 8 *Parus* species studied was 0.309, which was almost twice the mean genetic distance of the Passeriformes (0.148). Genetic distance between *Aegithalos caudatus* and *A. concinnus* was 0.3211, which is somewhat higher than in the *Parus* species.

3) Genetic relationships in Asian *Parus*

By means of the UPGMA procedure, a genealogic dendrogram (Fig. 1) was constructed, based on values for inter-specific distances within the species here analyzed. The dendrogram shows that the 8 species of *Parus* can be classified into four groups, the first group consisting of *Parus montanus*, the second *P. ater*, *P. venustulus* and *P. varius*, the third *P. major* and *P. monticolus*, and the fourth *P.*

Fig. 1. Dendrogram of the East Asian Paridae, *Panurus*, *Aegithalos* and *Sitta* based on Nei's genetic distance at 23 loci derived by the UPGMA method.

spilonotus and *P. holsti*. The genetic distance between *P. ater*-*P. venustulus*-*P. varius* group and *P. major*-*P. monticolus* group is 0.2770. The value between *P. spilonotus*-*P. holsti* group and *P. ater*-*P. venustulus*-*P. varius*-*P. major*-*P. monticolus* group (0.3496) and that between these groups and *P. montanus* (0.3943) are greater than the genus level genetic distance for the Passeriformes (0.315).

As to the genetic distances among *Aegithalos*, *Sitta*, *Panurus* and the 8 species of *Parus*, the closest is found to be between *Aegithalos* and *Sitta*, and *Panurus* is closer to *Aegithalos* and *Sitta* than *Parus*. On the other hand, all *Parus* species are more distant to *Aegithalos*, *Sitta* and *Panurus* than to each other.

DISCUSSION

1) Genetic variation and differentiation intra-species of East Asian *Parus*

In this study, the mean expected heterozygosity of the 8 *Parus* species (0.033) is

relatively lower than the value for the Passeriformes (0.045) (Table 5), and is 1.8 times the mean observed heterozygosity (0.018). However, we examined only a few individuals of *P. montanus* (1 individual) and *P. spilonotus* (2 individuals), the mean expected heterozygosity of the 6 *Parus* species except these two species (0.040) is almost same of the value for the Passeriformes. We obtained all of the samples from dealers, and there is a possibility that the samples of each species were collected from only one population.

2) Genetic variation and differentiation inter-species of East Asian *Parus*

Hellmayr (1903) recognized 11 subgenera for *Parus* by plumage pattern. According to him, the eight species investigated in this study belong to 6 subgenera: *Poecile* (*P. montanus*), *Periparus* (*P. ater*), *Pardaliparus* (*P. venustulus*), *Sittiparus* (*P. varius*), *Machlolophus* (*P. spilonotus*, *P. holsti*) and *Parus* (*P. major*, *P. monticolus*). *P. spilonotus* and *P. holsti* in the subgenus *Machlolophus* have a prominent crest. *P. varius* of the subgenus *Sittiparus* is monotypic, whereas *P. major* and *P. monticolus* were placed in the subgenus *Parus*. Both *P. ater* (subgenus *Periparus*) and *P. venustulus* (subgenus *Pardaliparus*) have a pale color patch on the neck or upper back, but the underparts are white or pale color in *P. ater* and bright yellow in *P. venustulus*. *P. montanus*, together with *P. palustris*, is in the own subgenus *Poecile*. This study supports a close relationship between *P. major* and *P. monticolus* and that between *P. spilonotus* and *P. holsti* respectively, in agreement with Hellmayr's classification. On the other hand, *P. ater*, *P. venustulus* and *P. varius*, which are probably closely related as suggested by the present study, were placed in three separate subgenera in Hellmayr's classification.

Wolters (1982) classified the family Paridae into 11 genera. He considered the subgenus *Sittiparus* (containing *P. varius*), *Periparus* (containing *P. ater*) and *Pardaliparus* (containing *P. venustulus*) forming a genus *Periparus*, separated from genera *Poecile* and *Parus*. These three subgenera formed a closely related group in our study, which agrees with Wolters's classification.

Since the genetic distance between the *P. spilonotus*-*P. holsti* group and the *P. ater*-*P. venustulus*-*P. varius*-*P. major*-*P. monticolus* group, and the distance between these two groups and *P. montanus*, are greater than the genus level genetic distance for Passeriformes, the *P. spilonotus*-*P. holsti* group and *P. montanus* may have diverged from the other groups relatively early in this family history.

Sheldon *et al.* (1992) and Slikas *et al.* (1996) studied phylogeny of the Paridae using DNA-DNA hybridization and stated that "the genetic divergence among tit species is much greater than expected for such similar-looking birds" and "the genetic distance between members of the *Parus*/*Cyanistes* subgenetic clade and other parid are remarkably large for bird congeners". Following Slikas *et al.* (1996) Check-list of North-American Birds (AOU. 1998) ranked *Poecile* and *Baeolophus* as separate genera. In our study, *Poecile* (*P. montanus*) and *Machlolophus* (*P. spilonotus* and *P. holsti*) showed the genus level genetic distances to other groups of the Paridae examined and we believe that these two genera should be recognized.

However the placement of *P. montanus* was not confirmed since only one individual specimen was available for this study. Data of another individuals are needed to determine the exact phylogenetic position of this species. As we obtained all of the samples from dealers, there is a possibility that the samples of each species

were collected from only one population. Also the data of other samples from several populations are needed to determine the phylogeny of the East Asian *Parus* species.

3) Relationships of *Aegithalos*, *Sitta* and *Panurus*

Aegithalos was at one time classified in the family Paridae (Gadow 1883, Hellmayr 1903, Mayr & Amandon 1951), but is now treated as a separate family (Snow 1967). Stresemann (1923) called attention to the fact that the Aegithalidae has some characteristics (presence of a complete juvenile-molt, nest structure, naked hatching, etc.) which differ from those of the Paridae. The genetic distance between *Aegithalos* and *Parus* appears to be at familial level (Table 5) and the present classification is supported by our study.

Sheldon & Gill (1996) studied phylogeny of song birds using DNA-DNA hybridization and concluded that the sister group of the seed-caching Paridae is the Remizidae (including Verdin *Auriparus*), whereas the Paridae may not be closely related to the Aegithalidae, Certhiidae and Sittidae which were placed close to them in traditional classification. Our study also shown that *Parus* are not so closely related to *Sitta*, *Aegithalos* and *Panurus*.

In this study, *Panurus* is found to be relatively closely related to *Aegithalos* and *Sitta*. *Panurus* was classified in *Parus* by Linné (1758), though most recent classifiers considered it to be related either to the Timaliidae or Paradoxornithidae (= Panurinae) (e.g., Mayr & Amandon 1951, Vaurie 1959, Deignan 1964, Beaman 1994). Gadow (1883) included *Parus*, *Aegithalos*, *Psaltriparus*, *Auriparus*, *Panurus*, *Regulus* and several other genera in his family Paridae, but remarked that "*Panurus* does not belong to the Paridae, but perhaps to the Fringillidae." Stresemann (1923) stated *Panurus*, *Paradoxornis* and the Aegithalidae (*Aegithalos*, *Psaltriparus* and *Psaltia*) share certain cranial characteristics, nest structure and naked hatchings. While our study suggests that *Panurus* may have a close family level genetic distance with *Aegithalos* and *Sitta*, data of the Paradoxornithidae and the Timaliidae are needed to determine the phylogenetic relationships of *Panurus*.

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和文抄録 Japanese Summary of Papers in English

ニホンジカが高密度に生息する森林の植生構造と鳥類群集

(Breeding Bird Community and Vegetation Structure in a Forest
with a High Density of Sika Deer. 48: 197-204)

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ニホンジカが高密度に生息する紀伊半島の森林において、繁殖期の鳥類群集と植生構造の調査を行った。調査を行った森林はブナが基底面積で50%近くを占め、それにオオイタヤメイゲツやトウヒなどが混じる針広混交林で、高さ1.5m以上の全樹木密度は1091本/ha、基底面積は45m²/haであった。植生の構造は、(1)低木密度が低い、(2)草本の密度と丈が低い、(3)枯死木の密度が高い、という特徴があり、植被の量が森林の上層部から下層部まで全体にわたって少なかった。これらの植生の特徴は、いずれもシカによる草本や樹木実生および樹皮の採食が大きな原因の一つであると考えられた。

94年から98年までの5年間、テリトリー・マッピング法によって、調査地内で繁殖する鳥の種類と密度を調べた結果、平均種数は20、平均番密度は409/100haで、年によって大きな変化は見られず安定していた。ヒガラがもっとも多く(29%)、シジュウカラ、ゴジュウカラ、ルリビタキ、ミソサザイの密度が高かった(8-10%)。調査地において繁殖する鳥の種数と密度は、シカの影響が小さいと思われる森林で行われた報告と比較すると少なかった。これは、草本層に営巣する種が繁殖せず、また低木層に営巣する鳥の種数と密度が低いためであり、植生の特徴の(1)と(2)に関係づけられた。一方、樹洞で営巣する鳥の種数と密度は他の森林と変わらず、これは植生の特徴(3)に関係づけられた。従って、森林に高密度に生息するニホンジカは、採食によって植生を衰退させるばかりでなく、そこに生息する鳥類群集の構造までも単純化していることが分かった。

アイソザイムによる東アジア産シジュウカラ科鳥類 および近縁鳥類の類縁関係の研究

(A Study on Genetic Differentiation and Phylogenetic Relationships among
East Asian Titmice (family Paridae) and Relatives. 48: 205-218)

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東アジアに生息するシジュウカラ科鳥類およびその近縁鳥類の類縁関係について調査するため、シジュウカラ属の6亜属8種（シジュウカラ科）、エナガ属2種（エナガ科）、ゴジュウカラ属1種（ゴジュウカラ科）およびヒゲガラ属1種（ダルマエナガ科）の計12種59個体について、15酵素・23遺伝子座の電気泳動分析を行い、遺伝的距離を求め、UPGMA法により系統樹を作成した。

その結果、東アジア産のシジュウカラ科8種はコガラの系統（コガラ）、ヒガラの系統（ヒガラ、キバラガラ、ヤマガラ）、シジュウカラの系統（シジュウカラ、キバラシジュウカラ）、タイワンシジュウカラの系統（セボシカンムリガラ、タイワンシジュウカラ）の4つの系統に分かれた。セボシカンムリガラはタイワンシジュウカラと遺伝的に近縁で、シジュウカラやキバラシジュウカラとは類縁が遠かった。また、エナガ属、ゴジュウカラ属およびヒゲガラ属は科レベルの値としては比較的小さな遺伝的距離で結びつき、このグループにシジュウカラ属が結びつく結果となった。

今回の実験では、コガラやセボシカンムリガラなど十分な個体数が得られなかった種があり、また、鳥獣商からサンプルを入手したため、複数個体を扱った種でも標本の抽出に偏りが生じている可能性がある。今後、複数の個体群からサンプルを収集し、今回収集できなかった種についても実験を行うことで、東アジア産のシジュウカラ科鳥類の類縁関係がさらに明らかになると考えられる。