

# Influence of the Last Two Glacial Periods and the Late Pliocene on the Latitudinal Population Structure of Resident Songbirds in the Far East

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**Abstract.** In order to investigate the effect of past climatic cycles on the latitudinal population structure in the Far East, we estimated the timing of genetic divergence between populations of Honshu and Taiwan in eight species of resident songbirds by the mitochondrial cytochrome b sequences. The estimated divergence times were highly concentrated on two periods: 0.09–0.17 million years ago (MYA) for four species and 2.4–3.1 MYA for the other four species. The shallow divergence was synchronous with the last two glacial periods (0.01–0.20 MYA) in the late Pleistocene and the deep divergence with the late Pliocene. These results suggest that 1) a substantial level of gene flow is lacking between the two regions, Honshu and Taiwan, even in the species with no morphological differences at least in the present, probably throughout the Holocene, and 2) the latitudinal population structure of resident birds in the Far East should be affected by the two epochs, that is the last two glacial and the late Pliocene epochs. On the other hand, all the genetic signals that might have been accumulated during the early Pleistocene must have been eliminated during the last two glacial periods, due to the influence in the late Pleistocene stronger than that in the early Pleistocene. This is in striking contrast to longitudinal division of bird populations, of which previous genetic researches have not yet shown any marked influence of the late Pleistocene though it had been long suspected. Subspecies-groups of birds in the Far East likely diverged older than those in North America and a little older even compared with sister species pairs in North America. This might suggest morphological evolution is slower in the Far East than in North America.

**Key words:** comparative phylogeography, genetic divergence, Japan, mitochondrial cytochrome b, resident songbirds, Taiwan.

## Introduction

Past climatic cycles have caused changes in the population size and distribution of many species of birds. Such a history of populations is reflected in the genetic population structure at present (e.g., Avise, 2000). Biogeography of multiple co-distributed species using molecular methods is termed as comparative phylogeography (Bermingham & Moritz, 1998). This method has been most often applied to test the late Pleistocene origin hypothesis of bird species proposed by Rand (1948), which assumes that the glacial

cycles in the Pleistocene caused allopatric speciation by subdividing populations into disconnected or isolated refugia. In Ice Ages of the Pleistocene, the habitats of forest birds in the Northern Hemisphere was pushed southwards by developed glaciers and divided into some separated refugia distributed in the east-west direction. Contrary to the hypothesis, Klicka and Zink (1997, 1999) first suggested that most North American avian speciation events occurred long before the most recent glacial cycles by examining mitochondrial DNA (mtDNA). Johnson and Cicero (2004), however, re-examined their claim

by comparing between only pairs of species that are considered as sister taxa, and suggested that most sister species of birds in North America diverged during the Pleistocene, though not only during late Pleistocene.

While phylogeographical relationships between eastern and western populations of birds have been well investigated both in North America and Eurasia (Hewitt, 2004; Weir & Schluter, 2004), few genetic research has yet been published on such relationships between north and south populations of birds. Movements of populations to the north or south should be most conspicuous during the Pleistocene epoch, which contains at least five glacial periods and four interglacial periods. Though the glaciers are known to be less developed in Asia than in Europe and North America, the forests were pushed southwards even in the Far East (Frenzel, 1968). Temperature during the Pliocene epoch was warmer than that at present, and the sea level during the warmest interval may have been 30 m higher than that at present (Adams, 1997). The climatic cycles in the last two glacial and late Pliocene epochs might affect the latitudinal distribution and population division of birds.

Honshu Island of Japan and Taiwan Island lie north and south, respectively, in the east end of Eurasia and both are not too far from each other for bird dispersal, lying at a distance of ca. 1400 km with the intermediates of Kyushu Island and the Ryukyu Islands. On the other hand, Honshu and Taiwan belong to the different biogeographical regions (Palearctic and Oriental regions) and different climatic zones (temperate and tropical zones), both of which act as the barriers of gene flow. While not many (only about 9%) bird species are generally distributed in more than one biogeographical regions of the world, 38.5% of Palearctic bird species also occur in the Oriental region (Newton, 2003). This may suggest relatively strong relationship between the Palearctic and Oriental regions for birds at species level. There are two factors that may accelerate the gene flow in the Pleistocene. Firstly, the northern limit of broadleaf forests as the main habitat of

the birds receded southwards to southern end of Kyushu in the last glacial maximum, only about 0.02 million years ago (MYA; Frenzel, 1968). Secondly, Honshu and Taiwan were nearly connected each other by Ryukyu Arc as a land bridge during 1.6–1.0 MYA and 0.2–0.025 MYA (Kimura, 2000).

OSJ (2000) listed 41 common resident breeders of passerine birds in Honshu. Among them 17 species (41%) are shared with Taiwan. We classify these 17 species into three categories according to the level of morphological differences between populations in the two regions. Five species (29%) show no difference, eight species (47%) show subspecies level difference, and four species (24%) show subspecies-group level difference (Table 1). We speculated that the levels of morphological differences reflect degree of genetic differences.

Control region of mitochondrial DNA (mtDNA) in birds had been regarded as the most suitable genetic marker for investigating intra-specific genetic variations, because its evolutionary rate was considered as faster than any other regions of the mtDNA (14–20%/MY; Baker & Marshall, 1997). However, it is not always the most variable region of mtDNA, and its evolutionary rate differs largely depending on the lineages (estimated more than 100 times; Ruokonen & Kvist, 2002). We thus use mitochondrial cytochrome b (cyt b) region. Its evolutionary rate is assumed to be constant, 2%/MY, in all passerine birds (Moore & DeFilippis, 1997) and it is suitable for estimating coalescence time of populations. Here, we analyzed complete cyt b sequence for the eight species ranging Honshu to Taiwan as shown in Table 1 in order to estimate their divergence time in the Far East and we are going to discuss the influence of past climatic cycles on the latitudinal population structure.

## Materials and Methods

### Analyzed species

As shown in Table 1, eight target species are classified into three categories by the level of

Table 1. Seventeen resident passerine species ranging both in Honshu and Taiwan.

Species	English Name	Subspecies (subspecies group)	
		Honshu	Taiwan
<i>Motacilla cinerea</i>	Grey Wagtail	<i>robusta</i>	<i>robusta</i>
<i>Cinclus pallasii</i>	Brown Dipper	<i>pallasii</i>	<i>pallasii</i>
<i>Monticola solitarius</i>	Rock Thrush	<i>philippensis</i>	<i>philippensis</i>
* <i>Passer montanus</i>	Tree Sparrow	<i>saturatus</i>	<i>saturatus</i>
<i>Passer rutilans</i>	Russet Sparrow	<i>rutilans</i>	<i>rutilans</i>
<i>Motacilla alba</i>	Pied Wagtail	<i>lugens</i>	<i>leucopsis</i>
* <i>Hypsipetes amaurotis</i>	Brown-eared Bulbul	<i>amaurotis</i>	<i>nagamichii</i>
<i>Troglodytes troglodytes</i>	Eurasian Wren	<i>fumigatus</i>	<i>taivanus</i>
<i>Prunella collaris</i>	Alpine Accentor	<i>erythropgygia</i>	<i>fennelli</i>
* <i>Cisticola juncidis</i>	Fan-tailed Warbler	<i>brunniceps</i>	<i>tinnabulans</i>
* <i>Parus varius</i>	Varied Tit	<i>varius</i>	<i>castaneiventris</i>
* <i>Zosterops japonicus</i>	Japanese White-eye	<i>japonicus</i>	<i>simplex</i> ( <i>batanis</i> in Orchid I.)
* <i>Corvus macrorhynchos</i>	Jungle Crow	<i>japonensis</i>	<i>colonorum</i>
<i>Zoothera dauma</i>	Ground Thrush	<i>aurea</i> (“ <i>aurea</i> ” group)	<i>horsfieldi</i> (“ <i>dauma</i> ” group)
<i>Parus ater</i>	Coal Tit	<i>insularis</i> (“ <i>ater</i> ” group)	<i>ptilosus</i> (“ <i>ptilosus</i> ” group)
* <i>Sitta europaea</i>	Eurasian Nuthatch	<i>amurensis</i> (“ <i>europaea</i> ” group)	<i>sinensis</i> (“ <i>sinensis</i> ” group)
* <i>Garrulus glandarius</i>	Eurasian Jay	<i>japonicus</i> (“ <i>japonicus</i> ” group)	<i>taivanus</i> (“ <i>bispecularis</i> ” group)

Species are classified into three groups according to the level of morphological differences between the two regions. Asterisks indicate analyzed species in this study.

morphological differences between Honshu and Taiwanese populations. The tree sparrow *Passer montanus* is widespread nearly throughout Eurasia, and Honshu and Taiwan share the same subspecies *P. m. saturatus*, which is distributed from Taiwan through Japan and southern Korea to Sakhalin.

The brown-eared bulbul *Hypsipetes amaurotis*, the varied tit *Parus varius*, and the Japanese white-eye *Zosterops japonicus* are distributed only in small area of islands in the Far East. A number of subspecies have evolved in these species and different subspecies settles in each of Honshu and Taiwan. The jungle crow *Corvus macrorhynchos* and the fantailed warbler *Cisticola juncidis* have wider distribution area southwards than the former three species.

The Eurasian nuthatch *Sitta europaea* and the Eurasian jay *Garrulus glandarius* show a wide distribution from Europe to Asia and large geographical variations with 17 subspecies in three subspecies groups (Harrap & Quinn, 1996) and 33 subspecies in five subspecies groups (Madge & Burn, 1994), respectively. In both species, dif-

ferent subspecies belonging to different subspecies groups are segregated to Honshu and Taiwan. The *europaea*-group of *Sitta europaea* is widely distributed in northern Eurasia from Scandinavia to Japan, but *sinensis*-group only in China (except for the far northeast) and Taiwan (Harrap & Quinn, 1996). Distribution of *japonicus*-group of *Garrulus glandarius* is restricted to Japan (except for Hokkaido), while *bispecularis*-group has wider distribution from the Himalayas to Taiwan (Madge & Burn, 1994).

### Sampling

Samples analyzed in this study are shown in Table 2. These samples were mainly collected from wild populations in Honshu and Taiwan during 2001–2003, but complemented by two tissue banks at National Science Museum, Tokyo, and Taiwan Endemic Species Research Institute. Sequence data of the Japanese populations in three species, *Passer montanus*, *Hypsipetes amaurotis*, and *Zosterops japonicus*, were derived from Nishiumi and Kim (2004). Two to four individuals from each region were analyzed

Table 2. Locality, tissue depository, and GenBank accession numbers of *cyt b* gene sequences for specimens analyzed in this study.

Species	Subspecies	Locality		Date of collection	Depository*	Tissue Number	Genbank Accession No.
<i>Passer montanus</i> Tree Sparrow	<i>saturatus</i>	Japan	Osaka	25 Apr 1991	NSMT	AD585	AB159158
		Japan	Tokyo	6 Sep 1995	NSMT	AD1633	AB159159
		Taiwan	Nantou	14 Mar 1999	NSMT	AD2930	AB239493
		Taiwan	Nantou	26 Apr 1998	TESRI	309	AB239494
		Taiwan	Nantou	14 Apr 1999	TESRI	754	AB239495
<i>Hypsipetes amaurotis</i> Brown-eared Bulbul	<i>amaurotis</i>	Japan	Tokyo	5 Mar 1997	NSMT	AD1722	AB159161
		Japan	Niijima Isl.	21 Jun 1997	NSMT	AD2339	AB159162
	<i>nagamichii</i>	Taiwan	Orchid Isl.	8 Mar 1999	NSMT	AD2883	AB239496
		Taiwan	Orchid Isl.	8 Mar 1999	NSMT	AD2885	AB239497
		Taiwan	Orchid Isl.	9 Mar 1999	NSMT	AD2886	AB239498
<i>Cisticola juncidis</i> Fan-tailed Warbler	<i>brunniceps</i>	Japan	Ibaraki	5 Jul 1988	NSMT	AD185	AB239499
		Japan	Ibaraki	7 Jul 1989	NSMT	AD187	AB239500
		Japan	Osaka	24 Jul 1993	NSMT	AD3627	AB239501
	<i>tinnabulans</i>	Taiwan	Chang-hua	13 Mar 1999	NSMT	AD2905	AB239502
		Taiwan	Nantou	13 Mar 1999	NSMT	AD2911	AB239503
		Taiwan	Nantou	13 Mar 1999	NSMT	AD2913	AB239504
<i>Parus varius</i> Varied Tit	<i>varius</i>	Japan	Gunma	5 Dec 1996	NSMT	AD1682	AB239505
		Japan	Tokyo	30 Apr 1994	NSMT	AD1587	AB239506
		Japan	Kanagawa	3 Jul 1997	NSMT	AD1965	AB239507
		Japan	Chiba	30 Apr 1999	NSMT	AD3024	AB239508
	<i>castaneiventris</i>	Taiwan	Kaohsiung	26 Feb 1999	TESRI	1397	AB239509
		Taiwan	Kaohsiung	27 Feb 1999	TESRI	1398	AB239510
<i>Zosterops japonicus</i> Japanese White-eye	<i>japonicus</i>	Japan	Ibaraki	14 Feb 1995	NSMT	AD1631	AB159165
		Japan	Ibaraki	6 Feb 1997	NSMT	AD1697	AB159166
	<i>simplex</i>	Taiwan	Taitung	6 Mar 1999	NSMT	AD2846	AB239512
		Taiwan	Taitung	6 Mar 1999	NSMT	AD2848	AB239513
	<i>batanis</i>	Taiwan	Orchid Isl.	10 Mar 1999	NSMT	AD2843	AB239511
		Taiwan	Orchid Isl.	9 Mar 1999	NSMT	AD2865	AB239514
<i>Corvus macrorhynchos</i> Jungle Crow	<i>japonensis</i>	Japan	Tokyo	30 Apr 1998	NSMT	AD1585	AB239515
		Japan	Tokyo	2002	NSMT	AD4063	AB239516
	<i>colonorum</i>	Taiwan	Nantou	1994	TESRI	491	AB239517
		Taiwan	Taichung	Sep 1997	TESRI	668	AB239518
		Taiwan	Taichung	Nov 1997	TESRI	704	AB239519
<i>Sitta europaea</i> Eurasian Nuthatch	<i>amurensis</i>	Japan	Yamanashi	12 Sep 1991	NSMT	AD1539	AB239520
		Japan	Yamanashi	19 Sep 1993	NSMT	AD1604	AB239521
	<i>sinensis</i>	Taiwan	Kaohsiung	3 Feb 1999	TESRI	593	AB239522
		Taiwan	Nantou	1 Mar 2000	NSMT	AD3354	AB239523
<i>Garrulus glandarius</i> Eurasian Jay	<i>japonicus</i>	Japan	Nagano	15 Feb 2000	NSMT	AD3495	AB239524
		Japan	Nagano	13 Mar 1997	NSMT	AD3496	AB239525
		Japan	Nagano	10 Mar 1996	NSMT	AD3497	AB239526
		Japan	Miyagi	2 Mar 1997	NSMT	AD3591	AB242559
	<i>taivanus</i>	Taiwan	Nantou	17 Jul 1997	TESRI	16	AB239527

\* NSMT, National Science Museum, Tokyo; TESRI, Taiwan Endemic Species Research Institute.

for eight species except for only one for the Taiwanese *Garrulus glandarius*. The most samples were from blood but the others were from the breast muscle preserved in pure ethanol. As for blood about 10–20  $\mu\text{L}$  was put into a 1.5 mL tube with 500  $\mu\text{L}$  Queens' lysis buffer (Seutin *et al.*, 1991) for preservation at room temperature during the field trip. DNA was extracted using the standard phenol-chloroform procedure at laboratory.

### Cytochrome b analysis

Polymerase chain reaction (PCR) amplifications of entire mitochondrial cyt b and partial NADH dehydrogenase subunit V (ND5) were performed using primers as follows (H=heavy strand; L=light strand; numbers give position of 3'-end in *Gallus gallus* mitochondrial genome (cf. Desjardins & Morais, 1990)): mt-F (H-16065), 5'-GGA GTC TTC AGT TTT TGG TTT ACA AGA C-3' (Helbig & Seibold 1999) and L14080ND5P, 5'-TCA ACY CAY GCM TTC TTC AAA GC -3' (modified from Sorenson *et al.*, 1999). These two primers gave a product of ca. 2.0 kb.

The primers mt-F and L14080ND5P were also used as sequencing primers. For some species, species-specific sequencing primers at a position of L-14764 were also used; L14764ND5N (5'-TGA TAC AAA MTR CTA GGM CCR GAA GG-3') for *Passer montanus* and primer L14764ND5HCZ (5'-TGA TTT AAR CTM MTA GGA CCA GAA GG-3') for *Hypsipetes amaurotis* and *Zosterops japonicus*.

### Amplification and sequencing

PCR was performed in volumes of 10  $\mu\text{L}$  containing 10 ng of total genomic DNA, 0.2 mM each of dNTPs, 1.5 mM  $\text{MgCl}_2$ , 0.4  $\mu\text{M}$  of each primer, and 0.2 units of Taq DNA polymerase (Takara Ex-taq). Amplifications were performed using Takara PCR Thermal Cycler MP (Takara) under the following conditions: 30 sec at 94°C, 30 sec at 52°C, and 90 sec at 72°C (35 cycles). Before the cyclic reactions the samples were incubated at 94°C for 3 min and after completion at

72°C for 5 min. We used 2  $\mu\text{L}$  of the reaction to run on a 1.5% agarose gel in 0.5 $\times$  TBE buffer to check the success of the reaction. The remainder of the PCR product was purified using ExoSAP-IT (Amersham Bioscience).

PCR products were sequenced with the BigDye Terminator Cycle Sequencing v3.1 Cycle sequencing Kit (Applied Biosystems) on an ABI PRISM<sup>TM</sup> 3100-Avant (Applied Biosystems). Sequencing reactions were performed in volumes of 10  $\mu\text{L}$  containing 50 ng of PCR product, 1.5  $\mu\text{L}$  of Terminator Ready Reaction Mix, 1.25  $\mu\text{L}$  of 5 $\times$  Sequencing Buffer, and 1.6 pmol of the sequencing primer. The reactions were conducted under the following conditions using Takara PCR Thermal Cycler MP: 10 sec at 96°C, 5 sec at 50°C, and 4 min at 60°C (25 cycles). Before the cyclic reactions the samples were incubated at 96°C for 1 min. The products were purified using ethanol/EDTA/sodium acetate precipitation according to the BigDye Terminator v3.1 Cycle Sequencing Kit manual.

### Calculation of genetic distance and estimation of time since divergence

Uncorrected pairwise divergence was used because the intra-specific difference of cyt b in birds is less than 10%, where even transitional substitutions at the third codon positions are far from saturation (Hackett, 1996). We used net nucleotide divergence between two populations ( $p_{\text{AB}(\text{net})}$ ) for estimating divergence time of populations:  $p_{\text{AB}(\text{net})} = p_{\text{AB}} - 0.5(p_{\text{A}} + p_{\text{B}})$ , where  $p_{\text{A}}$  and  $p_{\text{B}}$  are values for mean nucleotide diversity among individuals within populations A and B, respectively, and  $p_{\text{AB}}$  is the mean nucleotide divergence between individuals of these two populations (Johnson & Cicero, 2004). When we lack one of mean nucleotide diversity value within population because of the only one analyzed sample (e.g., Taiwanese population of *Garrulus glandarius*), we provisionally calculated net nucleotide divergence assuming the same value of intra-population diversity as that of the other group. The evolutionary rate of cyt b is assumed to be constant in 2%/MY followed by Moore and

Table 3. Genetic variation in 1143–1146 bp of cyt b of eight songbird species common to Honshu and Taiwan and the estimated divergence time.

Level of morphological difference	Species	Genetic difference (bp)				Sequence divergence (%)		Divergence time (MYA)
		within Honshu	within Taiwan	between regions		$p_{AB}$	$p_{AB}$ (net)	
				Av. (Range)	Net			
None	<i>Passer montanus</i>	2	0.67	3.3 (2–5)	2	0.29	0.17	0.09
Subspecies	<i>Hypsipetes amaurotis</i>	4	0.67	4.3 (3–6)	2	0.38	0.17	0.09
	<i>Cisticola juncidis</i>	2	1.33	5.7 (4–8)	4	0.50	0.35	0.17
	<i>Corvus macrorhynchos</i>	6	2.67	6.7 (6–7)	2.33	0.58	0.20	0.10
	<i>Parus varius</i>	1	0	70.5 (69–71)	70	6.2	6.1	3.1
	<i>Zosteropus japonicus</i> (Orchid Isl.)	1	0	56.5 (56–57)	56	4.9	4.9	2.4
				17.5 (17–18)	17	1.5	1.5	0.74
Ssp.-group	<i>Sitta europaea</i>	2	0	62 (61–63)	61	5.4	5.3	2.7
	<i>Garrulus glandarius</i>	1	n/a	59 (59)	58	5.2	5.1	2.5
	mean	2.38	0.67	31.7	30.3	2.8	2.6	1.3

De Filippis (1997). We also reanalyzed data from Nishiumi and Kim (2004) by this calculation for comparison.

## Results and Discussion

### Cytochrome b sequences

A total of 42 sequences of eight target species were examined. Thirty six samples were newly sequenced with the accession numbers of AB239493–AB239527 and AB242559 (Table 2). The other six sequencing data of Japanese birds were derived from Nishiumi and Kim (2004). The length of cyt b genes was 1143 bp for all of the analyzed species except for one species, *Passer montanus*, which exceed by three bp. Their amino acid sequences are attended with a glutamine at the end.

### Intra-populational genetic variation

The results on genetic comparisons are listed in Table 3. The value of genetic difference within the Honshu population was higher than that within Taiwanese one in every seven species compared. The mean value of intra-populational variation in Honshu, 2.38 bp, is larger than that in Taiwan, 0.67 bp. The mean value in Korea was calculated as 0.83 bp from six resident passerine

species (Table 4). The intra-populational genetic variations of the resident passerines in Honshu tend to be relatively large in the Far East. This suggests that long term population size might be high in Honshu or that the populations in Honshu might be mixture of old (relict) and new lineages. The other explanation of the difference in the genetic variation might be sampling bias, as for example by collecting individuals from subdivided populations in Honshu. As shown in “geographical origin” in Table 2, however, most samples were obtained from close localities. Further researches are required for explanation of the observed high values of intra-populational genetic variation in Honshu.

### Inter-populational genetic difference and recent gene flow

Mean sequence divergences between individuals from the Honshu and Taiwanese populations were widely dispersed with ranging from 3.3 (*Passer montanus*) to 70.5 bp (*Parus varius*) for nine subspecies pairs of eight species examined (Table 3). These values of genetic differences include the ancestral intra-populational diversity, and should be adjusted by subtracting the ancestral diversity to estimate divergence times between the populations. Net sequence divergences

Table 4. Genetic variation in cyt b of eight species of resident songbirds common to Honshu and Korea and the estimated divergence time.

Level of morphological difference	Species	Genetic difference (bp)				Sequence divergence (%)		Divergence time (MYA)
		within Honshu	within Korea	between regions		$p_{AB}$	$p_{AB}$ (net)	
				Av. (Range)	Net			
None	<i>Lanius bucephalus</i>	4	2	2.5 (1–5)	0.5	0.22	0.04	0.022
	<i>Passer montanus</i>	2	–	4 (3–5)	2	0.35	0.17	0.087
	<i>Hypsipetes amaurotis</i>	4	1	3.5 (2–5)	1	0.31	0.09	0.044
	<i>Zosteropus japonicus</i>	1	1	1 (0–2)	0	0.09	0	0
	<i>Cettia diphone</i>	2	1	1.5 (1–2)	0	0.13	0	0
Subspecies	<i>Aegithalos caudatus</i>	2	0	1 (0–2)	0	0.09	0	0
	<i>Emberiza cioides</i>	2	0	1 (1)	0	0.09	0	0
	mean	2.43	0.83	2.1	0.5	0.18	0.04	0.022

All data are derived from Nishiumi and Kim (2004).

between regions were more various with ranging from 2 to 70 bp (0.17–6.1%) and the estimated divergence times range from 0.09 to 3.1 MYA with mean value of 1.3 MYA. This mean value is much higher than that of Honshu and Korea, 0.022 MYA (Table 4), estimated from data of Nishiumi and Kim (2004).

One of the most remarkable finding is the presence of genetic differences between Honshu and Taiwanese populations in all eight species examined, which is contrasting with absence of such differences between Honshu and Korean populations in four of seven examined species. This result suggests that substantial level of gene flow lacks between Honshu and Taiwan at present, probably throughout the Holocene, which may be caused by isolation by geographical distance and/or local adaptation to each climatic zone in comparison to the relationship between Honshu and Korean populations.

#### Estimated divergence time and influence of past climatic changes

In a half of the species (four of eight species), sequence divergence between the Honshu and Taiwanese populations was shallow with ranging 0.17–0.35% in net value (Table 3). The estimated divergence time was 0.09–0.17 MYA, which synchronized with the last two glacial periods (be-

ginning at ca. 0.1 and ca. 0.25 MYA, respectively). This suggests strong effect of glacial periods on mixing the populations of Honshu and Taiwan. We can speculate that the cold climate during the glacial periods pushed the Honshu population towards south, and land bridge in Ryukyu Arc which appeared due to the reduced sea-level facilitated the contact with the Taiwanese population.

In the other half of the species, sequence divergence between the Honshu and Taiwanese populations was deep with ranging 4.9–6.1% in net value (Table 3). The estimated divergence time was 2.4–3.1 MYA, which just coincides with the late Pliocene. In general, the Pliocene (5.4–2.4 MYA) has rather warmer in climate than at present and sea levels may have been as much as 30 m higher than at present during the warmest intervals. The peak phase of warmth were mostly during the interval between 3.4–2.7 MYA, which was followed by gradual cooling, and the glacial events between 2.5–2.3 MYA were the most intense (Cronin *et al.*, 1994). The warmest climate may have caused that the Taiwanese birds living in evergreen forests extended their distribution northward to the Japanese islands, while the next cooling divided the population, and remarkably the populations of Honshu and Taiwan have never experienced substantial

gene flow since then. The deep divergence, which has persisted throughout the Pleistocene climatic cycles, implies special influence of the warmest late Pliocene period for the four species on the population structure.

The two typical figures of the populational relationship of the resident birds between Honshu and Taiwan were suggested: some species had gene flow in the last two glacial periods but never since then, and the others did in the late Pliocene but never since then. All of genetic signals that might have been accumulated during the early Pleistocene must have been eliminated during the last two glacial periods, implying much stronger influence in the late Pleistocene than in the early Pleistocene. This is in marked contrast to the longitudinal division of populations, of which any genetic researches have not shown strong influence of the late Pleistocene (e.g., Klicka & Zink, 1997, 1999; Avise & Walker, 1998), though it had been long speculated since Rand (1948).

As an exception of the distinct division of divergence time in the late Pleistocene and late Pliocene, divergence time of Orchid Island population (locating south-east off the main island of Taiwan) with Honshu in *Zosteropus japonicus* was estimated as 0.74 MYA in the mid Pleistocene, while that of Taiwanese main island population was 2.4 MYA (Table 3). Because distribution of this Orchid Island subspecies *Z. j. batanis*, from Green Island of Taiwan to the Batan Islands of the Philippines, is unique among bird subspecies, the genetic relationship with other subspecies might be also unique.

### Morphological difference and divergence time

*Passer montanus*, distributed as the same subspecies in Japan and Taiwan, showed shallow genetic divergence (0.17% in net value). Three of the five species with subspecies level differences in the two regions showed shallow, but the other two species showed deep divergence (Table 3). Both species with the subspecies-group level differences (*Sitta europaea* and *Garrulus glandarius*) showed deep divergence (Table 3). This suggests that morphological difference roughly re-

lates to genetic divergence, namely the time since the populations separated, but the relation is not direct among the species of songbirds.

Ball and Avise (1992) demonstrated that most subspecies of North American birds are genetically very close (0.03–0.48% in mtDNA) but subspecies-group pairs show relatively deep mtDNA divergence (0.8–1.2%). When comparing with these North American birds, Far Eastern birds much clearly separated into two categories which show shallow and deep divergences. Then Far Eastern subspecies-groups diverged older than those of North America and a little older even compared with sister species pairs of North America in the mean value as shown later. This suggests that morphological evolution of songbirds might be slower in Far East than in North America.

Sister species pairs of North American birds show relatively shallow mtDNA divergences (0.5–3.8%, 0.90 MYA in mean value) for arctic species, relatively deep divergence (0.2–9.5%, 1.75 MYA in mean value) for temperate species, and deep divergence (0.4–7.0%, 1.89 MYA in mean value) for tropical montane species (Weir & Schluter, 2004; Lovette, 2005). This suggests that the effects of the late Pleistocene glacial cycles on populational division were somewhat stronger in the species of high latitudes, which may be directly affected by glacier, than in those of low latitudes. For latitudinal divergence, we have no knowledge on the effects of latitudes yet.

### Species distribution and divergence time

*Passer montanus*, which showed the shallow divergence, is distributed wide area of Eurasia, but the subspecies *P. m. saturatus* is only in the Far East, from Taiwan to Sakhalin and southern Korea. Among the five species with subspecies level differences between Honshu and Taiwan, two species (*Cisticola juncidis* and *Corvus macrorhynchos*) are distributed widely in southern Eurasia. All of them showed shallow divergence. Among the other three of the five species, which distributed only in the Far East, one species showed shallow divergence but two did



deep. We can speculate all of these species originated from south, but the divergence is different among species. Thus we can not find any relationship between species distribution and divergence time. On the other hand, we found a certain relationship in the two species with subspecies group differences, *Sitta europaea* and *Garrulus glandarius*. Both species are distributed widely in Eurasia but not in the Ryukyu Islands, which is different from the other species mentioned above, and both showed deep divergence. Each subspecies group of the two species probably originated from different lineages (from northern and southern Eurasia) and they adapted to each of their environments, subtropical forests and temperate forests. They might not contact each other or they might keep their separation even though they had experience of contact. Species classification on such deeply diverged taxa should be revised if they had experience of contact during Pleistocene.

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極東のスズメ目留鳥における  
緯度方向の集団構造に最終氷期と鮮新世後期が与えた影響

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過去の気候サイクルは多くの生物種の個体数や分布の変動の原因となってきた。現在の種の遺伝的な集団構造からその影響を調べることができる。このような研究は、鳥類では北アメリカやユーラシア大陸での東西方向の集団分化についてよくおこなわれてきたが、南北方向の分化についてはまだほとんど研究されていない。そこで、8種の定住性鳥類についてミトコンドリアDNAチトクロームb領域の塩基配列を調べ、本州と台湾の集団間の分岐年代を評価することで、極東域における南北の集団分化について過去の気候サイクルの影響を調査した。その結果、両集団の分岐年代は2つの期間に集中していることがわかった。8種中4種は遺伝的分化が浅く、9万年前から17万年前に分岐したと評価され、残り4種は遺伝的分化が深く、240万年前から310万年前に分岐したと評価された。前者の分岐年代は更新世後期にあたる最終氷期からその前の氷期、すなわち1万年前から20万年前までと一致し、後者は鮮新世後期と一致した。このことから、1) 日本と台湾に分布する留鳥類は、形態的に区別できない種においてさえ、少なくとも現在は（おそらくは完新世全体を通して）実質的な遺伝子流動が両地域間で起こっていないこと、および2) 極東域における留鳥の南北の集団構造は2つの時期、すなわち最終氷期と鮮新世後期に強く影響を受けたことが示唆された。他方、前期更新世の間に蓄積されたであろう遺伝的シグナルはすべて最終氷期の間に消失していた。これは前期更新世の影響が最終氷期と比べて弱かったことを意味する。この結果は、予測された最終氷期の重要性が大陸の東西での分化の研究では否定されてきたことと対照的である。また、極東における鳥類の亜種グループは北アメリカの亜種グループと比べて分岐の時期がより古く、北アメリカの姉妹種間の分岐と比べてさえも少し古いことが示唆された。極東での鳥類の形態進化の速度が北アメリカと比べて遅い可能性が考えられる。