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ORIGINAL ARTICLE

Quaternary-related genetic differentiation and parallel population dynamics of the Eurasian Jay (*Garrulus glandarius*) in the circum-Japan Sea region

Daisuke Aoki[1] · **Gohta Kinoshita**[2] · **Alexey P. Kryukov**[3] · **Isao Nishiumi**[4] · **Sang-im Lee**[5] · **Hitoshi Suzuki**[6]

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Abstract

The circum-Japan Sea region (CJSR) greatly impacted animal diversity in the Eastern Palearctic during the Quaternary. However, its role in avian diversification has been underestimated because of the high dispersal capabilities of birds over the sea. We investigated the phylogeographic and demographic history of the Eurasian Jay (*Garrulus glandarius*), focusing on CJSR populations. We sequenced a total of 1744 bp of mitochondrial DNA (cytochrome *b* and control region) from 73 samples. Together with the database sequences, we reconstructed a phylogenetic tree for the Eurasian Jay over the Palearctic. The earliest phylogenetic divergence was inferred to be related to geological vicariance between the Japanese archipelago and Eurasian mainland around the Pliocene–Pleistocene boundary. Several demographic analyses have suggested that there are two divergent subspecies across the CJSR, *G. g. brandtii* on the mainland, Sakhalin, and Hokkaido and *G. g. japonicus* in the archipelago. These simultaneously experienced population contractions to independent refugia and subsequent expansions around the last glacial period, i.e., parallel population dynamics. We suggest that the two landmasses of the CJSR were important for generating and preserving the phylogenetic structure of the Eurasian Jay.

Keywords The circum-Japan Sea region · *Garrulus glandarius* · Mitochondrial DNA · Parallel population dynamics · Phylogeography

Zusammenfassung

Quartäre genetische Differenzierung und parallele Populationsdynamik des Eichelhähers *Garrulus glandarius* in der Region um das Japanische Meer Die Region um das Japanische Meer (engl: Circum-Japan Sea Region; CJSR) hatte während des Quartärs einen starken Einfluss auf die Vielfalt der Tiere in der östlichen Paläarktis. Aufgrund der hohen Ausbreitungsfähigkeit der Vögel über das Meer wurde ihre Bedeutung für die ornithologische Diversität allerdings unterschätzt. Wir untersuchten die phylogeografische und demografische Geschichte des Eichelhähers *Garrulus glandarius*, wobei wir uns auf die CJSR-Populationen konzentrierten. Wir nahmen eine Neusequenzierung von insgesamt 1744 bp mitochondrialer DNA (Cytochrom *b* und Kontrollregion) aus 73 Proben vor. Zusammen mit Sequenzen aus Datenbanken rekonstruierten wir einen phylogenetischen Stammbaum für den Eichelhäher in der Paläarktis. Die früheste phylogenetische Aufspaltung stand demnach in Relation zu geologischen Vikarianzereignissen zwischen dem japanischen Archipel und dem eurasischen Festland, etwa zur Pliozän-Pleistozän-Grenze. Verschiedene demografische Analysen lassen vermuten, dass es in der CJSR zwei getrennte Unterarten gibt: *G. g. brandtii* auf dem Festland, auf Sachalin und Hokkaido sowie *G. g. japonicus* auf dem Archipel, deren Populationen sich gleichzeitig in individuelle Refugien zurückgezogen und in der Folge etwa zur Zeit der letzten Glazialperiode wieder ausgebreitet haben, also eine parallele Populationsdynamik zeigen. Wir nehmen an, dass

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Extended author information available on the last page of the article

die beiden Landmassen der CJSR eine wichtige Rolle bei der Entstehung und Aufrechterhaltung der populationsgenetischen Struktur des Eichelhähers innehatten.

Introduction

The Quaternary climatic oscillations are regarded as one of the prominent causes affecting species distribution and population genetic diversity in several regions in the Holarctic. There are several potential refugial areas for terrestrial vertebrates in the Eastern Palearctic (Waltari and Cook 2005; Hirata et al. 2014; Řičánková et al. 2014). One strong candidate is the circum-Japan Sea (East Sea) region (CJSR), which consists of the southern Russian Far East, northeastern China, the Korean Peninsula, and Japan. This region is increasingly perceived as a refugial area for terrestrial vertebrate species during the Quaternary (Haring et al. 2007; Hope et al. 2010; Saitoh et al. 2010; Kinoshita et al. 2015; Li et al. 2016). It is believed that the formation of refugia in other Holarctic regions and population fragmentation in the Pleistocene drove the current genetic divergence between closely related lineages (Weir and Schluter 2004). Regarding the CJSR, genetic divergence in this region is estimated to be older than in other Holarctic regions, specifically around the Pliocene–Pleistocene boundary (Saitoh et al. 2010; Päckert et al. 2015). Therefore, ancient genetic divergence should have been preserved through the Quaternary climatic oscillations (Saitoh et al. 2010), yet the historical scenario of this phylogenetic process is not completely understood.

The CJSR is a unique geographic region comprising two landmasses along a north–south axis: the east coast of the Eurasian continent and its counterpart, an arc of land-bridge islands (Fig. 1). Several geographical barriers are present in the CJSR, including the Japan Sea, which divides the two landmasses, shallower straits in the northern part [the Tatar (Mamiya) Strait (over 12 m in depth) and the La Pérouse (Soya) Strait (over 55 m in depth)], and two deep, wide straits located farther south [the Tsugaru and Korea (Tsushima) Straits (both over 130 m in depth)]. It is thought that the colonization of terrestrial species between the two landmasses was severely restricted in glacial periods when the sea level dropped dramatically and land bridges formed over the deeper straits, whereas the northern shallower straits were not strongly influential because of frequent land bridge formation (Dobson and Kawamura 1998; Hikida 2003; McKay 2012). Therefore, vicariance due to the formation of deeper straits that segregated animal populations on the two landmasses is thought to be important for population differentiation and speciation in the CJSR (Hikida 2003; McKay 2012). Although sea level changes during glacial periods are believed to have had little impact on the distributions of volant species (Morioka and Sakane 1980, but see Nishiumi et al. 2006; Saitoh et al. 2010; Kryukov et al. 2012; Weißensteiner 2013), similar differentiation patterns across the Japan Sea were recently suggested for many bird taxa (Saitoh et al. 2015). Holt et al. (2013) treated this region as an independent zoogeographic region specifically for birds and suggested that the phylogenetic differentiation rate was

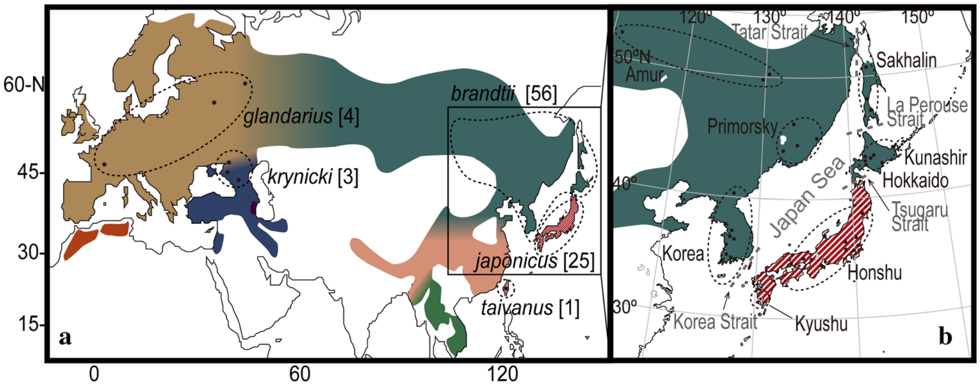

Fig. 1 Sampling localities for Eurasian Jays examined in this study. Colors correspond to the eight subspecies groups. See Online Resource 1 for the distributions of the subspecies and definitions of the subspecies groups. Distribution boundaries where hybridization has been recorded are indicated by color gradients. **a** Summary of sampling localities respective to each subspecies including sequences newly obtained for this study and database sequences. Dotted circles represent subspecies assigned to samples used, and numbers within parentheses indicate sample numbers for each subspecies. **b** Magnification of **a** indicating the circum-Japan Sea region (CJSR). Dots indicate sampling localities; dotted circles indicate assigned geographical regions. Distribution map of subspecies groups redrawn after dos Anjos (2009)

higher in this region. It is plausible that the CJSR played an important role in shaping the phylogeographic structure of avian species.

The Eurasian Jay (*Garrulus glandarius*) is a Eurasian corvid (family Corvidae), with an extensive range that spans from Europe, extending from northern Africa and the Middle East through Siberia, to the southern Russian Far East, Japan, and south to China and continental Southeast Asia (Fig. 1). It shows considerable geographic variation in morphology over its wide range; 34 subspecies are currently accepted (Dickinson and Christidis 2014; Gill and Donsker 2018) and are often divided into five or eight subspecies (racial) groups (Goodwin 1986; dos Anjos 2009; del Hoyo and Collar 2016). Members of a subspecies group share phenotypes that are reasonably distinct from other subspecies groups, which suggests the possibility of reciprocal monophyletic relationships (del Hoyo and Collar 2014) (see Online Resource 1 for the description and delineation of the subspecies and subspecies group assignments). Although a complex phylogeographic history is thought to have led to the high degree of intraspecific variation in this species, few studies have examined this. Akimova et al. (2007) confirmed deep genetic differentiation between two subspecies in the CJSR, *brandtii* on the mainland and *japonicus* in the Japanese archipelago, using mitochondrial DNA. However, their study included only a few samples from the CJSR and did not consider the phylogeographic process.

To investigate the phylogeographic relationships among the Eurasian Jay populations in the CJSR in more detail, we conducted phylogenetic analyses with additional samples collected from a wide selection of localities in this region. One of the main aims of this study was to understand how the CJSR affected the diversification of the Eurasian Jay through the inference of historical events. As a second aim, the demographic history that resulted in the present genetic structure within divergent groups of the Eurasian Jay in the CJSR was also inferred. Overall, we attempted to understand how the CJSR acted on bird species diversification and preservation.

Materials and methods

Sampling and laboratory methods

Liver and muscle tissues or toepads of skin specimens stored in ethanol were used for DNA extraction. In total, 72 tissues of individuals collected from the CJSR (Fig. 1b) and one tissue from Krasnodar, Russia were used to determine the complete *Cytb* and partial control region (CR) nucleotide sequences. Additionally, we downloaded sequences of another nine samples of *G. g. japonicus*, seven samples of Eurasian Jays from other Palearctic regions, including one sample of *G. g. taivanus* (Taiwan), three samples of *G. g. krynicki* (Caucasus), and three samples of *G. g. glandarius* (Europe) (Fig. 1a), and seven outgroup species (*G. lidthi*, *G. lanceolatus*, *Pica pica bactriana*, *P. p. jankowskii*, *Pyrrhocorax pyrrhocorax*, *Cyanopica cooki*, and *Cy. cyanus pallescens*) from the GenBank international DNA database. Details are listed in Online Resource 2. Total genomic DNA was extracted using the standard phenol–chloroform procedure (Sambrook and Russell 2001) or with the QIAamp DNA Mini Kit (QIAGEN), following the manufacturer's instructions. The polymerase chain reaction (PCR) for complete sequence determination of *Cytb* was performed by dividing it into "upper" and "lower" regions: ND5-Gar1 + (5′-GGATCATTTGCCCTATCAATC-3′) and Cytb-Gar1− (5′-TGTCTCGTGTAGGAAGGTCAGA-3′) for the upper and Cytb-Gar2+ (5′-ATTTTTCGCCTTCCATTTCC-3′) and Thr-Gar2- (5′-GCCTTCAATCTTTGGTTTACAA-3′) for the lower regions, which were all designed for this study using Primer3Plus web interface (Untergasser et al. 2007). The partial sequences of the CR were amplified using primers with sequences modified from Haring et al. (2007); CR-Gar+ (5′-AGTGCAGCGCAGGAGTTATC-3′) and Phe-Gar− (5′-CAGCCGTCTTGACATCTTCA-3′). PCR was performed in a final volume of 20 μL of admixture consisting of 1 μL of template DNA, 2.5 mM $MgCl_2$, 0.2 mM dNTPs, 0.05 μM of each set of primers, 1 × *Taq* Polymerase buffer, and 0.5 units AmpliTaq Gold 360 Master Mix kit [Applied Biosystems (ABI)]. The PCR was started with an initial cycle of denaturation at 95 °C (2 min), followed by 35 cycles of denaturation at 95 °C (30 s), annealing at 59.8 °C (30 s), and extension at 72 °C (1 min), and ended with a cycle of extension at 72 °C (10 min). The sequencing reactions of the PCR products were performed using the BigDye Terminator Cycle Sequencing kit v3.1 (ABI) with the corresponding primers, and automated sequencing was run on an ABI3130 Genetic Analyzer (ABI). Sequences were individually aligned for each gene by eye using ProSeq v. 3.5 software (Filatov 2009) and the MAFFT web server with the L-INS-i algorithm (Katoh et al. 2005; Katoh and Toh 2008). We did not detect any double peaks for sequences in either locus or any stop codon in the protein-coding region of *Cytb*.

Phylogenetic tree

We performed a reconstruction of the phylogenetic tree and estimation of divergence times using a partitioned dataset of *Cytb* and CR sequences by the Bayesian inference method, using the BEAST v. 2.4.5 software (Bouckaert et al. 2014). To generate an XML input file with settings specific to our dataset, we used the program BEAUti v. 2.4.5 (provided in the BEAST package). The two gene partitions were linked under "Link Trees" because they are in the same mitochondrial genome and should share the same tree topology; other settings were left unlinked. Each gene partition was individually assigned using the best-fitting substitution model as determined by the Bayesian information criterion (BIC) implemented in the MEGA v. 7.0 software (Kumar et al. 2016); HKY (Hasegawa et al. 1985) + G ($\alpha = 0.28$) and HKY + G ($\alpha = 0.44$) were selected for *Cytb* and the CR, respectively. The clock model was also set for each gene partition; Strict Clock was selected for *Cytb* and Relaxed Clock Log Normal was selected for the CR, with the clock rate estimated under *Cytb*. We used 1.05%/lineage/Mya as a molecular clock rate for *Cytb*, corresponding to the avian "standard" molecular clock rate of 2.1% sequence divergence per million years (Weir and Schluter 2008) because no fossil calibration was available for the Eurasian Jay. We assumed a coalescent exponential population as a tree prior for linked trees. Three independent Markov chain Monte Carlo (MCMC) analyses were run for 5×10^7 generations with log files and tree files sampled every 1000 and 2000 generations, respectively. The three independent output log files were combined with the first 10% of each of the sampled parameters discarded as burn-in using the LogCombiner v. 1.8.4 software (http://beast.community/logcombiner). The combined output log file was analyzed using the Tracer v. 1.6 software (Rambaut et al. 2014) to evaluate the convergences of parameters to stationary posterior distributions with effective sample sizes (ESS) larger than 200. We finally combined the three tree files with 10% each of sampled trees discarded as burn-in using LogCombiner v.1.8.4 software, and the combined trees were summarized using the TreeAnnotator v. 1.8.4 software (http://beast.community/treeannotator), with "Maximum clade credibility tree" and "Mean heights" as settings, and displayed using the FigTree v. 1.4.3 software (http://tree.bio.ed.ac.uk/software/figtree/).

Historical demography

As a result of low sample sizes and considering shared paleogeography, a sample from Kunashir Island and a sample from Kyushu were combined with geographical populations from Hokkaido and Honshu, respectively, in the data analyses. Nucleotide diversity (π) and the number of polymorphic sites were calculated for concatenated sequences in each of the CJSR subspecies (*brandtii* and *japonicus*) as well as the geographical populations assigned for *brandtii* (Fig. 1b). Mismatch distribution analysis (Li 1977; Harpending 1994; Rogers 1995) and neutrality tests (Tajima 1989; Fu 1997) were conducted for each locus separately in the focal subspecies and in the subgroups inferred by the phylogenetic trees. Mismatch distribution analysis was evaluated using the sum-of-squares deviation (SSD) and the raggedness index (rag.) to determine whether the focal population had experienced a sudden expansion (Rogers 1995). Tau (τ) values were calculated as a relative index of the time since population expansion; τ is expressed as $\tau = 2\,\mu L t$, where μ is the substitution rate, L is the length of the sequence, and t is the time since expansion. Using this equation and a molecular evolutionary rate of *Cytb* $\mu = 1.05\%$/lineage/Mya, we calculated the time since expansion t for τ of *Cytb*. The subgroup structure was also inferred by pairwise Φ_{ST} values among assigned populations, using concatenated sequences of *Cytb* and the CR with a substitution model selected according to BIC in the model test using MEGA v. 7.0; we selected the Tamura and Nei model (Tamura and Nei 1993) with a gamma correction + G ($\alpha = 0.05$). We used the program ARLEQUIN v. 3.5.2 (Excoffier and Lischer 2010) to conduct these statistical analyses.

We performed an extended Bayesian skyline plot (EBSP), as implemented in BEAST 2.4.5 (Heled and Drummond 2008; Bouckaert et al. 2014), separately for the two subspecies and for the two subgroups inferred from the phylogenetic trees. HKY was selected as the substitution model for *Cytb* and the CR of the entire sample set using MEGA v. 7.0. The molecular clock rate of *Cytb* (1.05%/lineage/Mya) was applied as a Strict Clock rate, whereas the rate for the CR was set to be estimated. An XML input file was generated using the program BEAUti v. 2.4.5 with settings identical to those used to reconstruct Bayesian inference phylogenetic trees, with the following exceptions: coalescent extended Bayesian skyline was selected for each of the gene trees in priors. EBSP results were visualized using the R v. 3.3.1 software (R Core Team 2016).

Results

Phylogeographic pattern and divergence time estimates

For all 72 of the newly sequenced Eurasian Jay samples from the CJSR, 1143 bp of the *Cytb* gene was unambiguously resolved and the partial CR resulted in an alignment of 601 bp including 4 indel sites. The concatenated sequences of the two mtDNA regions yielded 54 haplotypes with 115 polymorphic sites.

We propose that the Bayesian inference tree determined by BEAST using mtDNA sequences supported the monophyly of genus *Garrulus* where a sister-group relationship exists between the Eurasian Jay and the monophyletic group of two "relict" *Garrulus* species (*G. lidthi* and *G. lanceolatus*). The coalescence time estimates of *Garrulus* dated back to 7.98 million years ago (Mya) (95% highest possibility density, HPD 6.48–9.55 Mya), around the late Miocene (11.6–5.33 Mya).

Five distinct clades were recognized within the Eurasian Jay clade, perfectly corresponding to subspecies and supported by posterior probabilities (Fig. 2). The earliest split

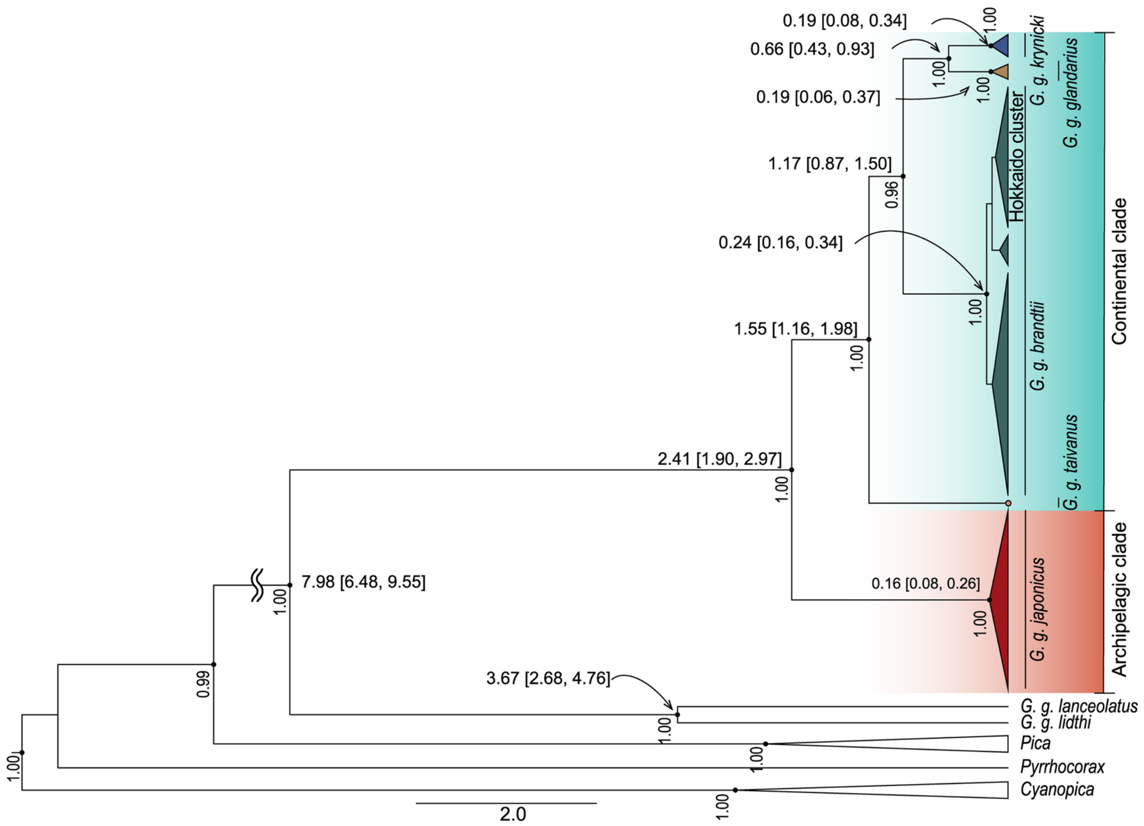

Fig. 2 Bayesian inference phylogenetic tree using sequences of mitochondrial *Cytb* and control region (CR) for 81 individuals of the Eurasian Jay and its related Corvidae outgroup species. Posterior probabilities are provided vertically by nodes, but not shown for minor clades within the five main clades of the Eurasian Jay. Horizontally aligned numbers indicate divergence time estimates calculated using a rate of 1.05%/lineage/Mya; 95% highest posterior density intervals indicated in square brackets. Because major clades corresponded to the subspecies classification, they were named after them. The earliest divergent clades of the Eurasian Jay were named the "archipelagic clade" and "continental clade". Hokkaido samples are specified

between *japonicus* (archipelagic clade) and the remaining subspecies, which are distributed widely over the Eurasian continent (continental clade), was estimated to be 2.41 Mya (95% HPD 1.90–2.97 Mya), around the Pliocene–Pleistocene boundary. The subsequent split within the continental clade was *taivanus*, followed by *brandtii* with coalescence dates of 1.55 Mya (95% HPD 1.16–1.98 Mya), and 1.17 Mya (95% HPD 0.87–1.50 Mya), respectively. Finally, the western subspecies (*glandarius* and *krynicki*) diverged, dating to 0.66 Mya (95% HPD 0.43–0.93 Mya). The estimated time to the most recent common ancestor (tMRCA) of each subspecies was dated to around the Middle (0.77–0.12 Mya) to the Late Pleistocene (0.12–0.01 Mya) (Fig. 2). Although within-clade phylogenetic structure was not statistically supported in *brandtii*, Hokkaido appeared as a different cluster from other geographical regions (Fig. 2). Hereafter, the putative genetic subgroups are defined as the Hokkaido subgroup (Hokkaido population) and mainland subgroup (the remainder of *brandtii* including the Sakhalin population). We observed no geographical structure in *japonicus*.

Genetic diversity and demographic history of genetic groups

The Primorsky population exhibited the highest nucleotide diversity (0.0028), followed by the Korean and Amur River range (Amur) populations (0.0022 and 0.0020, respectively). Compared to the continental populations, nucleotide diversity of island populations was slightly less variable (Hokkaido, 0.0018; Sakhalin, 0.0012). The nucleotide diversity was lower in *japonicus* (0.0020) than in *brandtii* (0.0027) (Table 1).

Tajima's *D* and Fu's *Fs* values calculated individually for each locus were mostly negative for the two divergent clades (*japonicus* and *brandtii*) and the two subgroups (mainland and Hokkaido) of *brandtii*, indicating sudden population expansions for the two clades and subgroups (Table 2). However, the negative Tajima's *D* values were not supported in *Cytb* and the CR of *japonicus* or the CR of *brandtii* and Hokkaido subgroup. The SSD and rag. values produced by the mismatch distribution analyses did not reject the null

Table 1 Sample size (n) and diversity estimates for assigned geographical regions

	n	No. haplotypes	No. of polymorphic sites	Nucleotide diversity (π)
japonicus	25	19	22	0.0020
brandtii	56	41	44	0.0027
Korea	17	13	18	0.0022
Primorsky	10	10	20	0.0028
Sakhalin	4	4	4	0.0012
Amur	4	4	7	0.0020
Hokkaido	21	14	19	0.0018

hypothesis that populations experienced sudden expansions for any genetic group (*japonicus* or *brandtii* as well as mainland or Hokkaido subgroups). Time since expansion, estimated on the basis of the τ values for *Cytb*, ranged from 44 thousand years ago (kya) to 84 kya, corresponding to the last glacial period. The time since expansion for the Hokkaido subgroup was estimated to be later (44 kya, 95% confidence interval, CI 3–94 kya) than the entire clade or mainland subgroup owing to its smaller *Cytb* τ value.

Among six geographical regions (Table 3), Φ_{ST} values were high ($\Phi_{ST} = 0.99$) between Honshu–Kyushu (*japonicus*) and all of the regional populations of *brandtii* (Korea, Primorsky, Sakhalin, Amur, and Hokkaido–Kunashir). The Φ_{ST} values between the Hokkaido–Kunashir population and populations of the mainland subgroup of *brandtii* were higher (ranging from 0.35 to 0.51, $p < 0.05$) than between regional populations within the mainland subgroup ($\Phi_{ST} < 0.13$, $p > 0.05$).

Demographic history of a population in the CJSR determined by EBSP

EBSP clearly indicated population expansions in each subspecies and the *brandtii* subgroups (Fig. 3), supported by the "sum(indicators.alltrees)" parameter in Tracer, which confidently rejected a constant population (not shown), but suggested one or two demographic changes (all the medians and modes equaled 1), and 95% central posterior density (CPD). The time of the population expansion remained unclear, but was estimated to be before or around the Last Glacial Maximum (LGM; 0.02 Mya).

Table 2 Statistics for demographic analyses of genetic groups inferred by phylogenetic analyses and pairwise Φ_{ST}

Genetic groups	Loci	Tajima's *D*	Fu's *Fs*	SSD	Raggedness index	τ (95% CI^{*a})	*t* [kya] (95% CI)
japonicus	Cyt*b*	−1.52*	−14.6**	0.0033	0.042	1.5(0.2–4.7)	62 (0–196)
	CR	−0.78	−10.8**	0.0010	0.044	2.3(1.2–3.9)	
brandtii	Cyt*b*	−2.18**	−22.4**	0.0040	0.073	1.8 (1.2–2.5)	73 (50–103)
	CR	−1.20*	−20.7**	0.0022	0.034	3.0 (2.2–3.8)	–
Subgroups							
Mainland	Cyt*b*	−1.78**	−13.0**	0.0070	0.077	2.0 (1.4–2.9)	84 (57–120)
	CR	−1.53**	−15.3**	0.0090	0.060	2.4 (1.5–3.3)	–
Hokkaido	Cyt*b*	−1.93**	−5.2**	0.0097	0.116	1.1 (0.07–2.3)	44 (3–94)
	CR	−1.12	−2.8*	0.0012	0.037	2.3 (1.0–3.5)	–

**$p < 0.05$; *$0.05 \leq p < 0.1$

^{*a}95% confidence interval (CI)

Table 3 Matrix of pairwise Φ_{ST} values among different populations of the Eurasian Jay

Population	Honshu–Kyushu	Korea	Primorsky	Sakhalin	Amur	Hokkaido–Kunashir
Honshu–Kyushu	–					
Korea	**0.99***	–				
Primorsky	**0.99***	0.04	–			
Sakhalin	**0.99***	0.13*	0.07	–		
Amur	**0.99***	0.08	−0.06	0.07	–	
Hokkaido–Kunashir	**0.99***	0.35*	0.37*	0.51*	0.4*	–

Significant Φ_{ST} values are indicated by *($p < 0.05$). Bold values indicate pairwise Φ_{ST} values between *japonicus* and *brandtii* subspecies; all the others indicate interpopulation comparisons for *brandtii*

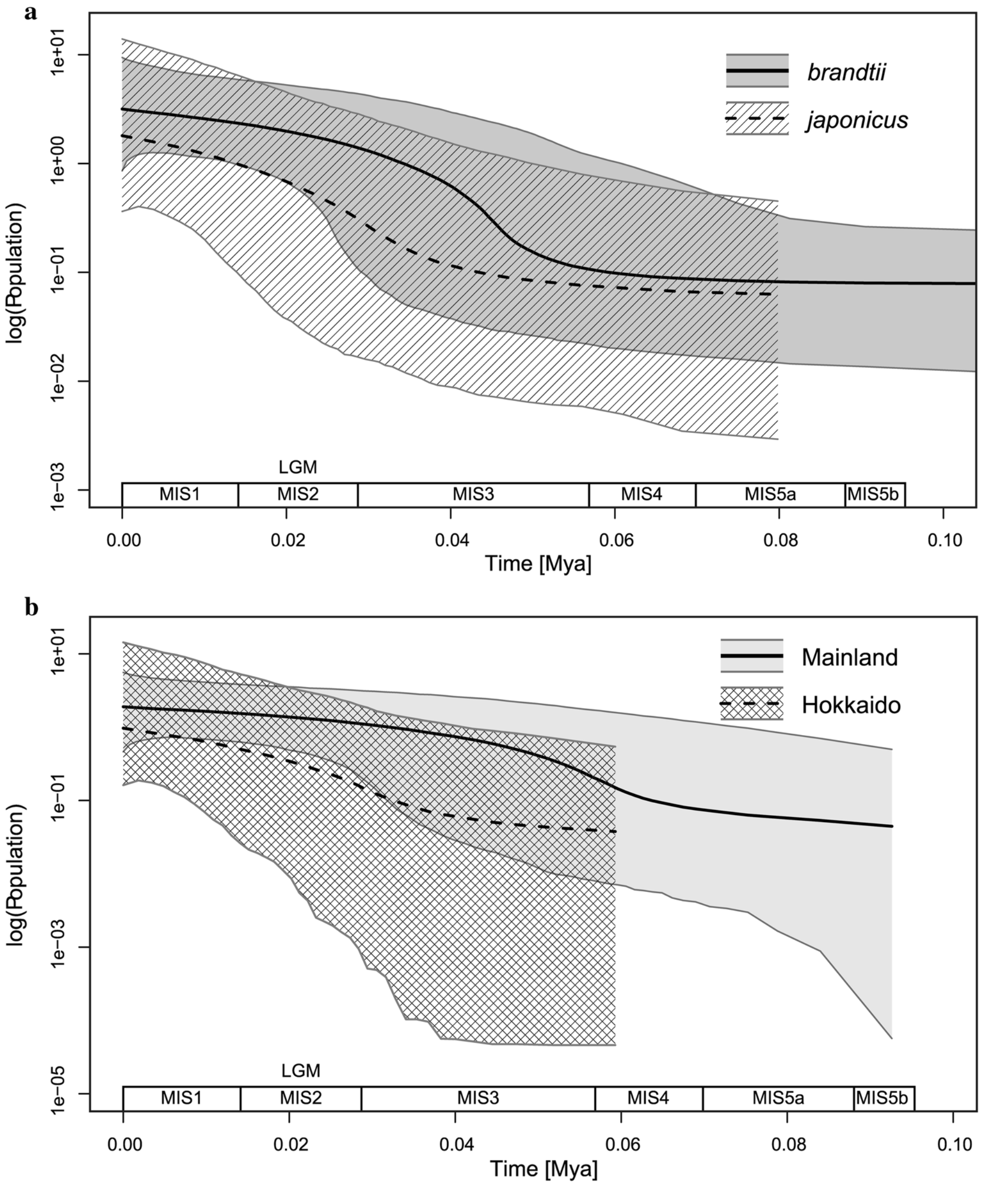

Fig. 3 Extended Bayesian skyline plot for **a** two subspecies of the Eurasian Jay in the CJSR and **b** the two subgroups of *brandtii*, using a molecular clock rate of *Cytb* (1.05%/lineage/Mya). Estimated means are indicated by black solid and black dashed lines. Areas bounded by gray lines and filled with gray color or hatching indicate 95% central posterior densities. MIS2 (Marine Isotope Stage 2) corresponds to the Last Glacial Maximum

Discussion

Divergence pattern in relation to paleogeography

A Bayesian inference phylogenetic tree was constructed using two mtDNA loci in samples from the five subspecies of the Eurasian Jay. Five distinct, divergent clades perfectly matched the five subspecies, which may reflect the considerable plumage divergence among the subspecies. The earliest divergence of the Eurasian Jay in our samples appeared as the split between the archipelagic (*G. g. japonicus* from Honshu to Kyushu) and continental (Far East Russian *G. g. brandtii*, Taiwanese *G. g. taivanus*, Caucasian *G. g. krynicki*, and European *G. g. glandarius*) clades, and was estimated to have occurred around the Pliocene–Pleistocene boundary. Because we did not include samples of many other subspecies, including Sino-Himalayan *bispecularis* and *sinensis*, Indochinese *leucotis*, and African *cervicalis*, we cannot preclude the possibility that these subspecies lineages form the basal group in our phylogenetic tree. However, the strong effect of the CJSR on diversification is still visible in the phylogenetic tree, specifically between the continental and archipelagic clades, as an ancient evolutionary split of the Eurasian Jay. Although the complex diversification of phenotypic traits among the subspecies cannot be fully explained by the phylogenetic trees obtained in this study, we found prominent endemism of *japonicus* in this species. This is supported by the presence of many endemic or basal lineages of bird and mammalian species in the Japanese archipelago that are highly diverged from their continental counterparts (Saitoh et al. 2010; Nishiumi and Kim 2015; Kayvanfar et al. 2017; Sato 2017). However, *brandtii*, the neighboring subspecies geographically, was not closest to *japonicus* phylogenetically. This warrants more comprehensive studies, including samples of other subspecies to infer the processes involved in the divergence within the continental clade following the split of *japonicus*.

Assuming that 1.05%/lineage/Mya is an appropriate molecular clock to apply to the Eurasian Jay, the divergence time between the continental and archipelagic clades was estimated to be 2.41 Mya (95% HPD 1.90–2.97 Mya). This range is very significant as a geological epoch, during which the Korea Strait was separated from the Japanese archipelago and Eurasian continent, specifically at 3.2, 2.9, 2.4, and 1.9 Mya (Tada 1994; Kitamura and Kimoto 2006; Gallagher et al. 2015). Many mammalian species are believed to have diverged as a result of geological vicariance due to the disappearance of the land bridge between the continent and archipelago, which led to population segregation and genetic divergence among populations around 2.4 Mya (Mckay 2012). Therefore, despite the uncertainty of our time estimation, we can safely conclude that two clades of Eurasian Jay diverged as a result of one continent–archipelago separation event in the CJSR around this geological time.

Geological vicariance as a trigger for bird species divergence in the CJSR has not been examined thoroughly. A recent phylogenetic study found that trans-Japan Sea differentiation among avian species separated by various degrees of genetic distance dated throughout the Quaternary epoch (Saitoh et al. 2015). In fact, similar trans-Japan Sea differentiation patterns with similar divergence times have been observed among sister taxa; examples include 2.8 Mya (2.0–3.6 Mya) between *Emberiza variabilis* and *E. tristrami* (Päckert et al. 2015), 1.7 Mya (1.2–2.2 Mya) between *E. spodocephala personate* and *E. s. spodocephala/sordida* (Päckert et al. 2015), 2.06 Mya between *Locustella amnicola* and *L. fasciolata* (calculated on the basis of the genetic divergence of 5.78% [Drovetski et al. 2004] and a molecular evolutionary rate of NADH dehydrogenase 2 of 2.8%/divergence/Mya [Norman et al. 2007]), and 2.5 Mya (1.8–3.3 Mya) between *Phylloscopus xanthodryas* and *P. borealis/examinandus* (Saitoh et al. 2010). This consistency indicates that geological vicariance should serve as a common driver for population differentiation or speciation in bird species in this region. These data, along with our results, suggest the importance of the CJSR in the divergence of avian species.

Recent demography of two divergent lineages in the CJSR

Although the two neighboring populations across the Japan Sea, *brandtii* and *japonicus*, were not in a sister relationship, it is noteworthy that their population dynamics inferred from our data resemble each other. Recent demographic events could have contributed greatly to the present genetic diversity of each subspecies while they retained genetic divergence. The Bayesian inference phylogenetic tree suggests that the two clades in the CJSR, *brandtii* and *japonicus*, did not show clear within-subspecies differentiation and displayed lower intragroup genetic diversity in the mtDNA, with their tMRCAs dated around the Middle to Late Pleistocene. The mismatch distribution and neutrality tests supported a sudden population expansion for each clade. The time since expansion inferred by τ and EBSP was estimated around the last glacial period for each subspecies. We have two possible scenarios for the timing of expansion in these two populations. One possibility is that Eurasian Jay populations in the CJSR experienced expansions during one of the relatively warmer periods within the last glacial period, including Early Marine Isotope Stage (MIS) 3 (ca. 50 kya) or MIS 5a (ca. 80 kya) (Igarashi and Oba 2006; Hanazaki et al. 2017). It is also possible that we improperly estimated the time of expansion by applying an inappropriate molecular evolutionary rate. Slightly deleterious mutations are believed to be eliminated over considerable timescales (Ho et al. 2011). The molecular evolutionary rate μ for a short timescale must therefore take uneliminated mutations into account, making μ higher than the rate calculated over a long timescale (e.g., 1.05%/lineage/Mya). Hanazaki et al. (2017) estimated evolutionary rates of *Cytb* in rodent species of 5 and 11%/lineage/Mya for expansion events in 60 and 10 kya, respectively. Therefore, as one possibility, we suggest that population expansions occurred in response to sudden global warming and biotic transition after the LGM. Nevertheless, note that the demographic histories of the two divergent populations were thought to be simultaneous and hence parallel across the Japan Sea.

We can speculate on possible refugial areas for *brandtii* and *japonicus* based on the unique ecology of the Eurasian Jay; *Garrulus* species are highly dependent on caches to survive through the winter, especially of acorns, which are produced by *Quercus* species (Goodwin 1986; Cramp 1994; de Kort and Clayton 2006; dos Anjos 2009). The Eurasian Jay migrates in response to the failure of acorn crops (Goodwin 1986; Cramp 1994). *Quercus* plants dominated southern Korea and southwestern Japan even during LGM, in contrast to northern areas, which they later colonized during the Holocene (Harrison et al. 2001; Chung et al. 2006, 2010; Okaura et al. 2007; Bazarova et al. 2008; Mokhova et al. 2009; Razzhigaeva et al. 2010; Chen et al. 2012; Iwasaki et al. 2012). Southern continental and archipelagic areas in the CJSR, therefore, presumably served as refugia for *brandtii* and *japonicus*, respectively. Generally, regions that served as refugia should hold higher genetic diversity than those colonized in later periods (Hewitt 1996). Nucleotide diversities were high in the Primorsky population, followed by the Korean population of *brandtii*, indicating that these regions might have acted as refugia for *brandtii*. As a result of the limited number of samples, we were not able to identify possible refugial areas for *japonicus*. Note that the similar pattern (divergent populations in refugia on the continent and archipelago that simultaneously experienced population expansions) has been documented in other bird species, including the Arctic Warbler species complex (*Phylloscopus xanthodryas* and *P. borealis/examinandus*) (Saitoh et al. 2010) and *Emberiza spodocephala* (Weißensteiner 2013). We would like to emphasize that two landmasses provided separate potential refugial areas for the two divergent populations.

The origin of *brandtii* populations in Hokkaido is also controversial concerning refugia formation. Population restrictions in the south of the CJSR should assume that the Hokkaido *brandtii* population was colonized from southern continental refugia of *brandtii* via Sakhalin around the last glacial period. This is supported by the unclear separation between Hokkaido and continental-Sakhalin populations in the Bayesian inference tree (Fig. 2) and unimodal mismatch distribution of all *brandtii* (Table 2). The expansion of the Hokkaido population was estimated to occur after that of the continental population on the basis of τ of the mismatch distribution and EBSP, not rejecting this possibility. Independent refugia in Hokkaido might also be a possibility, indicated by the appearance of the Hokkaido population as a genetic subgroup within *brandtii*. During the LGM, the cold and dry grassland and mire vegetation are thought to have extended to Hokkaido; however, a recent palynological study suggested possible *Quercus* refugia on the island (Igarashi et al. 2003; Igarashi 2016). In addition, it has been reported that Hokkaido populations of some mammals possess unique and ancient phylogenetic lineages diverged in the Middle Pleistocene, suggesting an independent demographic history from continental populations during the last glacial period (the Russian Flying Squirrel, Oshida et al. 2005; the Mountain Hare, Kinoshita et al. 2012; the Sable, Kinoshita et al. 2015). Either interpretation, or a combination of these, is possible considering the limitations of our data. Nevertheless, the Hokkaido population seems to have originated as a consequence of an expansion of *brandtii* via Sakhalin around the Middle to the Late Pleistocene, according to the time estimation of the tMRCA of *brandtii*.

Our results suggest that the geological structure of the CJSR had a considerable influence on the early stage of the evolutionary history of the Eurasian Jay. We also revealed that the CJSR provided a place for the formation of refugia and preserved the phylogenetic structure of this species across the Japan Sea.

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Compliance with ethical standards

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Affiliations

Daisuke Aoki[1] · Gohta Kinoshita[2] · Alexey P. Kryukov[3] · Isao Nishiumi[4] · Sang-im Lee[5] · Hitoshi Suzuki[6]

✉ Daisuke Aoki
aokid@eis.hokudai.ac.jp

[1] Division of Biology, Department of Biological Sciences, School of Science, Hokkaido University, N10W8, Kita-ku, Sapporo 080-0180, Japan

[2] Laboratory of Forest Biology, Division of Forest and Biomaterials Science, Graduate School of Agriculture, Kyoto University Kitashirakawa-oiwake, Sakyo-ku, Kyoto 606-8502, Japan

[3] Laboratory of Evolutionary Zoology and Genetics, Federal Scientific Center of the East Asia Terrestrial Biodiversity, Far Eastern Branch of the Russian Academy of Sciences, Vladivostok 690022, Russia

[4] Department of Zoology, National Museum of Nature and Science, 4-1-1 Amakubo, Tsukuba, Ibaraki 305-0005, Japan

[5] School of Undergraduate Studies, Daegu Gyeongbuk Institute of Science and Technology, Daegu 42988, South Korea

[6] Laboratory of Ecology and Genetics, Graduate School of Environmental Earth Science, Hokkaido University, N10W5, Kitaku, Sapporo 060-0810, Japan