

Comparative Phylogeography of Two Crow Species: Jungle Crow *Corvus macrorhynchos* and Carrion Crow *Corvus corone*

Alexey Kryukov^{1*}, Liudmila Spiridonova¹, Sumio Nakamura²,
Elisabeth Haring^{3,4}, and Hitoshi Suzuki⁵

¹Laboratory of Evolutionary Zoology and Genetics, Institute of Biology and Soil Science, Far East Branch
Russian Academy of Sciences, Vladivostok 690022, Russia

²Ornithological Society of Japan, Shinjuku, Tokyo 169-0075, Japan

³Central Research Laboratories, Museum of Natural History, Burgring 7, 1010 Vienna, Austria

⁴Department of Evolutionary Biology, University of Vienna, Althanstrasse 14, 1090 Vienna, Austria

⁵Laboratory of Ecology and Genetics, Graduate School of Environmental Earth Science,
Hokkaido University, Sapporo 060-0810, Japan

The jungle crow *Corvus macrorhynchos* Wagler, 1827, and the carrion crow *Corvus corone* L., 1758, are two closely related species with similar ecological requirements that occupy wide distribution ranges in the Palearctic. We studied patterns of their genetic variation by using sequences of the mitochondrial *cytochrome b* gene. *Corvus macrorhynchos* demonstrates a low level of variation and differentiation throughout its range, except for a highly diverged population of Cheju Island (Korea). The haplotype network shows two haplogroups. The island group comprises populations of Sakhalin, Hokkaido, Honshu, and Kyushu, while the haplotypes of Taiwan and Ryukyu Islands proved to be closer to the mainland group, which also includes populations from the Primorye, Khabarovsk, Amur, and Magadan regions in the Russian Far East. This pattern allowed us to develop a phylogeographic hypothesis regarding the two modes of settling of the island populations. Concerning *C. corone*, the presence of two distinct haplogroups was confirmed within the range of *C. c. orientalis*. Both haplogroups are found within the same populations in Kamchatka and North Sakhalin, which implies secondary contacts there. Populations of *C. corone* are found to be rather stable in the western parts of its range, while in the Far East populations experienced recent growth, as was observed for *C. macrorhynchos* in general. The two species appear to have passed through different evolutionary scenarios.

Key words: *cytochrome b*, mtDNA, haplotype, molecular phylogeny, population growth, *Corvus*

INTRODUCTION

Analysis of the patterns of genetic diversity throughout wide ranges of species enables the understanding and interpretation of phylogeographic processes. In some cases it is possible to trace the formation of species ranges and locate speciation centers. The Quaternary period is very important for such analyses, as periodical climatic oscillations shaped the current biodiversity. Such processes have been studied rather well for fauna of Europe (Hewitt, 1996, 2000; Taberlet et al., 1998) and North America (Klicka and Zink, 1997; Avise and Walker, 1998), while for Eastern Asia they are still poorly known. Comparative phylogeography forms bridges between historical processes in the biosphere and origin of local biodiversity.

Phylogeography of birds—particularly of the widely distributed Palearctic species—remains poorly studied, with only a handful of papers published on the subject. While in some cases no intraspecific structure was detected, in others phylogeographic breaks were found, e.g., in great spotted woodpecker *Dendrocopos major* (Zink et al., 2002), the wagtails *Motacilla flava* and *M. citriola* (Pavlova et al., 2003), great tit *Parus major* (Päckert et al., 2005), winter wren *Troglodytes troglodytes* (Drovetski et al., 2003), red-breasted flycatcher *Ficedula parva* and skylark *Alauda arvensis* (Zink et al., 2008), pine grosbeak *Pinicola enucleator* (Drovetski et al., 2010), and Arctic warbler *Phylloscopus borealis* (Saitoh et al., 2010), as well as several corvid species (Kryukov and Suzuki, 2000; Kryukov et al., 2004; Haring et al., 2007).

The corvids (Corvidae) are a bird family comprising ~113 currently accepted species with highly variable appearance and frequently high population numbers. Many species are widely distributed, and therefore present particularly interesting subjects for phylogeographic pattern studies (Avise, 2000). Over the past several years there have

* Corresponding author. Tel. : +7-423-2311392;

Fax : +7-423-2310193;

E-mail: kryukov@ibss.dvo.ru

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been a number of reports devoted to intraspecific variation of corvids: raven *Corvus corax* (Omland et al., 2000, 2006; Feldman and Omland, 2005; Baker and Omland, 2006), crow *Corvus corone* (Kryukov and Suzuki, 2000; Kryukov and Odati, 2000; Haring et al., 2007; Haas and Hansson, 2008; Haas et al., 2009; Wolf et al., 2010), jungle crow *Corvus macrorhynchos* (Iwasa et al., 2002), other species of genus *Corvus* (Haring et al., 2012), magpie *Pica pica* (Lee et al., 2003; Kryukov et al., 2004, 2005), azure-winged magpie *Cyanopica cyanus* (Fok et al., 2002; Kryukov et al., 2004, 2005), Siberian jay *Perisoreus infaustus* (Uimaniemi, 2004) and jay *Garrulus glandarius* (Akimova et al., 2007). Despite the considerable volume of studies, a number of questions concerning several widely distributed corvid species remain open. In particular, intraspecific diversity and differentiation in the eastern parts of distribution ranges remain poorly investigated.

The jungle crow *Corvus macrorhynchos* Wagler, 1827 is distributed in South and East Asia (from Indonesia in the South to the Magadan region of the Russian Far East in the North). It occupies forested landscapes, and is frequently found near human settlements. The species has not been studied genetically, and only rather preliminary results are presently available in the studies of Kryukov and Suzuki (2000) and Iwasa et al. (2002). In those reports, no clear phylogeographic pattern was revealed other than that of the rather distinct haplotypes found in birds from Ryukyu Islands. *Corvus macrorhynchos* is treated traditionally as single species with eleven subspecies (Dickinson, 2003), and we follow that convention. Some ornithologists, however, divide it into two species (each comprising further subspecies): the northern *Corvus [macrorhynchos] japonensis* and the southern *Corvus [macrorhynchos] levaillantii* (Martens and Eck, 1995), or even into three species by recognizing also *Corvus [macrorhynchos] culminatus* from Indostan (Rasmussen and Anderton, 2005). Although this classification appears reasonable it remains to be tested by phylogenetic (e.g., molecular, morphological, anatomical) analyses. Within the northern group, on which the present study is focused, subspecies subdivision should be reconsidered.

The Eurasian crow *Corvus corone* L., 1758 is one of the commonest bird species, widely distributed in Palearctic, similar in habitation with the *C. macrorhynchos*. Its taxonomic subdivision remains controversial as well. It is most commonly regarded as a single species—a convention we follow (Dickinson, 2003). Other authors consider *C. corone* s.l. an ex-conspecies with two or more semi-species, and thus propose species rank for the hooded crow *Corvus cornix* and leave subspecies ranks for both carrion crow taxa: western *C. corone corone* and eastern *C. corone orientalis* (Stepanyan, 2003; Parkin et al., 2003). The ambiguous status of these forms is reflected in the recent checklist of the Russian birds (Koblik et al., 2006): European carrion crow *C. (corone) corone*, Eastern carrion crow *C. (corone) orientalis*, and hooded crow *C. (corone) cornix*. We do not consider here several remaining subspecies closely related to *C. c. cornix*.

Within the wide transpalearctic range of *C. corone* we found unexpectedly two radically distinct groups of mitochondrial haplotypes (Kryukov and Suzuki, 2000). One of these corresponds to populations distributed over a wide area extending from Western Europe to Siberia. It includes

black-colored (carrion crows: ssp. *corone* and *orientalis*) as well as black-gray crows (hooded crows: ssp. *cornix*). The other group comprises populations of Primorye, Sakhalin, and the Japanese Islands within the range of the black carrion crow *C. corone orientalis*. Thus, there is a clear discordance between genetic differentiation and subspecies assignment, which is based on plumage color and distribution. While our first study (Kryukov and Suzuki, 2000) was based on only a short region of the mitochondrial (mt) *cytochrome b* (*cyt b*) gene and a small number of samples, a wide comparative phylogeographic analyses of ten corvid species based on sequences of the mt control region (Haring et al., 2007) confirmed the results. However, the location of the boundary between the two haplogroups as well as their interrelationships remained unresolved.

The aim of the present investigation was to study and compare in detail intraspecific diversity and phylogeographic patterns of the two ecologically similar Palearctic species, *C. macrorhynchos* and *C. corone*, in their eastern distribution ranges. We evaluate whether mt haplogroups correspond to described subspecies of *C. macrorhynchos* and reconsider distribution ranges. For *C. corone* the major goal of the present analysis was to investigate in more detail the region where the two haplogroups detected in previous studies (Kryukov and Suzuki, 2000; Haring et al., 2007) were thought to come into contact. Additionally, we suggest a number of hypotheses concerning colonization history of several taxa. As in our previous investigations (Kryukov and Suzuki, 2000; Iwasa et al., 2002), we used the mt *cyt b* gene which was successfully employed for intra-species level analyses in many kinds of organisms and in particular for population studies of corvids.

MATERIALS AND METHODS

Sampling

We used 160 tissue samples of *Corvus macrorhynchos* and 56 of *Corvus corone* s.l. (Supplementary Table S1 online). For *C. macrorhynchos* sample localities are depicted in Fig. 1 and for *C. corone* in Fig. 2. We included primarily samples from the populations of the northern group of *C. macrorhynchos* (*C. [macrorhynchos] japonensis*). Moreover, we included two samples from Laos, presumably belonging to the southern group *C. [macrorhynchos] levaillantii* not shown on the map. For visualization of geographic differentiation and affinities in trees and the network the following geographic groups were defined: “Magadan” (site 1 in Fig. 1), “Amur region” (2 and 3), “Khabarovsk region” (4–6), “Primorye” (7–11), “Korean Peninsula” (12–13), “Cheju Island” (14), “North Sakhalin” (15–18), “South Sakhalin” (19–22, plus sample 23 of Kuril Islands), “Hokkaido” (24), “Honshu” (25), “Kyushu” (26), “Ryukyus” (27), “Taiwan” (28), and “Laos” (not depicted). The division for North and South Sakhalin populations was undertaken according to the Schmidt Line, the most important biogeographic border on the Sakhalin Island (Miyabe and Tatewaki, 1937). The abovementioned combined populations were used for calculating population parameters and distances as well. For these calculations, the Magadan and Kurils populations were excluded due to excessively small sample sizes. Additionally, the combined samples “Sakhalin” (points 15–22) and “South Far East mainland” (2–13) were used.

For *C. corone* we concentrated on the eastern part of the range (*C. c. orientalis*) where the two distinct mitochondrial (mt) haplotypes (Kryukov and Suzuki, 2000) might come into contact. The following localities were combined into geographic groups: “Europe” (sites 1–3 in Fig. 2), “Siberia and Kamchatka” (4–8), “Khabarovsk



Fig. 1. Distribution range for *Corvus [macrorhynchos] japonensis*, with sampling sites.

and Primorye regions” (9–13), “North Sakhalin” (14–17), “South Sakhalin and Kurils” (18–21), Hokkaido (22) and Korea (23). These populations were used for calculating population parameters and distances and in the network. In addition we calculated parameters for the two distinct mt haplogroups: East and West.

As an outgroup for both species, we used the sequence of the rook *Corvus frugilegus* L. from GenBank No Y16885.

DNA procedures

DNA was extracted from liver samples fixed in ethanol with a salt-extraction method (Aljanabi and Martinez, 1997), or with the DNeasy Tissue Kit (Qiagen) following the manufacturer’s instructions. The complete mt *cyt b* gene was amplified using the following primers: L14827: 5’-CCACACTCCACACAGGCCTAATTAA-3’ binding in the *ND5* gene, and H16065: 5’-GGAGTCTTCAGTCTCTG-GTTTACAAGAC-3’ binding in the *tRNA-thr* gene (Helm-Bychowski and Cracraft, 1993). PCR reactions were performed with a UNO II - Thermoblock 48 (Biometra, Germany) in a volume of 20 ml containing 67 mM Tris-HCl, pH 8.8; 16.6 mM (NH₄)₂SO₄; 0.01% Tween 20; 2.5 mM MgCl₂; 0.1 μM dNTP mix; 0.2 mM of each primer (Sintol, Russia), 1 unit of *Taq*-polymerase, and 40–60 ng template DNA. PCR was performed under the following conditions: an initial denaturation step of 94°C for 2 minutes, followed by 35 cycles of 94°C (60 s), 39°C (30 s) and 72°C (2 min), and concluded with a final extension of 72°C (6 min). The resulting PCR products had a length of approximately 1200 bp.

The sequence of the amplified products was determined using an ABI PRISM 3130 sequencer with the ABI PRISM®BigDye™ Terminator Cycle Sequencing kit v. 3.1 and the same primers used for amplification. Conditions of the sequencing reaction were pre-denaturation at 95°C (1 min), followed by 25 cycles of denaturation at 95°C (30 s); annealing at 55°C (10 s) and extension at 60°C (3 min).

Data analysis

Forward and reverse sequences were combined using the Staden software (Bonfield et al., 1995). Alignment and editing of sequences was performed in BioEdit v.6.0.7.1 (Hall, 1999). Some ambiguous sites were excluded from the analyses. Thus, the final

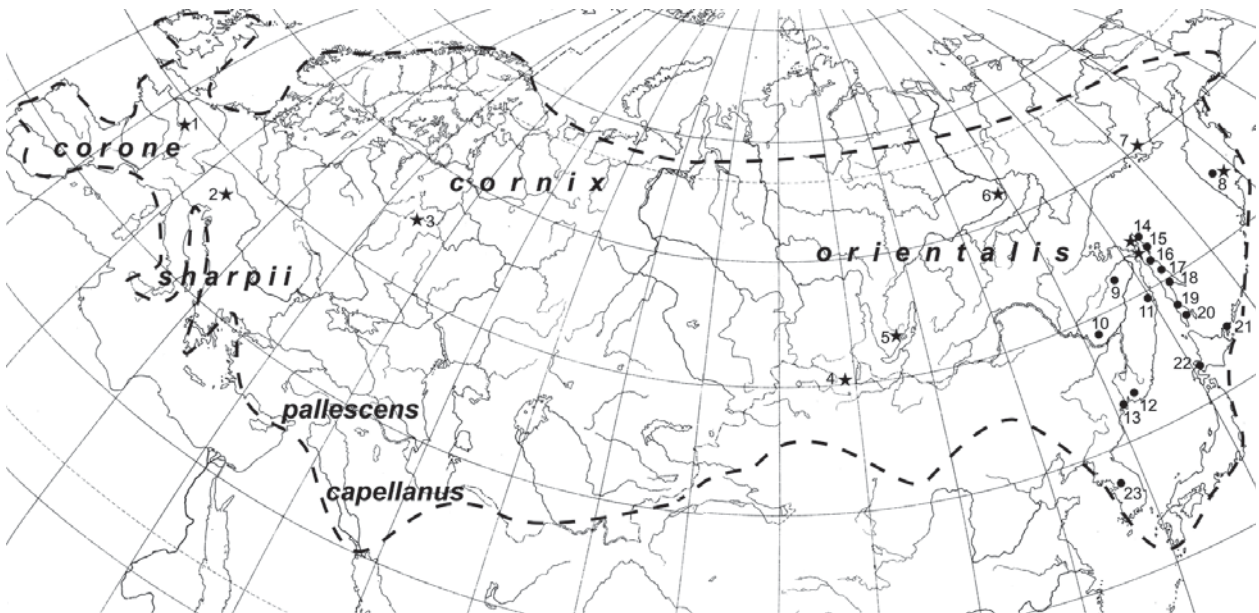


Fig. 2. Distribution range for *Corvus corone* s.l., with sampling sites. Stars represent findings of haplotypes from the western group, circles represent those from the eastern group.

lengths of sequences used were: 1140 for *C. macrorhynchos* and 1143 for *C. corone*. Phylogenetic trees (NJ, ME, ML and MP) were calculated in MEGA v.5 (Tamura et al., 2011). The optimal substitution model with the smallest Bayesian Information Criterion for ML was chosen using the same software. The selected model for the both species was HKY + G, $G = 0.13$ for *C. corone* and $G = 0.05$ for *C. macrorhynchos*. Transition/transversion bias (R) was 8.12 for *C. corone* and 20.85 for *C. macrorhynchos*. Statistical bootstrap support of nodes was calculated with 1000 replicates. MEGA5 was also used for computing genetic distances between sequences, considering all codon positions and both kinds of substitutions.

Median joining networks of haplotypes were constructed in Network v.4.6.0.0 (Bandelt et al., 1999). Such networks are in many cases more informative for revealing intraspecies affinities and homoplasies. DNA polymorphism was estimated with DNASP v.5.10.01 package (Librado and Rozas, 2009). The same was used to calculate demographic indexes, such as D (Tajima, 1989), raggedness index r (Harpending, 1994), and mismatch distribution of nucleotide differences (Rogers, 1995). For the polymorphism and population parameters analyses we used the geographic groups defined above, excluding populations with less than eight individuals. For calculating p-distances between populations and constructing trees, we used the MEGA5 partial deletion option.

RESULTS

We obtained and aligned sequences of the mitochondrial *cyt b* gene for 160 samples of *C. macrorhynchos* and 56 *C. corone*. Phylogenetic trees constructed with NJ, ME, ML and MP methods have similar topology for each species, respectively.

The ML phylogenetic tree of *C. macrorhynchos* (Supplementary Fig. S1) reveals a clear and highly supported (90%) distinctness of the samples from Cheju Island (South Korea). The remaining sequences are united in one clade without major substructuring. Even the samples from Laos are part of this group. Pairwise genetic p-distances between conventional geographic populations are small, as demonstrated by mean distances and net mean distances in Table 1. Exceptions are the population of Cheju Island that demonstrates higher divergences ranging from 0.55 to 0.77% for mean distances and from 0.46 to 0.70 for net mean distances, and to some extent the population from Ryukyu.

Median joining network was constructed to present optimally the connections between haplotypes of *C. macrorhynchos* (Fig. 3). The cycles reflecting homoplasies are not too pronounced in the network (there are only four small cycles)

and do not alter the general pattern of interrelation of the haplotypes. The network is conditionally divided into two groups, designated as “island” and “mainland” groups. Haplotypes of the two groups are differentiated by at least three substitutions. The highest number of haplotypes (20) was found in Sakhalin Island, which comprises the largest sample size as well. Most haplotypes were found only in Sakhalin samples, only six are shared with individuals from the mainland, Taiwan, and Japanese Islands. The most common haplotype from Sakhalin forms a star-like pattern together with 14 very similar haplotypes from Hokkaido, Honshu, and Kyushu Islands, and other Sakhalin haplotypes, each differing from the central haplotype by one substitution only. Other haplotypes from Hokkaido and Honshu differ by three substitutions at most. In contrast, the haplotypes of Ryukyu and Taiwan Island show affinity to those of the mainland. The Ryukyu haplotypes are close to each other and distant to the others by 4–5 substitutions. One haplotype of the birds from Taiwan is identical to a common haplotype occurring in samples from Far East Mainland and Sakhalin, while another differs from a Far East haplotype by one substitution only. The haplotype found in all four birds from Cheju Island proved to be distinct, differing from any other by at least six substitutions. On the other hand four haplotypes from continental Korea originating from the south and the center of the peninsula are nested with the “mainland group”. Two of them are shared with the mainland birds.

In the ML tree (Supplementary Fig. S2) and network (Fig. 4) of *C. corone*, two highly supported clades are apparent; those correspond to the two previously described western and eastern haplogroups (Haring et al., 2007). The western group includes samples from the populations of Western carrion crow *C. c. corone* from France and Austria, of the hooded crow *C. c. cornix* and Eastern carrion crow *C. c. orientalis* from populations spanning a region from Tuva to Kamchatka and North Sakhalin (Fig. 2). The eastern clade comprises populations from the southeastern part of the range: Amur region, the Khabarovsk and Primorsky territories, Sakhalin and the Japanese Islands, and Korea. The p-distance between these main clades is 2.7%, while within clades an average of 0.59% (West) and 0.51% (East) is observed. Within the main clades some subdivision into two or more subclades is apparent, but there is no geographic

Table 1. p-distances between populations of *C. macrorhynchos*, in %. Net mean distances between populations above diagonal, mean distances between populations below. Sample sizes are in parentheses. p-distances corresponding to Cheju Island population are in bold.

Population	1	2	3	4	5	6	7	8	9	10	11	12	13	Points at the map
1. Amur region (12)		0.033	0.011	0.054	0.179	0.282	0.193	0.193	0.316	0.072	0.463	0.012	0.228	2 – 3
2. Khabarovsk reg. (13)	0.289		0.009	0.052	0.139	0.231	0.155	0.160	0.294	0.049	0.469	0.024	0.219	4 – 6
3. Primorye (26)	0.207	0.294		0.037	0.148	0.250	0.158	0.163	0.284	0.024	0.468	0.000	0.205	7 – 11
4. North Sakhalin (29)	0.288	0.375	0.301		0.043	0.219	0.042	0.060	0.287	0.042	0.466	0.046	0.215	15 – 18
5. South Sakhalin (40)	0.379	0.428	0.378	0.311		0.027	0.005	0.011	0.394	0.148	0.543	0.170	0.304	19 – 22
6. Hokkaido (4)	0.504	0.541	0.501	0.409	0.283		0.024	0.058	0.497	0.260	0.629	0.278	0.409	24
7. Honshu (9)	0.427	0.478	0.422	0.344	0.263	0.314		0.001	0.380	0.156	0.580	0.185	0.317	25
8. Kyushu (5)	0.364	0.420	0.363	0.299	0.215	0.285	0.240		0.404	0.167	0.579	0.184	0.316	26
9. Ryukyus (4)	0.443	0.509	0.440	0.492	0.555	0.680	0.575	0.535		0.298	0.702	0.307	0.439	27
10. Korean Peninsula (5)	0.252	0.317	0.233	0.289	0.362	0.496	0.404	0.351	0.430		0.465	0.061	0.202	12 – 13
11. Cheju Island (4)	0.576	0.641	0.580	0.617	0.660	0.768	0.731	0.667	0.746	0.561		0.482	0.526	14
12. Taiwan (4)	0.137	0.261	0.179	0.262	0.353	0.482	0.402	0.338	0.417	0.224	0.548		0.219	28
13. Laos (2)	0.399	0.497	0.405	0.454	0.509	0.636	0.556	0.491	0.570	0.386	0.614	0.373		–

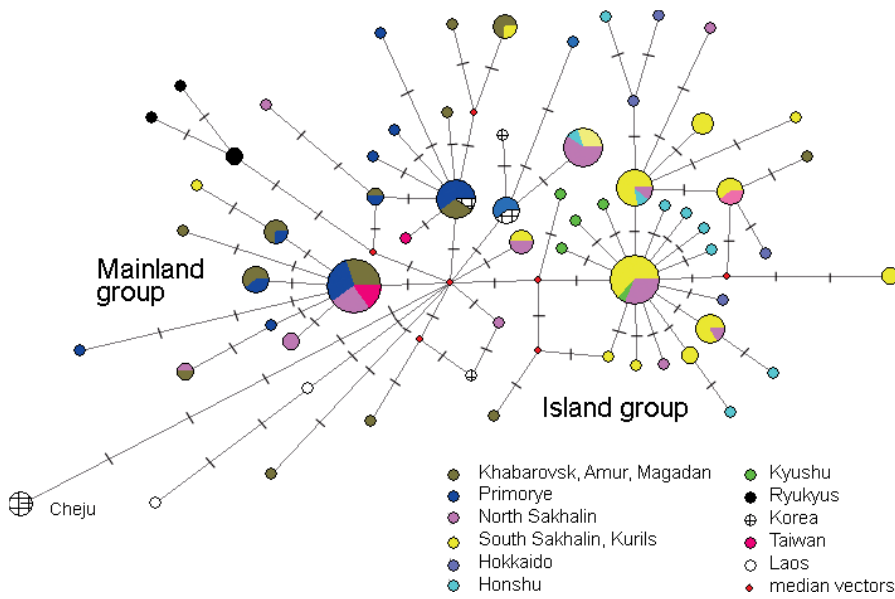


Fig. 3. Median-joining network for *Corvus [macrorhynchos] japonensis* including the two presumably *Corvus [macrorhynchos] leuallantii* individuals (Laos samples) based on the complete *cyt b* gene.

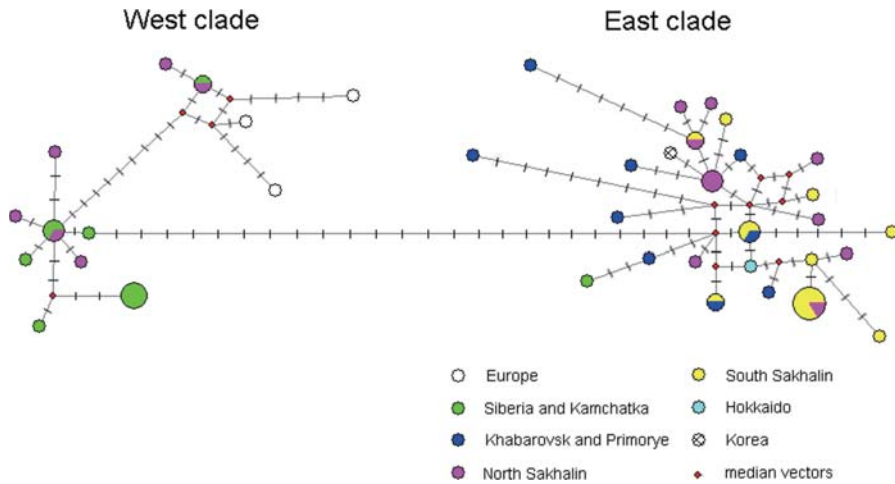


Fig. 4. Median-joining network for *Corvus corone* based on the complete *cyt b* gene.

pattern, other than a weak affinity of the Moscow, French, and Austrian populations within the western clade. Interestingly, birds from Kamchatka and North Sakhalin are present in both clades, while those from South Sakhalin are found exclusively in the eastern clade.

A number of calculations were performed in order to determine demographic parameters (Table 2). Tajima's *D* test was negative for nearly all the populations of *C. macrorhynchos*, although not statistically significant except for the Far East mainland population. For the Korean population it was positive, but also not significant. Values of raggedness statistics *r* were small for all populations. Mismatch distribution of nucleotide differences for all of populations showed clearly unimodal pattern (Fig. 5A, B), even for the Honshu population, which had a higher raggedness index. For Amur and Khabarovsk populations as well as for the South Far East mainland combined samples the distribution was unimodal as well (not shown).

For *C. corone*, Tajima's *D* test was positive for North Sakhalin and negative for other populations, but not significant for any of them (Table 2). Raggedness statistics *r* values were small for all populations. Mismatch distribution curves were unimodal for South Sakhalin (Fig. 5D) as well as for the Eastern clade. For the Western clade (not shown) and North Sakhalin population (Fig. 5C) it is multimodal, but for different reasons (see Discussion).

Table 2. Characters of DNA polymorphism and population dynamic tests for the both crow species.

	Region	<i>N</i>	<i>S</i>	<i>k</i>	$\pi \pm SD$	<i>h</i>	<i>Hd</i> \pm SD	<i>D</i>	<i>r</i>
<i>Corvus macrorhynchos</i>	Amur	12	8	1.848	0.178 \pm 0.051	5	0.742 \pm 0.116	-1.207; <i>P</i> > 0.10	0.066
	Khabarovsk	13	16	3.923	0.344 \pm 0.050	9	0.923 \pm 0.057	-1.005; <i>P</i> > 0.10	0.092
	North Sakhalin	29	18	3.334	0.296 \pm 0.023	13	0.906 \pm 0.030	-0.915; <i>P</i> > 0.10	0.054
	South Sakhalin	40	20	2.668	0.230 \pm 0.026	13	0.892 \pm 0.028	-1.431; <i>P</i> > 0.10	0.031
	Primorye	26	18	2.566	0.225 \pm 0.029	13	0.895 \pm 0.039	-1.617; <i>P</i> > 0.05	0.038
	Honshu	9	14	3.444	0.302 \pm 0.047	9	1.000 \pm 0.052	-1.590; <i>P</i> > 0.05	0.136
	Korea	9	10	4.167	0.365 \pm 0.047	5	0.806 \pm 0.120	0.616; <i>P</i> > 0.10	0.203
	Sakhalin in total	69	29	3.234	0.284 \pm 0.020	20	0.910 \pm 0.018	-1.473; <i>P</i> > 0.10	0.015
	South Far East mainland	56	30	2.786	0.269 \pm 0.023	21	0.901 \pm 0.024	-1.877; <i>P</i> < 0.05	0.028
	TOTAL	160	62	3.930	0.379 \pm 0.015	60	0.956 \pm 0.007	-1.975; <i>P</i> < 0.05	0.019
<i>Corvus corone</i>	North Sakhalin	18	52	16.659	1.497 \pm 0.195	16	0.980 \pm 0.028	0.396; <i>P</i> > 0.10	0.020
	South Sakhalin	13	25	5.821	0.539 \pm 0.099	9	0.872 \pm 0.091	-1.205; <i>P</i> > 0.10	0.072
	East clade	37	44	5.315	0.498 \pm 0.048	24	0.961 \pm 0.018	-1.774; <i>P</i> > 0.05	0.016
	West clade	19	24	5.848	0.590 \pm 0.093	12	0.918 \pm 0.047	-0.579; <i>P</i> > 0.10	0.028
	TOTAL	56	76	15.166	1.532 \pm 0.104	36	0.974 \pm 0.010	-0.331; <i>P</i> > 0.10	0.007

N: sample size; *S*: number of polymorphic sites; *k*: average number of nucleotide differences; π : nucleotide diversity ($\pi \times 100$); SD: standard deviation; *h*: number of haplotypes; *Hd*: haplotype diversity; *D*: Tajima's test statistics, and its significance *P*; *r*: raggedness statistics. Total sample for each species includes the small populations also.

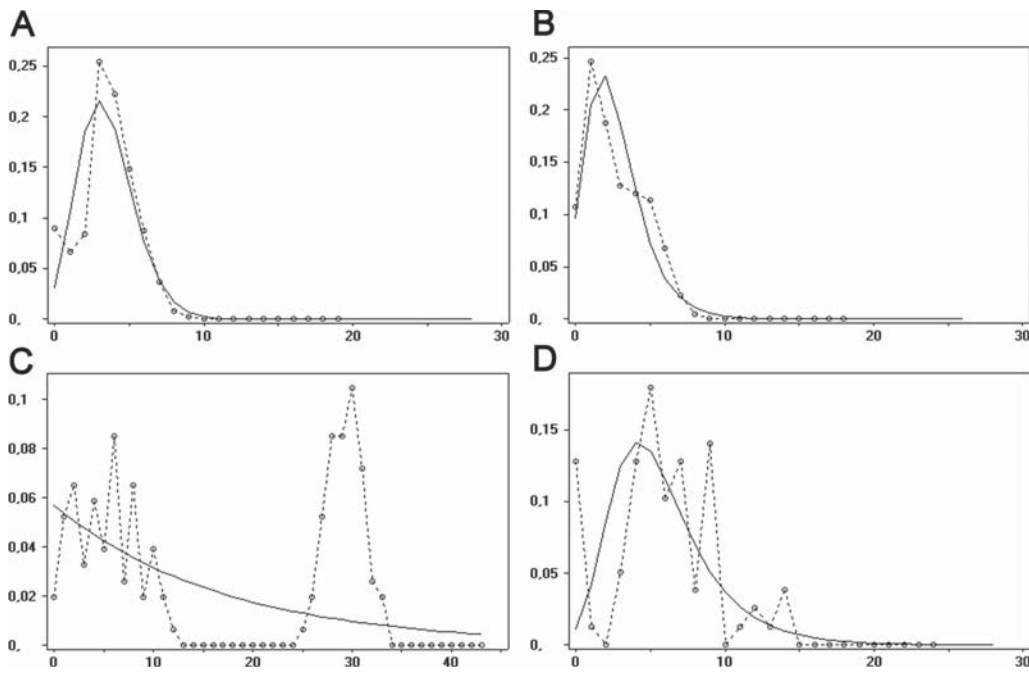


Fig. 5. Mismatch distribution of nucleotide differences for populations of *Corvus [macrorhynchos] japonensis*: North Sakhalin (A), South Sakhalin (B), *Corvus corone*: North Sakhalin (C), South Sakhalin (D). X axis: number of pairwise differences between sequences; Y axis: frequency. Solid lines: expected distributions; dashed lines: observed distributions.

F_s values were negative for all samples but not significant (not shown).

Genetic variation within populations is similar for all populations of both species (Table 2). However a lower number of polymorphic sites (8), low nucleotide diversity (0.18%) and haplotype diversity (0.74%) were found in the western marginal population of *C. macrorhynchos* in the Amur region (sites 2 and 3 at the map Fig. 1). In *C. corone*, nucleotide diversity is the highest in North Sakhalin because of admixture of the two haplogroups mentioned above. Within populations, nucleotide diversity of *C. corone* was higher compared to *C. macrorhynchos* from the same geographic regions, while haplotype diversity was similar in populations of the two species. Overall mean p-distances for *C. macrorhynchos* was 0.38%, for *C. corone* 1.53%.

DISCUSSION

Genetic diversity and differentiation

The haplotype network of jungle crow forms a rather complicated pattern (Fig. 3). The haplotypes from mainland populations from Magadan in the North to Korea in the South as well as island populations from Ryukyu, Taiwan, and Cheju are combined in the “mainland” group. The “island” group comprises haplotypes of Sakhalin, Hokkaido, Honshu, and Kyushu. While in this group only one haplotype was found in a mainland sample, several birds from the islands appear in the mainland group. This suggests that gene flow predominantly occurs from the mainland to the islands rather than in the opposite direction. However, incomplete lineage sorting may also contribute to this pattern. Sakhalin may have served as a source for the populations of the Kuril Islands, as well as Hokkaido, Honshu, and Kyushu Islands and the star-like pattern suggests a recent

population expansion as revealed also by Tajima’s D and mismatch distribution (see below).

The populations from Taiwan, the Ryukyus, and Cheju Island show genetic affinity to those of the mainland. The subspecies *C. m. colonorum* distributed in China and Taiwan is represented in our study only by samples from Taiwan. These are not clearly differentiated genetically from the mainland populations, suggesting a rather recent settling of the island and/or current genetic exchange. In contrast, the Ryukyu population (Amami-Oshima Island) is genetically distinct. Based on the network structure it can be assumed to have originated on the mainland rather than the adjacent

northern part of the Japanese Islands. Unfortunately we had no samples from the extreme South of the Ryukyu archipelago, i.e. Sakishima Islands, where another distinct subspecies *C. m. osai* occurs.

The genetic separation of the Cheju Island population was unexpected considering its closeness to the Korean Peninsula (only 100 km away). The genetic distance is much higher than that between other populations (almost twice, Table 1), higher even than that between the birds from Laos and the mainland group. This implies a long period of isolation of the Cheju population. We refrain from estimating corresponding divergence time, as we lack fossil calibration of the substitution rate. Application of the commonly used mutation rate of 2% per MY (Klicka and Zink, 1997) would result in divergence time of 300 KY BP, far exceeding the reported timing of Cheju Island separation (12 KY, Ohshima, 1990). One explanation for the genetic divergence of these island populations (Cheju and Ryukyu) might be the residential status of *C. macrorhynchos* on those southern islands, in contrast to the mainland and northern islands where climate drives birds to seasonal migrations.

The present study partly confirms the results of our previous work, based on merely 41 samples from 12 populations and a short sequence (336 bp; Iwasa et al., 2002). Due to the limited sampling the phylogenetic tree in that study was poorly resolved. Data presented here illustrate more clearly the distinctness of the crows from the Ryukyu Islands, a division into a mainland group and an island group and the position of the Laos population within the mainland group.

For *C. corone*, among seven sampling sites from Sakhalin both haplogroups were detected only in two northernmost ones, while the southern populations possess exclusively

haplotypes of the group East (Fig. 2). In our earlier study based on the mt control region western haplotypes detected in North Sakhalin and Kamchatka belong to a subclade within group West that seems to be restricted to East Siberia (see Haring et al., 2007). We concluded that haplotypes of the second subclade within the western group spread no further to the east than to the Daurian region. The tree based on *cyt b* presented here contradicts that conclusion, as the western clade is divided into two subclades that do not correspond to the geographic picture obtained earlier, and the birds from North Sakhalin are found in both clades. This could be due either to excessively small distances between *cyt b* haplotypes within this group, or to birds carrying the western subclade having spread as far as Kamchatka. In both places where the two haplogroups co-occur we suggest secondary contact rather than independent origin of the two same haplogroups in two distant localities. Another contact zone may be located in Amur River basin in East Siberia.

Hybrid zones among *C. corone* subspecies displaying distinct plumage color are well studied and hybrids are easy to recognize. However, the boundary of the two haplogroups does not correlate with subspecific differentiation, and thus “hybrids” between the haplogroups cannot be identified by their plumage. Further studies are needed to evaluate whether the two haplogroups correspond to populations that are distinct in other ways as well (morphological, ecological), and to assess possible gene flow. The present data identify North Sakhalin as the best choice for analyzing possible gene flow based on comparisons of nuclear and mt markers.

Taxonomy

Taxonomic considerations based solely on molecular data are usually not sufficient and need to be complemented by other data (e.g., morphological, ecological). Therefore, we may only show incidences supporting one or another view. In the case of *C. macrorhynchos*, divergence of haplotypes of the Ryukyu birds supports the subspecies status of *C. m. connectens*. Although sample size is still too small and the results have to be interpreted with caution, one can assume this subspecies may have diverged in longer-lasting isolation. A separation of Cheju populations has been reported for other animals as well, i.e. for striped field mouse *Apodemus agrarius* (Yoon et al., 2004). In that case, divergence reached species rank; moreover, the island population was considered ancestral to the mainland ones. Our results indicate that the Cheju crows may be regarded as a distinct subspecies; however, comprehensive morphological and ecological investigations are required to make a final determination.

All the studied mainland populations from Magadan to Korea and Taiwan possess similar haplotypes. With the exception of the Taiwan population, these populations correspond to *C. m. mandschuricus*. The birds from Taiwan represent the Chinese subspecies *C. m. colonorum*, which is not differentiated from *C. m. mandschuricus* in our data set. However, because of absence of any samples from continental China further considerations concerning *C. m. colonorum* remain speculative. The haplotypes of birds from Laos proved to be somewhat differentiated (Fig. 3, Fig. S1), but since we lack samples from major parts of the distribu-

tion range of *C. [macrorhynchos] levaillantii* our data do not allow us to evaluate whether the Laos population is representative of this form. The subspecies *C. m. japonensis*, however, is clearly confirmed by the close affinity of mt haplotypes of Sakhalin, Hokkaido, Honshu and Kuril crows. One could conclude that the proposed occurrence of the two subspecies—*C. m. mandschuricus* and *C. m. japonensis*—on Sakhalin (Nechaev, 1991) is supported by our data. Assuming that the mainland haplogroup represents *C. m. mandschuricus* the presence of mainland haplotypes in some of the Sakhalin birds could be interpreted in this way. However there is no evidence of correlation between haplogroups and the two forms reported by Nechaev (1991). Moreover, levels of nucleotide and haplotype variation of Sakhalin do not exceed those of other small populations (Table 2). This observation may mean rather high gene flow within the Island. Close affinity of North Sakhalin haplotypes and those from Khabarovsk region agrees with ornithologists' observations of presumed occurrence of *C. m. japonensis* in low Amur basin, Hungari and Udomi rivers (Spangenberg, 1960). In that territory, commonly occupied by *C. m. mandschuricus*, large birds with large bills were recorded suggesting the presence of *C. m. japonensis*.

In the case of *C. corone*, we demonstrated that the two distant haplogroups co-occur in the eastern part of the species' distribution range corresponding to the range of the subspecies *C. corone orientalis* (Dickinson, 2003). Genetic data cannot provide arguments for taxonomic consequences without additional data from nuclear markers. It is not possible to treat the two haplogroups as subspecies as their ranges overlap in Kamchatka and North Sakhalin. The fact that *C. c. orientalis* possess both haplogroups presents further problems and supports the conservative treatment of *C. corone* as a single species (Dickinson, 2003).

Demography and population dynamics

A number of demographic indicators obtained by us, such as negative *D* values, low values of *r* indexes, and unimodal mismatch distribution of nucleotide differences, point towards recent population growth in all populations of *C. macrorhynchos* (Table 2, Fig. 5A, B). Numerous ornithologists have reported a widespread, continuing increase of *C. macrorhynchos* population sizes. Its range is expanding as well. In recent years, *C. macrorhynchos* was recorded in Magadan region, Ola settlement during the nesting period (G. Atrashkevich, pers. comm. regarding collecting of two young females on 18.09 and 1.10.2009). Earlier yet, this species was observed as a rare visitor at Kronoki settlement in Kamchatka (Artukhin et al., 2000). In the Northwest, the distribution range of *C. macrorhynchos* is expanding as well. An adult bird was collected near the town of Svobodny, between Zeya and Selemdzha rivers, on 1.06.1961 (V. Nechaev, pers. comm.); it was recorded there for the first time after several years of careful observations. This finding was indicated by Stepanyan (2003) to be the westernmost point of the range. However, in 1997 *C. macrorhynchos* was found west of that location, in Gazimur river valley (119°09' E) (Goroshko, 2004). In 2009, a male bird was observed for the first time 40 km further to the west in the city of Chita (O. Goroshko, pers. comm.). Therefore, observations of ornithologists confirm our molecular genetic data regarding

the recent population expansion, indicating that this expansion is still going on. Moreover, the lowest nucleotide diversity in the westernmost marginal population in Amur region (Table 2) fits the expectation of leading edge expansion resulting in loss of alleles (Hewitt, 2000).

Invading new territories may even drive *C. macrorhynchos* to form mixed pairs with *C. corone*, as has been observed occasionally in Magadan region (G. Atrashkevich, pers. comm.) and Chita (O. Goroshko, pers. comm.). In that context it is worth noting that the range of habitats of the two species is influenced by the presence or absence of the other species. While *C. macrorhynchos* prefers forests, *C. corone* dominates in more open habitats (Higuchi, 1979; Nazarov et al., 1990). In cities however, both species often co-occur and build mixed flocks in wintering time. Thus, for both species expansion of distribution ranges might be connected with and influenced by human activities as well as global climate change.

For *C. corone*, population growth can be assumed for South Sakhalin, as well as for the Eastern and Western clades in total, although those populations' *D* values were not significant. The multimodal pattern observed in North Sakhalin reflects the presence of two distinct haplogroups rather than population stability. Moreover, the higher nucleotide diversity of *C. corone* compared to *C. macrorhynchos*, in view of their similar levels of haplotype diversity (Table 2), may indicate that the coalescence time of the former is somewhat longer.

CONCLUSIONS

In general, the history of the two species, although both mediated by the same Pleistocene climate oscillations, appears different. *Corvus macrorhynchos* has experienced population growth in the recent past and is still expanding its range, at least in the North and Northwest. This is deduced from homogeneity of haplotypes within most of its range, and the lowest level of nucleotide diversity within the marginal northwest population in the western Amur region. Additionally, negative values of *D* test and unimodal mismatch distributions imply population growth, which is also supported by direct observations by zoologists. According to the phylogeographic pattern obtained, settling of the Japanese Islands from the mainland may have occurred in either of two ways: one from Sakhalin and Hokkaido, and the other from the south via Taiwan or, less likely, from the Korean peninsula. The population of Cheju Island is unexpectedly highly diverged due to assumed isolation and absence of gene flow from the continent.

Existence of two deeply differentiated groups of mitochondrial haplotypes in *C. corone* implies long lasting geographic isolation. These two groups are characterized by higher intrapopulation nucleotide diversity compared to *C. macrorhynchos* suggesting that the former species retained larger population size throughout the last glaciation. Both haplogroups can be assumed to have survived in more than one refuge area. Moreover, after their resurgence from refuges, they made several secondary contacts in Kamchatka and North Sakhalin. Analysis of contact areas using nuclear genes as well as additional sampling is required in order to obtain a more detailed picture of the evolution of these species and resolve the taxonomic problems.

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