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ANIMAL GENETICS

Genetic Diversity of the Carrion and Jungle Crows as Evidenced by RAPD–PCR Analysis

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Abstract—RAPD–PCR analysis of the genetic diversity of the carrion crow (*Corvus corone*) and jungle crow (*C. macrorhynchos*) living in the continental parts of their species ranges and on some Russian and Japanese Far Eastern islands has been performed. Taxon-specific molecular markers have been found for each species. The genetic diversity of the carrion crow is considerably less than that of the jungle crow at the same genetic distance ($P_{95} = 68.2\%$, $D_N = 0.27$ and $P_{95} = 88.4\%$, $D_N = 0.24$, respectively). In both species, the genetic polymorphism of island samples is almost two times greater than that of continental samples (62 and 31.8%, respectively, for *C. corone* and 81.5 and 47.2%, respectively, for *C. macrorhynchos*). In addition, differences in genetic diversity between males and females ($P_{95} = 55.1$ and 72.1, respectively) has been found in the carrion crow but not in the jungle crow. The gene diversity of *C. macrorhynchos* is greater than that of *C. corone*: the mean numbers of alleles per locus are 2 and 1.81, effective numbers of alleles are 1.62 and 1.43, and the mean expected heterozygosities are 0.39 and 0.30, respectively. The phenograms and phylograms significantly segregate the clusters of the carrion and jungle crows. The clustering patterns of carrion crows corresponds to the intraspecies taxonomic and geographic differentiation: subspecies *C. c. corone* and *C. c. orientalis* living in the western and eastern parts of the species range, respectively, form different subclusters. The cluster of the jungle crow does not exhibit differentiation into subspecies *C. m. mandshuricus* and *C. m. japonensis*; molecular genetic differences between them are small.

INTRODUCTION

Family Corvidae is one of the most numerous among birds and is widely distributed over the world. According to the results of DNA–DNA hybridization, Corvidae originated from Australia and spread into Eurasia 20–30 Myr ago [1]. Tribe Corvini consists of 25 genera comprising 113 species, 38 of which constitute genus *Corvus*, which is one of the youngest in evolutionary terms [1]. Both the jungle crow (*Corvus macrorhynchos* Wagler, 1827) and the carrion crow (*C. corone* Linnaeus, 1758) are widely distributed and successful species.

The jungle crow inhabits a considerable part of central and eastern Asia with a wide variety of climatic zones (Fig. 1). Its range includes a continental part and island isolated populations; therefore, *C. macrorhynchos* is of special interest as a model object for studying microevolutionary processes occurring in restricted, isolated areas. The ranges of the jungle and carrion crows overlap in a considerable part of eastern Asia; however, there is a strict reproductive isolation between them. The range of the carrion crow is disrupted; it includes almost entire Western Europe, as well as Eastern Siberia, Mongolia, northern China, and Japan. In Western Siberia and Western Europe, its range contacts with the range of the hooded crow (*C. cornix*). Narrow, stable hybrid zones are formed throughout the boundaries between the ranges of the carrion and hooded crows [2]. During the past 10–20 years, the numbers of these crow species and, hence, the biocenotic role of this group have increased in all urbanized biotopes. This "burst" of their numbers is especially important for the jungle crow [3].

The morphological variability of the jungle crow is mainly expressed in varying body size, beak size, and development of long feathers on the throat [4]. Different authors distinguish 8 to 15 subspecies of *C. macrorhynchos* [4, 5]. Here, we consider two subspecies of the jungle crow, *C. m. japonensis* and *C. m. mandshuricus*, which mainly differ in beak size. The carrion crow is subdivided in only two subspecies, the European *C. c. corone* and the eastern Asian *C. c. orientalis* [4]. The eastern Asian form is slightly larger but otherwise does not differ from the European subspecies. Analysis of the nucleotide sequences of the cytochrome *b* gene located in mtDNA demonstrated that the carrion crow has two haplotypes of this gene, one belonging to the European carrion crow, as well as the hooded crow and, to a lesser extent, eastern Asian carrion crow, and the other being specific for *C. c. orientalis* from the southeastern part of its range [6].

The biology of the carrion and jungle crows has been well studied [7–9, etc.]. Both species form mixed flocks in autumn and winter, but they considerably dif-

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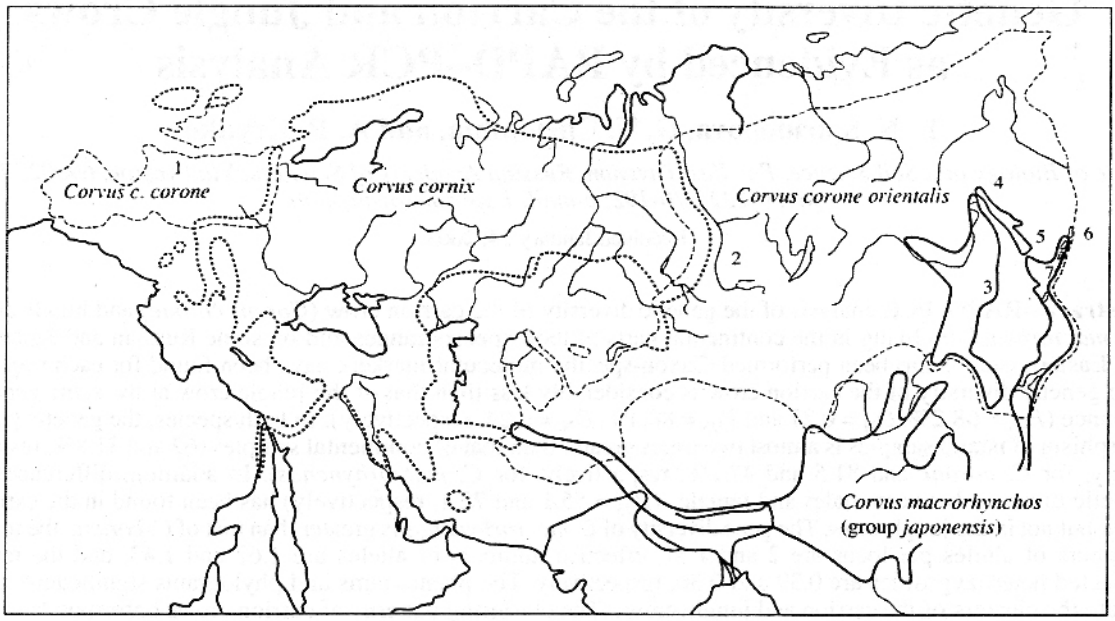

Fig. 1. Species ranges of *C. corone* and *C. macrorhynchos* and the sites of sampling (the numbers of the sites are the same as in Table 1).

fer in behavior and occupy different ecological niches [3, 9]. These species are monogamous and form constant pairs for long periods of time. The migration patterns of the jungle and carrion crows are different. The former is relatively settled and only migrates over short distances to wintering places [7], whereas the migration root of the latter species is as long as 2×10^3 km [8]. These biological characteristics have inevitably affected the genetic structures of the two species.

The purpose of this study was to estimate the characteristics of the genetic diversity and phylogenetic relationships of the two species of Corvidae, the jungle crow (*C. macrorhynchos*) and the carrion crow (*C. corone*). To do this, we set the following tasks: (1) to determine the characteristics of genetic diversity in both species; (2) to estimate the subspecies and geographic differentiation (i.e., to compare the continental and island populations); (3) to estimate the distribution of genetic diversity between males and females; (4) to find taxon-specific molecular markers and reconstruct the phylogenetic relationships of the carrion and jungle crows.

MATERIALS AND METHODS

The material comprised 15 carrion crows and 11 jungle crows (Table 1; Fig. 1). As an external control group, the magpie (*Pica pica*), a remote phylogenetic relative of crows, was used. DNA was isolated from fresh liver by the standard method [10] using proteinase K, followed by treatment with phenol–chloroform–isoamyl alcohol mixture, and repeated precipitation with isopropyl and ethyl alcohols. The total cell DNA preparations were used as a template for RAPD–PCR analysis as described earlier [11].

We used ten decameric oligonucleotide primers (Operon Technologies Inc., United States) differing in both the sequences and the (G + C) content (60–70%) and two specific primers for determination of sex [12] (Table 2). The latter primers were necessary because it was impossible to identify the birds' sex when we took material from chicks. The RAPD–PCR products were analyzed using electrophoresis in 2% agarose gel containing 0.5 μg/ml ethidium bromide dissolved in a 1 × TBE buffer solution. The *Pst*I hydrolysate of phage λ DNA was used as a molecular-weight marker. The resultant electrophoregrams were used to construct binary matrices in which the presence and absence of a given band were denoted by symbols 1 and 0, respectively.

To estimate genetic variation within the samples on the basis of the summary matrix, we used the POPGENE [13] and TEPGA [14] software. The following parameters were calculated: the genetic similarity (S), unbiased genetic distance (D_N) [15], the proportions of polymorphic loci for the 95 and 99% confidence limits (P_{95} and P_{99}), gene diversity (h), mean number of alleles per locus (n_a), effective number of alleles (n_e), mean expected (H_e) and unbiased (i.e., corrected for the sample size) (H_e^*) heterozygosities [16], and Shannon's index of sample heterogeneity (I).

The genetic variation between samples was estimated by the index of population subdivision (G_{st}) and the number of migrants per generation (Nm) between local populations: $Nm = 0.5(1 - G_{st})/G_{st}$ [13]. The total genetic variation (H_T), mean genetic diversity in a sample (H_S), and total genetic diversity between samples (D_{ST}) were calculated as described in [17]. The precise

Table 1. The list of samples studied

Species	Locality	Number of samples	Number of sampling site*
C. corone corone	Paris, France	4	1
C. c. orientalis	Arga, Krasnoyarskii krai	2	2
C. c. orientalis	Vladivostok, southern Primorskii krai	3	3
C. c. orientalis	Okha, northern Sakhalin	1	4
C. c. orientalis	Yuzhno-Sakhalinsk, southern Sakhalin	3	5
C. c. orientalis	Kunashir	1	6
C. c. orientalis	Sapporo, Hokkaido	1	7
C. macrorhynchos mandshuricus	Vladivostok, southern Primorskii krai	2	3
C. m. mandshuricus	Okha, northern Sakhalin	3	4
C. m. japonensis	Yuzhno-Sakhalinsk, southern Sakhalin	3	5
C. m. japonensis	Kunashir	2	6
C. m. japonensis	Sapporo, Hokkaido	1	7
Pica pica jankowskii	Vladivostok, southern Primorskii krai	1	3

* Corresponds to the site number indicated in Fig. 1.

test for population differentiation was calculated according to Raymond and Rousset [18].

The TREECON version 1.3 software package [19] was used to construct phylograms and phenograms.

RESULTS AND DISCUSSION

The screening performed earlier [20] allowed us to select primers that initiated the synthesis of predominantly major fragments, which were easily identifiable in electrophoregrams. In general, the RAPD–PCR analysis of the carrion and jungle crows demonstrated considerable similarity between the species; however, taxon-specific molecular markers were found. For example, primer OPC-09 identified, in DNA of each of subspecies *C. c. corone* and *C. m. macrorhynchos*, two species-specific fragments ($OPC\text{-}09_{555, 739}$ and $OPC\text{-}09_{493, 640}$, respectively); OPC-16 and OPC-12 primers identified one species-specific fragment each ($OPC\text{-}16_{957}$ and $OPC\text{-}12_{741}$) (Fig. 2).

The results of PCR using sex-specific primers demonstrated that the analyzed samples of carrion and jungle crows consisted of approximately equal numbers of males and females (Fig. 3). This allowed us to compare not only the intra- and interspecies diversity, but also some sex-related genetic differences.

The Diversity of RAPD–PCR Spectra

Each primer initiated the synthesis of a specific set of DNA fragments differing in molecular weight and in their belonging to major or minor fragments. Even visual analysis demonstrated differences between species with respect to genetic variation. The RAPD spectra of jungle crows were more variable than those of carrion crows, with intragenomic polymorphism being very high (Figs. 4, 5). The number of amplified bands in the RAPD spectra varied from 8 to 30; and the sizes of the fragments, from 260 to 4700 bp. In total, RAPD–PCR with ten primers allowed us to identify 327 characters in the jungle crow and 129 characters in the carrion crow, i.e., 33 and 12 RAPD loci per primer in an average DNA sample, respectively.

The degree of variation of RAPD spectra considerably varied for different primers. Some primers, e.g., OPF-02 and OPA-05 (data not shown), amplified the same sets of fragments in all birds; others detected either geographic (OPC-05) or individual (OPC-09) variation (Figs. 4, 5). For example, fragment $OPC\text{-}16_{1034}$ (Fig. 4) was found in all *C. c. orientalis* from the Far

Table 2. Primers used in the study

Primer	Nucleotide sequence (5' ⟶ 3')
OPA-05	AGGGGTCTTG
OPA-14	TCTGTGCTGG
OPC-02	GTGAGGCGTC
OPC-05	GATGACCGCC
OPC-08	TGGACCGGTG
OPC-09	CTCACCGTCC
OPC-10	TGTCTGGGTG
OPC-12	TGTCATCCCC
OPC-16	CACACTCCAG
OPF-11	TTGGTACCCC
2717F	GTAGAAGAAGATATTCTTGA
3088R	CCTCAGCACCAAACTTCAAA

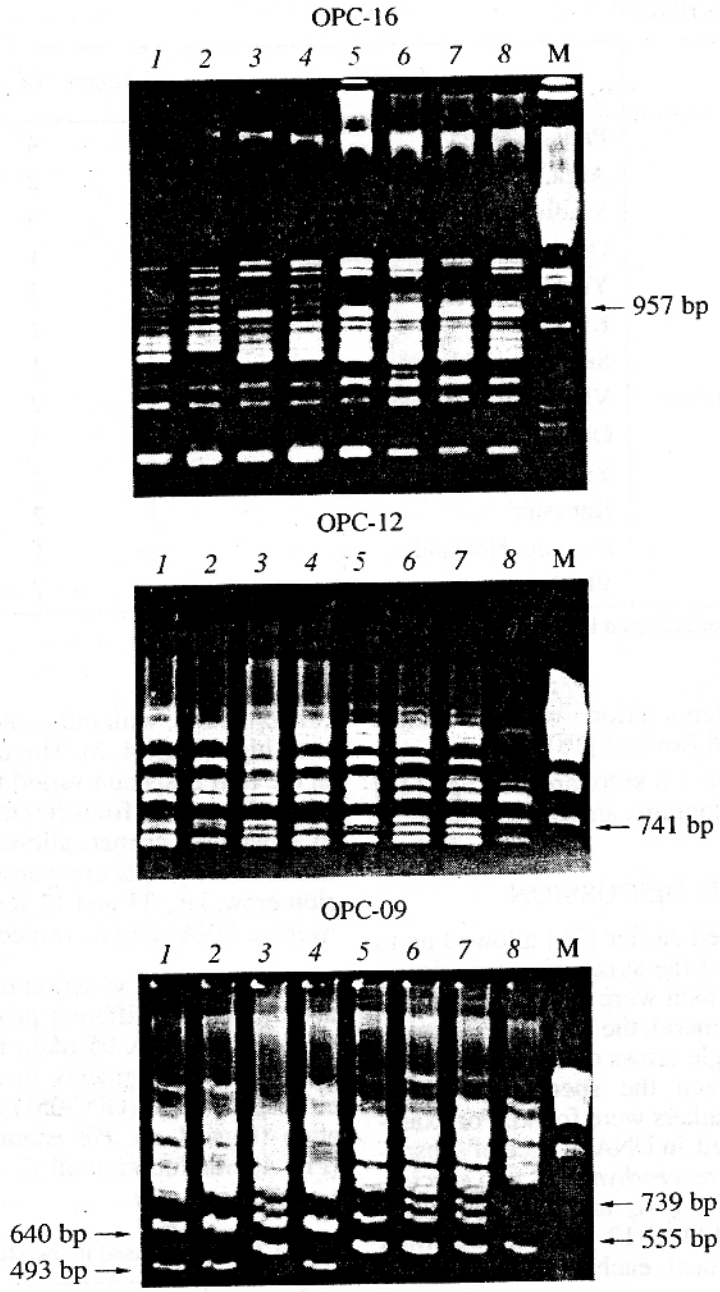

Fig. 2. RAPD–PCR spectra of DNA of (*1, 2, 4*) *C. macrorhynchos* and (*3, 5–8*) *C. corone* obtained using primers OPC-16, OPC-12, and OPC-09. M is the *Pst*I hydrolysate of phage λ DNA.

East but only in half *C. c. corone* from France and phenotypic *orientalis* from the Krasnoyarskii krai (Siberia). In other words, the crows studied exhibited frequency polymorphism with respect to this character. A block of three high-molecular-weight fragments (marked by braces in Fig. 4) identified by the same primer was found only in *orientalis* from the Far East. We found a diagnostic marker (OPC-05_{1487}) for *C. c. orientalis* from the Primorskii krai (Far Eastern Russia) (data not shown). The RAPD spectra of jungle crows exhibited a higher individual variation and were characterized by an increased intragenomic heterogeneity (Fig. 5). Along with fragments found in the majority of the crows, there were unique fragments that we found in only a few DNA samples or only some localities. For example, amplicons OPC-05_{3155} and OPC-09_{324} were found only in crows from Sakhalin (Fig. 5). We did not find subspecies markers for either jungle or carrion crows.

Gene Diversity

The analyzed samples of the two species of crows were characterized by a relatively high gene diversity (h). This value was higher for *C. macrorhynchos* than for

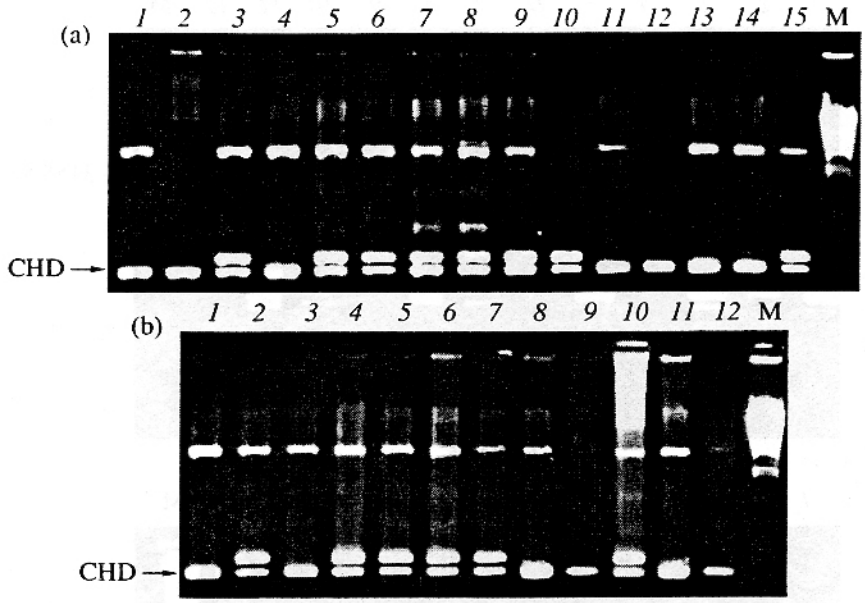

Fig. 3. Electrophoregrams of DNA of (a) *C. corone* and (b) *C. macrorhynchos* amplified using sex-specific primers. M is the *Pst*I hydrolysate of phage λ DNA.

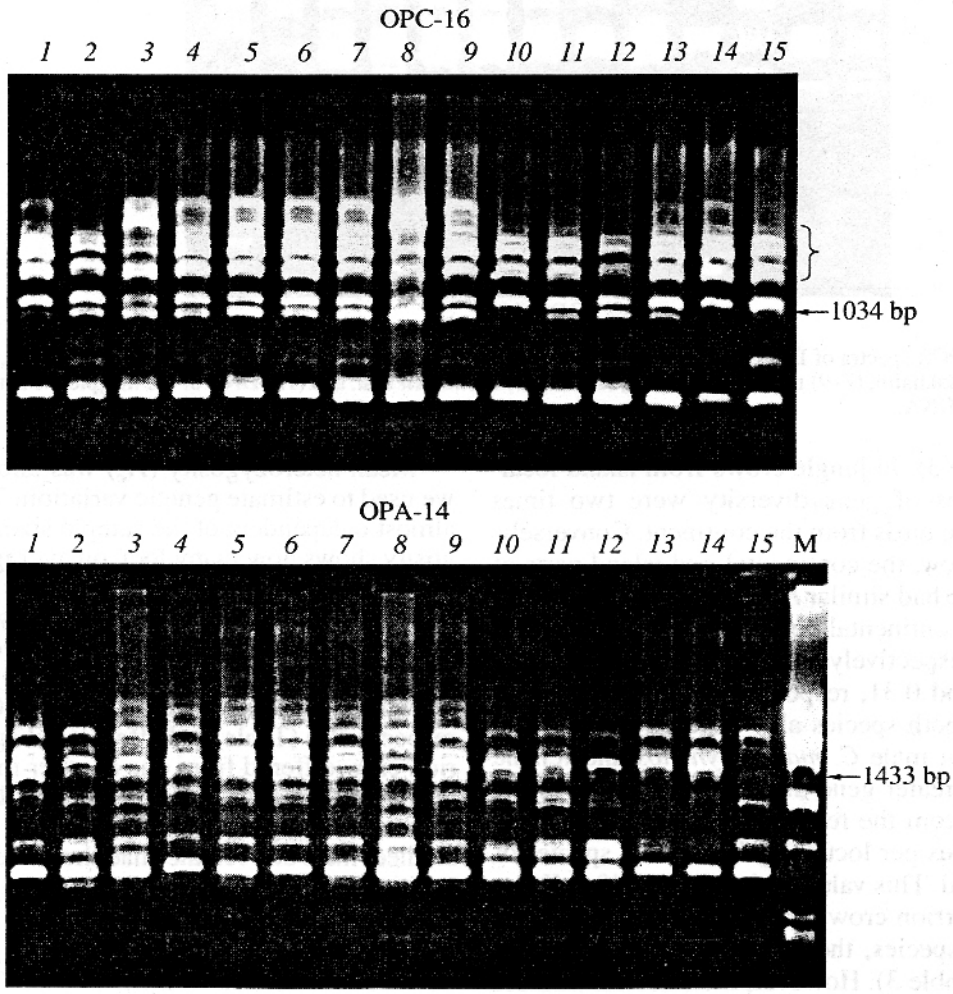

Fig. 4. RAPD–PCR spectra of DNA of *C. corone* from (*1*) Hokkaido, (2) Kunashir, (*3–6*) Sakhalin, (*7–9*) the Primorskii krai, (*10, 11*) the Krasnoyarskii krai, and (12–15) France. M is the *Pst*I hydrolysate of phage λ DNA.

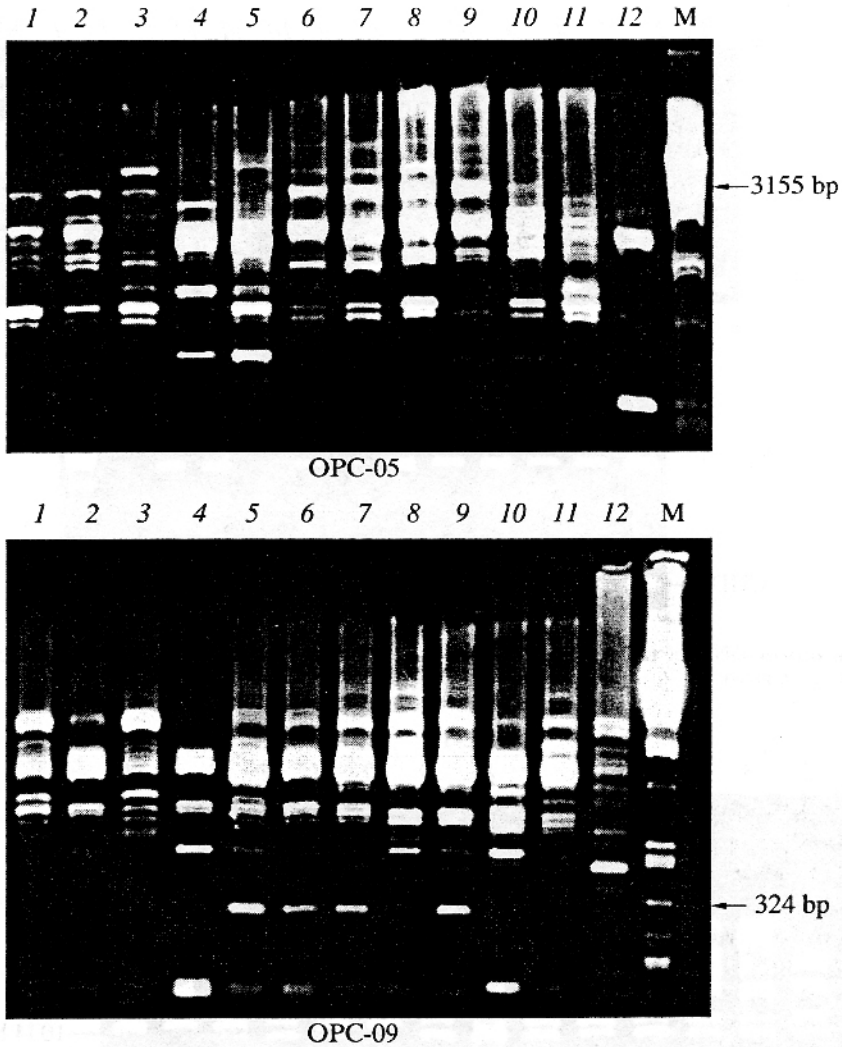

Fig. 5. RAPD–PCR spectra of DNA of *C. macrorhynchos* from island and continental populations: (*1*) Hokkaido, (*2, 3*) Kunashir, (*4–6*) southern Sakhalin, (*7–9*) northern Sakhalin, (*10, 11*) the Primorskii krai, and (*12*) DNA of the magpie. M is the *Pst*I hydrolysate of phage λ DNA.

C. corone (Table 3). In jungle crows from island localities, the degrees of gene diversity were two times higher than in the birds from the continent. Conversely, in the carrion crow, the continental and island parts of the species range had similar h values. Carrion and jungle crows from continental populations had h values of 0.13 and 0.15, respectively; and those from island populations, 0.15 and 0.31, respectively (Table 3). Males and females of both species also differed with respect to gene diversity: male *C. macrorhynchos* and female *C. corone* had greater gene diversity than birds of the opposite sexes from the respective species. The mean numbers of alleles per locus (n_a) in the two species of crows overlapped. This value varied from 1.40 in Western European carrion crows to 1.89 in Japanese jungle crows. In both species, the n_a was higher in females than in males (Table 3). However, the effective number of alleles (n_e) in male jungle crows was somewhat higher than in females of this species.

Mean heterozygosity (H_e) was another criteria that we used to estimate genetic variation. This parameter is almost independent of the sample size. Mean heterozygosity shows how many loci, on average, are heterozygous in an individual, population, or species. Its values were also higher in the jungle crow than in the carrion crow (0.375 and 0.288, respectively). Subspecies *C. m. japonensis* and *C. c. orientalis* had the highest H_e values in their respective species (0.365 and 0.241, respectively) (Table 3). Different subspecies of the carrion crow differed from one another in mean heterozygosity by two times; the differences between subspecies of the jungle crow were smaller. In both species studied, male and females had the same mean heterozygosities.

Genetic Variation

The proportion of polymorphic loci is among the parameters used to measure genetic variation. We esti-

Table 3. Parameters of genetic diversity of the two Corvidae species studied

Species	n_a	n_e	h	H_e (H_e^*)	I	$P_{95}(P_{99})$, %
C. corone	1.81 ± 0.39	1.43 ± 0.35	0.26 ± 0.18	0.2883 (0.2982)	0.3923 ± 0.2501	68.2 (79.1)
C. c. corone (n = 4)	1.40 ± 0.49	1.22 ± 0.34	0.13 ± 0.18	0.1195 (0.1365)	0.1740 ± 0.2668	31.8 (31.8)
C. c. orientalis (n = 11)	1.66 ± 0.47	1.36 ± 0.37	0.22 ± 0.19	0.2410 (0.2524)	0.3229 ± 0.2725	57.4 (65.8)
islands (n = 6)	1.46 ± 0.51	1.27 ± 0.37	0.15 ± 0.19	0.1704 (0.1859)	0.2208 ± 0.2769	62 (42.6)
continent (n = 9)	1.35 ± 0.48	1.23 ± 0.34	0.13 ± 0.18	0.2717 (0.2877)	0.3419 ± 0.2647	31.8 (37.2)
males (n = 7)	1.55 ± 0.51	1.29 ± 0.36	0.17 ± 0.19	0.2378 (0.2561)	0.2659 ± 0.2729	55.1 (55.1)
females (n = 8)	1.67 ± 0.48	1.38 ± 0.36	0.23 ± 0.19	0.2624 (0.2799)	0.3441 ± 0.2719	72.1 (72.1)
C. macrorhynchos	2.00 ± 0.00	1.62 ± 0.27	0.37 ± 0.11	0.3752 (0.3931)	0.5454 ± 0.1347	88.4 (97.4)
C. m. mandshuricus (n = 5)	1.60 ± 0.49	1.39 ± 0.39	0.23 ± 0.20	0.2557 (0.2841)	0.3341 ± 0.2893	60.8 (60.8)
C. m. japonensis (n = 6)	1.89 ± 0.31	1.49 ± 0.33	0.30 ± 0.16	0.3655 (0.3987)	0.4521 ± 0.2116	88.9 (88.9)
islands (n = 9)	1.95 ± 0.21	1.62 ± 0.31	0.31 ± 0.14	0.3655 (0.3987)	0.4760 ± 0.1880	81.5 (88.9)
continent (n = 2)	1.37 ± 0.48	1.25 ± 0.34	0.15 ± 0.20	0.2557 (0.2841)	0.2208 ± 0.2919	47.2 (60.9)
males (n = 5)	1.78 ± 0.41	1.50 ± 0.37	0.29 ± 0.19	0.3350 (0.3723)	0.4277 ± 0.2567	78.3 (78.3)
females (n = 6)	1.80 ± 0.41	1.43 ± 0.33	0.26 ± 0.17	0.3381 (0.3688)	0.3976 ± 0.2389	79.9 (79.9)

mated the general genetic polymorphism of each sample as the total proportion of RAPD characters for which variation was found among the total number of amplicons. If we used different confidence levels of the criterion for polymorphism, the respective proportions of polymorphic loci (P_{95} and P_{99}) differed for the jungle crow to a higher degree than for the carrion crow (Table 3). Such considerable differences between these species with respect to the degree of genetic polymorphism and the proportion of rare alleles may be explained by the more settled life of jungle crows compared to carrion crows, stronger influence of island isolation, and, hence, higher rates of fixation of gene mutations.

Of special interest was the fact that genetic polymorphism of both species studied was almost two times higher for island samples than for continental ones (Table 3). The increased polymorphism of island populations was earlier described in chaffinches (*Fringilla coelebs*), with specific haplotypes being fixed in each locality studied [21]. In addition, the differentiation between the continental and island chaffinch populations, as well as between different island populations, was very high (D = 4.5 and 3.5%, respectively) [21].

The genetic characteristics of male and female carrion and jungle crows were different, although it is always difficult to differentiate between males and females of these species under natural conditions on the basis of phenotypic characters. For example, the values of genetic polymorphism, heterozygosity, gene diversity and the number of alleles per locus are similar in male and female jungle crows. In carrion crows, these parameters are considerable higher in females than in males (Table 3).

Precise differentiation test [18] for all loci did not show substantial differences between subspecies of the carrion (χ^2 = 292.29, $d.f.$ = 258, p = 0.069) and jungle (χ^2 = 285.48, $d.f.$ = 378, p = 0.999) crows (Table 4). However, differences between continental and island *C. corone* were significant (χ^2 = 420.08, $d.f.$ = 258, p = 0.000). The genetic diversities of the analyzed localities of both species estimated by Shannon's index of sample heterogeneity (I) were insignificant. They varied from 0.1740 to 0.4521 in *C. c. corone* and *C m. japonensis*, respectively. Possibly, the weak subspecies and geographic differentiation of the carrion and jungle crows resulted from gene flows between populations.

The estimation of the genetic subdivision of populations (G_{st}) demonstrated greater differentiation between female and male jungle crows (i.e., fixation of different alleles in different sexes), as well as between island and continental localities of carrion crows, than between their subspecies (Table 4). The intraspecies similarity (S) was the same for both species; however, the total genetic variation (H_T) of the jungle crow was considerably greater than that of the carrion crow (Table 4). Unfortunately, the small sample sizes prevented us from making more definite conclusions and generalizations.

Intraspecies Genetic Discreteness

Figure 6 shows the distribution of pairwise genetic distances in carrion and jungle crows. As far as we could judge from these histograms, constructed for small samples, subspecies of both the jungle and carrion crows differed in the degree of genetic heterogeneity. The histograms for the eastern Asian subspecies of the carrion crow and the Japanese subspecies of the jungle crow displayed a distribution close to normal. These subspecies appeared to be more homogeneous than the western subspecies of the carrion crow and the Man-

Table 4. Parameters of intraspecies genetic differentiation of the carrion and jungle crows

Species	H_T (±)	H_S (±)	D_{ST} (±)	G_{st}	Nm	S Bias (unbias)	D_N Bias (unbias)	*Ext* χ^2/*d.f.*/*p*
C. c. corone ($n = 4$)/*C. c. orientalis* ($n = 11$)	0.24 (0.03)	0.17 (0.03)	0.07 (0.03)	0.29	1.24	0.76 (0.78)	0.27 (0.25)	292.29/258/0.069
islands ($n = 6$)/continent ($n = 9$)	0.24 (0.03)	0.13 (0.01)	0.11 (0.02)	0.47	0.57	0.85 (0.86)	0.17 (0.15)	420.08/258/0.000
males ($n = 7$)/females ($n = 8$)	0.23 (0.03)	0.20 (0.01)	0.03 (0.02)	0.15	2.79	0.92 (0.95)	0.08 (0.06)	126.56/258/1.000
C. m. mandshuricus ($n = 5$)/*C. m. japonensis* ($n = 6$)	0.31 (0.02)	0.26 (0.01)	0.05 (0.01)	0.16	2.65	0.79 (0.82)	0.24 (0.20)	285.48/378/0.999
islands ($n = 9$)/continent ($n = 2$)	0.29 (0.02)	0.23 (0.01)	0.06 (0.01)	0.21	1.91	0.76 (0.80)	0.27 (0.22)	195.64/378/1.000
males ($n = 5$)/females ($n = 6$)	0.28 (0.02)	0.18 (0.01)	0.1 (0.01)	0.36	0.90	0.85 (0.89)	0.17 (0.11)	239.07/378/1.000

Note: *Ext*, exact test for population differentiation including the values of χ^2; *d.f.*, degrees of freedom, and *p*, probability. Mean square deviation is indicated in parenthesis.

churian subspecies of the jungle crow. In addition, the histograms for carrion and jungle crows (for both entire species and individual subspecies) are shifted relative to each other. Note that the genetic differences between subspecies and the general intraspecies differentiation were greater in *C. macrorhynchos*.

Phylogenetic and Phenogenetic Reconstructions

The phylo- and phenograms of the carrion and jungle crows exhibited the same topology having two distinct species clusters; therefore, we show only the NJ tree here (Fig. 7). The jungle crow proved to be closer to the base. The cluster of *C. macrorhynchos* did not

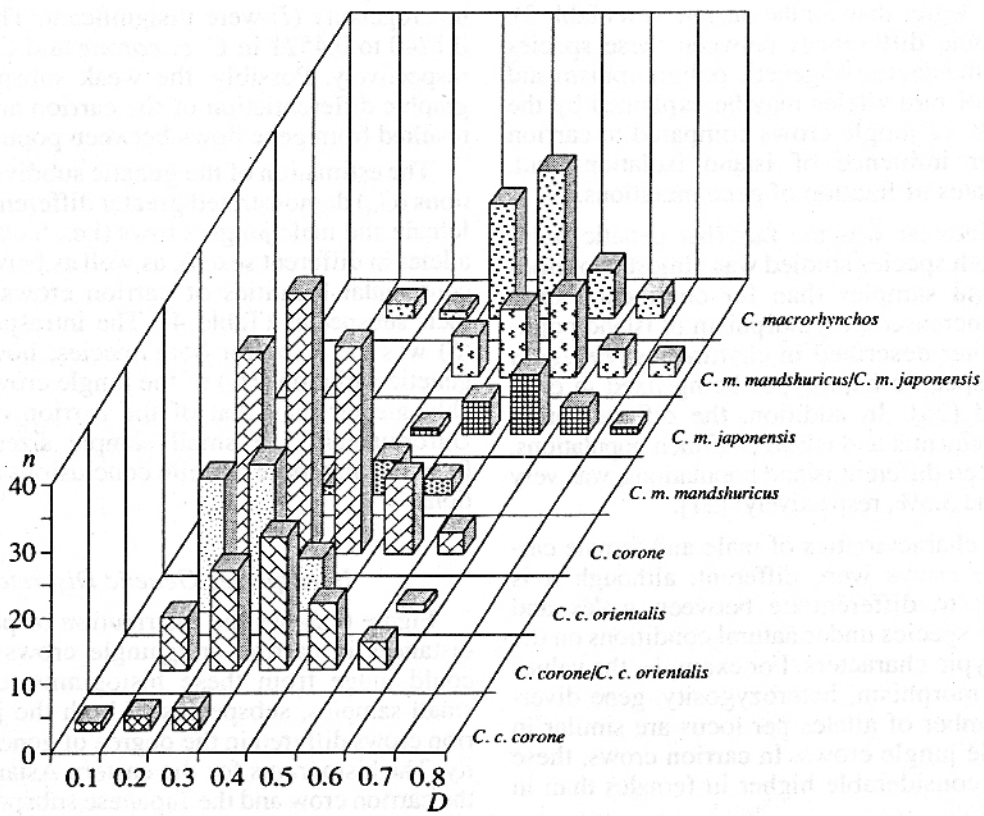

Fig. 6. The distribution histogram of pairwise genetic distances of carrion and jungle crows.

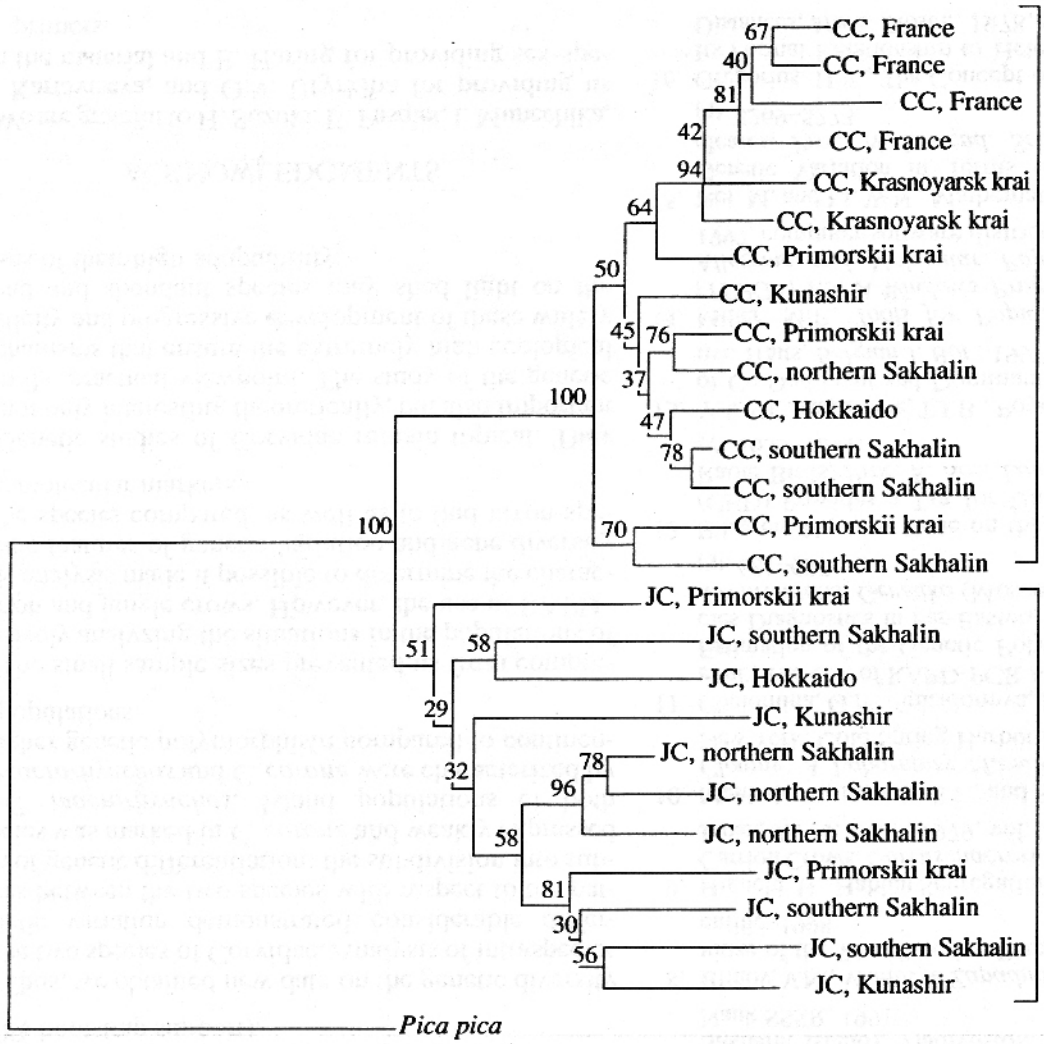

Fig. 7. Phylogenetic relationships between carrion crows (CC) and jungle crows (JC) from different localities. The NJ tree was constructed using the TREECON software.

exhibit subspecies differentiation and, interestingly, had no 100% bootstrap support of monophyletic origin. The values of bootstrap support of different clusters varied from 29 to 95%. The main subcluster comprised crows from southern Sakhalin, the Primorskii krai, and Hokkaido, as well as two birds from southern Sakhalin; the remaining individuals sequentially branched out of it. In general, the differentiation did not completely correspond to the subdivision into subspecies; however, this can be explained. On the one hand, the fact that the birds from the Primorskii krai and southern Sakhalin fell within the same group may have resulted from the vagueness of their migration routes (we did not find published data on the migration routes of *C. macrorhynchos* subspecies traced with the use of banding). On the other hand, the birds caught in Sakhalin were identified accurate to species, whereas both subspecies live in the island (although *C. m. japonensis* mainly inhabits its southern part; and *C. m. mandshuricus*, the northern part). Unfortunately, the relationship between the subspecies in the contact zone of their ranges has not been studied, and there are no distinct morphological criteria to identify their hybrids. The estimates of gene fixation and the number of migrants per generation indicated a considerable gene flow between *C. m. mandshuricus* and *C. m. japonensis* (identified by the sampling sites): $G_{st} = 0.16$, $Nm = 2.65$ (Table 4).

The cluster of *C. corone* is divided into several subclusters (Fig. 7), although not all differences between them are large or significant (37 to 94%). One subcluster comprises only Far Eastern crows (*C. c. orientalis*); and another, crows from France (*C. c. corone*). Crows from the Krasnoyarsk krai (phenotypic *C. c. orientalis*) are located between these two subclusters in Fig. 5; however, the high bootstrap support (94%) indicates that they are close to the European *C. c. corone*. On the one hand, this can be explained by the fact that phenotypically pure carrion crows from the Krasnoyarskii krai may be regarded as genotypic hybrids between

eastern Asian carrion crows and hooded crows according to the results of our RAPD–PCR analysis, because their DNA contained some fragments that were characteristic of the phenotypic hybrids from the Siberian hybrid zone and were absent in genetically pure forms from Far East (the data will be reported in our next article). On the other hand, French crows (phenotypically pure *C. c. corone*) may also be genotypic hybrids between the western form of the carrion crow and the hooded crow. These suggestions agree with the notion that the areas of introgressive hybridization of most hybrid zones go far beyond their phenotypic boundaries [22, 23]. When comparing the carrion crow subspecies *C. c. corone* and *C. c. orientalis*, we found a slightly greater fixation of gene differences than in the jungle crow: $G_{st} = 0.29$, $Nm = 1.24$ (Table 4). Some differences in estimated gene fixation between subspecies of the carrion crow may result from the disruption of the species range, so that *C. c. corone* and *C. c. orientalis* have different geographic range. This disruption is a relatively strong obstacle to the gene flow between them, whereas there is no such a barrier in the jungle crow, whose species range is continuous. However, the results of the bootstrap analysis of the NJ tree demonstrated a monophyletic origin of the carrion crow (a 100% bootstrap support).

Thus, we obtained new data on the genetic diversity of the two species of Corvidae. Analysis of intraspecies genetic variation demonstrated considerable differences between the two species with respect to the pattern of genetic differentiation: the subdivision into subspecies was marked in *C. corone* and weakly expressed in *C. macrorhynchos*. Island populations of both *C. macrorhynchos* and *C. corone* were characterized by a higher genetic polymorphism compared to continental populations.

The small sample sizes prevented us from comprehensively analyzing the situations in the populations of carrion and jungle crows. However, the use of RAPD–PCR analysis made it possible to determine the characteristic features of genetic variation and gene diversity of the species compared, as well as to find taxon-specific molecular markers.

Genetic studies of Corvidae remain topical. They are not only interesting theoretically, but also important from the practical viewpoint. The study of the genetic mechanisms that ensure the extremely high ecological plasticity and progressive development of these widely spread and abundant species may shed light on the causes of their high adaptability.

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