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MOLECULAR GENETICS

Peculiarities of RFLP of Highly Repetitive DNA in Crow Genomes

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Abstract – We present a study of the structural organization of highly repetitive DNA in genomes of hooded crow *Corvus cornix* L., carrion crow *C. corone* L., and jungle crow *C. macrorhynchos* Wagl. RFLP and blot-hybridization with ^{32}P-labeled *Msp* I fragment from hooded crow nDNA suggest the interspecific structural conservatism of the most repetitive DNA. The family of repeats we studied had tandem organization and the same (210 bp) period of reiteration for a set of restriction enzymes. However, in parallel to the general similarity of restriction patterns, there are species-specific peculiarities. The repetitive family revealed (*Alu* I, *Bsu*R I, and *Msp* I fragments) has quantitative RFLP of nDNA and interspecific differences in the extent of the multimer "ladder" pattern of *Msp* I fragments. The latter is more pronounced in nDNA of carrion crow than in that of phylogeneticaly distant jungle crow and closely related hooded crow. This suggests a recent amplification event for highly organizated homological repeats in crow genomes.

INTRODUCTION

Natural hybridization between hooded and carrion crows is well known. However, the genetic nature and genome organization of birds in general is insufficiently studied. Earlier, it was stated that hooded and carrion crows (as well as their hybrids) had the same cleavage patterns when restriction enzymes *Bsp*R I, *Alu* I, and *Sau* 3A were used [1, 2]. *Alu* I released from the genomes of approximately 6% of DNA, organized into extensive clusters more than 2 kb in length. Electrophoregrams observed under conditions of partial hydrolysis revealed a multimer series of fragments. The most mobile fragments were well distinguished from the total nDNA. Digestion of nDNA with *Bsp*R I yielded multiple bands. Dominating bands had more a complicated, but also regular distribution. Their sizes might suggest homology between *Alu* I and *Bsp*R I fragments. Moreover, in the case of *Bsp*R I, interspecies differences of quantitative character were observed. The presence of an easily identifiable satellite component allowed the obtaining of a molecular probe for testing the divergence extent in species and hybrid forms. Using satellite DNA as a molecular marker is the conventional method in evolutionary studies.

In our study, we tried to reveal more distinct interspecific differences and to determine the molecular phylogenetic marker for various *Corvidae* species. Our aims were: (1) to perform restriction analysis using a wide spectrum of restriction enzymes; (2) to identify a restriction DNA fragment suitable for testing highly repetitive DNA in crow genomes; (3) to perform blot-hybridization of DNA restriction fragments from three crow species with ^{32}P-labeled DNA probe; and (4) to compare qualitative and quantitative characteristics of restriction patterns and blot-hybridization results.

MATERIALS AND METHODS

Nestlings and adult birds of hooded crow were obtained in the Novosibirsk region and those of carrion and jungle crows, on Kunashir island in Sakhalin oblast'. The above populations of hooded and carrion crows are so distant that the possibility of change of genes via the hybrid zone between them is virtually excluded [3].

DNA from fresh or fixed in ethanol liver was isolated using a common method or Antonov's method [4], respectively. In restriction analysis, we used a standard set of restriction enzymes (*Eco*R I, *Hind* III, *Pst* I, *Msp* I, *Pvu* II, *Alu* I, *Bsu*R I, *Sal*G I, and *Bam*H I) as described above [1].

Isolated by electroelution from polyacrylamide gel, a 210 bp *Msp* I fragment from the genome of hooded crow was labeled (^{32}P) and used as a probe in blot-hybridization. The specific radioactivity of a probe labeled by nick-translation [6] was approximately 10^6 cpm per 1 μg.

RESULTS AND DISCUSSION

Restriction analysis. We did not succeed in effectively digesting crow nDNA with restriction enzyme *Sal*G I (Fig. 1*A*). However, restriction patterns for the remaining eight restriction enzymes demonstrate the

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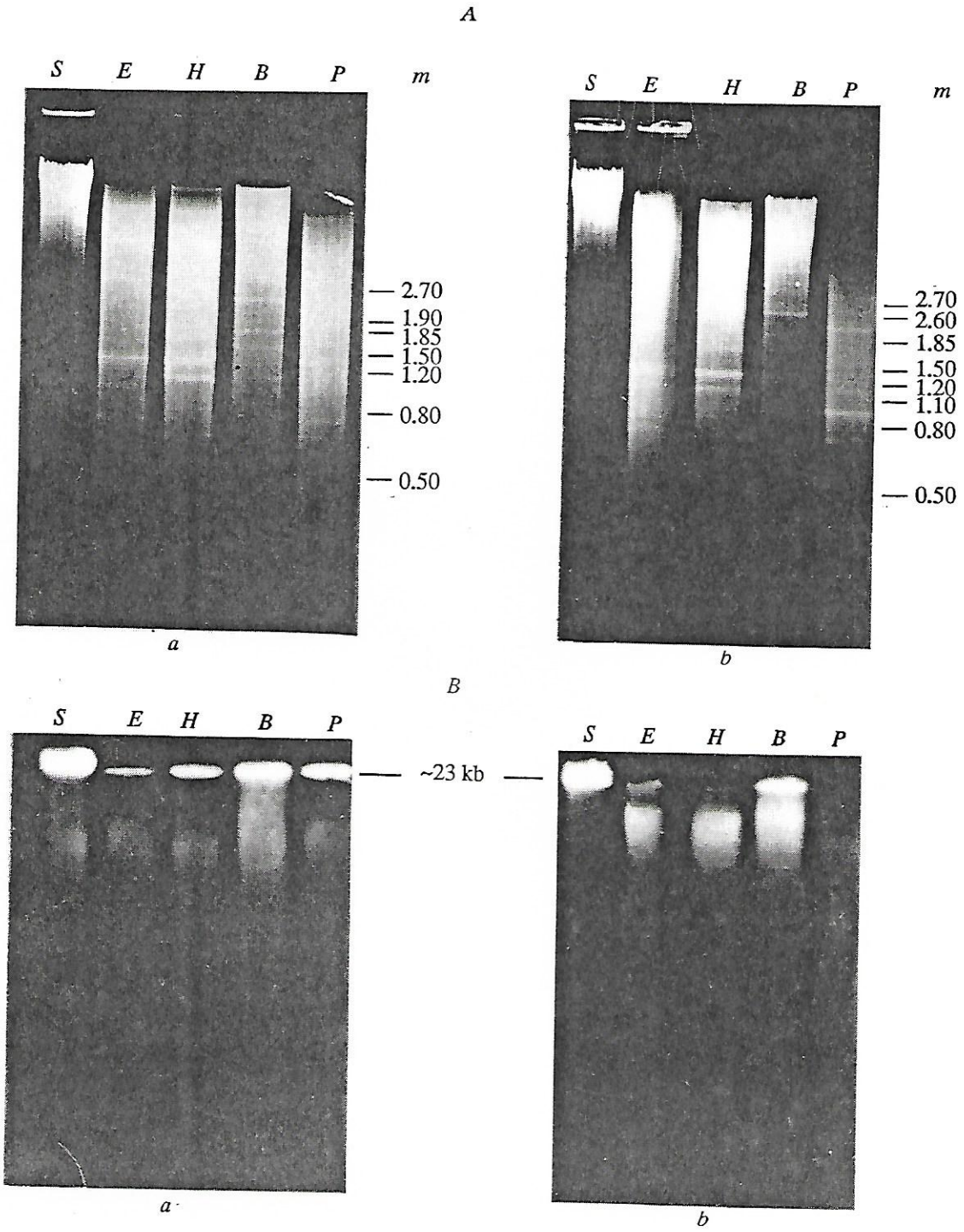

Fig. 1. nDNA isolated from (*a*) carrion and (*b*) jungle crows digested with *Sal*G I (*S*), *Eco*R I (*E*), *Hind* III (*H*), *Bam*H I (*B*), and *Pvu* II (*P*), fractionated on 1 % agarose gel (*A*) and subjected to blot-hybridization (*B*) with the ^{32}P-labeled *Msp* I fragment from hooded crow genome. Molecular weight marker (*m*), phage λ DNA digested with *Pst* I.

absence of differences between the hooded and carrion crows. Jungle crow shows a marked divergence from both, suggesting its morphological isolation. Restriction enzymes *Pvu* II and *Bam*H I reveal the most essential differences. In the genome restricts of hooded and carrion crows, we observed four weak *Pvu* II bands (molecular weights: 2.6, 1.5, 0.8, and 0.5 kb), equidistant of each other, and another series of weak *Bam*H I bands, without periodicity in organization and size distribution. The most intensive of them have the size 2.7 and 1.9 kb. Digested into discrete fragments, DNA of jungle crow forms more contrast bands with the other

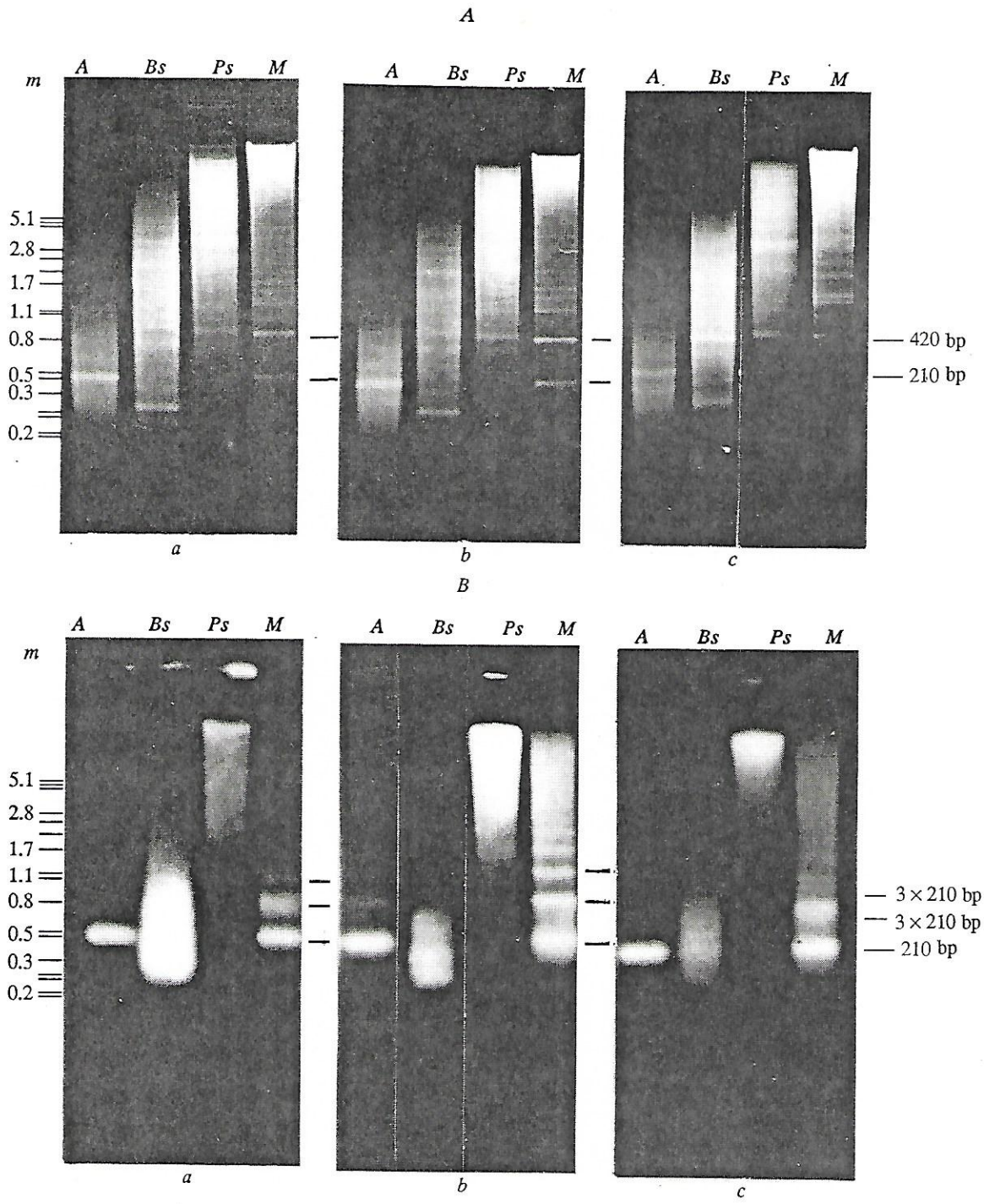

Fig. 2. nDNA isolated from *C. cornix* (*a*), *C. corone* (*b*), and *C. macrorhynchos* (*c*) digested with *Alu* I (*A*), *Bsu*R I (*Bs*), *Pst* I (*Ps*), fractionated on 1.8 % agarose gel (*A*) and subjected to blot-hybridization (*B*) with the ^{32}P-labeled *Msp* I fragment from hooded crow genome. Molecular weight marker (*m*), phage λ DNA digested with *Pst* I.

sizes. The most intensive of these are 2.7 and 1.1 kb for *Bam*H I and 2.6, 1.1, 0.8, and 0.5 kb for *Pvu* II-hydrolysates.

Restriction enzymes *Alu* I, *Bsu*R I, *Pst* I, and *Msp* I reveal insignificant interspecies differences in number, distribution character of discrete bands, and some varieties of repetitive DNA in certain bands (Fig. 2*A*). Fragments 420 and/or 210 bp are observed in all the samples. The most even distribution in lanes is characteristic of *Bsu*R I fragments, though they do not contain a high-molecular-weight fraction. *Alu* I-hydrolysates contain exceptionally low-molecular-weight fragments,

whereas the majority of *Msp* I and *Pst* I fragments are concentrated in the high-molecular-weight region of the spectrum.

Restriction enzymes *Eco*R I and *Hind* III show almost identical restriction patterns for all three species. The major component of hydrolysates is a 1.5 kb fragment in the case of *Eco*R I and a triplet of *Hind* III sequences: 1.85, 1.5, and 1.2 kb.

Blot-hybridization. We performed blot-hybridization of a ^{32}P-labeled *Msp* I fragment from hooded crow genome DNA and hydrolysates of total nDNA from three *Corvidae* species (Figs. 1*B* and 2*B*). Our results suggest that the restriction enzymes used enable us to identify at least two different families of repetitive DNA in birds. One of them shows good hybridization with the probe. This family includes *Msp* I, *Alu* I, and *Bsp*R I fragments with sizes divisible by 210 bp. The above sequences may be nonidentical, but have a high degree of homology (according to the intensity of signals in the hybridization).

Most of the *Alu* I restricts are represented by 210 bp fragments. Dimers 420 bp in size can also be observed, but in markedly lower quantities.

Unlike *Alu* I, the multimer "ladder" pattern of the *Msp* I tandem is more extensive. Mono-, di-, and trimers can be observed in hybridization patterns of hooded and jungle crows. In the case of carrion crow, the number of multimer fragments reaches 10 - 11. However, in each case, the most intensive bands are of mono- and dimer. We have no reason to consider this result an artifact caused by incomplete hydrolysis of carrion crow DNA, though such a possibility exists. Moreover, analogous examples of interspecies differences are well known. For example, satellite DNA of two close species of forest mouse, *Apodemus sylvaticus* and *A. flavicollis*, have identical patterns of digestion with restriction enzymes, but in the length of multimer, the "ladder" pattern of *Hind* III fragments in hybridization with corresponding ^{32}P probe is easily divisible [7].

The majority of *Bsu*R I fragments are in the low molecular weight range, which cannot be divided effectively under the conditions described (Fig. 2). It is probably for this reason that corresponding discrete bands in hybridization have blurred contours. However, it is *Bsu*R I sequences that have the highest interspecies quantitative polymorphism. Efficiency of molecular probe hybridization with *Bsu*R I fragments decreases markedly in the row hooded – carrion – jungle crow. In the genome of the former, it even exceeds the hybridization level of *Msp* I nDNA restricts.

We also performed an intragenomic comparison of probe hybridization with *Msp* I, *Alu* I, and *Bsp*R I sequences. In the hooded crow genome, the signal intensity decreases in the row *Bsu*R I, *Msp* I, and *Alu* I. The highest intensity of hybridization in the case of carrion and jungle crow is observed for *Msp* I restricts. Whereas the signal intensity of *Bsp*R I fragment is higher than that of *Alu* I in carrion crow, quite the reverse is observed for jungle crow (incidentally, the level of hybridization with *Bsu*R I is the lowest among all variants compared). Thus, the patterns we observed are species-specific. Unfortunately, in the absence of dot analysis, these results have only a preliminary character.

When electrophoresed on 1% agarose gel, patterns of blot-hybridization (and restriction as well) are identical for hooded and carrion crows. The absence of hybridization between labeled *Msp* I probe with discrete *Pst* I, *Eco*R I, *Hind* III, *Pvu* II, *Bam*H I, and *Sal*G I fragments suggests that the above restriction enzymes release the sequences of an unrelated family by cleavage. Those discrete *Alu* I, *Msp* I, and *Bsp*R I fragments of nDNA that show no signal in hybridization must also belong to another family.

CONCLUSION

Thus, we used a wide spectrum of restriction enzymes to study the organization of highly repetitive DNA in the genomes of three bird species of the *Corvidae* family. The data obtained testify to the correlation between the similarity level of restriction patterns for total nDNA of crows and phylogenetic relations inside *Corvidae*. Closely related species (hooded and carrion crow) show a similar series of restriction fragments, whereas in phylogeneticaly distant species (jungle crow), the spectrum of fragments markedly differs.

The method of nDNA RFLP and blot hybridization enabled us to reveal the heterogeneity of a highly repetitive nDNA fraction in crows. We demonstrated that the repeat family determined contains regular restriction sites for *Msp* I, *Alu* I, and *Bsu*R I; has tandem organization; and the sizes of its restriction fragments are divisible by 210 bp. The above fraction of repeats is rather conserved, as it shows a high extent of similarity in isolated, phylogeneticaly distant species (jungle crow, as compared to hooded and carrion crows). We also revealed another series of repeats that does not show hybridization with the molecular probe we used. These repeats contain both evolutionarily mobile (*Pvu* II, *Bam*H I) and conserved (*Eco*R I, *Hind* III) restriction sites and do not have tandem organization.

Analysis of the blot-hybridization results for the above family of repeats also revealed interspecific differences in the nDNA of the birds studied. The differences are of two kinds: quantitative differences are pronounced in the intensity of the hybridization signal and qualitative, in a multiplicity of restriction fragments. The first kind of difference suggests the species-specificity of the content of the *Msp* I, *Alu* I, and *Bsu*R I fragments. The second lies in a more pronounced "ladder" pattern of *Msp* I fragments in the genome of carrion crow than in jungle, and even in hooded crow. This allows the use of the *Msp* I probe of hooded crow nDNA for genetic analysis of phenotypicaly different

individuals from the hybridization zone of *C. cornix* and *C. corone*.

When analyzing species-specific peculiarities of RFLP of tandem highly repetitive DNA in the birds studied, as well as repeats in the mammals described earlier (*Bsp*-repeats in *Carnivora*, *Canidae* [8, 9]), the following regularities can be found.

Interspecies comparisons in both *Corvidae* and *Canidae* reveal quantitative variations in the content of repeats. Quantitative differences in the series of restriction bands can mark some closely related species (e.g., *C. cornix* and *C. corone*). This suggests amplification events that happened during the process of divergence from the common ancestor.

The only qualitative difference we found in this work is an extensive length of multimer *Msp* pattern in *C. corone* as compared to *C. cornix*. The same restriction patterns, also revealed in *Canidae* species, must illustrate a multistage evolution process including selective amplification processes [9, 10]. The similar molecular mechanism must have been used during the divergence of close crow species.

The data obtained for two classes of vertebrates (birds and mammals) confirms the theory that evolution of repetitive DNA, typical for the eukaryotic genome, has a species-specific character. Our data allows us to detect the most general molecular mechanisms of the evolution of repeats, and, therefore, the evolution of the eukaryotic genome.

ACKNOWLEDGMENTS

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