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ANIMAL GENETICS

Comparative Compositional Mapping of Chicken and Quail Chromosomes

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Abstract—The distribution of various isochore families on mitotic chromosomes of domestic chicken and Japanese quail was studied by the method of fluorescence in situ DNA–DNA hybridization (FISH). DNA of various isochore families was shown to be distributed irregularly and similarly on chromosomes of domestic chicken and Japanese quail. The GC-rich isochore families (H2, H3, and H4) hybridized mainly to microchromosomes and a majority of macrochromosome telomeric regions. In chicken, an intense fluorescence was also in a structural heterochromatin region of the Z chromosome long arm. In some regions of the quail macrochromosome arms, hybridization was also with isochore families H3 and H4. On macrochromosomes of both species, the pattern of hybridization with isochores of the H2 and H3 families resembled R-banding. The light isochores (L1 and L2 families) are mostly detected within macrochromosome internal regions corresponding to G bands, whereas microchromosomes lack light isochores. Although mammalian and avian karyotypes differ significantly in organization, the isochore distribution in genomes of these two lineages of the warm-blooded animals is similar in principle. On macrochromosomes of the two avian species studied, a pattern of isochore distribution resembled that of mammalian chromosomes. The main specific feature of the avian genome, a great number of microchromosomes (about 30% of the genome), determines a compositional specialization of the latter. This suggests the existence of not only structural but also functional compartmentalization of the avian genome.

INTRODUCTION

Compositional mapping consists in determining the distribution along chromosomes of extended (at least 300 kb) DNA fractions with homogeneous nucleotide composition, referred to as isochores [1]. Of particular interest is comparing the compositional and cytogenetic chromosome maps and correlating isochore fractions with morphological (centromeres and telomeres) and cytochemical (G/R bands) markers.

In most animal species, DNA fractions differing in the buoyant density are obtained by centrifugation of DNA fragments in sucrose gradient. This reflects DNA heterogeneity for the nucleotide composition: the GC-rich sequences have higher buoyant density than AT-rich ones. In human, two "light" (L1, c = 1.698 and L2, c = 1.700) and three "heavy" (H1, c = 1.704; H2, c = 1.708; and H3, c = 1.712) isochore families were found [1–5]. In the mouse genome, the families L1, L2, H1, and H2 are present [6]. The light isochores comprise about 63% of the mammalian genome [3]. In the cold-blooded animals, only the light isochore families, L1 and L2, are presented [4].

In several avian species belonging to different taxa, the genome composition was shown to be homogeneous [6]. Unlike mammals, birds have a family of heavy isochores H4 (c = 1.712) [6]. In addition, heavy isochore fractions contain a GC-rich satellite characteristic of birds and reptiles [6].

In mammals, different isochore families are irregularly distributed. The heaviest H3 isochores were localized to T bands, where concentration of genes, especially of housekeeping genes and oncogenes is the highest. In general, the light isochore fractions tend to be located in G bands, where gene concentration is much lower than in R bands and mostly tissue-specific genes are present, while heavy isochores are mainly associated with R bands [7, 8].

The avian karyotype has some morphological features that are interesting with respect to the compositional mapping. Microchromosomes constitute about 30% of the avian genome. They are not only much shorter than macrochromosomes, but also possess specific cytological and biochemical features [9]. Microchromosomes are R-positive and GC-rich [10]. Unlike macrochromosomes, microchromosomes contain many

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CpG islands as shown by DNA–DNA in situ hybridization, which testifies indirectly to microchromosome enrichment with genes [11]. In chicken, this part of the genome proved to rich with already mapped genes [12]. Another distinctive feature of microchromosomes is a lower level of methylation of the GC base pairs and a higher level of histon acetylation as compared to macrochromosomes, which is also typical of the functionally active chromatin [13]. Thus, the avian genome is characterized by structural and, presumably, structural–functional compartmentalization is characteristic of the avian genome. Since compositional mapping makes it possible to determine the functional role of genome regions [1], the problem of functional compartmentalization of the avian genome may be clarified using this approach.

MATERIALS AND METHODS

The preparations of mitotic chromosomes were obtained from cells of the 96-h embryos of Brown Leghorn chicken and Japanese quail by the standard procedure [14].

DNA probes containing chicken isochore fractions were described previously [15].

The DNA–DNA in situ hybridization was conducted according to the standard technique [16] with some modifications. The mitotic chromosome preparations were treated with a hybridization mixture (200 ng of labeled DNA probe was placed under each cover glass) and incubated in a dry-air temperature-controlled moist chamber for 16 h at 37°C. The avidin-FITC fluorescence system was used for signal detection. Afterwards, the preparations were stained with a propidium iodide solution (2 μg/ml, Sigma) in the Vectashield antifade (Vector) and analyzed using a Luman fluorescence microscope (magnification ob. 100×, oc. 10×), CCD CHIPER chambers, and the Ista Video Test-FISH 1.0 software.

The distribution of hybridization signals along chicken and quail chromosomes was determined as follows:

Flpter was measured for each hybridization signal;

based on the measurement results, the coordinates of hybridization signals were plotted on standard idiograms of differential RBG banding of chicken chromosomes [17];

the theoretically expected number of the biotinylated complexes was calculated based on the assumption of their uniform distribution along chromosome length;

the theoretically expected and observed values were compared using the χ^2 test.

RESULTS

The heaviest GC-richest fraction of chicken genome, Fr 7, which contains almost exclusively the H4 isochore family, was distributed along chromosomes as follows (Figs. 1a, 1b):

in both chicken and quail, numerous microchromosomes, mostly the shortest ones, were almost completely covered with hybridization signals;

in both of the avian species, a somewhat lower number of microchromosomes were partially covered with signals, primarily, in the telomeric regions;

in the majority of chicken and quail macrochromosomes, telomeres were revealed as positive regions of hybridization;

in chicken, a large fluorescent band was detected in a region occupied by the constitutive GC-rich heterochromatin in the long arm of the Z chromosome. In quail, this region exhibited weaker fluorescence.

The DNA fraction Fr 6 ranking second in buoyant density and containing H3 and H4 isochore families, occupied the same chromosome regions as described above in both avian species. In addition, a weak labeling was detected in the interstitial macrochromosome regions (Figs. 1c, 1d).

Fraction 5 represented by the H3 and H2 isochore families was localized to the same chromosome regions as fraction 6 in both chicken and quail (Figs. 1e, 1f). As judged from the hybridization signals, the distribution of both DNA fractions along chicken macrochromosomes is in a good agreement with the R/G banding pattern (Fig. 2).

Fractions 3 and 4 contain isochore families L2, H1 and H1, H2, respectively. The microchromosomes contained mostly DNA of these fractions. The small and medium-sized microchromosomes were completely covered with hybridization signals. On the large microchromosomes, signals were primarily detected in the telomeric regions. Both chicken and quail macrochromosomes contained hybridization sites in telomeric and some interstitial regions with a less pronounced band specificity (Figs. 1g, 1f, 3a, 3b).

Finally, fractions 2 and 1, which contained mostly light isochore families, L1 and L2, were revealed in neither microchromosomes nor telomeric regions of macrochromosomes in chicken and quail. Instead, they were shown to be distributed along macrochromosomes to provide a pattern more or less similar to that of the G-banding (Figs. 3c–3f).

DISCUSSION

The avian genome organization specific for warm-blooded animals, namely, chromosome heteromorphism, which is a kind of karyotype compartmentalization, has long attracted attention of researchers. At different times, main structural and functional features of micro- and macrochromosomes have been characterized. The microchromosomes are relatively gene rich, their DNA replicates early during the cell cycle, and they have high concentration of CpG islands, low meth-

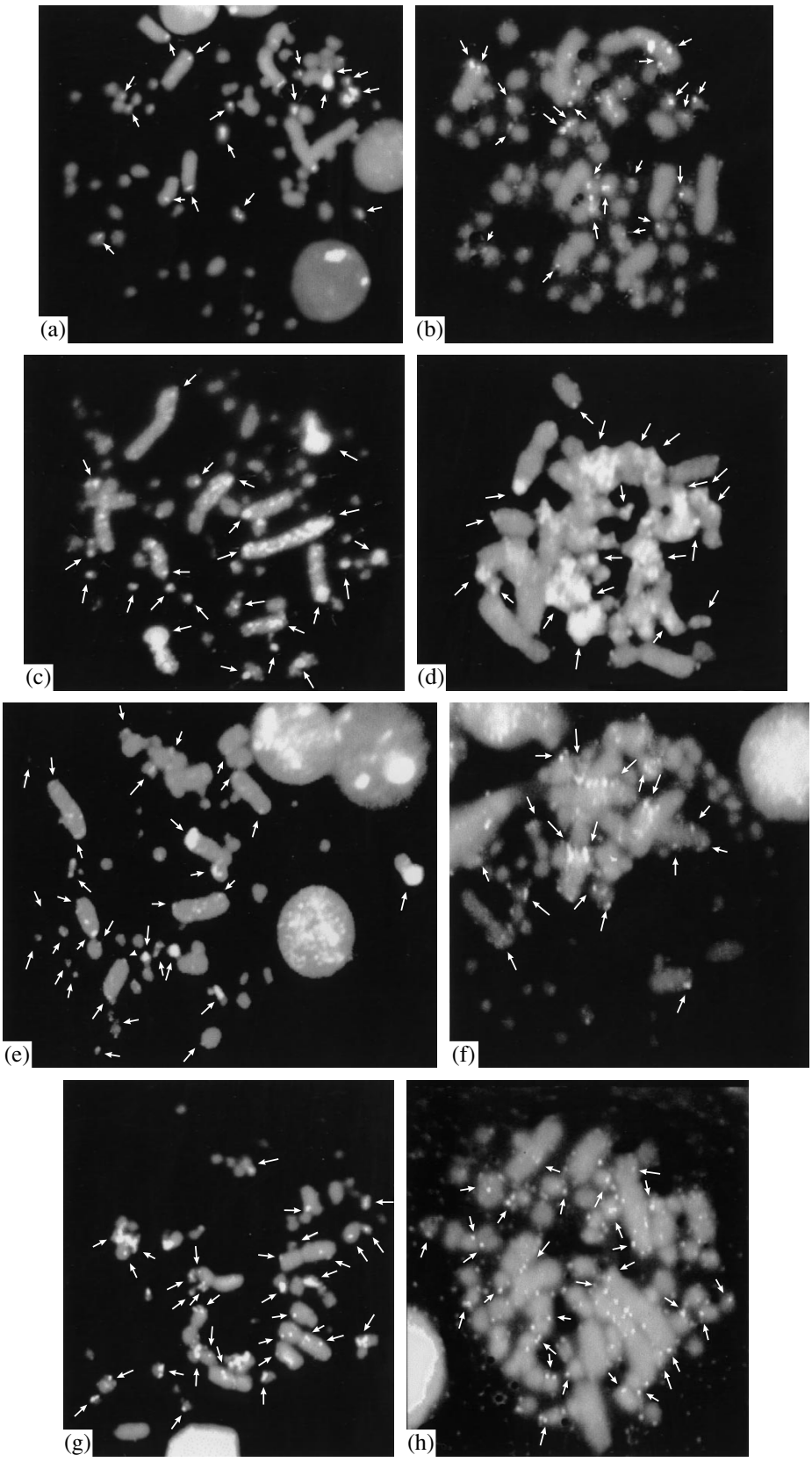

Fig. 1. Mitotic chromosomes of the domestic chicken (a, c, e, g) and Japanese quail (b, d, f, h) after in situ hybridization of their DNA with heavy fractions of chicken DNA: Fr 7 (a, b), Fr 6 (c, d), Fr 5 (e, f), and Fr 4 (g, h). Arrows mark the regions of specific hybridization.

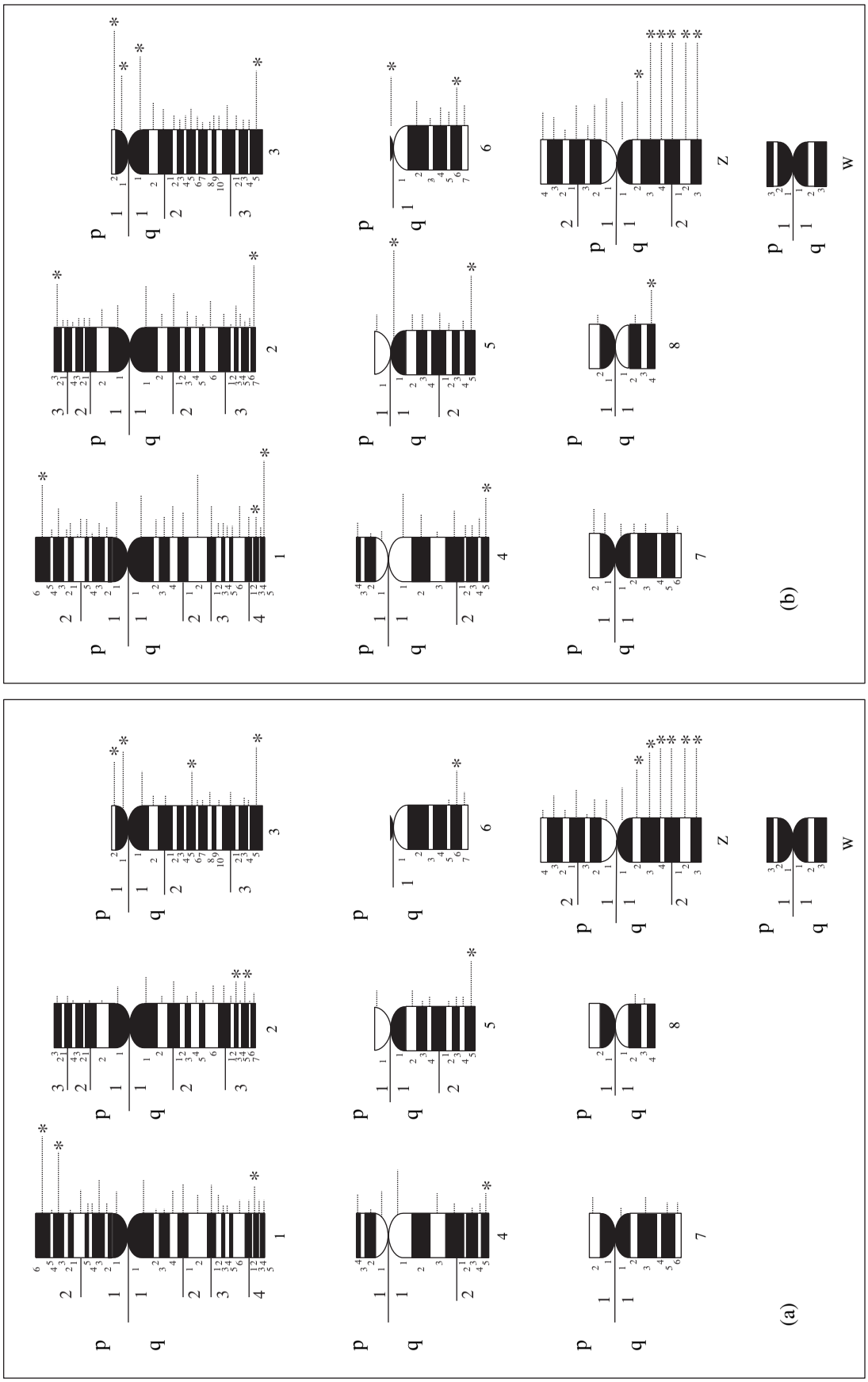

Fig. 2. A scheme of distribution of the biotinylated complexes along the chicken chromosomes after their DNA in situ hybridization with the isochore fractions Fr 6 (a) and Fr 5 (b). Asterisks mark the regions of significant specific hybridization ($P < 0.05$).

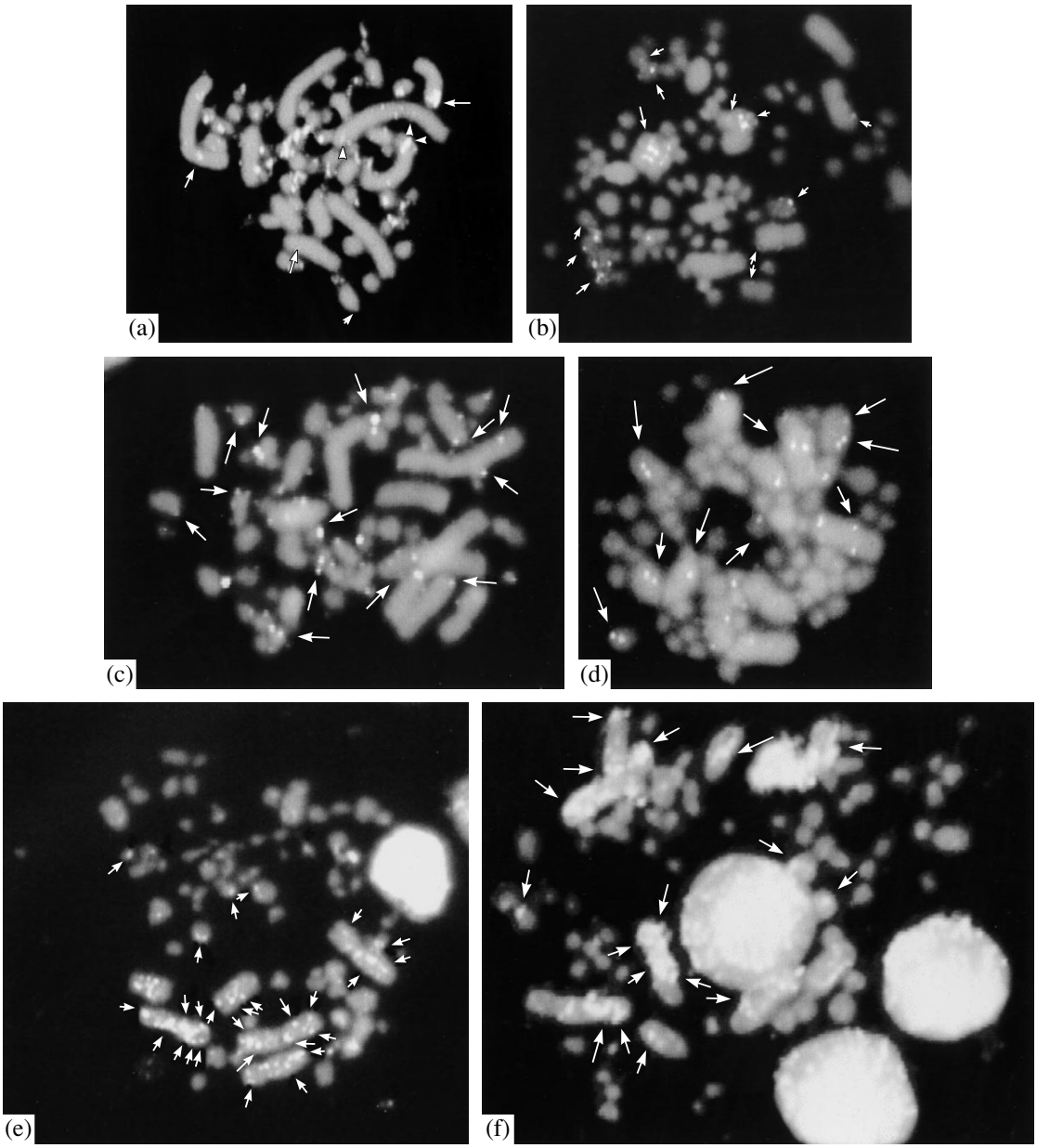

Fig. 3. Mitotic chromosomes of the domestic chicken (a, c, e) and Japanese quail (b, d, f) after in situ hybridization of their DNA with chicken DNA fractions: Fr 3 (a, b), Fr 2 (c, d), and Fr 1 (e, f). Arrows mark the regions of specific hybridization.

ylation of GC base pairs, and high histon acetylation [12, 13].

Using compositional mapping, we have shown that microchromosomes are rich with heavy isochores (Fig. 1) in both avian species studied, chicken and quail. This suggests that microchromosomes resemble T bands of mammalian chromosomes. In the context of this study, microchromosomes may be a specific class of T bands, which lacks light isochores of the L1 and L2 families (Fig. 3). These features of microchromosomes are similar to those of macrochromosome telomeric regions. Hence, our results confirm the hypothesis of the functional specialization of avian microchromosomes.

Note that the distributions of the lightest fraction (Fr1) and the heaviest one (Fr7) are opposite in both avian species (Figs. 1 and 3).

The heavy isochores provided a strong hybridization signal in the heterochromatic region of chicken Z chromosome (Fig. 1), which may be due to either the presence of the GC-rich satellite DNA sequences in the heavy isochore fractions [6] or insufficient suppression of nonspecific hybridization with repeated nucleotide sequences.

Note that the distribution of the isochore fractions is virtually identical in chicken and quail, which is most likely a result of close taxonomic positioning of these

species and similarity of their karyotypes [10]. The only distinction revealed, weak labeling of the heterochromatic region of Z chromosome in quail, is quantitative and may be explained by different composition of the satellite DNA in this region in the two avian species.

Of interest is the fact that a pattern of the DNA fraction distribution (Fr 3–Fr 6) along the macrochromosomes imitates differential R banding in both chicken and quail, i.e., the predominant location of these fractions in R bands provides an imperfect pattern of differential banding (Figs. 1, 3).

In the two avian species studied, macrochromosomes proved to be similar to the mammalian chromosomes with respect to heavy isochore location. The heaviest isochores were mostly detected in the telomeric regions, whereas the lighter ones were in positive RBG bands of the minor sites.

Although the mammalian and avian species significantly differ in their karyotype organization, the distribution of isochore families was essentially similar in the genomes of these two lineages of warm-blooded animals. The major feature of the avian genome organization is the presence of numerous microchromosomes (about 30% of the genome) resembling mammalian T bands, which exhibit highest functional activity. The avian microchromosomes contain the heaviest isochores, which also testify to a possible functional compartmentalization of the avian genome.

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