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Lampbrush Chromosomes of the Japanese Quail (*Coturnix coturnix japonica*): A New Version of Cytogenetic Maps

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Abstract—Avian oocyte chromosomes are transfomed into giant transcriptionally active lampbrush chromosomes (LBCs) at meiosis 1 diplotene. These chromosomes are a convenient tool for high-resulution cytogenetic analysis. Using differential staining with fluorochromes DAPI and CMA3, we have constructed detailed cytological maps for lampbrush macrochromosomes 1–5 and ZW of the Japanese quail *Coturnix coturnix japonica*. We also performed a comparative analysis of mitotic chromosomes and LBCs corresponding to them. We estimated the decondensation coefficient during LBC formation and determined the centromere indices for mitotic and diplotene chromosomes and thus found that different chromosomes and chromosomal regions demonstrate unequal degrees of decondensation.

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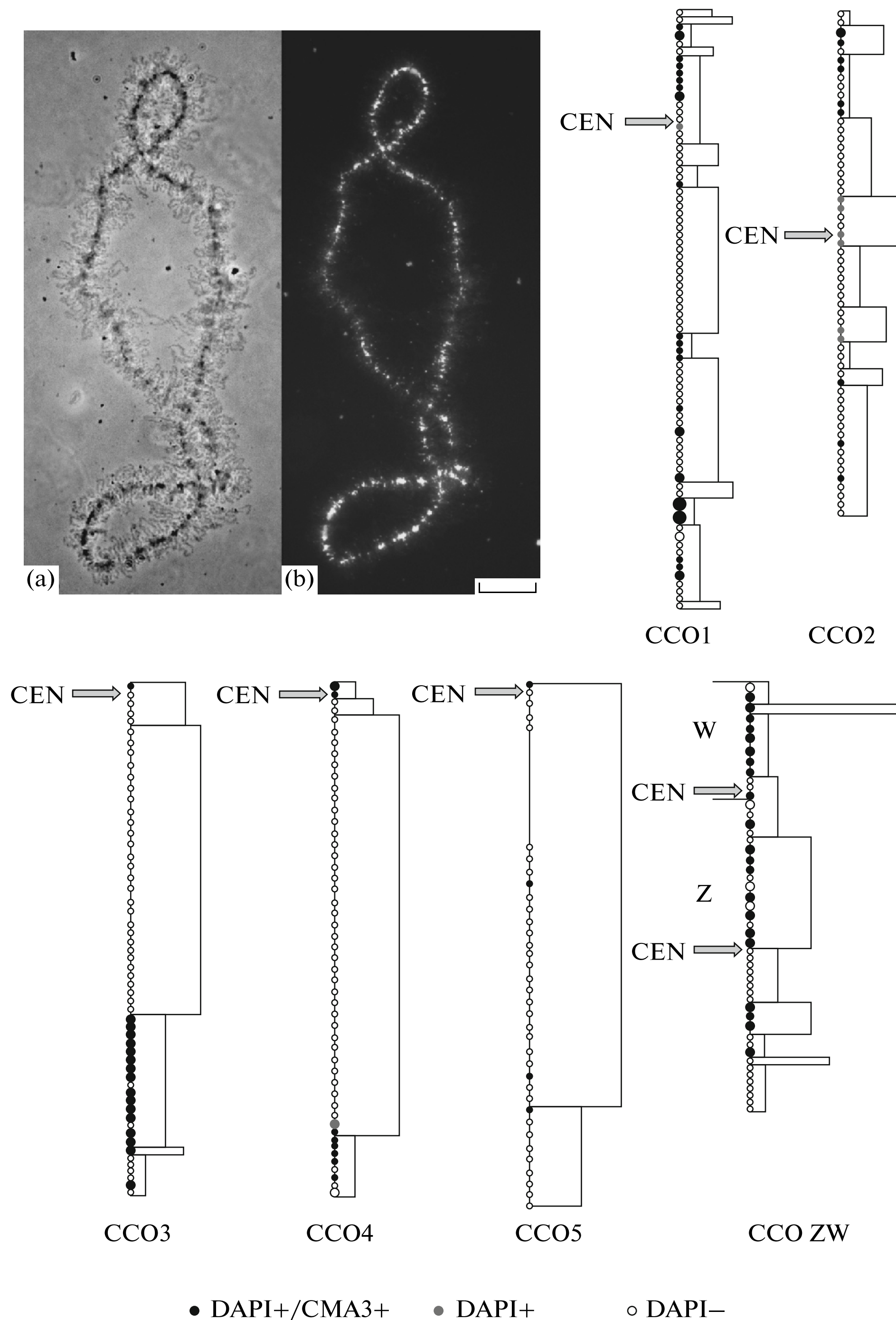
Japanese quail *Coturnix coturnix japonica* is a species steadily gaining popularity in connection with hypoallergenic meat and eggs. In addition, this species is actively used as a model object for studies in neurobiology, developmental biology, immunology, and biomedicine. Together with genetic mapping, an effective approach for studying genomes of different species is the transfer of data on the composition of syntenic linkage groups of the best studied objects onto the maps of poorly studied and most often closely related species of interest. The method providing such a possibility is fluorescence in situ hybridization (FISH), and the objects are commonly mitotic metaphase chromosomes. Domestic chicken (*Gallus gallus domesticus*) and Japanese quail belong to one family, Phasianidae, and have the same number of chromosomes ($2n = 78$), most of which are orthological [1]. The chicken genome and karyotype have been studied extensively [2–4], which permits fragments of the chicken genome to be used for analyzing chromosomes of other species. However, a small physical size of avian mitotic chromosomes (0.2–8 μm) impedes exact localization of sequences: both their mutual arrangement and the position relative to the centromeric regions.

As demonstrated in a number of studies, a promising tool for a high-resolution cytogenetic analysis are giant lampbrush chromosomes (LBCs). LBCs are diplotene bivalents that are highly decondensed due to active transcription in the nuclei of growing oocytes in numerous animals, including birds [5]. The use of LBCs allows one to avoid errors that occur because of a low resolution of the comparative cytogenetic analysis in the case of mitotic chromosomes [6].

The main problem in using LBCs for physical mapping is the lack of adequate cytological maps. Attempts to describe Japanese quail LBCs were undertaken earlier [7–9]. However, experience shows that the available maps are unfit for analyzing fluorochrome-stained chromosomes; moreover, they were constructed only for four largest macrochromosomes and the sex bivalent. We have developed a new version of maps for lampbrush macrochromosomes 1–5 and the ZW sex bivalent to identify quail LBCs and their regions during FISH on LBCs (Figure). To this end, we stained LBCs simultaneously with an AT-specific fluorochrome, DAPI, and a GC-specific fluorochrome, chromomycin A3 (CMA3). As applied to LBCs, this method reveales additional heterogeneity of staining along the chromosome length, which is an auxiliary marker when constructing cytogenetic maps of chromosomes: some chromomeres appear to be only DAPI-positive (enriched with AT pairs), and others are stained only with CMA3 (enriched with GC pairs), whereas a part of chromomeres are both DAPI- and CMA-positive. LBCs were microsurgically isolated from the nuclei of growing oocytes 0.5–1.5 mm in diameter following the protocol presented on the site http://projects.exeter.ac.uk/lampbrush/protocols.htm. The maps of quail DAPI/CMA3-stained LBCs 1–5 and ZW (Figure) show the number of chromomeres in each chromosome, the size and staining pattern of each chromomere, and the variation along the length of the lateral loops. The position of the centromere was mapped on the basis of immunocy-



Cytological maps of Japanese quail lampbrush macrochromosomes constructed on the basis of their individual chromomere–loop pattern. CCO1–5, chromosomes 1–5; CCO ZW, sex bivalent ZW. The centromere position is indicated by arrows. Square brackets reflect the average length of the lateral loops. Chromomeres intensively stained with DAPI and CMA3 are black; chromomeres stained only with DAPI are grey; chromomeres poorly stained with both dyes are white. (a, b) Microphotos of lampbrush chromosome 1: (a) phase contrast, (b) DAPI/CMA3 staining. Scale bar 10 μm.

tochemical LBC staining with K828 antibodies against the STAG2 protein of the cohesin complex [10].

We also performed comparative analysis of mitotic chromosomes and LBCs corresponding to them in the Japanese quail karyotype. Mitotic chromosomes were obtained from embryo fibroblasts according to the standard procedure. The method of heterologous FISH with chromosome-specific probes was used to exactly identify LBCs and their regions. As probes, we used chromosomal paints for chicken chromosomes 1–5 [4] and 35 Bac clones from the Wageningen chicken BAC library [2]. The sex bivalent is exactly

Absolute lengths and centromere indices of quail chromosomes at the stages of metaphase of mitosis and diplotene of meiosis (lampbrush stage)

Chromosome	Absolute length, l, μm		Degree of decondensation, $l_{LB}/l_{metaphase}$	Relative length		Centromere index, $p/l \times 100\%$	
	metaphase	LB		metaphase	LB	metaphase	LB
1	8.7 ± 0.54	109.8 ± 8.92	12.6	1	1	27.6 ± 0.21	21.1 ± 0.75
2	6.9 ± 0.45	87.4 ± 8.19	12.8	0.79	0.80	40.0 ± 0.20	48.7 ± 2.22
3	5.6 ± 0.38	91.5 ± 6.89	16.3	0.64	0.83	5.3 ± 0.20	1.0 ± 0.09
4	4.8 ± 0.27	88.6 ± 4.48	18.5	0.55	0.80	10.7 ± 0.37	1.4 ± 0.14
5	3.3 ± 0.21	92.7 ± 11.88	28.1	0.38	0.84	4.7 ± 0.25	1.4 ± 0.18
Z	4.8 ± 0.29	55.9 ± 8.94	11.6	0.55	0.5	49.3 ± 0.16	42.7 ± 2.33
W	2.6 ± 0.13	14.1 ± 1.69	5.4	0.30	0.13	5.7 ± 0.28	5.9 ± 0.86

identified by appearance: the only chiasma connecting chromosomes Z and W confers the univalent form to this LBC.

Using programs VideoTesT-Karyo-1.3 and UTH-SCSA Image Tool 3.0, we measured the absolute and relative lengths of Japanese quail mitotic and lampbrush chromosomes and determined their centromere indices (table). The relative lengths of the mitotic and lampbrush chromosomes were found to differ: chromosomes 3, 4, and 5 in the metaphase of mitosis are 0.64, 0.55, and 0.38, respectively, relative to the length of chromosome 1. At the LB stage, the lengths of these chromosomes constitute approximately equal proportions of the length of chromosome 1. By calculating the ratios of the lengths of LBCs to the length of corresponding mitotic chromosomes it is possible to estimate the coefficient of decondensation at the LB stage. Different chromosomes demonstrate unequal levels of decondensation. In particular, LBC 5 is approximately 2.5 times more decondensed than chromosomes 1, 2, and Z, and the W chromosome containing a large amount of heterochromatin is extended only fivefold in the LB state.

In many cases, the centromere indices of quail chromosomes observed in the metaphase of mitosis are not preserved in the LB state, suggesting unequal decondensation of their short and long arms. The most illustrative examples are chromosome 2 (from subcentromeric this chromosome becomes metacentric) and chromosome 4 (submetacentric ⟶ acrocentric). Note that the short heterochromatic arms in chromosomes 3, 4, and 5 constitute only 1–1.5% of the LBC length and correspond to the terminal DAPI+/CMA+ chromomere, while the highly heterochromatinized W chromosome retains its proportions.

It was already found in a number of works that the degree of decondensation of chromosomes in meiosis can vary [6, 11]. We suppose that it is heterochromatic regions that remain to be condensed in the LB state, thus determining unequal degrees of chromosome decondensation.

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