Russian Journal of Genetics, Vol. 38, No. 9, 2002, pp. 1054–1059. Translated from Genetika, Vol. 38, No. 9, 2002, pp. 1246–1251.

ANIMAL GENETICS

Lampbrush Chromosomes in the Japanese Quail *Coturnix coturnix japonica*: Cytological Map of Macrochromosomes and Meiotic Crossing-over Frequency in Females

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Received September 25, 2001

Abstract—Cytological map of lampbrush macrobivalents of the Japanese quail (*Coturnix coturnix japonica*) were constructed. Investigation of chiasmata allowed to estimate the frequency of reciprocal genetic recombination (crossing over) in Japanese quail female meiosis. The total chiasma number in bivalents of Japanese quail oocyte nuclei was determined to be 53–58. Macrobivalents 1–5 and Z of the Japanese quail had on average 3.3 chiasmata per bivalent, and microbivalents, 1.0–1.1 chiasmata per bivalent. The chiasmata (crossover) frequency in Japanese quail females was lower than in chicken. In macrochromosomes of Japanese quail females, one crossover occurred per 43.9 Mb, and in chicken, per 30.0 Mb. Judging from chiasma frequency, the genetic length of the Japanese quail genome is likely to be 2650–2900 cM. Crossover frequency in the species was 0.023 per Mb in macrobivalents and 0.07–0.08 Mb in microbivalents and for the total genome, 0.041 crossing over per Mb. The genetic length of one Mb (recombination rate θ) in female Japanese quails was 1.14 cM in macrochromosomes, 3.60–4.12 cM in microchromosomes, and about 1.96–2.15 cM averaged over the genome.

The Japanese quail (*Coturnix coturnix japonica*) is the first bird species whose lampbrush chromosomes (LBCs) were investigated by modern methods of light and electron microscopy [1–3]. These and later studies of LBCs of the quail and other birds showed that LBCs can be a model for investigation of chromosome organization by high-resolution FISH [4–7], physical mapping of cloned sequences [8–13], investigation of transcription during oogenesis [1, 5, 7–9, 14–16], investigation of the frequency of meiotic genetic recombination (crossing over) in birds, and determination of genetic linkage between chromosomal markers and physically mapped loci [13, 17–19]. Such studies require cytological map of LBCs which would present loop and chromomere patterns typical of each bivalent and an elaborated notation of chromosome markers. Use of such maps allows rapid identification of bivalents in a cytological slide and description of FISH signals and chiasmata (reciprocal genetic recombination sites) along a chromosome [10, 12, 13, 18–21]. By now, the chicken is the only higher vertebrate for which cytological LBC maps have been constructed [20]. In the present work, we have constructed maps of four lampbrush macroautosomes and the sex bivalent of the Japanese quail and calculated the meiotic recombination frequency in Japanese quail females.

MATERIALS AND METHODS

Lampbrush chromosomes of *C. c. japonica* were isolated from oocytes by the microsurgical procedure elaborated by Gaginskaya *et al.* [2] and Hutchison *et al.* [21, 22]. Nuclei were isolated from oocytes of 0.5–2.5 mm in diameter in a medium containing 83 mM KCl, 17 mM NaCl, 6.5 mM Na_2HPO_4, 3.5 mM KH_2PO_4, pH 7.0–7.4; purified from cytoplasm; and placed into a chamber for chromosome dispersion filled with the medium for nucleus isolation diluted by a factor of 1.33 and supplemented with paraformaldehyde to the final concentration of 0.1%. For convenience of in situ hybridization of nucleic acids on LBCs, chromosomes were isolated from nuclei in multiwell chambers (Fig. 1) glued to an object slide with a paraffin-vaseline mixture [23] or caoutchouc cement [10].

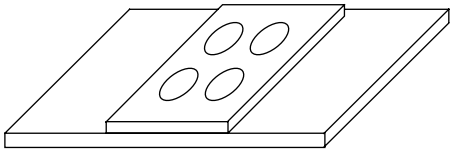

Fig. 1. Multiwell chamber for isolation of avian lampbrush chromosomes.

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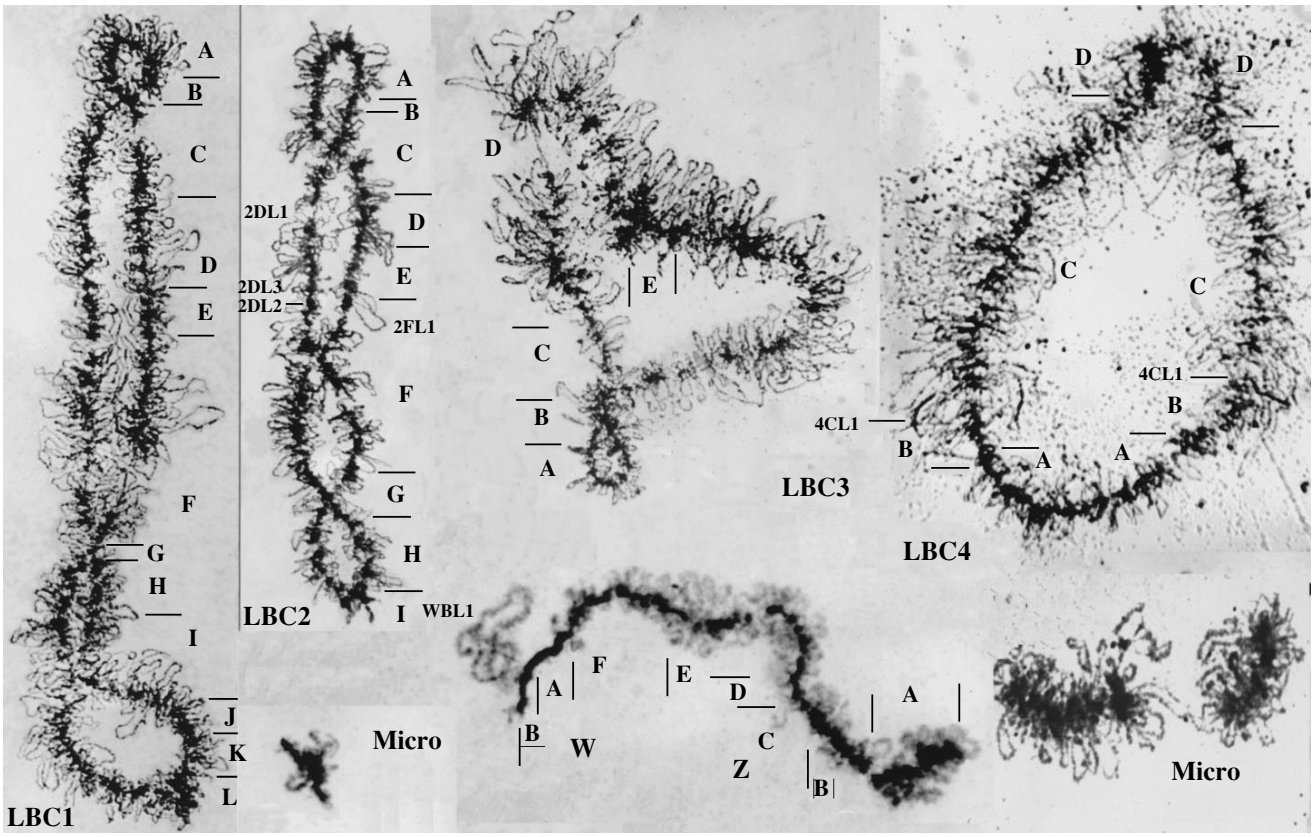

Fig. 2. Lampbrush chromosomes of the Japanese quail stained with Coomassie R-250. For marker description, see text.

Oocyte nuclei were disrupted with tungsten needles onto the object slide and lampbrush chromosomes were sedimented by 10–15 min centrifugation at 700–1000 g. The slide was fixed with 2% glutaric aldehyde (Merck) for 2–5 min, washed with alcohol solutions (70–96–96%) and stained with Coomassie R250 (Merck) or fluorescent dye DAPI (Serva). Chromosome measures were taken from ×1000 photo images using a curvimeter. Lengths of double bridges (loops extended at the base) were not taken into account when measuring the lengths of bivalent axes and coordinates of marker structures.

RESULTS AND DISCUSSIONS

Lampbrush chromosomes isolated from Japanese quail oocytes of 0.75–1.5 mm in diameter are shown in Fig. 2. Bivalents differ in length, morphology, and distribution of lateral loops along chromosomes.

Bivalent 1. In the pattern of distribution of chromosome domains with different modal lengths of lateral loops, bivalent 1 of the Japanese quail is very similar to that of the chicken. The chiasma frequency in the bivalent is 6.3 ± 0.94. We subdivided the bivalent into 12 domains (domains A–L, Figs. 2 and 3). Domain 1A starts with a chromomere whose short loops are usually coiled. This chromomere and the proximally adjoining area is a crossover hot spot: bivalent 1 of the quail is "locked" there with a terminal chiasma in 90% of samples. The terminal chromomere (1A1) is adjacent to 4–5 chromomeres with relatively long loops: 1AL2–1AL5/1AL6. The next two chromomeres (1BL1 and 1BL2) with coiled loops form domain 1B. The next domain, 1C, consists of 7–9 chromomeres, usually adjacent, with loops of medium length. The distal boundary of the domain is loop 1CL1. Domain 1D is a cluster of 5–6 chromomeres with relatively long loops. We designated the first of them as 1DL1. Domain E (the conjectured location of the centromere) consists of chromomeres intensely Coomassie-stained. Loops of these chromomeres are notably shorter than in the neighboring domains of chromosome 1 in the Japanese quail. Domain 1F consists of two subdomains: 1F1 and 1F2. Subdomain 1F1 contains four chromomeres with loops of medium length, and 1F2 contains four to five chromomeres with long loops. Subdomain 1F2 is adjacent to a loopless cluster of chromomeres intensely Coomassie-stained. The last of them sometimes bears a small but intensely stained pair of loops (Domain 1G). The next domain is 1H. Its five to seven chromomeres have loops of medium length. Domain 1I consists of seven to nine chromomeres with relatively long loops. The distant part of this domain often contains an intensely stained chromomere with coiled loops. Domain 1J is a cluster of loopless chromomeres, most

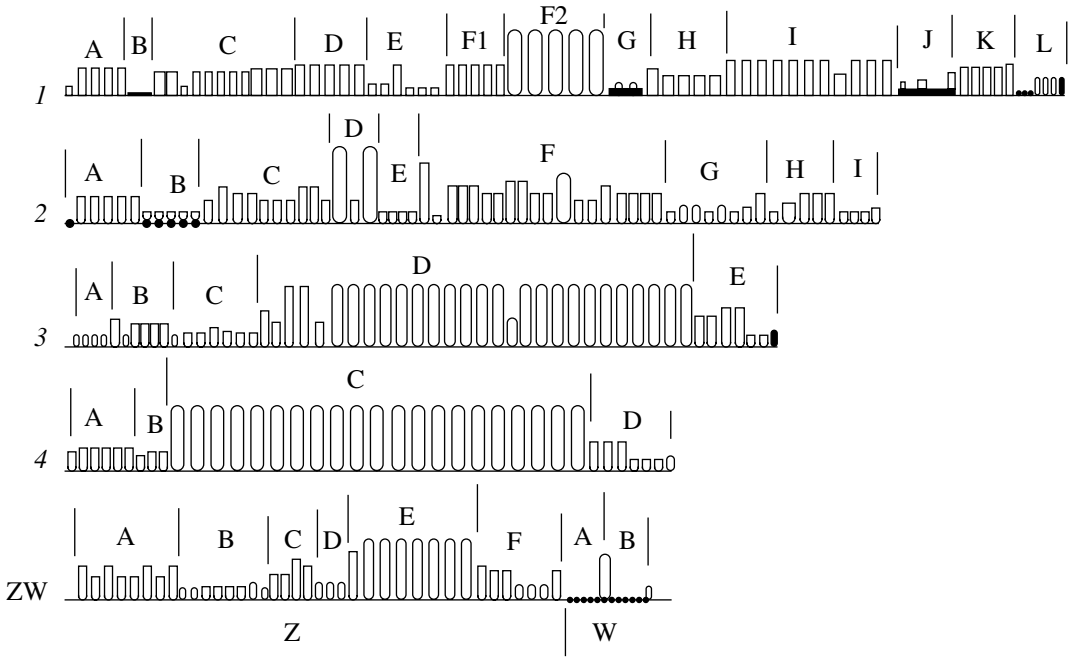

Fig. 3. Maps of macrobivalents of the Japanese quail.

clearly observed in bivalents from large oocytes. Domain 1K is a small cluster of looped chromomeres. It is adjacent to subterminal domain 1L, whose proximal part lacks loops and several subterminal chromomeres have short lateral loops.

Bivalent 2. The pattern of lateral loops in bivalent 2 of the Japanese quail is diverse and shows distinct chromosome domains. A characteristic marker of bivalent 2 of the quail is two long loops of domain D (2DL1 and 2DL3). Bivalent 2 on average contains 4.80 ± 0.7 chiasmata. According to the centromere index, the centromere is supposed to occur in domain 2E. Long loop 2FL1 and small loop 2FL2 with dark matrix occur at the boundary of the subcentromere region. Unlike bivalent 2 of the chicken, light microscopy does not reveal the specific spherical protein body (spaghetti marker) (see [6]) in the subtelomere region (2B) of the long arm of bivalent 2 in the quail.

Bivalent 3 of the Japanese quail is undoubtedly homeologous to bivalent 3 of the chick [20]. It is subdivided, in the same manner, into two parts: short-looped (domains 3A–3C) and long-looped (3D). Bivalent 3 of the chick contained on average 3.1 ± 0.67 chiasmata.

Bivalent 4 of the Japanese quail is subdivided into four domains, of which 4A, 4B, and 4D have relatively short loops, and 4C has long loops, the first of which (4CL1) is distinguished by dark matrix. The bivalent contains on average 2.8 ± 0.38 chiasmata.

Sex bivalent ZW of the quail is readily identifiable. If differs from that of the chicken by a longer W chromosome. This bivalent was formerly described in detail by Solovei *et al.* [21]. Loop WBL1 on chromosome W is prominent in size.

The experimentally confirmed correspondence of the number and locations of chiasmata to the number and locations of reciprocal genetic exchange events (crossovers) (see [24] for review) is the basic principle of cytogenetic analysis of genetic recombination. Application of this principle to data on chiasma location on bivalents allows estimation of the frequency reciprocal exchanges, investigation of genetic interference, and construction of maps of genetic linkage of chromosomal markers [17–19, 24–28]. To determine crossover frequency in oogenesis in *C. c. japonica* and calculate the crossover-based genetic length of the quail macrochromosome linkage groups in quail macrochromosomes, we calculated the chiasma frequencies in each of lampbrush macrobivalents and on microbivalents (Table 1). Note that microbivalents are poorly preserved on slides. For this reason, exact calculation of chiasmata in microbivalents is difficult. Those we identified contained one or two chiasmata, with one chiasma observed in the overwhelming majority of cases. Comparison of the data on chiasma frequency in quail oogenesis with the corresponding data formerly obtained for the chicken [24] and turkey [29] shows that crossover frequency in the quail is lower than in any of the *Galliformes* investigated (Table 1).

General recombination characteristics of the genomes of chicken, quails, and mammals can be estimated from chiasma frequencies in meiosis (Table 2). Generally, crossover frequency in meiosis in the quail is lower than in the chicken. In the quail genome, one crossover occurs per 23.3–25.4 Mb: in macrochromo-

Table 1. Chiasma frequency and linkage group lengths in females of three species of order Galliformes

Chromosome	Chiasma frequency $x \pm s_x$			Linkage group length, cM $x \pm s_x$		
	Coturnix c. japonica	*Meleagris gallopavo**	*Gallus g. domesticus*	*Coturnix c. japonica*	*Meleagris gallopavo**	*Gallus g. domesticus*
1	6.3 ± 0.94	8.0 ± 0.32	7.7 ± 0.11	313 ± 47.2	405 ± 17.5	386 ± 5.3
2	4.8 ± 0.7		6.1 ± 0.09	238 ± 34.9		304 ± 4.3
3	3.1 ± 0.67	4.0 ± 0.26	4.5 ± 0.09	154 ± 33.4	200 ± 13.0	227 ± 4.7
4	2.8 ± 0.38	2.4 ± 0.26	3.9 ± 0.13	143 ± 18.9	120 ± 13.0	195 ± 6.5
5	~2	~2	2.9 ± 0.11	~100	~100	144 ± 5.5
2q		3.0 ± 0.26			150 ± 13.0	
ZW bivalent	1.0 ± 0.0	1.0 ± 0.0	1.0 ± 0.0	50	50	50
Macro 1–5	19	19.5	25.1	948	975	1256
Micro 6–10	~10	~10	~10	~500	~500	~500
Total per oocyte	53–58	54–59	59–64	2650–2900	2700–2950	2950–3200

* After [29].

Table 2. Recombination frequencies in meiosis in the quail, chick, and man

	Coturnix coturnix japonica, females			*Gallus gallus domesticus*, females			*Homo sapiens*, males
	macro	micro	total	macro	micro	total	total
Chromosome number (*n*)	5 + Z	33 + W	39	5 + Z	33 + W	39	22 + X(Y)
1C DNA amount (pg)	0.91	0.49	1.4*	0.81	0.44	1.25	3.5*
DNA amount, Mb	878.15	472.85	1351	784.06	422.19	1206.25	3377.5
DNA per chromosome, Mb	146.4	13.9	34.6	130.7	12.4	30.9	146.8
Chiasmata per nucleus	20.0	34–39	53–58	26.1	34–39	59–64	52.33**
Chiasmata per chromosome	3.3	1.0–1.1	1.6	4.35	1.0–1.1	1.6	2.3
Length of genetic maps, cM	1000	1700–1950	2650–2900	1305	1700–1950	2950–3200	2616.5
Crossover per 1 Mb	0.023	0.07–0.08	0.041	0.033	0.07–0.08	0.051	0.015
Genetic length (cM) of 1 Mb	1.14	3.60–4.12	1.96–2.15	1.66	4.03–4.62	2.44–2.65	0.77

* After [36]; **after [25, 30].

somes, per 43.9 Mb, and in microchromosomes, per 12.1–14.0 Mb. In the chicken genome, one crossover occurs per 18.8–20.4 Mb: in macrochromosomes, per 30.0 Mb, and in microchromosomes, per 10.8–12.4 Mb.

In meiosis of human males, one crossover occurs per 64.5 Mb [30]. Apparently, high crossover frequency is a common feature of microchromosomes in all species [31]. We know two factors which may determine the high recombination rate in avian microchromosomes: GC abundance in microchromosomal euchromatin [32–34] and the presence of recombination hot spots [18, 35].

To sum up, note that in previtellogenesis, LBCs are approximately 30 times as long as the corresponding chromosomes at the metaphase stage of mitosis [20], whereas the number of structures recognized there (loops and chromomeres), allowing FISH mapping of genes and other sequences, exceeds the number of G and RBA bands in so-called high-resolution differential banding of prometaphase mitotic chromosomes [12, 13, 19, 34]. Thus, we can regard the maps of avian LBCs constructed by us ([20] and the present communication]) as superhigh-resolution cytological (physical) maps of avian chromosomes.

This study was supported by the Russian Foundation for Basic Research, project no. 00-04-49327.

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