ANIMAL
GENETICS

Crossing Over in Chicken (*Gallus gallus domesticus*) Oogenesis: Periodic Arrangement of Chiasmata over Chromosomes

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Abstract—The periodic occurrence of chiasmata was studied in lampbrush chromosomes of the chicken (*Gallus gallus domesticus*). It was shown that the most probable interference distance in chicken macrobivalents 1–3 corresponded to 24.48 Mb. The distance at which absolute interference is observed in chicken macrochromosomes varies from 5.75 to 9.02 Mb.

INTRODUCTION

According to Mather's hypothesis [1], chiasmata are regularly distributed over a chromosome, starting from the centromere. The first chiasma appears at a certain distance from the centromere (differential distance), which correlates with the chromosome arm length. Subsequent chiasmata form so that the mean distances between them (interference distances) are uniform for all chromosomes of a species.

Interference distance can be evaluated by studying the distribution of homological recombination events (chiasmata) [2, 3], recombination nodules [4, 5], and foci of the MLH1 protein (responsible for repair on unpaired bases) related to late recombination nodules [6, 7]. In particular, the distribution of MLH1 foci over the length of the synaptonemal complexes (SCs) of oocyte chromosomes in the chicken *Gallus gallus domesticus* shows that the modal interference distance equals 30–70% of the SC length at least in chromosomes 6–13 [7].

Formerly, we showed that chicken lampbrush chromosomes (LBCs) were a convenient model for studying crossing over in bird oogenesis [8–11]. The number and location of chiasmata can be determined quite precisely owing to the size of each macrobivalent and abundance of markers. This model was applied to the analysis of the periodic distribution of chiasmata over of lampbrush bivalents 1–3 in *Gallus gallus domesticus*. We showed the presence of the most probable (interference) distance between chiasmata, corresponding to ca. 24.48 Mb. The distance at which absolute interference in chicken macrochromosomes was observed was ca. 5.75–9.02 Mb.

MATERIALS AND METHODS

Experiments were performed with domestic chicken (*Gallus gallus domesticus*), commercial hybrid ISA brown. Permanent lampbrush chromosome preparations were obtained by the Kropotova and Gaginskaya method [12] with minor modifications as in [11]. Chromosomes and chromosome regions were identified with reference to cytologic maps of chicken lampbrush chromosomes [11, 13]. Chiasmata are generally easily recognized in well-spread lampbrush chromosomes, because homolog axes of neighboring chiasmata are twisted out of line because of torsion tension [9]. In this work, we studied the distribution of chiasmata over 100 bivalents 1, 100 bivalents 2, and 57 bivalents 3 of the chicken. A total of 1883 chiasmata were recorded on chromosomes 1–3. The periodic distribution of chiasma frequency along bivalents 1–3 was studied by spectroscopic methods [14]. The data were processed with the use of the STATISTICA 5.0 software package.

RESULTS

Plots of chiasma distribution on chromosomes subdivided into 2 μm intervals are shown in Figs. 1a, 1c. The distribution of interstitial chiasmata has an oscillating pattern. The results of processing of the periodic arrangement of chiasmata over chromosomes with STATISTICA 5.0 are presented in Figs. 2–4 as periodograms, where the period length is plotted on the X axis, and the Y axis shows the intensity (*I*), meaning the sum of squared *a* and *b*, determined as follows:

$$a(\alpha) = 1/\sqrt{n\pi} \sum_{t=1}^{n} u_t \cos \alpha t,$$
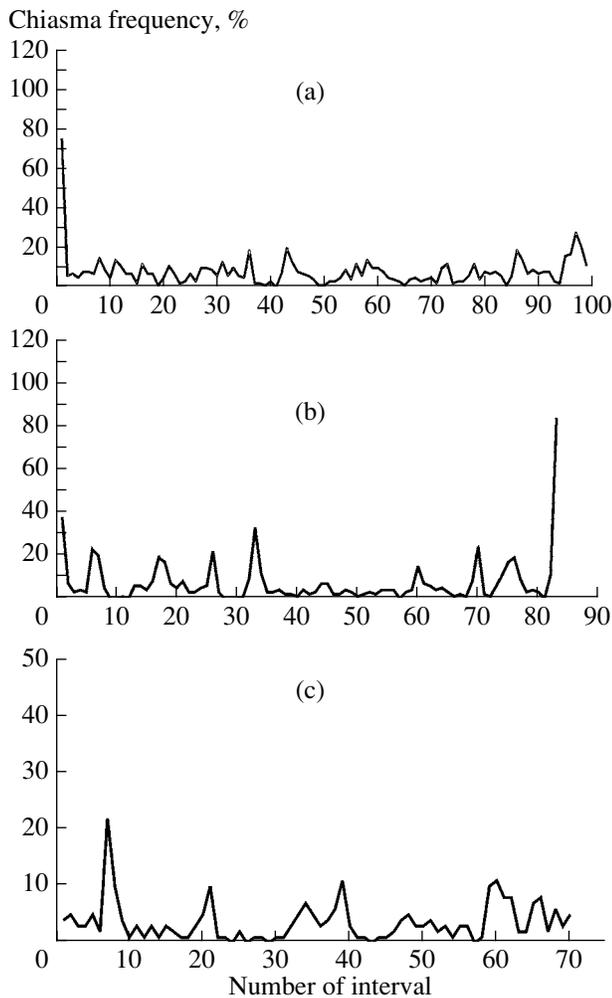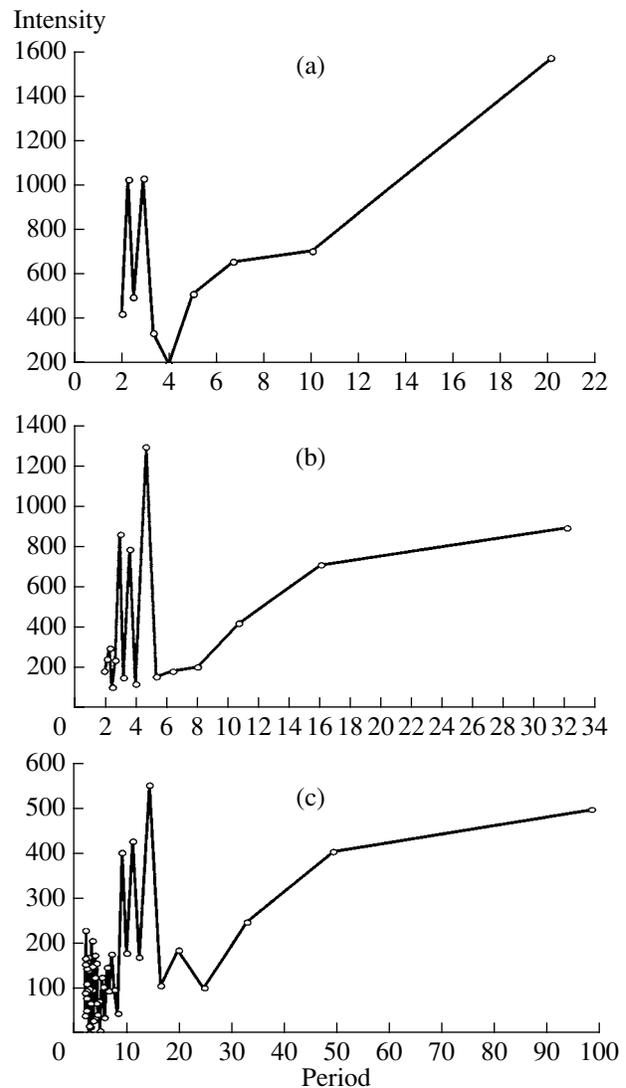
Chiasma frequency, %

Fig. 1. Chiasma distribution over lampbrush chromosomes:
(a) 1; (b) 2; and (c) 3.

$$b(\alpha) \;=\; 1/\sqrt{n\pi} \sum_{t=1}^{n} u_t \sin \alpha t,$$

where u_t is the series under study; α, harmonic variation frequency; t, period.

The functions producing a and b are covariances of the data series under study with $\cos\alpha t$ or $\sin\alpha t$. It is expected that, if the series under consideration, u_t, contains a harmonic component with the period $1/\alpha$, the intensity I should be relatively high. Thus, I evaluates the degree of correlation of the parameters of the series with all existing harmonic variations. The program detects this correlation between our data and the harmonics it generates.

During scanning the spectrum, the intensity remains low until the period corresponds to a true periodic component of the series at which the intensity shows a high peak. The set of such peaks should determine the set of periodic components.

Intensity

Fig. 2. Periodogram of the distribution of chiasma frequency over lampbrush chromosome 1 for intervals of (a) 12 μm, (b) 6 μm, and (c) 2 μm.

The periodogram of the distribution of chiasma frequency at 12 μm intervals for chromosome 1 shows three major peaks, corresponding to three cycles with periods 26, 30, and 240 μm (Fig. 2a). The last value corresponds to the regular variations of the length corresponding to the whole chromosome; therefore, it is of no interest. To find regular variations with smaller periods, we performed spectrum analysis of chiasma distribution over smaller intervals. The periodograms of spectra with 6 μm (Fig. 2b) and 2 μm (Fig. 2c) intervals showed four major peaks each, including that corresponding to the cycle covering the whole chromosome. However, in both cases peaks corresponding to the period 27 μm (for chiasma frequency distribution at 6 μm intervals; Fig. 2b) and to 28 μm (for distribution at 2 μm intervals; Fig. 2c) are prominent. The intensity reaches its maximum ($I = 1300$) at the period length
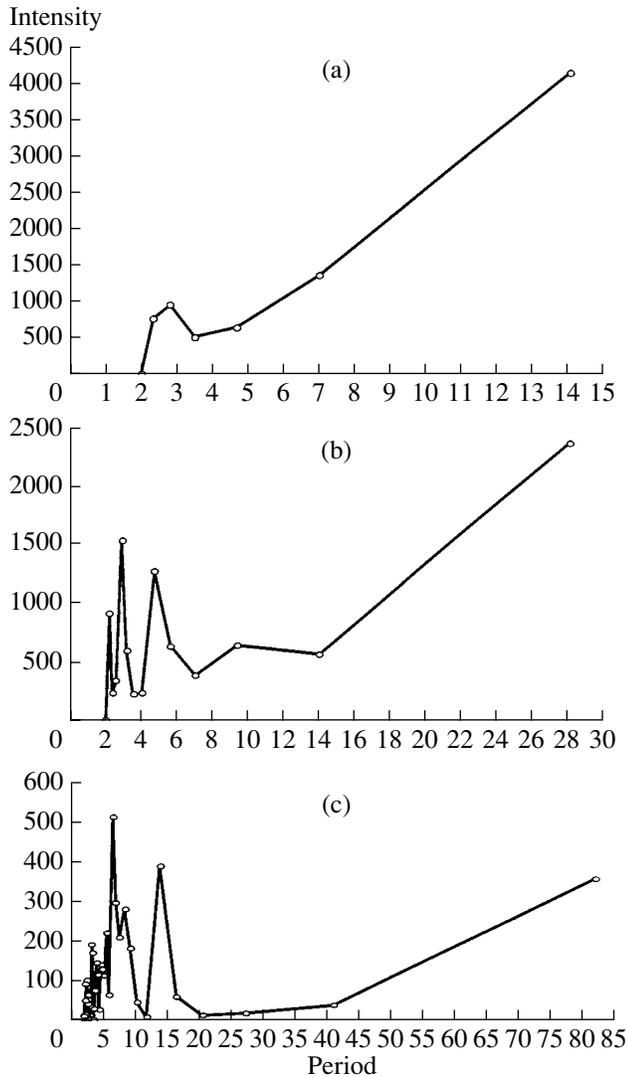
Fig. 3. Periodogram of the distribution of chiasma frequency over lampbrush chromosome 2 for (a) intervals of 12, (b) 6, and (c) 2 μm.

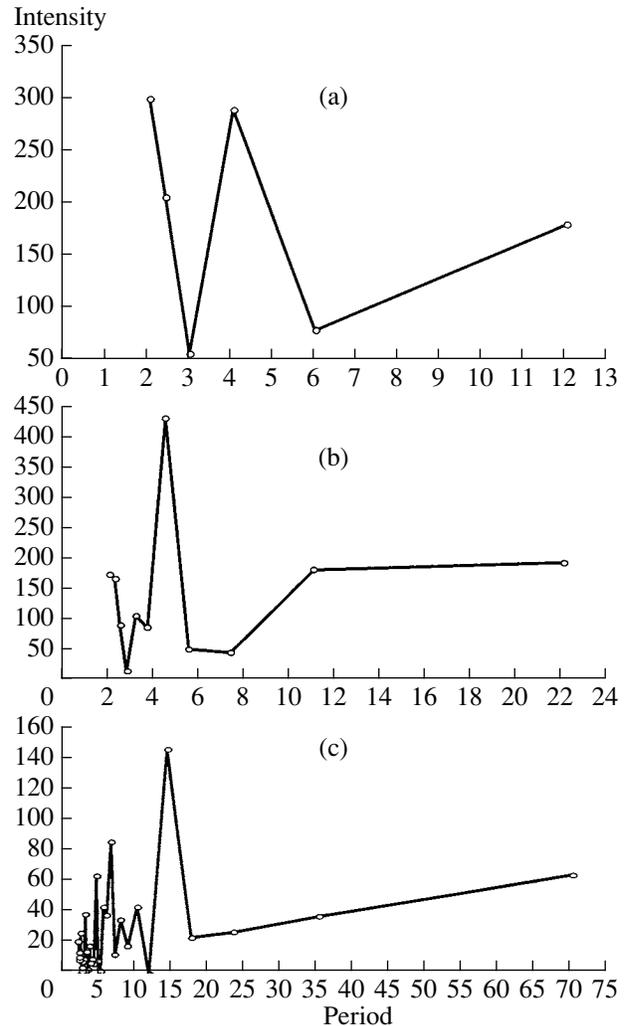
Fig. 4. Periodogram of the distribution of chiasma frequency over lampbrush chromosome 3 for (a) intervals of 12, (b) 6, and (c) 2 μm.

27 μm, with the chromosome split into 6 μm intervals (Fig. 2b). Thus, we conclude that regions with high and low probabilities of chiasma formation at the period 27 μm alternate on lampbrush chromosome 1.

Similar regularities are observed on chromosome 3 (Figs. 4a, 4c). It shows regular variations of chiasma frequency with period lengths 24, 48, and 144 μm. With intervals 6 and 2 μm (Figs. 4b, 4c), regular variations with periods 27 and 28 μm are observed. The maximum intensity was observed for the period 27 μm ($I = 425$ with 6 μm intervals).

Chromosome 2 was also subdivided into 12, 6, and 2 μm intervals. The corresponding periodograms are shown in Figs. 3a, 3c. Two major peaks corresponding to two cycles with periods 33 and 168 μm are observed with 12 μm intervals (Fig. 3a). The periodograms of chiasma frequency distribution with 6 μm (Fig. 3b) and

2 μm (Fig. 3c) intervals are more complicated. They show two major regular variations with periods 15 ($I = 1510$) and 28 ($I = 1250$) or 14 ($I = 530$) and 28 μm ($I = 400$) with intervals 6 and 2 μm, respectively. The maximum intensity is observed with the smaller period. However, taking into account the data on chromosomes 1 and 3 (period length 27 μm) and the facts that the distance between the peaks equals the period length and their intensities differ insignificantly, we suggest regular variations with a period 27.5 ± 0.55 μm. The accessory peak, corresponding to a 14–15 μm period can be attributed to the so-called echo effect, which assumes that a series containing a major periodic variation with period t includes periodic terms with periods $1/2t$, $1/3t$, etc. [15].

Thus, we discovered regular chiasma frequency variations with periods about 27.5 μm in all the three chromosomes examined.
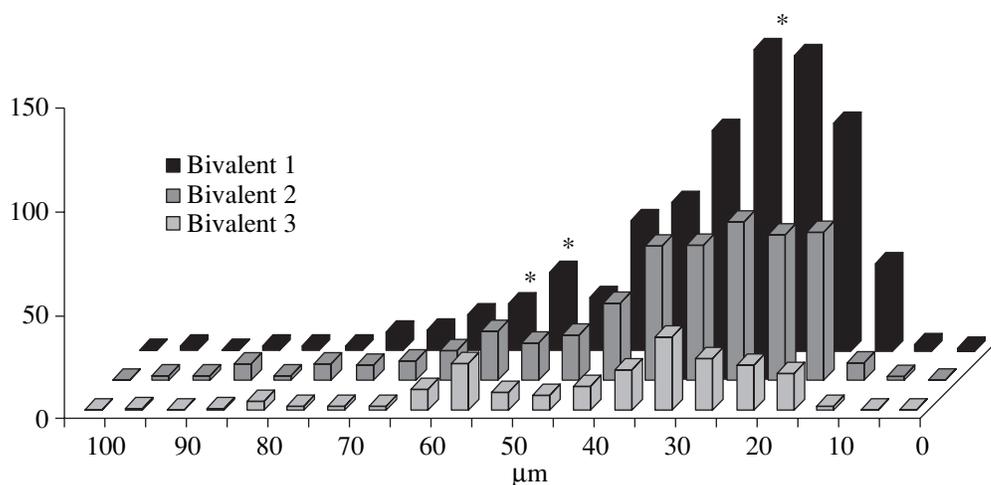
Fig. 5. Distribution of interchiasmal distances over lampbrush chromosomes of chickens *Gallus gallus domesticus*. Interchiasmal distances are plotted over the X axis; chiasma frequency, over the Y axis.

Interchiasmal Distances in Chicken Macrochromosomes 1–3 and the Absolute Interference Value

To determine the minimal, average, and most probable (modal) distance between chiasmata, we analyzed variation of distances between chiasmata in individual bivalents 1–3. The bar graph presented in Fig. 5 shows that the modal distance between chiasmata is 26–30 µm. The mean distance between neighboring crossings in female chicken macrochromosomes 1–3 was calculated to be 30.33 ± 1.28 µm.

The minimal distance between chiasmata (ix_{min}) characterizes the level of absolute interference, the distance at which a single crossover event suppresses all crossovers in the chromosome [16, 17]. In our experiments, the minimal distance between chiasmata (ix_{min}) on chromosome 1 was 6 µm; on chromosome 2, 10 µm; and on chromosome 3, 9 µm.

DISCUSSION

The material of a meiotic chromosome has several levels of packing [17]. Data on the size of chicken macrochromosomes obtained by genome sequencing (for chromosome lengths, see table) together with our cytological data on mean sizes of chicken lampbrush chromosomes demonstrate that one micrometer of a chicken LBC corresponds to 0.89 Mb. The lengths of absolute genetic interference regions in chicken macrochromosomes are: 6 × 0.959 = 5.75 Mb for chromosome 10 × 0.902 = 9.02 Mb for chromosome 2, and 9 × 0.779 = 7.01 Mb for chromosome 3. These data are in good agreement with absolute interference distance calculations made for meiotic chromosomes of male mice [19].

Our study of the periodic arrangement of chiasmata over chicken bivalents 1–3 showed that the most prob-

able distance between crossovers (chiasmata) in these chromosomes averaged 24.48 (27.5 ± 0.89) Mb. This corresponds to the interval between chromosome regions where conditions for formation of late recombination nodules exist, remain, or appear de novo, which seems to correspond to the time of appearance of crossover-related hemichiasmata [20].

Hypotheses concerning the causes of the periodic chiasma arrangement can be combined into two groups. The first group assumes that sequences with elevated recombination frequencies occur in chicken genomic DNA (or, at least, in its macrochromosome component) at 23–27 Mb intervals (26–30 µm, as indicates with stars in Fig. 5). This is in agreement with the presence of a small peak in the bar graphs of Fig. 5, whose position corresponds to double modal distance between chiasmata (56–60 µm).

"Functional" hypotheses assume that a recent crossover alters the properties of the neighborhood within a certain (interference) distance, hampering the operation of the recombination complex. This may be caused by deficiency of components required for assembly of recombination complexes (late nodules or recombinomers) or excess of factors hampering recombination in the vicinity of accomplished crossovers [21]. It is also conceivable that a change of spatial arrangement of the chromosome occurs around a hemichiasma and hampers crossover initiation in neighboring areas [22–24]. Also, intermediates required for recombinase operation are scarce in each of the bivalents [20], so that the effect of an accomplished crossover vanishes only at the mean distance 26.99 ± 1.14 Mb (30.33 ± 1.28 µm × 0.89 Mb).

Parameters and recombination properties of chicken macrochromosomes 1–3

Chromosome	L_{lbc}, μm	L_{dna}, Mb	L_{dna}/L_{lbc}, Mb/μm	X_{min}		Regular chiasma emergence (period length)	
1	196	188	0.959	6 μm	5.754 Mb	27 μm	25.893 Mb
2	164	148	0.902	10 μm	9.020 Mb	27.5 μm	24.805 Mb
3	140	109	0.779	9 μm	7.011 Mb	27 μm	21.033 Mb
Mean			0.890				

Note: L_{lbc}, mean length of a bivalent at the lampbrush stage; L_{dna}, mean length of a bivalent, Mb, according to [18]; X_{min}, minimal interference distance.

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