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Sex-specific recombination maps for individual macrochromosomes in the Japanese quail (*Coturnix japonica*)

Lucía del Priore · María Inés Pigozzi

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Abstract Meiotic recombination in the Japanese quail was directly studied by immunolocalization of mutL homolog 1 (MLH1), a mismatch repair protein of mature recombination nodules. In total, 15,862 crossovers were scored along the autosomal synaptonemal complexes in 308 meiotic nuclei from males and females. Crossover frequencies calculated from MLH1 foci show wide similitude between males and females with slightly higher number of foci in females. From this analysis, we predict that the sex-averaged map length of the Japanese quail is 2580 cM, with a genome-wide recombination rate of 1.9 cM/Mb. MLH1 focus mapping along the six largest bivalents showed few intersex differences in the distribution of crossovers along with variant patterns in metacentric and acrocentric macrobivalents. These results provide valuable information to complement linkage map analysis in the species while providing insight into our understanding of the mechanisms of crossover distribution along chromosome arms.

Keywords Birds · Meiosis · Crossing over · MLH1 foci · Poultry genome · Japanese quail

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L. del Priore · M. I. Pigozzi (✉)
INBIOMED (CONICET-UBA), Facultad de Medicina,
Universidad de Buenos Aires,
Paraguay 2155 Piso 10, C1121ABG Buenos Aires, Argentina
e-mail: mpigozzi@fmed.uba.ar

Abbreviations

MLH1	mutL homolog 1
SC	Synaptonemal complex
cM	Centimorgan
SMC3	Structural maintenance of chromosomes 3
CREST	Calcinosis, Raynaud phenomenon, esophageal dysmotility, sclerodactyly, and telangiectasia
RN	Recombination nodule

Introduction

The Japanese quail (*Coturnix japonica*) is a well-established animal model in biology and a bird used for intensive egg and meat production. In common with its close relative species, the chicken, it belongs to the family Phasianidae in the order Galliformes, and the two species have diverged 35–46 million years ago (van Tuinen and Dyke 2004; van Tuinen and Hedges 2001; Kan et al. 2010). The Japanese quail has a karyotype of $2n=78$ chromosomes comprising a few morphologically distinct macrochromosomes and numerous cytologically indistinguishable microchromosomes. Highly conserved chromosome homology has been revealed between this species and the chicken using different approaches such as sequence mapping (Shibusawa et al. 2001; Galkina et al. 2006; Kayang et al. 2006; McPherson et al. 2014), chromosome painting (Guttenbach et al. 2003; Shibusawa et al. 2004), and linkage analysis (Kayang et al. 2006; Sasazaki et al. 2006). Recently, a draft

genome sequence has been obtained for the Japanese quail that will provide genetic and genomic reference information to facilitate research on this species (Kawahara-Miki et al. 2013). In connection with the interest on the Japanese quail a as commercial and biological source, genetic maps with amplified fragment length polymorphisms (AFLPs) and microsatellites have been developed (Roussot et al. 2003; Kayang et al. 2004, 2006). These linkage maps opened the way to comparative works with the chicken and to quantitative trait loci (QTL) detection for a variety of traits as well as to fine mapping of complex traits (Minvielle et al. 2005; Frésard et al. 2012). In spite of the importance of the species in production and research, genetic maps and genomic studies are comparatively underdeveloped compared to the chicken (Roussot et al. 2003; Kayang et al. 2004, 2006; Frésard et al. 2012). Additional studies are then desirable to improve estimates of genetic map length and to give further insights about the genetic distances between physical chromosome positions in this species.

Precise approach to crossovers between closely linked genes can be provided by genetic linkage studies, but this method requires the analysis of large data sets, well-controlled crosses, or well-characterized pedigree records. In addition to the complexity to generate a linkage map of one species, the methodology has some drawbacks such as overestimation of the total map length due to the addition of short map intervals or underestimation of map length due to the lack of markers in some chromosome regions (reviewed in Barnes 2003; Hultén and Tease 2006). An alternate approach for crossover mapping has been developed on the basis of the cytological localization of recombination sites along the pachytene chromosomes using antibodies to mutL homolog 1 (MLH1), a mismatch repair protein of mature or late recombination nodules (RNs). Similarly to RNs observed with electron microscopy, MLH1 protein appears as discrete foci detectable by immunofluorescence along the linear synaptonemal complexes (SCs), which represent synapsed bivalents at pachytene (Sherman and Stack 1995; Barlow and Hultén 1998; Anderson et al. 1999; Froenicke et al. 2002; Koehler et al. 2002). Crossover maps based on MLH1 focus counts have been obtained in humans, mice, and other mammals (Froenicke et al. 2002; Sun et al. 2004; Borodin et al. 2007, 2008, 2011; Basheva et al. 2008; Garcia-Cruz et al. 2011; Pan et al. 2012; Vozdova et al. 2013; Mary et al. 2014). These maps have revealed subspecies differences in genome-scale recombination rates in mice (Dumont and Payseur 2011a); they were employed to investigate the evolution of recombination in mammals (Dumont and Payseur 2011b; Segura et al. 2013) and gave insight on the mechanisms of crossover interference (de Boer et al. 2006; Lhuissier et al. 2007; Borodin et al. 2008), among other important biological problems.

In birds, antibodies to the mammalian MLH1 protein recognize foci along SCs during pachytene (Pigozzi 2001). MLH1 focus mapping on oocytes gave an excellent prediction of the total map length in the chicken, later confirmed after extensive linkage mapping in this species (Pigozzi 2001; Groenen et al. 2009). Also, MLH1 mapping in males and females gave additional evidence about the existence of similar recombination rates in both sexes among birds (Calderón and Pigozzi 2006), as previously suggested by RN counts in chickens and pigeons (Rahn and Solari 1986; Pigozzi and Solari 1999). In summary, broad research in mammals, birds, and plants has shown that MLH1 foci (and RNs) provide an unbiased estimate of the total genome map length, as well as the frequency and distribution of crossovers in individual chromosomes.

In a previous report, we found that the number of MLH1 foci in quails is similar in males and females and compare the global recombination rates in this species with the chicken and other birds (Calderón and Pigozzi 2006). However, the distribution of MLH1 foci along individual bivalents and comparative crossover maps in males and females were not investigated. Taking into account the current efforts to build a linkage map for the species and considering that MLH1 focus frequencies provide a mean to relate crossover frequencies with chromosome structure, we quantify here the variations of MLH1 focus distributions along the largest SCs in male and female Japanese quails. Because each MLH1 focus corresponds to one crossover, the frequency of foci on defined chromosomal segments can be converted to centimorgan (cM) values. This reasoning has been the basis for the construction of crossover maps based on direct (cytogenetic) meiotic analysis where the cM values are shown along pachytene chromosomes, similarly to the information depicted by linkage maps based on pedigree analysis (Anderson et al. 2004; Chang et al. 2007; Hultén and Tease 2006). Among the advantages of this method are that estimations of crossover distances along chromosomes are not dependent of marker density or high number of recombinant types; they provide data of several meiosis per individual, and the

total amount of crossovers in one meiotic nucleus can be scored at the same time.

Using immunolocalization of MLH1 foci, we found that the total genetic map for the autosomal linkage groups of the Japanese quail is 2515 in males and 2655 in females, with the difference partially explained by higher recombination rates along chromosome 6 in oocytes. Detailed MLH1 focus mapping along the six largest bivalents reveals wide intersex similitude of crossover distribution and also sex-specific differences along chromosomes 5 and 6. The results of this study offer new information of genetic distances along the largest chromosomes in the Japanese quail, while providing valuable data to complement ongoing linkage studies in this species.

Materials and methods

Birds Data in the present analysis were obtained from two adult males and three females from the same commercial stock. MLH1 foci are found during pachytene, and we used 2-day-old females because the peak of pachytene oocytes occurs at this age in the Japanese quails. Handling and euthanasia of birds were performed as per protocol approved by the Animal Care and Use Committee of the University of Buenos Aires School Of Medicine (EXP-UBA 25477/10) following all institutional and national guidelines for the care and use of laboratory animals.

Meiotic chromosome preparations SC spreads from oocytes and spermatocytes were prepared according to methods previously described (Pigozzi 2001; Goday and Pigozzi 2010). Immunostaining of MLH1 (BD Pharmingen), structural maintenance of chromosomes 3 (SMC3) (Chemicon, Millipore), and centromere proteins with CREST serum (Immuno Concepts), as well as image capture and analysis, was performed as described in Calderón and Pigozzi (2006). Part of the testicular material from adult males was subjected to the air-drying procedure (Evans et al. 1964) and stained with Giemsa for light microscopy analysis of chiasmata.

SC measurements and MLH1 focus counts We only analyzed SC spreads that fulfilled the following standards: (1) the signal to background ratio for both MLH1 and SMC3 was sufficiently high to allow unambiguous identification of the MLH1 foci and tracing of all SCs from one end to the other, (2) the bivalents displayed full synapsis with no obvious stretching, and (3) the centromeres, represented by CREST signals, were clearly labeled. We retained only cells with at least one MLH1 focus on each SC, excepting the possibility of one microbivalent without focus. Digital images were obtained using a cooled CCD camera (Olympus DP73), coupled to a Zeiss Axiophot microscope. Two color images were acquired for each nucleus using appropriate filter sets (Carl Zeiss): one image for the SCs and centromeres and the second for MLH1 foci. Images were adjusted for brightness and contrast if necessary and merged with Photoshop CS2 software (Adobe). SC lengths and the position of the centromere and MLH1 foci were scored using the version 3.3 of the MicroMeasure program (http://www.colostate.edu/Depts/Biology/MicroMeasure/) which records absolute and relative distances from the centromere on digitized images. After compiling the focus data, the genetic map length of each bivalent was calculated by determining the average number of MLH1 foci per SC and then multiplying by 50.

Preparing recombination maps on pachytene bivalents The construction of crossover frequency histograms from RNs or MLH1 foci has been explained in detail in several publications (Sherman and Stack 1995; Pigozzi and Solari 1999; Borodin et al. 2008). In order to compare focus distribution at equivalent chromosome segments in males and females, the sex-averaged SC length was used to calculate the absolute position of each MLH1 focus on macrobivalents 1 to 6. These data were pooled for each arm and graphed to represent frequency histograms for males and females.

Mitotic chromosomes Mitotic chromosomes were obtained from the bone marrow of the females used for SC spreads and from whole embryos at 6 days of embryogenesis. In both cases, cells were harvested after 1 h of culture in Eagle's minimal essential medium with 0.05 μg colchicine (Sigma-Aldrich)/ml. Cells were exposed to 0.56 % KCl hypotonic solution for 20 min and then fixed in 3:1 methanol and glacial acetic acid according to standard procedures followed by Giemsa staining. In order to compare the average relative lengths and centromere position in mitotic chromosomes and SCs, the six largest autosomal pairs in each metaphase

were measured using MicroMeasure. The relative length of each chromosome pair was calculated dividing their average length by the total length of the six largest autosomal pairs in the same metaphase. Measurements of mitotic chromosomes of the same pair were averaged before calculating their relative lengths since this program is designed to measure haploid sets. Centromeric indexes (length of the short arm divided by total chromosome/SC length) were also obtained from these measurements.

Results and discussion

Meiotic (SC) vs. mitotic karyotype

Figure 1a–c shows examples of the immunostained spreads and mitotic metaphases used to obtain average relative lengths and centromeric indexes of the largest mitotic pairs and their SCs. The two largest SCs showed distinguishable short arms, while bivalents 3 to 6 had negligible short arms, in agreement with the morphological features of the mitotic chromosomes (Fig. 1c). Measurements of the six largest autosomes in mitotic methaphases and SC spreads showed that the largest SCs in the Japanese quail are three to five times longer than their mitotic counterparts, although their relative lengths and centromeric indexes show remarkable agreement (Fig. 1d). The morphological correspondence of mitotic and SC karyotypes in birds has also been observed in a wide variety of avian taxa from ratites to the more recently evolved passerine birds (Pigozzi and Solari 1997, 1998). Moreover, in addition to similar arm ratios in mitotic chromosomes and pachytene bivalents, it has been shown that there is a direct relationship between the positions of interstitial sequences along the pachytene (SC) and the mitotic Z chromosome in the zebra finch (Pigozzi 2008). These observations contrast with those from mice, humans, and other mammals, where several SCs are shorter or longer than expected from their relative lengths in mitosis (Froenicke et al. 2002; Sun et al. 2004; Borodin et al. 2008). Discrepancies between mitotic chromosomes and SCs probably lie in the differential organization in mitosis and meiosis of repetitive sequences enriched in G and R bands and those forming heterochromatin of mammalian chromosomes (Zickler and Kleckner 1999; Hernández-Hernández et al. 2008; de la Fuente et al. 2014). The chicken and possibly most birds possess comparatively fewer repetitive sequences and heterochromatin than mammals, especially in the largest chromosomes (International Chicken Genome Consortium 2004), so the regional variations in chromatin loop sizes along the lateral elements of the SCs which distort the relative lengths of mitotic and meiotic (pachytene) chromosomes may be less obvious in the macrobivalents of birds. More studies, however, are necessary to confirm or dispute the existence of a more homogeneous distribution of chromatin along meiotic chromosomes in birds compared to mammals.

MLH1 focus stability during pachytene

Proteins involved in meiotic recombination are widely conserved in different organisms, and they are loaded in a sequential manner as recombination interactions are solved (reviewed in Gerton and Hawley 2005). This sequential order of events might lead to the idea that crossovers are underestimated in MLH1 focus counts due to the temporary nature of MLH1 protein foci during late prophase stages. To test the assumption that MLH1 foci are representative of crossover events in the Japanese quails, the number of MLH1 foci on SC 1 was compared with the number of chiasmata on the corresponding bivalent during diakinesis/metaphase I in males (Fig. 2). Counts were limited to bivalent 1 to avoid possible errors interpreting the configuration of chiasmata or misidentification of the bivalents which are shorter at metaphase I. We found a strong agreement in the average numbers of chiasmata (5.4±0.15, N=39) and MLH1 foci (5.4±0.071, N=159, see results below) with no significant differences between these average values (t=0.32, df=196; P=0.7473). Furthermore, the range of chiasmata (4–7) and the range of MLH1 foci are very similar (4–8), and bivalents with five or six crossovers were observed more often for MLH1 foci than for chiasmata. These results indicate that the criteria used here to select the cells for MLH1 focus counts overcome the possible underestimation of crossover numbers due to the transient nature of MLH1 foci during pachytene. In the chicken, MLH1 foci also seem to mark all the crossover events, considering the agreement of global recombination frequencies obtained by this cytological method and the linkage map (Pigozzi 2001; Groenen et al. 2009). Altogether, these lines of evidence support the idea that the temporal loading of MLH1 protein onto DNA sites of recombinational

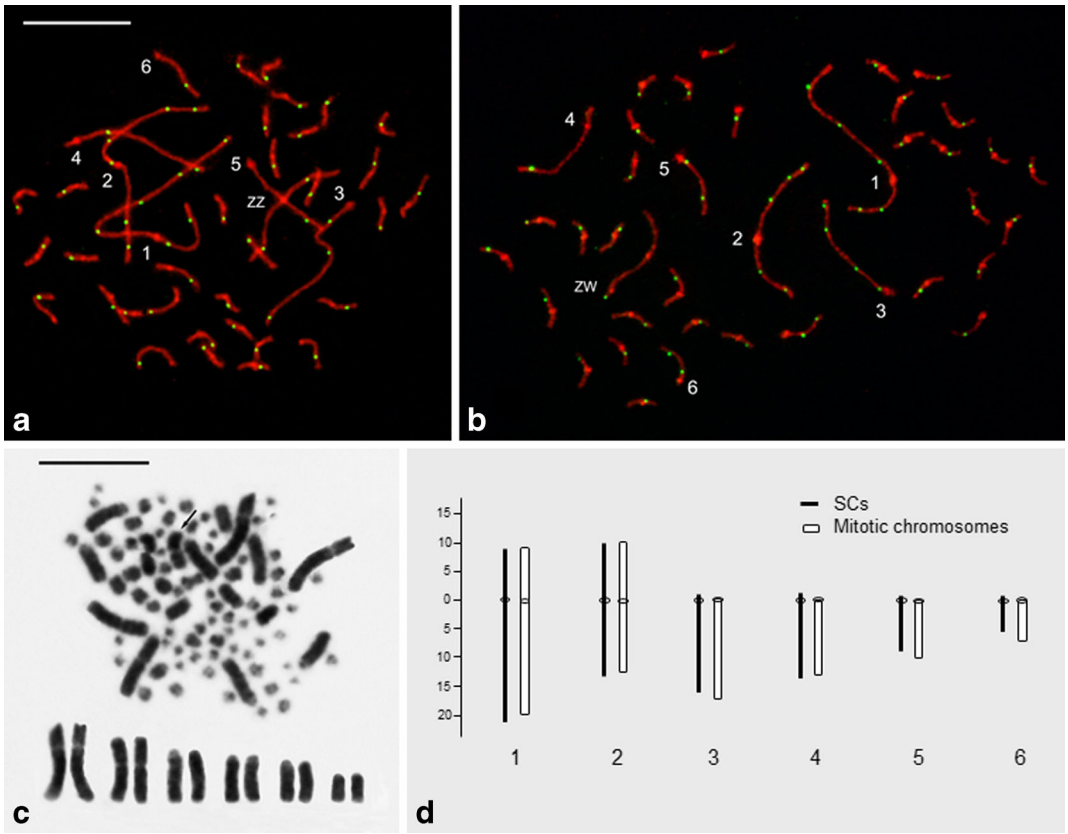

Fig. 1 Comparison of mitotic and meiotic chromosomes in the Japanese quail. Pachytene male (**a**) and female (**b**) germ cells showing the immunostained SCs and centromeres (*red*) and MLH1 foci (*green*). The *number next to the centromeric signals* identifies the largest autosomal synaptonemal complexes. **c** Mitotic metaphase from a female embryo and the six largest autosomes arranged in pairs. The *arrow* points to the Z chromosome; the W chromosome cannot be identified with certainty without additional techniques. *Bars*=10 μm. **d** Comparative ideograms of synaptonemal complexes and mitotic chromosomes. The *scale* indicates the relative length represented by each chromosome or chromosome arm in respect to the total length of the six largest autosomal pairs. Relative lengths and centromeric indexes are means from 60 SC sets (30 from each sex) and 20 mitotic metaphases

repair does not lead to uncounted recombination events in birds; therefore, MLH1 foci represent the same set of crossovers marked by chiasmata in diplotene and by RNs during pachytene (Pigozzi and Solari 1999).

Genomic map lengths using MLH1 mapping

Statistical tests revealed that there were no significant effects of individual on the variation of MLH1 focus numbers, in both males and females ($P>0.05$, by t test and Kruskal-Wallis test, respectively). Consequently, MLH1 focus data from different individuals were pooled and used for comparisons between sexes. Altogether, 11,666 autosomal SCs were examined for the presence of MLH1 foci in the total 308 meiosis analyzed in both sexes (Table 1). The sex pair was not included in this analysis because the ZW bivalent is highly heteromorphic and recombination is restricted to a single terminal event while the ZZ bivalent recombines freely (Fig. 1a). SCs without foci were rare: no macrobivalents were found without MLH1 foci, and microbivalents lacking a focus represented less than 1 % of the total SCs. This frequency of microbivalents without foci is comparable to that observed in the chicken (Pigozzi 2001) and can be ascribed to immunostaining failure of these particular bivalents since at least one crossover is needed to ensure correct segregation at later stages of meiosis. Based on the average number of foci in autosomal bivalents of males and females, we estimate that the total sex-averaged map length for the Japanese quail is 2580 cM (51.6 MLH1 foci × 50 cM/focus). To our knowledge, these data represent the highest number of crossover

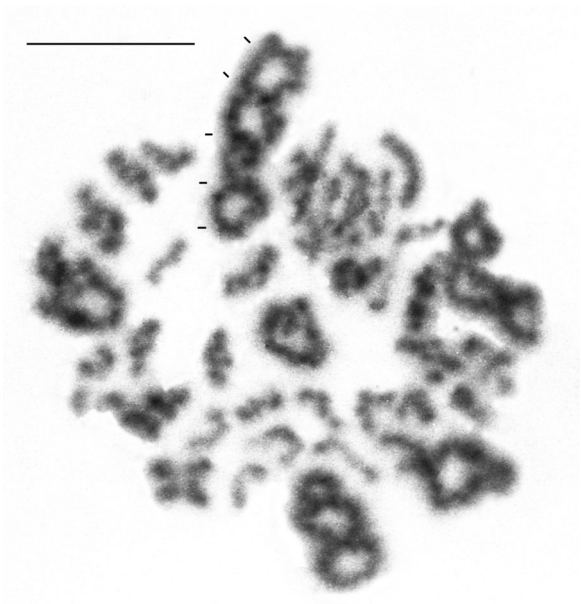

Fig. 2 Metaphase I spermatocyte of the Japanese quail. The five chiasmata observed on the largest bivalent are indicated by *short lines*. *Bar*=10 μm

observations in a single species of bird using cytological methods. The difference in map length between males and females is relatively small (150 cM), and it is explained by the presence of two foci in bivalent 6 and in one unidentified microbivalent, more frequently in oocytes than in spermatocytes (see Table 2).tgrouptgroup

In a previous report, we found that the sex-averaged number of MLH1 foci in the Japanese quail was 55.8 (Calderón and Pigozzi 2006), four foci more than in the present study. This difference is not restricted to one or few bivalents, but instead, we found that the average number of foci was slightly lower in all the macrobivalents in the animals examined in the present study. Because the presence of multiple chromosomal rearrangements in heterozygosity in most or all animals causing diminution of crossover rates in all macrobivalents seems unlikely, we are inclined to think that the difference represents real differences in recombination rates between the quail stocks employed in each case. Differences in recombination rates have been observed in mapping populations in chickens, with significantly higher rates in purebred domestic animals (Groenen et al. 2009). Intraspecific variations of recombination rates in the absence of chromosomal polymorphisms have also been observed between different strains of *Mus musculus* (Koehler et al. 2002; Dumont et al. 2009), as well as in other animals (Andersson and Sandberg 1984). Further investigations on recombination using inbred lines may clarify if recombination frequencies also vary among Japanese quail populations.

Table 1 Average number of MLH1 foci and total genetic lengths of the Japanese quail genome in males and females

	Average	Range	cM	*N*
Males	50.3±2.2*	46–67	2513.4	161
Females	53.1±3.8*	45–57	2653.0	147
Average	51.6±3.4	45–67	2580	308

*$P<0.0001$, significantly different, by Mann-Whitney test

Assuming that the haploid genome size in the Japanese quail is about 1320 Mb (Nakamura et al. 1990; Kawahara-Miki et al. 2013), the global recombination rate across the genome is 1.9 cM/Mb, lower than the 2.6 cM/Mb ratio observed in the chicken (Groenen et al. 2009). The difference cannot be explained by large differences in DNA content or by different chromosome numbers since both parameters are very similar in these species (Gregory 2014; Shibusawa et al. 2001). Comparison of recombination rates between the chicken and other avian species with conserved synteny also shows that recombination rates are higher in the chicken (Backström et al. 2006; Dawson et al. 2007), suggesting that its linkage map may not be suitable as a reference to estimate genetic distances in all birds, even in the presence of large syntenic regions.

The total map length of the autosomal set from the present MLH1 analysis (2580 cM) is considerably longer than estimates based on linkage studies in the Japanese quail. The most extensive linkage map integrates two microsatellite and AFLP genetic maps encompassing 15 linkage groups and 20 unlinked markers (Kayang et al. 2006), and its projected length is 1904 cM. The discrepancy in map length compared with both our estimates and estimates from lampbrush chromosome analysis (Rodionov and Chechik 2002) indicates that linkage maps for the species currently lack enough marker density across most linkage groups. It is likely that the discrepancy with the cM length from MLH1 foci will decrease as more markers are added.

Sex-specific recombination maps for male and female chromosomes

From the sample of 308 cells analyzed for the total number of MLH1 foci, we scored MLH1 focus

Table 2 Comparison of crossover frequencies and map lengths using MLH1 foci, lampbrush bivalents, and linkage mapping

	MLH1 map				LBC map[a]	Linkage map[b]
	Males		Females		Females	Sex-averaged
SC	MLH1 foci±SD	cM	MLH1 foci±SD	cM	cM	cM
1	5.3±1.0	266	5.5±1.2	275	313	274.8
2	4.2±0.9	210	4.5±1.0	223	238	174.4
3	2.9±0.7	144	3.0±0.8	151	154	48.4
4	2.4±0.5	122	2.7±0.7	133	143	20.8
5	1.9±0.4	96	1.9±0.5	97	~100	42.1
6	1.1±0.4*	57	1.6±0.5*	79	–	96.1
1–6	17.9	896	19.2	958	–	656.6
7–38	32.4	1620	33.9	1695	–	–

[a] After Rodionov and Chechik 2002

[b] After Kayang et al. 2006

*$P<0.0001$; $t=8.582$, means differ significantly

positions along SCs 1–6 to obtain the average number of foci on each SC and individual frequency histograms in each sex (Table 2; Fig. 3). The average number of MLH1 foci in the four largest bivalents is similar to estimates from chiasmata in lampbrush bivalents (Rodionov and Chechik 2002; Schmid et al. 2005), with the difference being slightly more than one crossover (66 cM). As previously pointed out, linkage mapping is at a relatively early stage of construction in the Japanese quail, explaining the lack of agreement with the cM lengths found in the present analysis. The only exception is the largest chromosome pair (CJA01). Genetic maps of quail chromosome 1 from two different analyses give comparable genetic lengths for CJA01—274.8 and 284.5 cM (Kayang et al. 2006; Frésard et al. 2012)—similar to the cM length obtained here by MLH1 focus mapping (Table 2).

Two features of crossover distribution are particularly well shown by MLH1 focus analysis of recombination on meiotic bivalents: distribution along chromosome arms and variation between sexes. The present analysis shows that bivalents in males and females reproduce a distribution of MLH1 foci that is generally observed in other organisms: metacentric/submetacentric chromosomes show a pronounced recombination peak near to the telomeric or subtelomeric regions, a deficiency near to the centromere and a bimodal or a multimodal distribution along the chromosome arms (Fig. 2). On the other hand, no MLH1 foci were found in the short arms of the chromosomes 3, 4, 5, and 6, which are all acrocentric with very short arms mainly formed by heterochromatin. In these bivalents, there are pronounced recombination peaks close to the centromeres (within 1 μm or less), similar to those at telomeric regions, contrasting with the lower frequency of foci at centromeric regions of bivalents 1 and 2. This difference of recombination levels around the centromeres in meta/submetacentric vs. acrocentric bivalents in the Japanese quail probably reflects diverse patterns of crossover formation in chromosomes with very short arms compared to metacentric or submetacentric bivalents. We favor this explanation over the frequent view that heterochromatin might be the basis of crossover paucity around centromeres because in the Japanese quail, heterochromatic blocks at the centromeric regions of chromosomes 1 and 2 are not particularly larger than those on acrocentric chromosomes 3 to 6 (Stock and Bunch 1982; Schmid et al. 1989). Moreover, in pigeons, zebra finch, and chickens, the acrocentric macrochromosomes also show high recombination levels around the centromeres compared to metacentric chromosomes (Pigozzi and Solari 1999; Calderón and Pigozzi 2006; Pigozzi, unpublished observations) in spite of similar amounts of centromeric heterochromatin in all macrochromosomes judging from C-banding patterns in these species. While the presence of large blocks of heterochromatin is a likely explanation of recombination suppression around the centromere in certain organisms (Sherman and Stack

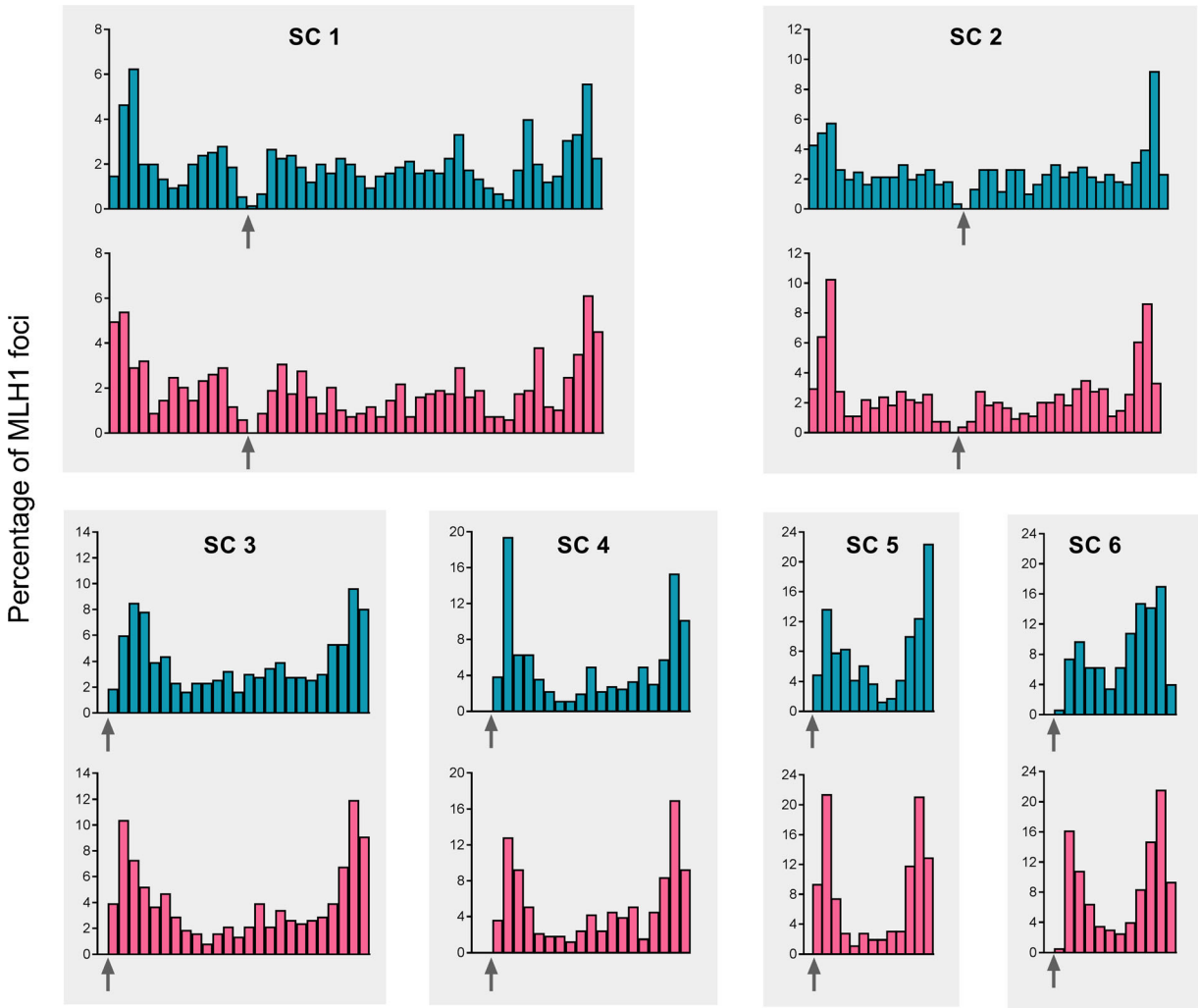

Fig. 3 Crossover distribution in the Japanese quail. Each histogram represents the distribution of MLH1 foci along the six largest autosomal bivalents in males (*top*) and females (*bottom*). For each autosome, the *x*-axis indicates the relative positions of the MLH1 foci on the SC, from the p (*left*) arm to the q (*right*) arm. The bin width in each histogram represents a fraction of the total length of each SC, and it is equivalent to about 5.5 μm. The *arrows* indicate the location of the centromeres

1995), variations of recombination patterns at centromeric regions in birds are more consistent with an effect of chromosome morphology on the process of crossover formation, as suggested from crossover distribution analysis in a number of mammals showing Robertsonian polymorphisms (Bidau et al. 2001; Merico et al. 2003; Borodin et al. 2007, 2008).

In addition to these general patterns present in males and females, histograms of SC 4 show an interstitial region with a scarce amount of foci in both sexes. It can be predicted then that markers located at this region, spanning nearly 20 % of the long arm, will appear closer than they actually are as a product of lower recombination rates in both sexes. In spite of this general similarity of crossover patterns in males and females, some differences can be pointed out, for example, on the short arm of SC2 and at the proximal segments of SCs 5 and 6. Consequently, we built cumulative frequency plots of MLH1 foci along SCs 1–6 since this type of representation offers a better comparison of frequency distributions and also allows for the statistical estimation of similarities. To produce cumulative distributions, the frequency of MLH1 foci was added along each SC starting from the tip of the short arm with distances expressed as a percentage of the SC length (Fig. 4). The analysis revealed that the distribution of MLH1 foci

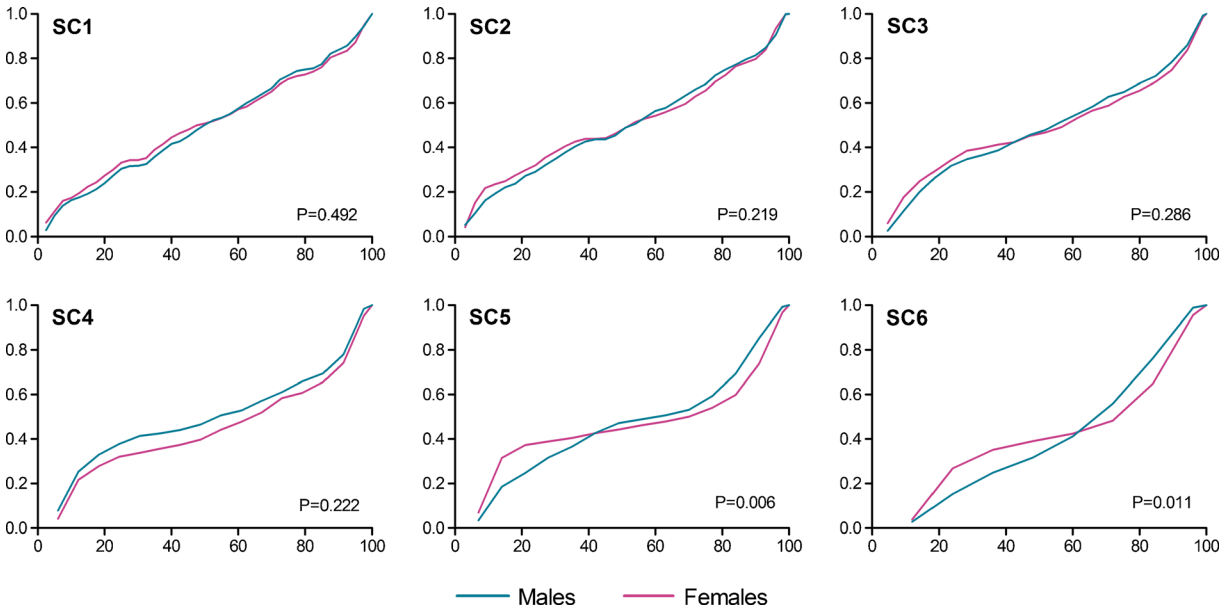

Fig. 4 Cumulative frequency plots of foci in males and females. Shown are the cumulative frequencies of foci as a function of the distance to the telomeric end of the long arm of the SC. The distance is expressed as a percentage of the SC length on which the focus was located. For each bivalent, the *P* value represents the probability that MLH1 focus positions in males and females stem from the same distribution (Kolmogorov-Smirnov two-sample test). See the text for further explanations

in chromosomes 1 to 4 does not differ significantly between sexes while statistically significant differences were found for chromosomes 5 and 6 (Fig. 4). The average number of MLH1 foci on bivalent 5 does not differ significantly between males and females, but the difference is statistically significant for chromosome 6 (Table 2). In females, bivalent 6 showed two foci in 80 % of the analyzed cells, while only 16 % had two foci in males. When a single focus is present on this SC, it is more displaced toward distal positions in females than in males, explaining the presence of a steeper segment of the curve in females (Fig. 4). The difference in focus number and distribution on bivalent 6 may represent real differences between males and females. However, it is not possible to rule out the presence of a chromosomal rearrangement in one or both males that may have caused a crossover diminution on this particular bivalent. Irregular configurations of the SCs were not detected in spermatocytes, but a small deletion or a paracentric inversion may have been unnoticed if synaptic adjustment or heterosynapsis takes place in quail spermatocytes.

From the present analysis, we conclude that intersex differences in recombination frequencies and distributions are relatively small in the Japanese quail and they will not affect substantially the genetic (cM) distances between markers in most chromosomal segments even if distances were calculated from crossovers in one sex. The existence of equal or very close amounts of crossing over in male and female birds seems to be shared by avian species from different orders, as shown by cytological and molecular data in chicken, pigeons, and zebra finches (Pollock and Fechheimer 1978; Rahn and Solari 1986; Rodionov et al. 1992; Pigozzi and Solari 1999; Calderón and Pigozzi 2006; Groenen et al. 2009). An exception has been reported in a passerine bird, with approximately twofold higher rate of recombination in females than in males (Hansson et al. 2005), even though it remains to be established if this difference is present genome wide.

Concluding remarks MLH1 focus maps provide valid estimates of genomic and chromosomal recombination rates that are not dependent on assumptions that often lead to map inflation in linkage studies (reviewed in Hultén and Tease 2006). Even though the MLH1-cM given cannot relate directly recombination rates with genome sequences, the cumulative distributions calculated from MLH1 focus data can be used to predict the physical location of a genetically mapped marker on a chromosome arm and, combined with fluorescence in situ hybridization (FISH) serve to anchor, the genetic

map to cytological positions on pachytene bivalents (Anderson et al. 2004; Chang et al. 2007). Conversely, cM distances between sequences mapped by FISH on pachytene SCs can be calculated directly when the corresponding cM map based on MLH1 focus frequencies is available (Pigozzi 2008). In view of this versatile use of the MLH1-cM maps, we are currently interested to investigate if our map is a good predictor of the physical positions of genetically mapped markers in the Japanese quail.

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Conflict of interest The authors (María Inés Pigozzi and Lucía del Priore) declare no conflict of interest.

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