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Relationship between physical and genetic distances along the zebra finch Z chromosome

María Inés Pigozzi*
Instituto de Investigaciones en Reproducción, Facultad de Medicina, Universidad de Buenos Aires, Paraguay 2155 piso 10, C1121ABG, Buenos Aires, Argentina; Tel: +54 11 59509612; Fax: +54 11 59509612; E-mail: mpigozzi@fmed.uba.ar
**Correspondence*

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Abstract

Nine bacterial artificial chromosomes containing genes linked to the Z chromosome of the zebra finch (*Taeniopygia guttata*) were localized using FISH on synaptonemal complex spreads. Their positions were correlated with those previously reported on the mitotic Z chromosome, showing a linear relationship between positions along the mitotic chromosome and its synaptonemal complex. Distances in cM between the genes were calculated using a cytological map of the crossing-over based on the distribution of MLH1 foci along the ZZ synaptonemal complex (MLH1-cM map). It is shown that physical and genetic distances lack a linear relationship along most of the chromosome length, due to clustering of crossover events around the telomeres. This relationship departs strongly from that observed in the chicken Z chromosome and reflects the existence of different recombination rates and patterns among birds in spite of wide genomic conservation.

Abbreviations

BAC	bacterial artificial chromosome
CREST	calcinosis, Raynaud phenomenon, oesophageal dysmotility, sclerodactyly, and telangiectasia
FISH	fluorescence *in-situ* hybridization
FITC	fluorescein isothiocyanate
MLH1	mutL homologue 1
RN	recombination nodule
SC	synaptonemal complex
SMC3	structural maintenance of chromosome 3

Introduction

In the assembly of a genome project, large amounts of genomic data must be related to specific locations on chromosomes. In this context, linkage maps play a central role in aligning different types of maps describing the order of genes and the amount of recombination between them. However, linkage maps are less useful to predict physical positions of markers and genes on a chromosome, because recombination rates vary along the length of chromosomes and, as a result, genetic distances are not directly proportional to physical distances. Cytogenetic maps then become a valuable source to relate the linear order of markers in a linkage map with respect to chromosomal landmarks such as telomeres, centromeres, or nucleolar organizers. A powerful strategy to construct a cytogenetic map is to visualize the positions of genes or non-coding sequences directly by fluorescence *in-situ* hybridization (FISH). This methodology complements high-resolution physical mapping by chromosome walking or radiation hybrid maps, provides confirmation of anchored BAC contigs, and makes markers available

for assignment of a chromosome number to each linkage group. Localization of single-copy sequences by FISH is commonly applied on mitotic chromosomes, but meiotic bivalents prepared for observation of the synaptonemal complexes (SCs) have been employed in plants and also in mice and the chicken among vertebrates (Peterson *et al*. 1999, Froenicke *et al*. 2002, Pigozzi 2007). In addition to being good substrates for FISH, SC spreads offer the chance to observe directly the positions of crossover events along bivalents in whole nuclei. During meiosis, reciprocal exchanges between homologues can be cytologically detected at pachytene as electron-dense protein complexes called recombination nodules (RNs) or late nodules associated to the central space of the SCs. The number and distribution of RNs match the number and distribution of chiasmata or genetically mapped crossovers in a number of species (Carpenter 1975, reviewed in Zickler & Kleckner 1999). In certain organisms such as birds and plants, it has been possible to construct cytological maps of the crossovers by scoring the RNs along individual synaptonemal complexes (Rahn & Solari 1986, Sherman & Stack 1995, Pigozzi & Solari 1999a,b, Anderson *et al*. 2003). Because RN mapping in whole nuclei is labour intensive, an alternative is to use immunostaining of the mismatch-repair protein MLH1 that forms distinct foci on the synaptonemal complexes in a variety of organisms and is a component of RNs (Baker *et al*. 1996, Moens *et al*. 2002, Marcon & Moens 2003). In mice and humans, MLH1 foci are equivalent to chiasmata observed in metaphase I and to crossover events determined in linkage studies, representing all prospective reciprocal exchanges in these species (Froenicke *et al*. 2002, Sun *et al*. 2004). Similarly, the number of MLH1 foci or RNs associated with the synaptonemal complexes of the largest autosomal bivalents is equivalent to the number of chiasmata in chickens, quails, and pigeons (Pigozzi & Solari 1999a, Pigozzi 2001, Calderón & Pigozzi 2006). Because each RN or MLH1 focus represents one crossover that equals 50 centimorgans on a linkage map, their frequencies can be converted into cM distances to create RN- or MLH1-cM maps, respectively, for a given pachytene chromosome (Anderson *et al*. 2004, Chang *et al*. 2007).

Here the physical location of nine genes was established using FISH along the ZZ bivalent, using synaptonemal complex spreads from male zebra finches. Because these genes had previously been mapped on the mitotic Z chromosome (Itoh *et al*. 2006), it was possible to compare their positions in the mitotic and meiotic (SC) chromosomes. The genetic distances between these genes were determined using an MLH1-cM map, built from the analysis of MLH1 focus distribution during pachytene, and then related to their physical positions on the synaptonemal complex determined by FISH. The present analysis offers the first data about the relationship of genetic and physical distances in this bird and shows that this relationship differs strongly from that observed in the chicken Z chromosome.

Materials and methods

Synaptonemal complex spreads

SC spreads were done from adult male zebra finches *Taeniopygia guttata*. Testes were dissected out, placed into Hanks' balanced salt solution, and released from the tunica albuginea. Tubules were treated with a hypotonic extraction buffer (Peters *et al*. 1997) for 30 min and then gently disaggregated in 100 mM sucrose at pH 8.5 to give a homogeneous cell suspension. Amounts of about 30 μl of this suspension were dropped onto glass slides covered with a thin layer of 1% PFA, 0.1% Triton X-100. The slides were allowed to dry for about 1 h in a humid chamber and then were washed in 0.4% Photoflo. The spreads were used immediately for FISH and immunostaining or alternatively were kept at −70°C for later use.

Fluorescence in-situ hybridization and immunostaining

Probes specific for genes on the Z chromosome were prepared from BAC clones isolated from a zebra finch genomic library made by the Arizona Genomics Institute (www.genome.arizona.edu), and previously used for FISH on mitotic chromosomes from zebra finch fibroblasts (Itoh *et al*. 2006). A complete list of the isolated BACs and genes mapped along the zebra finch Z chromosome can be found in the paper cited. FISH and immunostaining to SC spreads were carried out according to methods previously described (Pigozzi 2007). Slides were examined under a Zeiss Axiophot microscope and images of well-spread pachytene nuclei with clear

FISH signals and SC labelling were captured using a CCD camera. Most probes were detected using FITC-labelled streptavidin or anti-digoxigenin. An antibody against the cohesin component SMC3 was used to label the synaptonemal complexes and detected with an anti-rabbit secondary antibody conjugated to a red fluorochrome. Two separate images were taken, one with the FITC filter for the probe signal and the other with the rhodamine/Cy3 filter to obtain the image of the SCs, and then merged using Adobe Photoshop.

The cytogenetic SC-FISH map

Linear distances between each FISH signal and the telomere of the short arm (pter) were measured using the program MicroMeasure (Reeves 2001). The relative distance between the FISH signal and pter is expressed as a fraction of the ZZ synaptonemal complex and is calculated by dividing the absolute distance from the telomere to the probe by the total SC length in each particular nucleus. Relative positions along a given SC are equivalent in spite of variations in the absolute SC length between nuclei and can be converted to μm distances using the standardized absolute length of each SC (Sherman & Stack 1995, Pigozzi & Solari 1999a). The arm location of each gene was previously reported on the mitotic Z chromosome (Itoh *et al*. 2006); therefore, centromere localization along with FISH was unnecessary in most cases. In fact, for most BACs the telomere of the short arm could be determined by looking at the position of the FISH signal on the SC before starting the measurement. One exception was BAC 223K19, containing the gene *ACO1*, because this sequence is located at a proximal position on the long arm with respect to the centromere that is coincident with the middle of the linear SC. In this case, centromeres were labelled with CREST serum simultaneously with FISH to locate *ACO1* in order to single out the telomere of the short arm (see Results).

Preparation of the MLH1-cM map

MLH1 focus data were obtained for the same males used for FISH following procedures that were previously described (Calderón & Pigozzi 2006). Briefly, the primary antibodies were mouse anti-MLH1 (BD, Pharmingen), rabbit anti-SMC3 (Chemicon) and human CREST serum that labels centromeric proteins. In order to build the frequency histogram the standard length of the ZZ bivalent in μm was calculated by multiplying the average relative length of its synaptonemal complex by the average absolute length of the whole SC complement. MLH1 focus positions were measured as relative distances in each nucleus and converted to absolute distances in μm on the standard ZZ bivalent. Foci numbers and their positions along the ZZ bivalent in each nucleus were scored using MicroMeasure from 103 SC spreads from three males. Pachytene spermatocytes used in the analysis showed the whole complement of SCs and the ZZ bivalent could be identified by its relative length and centromere position. The locations of 207 MLH1 foci scored in these nuclei were used to build a histogram with 0.25 μm intervals that shows the crossover frequency along the ZZ bivalent. Since one MLH1 focus corresponds to one crossover event, the SC interval that has on average one focus would be 50 cM long, which is the map distance between two loci that show on average one crossover per meiosis. MLH1-focus frequency in the histogram can be converted to centimorgans by multiplying the number of foci in each SC segment by 50, and then dividing by the total number of SCs observed. The cM values for adjacent intervals were added to generate a cumulative cM map along the ZZ bivalent. Once the cumulative cM map is available, it can be used to obtain the cM distance between the telomere of the short arm (pter) and any position along the bivalent. This procedure is based on the construction of recombination maps for maize and tomato pachytene bivalents that were built using the frequency of recombination nodules on the synaptonemal complexes in these species (Anderson *et al*. 2004, Chang *et al*. 2007).

Results

Centromere position on the meiotic (SC) and mitotic Z chromosome

Zebra finches have an asymmetric karyotype with macro- and microbivalents (2n=80), including 78 autosomes and the sex chromosomes in somatic cells (Pigozzi & Solari 1998). In addition, there is a germline restricted chromosome regularly present as a

heterochromatic univalent in males and a recombining bivalent in females (Pigozzi & Solari 2005). The Z chromosome is submetacentric and the fourth in size. However, in some zebra finches there is a pericentromeric inversion involving a large portion of the Z chromosome (Y. Itoh & A. Arnold, personal communication, 19 September 2007). In the present study, centromere localization showed that the ZZ synaptonemal complex was the fourth in size with paired kinetochores, indicating that individuals were not heterozygous for the inversion (Figure 1A). One of the males was heterozygous for an inversion in chromosome 6 (Itoh & Arnold 2005) and the other two males had an acrocentric pair 6. The centromeric index in SC spreads is 40.0, differing slightly from that in mitotic chromosomes, which is 42.0 (Itoh & Arnold 2005). This difference and the fact that sometimes the arm ratio is switched in pachytene bivalents compared with mitotic chromosomes (Solari 1980, Stack 1984, Sun *et al.* 2004) could raise doubts about the arm correspondence between the mitotic Z and its SC. For this reason, centromeres were immunostained in some of the SC spreads used for FISH. Figure 1B shows the signal for *SPIN* on the short arm of the ZZ synaptonemal complex in agreement with data from FISH on mitotic chromosomes (Itoh *et al.* 2006). FISH with *ACO1* provided further evidence of arm correspondence in mitotic and meiotic chromosomes (see below). These data demonstrate that MLH1-focus distribution on the ZZ synaptonemal complex obtained from immunostained SC spreads is specific for each arm, and therefore simultaneous FISH and MLH1 immunostaining is not essential to distinguish arms in this bivalent.

Comparative gene mapping in the mitotic and meiotic Z chromosome

The positions of the nine genes localized here on the ZZ bivalent were compared with those reported using FISH on the mitotic Z chromosome (Itoh *et al.* 2006). In SC spreads the probe gives a thread-like signal that crosses the synaptonemal complex of the ZZ bivalent at one point (Figures 1B and 2A–I). For most genes, the signal was clearly closer to one of the SC ends, identified as the tip of the short or the long arm depending on the BAC. In the case of

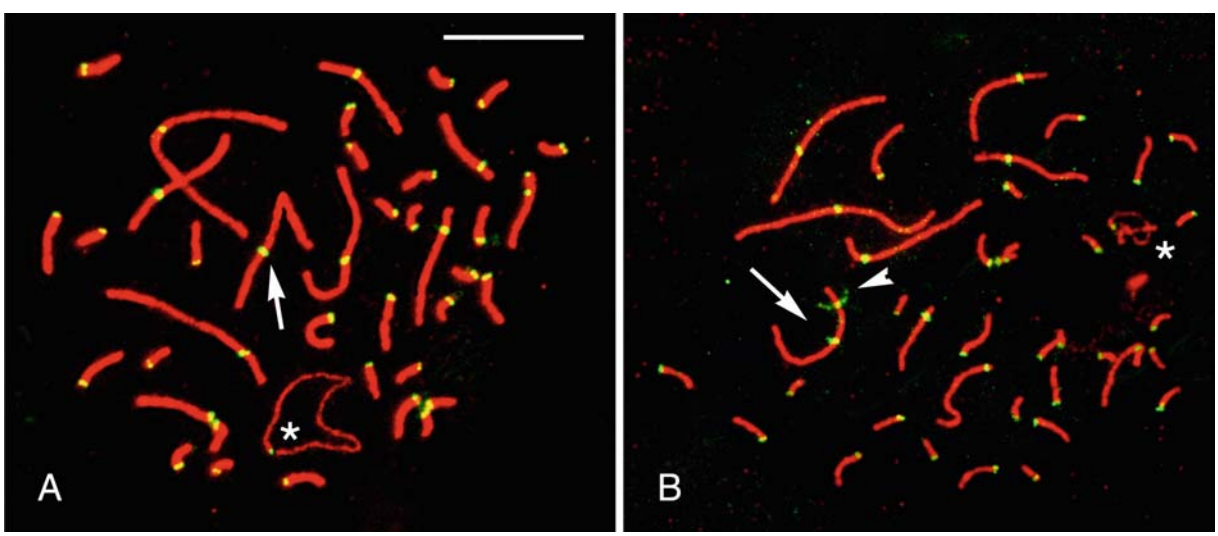

Figure 1. Centromere localization on the ZZ bivalent. **(A)** Complete SC set immunostained with anti-SMC3 antibody (red) and CREST serum (green). The arrow points at the ZZ bivalent. **(B)** FISH and immunostaining showing the signal of BAC 201H5 (*SPIN*) on the short arm of the ZZ bivalent (arrow). The arrowhead points at the thread-like signal crossing the ZZ synaptonemal complex near the tip of the short arm. Centromeres labelled with CREST serum appear as green dots on each SC. In each figure an asterisk marks the single axis of the germline restricted chromosome.

ACO1, centromeres were labelled with CREST serum along with the SCs to identify the end of the short arm with certainty, because this gene is located near the middle of the linear SC (Figure 2E). Centromere localization was avoided in the rest of the experiments to prevent any background that might impair clear visualization of the FISH signal. For each gene, the distance from the telomere of the

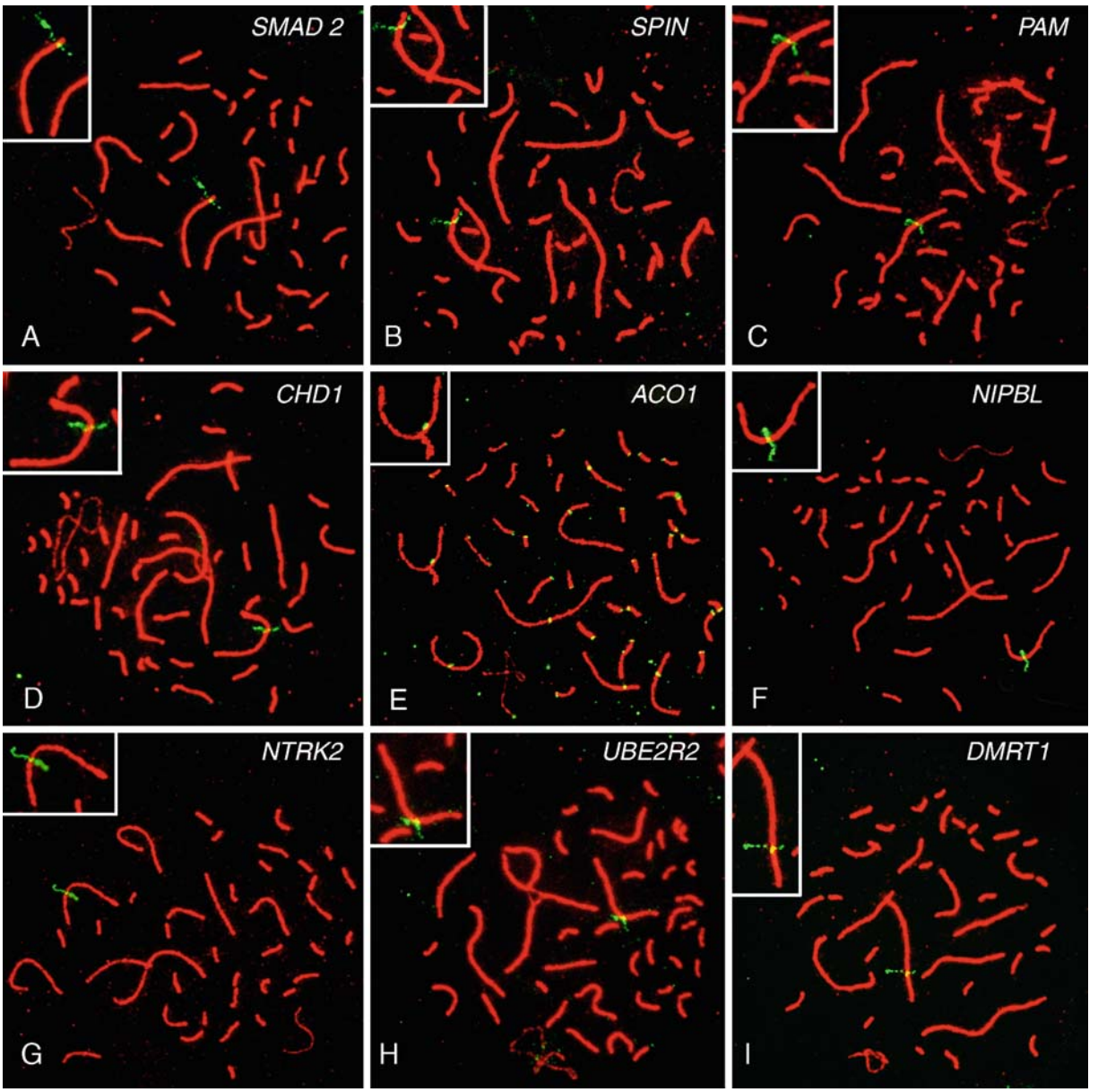

Figure 2. FISH and immunostaining in pachytene spermatocytes. Images of synaptonemal complex spreads showing the localization of nine different genes mapping on the ZZ bivalent. An enlarged view of the ZZ synaptonemal complex bearing the FISH signal (green) is shown at the upper left corner of each nucleus. The names in italic type correspond to the genes contained in each BAC used to prepare the probes. **(A–D)** Genes localized on the short arm. **(E)** FSIH showing the position of *ACO1* on the long arm of the ZZ bivalent along with immunostained centromeres with CREST serum (green dots). **(F–I)** genes mapping on the long arm.

Table 1. Gene positions along the SC and the mitotic Z chromosome

Gene	SC spreads		Mitotic chromosome[A]
	Distance (%) ± SD	N	Distance (%) ± SD
SMAD	6.8 ± 0.7	14	7.2 ± 1.8
SPIN	15.2 ± 1.2	15	18.5 ± 2.8
PAM	30.2 ± 1.9	11	28.9 ± 1.5
CHD	38.8 ± 2.6	17	38.6 ± 0.8
Centromere	40.0 ± 3.0	107	42.6 ± 1.1
ACO	47.5 ± 1.1	17	46.7 ± 1.1
NIPBL	55.0 ± 1.7	25	57.8 ± 1.9
NTRK2	66.5 ± 1.5[a]	23	67.7 ± 1.7
UBE2R2	69.1 ± 1.4[b]	15	68.3 ± 0.8
DMRT1	75.0 ± 2.3	20	75.4 ± 3.3

[A]Data from Itoh *et al*. (2006).
[a,b]Values with different superscript letters differ significantly from each other ($P < 0.0001$).

short arm to the signal was measured in at least 11 nuclei and expressed as a percentage of the total length of the ZZ synaptonemal complex (Table 1). The positions of *NTRK2* and *UBE2R2* are too close to discriminate their centromere–telomere orientation by double FISH on mitotic chromosomes (Itoh *et al*. 2006). Unfortunately, simultaneous localization of these probes on SC spreads did not give satisfactory results, but they showed clear signals when used individually (Figure 2G and H). A *t*-test showed that the average distances from pter for these two BACs are significantly different, with NTRK2 being more proximal with respect to the centromere (Table 1). Comparisons between the positions obtained using FISH on SCs and the respective positions obtained on the mitotic Z chromosome show an excellent linear relationship with a regression coefficient of 0.99 ($y = 0.988x + 1.17$). This is the first point-by-point comparison of positions along a mitotic chromosome and its corresponding SC in a bird, an important fact to support interpolation of physical locations between mitotic chromosomes and their SCs.

Genetic distances based on the MLH1-cM map

The location of the centromeres and MLH1 foci were measured on spreads showing full SC sets. In 103 pachytene spreads analysed, most ZZ bivalents showed almost invariably two foci, one on each arm, with an average of 2.01 foci (Figure 3A). In this sample, only one bivalent had three foci: one on the short arm and two on the long arm. Most foci (95%) were located in 20% of the synaptonemal complex length, clustered towards the telomeric regions of the bivalent as shown in the frequency distribution histogram (Figure 3B). In order to build this histogram, the relative distance from the centromere to each focus was converted to μm by multiplying the relative value by the standard length of the ZZ bivalent (see Materials and Methods). The frequency of MLH1 foci in each 0.25 μm interval of the histogram was converted to a centimorgan value and then added along the SC length from the telomere of the short arm to give a cumulative map of 100.5 cM. The number of centimorgans per 0.25 μm interval shows large variations, from 0 in several intervals around the centromere to a value of 15 cM at two intervals at subtelomeric regions. Owing to this particular distribution of crossover events, the cumulative frequency of cM values along the ZZ bivalent shows fast increments over the intervals closer to pter, then a large plateau with virtually 0 cM values and finally another peak towards the telomere of the long arm (Figure 3B). In the present analysis, these cM values were related to physical distances measured in μm or as a percentage of the SC length. However, rough estimates of cM values per Mb can be obtained along the Z chromosome of the zebra finch based on the conserved chromosome size and gene content with respect to its chicken homologue (Itoh *et al*. 2006). In the chicken, the Z chromosome represents about 7% of the haploid genome and it has around 70 Mb (Schmid *et al*. 2005, Wahlberg *et al*. 2007). Assuming an even distribution of DNA along the SC, each 0.25 μm interval of the zebra finch ZZ bivalent represents 1.25 Mb (70 Mb divided by 56 intervals) and the average recombination rate can be estimated as 1.43 cM/Mb, compared with 2.7 cM/Mb in the chicken (Wahlberg *et al*. 2007).

Each interval with its cM value calculated from the MLH1 focus map can be related to a specific relative position along the SC. Because the position of each BAC located by FISH is also expressed as a relative value with respect to the SC length, cM distances can be obtained from pter to each gene mapped along the Z chromosome. Figure 4 summarizes the physical map (SC-FISH map) obtained by FISH to the ZZ bivalent and the MLH1-cM map with the corresponding positions expressed as cM values. In this graph, the location of the pseudoautosomal

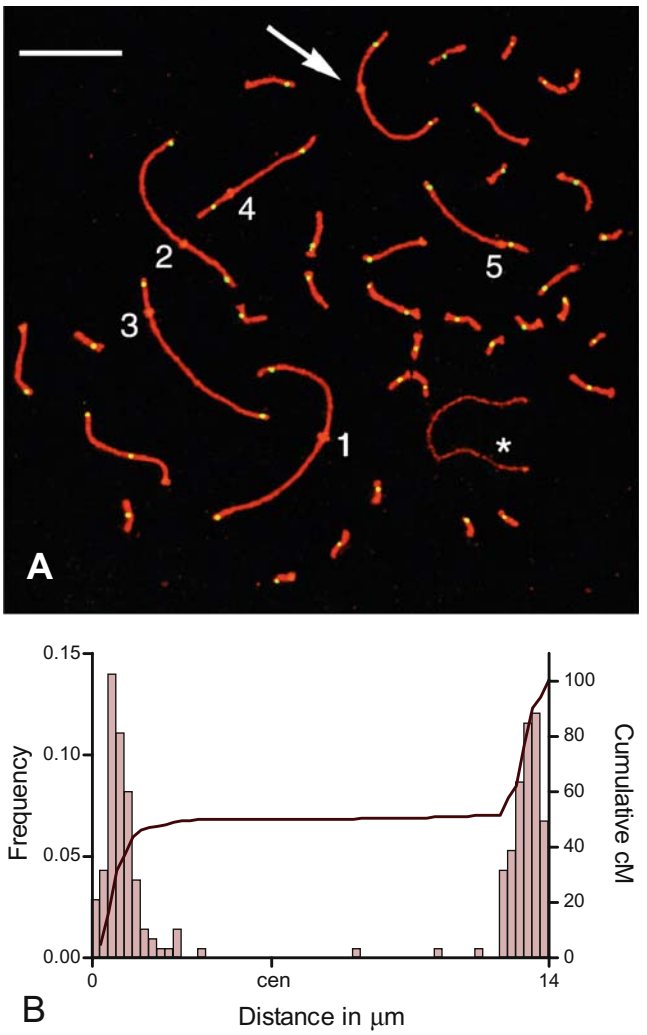

Figure 3. MLH1 foci on the ZZ bivalent. **(A)** Complete SC set immunostained with anti-SMC3 antibody and CREST serum (red) and anti-MLH1 antibody (green). The arrow points at the ZZ synaptonemal complex showing one MLH1 focus on each arm. Centromeres appear as red dots bulging outside the linear SCs. Asterisk: germ-line restricted chromosome. **(B)** Histogram showing the distribution of MLH1 foci along the ZZ synaptonemal complex, represented along the *x*-axis. Each interval is 0.25 μm long, the short arm is to the left and the position of the centromere is marked 'cen'. The line superimposed on the RN distribution gives the cumulative cM value for each interval along the chromosome. The map length in cM is represented on the right *y*-axis and adds to a total of 100.5 cM.

region (PAR) is based on the observation of a single recombination nodule or MLH1 focus near the tip of the short arm in the ZW bivalent (Pigozzi & Solari 1998, Calderón & Pigozzi 2006).

The plot of physical distances in μm obtained for each gene mapped along the SC against the cM distances calculated from the MLH1-cM map shows a strong lack of linearity (Figure 5). This relationship should be expected owing to the large variations in recombination rates that generate hot regions of crossing-over around the telomeres and a large stretch with 0 cM values in the middle. An immediate practical consequence for linkage map experiments is that many more meioses will be necessary to obtain accurate marker positions from the middle of the short arm to about 2 μm from the end of the long arm. This region with low recombination rates represents almost 50% of the

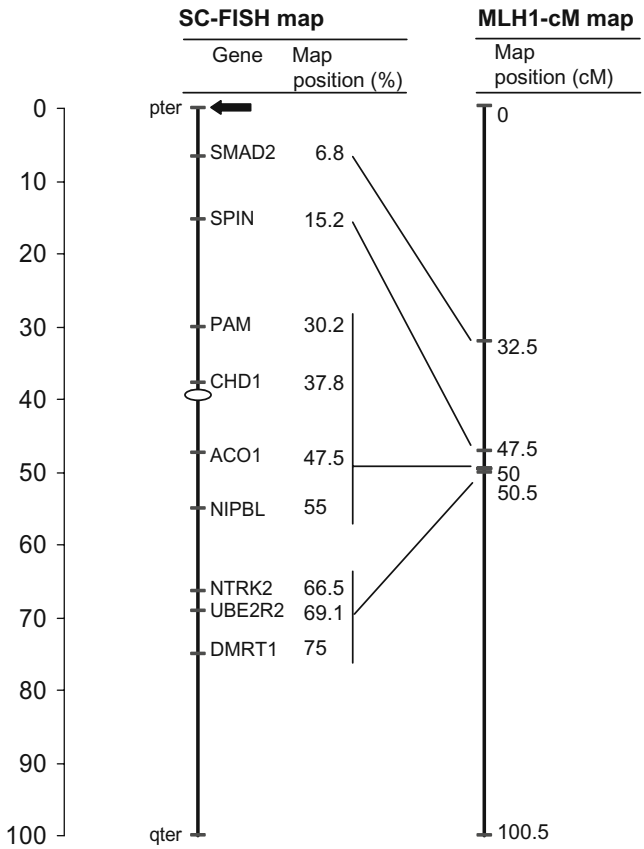

Figure 4. Physical map and cM distances along the ZZ bivalent. The SC-FISH map shows the physical positions of the nine genes mapped along the SC and the centromere position. Map positions are expressed as percent values of the total SC length according to the scale on the left. The black arrow at pter points at the location of the pseudoautosomal region, which is based on recombination nodule location in ZW pairs. The MLH1-cM map shows the cumulative cM distances from pter to the end of the long arm (100.5 cM). The horizontal ticks are the map positions of each gene and are linked by straight lines to the corresponding positions along the physical SC-FISH map. Note the clustering of markers in the region from 30% to 75% of the chromosome.

chromosome size or 35 Mb, calculated from approximate Mb values (see above).

Discussion

The ZZ synaptonemal complex and its relationship to the MLH1-cM map

In several avian species, including the zebra finch, relative lengths and centromere position on the largest SCs show good agreement with those from mitotic chromosomes (Rahn & Solari 1986, Pigozzi & Solari 1998, 1999a). This correspondence can be extended to interstitial positions as shown here comparing the relative positions of nine genes along the ZZ synaptonemal complex and those reported using FISH on the mitotic Z chromosome of the zebra finch (Itoh *et al*. 2006). This agreement regarding gene location and centromere index in mitotic chromosomes and their SCs differs strongly from observations in mammals. It has been shown that gene positions with respect to the centromere are different in mitotic compared to meiotic chromosomes in mice (Froenicke *et al*. 2002). Similarly, several differences were observed in relative length and arm ratios between mitotic chromosomes and their SCs in human spermatocytes (Sun *et al*. 2004). These discrepancies lie in the differential packing of the DNA along the axial elements of the highly specialized meiotic chromosomes. The main components involved in this differential distribution of the

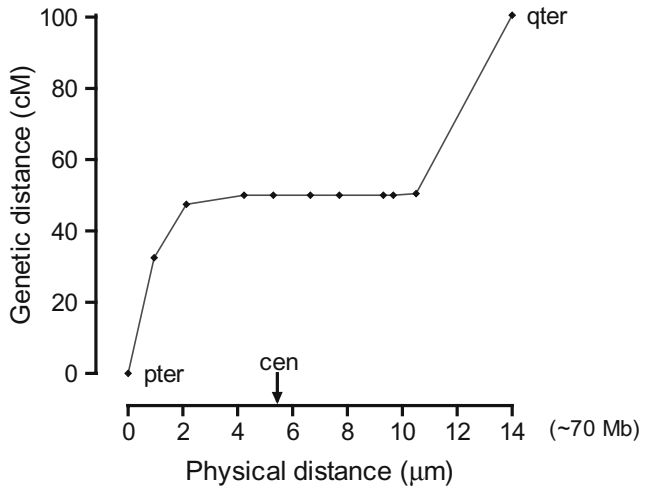

Figure 5. Relationship of physical and genetic distances along the Z chromosome. Dots represent the positions of the nine genes mapped by FISH and the tips of the synaptonemal complex (pter and qter). The arrow shows the position of the kinetochore (kc). The *x*-axis represents the whole SC length divided in μm intervals. The estimated size of the chromosome, ~70 Mbp, is shown at the end of the axis (see Results for further details).

DNA are repetitive sequences enriched in mammalian G or R bands and those sequences forming heterochromatin (Stack 1984, Zickler & Kleckner 1999, Hernandez-Hernandez *et al*. 2008). Since birds have comparatively less heterochromatin and repeat DNA than mammals (Organ *et al*. 2007), it is possible that their DNA is more evenly distributed along the SCs, leading to a better agreement of physical positions with mitotic chromosomes. Establishing this correspondence is valuable because, at least in the zebra finch, it makes possible extrapolation of cytological mapping data from the mitotic Z chromosome directly onto the ZZ synaptonemal complex. Because genetic distances can be charted directly onto synaptonemal complexes using an MLH1-cM map, physical positions along an SC can be translated into cM distances along a particular pachytene bivalent, as shown here for the Z chromosome. At present, the reverse procedure, i.e. predicting chromosomal location of genetically mapped markers, cannot be tested in the zebra finch because linkage maps are not yet available for this species. This kind of prediction has been possible using RN-cM maps for each linkage group in maize (Anderson *et al*. 2004) and for chromosome 1 in tomato (Chang *et al*. 2007). For maize chromosomes, a translator program is available online that converts the cM distances of markers on a linkage map to its physical location on an SC expressed in percent units (Lawrence *et al*. 2006). Considering these preceding results, it should be possible to assign the physical location of genetic markers along a zebra finch chromosome using the corresponding MLH1-cM map.

Different recombination patterns in the zebra finch and the chicken Z chromosome

Because the sequencing of the zebra finch genome is in progress, it is especially useful to have an estimate of the relationship of genetic and physical distances in this species. The present results on cM distances (MLH1-cM map) and physical mapping (SC-FISH map) along the zebra finch Z bivalent show clearly that tight linkage is not indicative of physical proximity along most of the Z chromosome. The MLH1-cM map built from 103 meioses shows 7 genes—from PAM to DMRT1—grouped within 0.5 cM, while physical mapping by FISH shows that they are located in a segment comprising 45% of the chromosome length. Conversely, markers that are physically close to telomeric regions appear separated by comparatively large cM distances from telomeres. Even though lack of proportionality between genetic and physical maps is expected due to variations of recombination levels along a chromosome, the results in the zebra finch are especially relevant because they depart from the general pattern of recombination among birds based on chicken

genomic analysis. In the chicken, recombination varies slightly over the chromosomes and linear regression equations can be generated for each linkage group to interpolate genetic distances between homologous loci mapped in other birds (Schmid *et al*. 2005, Dawson *et al*. 2007). Two main differences can be pointed out regarding crossing-over between the chicken and the zebra finch Z chromosome: the existence of higher crossover rates in the chicken, and stronger localization of crossovers in the zebra finch. The total map length of the zebra finch Z chromosome spans 100.5 cM, one-half that of the chicken Z chromosome, which is 200 cM (Wahlberg *et al*. 2007). A plot of genetic and physical distances in the chicken shows stepwise increments from pter (Wahlberg *et al*. 2007), differing markedly from the picture in the zebra finch (see Results). These differences in crossover frequency and distribution are not restricted to the ZZ bivalent but are also present in autosomal macrobivalents of these two species (Calderón and Pigozzi 2006). Moreover, differences in recombination rates have been observed between other passerines and the chicken at homologous loci (Backström *et al*. 2006, Dawson *et al*. 2007). The reasons for these differences are unknown, although diversified life histories of passerines and the chicken may be involved. To corroborate this hypothesis data on selection of beneficial traits and their coupling with recombination rates are necessary; therefore, full interpretation of these findings must await additional research.

On the other hand, practical consequences in linkage analysis based on parameters established from chicken data can be pointed out considering the substantial differences in this regard from other birds. Since the release of the genome sequence of *Gallus gallus*, the linkage map of this species has offered a framework for genome comparisons in other birds (Ellegren 2005, Backström *et al*. 2008). In these comparative genomic studies, reference markers derived from the chicken genome can be used to study avian genome evolution in a wide range of birds. The bases of these comparisons are the conservation of genome size and low rate of chromosomal rearrangements among birds (Derjusheva *et al*. 2004, Itoh *et al*. 2006, Gregory *et al*. 2007). However, as previously discussed, recombination patterns depart from this conservative scheme in a number of avian species (Calderón & Pigozzi 2006, Backström *et al*. 2006, Dawson *et al*. 2007). Consequently, linkage map efforts initially based on parameters derived from the chicken genome should give only an approximation to cM distances in other birds. A preliminary evaluation of recombination by an MLH1-cM map could facilitate the building of linkage maps in other birds, providing information about the minimum number of meioses necessary to give reliable positioning of markers and helping to design linkage map strategies fitted to particular crossover patterns.

Note added in proof

Recently, a whole-genome linkage map of the zebra finch was published based on SNP (single nucleotide polymorphism) analysis. This study estimates that the length of the Z-chromosome map is 32.8 cM, based on 61% coverage of this linkage group (Stapley et al 2008).

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