A BIRD´S-EYE VIEW OF CHROMOSOMES DURING MEIOTIC PROPHASE I

UNA VISTA PANORÁMICA DE LOS CROMOSOMAS EN LA PROFASE I DE LA MEIOSIS

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ABSTRACT

The present review aims to summarize the research carried out in relation to meiosis in birds, especially by observing the protein axes of the chromosomes in prophase I of meiosis. This line of research, initially developed in Argentina, has provided key data in the study of the evolution of sex chromosomes and the mechanisms involved in the frequency and distribution of crossing over in birds, among other topics. Some of these contributions, in addition to those made by other authors, are described also providing the general theoretical framework or the hypotheses that support them.

Key words: crossing over, meiosis, sex chromosomes, synaptonemal complex

RESUMEN

La presente revisión tiene por objetivo resumir las investigaciones realizadas en relación a la meiosis de las aves, especialmente mediante la observación de los ejes proteicos de los cromosomas en la profase I de la meiosis. Esta línea de investigación, desarrollada inicialmente en Argentina, ha aportado datos clave dentro del estudio de la evolución de los cromosomas sexuales y los mecanismos involucrados en la frecuencia y distribución del *crossing over* en las aves, entre otros temas. Algunas de estas contribuciones, además de las realizadas por otros autores, se describen proporcionando también el marco teórico general o las hipótesis que las sustentan.

Palabras clave: complejo sinaptonémico, cromosomas sexuales, entrecruzamiento, meiosis

During meiosis, a single event of DNA replication is followed by two rounds of cell divisions; as a result, haploid gametes are originated. Besides the function of reducing the DNA content by a half, key events of meiosis are the occurrence of chromosome synapsis and homologous recombination during the prophase of the first division. During prophase I, chromosomes remain in an organized, individualized state for an extended period and the biochemical complexes responsible for recombination at the DNA level are physically associated with underlying chromosome protein axes. After synapsis, these proteinic axes become part of the synaptonemal complex (SC) that tethers the homologs together until recombination intermediates are solved as crossovers (COs) or non-crossovers (Zickler and Kleckner, 2015). Because the SC is almost universally present among eukaryotes, labeling its protein components provides a framework to investigate the molecular factors involved in synapsis and recombination by cytological methods in a large variety of organisms. Electron microscopy and the use of immunofluorescent techniques in nuclei with preserved meiotic axes are especially useful for cytogenetic studies of early meiosis in organisms as different as yeasts and higher plants and animals (Ashley and Plug, 1998; Zickler and Kleckner, 1999; Rockmill, 2009; Stack and Anderson, 2009; Thomas and McKee, 2009; Ribagorda *et al.*, 2019; Cuñado, 2020). The formation of double-strand breaks (DSBs) that initiate recombination interactions, the number and distribution of crossover events, synaptic abnormalities in the presence of chromosome rearrangements, sex-chromosome specific features, and the time course of meiotic proteins in wild-type *vs.* meiotic knock-out organisms, are some of the features that can be analyzed by looking at meiotic chromosome axes or the SCs and their associated proteins (Ashley *et al.*, 1995; Baudat *et al.*, 2000; Merico *et al.*, 2003; Garcia-Cruz *et al.*, 2009; Phillips *et al.*, 2013; Anderson *et al.*, 2014; Sciurano and Solari, 2014). In the present work, the contributions of SC analyses to the knowledge on meiosis and cytogenetics in birds are reviewed. First, the main features of the mitotic karyotype of birds are described to give a background of the contributions of prophase chromosome studies to avian cytogenetics. An outline of the prophase I is presented in the second section to introduce specific terminology related to the chromosome axes and some of the proteins involved in synapsis and recombination. The third section features the behavior of the sex chromosomes in avian oocytes and a comparison between them and the XY pair of mammals. The last part summarizes the current knowledge on crossover rates in birds inferred from immunocytological analysis of pachytene chromosomes.

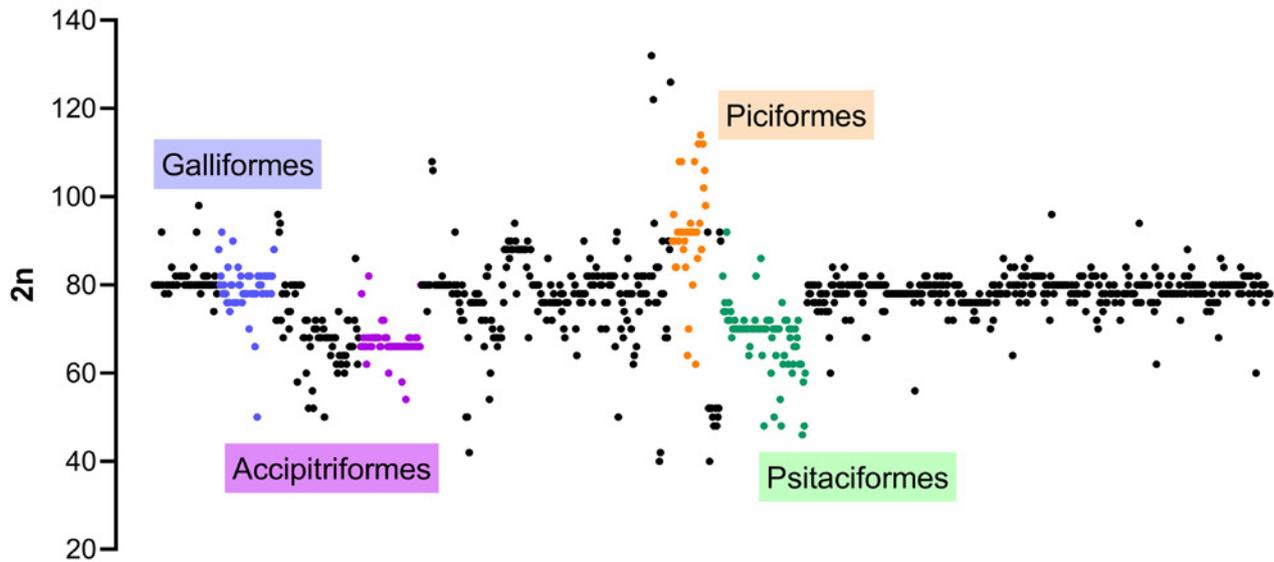## 1. Main features of the avian karyotype

Most avian karyotypes have diploid chromosome numbers between 78-82 (Figure 1), with the presence of numerous microchromosomes (~30 pairs) that are often undistinguishable even with a combination of cytogenetic and genomic methods (Damas *et al.*, 2018; Kretschmer *et al.*, 2018). As a consequence, the regularly sized chromosomes have been broadly analyzed but microchromosomes remain largely uncharacterized. In this context of apparent stasis, there are karyotype variations that, at least in some cases, correspond to birds of the same taxonomic group. For example, diploid numbers higher than 100 have been scored in several species of Piciformes, but they also occur in other orders (Degrandi *et al.*, 2020). Raptors (Accipitriformes and Falconiformes) tend to have smaller chromosome numbers, from 48 to 66, resulting from fusions of ancestral macro- and microchromosomes. Low diploid numbers are also present among Psittaciformes, but differently from birds of prey, there is a marked difference of size between macro- and microchromosomes (Kretschmer *et al.*, 2018).

Comparative FISH mapping using whole-chromosome painting probes and locus-specific probes, as well as genomic studies at the chromosome level ("chromonomics") show the existence of extensive chromosome conservation, with a comparatively small number of interchromosomal rearrangements in species with diploid numbers from 78 to 92 (Shetty *et al.*, 1999; Griffin *et al.*, 2007; Ellegren, 2010; Romanov *et al.*, 2014; Kretschmer *et al.*, 2018). Together with a conserved karyotypic structure, species of birds separated by more than 80 million years of evolution show a high degree of evolutionary stasis at the levels of nucleotide sequence and gene synteny (Zhang *et al.*, 2014a). The chicken karyotype has served as the model for comparative chromosome and genomic studies and it is considered to be close to the ancestral avian karyotype (Griffin *et al.*, 2007; Damas *et al.* 2018). Even though specific probes for each chicken microchromosome were designed (Masabanda et al., 2004), they are not stable and, as a consequence, it is not possible to identify all chicken chromosomes in mitotic metaphases or meiotic cell spreads. Moreover, in the last build of the chicken genome sequence, five linkage groups are still not assembled (Warren *et al.*, 2017), pointing out the difficulties that high chromosome numbers impose on both cytogenetic and genomic studies.

All birds studied so far show female heterogamety and, consequently, the sex chromosomes are named Z and W. In most cases, the Z chromosome is the 4th in size and the W chromosome is comparatively small and heterochromatic. The extent of morphological

differentiation of the sex chromosomes varies throughout the avian phylogeny: less differentiated sex chromosomes are found in the basal Palaeognathae, especially ratites, while highly heteromorphic sex pairs are present in the rest of contemporary birds included in Neognathae (Ansari et al., 1988; Pigozzi, 1999). The

cytogenetics of mitotic and meiotic chromosomes from ancestral avian groups, such as ratites and tinamous from South America, was essential to unravel the main steps of avian sex chromosome evolution (Fridolfsson et al., 1998; Pigozzi, 1999; Tsuda et al., 2007).

Figure 1. Diploid chromosome numbers in birds. Each dot represents the diploid number (2n) of a species. The groups displayed in colors illustrate orders in which most species have either the typical avian karyotype (Galliformes), or diploid numbers are often below or above the average. Data to build the graph were downloaded from the Bird Chromosome Database (BCD, 2021).

2. Visualization of prophase I events by immunocytology

Prophase I is divided into leptotene, zygotene, pachytene, diplotene and diakinesis. As chromosomes individualize and condense during early prophase (lepto-zygotene), the sister chromatids become organized along structures called axial elements (AEs). Axial elements from homologous chromosomes are "zippered" together by the insertion of the central region. At zygotene, the paired AEs are incorporated into the SC structure as part of the lateral elements (LEs). By the beginning of pachytene, the chromosomes achieve a state known as synapsis, when the four chromatids are aligned and held together by the SC. At this point, the SC consists of the paired LEs, and a central region comprised of transverse filaments distributed asymmetrically between the LEs and the central element, which runs midway through the central region. The meiotic axes components comprise axis-associated proteins, cohesin complexes

and cohesin regulators. Homologous proteins have been identified in different species and are particularly well characterized in *Saccharomyces cerevisiae*, *Mus musculus*, *Caenorhabditis elegans*, *Arabidopsis thaliana*, and *Drosophila melanogaster*. While the cohesin components have relatively high degree of homology, the axis-associated proteins are poorly conserved at amino-acid sequence level and were identified as homologs based on *in vivo* and *in vitro* data (Grey and de Massy, 2021).
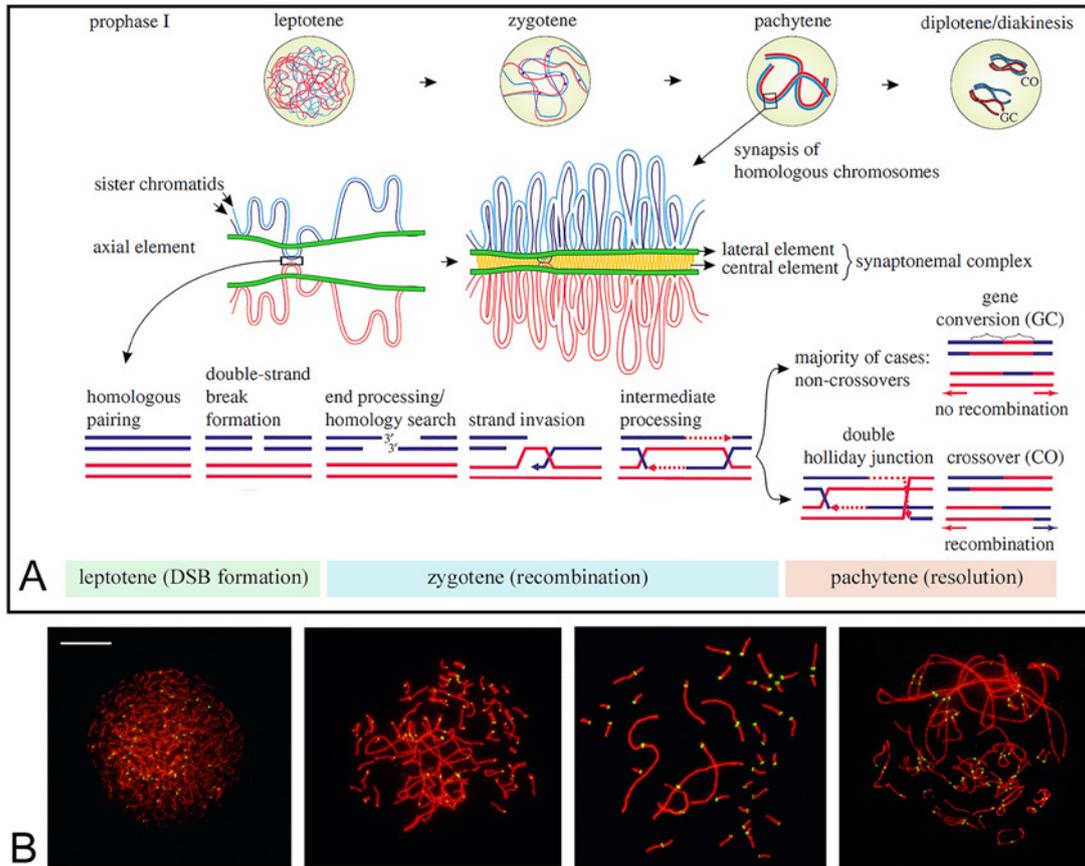
One of the main mechanisms by which homologous chromosomes are maintained together involves crossing over, which is the result of recombination events that are initiated by DSBs (Gerton and Hawley, 2005). DSBs are introduced by the Spo11 protein, a relative of archaeal topoisomerase VI (Keeney, 2001). These breaks occur during leptotene, in coincidence with axial element formation and homolog pairing. In most organisms, meiotic DSBs are processed to produce single-stranded, recombinase-bound ends that search

for homologous chromosomes and give rise to double Holiday junctions (dHJs) (Sun *et al.*, 1989; Schwacha and Kleckner, 1995). The resulting recombinational interactions are biased to occur between homologous chromosomes, in contrast to somatic recombination, which occurs almost exclusively between sister chromatids (Hunter, 2015). A large number of DSBs are introduced throughout the genome and most of them are repaired following a pathway that gives non-recombinant products (Lam and Keeney, 2014). A subset of DSBs however, are repaired by the formation of one-ended strand-exchange intermediates, called single-end invasions (SEIs), which are considered the earliest detectable crossover-specific joint molecules (Hunter and Kleckner, 2001; Zhang et al., 2014b). The formation of SEIs is coincident with chromosome synapsis, reflecting the interdependence between the initiation of synapsis and the initial differentiation of crossover and non-crossover pathways (Figure 2A). Different organisms rely on different methods for this process of homolog matching and genetic exchange. In yeast and mice, recombination is necessary for recognition and pairing of homologous chromosomes, while other organisms such as *Caenorhabditis elegans, Drosophila melanogaster*, are not entirely dependent on recombination for homologous chromosome synapsis and, instead, recognition and pairing involve *cis* elements, such as heterochromatin, centromeres and pairing centers (McKim *et al.*, 1998; Mahadevaiah *et al.*, 2001; Gerton and Hawley, 2005; Baudat *et al.*, 2013).
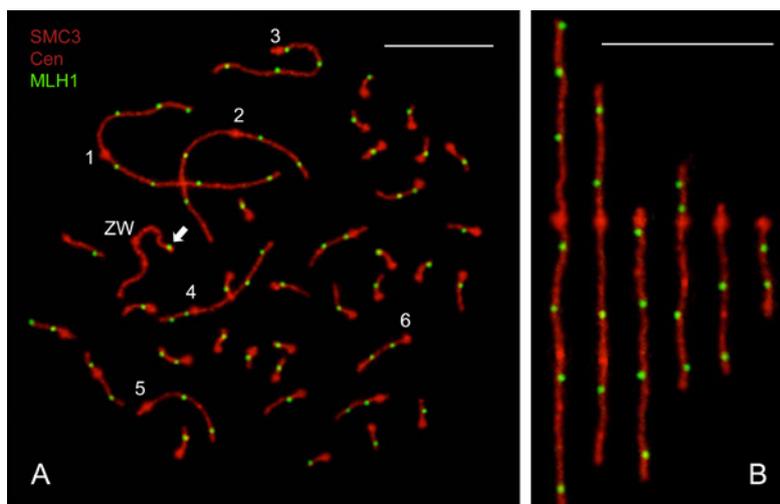
Homologous axes and the SC were initially observed by electron microscopy in sections or microspreadings of meiotic nuclei in a large variety of organisms, including yeast, insects, plants, and vertebrates (Solari, 1981; von Wettstein *et al.*, 1984; Stack and Anderson, 2009). Following the identification of SC protein components (see Fraune et al., 2012 for a review of the mammalian SC), it was possible to develop specific antibodies that delineate the meiotic axes during prophase I (Figure 2B). Immunostaining of meiotic axes and other proteins can be used in combination with FISH probes for centromeres, telomeres, or individual chromosome sites, to evaluate the progression of prophase I, the behavior of specific chromosomes, or the time course of events leading to CO designation and resolution (Ashley and Plug, 1998; Sciurano and Solari, 2014; Dia *et al.*, 2017). For example, the protein MEI4, accessory to the endonuclease Spo11, shows the precise moment of DSB formation at leptotene; the complex Rad51/Dmc1 is one reporter of DSB processing and single-strand DNA invading ends; detection of MSH4 and MSH5, implies stable dHJs determined to be COs and, the MutS homolog, MLH1, is a component of late (recombination) nodules so its detection labels the sites of the CO events at pachytene (Ashley *et al.*, 1995; Baker *et al.*, 1996; Lynn *et al.*, 2007; Kumar *et al.*, 2015). The mentioned

and many other proteins with key roles in synapsis and recombination are evolutionarily conserved. An advantage of this feature in cytology is that antibodies developed against a protein in a model species such as mice recognize the orthologue in other vertebrates or even in insects, opening the investigation of prophase I events in non-model organisms (Pigozzi, 2001; Viera *et al.*, 2004; Lisachov *et al.*, 2019). More specifically in birds, the immunolocalization of the protein MLH1 has been employed to count CO events on pachytene macro and microbivalents in SC spreads (Figure 3). As previously mentioned, the majority of meiotic COs follow pathways that are conserved in budding yeast, mammals and other organisms. The resolution of the produced dHJs requires the endonuclease activity of the MLH1-MLH3 DNA mismatch repair factor to be resolved exclusively into a CO product. In this conserved pathway, MLH1 protein tag a subset of CO events that show chiasmatic interference, while a second type of COs (non-interfering) follows a molecular pathway lacking MLH1 (reviewed in Pannafino and Alani, 2021). Therefore, the immunostaining for MLH1 labels most CO events, but a fraction escapes the detection with this methodology. Depending on the organism, non-interfering COs represent 5–30% of all CO events (de Boer *et al.*, 2006; Falque *et al.*, 2009). The presence of two classes of COs in birds is not confirmed, but both, recombination nodules and MLH1 foci, show CO interference (Pigozzi and Solari, 1997; Pigozzi, 2001). Comparative counts of CO markers at pachytene and diakinesis -MLH1 foci/ recombination nodules *vs.* chiasmata- in chickens, quails and pigeons show none or only slight differences within the same species supporting the view that MLH1 foci account for most CO in birds (Pigozzi and Solari 1999b; Pigozzi, 2001; Rodionov and Chechik, 2002; del Priore and Pigozzi, 2015).

SC spreads from birds are also useful to determine diploid numbers and to compare mitotic and meiotic (SC) sets. SC karyotyping revealed a germ-line restricted chromosome, first in the zebra finch (*Taeniopygia guttata*), and later in other songbirds (Torgasheva *et al.*, 2019), in a clear example that cytogenetic analyses of mitotic and meiotic chromosomes are not just a companion to genomic studies, but become necessary to provide a comprehensive understanding of any species genome.

Figure 2. Chromosome axes and main events during meiotic prophase I. A. Chromosome organization during meiotic prophase I is exemplified with two pairs of homologous chromosomes, each split into two sister chromatids (red and blue lines). Following synapsis, the axial elements (in green) become the lateral elements of the synaptonemal complex. The bottom part of the figure shows the events of meiotic recombination at the DNA level. Meiotic recombination starts with the formation of double-strand breaks (DSBs) at leptotene and is completed before the end of pachytene. Reproduced with modifications from Mirzaghaderi and Hörandl (2016); CC by 4.0. B. Immunofluorescence staining of synaptonemal complex and centromere components on chicken oocyte spreads. From left to right the stages are leptotene, zygotene, pachytene and diplotene. An antibody against the cohesin component SMC3 was used to visualize chromosome axes (red) and CREST serum to label centromere proteins (green). Reproduced from del Priore and Pigozzi (2012); Copyright© 2012 Karger Publishers, Basel, Switzerland.

Figure 3. Immunolocalization of recombination events at pachytene. A. immunostained chicken oocyte showing the complete set of synaptonemal complexes labeled with anti-SMC3 and the crossovers detected with anti-MLH1. The ZW pair has a single MLH1 focus located near the homologous end of the bivalent (arrow). The six largest autosomal bivalents have a number next to the centromere signal (red protruding marks). B. The synaptonemal complexes of the six largest bivalents were digitally straightened to enable the comparison in size and position of the centromeres. Bars = 10 µm.

3. The meiotic ZW pair: similarities and differences with the XY pair of mammals
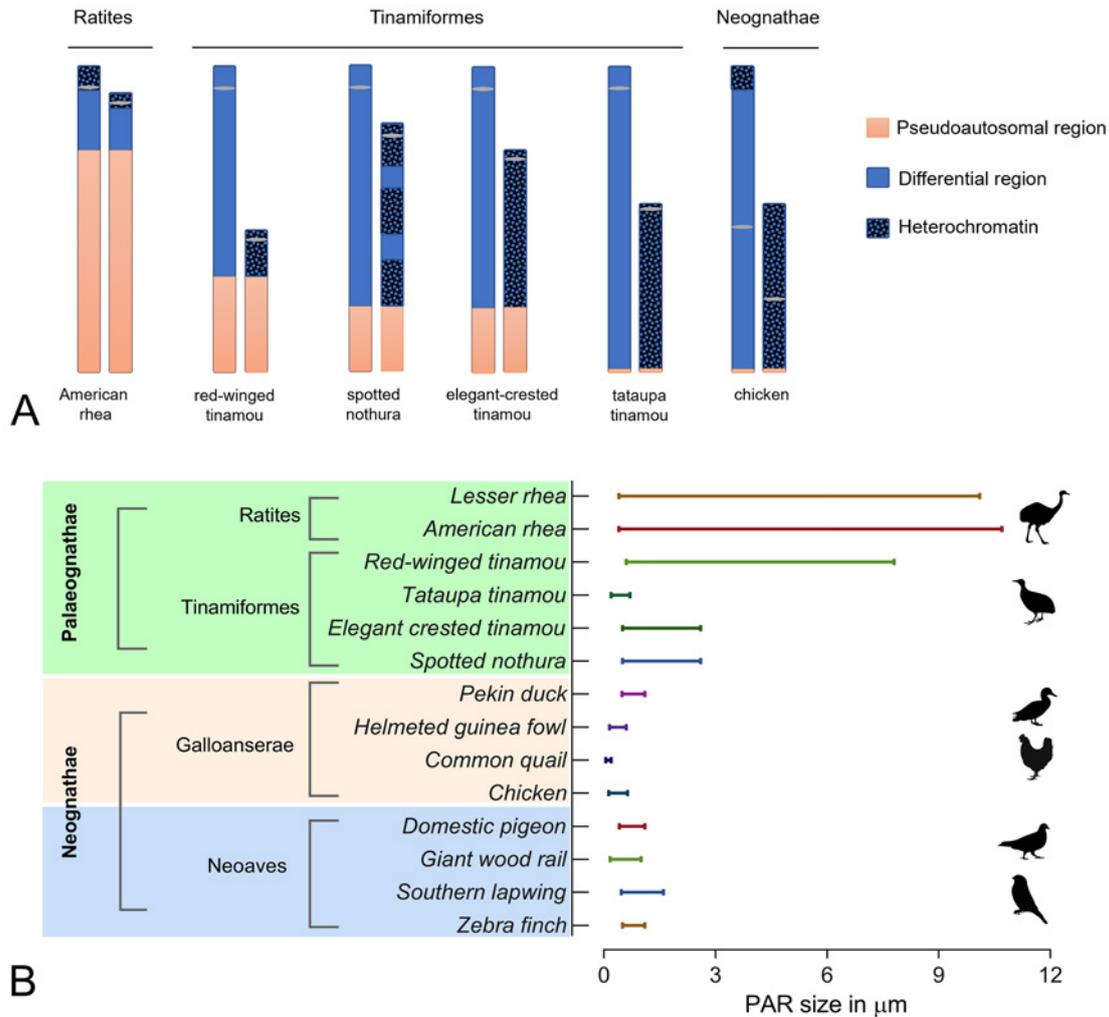
Like in mammals, female meiosis of birds starts during embryonic development. In the chicken, oocytes progress through early first prophase before hatching and most of them reach the pachytene stage around hatching day (Hughes, 1963; Smith *et al.*, 2008; Zheng *et al.*, 2009). A long and incompletely characterized diffuse stage is observed five days after hatching (Wylie. 1972); this stage is followed by the reemergence of individualized chromosomes in the form of lampbrush bivalents that are recognizable in the growing intrafollicular oocytes after 2–3 weeks of post-hatching development, remaining visible in the adult ovary of hens (Gaginskaya and Chin, 1980; Hutchison, 1987).

The synaptic and chiasmatic nature of the ZW pair was first demonstrated in the chicken after staining of SCs and recombination nodules for electron microscopy (Solari, 1977; Rahn and Solari, 1986a). Subsequent studies in other birds with highly heteromorphic sex pairs led to characterization of the meiotic behavior of the sex bivalent, consisting of: a) the formation of a fully synapsed bivalent despite lack of homology in most of the Z and W length; b) the occurrence of a single recombination event at subtelomeric position; c) the existence of a synaptic adjustment of the length of the Z and W axes, a process that now is known to occur in heteromorphic sex chromosomes of other organisms; and, d) the lack of heteropicnosis or condensation of the sex bivalent chromatin (Solari, 1992; Solari and Pigozzi, 1993; Pigozzi and Solari, 1999a). From the observations of the Z and W meiotic axes and the associated chromatin, it was inferred that the sex pair of birds and mammals shared some similarities -*e.g.*, the existence of a pseudoautosomal region, PAR- but also had fundamental differences accordingly to their evolution from different ancestral autosomes in each lineage (Solari, 1993; Bellott *et al.*, 2017).

The empirical and theoretical research about sex chromosome differentiation and the evolutionary forces that shape this process have been reviewed in depth by experts in the field (Rice, 1984; Charlesworth, 1991; Charlesworth *et al.*, 2005; Bachtrog *et al.*, 2014). Here, a brief background is provided for better understanding the role of the meiotic ZW pair analysis in the research on avian sex chromosome differentiation. Heteromorphic sex chromosome pairs are thought to have originated as autosomal homologs following the random acquisition of a mutation that transformed a gene into a sex-determining locus (Rice, 1984; Charlesworth, 1991; Charlesworth *et al.*, 2005; Bergero and Charlesworth, 2009). Recombination suppression may initially encompass only a small chromosomal segment surrounding that locus, but then progressively spreads along Y or W chromosomes. A

consequence of this recombination suppression on the Y/W chromosomes is the loss of almost all functional genes present on the ancestral chromosome except for a few loci, often with sex-specific functions, and the acquisition of repetitive sequences and heterochromatin in the non-recombining region of the Y/W (Daish and Grützner, 2019). The region of the sex chromosomes that retains homology is referred to as PAR because this chromosomal segment recombines in both sexes and is functionally not hemizygous in the heterogametic sex (Burgoyne, 1982; Ellis and Goodfellow, 1989). One way to demonstrate homology is to determine the extension of the recombining segment in the sex bivalent of the heterogametic sex, for example using cytogenetic markers of crossing over.

In contrast with mammals, where homomorphic XY pairs are not found, nearly homomorphic ZW pairs are present in all ratites, a group of palaeognathus birds that includes ostriches in Africa, rheas in South America, emus in Australia, cassowaries in Australia and New Guinea, and kiwis in New Zealand. Mapping of recombination nodules/MLH1 foci in SC spreads in both species of rheas showed the existence of homologous recombination over most of the W chromosome, and therefore the presence of a large PAR (Pigozzi and Solari, 1997; Pigozzi and Solari, 1999c; del Priore and Pigozzi, 2017). The homology in the ZW of rheas comprises over 70% of the Z chromosome length, as assessed by recombination nodule and MLH1 focus mapping. Instead, these recombination markers are restricted to a small terminal segment -less than 5% of the Z chromosome- in birds with highly differentiated sex chromosomes (Solari and Pigozzi, 1993; Pigozzi and Solari, 1998b; Pigozzi and Solari, 1999b). Along with ratites, Palaeognathae also includes the monophyletic group of tinamous, that are ground-dwelling birds found in Central and South America. Cytogenetics of female meiotic prophase and sequence comparison of the Z and W chromosome within this avian group revealed important insights into the sex chromosome evolution. Recombination nodule and MLH1 mapping in tinamous uncovered examples of intermediate stages of sex chromosome differentiation (Pigozzi and Solari, 1999c; Pigozzi and Solari, 2005; Pigozzi, 2011). In three of the four tinamid species where meiotic recombination was examined, the PAR is larger compared to neognaths, but shorter compared to ratites, while recombination is restricted to a small segment, comparable to the chicken, in the tataupa tinamou (Figure 4). These results implied different paths of genetic degeneration and the probable existence of different evolutionary strata in the ZW pair of birds. Analyses of sequence read depths between the Z/W relative to autosomes demarcated the PAR and the non-recombining differentiated region in 17 species spanning the entire avian phylogeny (Zhou *et al.*, 2014). The non-recombining regions between

Figure 4. Steps of the sex chromosome differentiation among living birds. A. Spectrum of sex chromosome morphologies in primitive and modern birds. The relative size of the pseudoautosomal region (PAR) in each ZW pair represents the segment with recombination nodules or MLH1 foci in the meiotic bivalent at pachytene. The differential regions are represented in blue, with a black pattern when heterochromatin is present in the C-banded mitotic chromosomes. The ZW pair of chicken is upside down to locate the homologous end towards the same side as in the other species. B. Comparative graph of the PAR sizes in Palaeognathae and Neognathae. Each line represents the size of the PAR in μm, from the homologous telomere of the ZW pair to the more distal recombination nodule or MLH1 focus in each species.

Z and W of many species exhibit a complex pattern of "evolutionary strata" resulting from the suppression of recombination in a stepwise and independent manner among some lineages. Another finding of this study is that the W chromosome sequence in some Neognathae is not completely degenerated and that they have relatively long PARs compared to the chicken where only 28 of the 685 ancestral genes remain on the W chromosome (Bellott *et al.*, 2017). Remarkably, recombination analysis in pachytene also predicts different PAR sizes among Neognathae, even within closely related species (Figure 5). For example, the position of the single recombination nodule in the ZW pair of the domestic quail indicates a smaller recombining segment compared to that of the chicken, and the recombining segment in Southern lapwing (*Vanelus chilensis*, Charadriiformes) is larger compared to that of other neognaths, a feature that was also reported for two other species of the same order (Lisachov *et al.*, 2017).
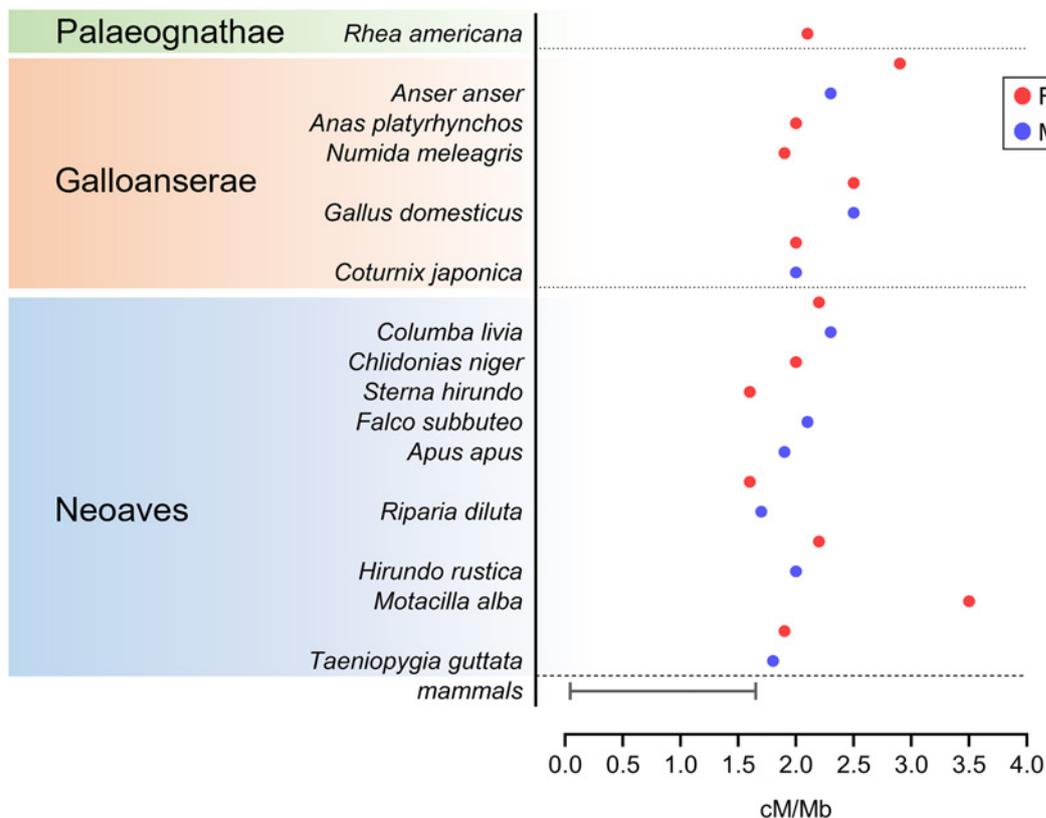
The sex chromosomes of birds and mammals originated independently from different ancestral autosomes after the two lineages diverged more than 300 million years ago (Daish and Grutzner, 2009; Zhou *et al.*, 2014). The ZW and XY pairs are not orthologous: genes that are sex linked in birds are autosomal in mammals, and vice versa (Nanda *et al.*, 1999; Bellott *et al.*, 2010). During this parallel evolution and resultant to the morphological differentiation process, birds and mammals developed different tolerance to the presence of unpaired, non-homologous segments of the sex chromosomes during the first meiosis. The differential regions of the X and Y chromosomes of mice and other therian mammals remain largely unsynapsed at prophase I, and their chromatin is

condensed forming the XY-body observed in pachytene spermatocytes; instead, the ZW pair of birds undergo complete synapsis and lack any sign of condensation at pachytene (Solari, 1974; Pigozzi, 2016). These differences may respond to different ways to deal with unsynapsed heterologous chromosome segments during gametogenesis. In eukaryotes, an ancestral and evolutionarily conserved response is induced when any pairing problem between homologous chromosomes results in asynapsis. This response involves the silencing and epigenetic modification of the unpaired segments, a phenomenon called meiotic silencing of unpaired chromatin (MSUC) (reviewed in Turner *et al.*, 2005). Its manifestation on the sex chromosomes is the meiotic sex chromosome inactivation or MSCI that occur during prophase I in mammalian spermatocytes due to the unsynapsed heterologous segments of the X and Y. While MSUC is an ancient response to unpaired DNA and part of a checkpoint, MSCI is restricted to the sex chromosomes of therian mammals (Turner *et al.*, 2005; Turner, 2007). A prominent epigenetic marker of MSCI is the histone variant γ-H2AX which decorates the chromatin domains of the asynaptic regions of the X and Y chromosomes at pachytene in marsupials and eutherians (Fernandez-Capetillo *et al.*, 2003; Turner *et*

al., 2004; Franco *et al.*, 2007). Failure of MSCI results in the activation of a pairing checkpoint that ultimately causes spermatocyte elimination by apoptosis, and therefore this phenomenon has direct relevance to the cause of genetic disease and fertility (Turner *et al.*, 2006; Sciurano *et al.*, 2012). In the chicken, the gene expression during the meiotic prophase could not find evidence of an inactivation of sex-linked loci (Guioli *et al.*, 2012). Unlike the mammalian XY, the H2AX phosphorylation in the ZW pair is compatible with the timing of DSB occurrence and processing and it is lost by the pachytene stage. Further, the presence of repressive marks such as H3K9me3 are limited to the W chromosome and the terminal heterochromatin of the Z chromosome (del Priore, 2011; Guioli *et al.*, 2012). Altogether these observations support the notion that MSCI is absent in the avian ZW pair.

The suggestion that heterologous synapsis in the avian ZW pair helps to avoid an unpaired DNA checkpoint in oocytes awaits further investigations. Looking at the synaptic process in heterozygotes for chromosome rearrangements and direct analyses of the checkpoint proteins present during meiotic prophase in birds can provide a means to test this hypothesis.

Figure 5. Genome wide recombination rates (RRs) in birds obtained by direct counts of crossovers at pachytene. The number of recombination nodules or MLH1 of foci were multiplied by 50 to obtain the genetic map length in cM and then divided by the genome size (in Mb) of each species to obtain the RRs. The range of the RRs in mammals was obtained from Dumont and Payseur (2008) and from Segura *et al.* (2013).

4. Cytogenetic recombination maps in birds

Recombination rates (RRs), that is, the number of recombination events per Mb per generation, are known to vary between species, between individuals, and even between sexes within the same species. The RRs can be described by the genome wide recombination (how many CO events occur per meiosis) and also by the variations of the CO locations within a genome (recombination landscape). There is a growing interest in comparative studies of the RRs across taxa with the aim to understand how they evolve and how they impact on other evolutionary processes within sexually reproducing organisms (Dumont and Payseur, 2008; Capilla *et al.*, 2016; Stapley *et al.*, 2017). RRs in birds have been studied by using polymorphic DNA markers on the same chromosome in crosses or pedigrees, as well as in genome-wide linkage disequilibrium investigations. (Aslam *et al.*, 2010; Ball *et al.*, 2010; Singhal *et al.*, 2015; Kawakami *et al.*, 2017). These approaches can provide kb-level resolution estimates of recombination, but using this information to compare average genome-wide RRs between taxa is difficult since independent analyses differ in marker density or genome coverage. A method to obtain the global RRs for direct comparisons is to score the number and distribution of cytological markers of crossing over, such as recombination nodules or MLH1 foci at pachytene (see section 2). In birds, recombination nodules were visualized using phosphotungstic acid staining of spread oocytes and spermatocytes at pachytene (Pigozzi and Solari, 1998a; Pigozzi and Solari, 1999a). Even though in certain birds like the domestic pigeon recombination nodules are well-preserved, in other species like the chicken or the zebra finch they are small and their staining differ between nuclei (Rahn and Solari, 1986b; Pigozzi and Solari, 1998b). For this reason, the cytogenetic studies of recombination are mainly represented by immunostaining of MLH1 foci. So far, recombination nodules or MLH1 foci have been scored in oocytes and/or spermatocytes of 14 species from eight orders that belong to the three main avian radiations: Palaeognathae, Galloanserae and Neoaves (Table 1; Figure 5). Even though the total number of species is scarce, some features can be pointed out. First, the average genome-wide RRs in birds are higher compared to mammals and vary within narrower limits: 1.6 cM/Mb and 3.5 cM/Mb for the lowest and highest RRs recorded in birds compared to 0.18 cM/Mb (in the elephant shrew) and 1.78 cM/Mb, in the Chinese muntjac, among mammals (Dumont and Payseur, 2008; Yang *et al.*, 2011). Another feature is that RRs show larger differences between species of the same order than between species of different orders (for example ducks and geese vs. guinea fowl and zebra finch), suggesting that RRs do not follow a phylogenetic trend. In mammals, different types of recombination analyses, including MLH1-focus counts, suggest the existence of a phylogenetic effect in RRs, with more closely related species having more similar average rates of recombination (Dumont and Payseur, 2008; Dumont and Payseur, 2011; Segura *et al.*, 2013). Overall, the evolutionary forces behind the observed distributions of genomic RRs in birds and mammals are not clear. Careful partition of the source variation at the individual, population or species level is needed for better understanding of the genetic and environmental components of RR variations. In order to obtain this information, empirical observations should be obtained in hypothesis-based studies, and in parallel, theoretical models on recombination should be developed to address empirical data (Dapper and Payseur, 2017).

The cytological observation of the crossing over also offers the opportunity to analyze the positions of COs along chromosome arms. In most species, COs are found anywhere along macrochromosome arms occurring at higher frequencies near telomeres. This CO distribution is found in the American rhea, the chicken and the domestic pigeon, among other birds (del Priore and Pigozzi, 2017; Malinovskaya *et al.*, 2019). Another, less common pattern of CO distribution, is a strong polarization of COs towards the chromosome ends, with scarce COs at mid chromosome regions. An extreme example of CO localization is found in the zebra finch (Estrildidae, Passeriformes) where as much as 80% of the total amount of recombination is concentrated on the 20% distal parts of the largest bivalents (Calderón and Pigozzi, 2006; Stapley *et al.*, 2010). Localized COs were also observed in the guinea fowl, a species that is related to domestic chicken, indicating that variable CO landscapes are not related to the phylogenetic position (del Priore and Pigozzi, 2020). The fact that COs are localized in the macro-SCs of the guinea fowl but show a more uniform distribution in the chicken also indicates that broad-scale recombination does not have a strong relationship with large-scale genomic variation, since both species share extensive identity in karyotype and syntenic blocks (Shibusawa *et al.*, 2004; Vignal *et al.*, 2019). At fine-scale, kb level, high recombination rates (hotspots) are related to CpG islands that are associated to gene promoter regions in birds. These regions have an open chromatin state that could favor the access of the recombination machinery (Singhal *et al.*, 2015; Kawakami *et al.*, 2017). However, unlike the recombination maps observed at cytological level, the distribution of genes does not follow a localized pattern, indicating that hotspots at fine scale do not correlate with broad-scale maps of crossing over. Epigenetic modifications acting to regulate the strength of hotspots might explain the differences in the fine *vs.* broadscale recombination landscapes, as well as differences in recombination observed between closely related species (Kawakami *et al.*, 2017).

Another aspect that can be approached by cytological examination of CO events is the presence and extent of heterochiasmy, that is the situation in which both sexes recombine, but at different rates. Variant recombination amount and diverse CO patterns between sexes are widely extended among vertebrates and many other eukaryotes (Lenormand, 2003; Sardell and Kirkpatrick, 2020). In birds, male *vs.* female recombination rates have relatively small discrepancy between sexes, but in a few cases, differences are more noticeable (Table 1; Figure 5). When intersex recombination differences were detected, the number of COs could be higher in either sex; in the case of different CO distributions in males and females, they could be located more frequently near the centromeres or towards the telomeres, depending on the species (Malinovskaya *et al.*, 2020 and references there in). A common feature underlying intersex recombination differences is the presence of longer SCs in the sex with higher recombination frequency (Lisachov *et al.*, 2017; Torgasheva and Borodin, 2017), in agreement with the interplay between meiotic axial length, arrangement of DNA loops and the frequencies of recombination intermediates that are solved as COs (Kleckner *et al.*, 2003; Ruiz-Herrera *et al.*, 2017).

Overall, the CO patterning observed in birds indicates that different mechanisms operate at wide range scale from the DNA level to the chromosomal level.

The karyotype of birds has low rates of interchromosomal changes, when compared to mammals and non-avian reptiles. Even though the intrachromosomal rearrangements are common, there are very few examples of interchromosomal rearrangements at the level of macrochromosomes and high degree of synteny, even between distantly related avian lineages (O'Connor *et al.*, 2018). This creates an apparent paradox between karyotype and genome conservation and the morphological, physiological and adaptive diversity of birds. A possibility that makes compatible these two facts is that the organization of the bird genome in numerous chromosomes favors genetic diversity by increasing recombination rates and increasing the capacity for random segregation due to the presence of microchromosomes (Ellegren, 2013). Cytological maps of broad-scale recombination supports the idea that karyotype homogeneity does not restrict the advent of recombination variants in macrochromosomes. In other words, variant recombination landscapes found in macrochromosomes (that is, restricted *vs.* non-restricted recombination) also create genetic variation, which contributes to the great phenotypic diversity observed among birds.

Table 1. Recombination rates estimated from recombination nodules (RNs) or MLH1-focus analysis in birds.

Common name	Latin name	Order	Sex	COs per autosomal set		Genetic map (cM)	C value (pg)	Genome size (Mb)	RR (cM/Mb)	Reference
				X	SD					
Greater rhea	*Rhea americana*	Rheiformes	F	58.8	4.4	2940	1.46	1428	2.1	del Priore and Pigozzi, 2017
Greylag goose	*Anser anser*	Anseriformes	F	72.6	7.8	3632	1.3	1408	2.9	Torgasheva and Borodin, 2017
			M	57.9	7.6	2897			2.3	Torgasheva and Borodin, 2017
Domestic duck	*Anas platyrhynchos*	Anseriformes	F	55.9	3.8	2795	1.54	1506	2.0	del Priore and Pigozzi, 2016
Guinea fowl	*Numida meleagris*	Galliformes	F	44.4	1.6	2220	1.22	1193	1.9	del Priore and Pigozzi, 2020
Chicken	*Gallus domesticus*	Galliformes	F	62.1	5.4	3105	1.25	1223	2.5	del Priore and Pigozzi, 2020
			M	61.5	5.6	3075			2.5	Malinovskaya *et al.,* 2019
Japanese quail	*Coturnix japonica*	Galliformes	F	55.3	2.1	2765	1.41	1379	2.0	del Priore and Pigozzi, 2015
			M	56.3	1.8	2815			2.0	del Priore and Pigozzi, 2015
Domestic pigeon	*Columba livia*	Columbiformes	F	62.7	4.9	3135	1.44	1408	2.2	Pigozzi and Solari, 1999a
			M	64.7	4.8	3235			2.3	Pigozzi and Solari, 1999b
Black tern	*Chlidonias niger*	Charadriiformes	F	43.1	5	2155	1.4	1369	2.0	Lisachov *et al.,* 2017
Common tern	*Sterna hirundo*	Charadriiformes	F	52	4.2	2600	1.4	1369	1.6	Lisachov *et al.,* 2017
Eurasian hobby	*Falco subbuteo*	Falconiformes	M	51.1	6.6	2555	NA	1242[a]	2.1	Malinovskaya *et al.,* 2018
Common swift	*Apus apus*	Apodiformes	M	51.4	4.3	2570	NA	1350[b]	1.9	Malinovskaya *et al.,* 2018
Barn swallow	*Hirundo rustica*	Passeriformes	F	55.6	6.3	2780	1.38	1252	2.2	Malinovskaya *et al.,* 2020
			M	49	4.5	2450			2.0	Malinovskaya *et al.,* 2020
Pale martin	*Riparia diluta*	Passeriformes	F	44.6	3.6	2230	1.45[c]	1418	1.6	Malinovskaya *et al.,* 2020
			M	48.9	2.4	2445			1.7	Malinovskaya *et al.,* 2020
White wagtail	*Motacilla alba*	Passeriformes	F	76.1	8.6	3805	NA	1072[d]	3.5	Semenov *et al.,* 2018
Zebra finch	*Taeniopygia guttata*	Passeriformes	F	45.7	0.4	2285	1.25	1223	1.9	Calderón and Pigozzi, 2006
			M	45.2	0.2	2260			1.8	Calderón and Pigozzi, 2006

COs crossovers; *X*, average; *SD*, standard deviation; RR, recombination rate.

[a, b, c] Genome sizes were derived from C values reported in Gregory (2021). Genome sizes are unknown for *F. subbuteo, A. apus* and *R. diluta*. The available genome size of the closest species was used; they are *F. eleonorae* (1.27 pg), *A. affinis* (1.38) and *R. riparia* (1.45), respectively.

[d] From *M. alba* genome assembly (https://www.ncbi.nlm.nih.gov/assembly/GCF_015832195.1/#/st).

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