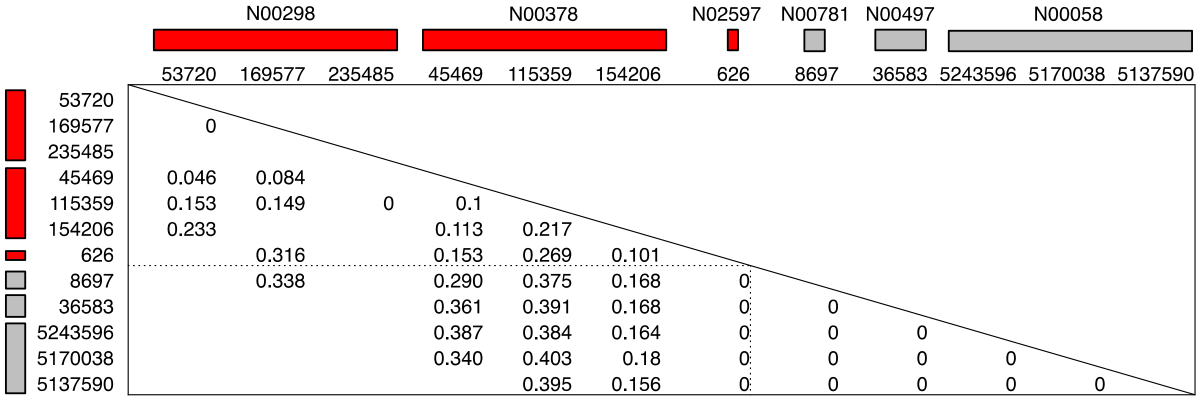
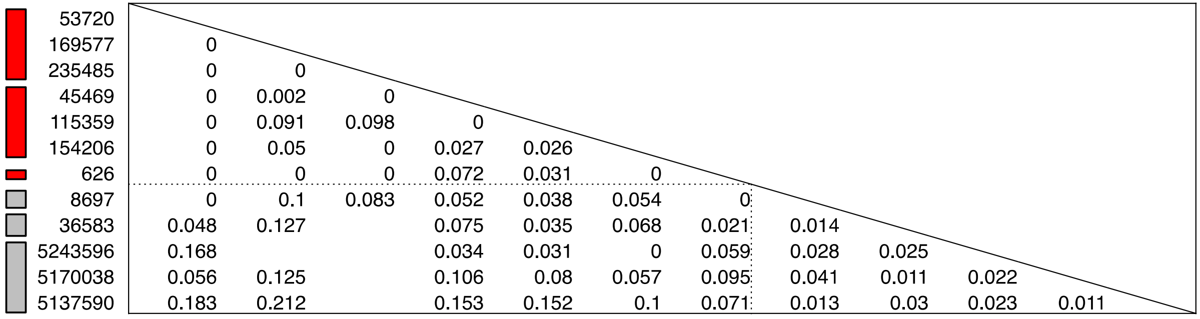
A) Female



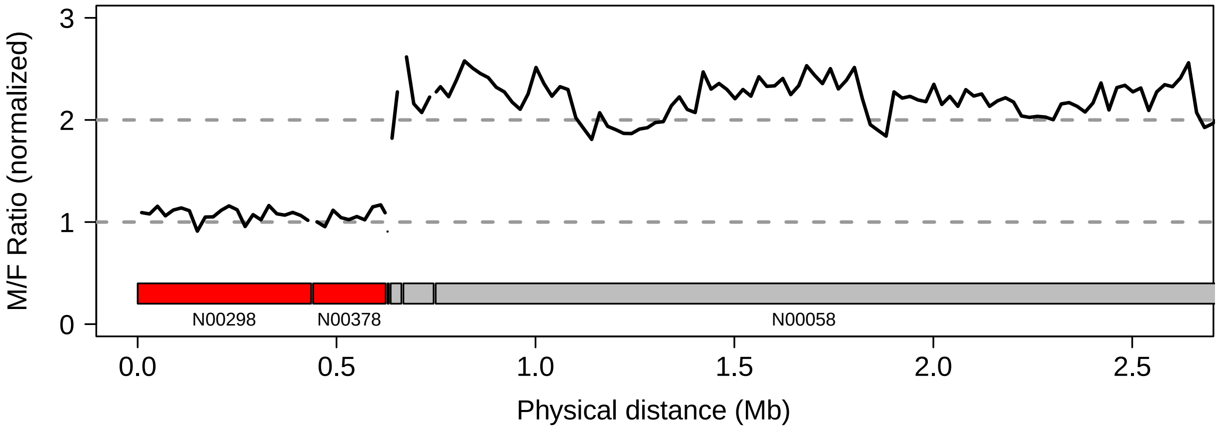
| | N00298 | | | N00378 | | | N02597 | N00781 | N00497 | N00058 | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | 53720 | 169577 | 235485 | 45469 | 115359 | 154206 | 626 | 8697 | 36583 | 5243596 | 5170038 | 5137590 |
| 53720 | | | | | | | | | | | | |
| 169577 | 0 | | | | | | | | | | | |
| 235485 | | | | | | | | | | | | |
| 45469 | 0.046 | 0.084 | | | | | | | | | | |
| 115359 | 0.153 | 0.149 | 0 | 0.1 | | | | | | | | |
| 154206 | 0.233 | | | 0.113 | 0.217 | | | | | | | |
| 626 | | 0.316 | | 0.153 | 0.269 | 0.101 | | | | | | |
| 8697 | | 0.338 | | 0.290 | 0.375 | 0.168 | 0 | | | | | |
| 36583 | | | | 0.361 | 0.391 | 0.168 | 0 | 0 | | | | |
| 5243596 | | | | 0.387 | 0.384 | 0.164 | 0 | 0 | 0 | | | |
| 5170038 | | | | 0.340 | 0.403 | 0.18 | 0 | 0 | 0 | 0 | | |
| 5137590 | | | | | 0.395 | 0.156 | 0 | 0 | 0 | 0 | 0 | |

B) Male

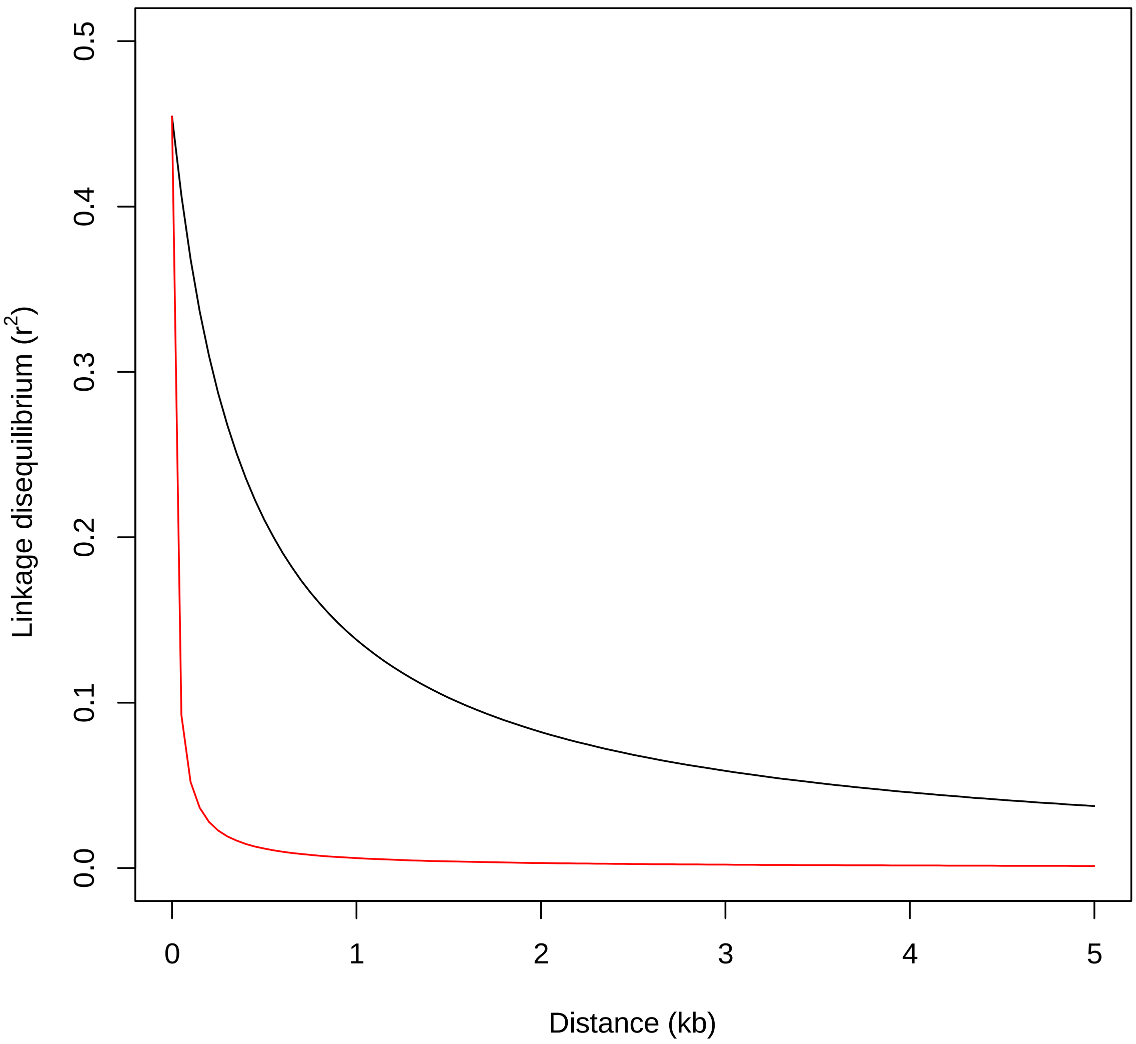


| | N00298 | | | N00378 | | | N02597 | N00781 | N00497 | N00058 | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | 53720 | 169577 | 235485 | 45469 | 115359 | 154206 | 626 | 8697 | 36583 | 5243596 | 5170038 | 5137590 |
| 53720 | | | | | | | | | | | | |
| 169577 | 0 | | | | | | | | | | | |
| 235485 | 0 | 0 | | | | | | | | | | |
| 45469 | 0 | 0.002 | 0 | | | | | | | | | |
| 115359 | 0 | 0.091 | 0.098 | 0 | | | | | | | | |
| 154206 | 0 | 0.05 | 0 | 0.027 | 0.026 | | | | | | | |
| 626 | 0 | 0 | 0 | 0.072 | 0.031 | 0 | | | | | | |
| 8697 | 0 | 0.1 | 0.083 | 0.052 | 0.038 | 0.054 | 0 | | | | | |
| 36583 | 0.048 | 0.127 | | 0.075 | 0.035 | 0.068 | 0.021 | 0.014 | | | | |
| 5243596 | 0.168 | | | 0.034 | 0.031 | 0 | 0.059 | 0.028 | 0.025 | | | |
| 5170038 | 0.056 | 0.125 | | 0.106 | 0.08 | 0.057 | 0.095 | 0.041 | 0.011 | 0.022 | | |
| 5137590 | 0.183 | 0.212 | | 0.153 | 0.152 | 0.1 | 0.071 | 0.013 | 0.03 | 0.023 | 0.011 | |

**Supplementary Figure 1.** Pairwise recombination fractions between SNP markers in the pseudoautosomal region (PAR) and neighbouring SNP markers on sex-specific part of Z chromosome for A) females and B) males. Red and grey rectangles indicate PAR and Z chromosome scaffolds, respectively. Numbers below scaffolds indicate SNP positions on scaffolds. The border between PAR and Z chromosome is indicated by dotted line. Only recombination fractions with LOD score support larger than 3 are shown.



**Supplementary Figure 2**. Male-to-female (M/F) coverage ratio for 20 kb windows in the distal part of the Z chromosome. Scaffolds are indicated below the plot. Coverage was normalized by the average M/F ratio of autosomal scaffolds.



**Supplementary Figure 3**. Relationship between level of linkage disequilibrium ($r^2$) and physical distance between pairs of markers (LD decay curve). Red is the PAR and black is the rest of the Z chromosome. The LD decay curve was estimated by nonlinear regression[1].

**Supplementary Table 1**. Significant two-point linkage between markers from the PAR and markers on the Z chromosome. For each marker from the PAR, linkage to the Z chromosome marker with the strongest LOD score support from three different scaffolds is listed.

| PAR marker | Linked Z marker | Female | | Male | | Sex-average | |
|---|---|---|---|---|---|---|---|
| (scaffold:position) | | Rec | LOD | Rec | LOD | Rec | LOD |
| N00298:53720 | N00781:8697 | 0.483 | 0.89 | 0 | 19.27 | 0.259 | 2.79 |
| | N00497:36583 | 0.500 | 0 | 0.048 | 23.51 | 0.265 | 3.55 |
| | N00058:5170038 | 0.500 | 0 | 0.056 | 20.33 | 0.279 | 2.84 |
| N00298:169577 | N00781:8697 | 0.338 | 3.28 | 0.1 | 4.73 | 0.258 | 1.57 |
| | N00497:36583 | 0.500 | 0 | 0.127 | 4.88 | 0.273 | 1.75 |
| | N00058:5170038 | 0.500 | 0 | 0.125 | 5.24 | 0.293 | 1.09 |
| N00298:235485 | N00781:8697 | 0.497 | 0.01 | 0.083 | 4.76 | 0.224 | 1.44 |
| | N00497:36583 | 0.470 | 0.11 | 0.256 | 0.80 | 0.457 | 0.02 |
| | N00058:5170038 | 0.459 | 0.22 | 0.229 | 2.48 | 0.313 | 0.60 |
| N00378:45469 | N00781:8697 | 0.290 | 8.58 | 0.052 | 22.9 | 0.154 | 6.70 |
| | N00497:36583 | 0.361 | 6.89 | 0.075 | 18.08 | 0.187 | 5.39 |
| | N00058:5170038 | 0.340 | 6.49 | 0.106 | 21.03 | 0.195 | 5.36 |
| N00378:115359 | N00781:8697 | 0.375 | 4.60 | 0.038 | 20.78 | 0.210 | 4.79 |
| | N00497:36583 | 0.391 | 4.24 | 0.035 | 21.97 | 0.205 | 5.66 |
| | N00058:5170038 | 0.403 | 3.47 | 0.080 | 20.65 | 0.227 | 4.51 |
| N00378:154206 | N00781:8697 | 0.168 | 11.35 | 0.054 | 12.61 | 0.117 | 10.89 |
| | N00497:36583 | 0.168 | 11.25 | 0.068 | 14.93 | 0.123 | 11.91 |
| | N00058:5170038 | 0.180 | 10.75 | 0.057 | 14.26 | 0.126 | 11.61 |
| N02597:626 | N00781:8697 | 0 | 27.09 | 0 | 17.16 | 0 | 26.49 |

|  |  |  |  |  |  |  |  |
|---|---|---|---|---|---|---|---|
|  | N00497:36583 | 0 | 30.10 | 0.021 | 13.50 | 0.008 | 24.24 |
|  | N00058:5170038 | 0 | 29.80 | 0.095 | 11.39 | 0.042 | 21.08 |

**Supplementary Table 2**. List of genes in the collared flycatcher PAR, their position in the Z chromosome assembly and male-to-female (M:F) expression ratio.

| Gene symbol | Gene | Ensembl ID | Starts (bp) | Ends (bp) | M:F ratio |
|---|---|---|---|---|---|
| Novel | | ENSFALG00000011552 | 7885 | 3068 | 0.76 |
| Novel | | ENSFALG00000011557 | 61860 | 15716 | 0.94 |
| Novel | | ENSFALG00000011561 | 138014 | 137043 | 0.81 |
| *TXNL1* | thioredoxin-like 1 | ENSFALG00000011580 | 163014 | 148969 | 0.97 |
| *WDR7* | WD repeat domain 7 | ENSFALG00000011623 | 168591 | 202971 | 0.91 |
| *ST8SIA3* | ST8 alpha-N-acetyl-neuraminide alpha-2,8-sialyltransferase 3 | ENSFALG00000011668 | 235245 | 238102 | 1.20 |
| Novel | | ENSFALG00000011567 | 243179 | 147380 | - |
| *ONECUT2* | one cut homeobox 2 | ENSFALG00000011678 | 247536 | 257035 | 0.76 |
| Novel | | ENSFALG00000011685 | 267155 | 262688 | 0.92 |
| *FECH* | ferrochelatase | ENSFALG00000011690 | 292084 | 284455 | 0.91 |
| *NARS* | asparaginyl-tRNA synthetase | ENSFALG00000011698 | 303598 | 298472 | 1.01 |
| *ATP8B1* | ATPase, aminophospholipid transporter, class I, type 8B, member 1 | ENSFALG00000011710 | 320466 | 306830 | 1.04 |
| *NEDD4L* | neural precursor cell expressed, developmentally down-regulated 4-like | ENSFALG00000011721 | 362872 | 427848 | 1.07 |
| *LMAN1* | lectin, mannose-binding, 1 | ENSFALG00000009746 | 458778 | 471081 | 1.04 |

| | | | | | |
|---|---|---|---|---|---|
| *CPLX4* | complexin 4 | ENSFALG00000009751 | 475285 | 483012 | 1.03 |
| *RAX* | retina and anterior neural fold homeobox | ENSFALG00000009755 | 491593 | 493031 | 0.82 |
| *GRP* | gastrin-releasing peptide | ENSFALG00000009759 | 503864 | 499306 | 1.07 |
| *SEC11C* | SEC11 homolog C | ENSFALG00000009763 | 511985 | 508340 | 0.95 |
| *ZNF532* | zinc finger protein 532 | ENSFALG00000009766 | 541001 | 517686 | 0.99 |
| *MALT1* | mucosa associated lymphoid tissue lymphoma translocation gene 1 | ENSFALG00000009773 | 578833 | 562895 | 0.97 |
| *ALPK2* | alpha-kinase 2 | ENSFALG00000009783 | 603034 | 608770 | 0.88 |
| Novel | | ENSFALG00000015559 | 610043 | 609952 | - |

**Supplementary Note 1.** Sex chromosomes in paleognath birds.

The deepest split within modern birds is that between Palaeognathae and Neognathae, with the latter containing >99% of all extant avian species. Cytogenetic work has shown that recombination in female meiosis occurs between large parts of the Z chromosome and the W chromosome in ratites [2-4], the group of flightless birds within Palaeognathae that includes ostrich, kiwi, emu and others. A recent RNA-seq study estimated that approximately two-thirds of the ostrich Z chromosome recombines in female meiosis [5] and this might also be the case in emus [6]. In these species the PAR thus constitute the majority of the Z chromosome (cf. [7]. Since the sex chromosomes in Palaeognathae and Neognathae seem fully homologous [8,9] and because Z-W differentiation started prior to the split of the two lineages 130-150 million years ago [5,10,11], sex chromosome evolution in Palaeognathae has been slow. In contrast, sex chromosome evolution in Neognathae has resulted in advanced Z-W differentiation. This difference may be related to an absence of dosage compensation in non-recombining regions of the ostrich Z chromosome, where male expression is on average twofold higher than female expression [5]. Without a compensatory mechanism, there might be strong selection against expansion of the non-recombining region because hemizygosity of sex-linked genes in the heterogametic sex should perturb gene interactions and networks. In non-recombining regions of neognath Z chromosomes, the degree of dosage compensation varies and may have evolved on a gene-by-gene basis, allowing recombination restriction to spread [12-15].

**Supplementary Note 2.** The flycatcher pseudoautosomal region.

Using the BUILD option for multipoint linkage analysis in CRIMAP, scaffold order N00298-N00378-“all scaffolds from the majority of the Z chromosomes that do not recombine in female meiosis” was supported by a LOD score >5. The order N00298-N00387-N02597-“Z chromosome” was supported by a LOD score >2. The LOD score support for orientation of

N00378 was >3 while for N00298 it was only >0.1. However, independent support for the inferred orientation of N00298 was provided by analyses of mapped mate-pairs from three libraries with insert sizes of 5 kb, 18 kb and 21 kb, respectively (SRA Accession numbers ERX093495, ERX093467 and ERX093470) [16]. Any read that mapped closer to an end than each library's insert size was considered and links between mates on different scaffolds were counted. Mate-pair links were only found between the end of N00298 and the start of N00378. We note that if the orientation of N00298 would be the opposite, it would not affect our recombination rate estimates much since most PAR recombination in females occurred in N00387.

## Supplementary Note 3. Evolution of the avian pseudoautosomal region.

Failure to identify the PAR in chicken [17] and zebra finch [18] genome sequencing projects and cytogenetic evidence for recombination being restricted to the terminus of a small Z-W pairing region in birds from other neognath lineages [19], suggests that a small PAR as identified in flycatcher may be a common feature of neognath sex chromosomes. However, since there are deep, multiple splits within Neognathae (which includes some 20 avian orders), in particular in the group of Neoaves, and because it is well known that sex chromosome evolution has occurred in parallel in different neognath lineages [20,21], it cannot be excluded that sex chromosomes of species from avian lineages yet to be investigated are only intermediately differentiated. It should be straightforward to test this by comparing male and female coverage over the Z chromosome in deep genome sequencing, analogous to what we demonstrate in Fig. 2. Because the end of the flycatcher Z chromosome harbouring the PAR corresponds to one of the ends of the distantly related chicken Z chromosome (**Fig. 3**), it is a strong candidate region for representing the PAR across different bird lineages.

In the absence of an assembled flycatcher W chromosome we cannot date the events leading to recombination restriction between sex chromosomes in this lineage. However, the fact that the male-to-female ratio in read coverage was consistently close to two outside the PAR (**Fig. 2**) indicates that, overall, the degree of any remaining Z-W sequence similarity in the region that most recently ceased to recombine was not sufficient for reads from the W chromosome to map to the Z chromosome. Thus, the situation with a very small PAR may have persisted for a long period of time in the lineage leading to flycatcher.

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