

FIG. S1. Gene synteny among the Z chromosomes. The alignment of coding sequence and plot were implemented by the python package jcv (MCscan). Only scaffolds (bars) longer than 50k were shown. As a showcase, the orientation of scaffold 813 of ostrich was corrected. The ~12M containing scaffolds 816, 79, 179, 347 and a part of scaffold 9 were removed from the ostrich. Mate-pair reads alignment for the breakpoint on the scaffold 9 is shown in S4.



FIG. S2. Alignments of mate-pair reads against the scaffold9 of ostrich. The breakpoint is located at the near-end of the scaffold (~5.6M). The upper panel shows the alignments of 10k mate-pair reads and the bottom panel is for 20k mate-pair reads.

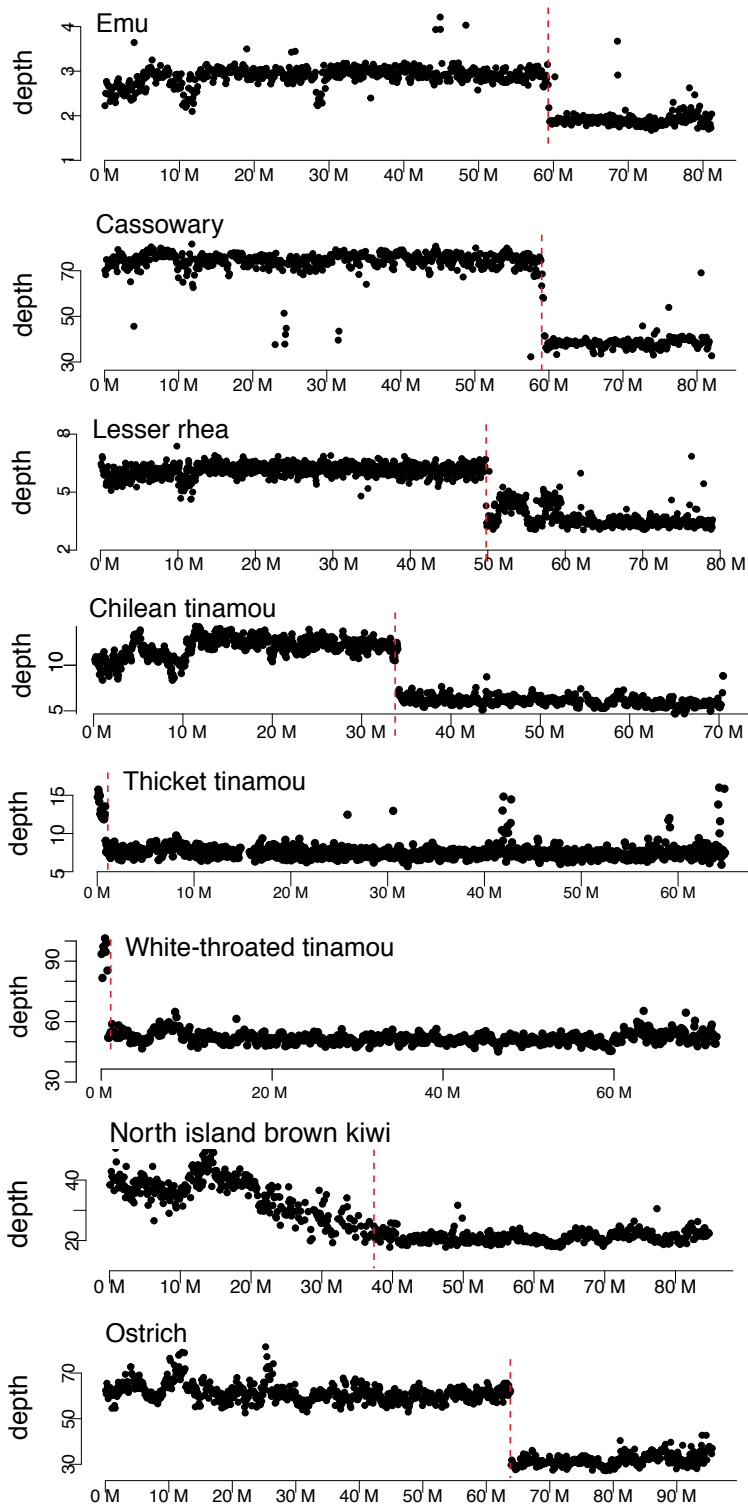


FIG. S3. Coverage of female sequencing reads on the Z chromosome. Each dot represents a 50k window. The red dashed line denotes the boundary of the pseudoautosomal region (PAR) and the differentiated region (DR).

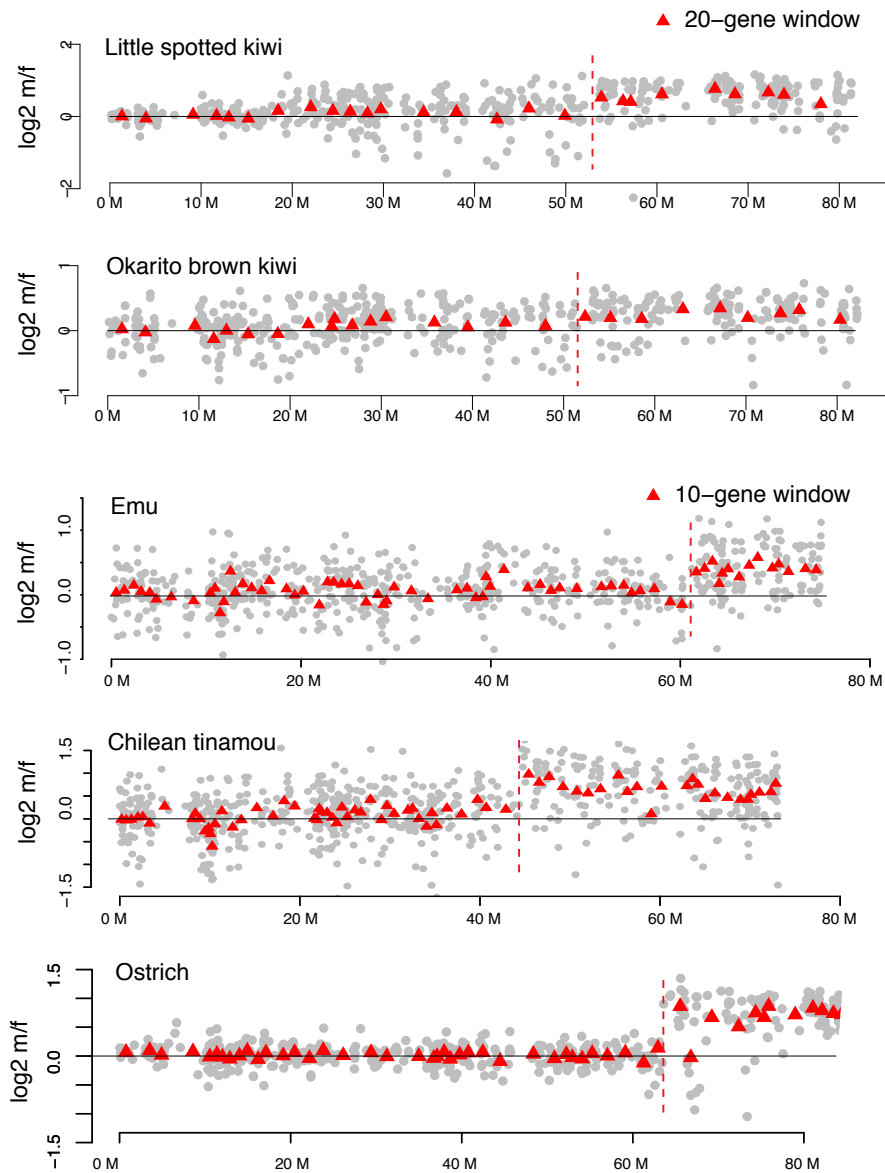


FIG. S4. Male-to-female (m/f) expression ratios for Z-linked genes. The red triangle represents the mean m/f expression ratio. The red dashed line denotes the boundary of the PAR and the DR based on the shift of m/f ratio.

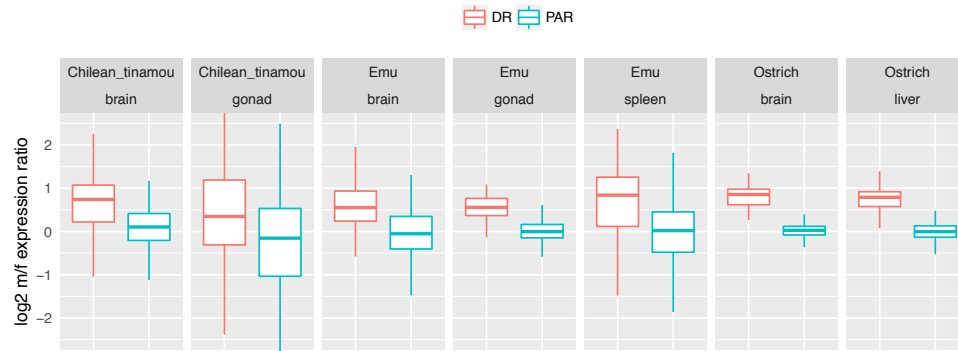


FIG. S5. Male-to-female expression ratios for DR- and PAR-linked genes. For Chilean tinamou, emu and ostrich, RNA-seq data of multiple tissues of both sexes are available. The m/f ratios (log2 transformed) of DR-linked genes are larger than 1 but less than 2, suggesting incomplete dosage compensation, but show limited variation within species.

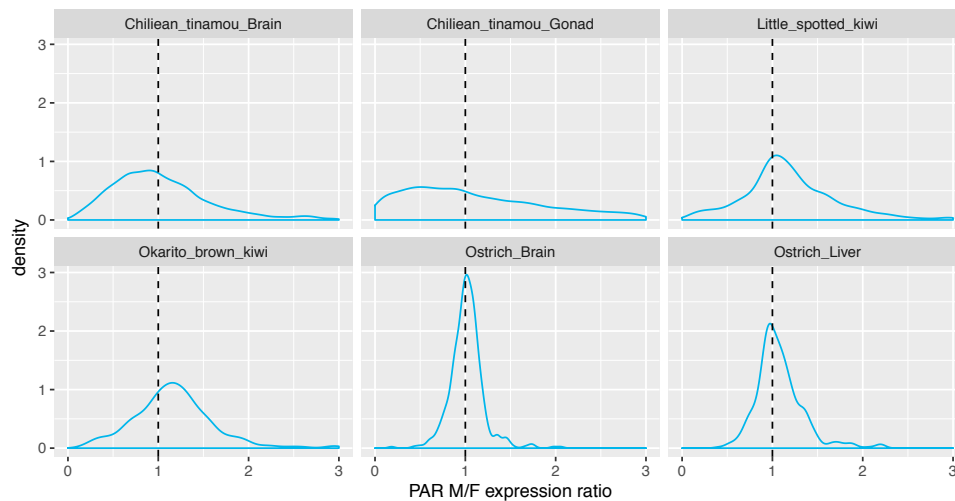


FIG. S6. Distribution of male-to-female expression ratios for PAR-linked genes. In most samples m/f expression ratios do most deviate from 1, suggest similar expression levels of PAR-linked genes between males and females. Only in Okarito brow kiwi, however, male expression levels is slightly higher than for females.

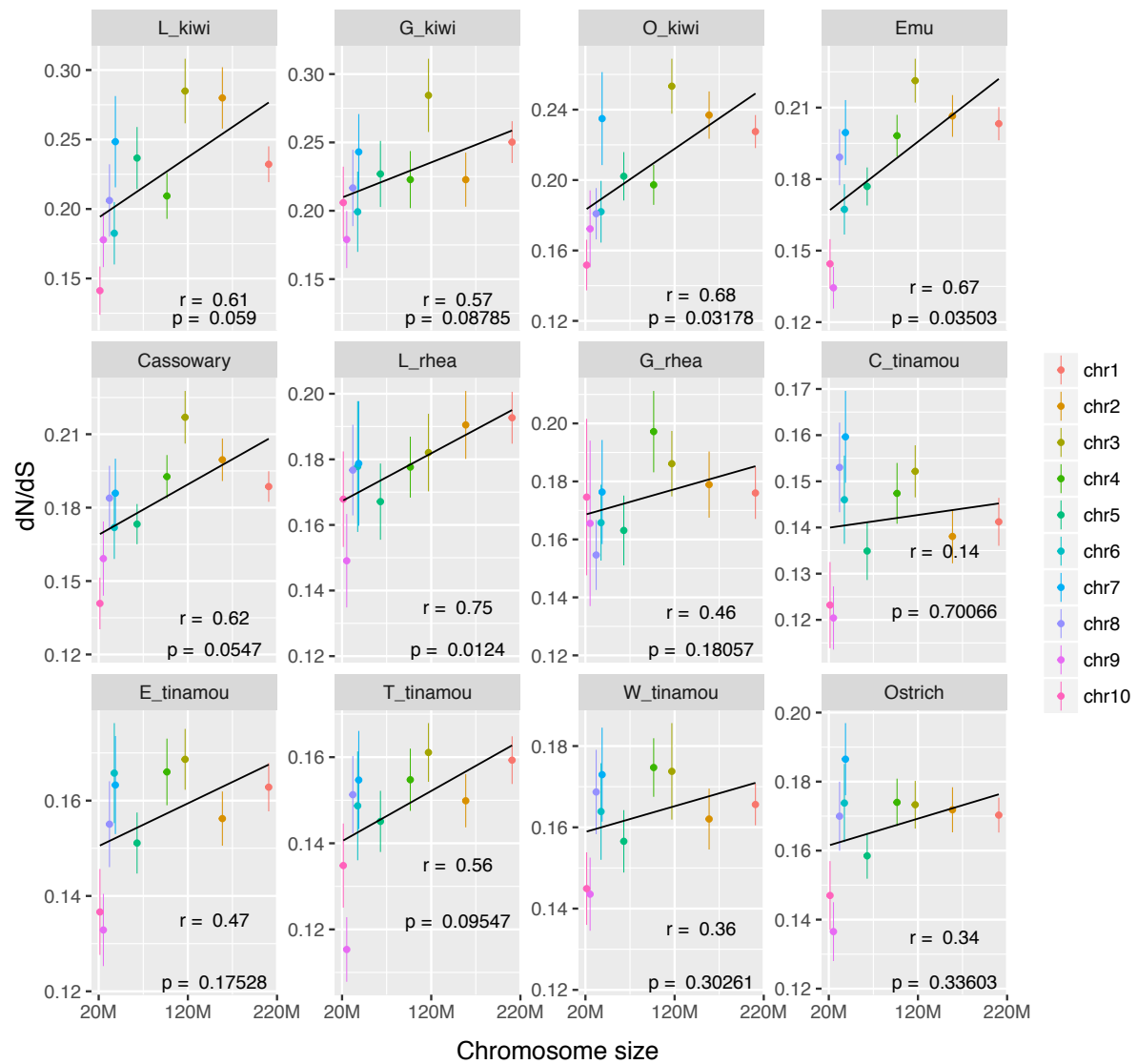


FIG. S7. Positive correlation of dN/dS ratios and chromosome size among macro-chromosomes. Among macro-chromosome (chr1 – chr10), chromosome size positively correlates with dN/dS ratios. The chromosome size of the Z is about 75M, between the sizes of chr4 (~97M) and chr5 (~63M). The 'r' stands for Pearson's correlation coefficient. Abbreviation for species names: L_kiwi, little spotted kiwi; G_kiwi, great spotted kiwi; O_kiwi, Okarito brown kiwi; L_rhea, Lesser rhea; G_rhea, Greater rhea; C_tinamou, Chilean tinamou; E_tinamou, elegant crested tinamou; T_tinamou, thicket tinamou; W_tinamou, white-throated tinamou.

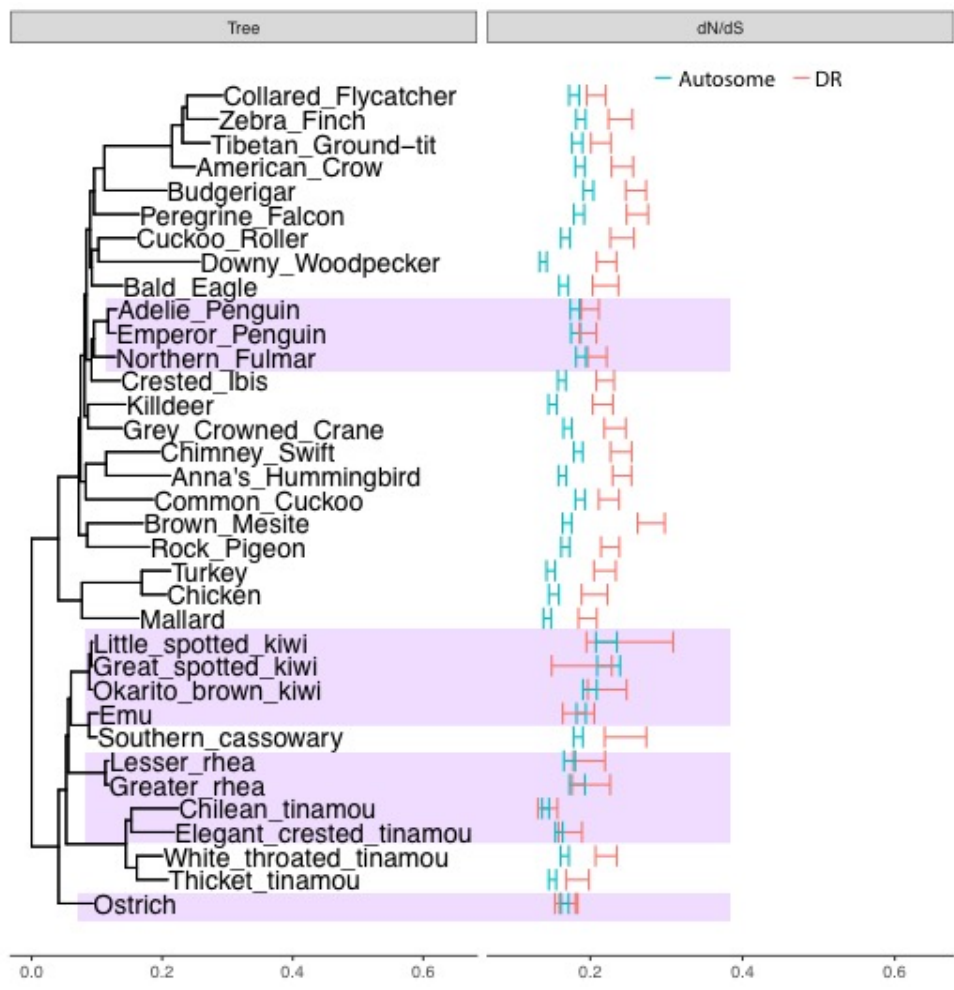


FIG. S8. A lack of faster-DR in most paleognaths. The PAR-linked genes were removed from the analysis. Species without faster-DR effect (permutation test, $P > 0.05$) were highlighted by purple colour. The faster-Z effect is no longer observed in Okarito brown kiwi, elegant crested tinamou and thicket tinamou after PAR-linked genes were removed.

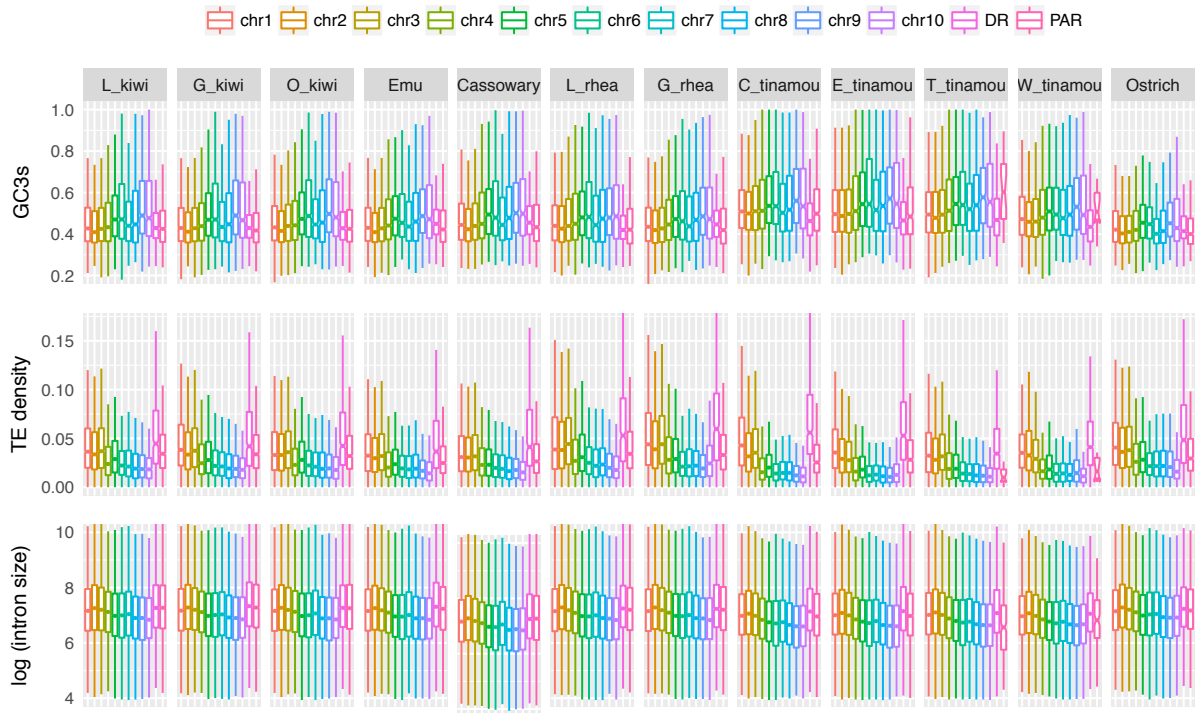


FIG. S9. Comparison of genomic feature among macro-chromosomes. GC3s (GC content of synonymous site of the third codon) and exon density show negative correlation with chromosome size, while TE (transposable element) density and intron size show positive correlation with chromosome size.

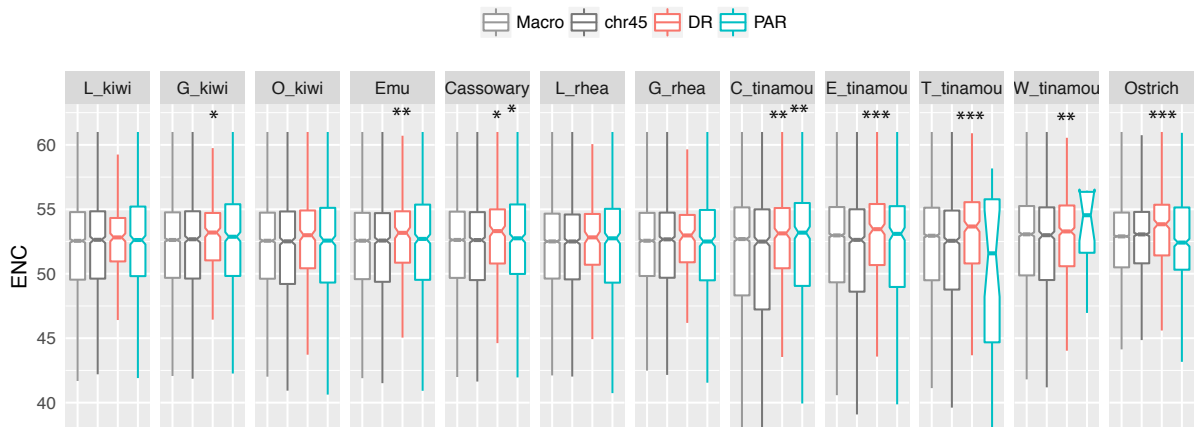


FIG. S10. Comparison of ENC between PAR/DR and autosomes. The ENC (Effective Number of Codons) values are higher in DRs for many species, but only for cassowary and Chilean tinamou ENC values are higher in PAR than for autosomes. Asterisks indicate the significant levels of PAR/DR vs. chr4/5 comparison (Wilcoxon sum rank test), * <0.05, ** <0.01, *** <0.001.

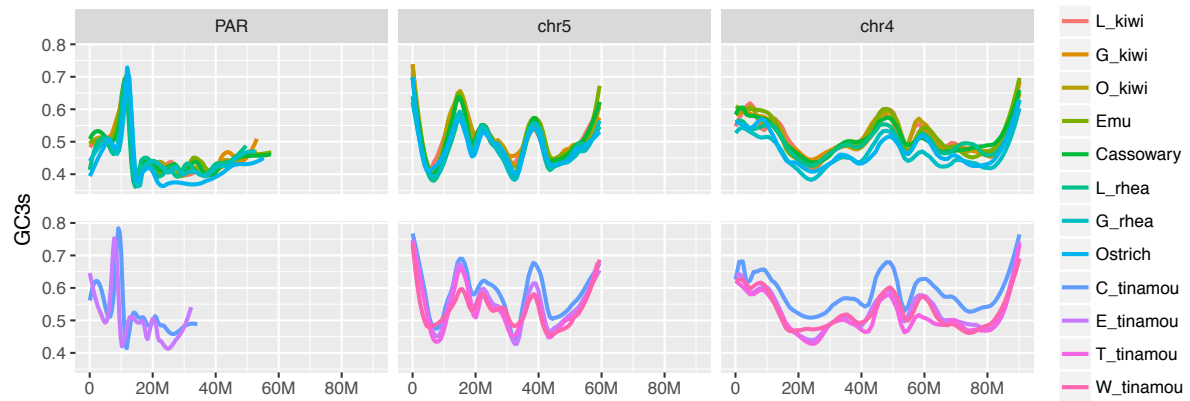


FIG. S11. Reduced GC3s on the PARs compared to chr5 and chr4. The location of the PAR-linked genes is based on the pseudo-chromosome Z, and the location of genes of chr4 and chr5 are based on the homologous genes of the chicken genomes. The abbreviation for species names is the same as in FIG S5.

Table S1. The length of pseudoautosomal region (PAR) and differentiated region (DR) in palaeognaths and selected neognaths

Species	PAR	DR	Reference
Little spotted kiwi	53,648,137	27,858,477	This study
Great spotted kiwi	53,103,935	27,917,301	This study
Okarito brown kiwi	53,052,411	29,311,255	This study
Emu	59,302,072	21,929,632	This study
Southern cassowary	59,264,808	22,782,997	This study
Lesser rhea	54,869,205	26,064,611	This study
Great rhea	52,553,322	29,742,736	This study
Chilean tinamou	34,050,901	36,483,903	This study
Elegant crested tinamou	32,217,551	33,168,615	This study
Thicket tinamou	250,000	71,263,047	This study
White-throated tinamou	685,144	62,454,206	(Zhou et al. 2014)
Ostrich	52,483,918	31,782,146	(Zhou et al. 2014), this study
Collared flycatcher	630,000	68,355,977	(Smeds et al. 2014)
Zebra finch	450,000	75,826,118	(Singhal et al. 2015)
Chicken	10,000	82,519,921	(Bellott et al. 2017)
Pekin duck	1,050,000	76,500,000	(Zhou et al. 2014)

Large PAR species are shaded in gray

Table S2. Correlation between chromosome sizes and genomic features

Species		GC3s	TE density	Intron size	Exon density	ENC	Intergenic size
L_kiwi	r	-0.86	0.9	0.83	-0.68	0.79	0.53
	p-value	0.00143	0.00033	0.0033	0.03091	0.00633	0.11574
G_kiwi	r	-0.86	0.93	0.81	-0.75	0.74	0.5
	p-value	0.00157	0.00009	0.00434	0.01307	0.01534	0.1394
O_kiwi	r	-0.86	0.9	0.74	-0.63	0.87	0.19
	p-value	0.00134	0.00045	0.01365	0.0532	0.00117	0.60513
Emu	r	-0.86	0.92	0.86	-0.71	0.9	0.71
	p-value	0.00158	0.00019	0.00125	0.02259	0.00035	0.02122
Cassowary	r	-0.87	0.92	0.84	-0.84	0.87	0.76
	p-value	0.00105	0.00013	0.00256	0.00209	0.00119	0.01071
L_rhea	r	-0.88	0.9	0.87	-0.77	0.77	0.62
	p-value	0.00069	0.00039	0.0011	0.00957	0.00951	0.05399
G_rhea	r	-0.91	0.92	0.82	-0.81	0.77	0.86
	p-value	0.00021	0.00017	0.00406	0.00439	0.00893	0.00124
C_tinamou	r	-0.79	0.95	0.89	-0.86	0.91	0.81
	p-value	0.00681	0.00003	0.00059	0.00131	0.00022	0.00447
E_tinamou	r	-0.9	0.94	0.91	-0.79	0.94	0.74
	p-value	0.00039	0.00006	0.00022	0.00681	0.00006	0.01391
T_tinamou	r	-0.89	0.97	0.91	-0.85	0.88	0.78
	p-value	0.00066	0	0.00023	0.0017	0.00068	0.00735
W_tinamou	r	-0.82	0.94	0.87	-0.8	0.8	0.71
	p-value	0.00396	0.00006	0.00119	0.00513	0.00555	0.02226
Ostrich	r	-0.54	0.95	0.75	-0.77	0.51	0.75
	p-value	0.10623	0.00004	0.01255	0.00933	0.1314	0.01212

Table S3. Location of Janes et al 2009 BAC sequence in the emu genome assembly.

GENBANK RECORD	CHROMOSOME (JANES ET AL 2009)	EMU GENOME LOCATION	EMU GENOME CHROMOSOME
EU200931	Autosome	not determined	not determined
EU200931	Autosome	not determined	not determined
ET041500	Autosome	not determined	not determined
ET041501	Autosome	not determined	not determined
ET041502	Autosome	not determined	not determined
ET041515	Autosome	not determined	not determined
ET041512	Autosome	not determined	not determined
ET041513	Autosome	not determined	not determined
AB002056	PAR	presumed assembly gap	Z (PAR) (1)
AB006694	PAR	scaffold_221:152067-154756	Z (DR)
AY095498	PAR	scaffold_13:6582073-6583283	Z (DR)
AB006695	PAR	scaffold_239:1028675-1030884	Z (PAR)
ET041507	PAR	scaffold_16:5843173-5843899	chr5
ET041520	PAR	scaffold_66:2150084-2282118	chr7
ET041521	PAR	scaffold_66:2150084-2282118	chr7
ET041516	PAR	scaffold_14:4429102-4300170	chr4
ET041517	PAR	scaffold_14:4429102-4300170	chr4
ET041508	PAR	scaffold_19:1980997-2079372	Z (DR)
ET041509	PAR	scaffold_19:1980997-2079372	Z (DR)
ET041510	PAR	scaffold_19:1980997-2079372	Z (DR)
ET041518	PAR	scaffold_106:822019-944625	chr8
ET041519	PAR	scaffold_106:822019-944625	chr8

(1) determined by alignment to other palaeognaths