Supplementary information

The rate of W chromosome degeneration across multiple avian neo-sex chromosomes

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This supplementary information includes **Tables S1-S5** and **Figure S1**.

Table S1. Information on samples and species used in this study.

Species	**Common name**	**Female sample**	**Male sample**
Alauda arvensis	Eurasian skylark	QL-1681-19_S46_L006	QL-1681-21_S47_L006
Alauda razae	Raso lark	QL-1681-95694_S52_L008	QL-1681-246_S51_L008
Calandrella cinerea	red-capped lark	TI-2672-Cbwil-146	TI-2672-Cbwil-143
Camaroptera brevicaudata	grey-backed camaroptera	TI-2672-Cbre-755	TI-2672-Cbre-802
Cisticola juncidis	zitting cisticola	QF-1504-CISJUN-2_S6_L002	QF-1504-RA5680_S5_L002
Eremophila alpestris	horned lark	QF-1504-H-19_S8_L003	QF-1504-H-88_S7_L003
Panurus biarmicus	bearded reedling	QF-1504-2KR32024_S2_L001	QF-1504-1ET92164_S3_L001
Sylvietta brachyura	northern crombec	SJ-2333-Sbra-553_S28_L002	SJ-2333-Sbra-878_S26_L002

Table S2. Sex-linked genome regions ("strata") found in previous studies across the studied species. See Main text for details. The genomic ranges and group numbers correspond to those in Figure 1.

Chromosome	Strata ID	Genomic range (Mb)	No. of genes	Present (X) or absent (-) in group (I-V)				
				I	II	III	IV	V
Z	chrZ	0.0–72.9	443	X	X	X	X	X
4A	chr4A-a	5.3–9.6	49	X	X	X	X	X
4A	chr4A-b	0.0–5.3	29	X	X	X	-	X
4	chr4	13.8–49.1	214	-	-	-	-	X
8	chr8	7.3–21.8	136	-	-	-	X	-
3	chr3-a	8.4–10.4; 18.1–24.1	72	X	X	X	-	-
3	chr3-b	10.4–14.0	26	-	X	X	-	-
3	chr3-c	5.8–8.4; 14.0–18.1; 29.8–88.0	400	-	-	X	-	-
5	chr5	9.1–45.4	289	-	-	X	-	-

Table S3. Node-specific losses (count and proportion) of W-linked gametologs. In Figure 3, data from all strata and species are shown (corresponding to all rows in this table. In Figure 4, the data is filtered to only include node values where each species is sex-linked (see Main text). The node numbers (2-8 in column "Node") and node ages ("nodeAge") follow that of Figure 1a. Values in column "Node" with species names are terminal nodes for each species. "Total" = original number of genes within this strata. "Gene count" = Number of W-linked gametologs present at each node. "Prop. remaining" = Proportion of W-liked gametologs compared to the original value.

Strata	Node	nodeAge	Total	Gene count	Prop. remaining
chr3-a	2	24	72	72	1
chr3-a	7	23	72	72	1
chr3-a	3	22	72	62	0.86
chr3-a	4	11	72	52	0.72
chr3-a	8	9	72	72	1
chr3-a	6	6	72	49	0.68
chr3-a	5	5	72	44	0.61
chr3-a	Alauda_arvensis	0	72	42	0.58
chr3-a	Alauda_razae	0	72	44	0.61
chr3-a	Calandrella_cinerea	0	72	43	0.6
chr3-a	Camaroptera_brevicaudata	0	72	72	1
chr3-a	Cisticola_juncidis	0	72	72	1
chr3-a	Eremophila_alpestris	0	72	46	0.64
chr3-a	Panurus_biarmicus	0	72	54	0.75
chr3-a	Sylvietta_brachyura	0	72	71	0.99
chr3-b	2	24	26	26	1
chr3-b	7	23	26	26	1
chr3-b	3	22	26	26	1
chr3-b	4	11	26	25	0.96
chr3-b	8	9	26	26	1
chr3-b	6	6	26	23	0.88
chr3-b	5	5	26	21	0.81
chr3-b	Alauda_arvensis	0	26	20	0.77
chr3-b	Alauda_razae	0	26	19	0.73
chr3-b	Calandrella_cinerea	0	26	20	0.77
chr3-b	Camaroptera_brevicaudata	0	26	25	0.96
chr3-b	Cisticola_juncidis	0	26	26	1
chr3-b	Eremophila_alpestris	0	26	20	0.77
chr3-b	Panurus_biarmicus	0	26	26	1

chr3-b	Sylvietta_brachyura	0	26	26	1
chr3-c	2	24	400	400	1
chr3-c	7	23	400	400	1
chr3-c	3	22	400	399	1
chr3-c	4	11	400	399	1
chr3-c	8	9	400	400	1
chr3-c	6	6	400	399	1
chr3-c	5	5	400	396	0.99
chr3-c	Alauda_arvensis	0	400	388	0.97
chr3-c	Alauda_razae	0	400	392	0.98
chr3-c	Calandrella_cinerea	0	400	396	0.99
chr3-c	Camaroptera_brevicaudata	0	400	398	1
chr3-c	Cisticola_juncidis	0	400	400	1
chr3-c	Eremophila_alpestris	0	400	395	0.99
chr3-c	Panurus_biarmicus	0	400	396	0.99
chr3-c	Sylvietta_brachyura	0	400	396	0.99
chr4	2	24	214	214	1
chr4	7	23	214	214	1
chr4	3	22	214	214	1
chr4	4	11	214	214	1
chr4	8	9	214	180	0.84
chr4	6	6	214	214	1
chr4	5	5	214	214	1
chr4	Alauda_arvensis	0	214	213	1
chr4	Alauda_razae	0	214	213	1
chr4	Calandrella_cinerea	0	214	214	1
chr4	Camaroptera_brevicaudata	0	214	169	0.79
chr4	Cisticola_juncidis	0	214	153	0.71
chr4	Eremophila_alpestris	0	214	212	0.99
chr4	Panurus_biarmicus	0	214	213	1
chr4	Sylvietta_brachyura	0	214	213	1
chr4A-a	2	24	49	46	0.94
chr4A-a	7	23	49	41	0.84
chr4A-a	3	22	49	44	0.9

chr4A-a	4	11	49	40	0.82
chr4A-a	8	9	49	37	0.76
chr4A-a	6	6	49	39	0.8
chr4A-a	5	5	49	38	0.78
chr4A-a	Alauda_arvensis	0	49	37	0.76
chr4A-a	Alauda_razae	0	49	36	0.73
chr4A-a	Calandrella_cinerea	0	49	38	0.78
chr4A-a	Camaroptera_brevicaudata	0	49	32	0.65
chr4A-a	Cisticola_juncidis	0	49	33	0.67
chr4A-a	Eremophila_alpestris	0	49	37	0.76
chr4A-a	Panurus_biarmicus	0	49	43	0.88
chr4A-a	Sylvietta_brachyura	0	49	38	0.78
chr4A-b	2	24	29	29	1
chr4A-b	7	23	29	29	1
chr4A-b	3	22	29	29	1
chr4A-b	4	11	29	24	0.83
chr4A-b	8	9	29	21	0.72
chr4A-b	6	6	29	21	0.72
chr4A-b	5	5	29	22	0.76
chr4A-b	Alauda_arvensis	0	29	20	0.69
chr4A-b	Alauda_razae	0	29	21	0.72
chr4A-b	Calandrella_cinerea	0	29	21	0.72
chr4A-b	Camaroptera_brevicaudata	0	29	21	0.72
chr4A-b	Cisticola_juncidis	0	29	16	0.55
chr4A-b	Eremophila_alpestris	0	29	18	0.62
chr4A-b	Panurus_biarmicus	0	29	29	1
chr4A-b	Sylvietta_brachyura	0	29	28	0.97
chr5	2	24	289	289	1
chr5	7	23	289	289	1
chr5	3	22	289	289	1
chr5	4	11	289	289	1
chr5	8	9	289	289	1
chr5	6	6	289	289	1
chr5	5	5	289	282	0.98

chr5	Alauda_arvensis	0	289	274	0.95
chr5	Alauda_razae	0	289	277	0.96
chr5	Calandrella_cinerea	0	289	289	1
chr5	Camaroptera_brevicaudata	0	289	288	1
chr5	Cisticola_juncidis	0	289	289	1
chr5	Eremophila_alpestris	0	289	285	0.99
chr5	Panurus_biarmicus	0	289	288	1
chr5	Sylvietta_brachyura	0	289	287	0.99
chr8	2	24	136	136	1
chr8	7	23	136	136	1
chr8	3	22	136	136	1
chr8	4	11	136	136	1
chr8	8	9	136	136	1
chr8	6	6	136	136	1
chr8	5	5	136	136	1
chr8	Alauda_arvensis	0	136	135	0.99
chr8	Alauda_razae	0	136	136	1
chr8	Calandrella_cinerea	0	136	136	1
chr8	Camaroptera_brevicaudata	0	136	136	1
chr8	Cisticola_juncidis	0	136	135	0.99
chr8	Eremophila_alpestris	0	136	136	1
chr8	Panurus_biarmicus	0	136	136	1
chr8	Sylvietta_brachyura	0	136	123	0.9
chrZ	2	24	443	52	0.12
chrZ	7	23	443	43	0.1
chrZ	3	22	443	46	0.1
chrZ	4	11	443	41	0.09
chrZ	8	9	443	38	0.09
chrZ	6	6	443	37	0.08
chrZ	5	5	443	37	0.08
chrZ	Alauda_arvensis	0	443	35	0.08
chrZ	Alauda_razae	0	443	35	0.08
chrZ	Calandrella_cinerea	0	443	37	0.08
chrZ	Camaroptera_brevicaudata	0	443	32	0.07

chrZ	Cisticola_juncidis	0	443	34	0.08
chrZ	Eremophila_alpestris	0	443	34	0.08
chrZ	Panurus_biarmicus	0	443	42	0.09
chrZ	Sylvietta_brachyura	0	443	38	0.09

Table S4. Haploinsufficiency score statistics. The column "HI score (median)" show the median haploinsufficiency score for each strata type (chrZ = "old"; all other strata="new"), for groups of genes with ("Z + W") and without ("Z only") a W-linked gametolog. The last two columns contain Mann-Whitney U test statistics of tests between the "Z only" and "Z + W" genes in each species and strata type. See Main text for details.

W-linked gametolog	**Strata type**	**Species**	**HI score (median)**	**Mann-Whitney U test statistics**	
				U	**P value**
Z only	new	Alauda_arvensis	45.945	19584	0.0035
Z + W	new	Alauda_arvensis	29.8	19584	0.0035
Z only	old	Alauda_arvensis	27.535	5427	0.0001
Z + W	old	Alauda_arvensis	12.45	5427	0.0001
Z only	new	Alauda_razae	43.51	16879	0.0302
Z + W	new	Alauda_razae	29.89	16879	0.0302
Z only	old	Alauda_razae	27	5596	0.0024
Z + W	old	Alauda_razae	12.76	5596	0.0024
Z only	new	Calandrella_cinerea	44.39	1946	0.0037
Z + W	new	Calandrella_cinerea	28.47	1946	0.0037
Z only	old	Calandrella_cinerea	27.895	5603	0.0003
Z + W	old	Calandrella_cinerea	12.45	5603	0.0003
Z only	new	Camaroptera_brevicaudata	37.345	3175	0.5396
Z + W	new	Camaroptera_brevicaudata	30.76	3175	0.5396
Z only	old	Camaroptera_brevicaudata	26.995	2515	0.0686
Z + W	old	Camaroptera_brevicaudata	14.16	2515	0.0686
Z only	new	Cisticola_juncidis	44.735	5173	0.0002
Z + W	new	Cisticola_juncidis	27.08	5173	0.0002
Z only	old	Cisticola_juncidis	27.535	5140	0.0009
Z + W	old	Cisticola_juncidis	12.85	5140	0.0009
Z only	new	Eremophila_alpestris	43.655	1890	0.0221
Z + W	new	Eremophila_alpestris	28.63	1890	0.0221
Z only	old	Eremophila_alpestris	27.84	4548	0.0009
Z + W	old	Eremophila_alpestris	12.56	4548	0.0009
Z only	new	Panurus_biarmicus	56.9	1029	0.0014
Z + W	new	Panurus_biarmicus	28.63	1029	0.0014
Z only	old	Panurus_biarmicus	28.085	6538	0.0000
Z + W	old	Panurus_biarmicus	12.45	6538	0.0000
Z only	new	Sylvietta_brachyura	35.175	549	0.5744

Z + W	new	Sylvietta_brachyura	24.25	549	0.5744
Z only	old	Sylvietta_brachyura	27.895	5250	0.0003
Z + W	old	Sylvietta_brachyura	12.45	5250	0.0003

Table S5. See Table S4 for descriptions of columns values, with the exception of "dN/dS (chicken vs. zebra finch)". This column contain median dN/dS values between chicken and zebra finch. See Main text for details.

W-linked gametolog	Strata type	Species	dN/dS (chicken vs. zebra finch)	Mann-Whitney U test statistics	
				U	P value
Z only	new	Alauda_arvensis	0.1566	24827	0.0003
Z + W	new	Alauda_arvensis	0.1083	24827	0.0003
Z only	old	Alauda_arvensis	0.1246	3271	0.1240
Z + W	old	Alauda_arvensis	0.0973	3271	0.1240
Z only	new	Alauda_razae	0.1566	21497	0.0102
Z + W	new	Alauda_razae	0.1102	21497	0.0102
Z only	old	Alauda_razae	0.1246	3967	0.0519
Z + W	old	Alauda_razae	0.0958	3967	0.0519
Z only	new	Calandrella_cinerea	0.1543	2549	0.0000
Z + W	new	Calandrella_cinerea	0.0804	2549	0.0000
Z only	old	Calandrella_cinerea	0.1246	3449	0.0979
Z + W	old	Calandrella_cinerea	0.0958	3449	0.0979
Z only	new	Camaroptera_brevicaudata	0.1543	4360	0.0002
Z + W	new	Camaroptera_brevicaudata	0.0970	4360	0.0002
Z only	old	Camaroptera_brevicaudata	0.1245	1039	0.7650
Z + W	old	Camaroptera_brevicaudata	0.1627	1039	0.7650
Z only	new	Cisticola_juncidis	0.1543	5165	0.0000
Z + W	new	Cisticola_juncidis	0.0916	5165	0.0000
Z only	old	Cisticola_juncidis	0.1246	3415	0.0166
Z + W	old	Cisticola_juncidis	0.0485	3415	0.0166
Z only	new	Eremophila_alpestris	0.1508	2444	0.0004
Z + W	new	Eremophila_alpestris	0.0854	2444	0.0004
Z only	old	Eremophila_alpestris	0.1246	2730	0.1320
Z + W	old	Eremophila_alpestris	0.0821	2730	0.1320
Z only	new	Panurus_biarmicus	0.1717	1102	0.0010
Z + W	new	Panurus_biarmicus	0.0894	1102	0.0010
Z only	old	Panurus_biarmicus	0.1302	4490	0.0098
Z + W	old	Panurus_biarmicus	0.0839	4490	0.0098
Z only	new	Sylvietta_brachyura	0.1543	838	0.0083
Z + W	new	Sylvietta_brachyura	0.0901	838	0.0083

Z only	old	Sylvietta_brachyura	0.1246	3277	0.1079
Z + W	old	Sylvietta_brachyura	0.0942	3277	0.1079

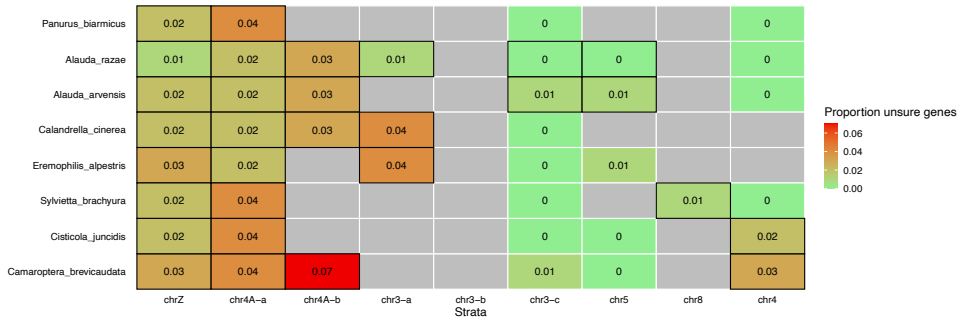

Figure S1. Proportion of “unsure” genes on the different strata for all eight species. Grey tiles without numbers represent strata and species combinations with no such genes (n = 0). Tiles with values ≤0.005 and were rounded down to ”0”.