

Table S1. Status of 78 W-linked protein-coding genes in focal species: summary. Results for eight species, including four distinct W-chromosome lineages in cuckoo finch, with associated hosts labeled as in Fig. 1. The set of 78 potential Z-W gametologs was compiled from previous avian studies (Smeds et al., 2015; Xu et al., 2019) and W-chromosome annotations of the focal species (see Methods). For five genes, we found no evidence of a W-linked copy in any of our focal species. Nineteen genes were present only as pseudogenes or “remnant pseudogenes” in one or more taxa, whereas 54 genes had a putatively functional copy in one or more taxa. Genes that may remain functional despite apparent loss-of-function mutations in comparison to the consensus annotation for the Z-linked gametolog (i.e., “reduced, putatively functional”) include: SMAD4-W, which in cuckoo finches has a stop codon in an exon that is variably expressed in alternative transcripts of the Z-linked gametolog; 2) ZNF131-W, which in cuckoo finches has an early stop codon that shortens the implied protein by 34 amino acids; 3) FCHO2-W, which is clearly a pseudogene in seven of the eight species we analyzed, but in *Serinus* is missing only one long exon that is variably expressed in alternative transcripts of the Z-linked gametolog; and 4) HINT1-W, which appears to be substantially shorter than HINT1-Z in all eight species in our analysis.

Species/lineage	Status of W-linked gametolog				
	full-length, functional (F)	reduced, putatively functional (f)	pseudogene (P)	remnant pseudogene (RP)	entirely lost (L)
<i>Agelaius</i> (Apho)	49	1	9	4	15
<i>Molothrus</i> (Mate)	49	1	11	4	13
<i>Serinus</i> (Scan)	49	2	7	7	13
<i>Taeniopygia</i> (Tgut)	36	1	8	7	26
<i>Lonchura</i> (Lstr)	38	1	6	4	29
<i>V. macroura</i> (Vmac)	43	1	7	5	22
<i>V. chalybeata</i> (Vcha)	41	1	12	4	20
<i>A. imberbis</i> _PB	28	3	10	7	30
<i>A. imberbis</i> _PR	28	3	10	6	31
<i>A. imberbis</i> _CE	27	3	11	6	31
<i>A. imberbis</i> _CJ	28	3	10	6	31

Gene	Apho	Mate	Scan	Tgut	Lstr	Vmac	Vcha	Aimb_PB	Aimb_PR	Aimb_CE	Aimb_CJ	Notes
UBE2R2	F	F	F	F	F	F	F	F	F	F	F	5
VCP	F	F	F	F	F	F	F	F	F	F	F	
ZFAND5	F	F	F	F	F	F	F	F	F	F	F	
ZFR	F	F	F	F	F	F	F	F	F	F	F	
ZNF131	F	F	F	F	F	F	F	f95	f95	f95	f95	
ZSWIM6	F	F	F	F	F	F	F	F	F	F	F	6
BTF3	F	F	F	F	F	F	F	L	L	L	L	
CDC37L1	F	F	F	P90	F	L	P90	L	L	L	L	
CERT1	F	F	F	L	L	F	F	F	F	F	F	
CTIF	F	F	F	RP10	RP13	L	L	L	L	L	L	
CWH18orf32	F	F	F	F	F	F	F	L	L	L	L	7
FAM219A	F	F	F	P47	P53	RP29	L	RP28	RP28	RP28	RP28	
FCHO2	P73	P83	f83	P51	RP6	P60	P60	L	L	RP9	RP9	
FEM1C	F	F	F	P71	P100	F	F	L	L	L	L	
HOMER1	F	F	P49	L	L	L	L	RP24	RP24	RP15	RP15	
KIF2A	F	F	F	F	P88	P45	P73	P46	P46	P46	P46	8
MACIR	L	L	F	L	L	L	L	L	L	L	L	
MAP1B	F	F	F	F	F	F	F	P100	P100	P100	P100	
MEX3C	F	F	F	P100	P85	F	F	P88	P88	L	L	
MRPS36	F	F	F	F	F	F	F	P88	P88	P88	P88	
PAIP1	F	F	F	P71	F	F	F	P100	P100	P100	P100	9
PIAS2	F	F	RP33	L	L	F	P93	P71	P71	P71	P71	
PRKAA1	F	F	F	P92	P92	F	F	P100	P100	P100	P100	
RPL17	F	F	F	P100	F	F	F	L	L	L	L	
SCAMP1	F	F	F	L	L	P84	P84	L	L	L	L	
SLC25A51	F	F	F	F	P100	F	100	P100	P100	P100	P100	10
SMAD7	F	F	F	L	L	L	L	L	L	L	L	
SNX18	L	L	F	F	F	F	F	L	L	L	L	

Notes:

- 1 DCAF12: a longer Z-linked annotation (Tgut, XP_030113358.1; 1,440bp over 9 coding regions) implies a non-functional W-linked gene in all taxa; thus, we used a slightly shorter annotation for the Z-linked gene (Tgut, XP_030113359.1; 1,371bp over 9 coding intervals) as the model for the W-linked gene. The latter annotation is consistent with annotations for a broad diversity of birds and is presumably the best consensus annotation for the gene.
- 2 HINT1-Z has three coding regions totaling 381bp, but applying the annotation for the Z-linked gene (Tgut, NP_001139032.1) implies a non-functional W-linked gene in all taxa. Thus, we infer that HINT1-W is reduced in length in comparison to the Z-linked gene, consistent with documented W-linked transcripts in Tgut (ACH45037.1) and Vcha (XM_053933340.1).
- 3 RASA1: first coding region lost from the W-linked gene in the cuckoo finch *Cisticola erythropus* lineage ("Aimb_CE") only.
- 4 Cuckoo finch SMAD4-W has a stop codon in one exon that is variably expressed in alternative transcripts for the Z-linked gametolog; compare XP_053824863.1 and XP_053824864.1 in Vcha. Thus, the shorter transcript may remain functional in cuckoo finch. The same exon appears to be missing from SMAD4-Z in the estrildid finches (Tgut and Lstr), a rare case of "degeneration" in a Z-linked gene (see also notes 8 and 9 below).
- 5 Cuckoo finch ZNF131-W has an early stop codon that shortens the implied protein by 34 codons in comparison to other songbirds; thus, the W-linked gene may remain functional but potential effects on phenotype/fitness are unknown.
- 6 FCHO2-W is clearly a pseudogene in seven of the eight species we analyzed, but in the canary, it is missing just one long exon that is variably expressed in transcripts for the Z-linked gametolog (compare XP_030114946.3 and XP_030114951.3 in Tgut). Thus, a shorter W-linked transcript may remain functional in canary.
- 7 FEM1C: the W-chromosome assemblies for Vcha and Vmac include two identical or nearly identical copies of this gene in close proximity, whereas the Tgut W-chromosome includes two incomplete copies of the gene.
- 8 MACIR: the Z-linked copy of this gene has a frameshift mutation in Lstr that presumably renders the gene non-functional; this is one of two examples we detected (see next note) of loss or pseudogenization of a Z-linked gene.
- 9 MEX3C: the genome assemblies for Tgut, Lstr, and Aimb lack a Z-linked copy of this gene, and we failed to assemble Z-linked copies of these genes from re-sequencing data for these species. The Z-linked gene is characterized by extreme GC-content (~72-74% in coding regions), perhaps making it difficult to assemble. However, we note that species missing the Z-linked gene also have non-functional or missing W-linked copies of this gene. See *Rissa tridactyla* XP_054042183 for shorter annotation ("MMAAML...").
- 10 RPL17: examination of alignments and phylogenies suggest an apparent case of gene conversion involving the transfer of two exons from RPL17-Z to RPL17-W in the *Vidua* lineage (see fig. S4).

Table S3. Summary of PAML results for different sets of sex-linked, autosomal and OXPHOS-related genes. dN and dN/dS estimates from the “VA” model (see fig. S9), which assumes different dN/dS rates for the outgroup (“out”), *Vidua* and *Anomalospiza* (“Aimb”), respectively, are shown along with the percentage difference in rate for *Anomalospiza* relative to *Vidua*. Differences greater than 30% are highlighted in blue, which corresponds to gene sets with significantly higher dN/dS in *Anomalospiza* than in *Vidua*. Results for the mitochondrial 12S, 16S and tRNA genes are expressed as substitutions per site (d), with rate differences tested using baseml rather than codeml. The best model (see fig. S9) and its $\Delta AICc$ value relative to the null model is shown with up and down arrows indicating whether the rate in *Vidua* (**V**) and/or *Anomalospiza* (**A**) is higher or lower, respectively, than in the outgroup. Gene sets are described in notes provided below the table.

gene set ¹	genomic location	#genes	dN (or d^* for non-coding genes)			dN/dS				best model	$\Delta AICc$
			Vidua	Aimb	±% in Aimb	out	Vidua	Aimb	±% in Aimb		
29W	W-chr	29	0.0023	0.0044	88%	0.1872	0.2136	0.3107	45%	A↑	18.3
29Z	Z-chr	29	0.0014	0.0035	145%	0.0597	0.0663	0.1322	99%	A↑	42.3
48Z	Z-chr	48	0.0044	0.0059	35%	0.1531	0.1897	0.2592	37%	V↑A↑	52.1
PAR	ZW-chrs	16	0.0043	0.0058	36%	0.1617	0.1876	0.2590	38%	A↑	15.8
Z-control	Z-chr	106	0.0068	0.0065	-5%	0.2162	0.2678	0.2493	-7%	A↑V↑	3.8
13mt	mt	13	0.0244	0.0307	26%	0.0309	0.0388	0.0516	33%	V↑A↑	70.4
Complex I: mt	mt	7	0.0345	0.0400	16%	0.0420	0.0563	0.0744	32%	V↑A↑	64.1
Complex I: nuc	nuc	37	0.0046	0.0103	125%	0.1824	0.1978	0.4842	145%	V↑A↑	58.5
Complex II: nuc	nuc	4	0.0048	0.0066	37%	0.0797	0.0590	0.0806	37%	null	–
Complex III: mt	mt	1	0.0138	0.0120	-13%	0.0240	0.0233	0.0157	-33%	V↓	0.38
Complex III: nuc	nuc	11	0.0017	0.0020	20%	0.2164	0.2064	0.2040	-1%	null	–
Complex IV: mt	mt	3	0.0078	0.0167	116%	0.0121	0.0108	0.0250	131%	A↑	12.9
Complex IV: nuc	nuc	13	0.0076	0.0124	63%	0.2166	0.2233	0.3733	67%	A↑	1.66
Complex V: mt	mt	2	0.0321	0.0406	26%	0.0307	0.0478	0.0554	16%	P↑	1.65
Complex V: nuc	nuc	18	0.0028	0.0070	153%	0.0993	0.1165	0.2297	97%	A↑	14.3
12S rRNA*	mt	1	0.0283	0.0664	135%	–	–	–	–	A↑	11.6
MRPS	Nuc	29	0.0070	0.0093	32%	0.2482	0.2586	0.3672	42%	A↑	5.64
16S rRNA*	mt	1	0.0340	0.0447	32%	–	–	–	–	A↑	1.83
MRPL	nuc	48	0.0068	0.0074	8%	0.1984	0.2191	0.2459	12%	P↑	3.03
mt tRNAs*	mt	22	0.0491	0.0754	53%	–	–	–	–	A↑	6.58
aaRS	nuc	22	0.0045	0.0053	16%	0.1818	0.1874	0.2097	12%	null	–
NADHD assembly	nuc	8	0.0073	0.0088	19%	0.2727	0.3162	0.3681	16%	P↑	1.88
COX assembly	nuc	16	0.0072	0.0064	-11%	0.2370	0.3080	0.2376	-23%	null	–
Co Q10 assembly	nuc	12	0.0046	0.0060	30%	0.2172	0.2140	0.2383	11%	null	–

gene set ¹	genomic location	#genes	dN			dN/dS				best model	ΔAICc
			Vidua	Aimb	±% in Aimb	Out	Vidua	Aimb	±% in Aimb		
A-control	nuc	102	0.0033	0.0034	5%	0.1529	0.1505	0.1637	9%	null	–
V-type ATPases	nuc	24	0.0020	0.0019	-6%	0.0995	0.0837	0.0865	3%	null	–
P-type ATPases	nuc	38	0.0026	0.0028	7%	0.1112	0.1062	0.1029	-3%	P↓	0.72
neuro-related	nuc	59	0.0025	0.0031	26%	0.1446	0.1242	0.1448	17%	V↓	4.25
social behavior	nuc	56	0.0032	0.0031	-4%	0.1047	0.1280	0.1118	-13%	V↑	12.6
egg production	nuc	18	0.0043	0.0048	11%	0.2507	0.2150	0.2332	8%	V↓A↓	2.49

Notes:

1 Gene sets:

29W = W-linked genes with putatively functional copies in all eight species analyzed

29Z = the Z-linked gametologs of the “29W” genes

48Z = Z-linked genes with lost or non-functional W-linked gametologs in one or more of the eight species analyzed

16PAR = genes in the pseudoautosomal region shared by the Z- and W-chromosomes

Z-control = a control set of 106 Z-linked genes not known to have a W-linked gametolog in any songbird

13mt = the 13 mitochondrial protein-coding genes combined

Complexes I-V mt/nuc = the mitochondrial and nuclear genes coding for components of respiratory complexes I through V

12S = the mitochondrial 12S ribosomal RNA gene

MRPS = nuclear-encoded small ribosomal subunit proteins

16S = the mitochondrial 16S ribosomal RNA gene

MRPS = nuclear-encoded large ribosomal subunit proteins

mt tRNAs = mitochondrial tRNA genes

aaRS = aminoacyl-tRNA synthetase genes

NADHD assembly, COX assembly, Co Q10 assembly = proteins involved, respectively, in the assembly of respiratory complexes I and IV and Coenzyme Q10

A-control = a control set of 102 autosomal genes

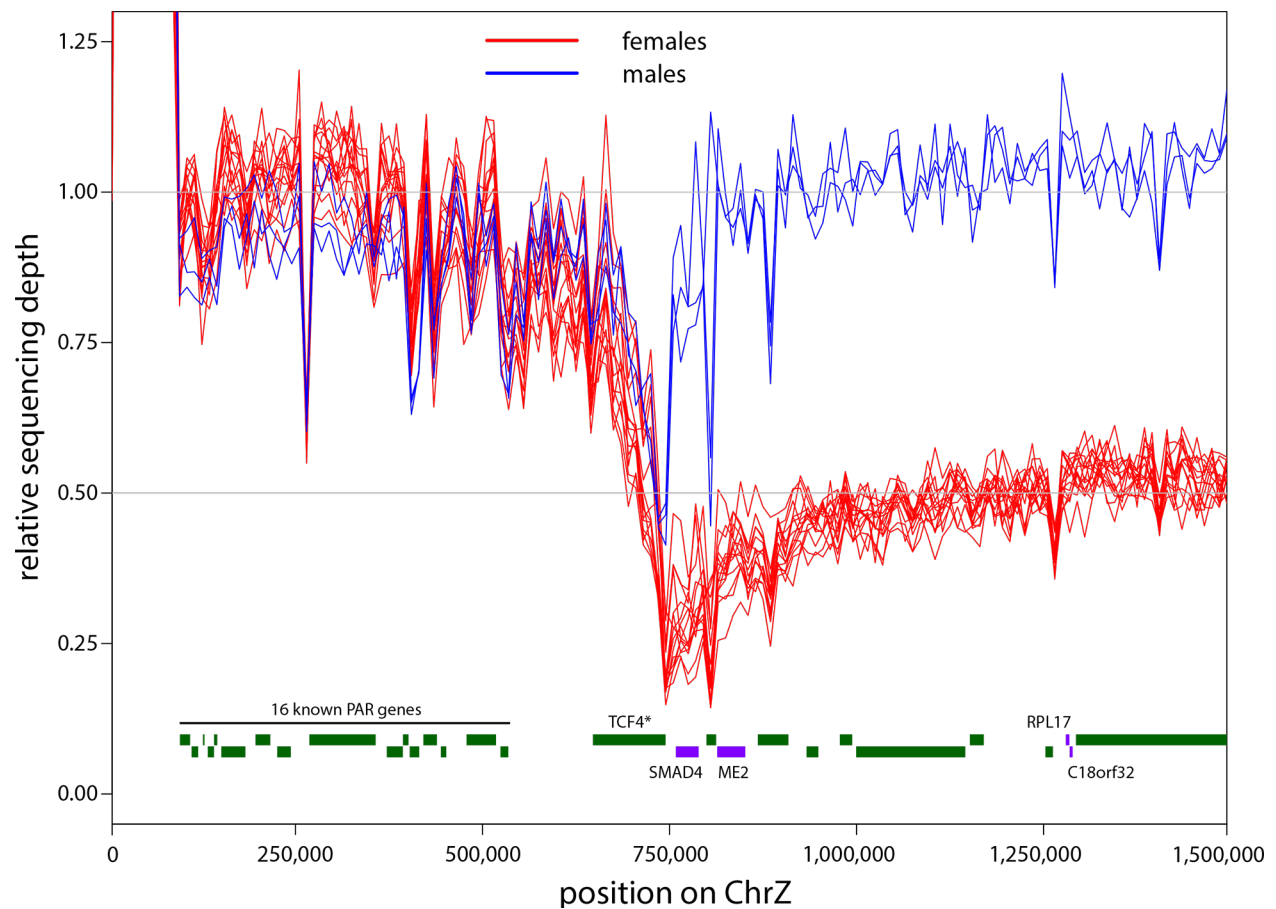
V-type ATPases = subunits of the vacuolar-type ATPase (<https://www.genenames.org/data/genegroup/#!/group/415>)

P-type ATPases = members of the P-type ATPase gene family (<https://www.genenames.org/data/genegroup/#!/group/414>)

neuro-related = neuro-related genes previously found to be accelerated in zebra finch relative to chicken (Nam et al. 2010)

social behavior = genes putatively involved in parental care and/or social behavior generally (e.g., Lopes & de Bruijn 2021; Lynch et al. 2019, 2020)

egg production = genes putatively associated with egg production in galliforms or anseriforms (Mishra et al. 2020; Bhavana et al. 2022)



Supplementary Fig. 1 | Male versus female sequencing depth across the pseudoautosomal region and adjacent portion of the Z-chromosome. Sequencing depth for 10 kbp windows, expressed as a function of median autosomal sequencing depth per sample, for four male and 15 female Cameroon indigobirds *Vidua camerunensis*. The location of annotated protein-coding genes is indicated in green or purple, the latter identifying genes with W-linked gametologs in at least some songbirds. We failed to assemble the TCF4 gene in most species, but this region is characterized by extreme GC content.