Supplementary Information

A genetic map of ostrich Z chromosome and the role of inversions in avian sex chromosome evolution

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Supplementary Material and Methods

Linkage analysis with CRIMAP

In order to build a genetic map in CRIMAP, two loci must be chosen as ordered loci in the first run. In the end, the resulting map length can vary depending on which two loci are chosen. It is recommended according to CRIMAP tutorial (https://www.animalgenome.org/bioinfo/resources/manuals/Embnetut/Crimap/manuatoc.html) to build the map choosing multiple pairs of loci from TWOPOINT analysis that have a large inter-locus distance (rec.fracs) and large confidence (LODs). Choosing loci that are far apart for the initial map has the advantage that the BUILD run may reach more distant loci on either side of the map. However, this may be associated with more errors in the relative positions of loci. For this reason, BUILD runs are best followed by FLIPSN runs, which iteratively evaluate different orders. Maps from these trials can be used as ordered loci for subsequent BUILD runs where all remaining loci are to be inserted.

In order to build the ostrich Z chromosome map, 10 pairs of loci were chosen based on TWOPOINT analysis. For each run, sex-specific maps were generated. BUILD was run in a series of steps as follows:

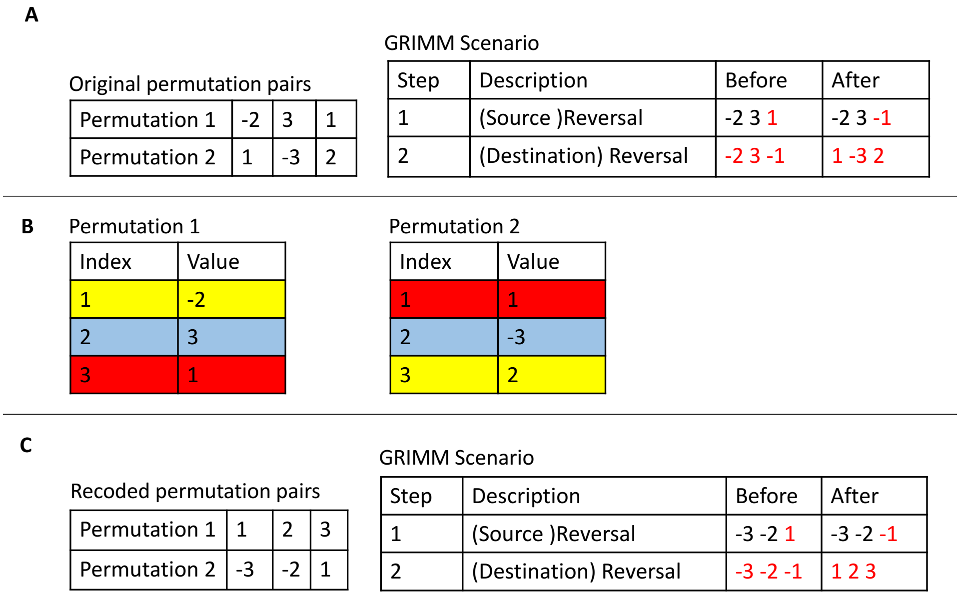
1. Run BUILD with LOD = 6. Use two loci from TWOPOINT analysis as ordered loci. Run FLIPS4 to iteratively evaluate different orders, rearranging four adjacent markers at a time, until no better order could be found. This is the frame work map.
2. Run BUILD with LOD = 5. Use the ordered loci obtained from the previous step as ordered loci. Run FLIPS4.

3. Run BUILD with LOD = 4. Use the ordered loci obtained from the previous step as ordered loci. Run FLIPS4.
4. Run BUILD with LOD = 3. Use the ordered loci obtained from the previous step as ordered loci. Run FLIPS4. This is the best order map.

Inversion analysis using GRIMM and baobabLUNA

Gene order and orientation are commonly modelled with a signed permutation as in the GRIMM algorithm (Tesler 2002). Three main goals in an inversion analysis are identifying the number of inversion events from an ancestral to a descendent sequence, the breakpoints of inversions and the order of inversion events. The number of inversion events is calculated based on parsimony principles, which means that the scenario associated with the least number of events is considered as the optimal scenario. Inferring the position of breakpoints is a more challenging task as multiple optimal inversion scenarios with different sets of breakpoints can exist. However, GRIMM reports only one scenario. In contrast, the program baobabLUNA (Braga 2009) uses an algorithm that give a summary of all possible parsimonious inversion scenarios.

For the GRIMM analysis, we used the ancestral lineage as the source genome and the descendent lineage as the destination genome (Table 1). In order to extend the GRIMM analysis, we used every pair of inferred ancestral and descendent sequences as input to baobabLUNA. All permutations were polarized according to the lizard state. The module analyzeTrace of baobabLUNA sorts every permutation to the sorted sequence of ancestral block numbers. In order to use analyzeTrace, we first reordered every descendent lineage based on the most recent ancestral state by recoding one permutation based on another, as described with a permutation example in Figure 1.



A

Original permutation pairs

Permutation 1	-2	3	1
Permutation 2	1	-3	2

GRIMM Scenario

Step	Description	Before	After
1	(Source)Reversal	-2 3 1	-2 3 -1
2	(Destination) Reversal	-2 3 -1	1 -3 2

B

Permutation 1

Index	Value
1	-2
2	3
3	1

Permutation 2

Index	Value
1	1
2	-3
3	2

C

Recoded permutation pairs

Permutation 1	1	2	3
Permutation 2	-3	-2	1

GRIMM Scenario

Step	Description	Before	After
1	(Source)Reversal	-3 -2 1	-3 -2 -1
2	(Destination) Reversal	-3 -2 -1	1 2 3

Figure 1. Recoding algorithm to obtain the baobabLUNA input. (A) Original permutation pairs with the GRIMM inversion scenario. (B) Tables indicating the indices of every element of a given permutation. (C) Recoded permutation pairs and their respective GRIMM scenario.

Figure 1A describes the original permutation pairs used in the GRIMM analysis. In order to recode the original permutations to the input of baobabLUNA analyzeTrace, the following steps were performed (Figure 1B):

1. For every index in permutation 1, check its absolute value. For example, the absolute value of index 1 is 2.
2. Find the index of that absolute value in permutation 2. The index of 2 is 3.
3. If the values of the indices have opposite signs, insert the minus sign in the second permutation. Otherwise, keep the signs the same.
4. Output the signed indices of permutation 1 and permutation 2 for that value. For example, for value 2, the indices 1 and 3 become 1 and -3 (Figure 1C).

After recoding every ancestral-descendent pair (Table 2), we used baobabLUNA to obtain all possible solutions of the inversion scenario (Table 3). We denote them with the set notation, each set containing the inverted block(s). The scripts for this analysis can be found in https://github.com/Homap/inversions_breakpoints_simulation.

Table 1. Input for GRIMM analysis.

Ancestral lineage (source genome)	Descendent lineage (destination genome)
Lizard 1,2,3,4,5,6,7,8,9,10,11,12,13,14,15,16,17,18	Palaeognathae-Neognathae ancestor 1,-2,-3,4,5,6,7,8,9,10,11,17,-14,-13,-12,18,-15,-16
Palaeognathae-Neognathae ancestor 1,-2,-3,4,5,6,7,8,9,10,11,17,-14,-13,-12,18,-15,-16	Galloanserae-Neoaves ancestor 1,-2,-3,4,5,6,12,13,14,-17,-11,-10,-9,-18,7,8,-15,-16
Galloanserae-Neoaves ancestor 1,-2,-3,4,5,6,12,13,14,-17,-11,-10,-9,-18,7,8,-15,-16	Galliformes ancestor 1,-2,-3,4,5,6,12,13,14,-17,-11,-10,-9,18,-8,-7,-15,-16
Galloanserae-Neoaves ancestor 1,-2,-3,4,5,6,12,13,14,-17,-11,-10,-9,-18,7,8,-15,-16	Passeriformes ancestor 1,-2,-3,4,5,6,12,13,14,-17,-10,-9,-18,16,15,-11,-8, -7
Palaeognathae-Neognathae ancestor 1,-2,-3,4,5,6,7,8,9,10,11,17,-14,-13,-12,18,-15,-16	Ostrich 1,-2,-3,4,5,6,7,8,9,10,11,17,-14,15,-18,12,16,-13
Galliformes ancestor 1,-2,-3,4,5,6,12,13,14,-17,-11,-10,-9,18,-8,-7,-15,-16	Quail 1,-5,-4,-2,-3,6,12,13,14,-17,-11,-10,-9,18,-8,-7,-15,-16
Passeriformes ancestor 1,-2,-3,4,5,6,12,13,14,-17,-10,-9,-18,16,15,-11,-8,-7	Flycatcher 1,-2,-3,4,5,6,12,13,14,-17,-10,-9,-18,16,15,-11,8,-7
Passeriformes ancestor 1,-2,-3,4,5,6,12,13,14,-17,-10,-9,-18,16,15,-11,-8,-7	Great tit 1,-2,-3,4,5,6,12,13,14,-17,-10,-9,-18,16,15,8,11,-7
Passeriformes ancestor 1,-2,-3,4,5,6,12,13,14,-17,-10,-9,-18,16,15,-11,-8,-7	Zebra finch -1,18,9,8,11,-15,-16,-2,-3,4,10,5,17,-14,-13,-12,-6,-7

Table 2. Input for baobabLUNA analysis.

Ancestral lineage	Descendent lineage
Lizard 1,2,3,4,5,6,7,8,9,10,11,12,13,14,15,16,17,18	Palaeognathae-Neognathae ancestor 1,-2,-3,4,5,6,7,8,9,10,11,17,-14,-13,-12,18,-15,-16
Palaeognathae-Neognathae ancestor 1,2,3,4,5,6,7,8,9,10,11,12,13,14,15,16,17,18	Galloanserae-Neoaves ancestor 1,2,3,4,5,6,-15,-14,-13,-12,-11,-10,-9,-16,7,8,17,18
Galloanserae-Neoaves ancestor 1,2,3,4,5,6,7,8,9,10,11,12,13,14,15,16,17,18	Galliformes ancestor 1,2,3,4,5,6,7,8,9,10,11,12,13,-14,-16,-15,17,18
Galloanserae-Neoaves ancestor 1,2,3,4,5,6,7,8,9,10,11,12,13,14,15,16,17,18	Passeriformes ancestor 1,2,3,4,5,6,7,8,9,10,12,13,14,-18,-17,11,-16,-15
Palaeognathae-Neognathae ancestor 1,2,3,4,5,6,7,8,9,10,11,12,13,14,15,16,17,18	Ostrich 1,2,3,4,5,6,7,8,9,10,11,12,13,-17,-16,-15,-18,14
Galliformes ancestor 1,2,3,4,5,6,7,8,9,10,11,12,13,14,15,16,17,18	Quail 1,-5,-4,2,3,6,7,8,9,10,11,12,13,14,15,16,17,18
Passeriformes ancestor 1,2,3,4,5,6,7,8,9,10,11,12,13,14,15,16,17,18	Flycatcher 1,2,3,4,5,6,7,8,9,10,11,12,13,14,15,16,-17,18
Passeriformes ancestor 1,2,3,4,5,6,7,8,9,10,11,12,13,14,15,16,17,18	Great tit 1,2,3,4,5,6,7,8,9,10,11,12,13,14,15,-17,-16,18
Passeriformes ancestor 1,2,3,4,5,6,7,8,9,10,11,12,13,14,15,16,17,18	Zebra finch -1,-13,-12,-17,-16,-15,-14,2,3,4,-11,5,-10,-9,-8,-7,-6,18

Table 3. Inversion scenarios obtained by baobabLUNA.

Inversion scenarios obtained by baobabLUNA
Lizard -> Palaeognathae-Neognathae ancestor {2}[*]{3}{12.13.14.15.17.18}{12.13.14.16.17.18}{12.13.14.15.16.17}{15.16.17} {2}{3}{12.13.14.17}{17}{15.17.18}{16.17.18} : GRIMM solution
Palaeognathae-Neognathae ancestor -> Galloanserae-Neoaves ancestor {7.8.16}{7.8.9.10.11.12.13.14.15} : GRIMM solution
Galloanserae-Neoaves ancestor -> Galliformes ancestor {14}{15.16} : GRIMM solution
Galloanserae-Neoaves ancestor -> Passeriformes ancestor {11.15.16.17.18}{11.12.13.14.15.16}{12.13.14.15.16} : GRIMM solution
Palaeognathae-Neognathae ancestor -> Ostrich {14.18}{14.15.16.17} : GRIMM solution
Galliformes ancestor -> Quail {2.3}{2.3.4.5} : GRIMM solution
Passeriformes ancestor -> Flycatcher {17} : GRIMM solution
Passeriformes ancestor -> Great tit {16.17} : GRIMM solution
Passeriformes ancestor -> Zebra finch {1}{2.3.4.5.6.7.8.9.10.11.14.15.16.17}{5.6.7.8.9.10}{5.6.7.8.9.10.11}{6.7.8.9.10}{2.3.4.5.6.7.8.9.10.11.12.13} : GRIMM solution {1.12.13.14.15.16.17}{1.2.3.4.5.11.12.13}{1.2.3.4.11.12.13}{1.2.3.4.12.13}{1.2.3.4.5.14.15.16.17}{6.7.8.9.10.11.12.13.14.15.16.17} {1.12.13.14.15.16.17}{5.6.7.8.9.10}{6.7.8.9.10}{1.2.3.4.12.13}{1.2.3.4.14.15.16.17}{5.6.7.8.9.10.11.12.13.14.15.16.17} {1.12.13.14.15.16.17}{1.2.3.4.5.11.12.13}{1.2.3.4.5.11.14.15.16.17}{6.7.8.9.10.12.13.14.15.16.17}{5.6.7.8.9.10}{5.6.7.8.9.10.11} {1.12.13.14.15.16.17}{6.7.8.9.10}{1.2.3.4.11.12.13}{1.2.3.4.11.14.15.16.17}{5.6.7.8.9.10.12.13.14.15.16.17}{5.6.7.8.9.10.11}

[*] Each set indicates the block or a series of adjacent blocks that is inverted every step.

Supplementary Tables

Table S1. Male pools used for SNP identification from (Adolfsson and Ellegren 2013).

Pool	NCBI Accession	Sample1	Sample2	Sample3
Ostrich Brain Male1	SRX142690	2_13_BM	3_15_BM	
Ostrich Brain Male2	SRX143226	4_16_BM	6_21_BM	
Ostrich Brain Male3	SRX145593	7_22_BM	9_26_BM	
Ostrich Gonad Male1	-*	1_12_GM	2_13_GM	3_15_GM
Ostrich Gonad Male2	-*	4_16_GM	5_21_GM	
Ostrich Gonad Male3	-*	6_22_GM	7_26_GM	
Ostrich Liver Male1	SRX151868	1_7_LM	2_12_LM	
Ostrich Liver Male2	SRX153141	3_13_LM	4_15_LM	5_6_LM
Ostrich Liver Male3	SRX154477	6_21_LM	7_22_LM	8_26_LM

* Male gonad samples were not used in Adolfsson and Ellegren 2013, hence, the data are not deposited in NCBI. The raw reads of male gonads are available upon request.

Table S2. SNP filtering steps for genetic mapping.

SNP filtering steps	**Comments**	**Number of SNPs removed**	**Number of SNPs**
SNPs submitted for genotyping			384
SNPs genotyped as reported in the vcf file	15 SNPs were not reported in the vcf file. 2 due to low call rate and 13 because they were monomorphic so they wouldn't show in vcf file.	15	369
Removing SNPs in scaffolds 1386 and 294		4	365
genotype quality < 30		3	362
SNPs heterozygote for all individuals		1	361
Indels or SNPs with more than two alleles		8	353
Removing (SNPs with sample call rate < 90% (Range: 11-89%) = 24 SNP)	22 SNPs were in the vcf, the last 2 SNPs were not in the vcf (colored red)		
SNPs with low call rate already removed in Indel removal	8 SNPs		
Final Number of SNPs with low sample call rate removed		14	339
SNPs removed because females were heterozygous in non-PAR region		6	**333**

Table S3. Individual filtering steps for genetic mapping.

Individual filtering steps	**Comments**	**Number of samples removed**	**Parents**	**Offspring**	**Number of samples**
Samples submitted for genotyping			44	214	258
Samples with number of inheritance errors > 5		9		205	
Samples with uncertain sex		9		196	
Samples with low genotype call rate = 4 samples	Already removed in inheritance error and uncertain sex steps	0		196	
Identical samples due to contamination, from different families	142_986 already removed in previous steps	1		195	
Identical samples with no explanations, from the same family	185_816, 185_929, 146_681, 146_809	2		193	
Families with number of markers in segregation distortion > 6	Family 56 – 2 parents and 5 offspring	7	42	188	**230**

Table S4. List of SNPs and genes covered by the genetic map.

No	Scaffold_SNPposition	*G.gallus* Gene ID	*S.camelus* Gene ID
1	superscaffold26_44551	ENSGALG00000002805	Sca_R015347
2	superscaffold26_57111	ENSGALG00000002805	Sca_R015347
3	superscaffold26_127813	ENSGALG00000002839	Sca_R015349
4	superscaffold26_751130	ENSGALG00000003087	Sca_R005880
5	superscaffold26_785762	ENSGALG00000003066	Sca_R005881
6	superscaffold26_1082202	ENSGALG00000003019	Sca_R005886
7	superscaffold26_1102598	ENSGALG00000003019	Sca_R005886
8	superscaffold26_1113307	ENSGALG00000003019	Sca_R005886
9	superscaffold26_3461665	ENSGALG00000002777	Sca_R005903
10	superscaffold26_3463963	ENSGALG00000002777	Sca_R005903
11	superscaffold26_3677222	ENSGALG00000002677	Sca_R005908
12	superscaffold26_3682803	ENSGALG00000002677	Sca_R005908
13	superscaffold26_4790876	ENSGALG00000001843	Sca_R005917
14	superscaffold26_4810948	ENSGALG00000001843	Sca_R005917
15	superscaffold26_5215322	ENSGALG00000014644	Sca_R005922
16	superscaffold26_5218700	ENSGALG00000014644	Sca_R005922
17	superscaffold26_10282269	ENSGALG00000014168	Sca_R007593
18	superscaffold26_10507222	ENSGALG00000005800	Sca_R007599
19	superscaffold26_11691141	ENSGALG00000002009	Sca_R015071
20	superscaffold26_11695127	ENSGALG00000002009	Sca_R015071
21	superscaffold26_11711236	ENSGALG00000002023	Sca_R015072
22	superscaffold26_11729013	ENSGALG00000002069	Sca_R015074
23	superscaffold26_12078493	ENSGALG00000002326	Sca_R015078
24	superscaffold26_12089137	ENSGALG00000002326	Sca_R015078
25	superscaffold26_12167171	ENSGALG00000002371	Sca_R015079
26	superscaffold26_12175653	ENSGALG00000002371	Sca_R015079
27	superscaffold26_12403910	ENSGALG00000002412	Sca_R009831
28	superscaffold26_12445606	ENSGALG00000002523	Sca_R009834
29	superscaffold26_12448133	ENSGALG00000002523	Sca_R009834
30	superscaffold26_12540349	ENSGALG00000002578	Sca_R009836
31	superscaffold26_13125126	ENSGALG00000003168	Sca_R009848
32	superscaffold26_13949850	ENSGALG00000003353	Sca_R009859
33	superscaffold26_13951571	ENSGALG00000003353	Sca_R009859
34	superscaffold26_13980754	ENSGALG00000003365	Sca_R009860
35	superscaffold26_14007530	ENSGALG00000003432	Sca_R009863
36	superscaffold26_14313172	ENSGALG00000013376	Sca_R009870
37	superscaffold26_14348417	ENSGALG00000013377	Sca_R009872
38	superscaffold26_14362307	ENSGALG00000013377	Sca_R009872
39	superscaffold26_14405256	ENSGALG00000003558	Sca_R009874
40	superscaffold26_14412022	ENSGALG00000003558	Sca_R009874
41	superscaffold26_14449954	ENSGALG00000021304	Sca_R009875
42	superscaffold26_14451167	ENSGALG00000021304	Sca_R009875
43	superscaffold26_14455695	ENSGALG00000021304	Sca_R009875
44	superscaffold26_14987729	ENSGALG00000003708	Sca_R009889
45	superscaffold26_15096314	ENSGALG00000003708	Sca_R009889
46	superscaffold26_15381119	ENSGALG00000003726	Sca_R009893
47	superscaffold26_15406896	ENSGALG00000003726	Sca_R009893
48	superscaffold26_15440957	ENSGALG00000003733	Sca_R009894

49	superscaffold26_15450690	ENSGALG00000003733	Sca_R009894
50	superscaffold26_15593125	ENSGALG00000003747	Sca_R009899
51	superscaffold26_15598338	ENSGALG00000003747	Sca_R009899
52	superscaffold26_15601723	ENSGALG00000003747	Sca_R009899
53	superscaffold26_15662822	ENSGALG00000003775	Sca_R009900
54	superscaffold26_15757822	ENSGALG00000003792	Sca_R009902
55	superscaffold26_16398849	ENSGALG00000014835	Sca_R009915
56	superscaffold26_16400861	ENSGALG00000014835	Sca_R009915
57	superscaffold26_16404183	ENSGALG00000014835	Sca_R009915
58	superscaffold26_16414650	ENSGALG00000014835	Sca_R009915
59	superscaffold26_16718019	ENSGALG00000014846	Sca_R015001
60	superscaffold26_16759866	ENSGALG00000014846	Sca_R015001
61	superscaffold26_16785314	ENSGALG00000014851	Sca_R014999
62	superscaffold26_16787479	ENSGALG00000014851	Sca_R014999
63	superscaffold26_17050967	ENSGALG00000014855	Sca_R014998
64	superscaffold26_17052813	ENSGALG00000014855	Sca_R014998
65	superscaffold26_17083966	ENSGALG00000023503	Sca_R014997
66	superscaffold26_17084513	ENSGALG00000023503	Sca_R014997
67	superscaffold26_17212085	ENSGALG00000014863	Sca_R014992
68	superscaffold26_17219158	ENSGALG00000014863	Sca_R014992
69	superscaffold26_17225443	ENSGALG00000014865	Sca_R014991
70	superscaffold26_17229657	ENSGALG00000014865	Sca_R014991
71	superscaffold26_17234708	ENSGALG00000014865	Sca_R014991
72	superscaffold26_17251315	ENSGALG00000014867	Sca_R014990
73	superscaffold26_17287868	ENSGALG00000014869	Sca_R014989
74	superscaffold26_17289001	ENSGALG00000014869	Sca_R014989
75	superscaffold26_17298508	ENSGALG00000014869	Sca_R014989
76	superscaffold26_19402555	ENSGALG00000014906	Sca_R007254
77	superscaffold26_20228145	ENSGALG00000014700	Sca_R007266
78	superscaffold26_20230788	ENSGALG00000014700	Sca_R007266
79	superscaffold26_20235348	ENSGALG00000014700	Sca_R007266
80	superscaffold26_20410837	ENSGALG00000014712	Sca_R007269
81	superscaffold26_20418429	ENSGALG00000014712	Sca_R007269
82	superscaffold26_20474443	ENSGALG00000014713	Sca_R007270
83	superscaffold26_20553918	ENSGALG00000014716	Sca_R007274
84	superscaffold26_20555257	ENSGALG00000014716	Sca_R007274
85	superscaffold26_20557374	ENSGALG00000014716	Sca_R007274
86	superscaffold26_20559480	ENSGALG00000014716	Sca_R007274
87	superscaffold26_20619747	ENSGALG00000014717	Sca_R007275
88	superscaffold26_23058553	ENSGALG00000014737	Sca_R007303
89	superscaffold26_23127061	ENSGALG00000014740	Sca_R007305
90	superscaffold26_23175751	ENSGALG00000014740	Sca_R007305
91	superscaffold26_23676705	ENSGALG00000014743	Sca_R007309
92	superscaffold26_24230300	ENSGALG00000014765	Sca_R007316
93	superscaffold26_24440101	ENSGALG00000014773	Sca_R007322
94	superscaffold26_24471470	ENSGALG00000014773	Sca_R007322
95	superscaffold26_24483208	ENSGALG00000014773	Sca_R007322
96	superscaffold26_24534316	ENSGALG00000014775	Sca_R007323
97	superscaffold54_101636	ENSGALG00000000273	Sca_R005537
98	superscaffold54_4351427	ENSGALG00000015288	Sca_R004908
99	superscaffold54_4357228	ENSGALG00000015288	Sca_R004908

100	superscaffold54_4536437	ENSGALG00000015301	Sca_R004904
101	superscaffold54_4543088	ENSGALG00000015301	Sca_R004904
102	superscaffold54_4584366	ENSGALG00000014682	Sca_R004902
103	superscaffold54_4594770	ENSGALG00000014682	Sca_R004902
104	superscaffold54_4600603	ENSGALG00000014682	Sca_R004902
105	superscaffold54_4602267	ENSGALG00000014682	Sca_R004902
106	superscaffold54_4604721	ENSGALG00000014682	Sca_R004902
107	superscaffold54_5010797	ENSGALG00000014673	Sca_R004897
108	superscaffold54_5024726	ENSGALG00000014672	Sca_R004896
109	superscaffold54_5037026	ENSGALG00000014672	Sca_R004896
110	superscaffold54_5045452	ENSGALG00000014670	Sca_R004895
111	superscaffold54_5060400	ENSGALG00000014670	Sca_R004895
112	superscaffold54_5063420	ENSGALG00000014670	Sca_R004895
113	superscaffold54_5071735	ENSGALG00000014670	Sca_R004895
114	superscaffold54_5581580	ENSGALG00000014664	Sca_R004889
115	superscaffold54_5789263	ENSGALG00000014661	Sca_R004887
116	superscaffold54_5822338	ENSGALG00000014661	Sca_R004887
117	superscaffold54_7118120	ENSGALG00000014657	Sca_R004882
118	superscaffold54_7265897	ENSGALG00000014657	Sca_R004882
119	superscaffold54_8682873	ENSGALG00000015641	Sca_R004874
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Table S5. Number of SNPs per scaffold.

Scaffold	**Human chromosome**	**Scaffold length (bp)**	**Number of genes**	**Number of SNPs**	**Number of SNPs/Mbp**
superscaffold26	5,18	25310599	54	96	3.79
superscaffold54	3, 5, 6, 8	29256470	40	83	2.84
superscaffold35	5	4625539	12	27	5.84
superscaffold36	5	9394175	11	23	2.45
superscaffold62	9	2917291	4	9	3.09
superscaffold67	9	5300260	7	15	2.83
superscaffold69-1	9	5978518	17	34	5.69
superscaffold83	9	782506	1	2	2.56
superscaffold88	9	624114	4	5	8.01
superscaffold93	9	4983591	7	10	2.01
superscaffold92	9	2882843	4	6	2.08
superscaffold63	9	1692925	11	23	13.59

Supplementary Figure Legends

Fig. S1. Superscaffolds from the optical-map improved ostrich Z assembly plotted against the scaffolds prior to optical mapping using nucmer anchors. For each superscaffold, the red bars indicate the position of SNPs used in the linkage mapping. Genes within each superscaffold are shown with grey rectangles with black borders. Nucmer anchors are shown by orange lines from the superscaffolds to scaffolds. The region within each superscaffold without alignment to scaffolds contain gaps and are indicated by N. The blue scaffolds in superscaffold54 belong to linkage group 2 and are removed from the Z chromosome.

Fig. S2. Construction of synteny blocks for ostrich and chicken Z genes. Top figure depicts the alignment of orthologous genes between ostrich and chicken Z. Bottom figure represents the grouping of genes into synteny blocks by the programme GRIMM_SYNTENY-2.02. Grey: genes with conserved gene orientation between the two species. Red: inverted genes in one species compared to the other.

Fig S3. A) Lep-MAP2 sex-averaged genetic map of Z chromosome. B) The sex averaged framework map of ostrich Z chromosome as obtained by CRIMAP 2.507 BUILD option. To the left are the genetic positions of the markers in cM and to the right, markers are indicated as superscaffold_position. The reverse orientation of superscaffolds 54 and 36 is also observed by this analysis. The order of superscaffolds 93, 63 and 92 are also confirmed. The only point of conflict in this map with the results from Lepmap2 are markers 54_5045452 and 54_8682873.

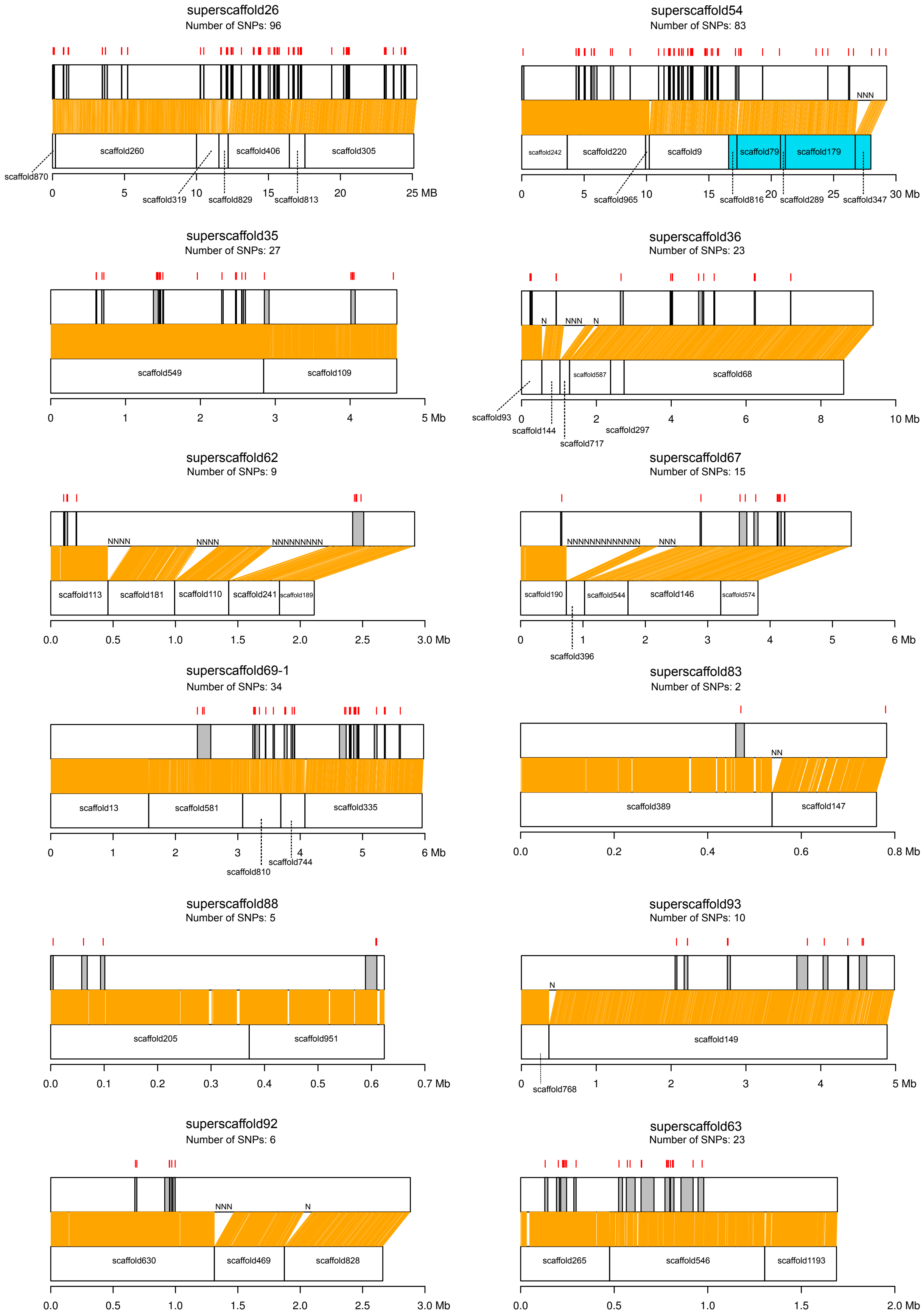
Fig. S4. Linkage group 2. A) male map B) female map

Fig. S5. Repeat density in 50 kb window across ostrich Z chromosome. Dashed line represents the PAR boundary (Figure generated by script from (Kapusta and Suh 2017)).

Fig. S6. GRIMM inversion scenarios for the ancestral states. Each arrow is a synteny block.

Fig. S7. GRIMM inversion scenarios for every pair of ancestral state and the species used in this study. Each arrow is a synteny block.

Fig. S8. Alignment of turtle and alligator genes against lizard chromosome 2 and alignment of genes located in lizard unassigned scaffolds and chromosome 2 to ostrich chromosome Z. Ostrich chromosome Z: 4.73 - 7.1 and 23.76 - 23.83 Mb align to lizard chromosome 1: 34.35-37.1 and 226.35 – 226.44 Mb with conserved order and orientation. Grey: genes with conserved gene orientation between the two species. Red: inverted genes in one species compared to the other.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scaffold951
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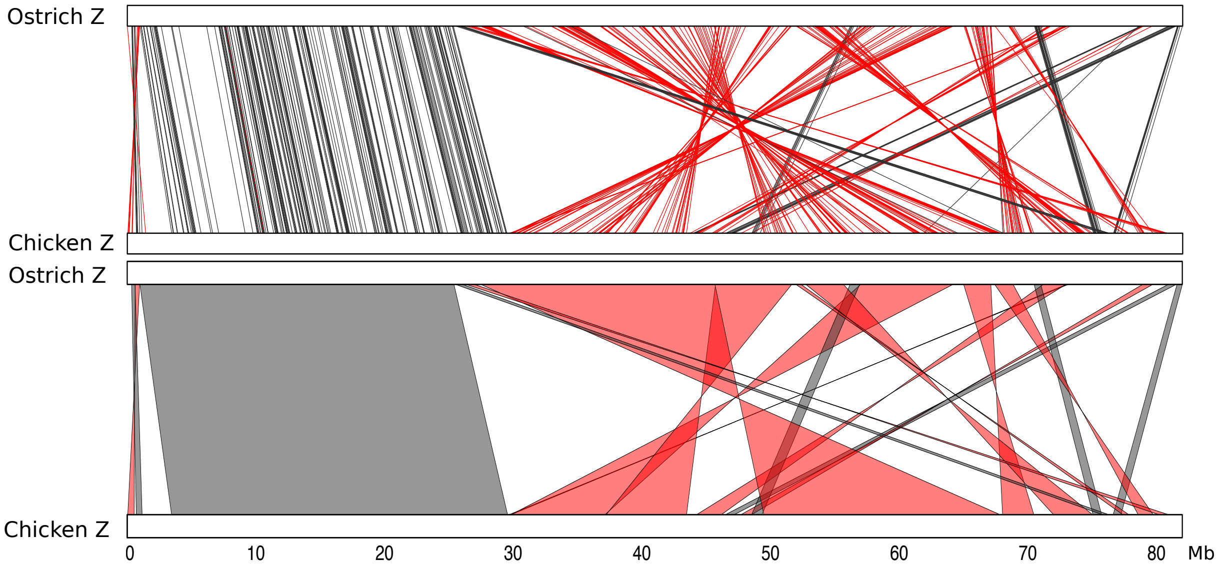
Figure S1.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Chicken Z
Ostrich Z
Chicken Z
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10
20
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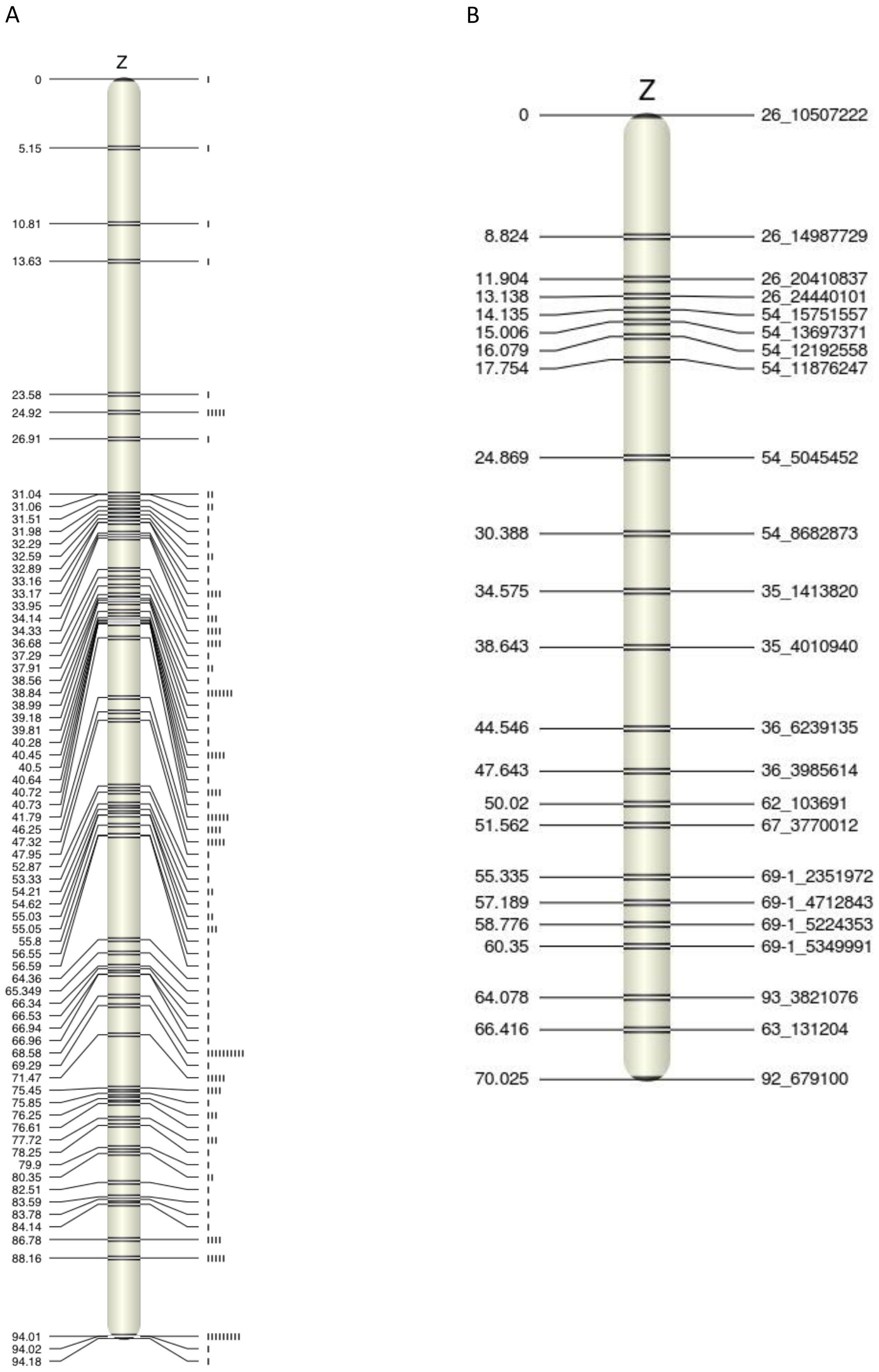
40
50
60
70
80
Mb

Figure S2.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34.14
34.33
36.68
37.29
37.91
38.56
38.84
38.99
39.18
39.81
40.28
40.45
40.5
40.64
40.72
40.73
41.79
46.25
47.32
47.95
52.87
53.33
54.21
54.62
55.03
55.05
55.8
56.55
56.59
64.36
65.349
66.34
66.53
66.94
66.96
68.58
69.29
71.47
75.45
75.85
76.25
76.61
77.72
78.25
79.9
80.35
82.51
83.59
83.78
84.14
86.78
88.16
94.01
94.02
94.18
B
Z
0 26_10507222
8.824 26_14987729
11.904 26_20410837
13.138 26_24440101
14.135 54_15751557
15.006 54_13697371
16.079 54_12192558
17.754 54_11876247
24.869 54_5045452
30.388 54_8682873
34.575 35_1413820
38.643 35_4010940
44.546 36_6239135
47.643 36_3985614
50.02 62_103691
51.562 67_3770012
55.335 69-1_2351972
57.189 69-1_4712843
58.776 69-1_5224353
60.35 69-1_5349991
64.078 93_3821076
66.416 63_131204
70.025 92_679100

Figure S3.

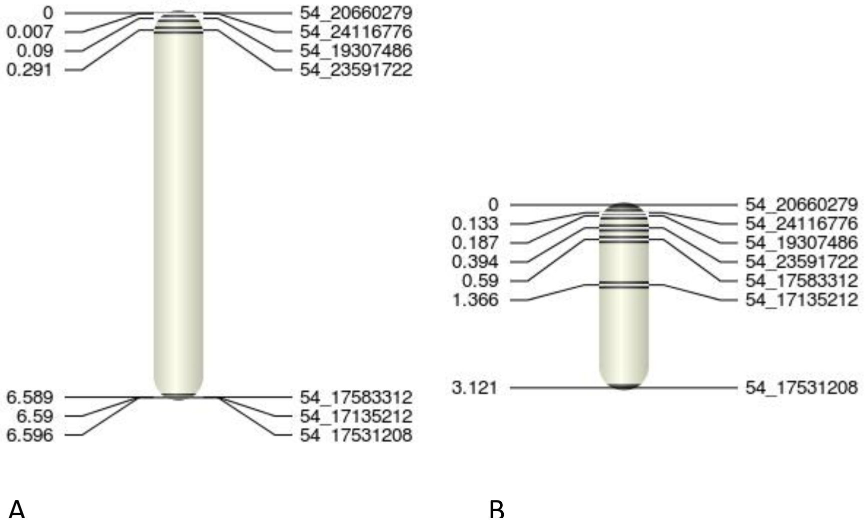
0
0.007
0.09
0.291
54_20660279
54_24116776
54_19307486
54_23591722
6.589
6.59
6.596
54_17583312
54_17135212
54 17531208
A
0
0.133
0.187
0.394
0.59
1.366
3.121
54_20660279
54_24116776
54_19307486
54_23591722
54_17583312
54_17135212
54_17531208
B

Figure S4.

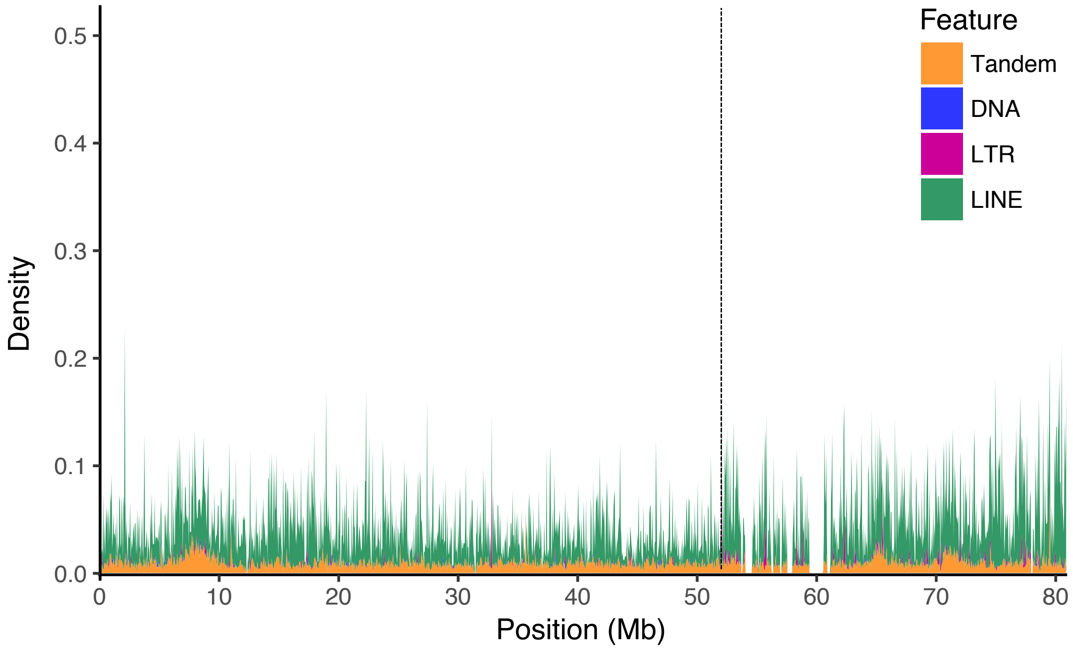
Density
0.5
0.4
0.3
0.2
0.1
0.0
0
10
20
30
40
50
60
70
80
Position (Mb)
Feature
Tandem
DNA
LTR
LINE

Figure S5.

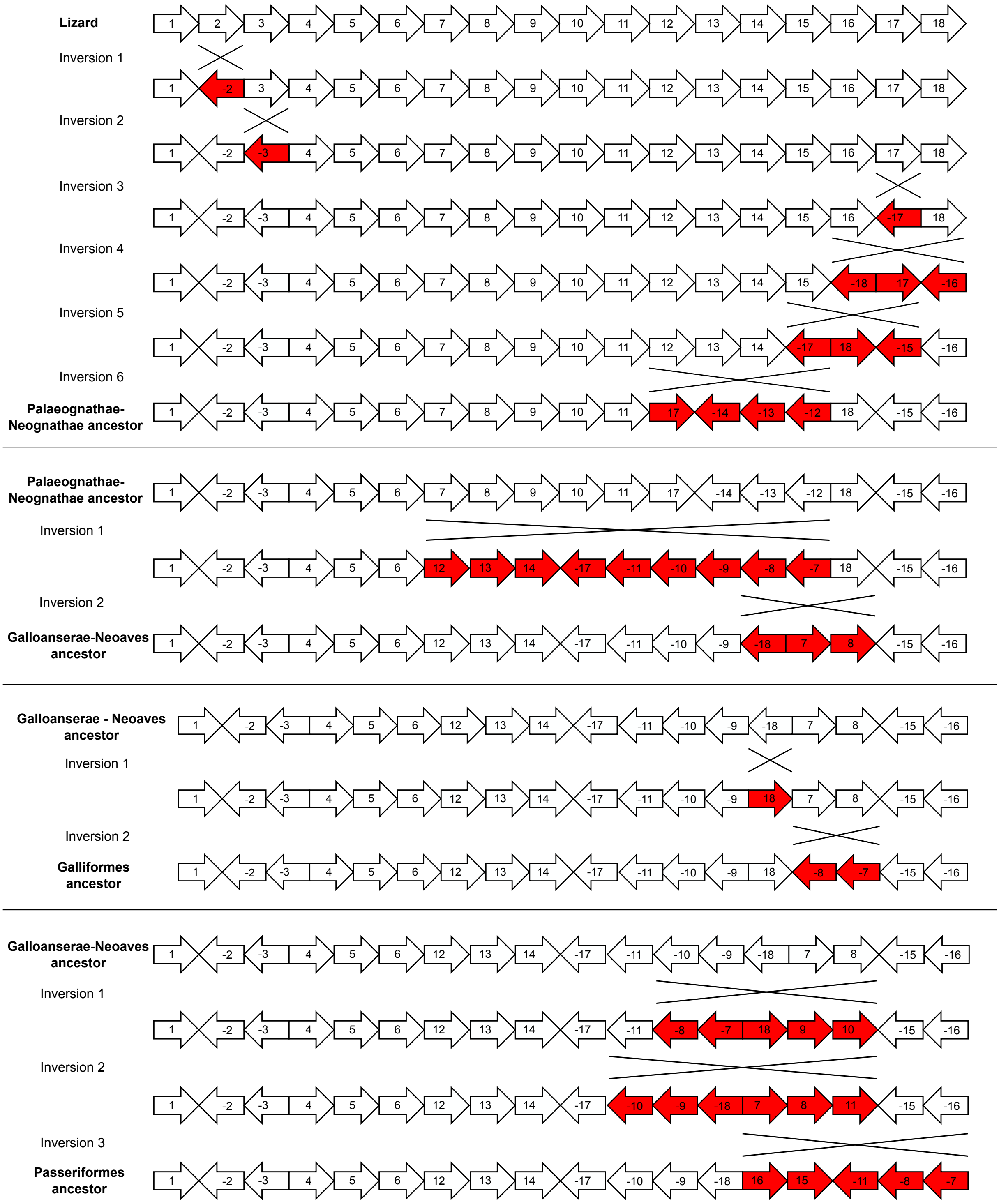
Lizard
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18
Inversion 1
1 -2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18
Inversion 2
1 -2 -3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18
Inversion 3
1 -2 -3 4 5 6 7 8 9 10 11 12 13 14 15 16 -17 18
Inversion 4
1 -2 -3 4 5 6 7 8 9 10 11 12 13 14 15 -18 17 -16
Inversion 5
1 -2 -3 4 5 6 7 8 9 10 11 12 13 14 -17 18 -15 -16
Inversion 6
Palaeognathae-Neognathae ancestor
1 -2 -3 4 5 6 7 8 9 10 11 17 -14 -13 -12 18 -15 -16
Palaeognathae-Neognathae ancestor
1 -2 -3 4 5 6 7 8 9 10 11 17 -14 -13 -12 18 -15 -16
Inversion 1
1 -2 -3 4 5 6 12 13 14 -17 -11 -10 -9 -8 -7 18 -15 -16
Inversion 2
Galloanserae-Neoaves ancestor
1 -2 -3 4 5 6 12 13 14 -17 -11 -10 -9 -18 7 8 -15 -16
Galloanserae - Neoaves ancestor
1 -2 -3 4 5 6 12 13 14 -17 -11 -10 -9 -18 7 8 -15 -16
Inversion 1
1 -2 -3 4 5 6 12 13 14 -17 -11 -10 -9 18 7 8 -15 -16
Inversion 2
Galliformes ancestor
1 -2 -3 4 5 6 12 13 14 -17 -11 -10 -9 18 -8 -7 -15 -16
Galloanserae-Neoaves ancestor
1 -2 -3 4 5 6 12 13 14 -17 -11 -10 -9 -18 7 8 -15 -16
Inversion 1
1 -2 -3 4 5 6 12 13 14 -17 -11 -8 -7 18 9 10 -15 -16
Inversion 2
1 -2 -3 4 5 6 12 13 14 -17 -10 -9 -18 7 8 11 -15 -16
Inversion 3
Passeriformes ancestor
1 -2 -3 4 5 6 12 13 14 -17 -10 -9 -18 16 15 -11 -8 -7

Figure S6.

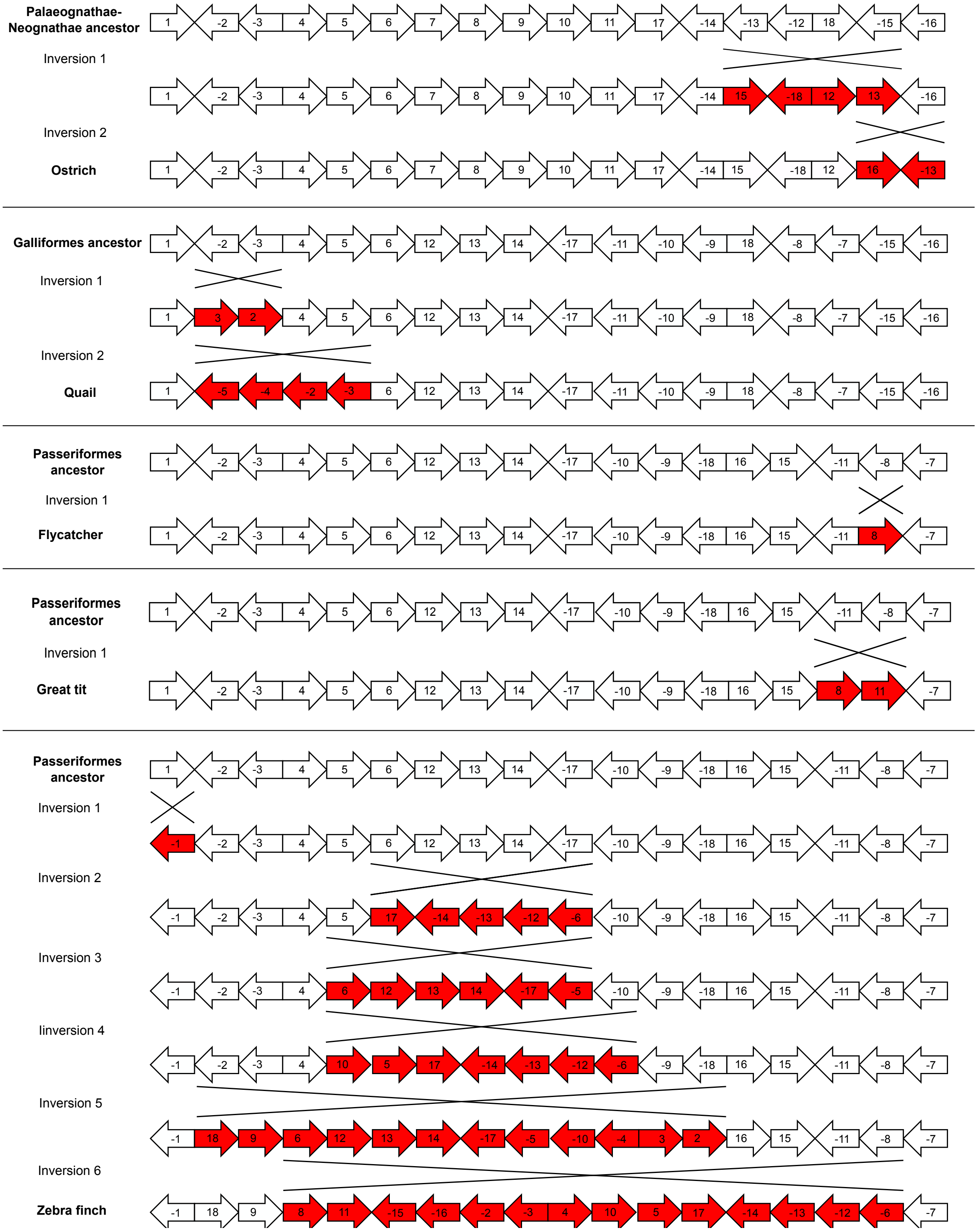
Palaeognathae-Neognathae ancestor
1 -2 -3 4 5 6 7 8 9 10 11 17 -14 -13 -12 18 -15 -16
Inversion 1
1 -2 -3 4 5 6 7 8 9 10 11 17 -14 15 -18 12 13 -16
Inversion 2
Ostrich
1 -2 -3 4 5 6 7 8 9 10 11 17 -14 15 -18 12 16 -13
Galliformes ancestor
1 -2 -3 4 5 6 12 13 14 -17 -11 -10 -9 18 -8 -7 -15 -16
Inversion 1
1 3 2 4 5 6 12 13 14 -17 -11 -10 -9 18 -8 -7 -15 -16
Inversion 2
Quail
1 -5 -4 -2 -3 6 12 13 14 -17 -11 -10 -9 18 -8 -7 -15 -16
Passeriformes ancestor
1 -2 -3 4 5 6 12 13 14 -17 -10 -9 -18 16 15 -11 -8 -7
Inversion 1
Flycatcher
1 -2 -3 4 5 6 12 13 14 -17 -10 -9 -18 16 15 -11 8 -7
Passeriformes ancestor
1 -2 -3 4 5 6 12 13 14 -17 -10 -9 -18 16 15 -11 -8 -7
Inversion 1
Great tit
1 -2 -3 4 5 6 12 13 14 -17 -10 -9 -18 16 15 8 11 -7
Passeriformes ancestor
1 -2 -3 4 5 6 12 13 14 -17 -10 -9 -18 16 15 -11 -8 -7
Inversion 1
-1 -2 -3 4 5 6 12 13 14 -17 -10 -9 -18 16 15 -11 -8 -7
Inversion 2
-1 -2 -3 4 5 17 -14 -13 -12 -6 -10 -9 -18 16 15 -11 -8 -7
Inversion 3
-1 -2 -3 4 6 12 13 14 -17 -5 -10 -9 -18 16 15 -11 -8 -7
Iinversion 4
-1 -2 -3 4 10 5 17 -14 -13 -12 -6 -9 -18 16 15 -11 -8 -7
Inversion 5
-1 18 9 6 12 13 14 -17 -5 -10 -4 3 2 16 15 -11 -8 -7
Inversion 6
Zebra finch
-1 18 9 8 11 -15 -16 -2 -3 4 10 5 17 -14 -13 -12 -6 -7

Figure S7.

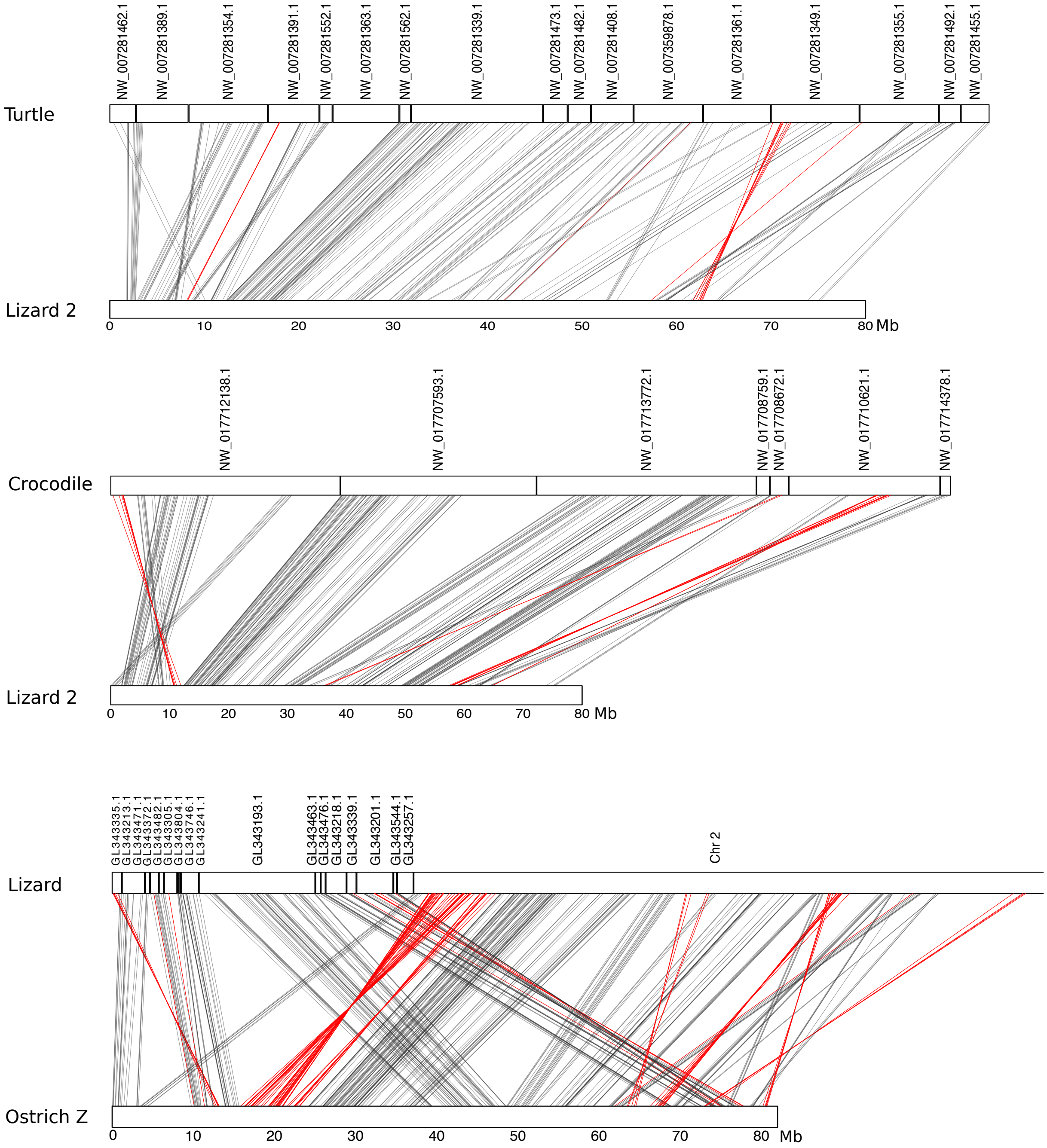
NW_007281462.1
NW_007281389.1
NW_007281354.1
NW_007281391.1
NW_007281552.1
NW_007281363.1
NW_007281562.1
NW_007281339.1
NW_007281473.1
NW_007281482.1
NW_007281408.1
NW_007359878.1
NW_007281361.1
NW_007281349.1
NW_007281355.1
NW_007281492.1
NW_007281455.1
Turtle
Lizard 2
0
10
20
30
40
50
60
70
80
Mb
NW_017712138.1
NW_017707593.1
NW_017713772.1
NW_017708759.1
NW_017708672.1
NW_017710621.1
NW_017714378.1
Crocodile
Lizard 2
0
10
20
30
40
50
60
70
80
Mb
GL343335.1
GL343213.1
GL343471.1
GL343372.1
GL343482.1
GL343305.1
GL343804.1
GL343746.1
GL343241.1
GL343193.1
GL343463.1
GL343476.1
GL343218.1
GL343339.1
GL343201.1
GL343544.1
GL343257.1
Chr 2
Lizard
Ostrich Z
0
10
20
30
40
50
60
70
80
Mb

Figure S8.

References

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