

## Supplementary material

**Supplementary Table 1.** Mean total sperm length of 31 males of *L. megarhynchos* (LM) and 16 males of *L. luscinia* (LL).

Individual	Sperm length ( $\mu\text{m}$ )	Individual	Sperm length ( $\mu\text{m}$ )
LM1	271.885	LL1	218.695
LM2	277.795	LL2	234.185
LM3	279.195	LL3	234.085
LM4	270.865	LL4	240.290
LM5	279.040	LL5	225.730
LM6	280.540	LL6	241.075
LM7	270.870	LL7	227.270
LM8	269.260	LL8	245.210
LM9	277.330	LL9	228.960
LM10	273.225	LL10	235.455
LM11	276.960	LL11	230.620
LM12	272.760	LL12	233.580
LM13	260.900	LL13	247.865
LM14	263.925	LL14	235.170
LM15	262.420	LL15	238.555
LM16	275.515	LL16	217.685
LM17	267.410		
LM18	279.705		
LM19	272.835		
LM20	280.975		
LM21	270.295		
LM22	273.670		
LM23	262.620		
LM24	275.385		
LM25	268.130		
LM26	276.680		
LM27	276.195		
LM28	271.450		
LM29	265.855		
LM30	270.085		
LM31	267.010		

**Supplementary Table 2.** A list of 12 Z-linked intronic loci analyzed in this study and their position on the Z chromosome.

Locus	Ensembl gene ID	Gene name	Position (Mb) <sup>1</sup>
<i>TG401</i>	ENSTGUG00000000401	SPTLC1	6.1
<i>SPINZ-2*</i>	ENSTGUG00000000468	SPIN1	7.5
<i>TG853</i>	ENSTGUG00000000853	TRPM3	16.9
<i>TG1015</i>	ENSTGUG00000001015	APC	21.2
<i>TG1505</i>	ENSTGUG00000001505	ACO1	31.9
<i>TG1925</i>	ENSTGUG00000001925	TARS	40.8
<i>GHR-5.2*</i>	ENSTGUG00000002265	GHR	44.1
<i>ADAMTS6*</i>	ENSTGUG00000002799	ADAMTS6	50.7
<i>PPWD1*</i>	ENSTGUG00000002834	PPWD1	50.9
<i>TG5287</i>	ENSTGUG00000005287	RFX3	64.5
<i>VLDLR-7</i>	ENSTGUG00000005386	VLDLR	64.7
<i>TG5729</i>	ENSTGUG00000005729	TNPO1	66.2

\* Sequences of these loci were taken from a previous study (Storchová *et al.* 2010). The rest of loci were sequences in this study.

<sup>1</sup> Genomic position in the Zebra Finch (*Taeniopygia guttata*) genome (assembly taeGut3.2.4, August 2008). The total length of the Z chromosome is 73 Mb.

**Supplementary Table 3.** Sequences of primers used for PCR amplification and sequencing of 8 Z-linked intronic loci.

Locus	Reference for primer sequences	Forward primer (5' - 3')	Reverse primer (5' - 3')
<i>TG401</i>	This study	TTTTCTCCAGCATTGCACTT	TTTTCTTCTTTCTCCAGATAGCG
<i>TG853</i>	This study	GCACAGAAATCATGGATAGAAAGA	CATGGCTTTATTGGAGTGGC
<i>TG1015</i>	This study	TTGAGGAACTTGTCTGCTGGC	GAAATGCAAGAGCACCATCA
<i>TG1505</i>	This study	CCCAACACCCGTGCTAACTA	AGACCTCCTTGAACATCCCA
<i>TG1925</i>	This study	TGAAAGACTGGAAGTTAAGAAGGAA	TCTCATTGAGGATGCGACAC
<i>TG5287</i>	This study	GAGTCTTTATCGAGAGCACTGTGA	AATAGCGCCAGAAAGTTTGC
<i>TG5729</i>	This study	GTTTGTCTCAAGAGGTGGC	CATGGTACATATCCCACGGA
<i>VLDLR-7</i>	Borge <i>et al.</i> 2005	CAGAAGTGGAGAATGCATAG	ACAGTCACATTCATAGCCAA

**Supplementary Table 4.** Polymorphism statistics for 12 Z-linked and 8 autosomal intronic loci.

Locus	Chr	L <sup>1</sup>	Species <sup>2</sup>	n <sup>3</sup>	S <sup>4</sup>	$\pi$ (%) <sup>5</sup>	$\theta$ (%) <sup>6</sup>	D <sub>xy</sub> (%) <sup>7</sup>	Rm <sup>8</sup>	R <sup>9</sup>
<i>ADAMTS6</i>	Z	483	LM	50	3	0.110	0.139	2.133	1	0.022
			LL	50	8	0.307	0.370	2.075	1	0.014
<i>GHR-5.2</i>	Z	404	LM	50	8	0.302	0.442	4.772	0	0.345
			LL	50	13	0.451	0.718	4.144	1	0.113
<i>PPWD1</i>	Z	574	LM	44	10	0.189	0.400	4.328	0	0.000
			LL	42	8	0.265	0.324	3.824	0	0.135
<i>SPINZ-2</i>	Z	822	LM	50	6	0.191	0.163	4.063	0	0.020
			LL	50	19	0.350	0.516	4.195	0	0.008
<i>TG401</i>	Z	754	LM	50	12	0.224	0.355	4.857	1	0.000
			LL	46	5	0.040	0.151	4.662	0	0.002
<i>TG853</i>	Z	631	LM	50	20	0.714	0.708	/	2	0.008
			LL	40	40	0.854	1.490	/	5	0.055
<i>TG1015</i>	Z	641	LM	42	10	0.285	0.363	3.896	0	0.000
			LL	44	12	0.457	0.430	3.315	0	0.016

<i>TG1505</i>	Z	521	LM	46	12	0.817	0.524	5.620	2	0.031
			LL	40	13	0.386	0.587	5.557	1	/
<i>TG1925</i>	Z	597	LM	31	3	0.032	0.126	7.041	0	/
			LL	26	17	0.745	0.746	6.829	1	0.023
<i>TG5287</i>	Z	558	LM	38	4	0.157	0.171	6.131	0	0.880
			LL	44	8	0.193	0.330	6.040	0	/
<i>TG5729</i>	Z	452	LM	40	1	0.022	0.052	2.666	0	/
			LL	22	6	0.215	0.364	2.605	0	0.092
<i>VLDLR-7</i>	Z	431	LM	46	9	0.381	0.475	5.407	1	0.007
			LL	42	12	0.455	0.647	5.602	1	0.225
<b>All</b>	<b>Z</b>	<b>572</b>	<b>LM</b>	<b>44.8</b>	<b>8.17</b>	<b>0.285</b>	<b>0.327</b>	<b>4.629</b>	<b>0.6</b>	<b>0.131</b>
			<b>LL</b>	<b>41.3</b>	<b>13.4</b>	<b>0.393</b>	<b>0.556</b>	<b>4.441</b>	<b>0.8</b>	<b>0.068</b>
<i>CKB-2</i>	A	453	LM	48	7	0.369	0.348	0.814	0	0.015
			LL	50	14	0.624	0.690	1.307	5	0.073
<i>ACTB-3</i>	A	291	LM	40	15	1.671	1.212	3.505	1	0.037
			LL	42	23	1.471	1.837	3.068	3	0.514
<i>GAPD-3</i>	A	133	LM	44	3	0.828	0.519	4.033	1	1.015

			LL	46	1	0.064	0.171	3.792	0	/
<i>ALASI-8</i>	A	377	LM	38	11	0.634	0.694	4.761	3	0.222
			LL	34	17	0.818	1.103	4.595	3	0.246
<i>GHI-2</i>	A	547	LM	40	18	0.667	0.774	5.495	4	0.135
			LL	48	26	0.936	1.071	5.891	7	0.289
<i>25374</i>	A	710	LM	48	16	0.681	0.508	3.744	2	0.009
			LL	46	35	1.160	1.122	4.057	5	0.005
<i>1152</i>	A	568	LM	50	13	0.461	0.511	4.092	4	0.045
			LL	42	12	0.399	0.491	3.999	3	0.053
<i>25149</i>	A	747	LM	44	28	1.056	0.862	4.974	7	0.038
			LL	46	48	1.327	1.464	4.840	12	0.121
<b>All</b>	<b>A</b>	<b>478</b>	<b>LM</b>	<b>44.0</b>	<b>13.9</b>	<b>0.796</b>	<b>0.679</b>	<b>3.927</b>	<b>3.1</b>	<b>0.217</b>
			<b>LL</b>	<b>44.3</b>	<b>22.0</b>	<b>0.850</b>	<b>0.994</b>	<b>3.944</b>	<b>5.4</b>	<b>0.217</b>

<sup>1</sup> Length of the sequence (bp).

<sup>2</sup> *L. megarhynchos* (LM), *L. luscinia* (LL).

<sup>3</sup> Number of analyzed haploid sequences.

<sup>4</sup> Number of segregating sites.

<sup>5</sup> Average number of nucleotide differences.

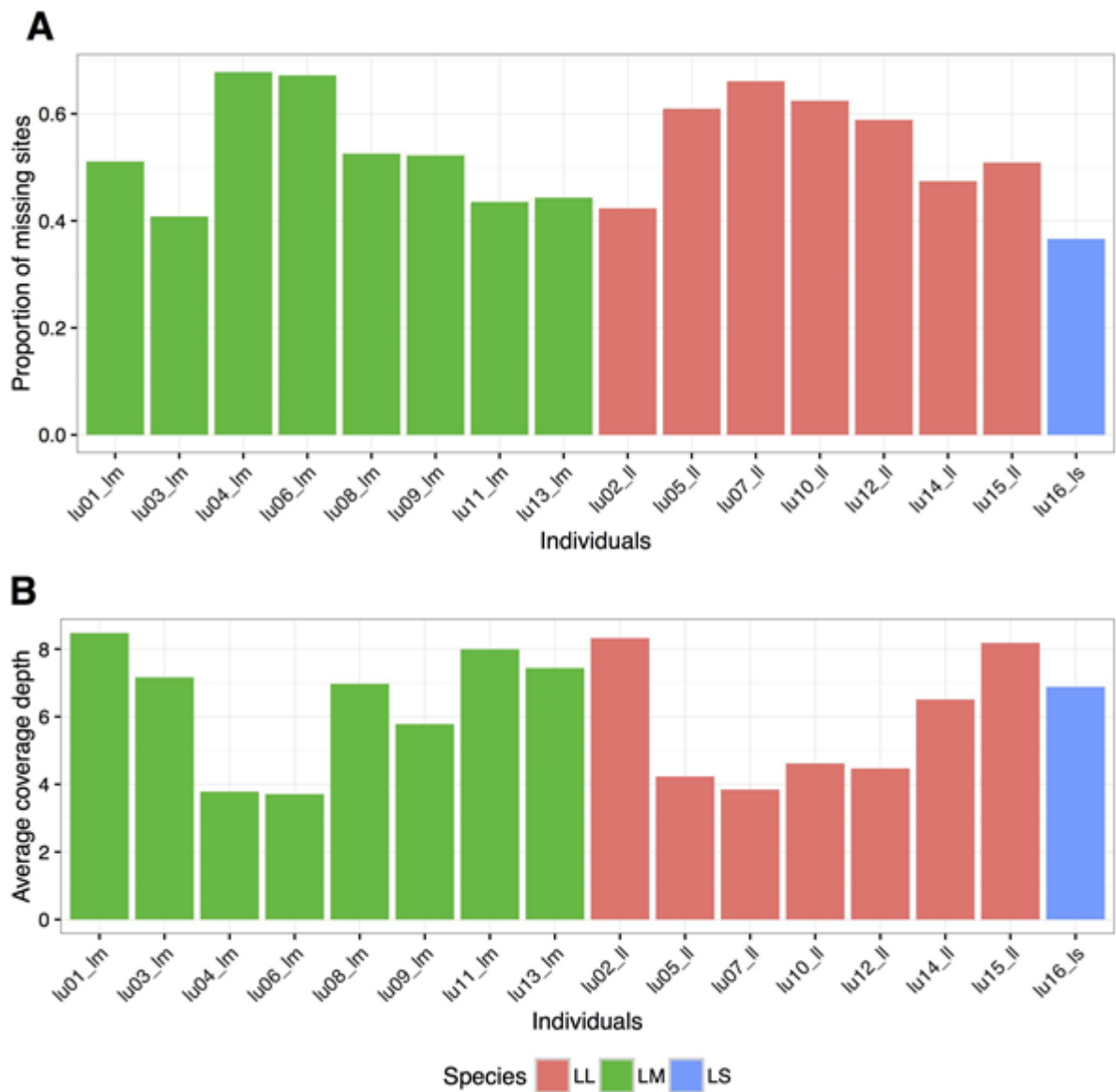
<sup>6</sup> Proportion of polymorphic sites.

<sup>7</sup> Absolute divergence to *L. svecica* measured as average pairwise divergence.

<sup>9</sup> The minimum number of recombination events.

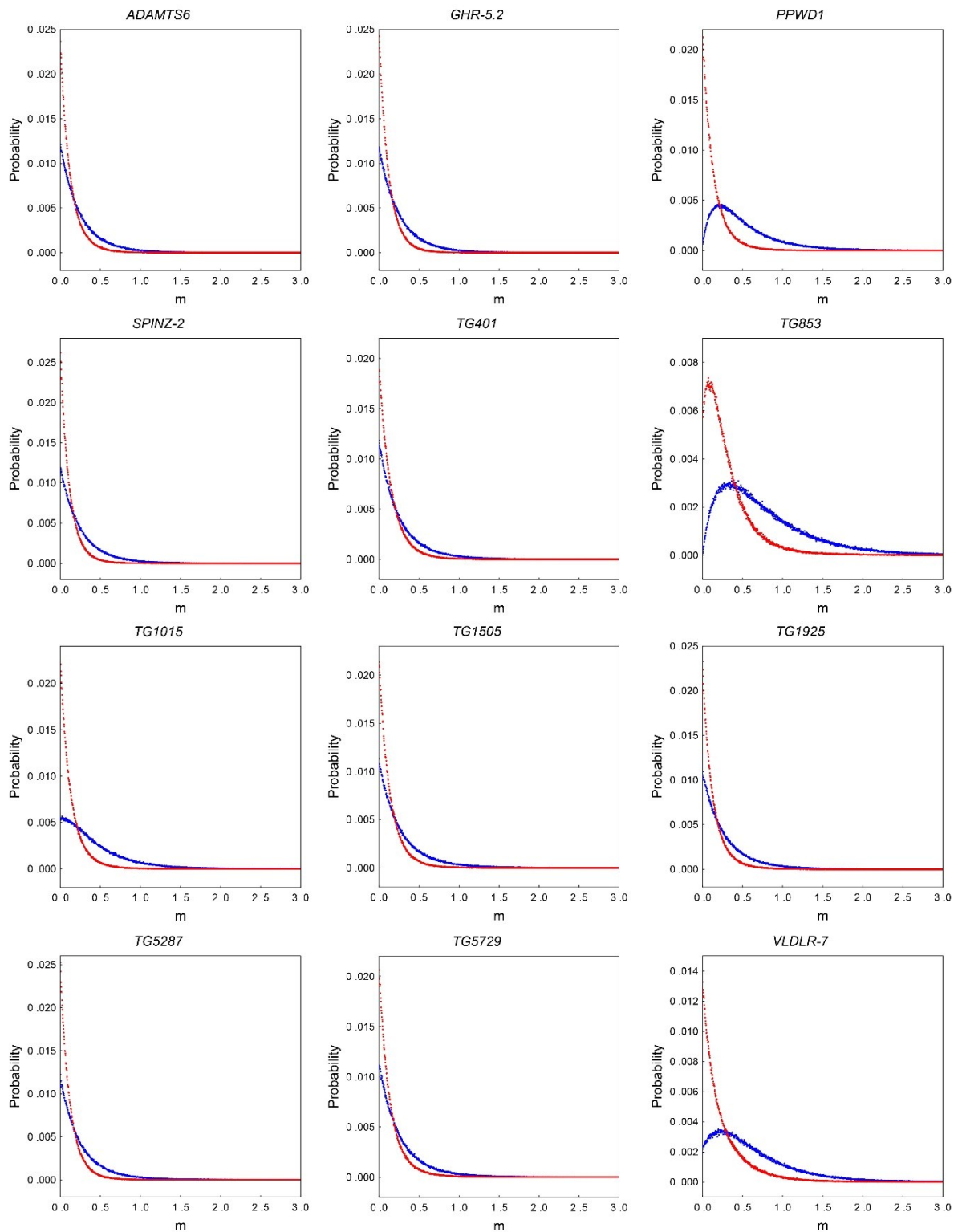
<sup>10</sup> The recombination parameter,  $R = 4Nr$ .

**Supplementary Figure 1.** Proportion of missing sites (A) and average coverage depth (B) per individual across individual samples. Samples of *L. megarhynchos* (LM) in green, *L. luscinia* (LL) in red, and *L. svecica* (LS) in blue.

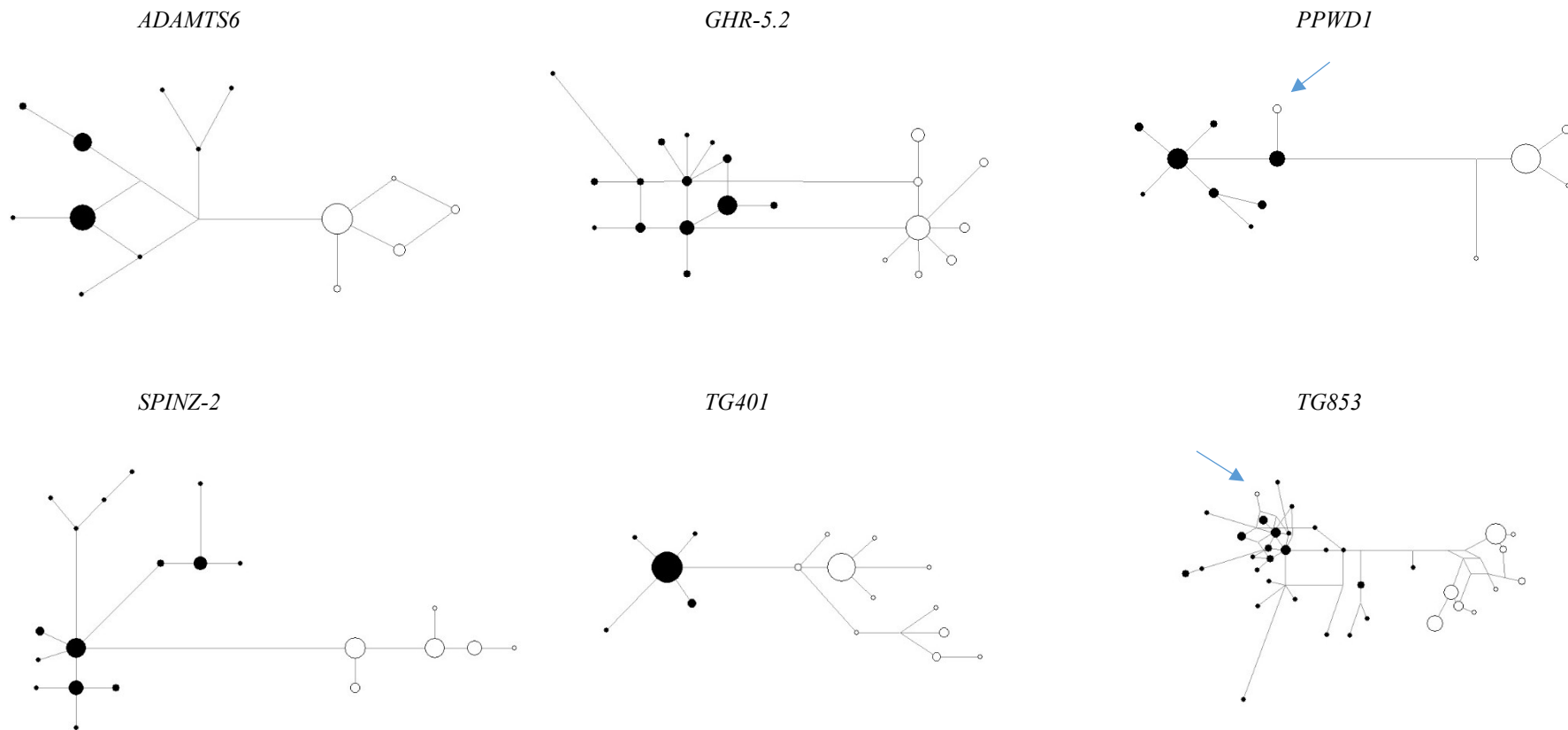




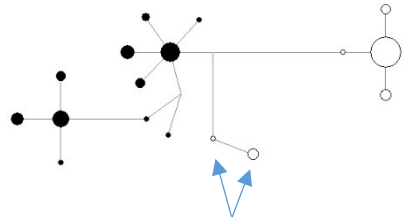
**Supplementary Figure 2.** Posterior probability distributions of migration parameters of the IM model for individual loci. Migration from *L. luscinia* to *L. megarhynchos* (m1, blue line), migration from *L. megarhynchos* to *L. luscinia* (m2, red line).



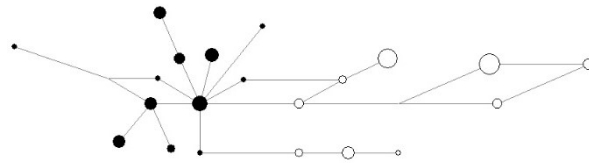
**Supplementary Figure 3.** Haplotype networks for 12 Z-linked intronic loci. Haplotypes coming from *L. megarhynchos* are represented by white circles and haplotypes from *L. luscinia* by black circles. Size of the circles is proportional to the number of haplotypes. Probable introgression events are marked by arrows. Interestingly, they seem to be mostly from *L. luscinia* to *L. megarhynchos* (white circles surrounded by the black ones). Haplotype networks were reconstructed with Network software using Median joining algorithm.



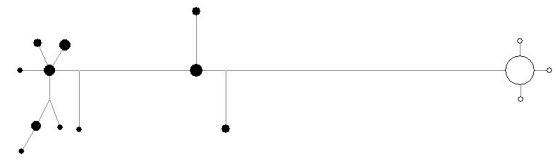
*TG1015*



*TG1505*



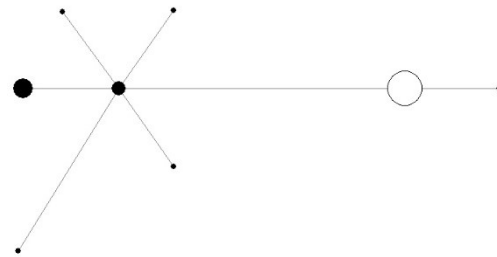
*TG1925*



*TG5287*



*TG5729*



*VLDLR-7*

