Supporting information

The genomic landscape of divergence across the speciation continuum in island-colonising silvereyes (*Zosterops lateralis*)

SUPPLEMENTARY METHODS

Library preparation

100 ng of genomic DNA from each individual was digested with 2.4 U of restriction enzyme *SbfI*-HF (New-England Biolabs Inc., Beverly MA, USA) at 37°C for 60 minutes, followed by an inactivation step of 80°C for 20 minutes. BestRAD *SbfI* adapters were then ligated to the overhanging ends of the products of the restriction reactions by adding 50 nM to each sample with 320 U T4 DNA Ligase (New-England Biolabs Inc.). Barcoding of samples was achieved with a set of index nucleotides within the BestRAD *SbfI* adapter sequences. Reactions were incubated at 20°C for 16 hours and then heat-inactivated by holding at 65°C for 20 minutes. The reactions were then pooled, cleaned with AMPure XP beads, and the products randomly sheared to a mean size of 500 bp by sonication, using a BioRuptor NGS (Diagenode). Following sonication, Dynabeads M-280 streptavidin magnetic beads (Life Technologies) were used to bind the biotinylated ends of the BestRAD Sbf1 adaptors. Sbf1-HF was used once more to release adapter bound fragments and AMPure XP beads were used to clean the samples. After the bead clean up, ends were treated with Blunt End-Repair Mix found in the NEBNext Ultra DNA Library Prep Kit (New-England Biolabs Inc.) to remove overhangs. NEBNext Adaptors for Illumina sequencing were then ligated to blunt ends following a two-step incubation; first incubated at 20°C for 15 minutes with Blunt/TA Ligase Master Mix (New-England Biolabs Inc.) and then at 37°C for 15 minutes with USER Enzyme (New-England Biolabs Inc.). Following size selection using AMPure XP beads to isolate fragments within the size range 300–700 bp, the library was PCR enriched using 10 uM of P1 index primer and 10 uM of Universal PCR Primer (NEB). First a test PCR was run using 5 ul of DNA with 15 PCR cycles. Depending on the brightness of the band produced, the final PCR cycle number was adjusted from 9-12 cycles. PCR products were cleaned using AMPure XP beads. Libraries were sequenced on three Illumina HiSeq4000 lanes (Illumina, San Diego, CA, USA) at the UC Davis Genome Center using paired-end 150-bp sequence reads.

SUPPLEMENTARY TABLES & FIGURES

Supplementary Table 1: List of parameters of the model with default values

Parameter	Definition	Value	Source
N_0	Initial number of breeders	400	Kikkawa and Wilson (1983)
ζ	Number of young fledging by clutch	1.9	Kikkawa and Wilson (1983)
S_S	Survival rate at summer	0.96	Sandvig et al. (2017)
S_a	Survival rate at autumn	0.76	Sandvig et al. (2017)
S_w	Survival rate at winter	0.63	Sandvig et al. (2017)
δ	Sex ratio	0.5	Kikkawa and Wilson (1983)
G	Generation time	3	Clegg et al. (2008)
n_L	Number of SNPs	46,853	Chromosome 5
σ_{dem}	Stochastic demographic variant	0.0013	*
θ	Recombination rate	2, 3, 6, 10, 19	†
m	Migration rate	0.0001, 0.001, 0.01	‡

* This value gives an average of 200 breeding pairs per generation, in line with the size of the breeding population observed on Heron Island (Kikkawa and Wilson 1983).

† Recombination rates observed for parts of the collared-flycatcher (*Ficedula albicollis*) genome (1). The value of 2 cM/Mb is the average for chromosomes > 100Mb, 3 cM/Mb is the genome-wide average; 6 cM/Mb the average for chromosome 17, 10 cM/Mb the average for chromosomes < 10Mb, and 19 cM/Mb the maximum average observed in a collared-flycatcher chromosome.

‡ A migration rate of 0.01 was empirically estimated among Heron Island and neighbouring islands (Brook and Kikkawa 1998). This was taken as the upper estimate of migration rate, but as the population comparisons considered here include more isolated islands, we also ran simulations assuming migration rates one and two magnitudes lower.

Supplementary Table 2: Distributional skew of F_{ST} values calculated for each autosomal chromosome. Only chromosomes with at least 10 x 50kb windows are reported. Comparisons where distributional skew is significantly higher when diverging with gene flow are highlighted in grey. Significance determined using a modified randomisation test.

Chromosome	Early Stage		Mid stage	Late stage	
	Gene flow (SI vs. CI)	No gene flow (SI vs. FP)	Gene flow (ML vs. HI)	Gene flow (GT vs. LF)	No gene flow (ML vs. LH)
1	2.66	3.21	1.51	2.13	1.31
1A	1.93	1.60	1.68	1.80	1.28
1B	-	-	-	-	-
2	2.63	1.82	0.94	2.32	1.28
3	2.48	2.03	1.63	1.64	1.41
4	2.31	1.44	1.57	1.67	2.43
4A	1.72	2.11	0.94	1.62	0.88
5	2.32	1.63	1.88	1.38	1.12
6	2.34	1.43	1.47	1.66	1.73
7	2.43	1.57	1.47	2.13	1.14
8	1.21	3.39	1.50	0.84	1.51
9	1.02	1.63	1.17	1.68	0.98
10	1.97	1.22	1.41	0.55	0.76
11	3.03	2.12	2.25	1.65	1.29
12	2.38	1.05	1.92	1.81	1.08
13	4.75	1.64	1.22	1.76	1.77
14	1.71	1.71	0.92	1.29	1.11
15	1.47	1.13	2.06	1.66	1.28
17	1.68	1.92	1.51	1.53	0.98
18	3.03	3.45	0.57	0.63	-0.13
19	3.38	2.10	0.77	0.31	1.25
20	1.05	2.84	1.38	2.80	1.31
21	3.14	1.21	1.73	1.23	0.76
22	1.67	1.07	1.69	2.44	1.05
23	1.48	0.90	1.35	1.65	-0.30
24	0.89	1.61	0.15	0.64	0.23
25	1.93	1.54	1.41	0.76	0.61
26	1.80	0.76	1.08	0.79	0.86
27	-	-	-	-	-
28	0.87	1.09	-1.12	0.08	0.51
LGE22	1.54	2.23	0.44	-0.01	0.07

Supplementary Table 3: Results of post hoc Dunn's multiple comparison test, showing significance of differences in genomic valley size when detecting genomic valleys using F_{ST} values summarised in 5kb and 100kb windows. Pairwise comparisons with significant differences are highlighted. Below diagonal = z-test-statistic; above diagonal = p-value.

5kb					
	SI vs. CI	**SI vs. FP**	**ML vs. HI**	**GT vs. LF**	**ML vs. LH**
SI vs. CI	-	0.040	0.428	0.482	0.241
SI vs. FP	-1.170	-	0.007	0.024	0.006
ML vs. HI	0.183	2.471	-	0.440	0.242
GT vs. LF	0.046	1.987	-0.151	-	0.235
ML vs. LH	0.702	2.489	-0.700	-0.723	-
100kb					
	SI vs. CI	**SI vs. FP**	**ML vs. HI**	**GT vs. LF**	**ML vs. LH**
SI vs. CI	-	0.500	0.121	0.080	0.081
SI vs. FP	0.000	-	0.111	0.072	0.071
ML vs. HI	-1.172	-1.224	-	0.006	0.004
GT vs. LF	1.405	1.461	2.511	-	0.442
ML vs. LH	1.403	1.471	-2.627	0.145	-
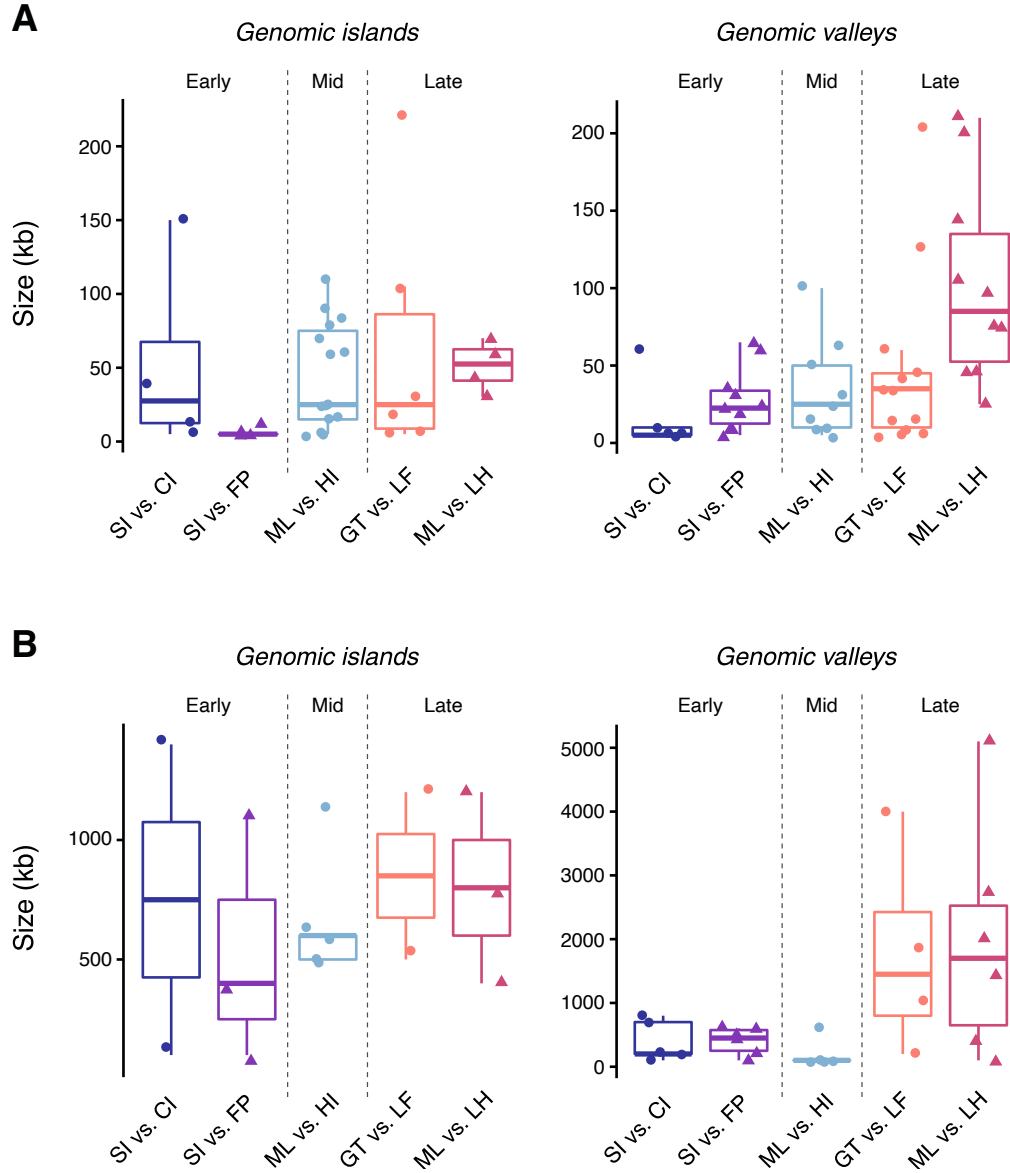
Supplementary Figure 1: Size (kb) of genomic islands and genomic valleys identified for each population comparison using F_{ST} values summarised in (A) 5kb windows; and (B) 100kb windows. Populations diverging with gene flow are indicated by circles and populations diverging in isolation by triangles.

REFERENCES

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