Supplementary figures and tables

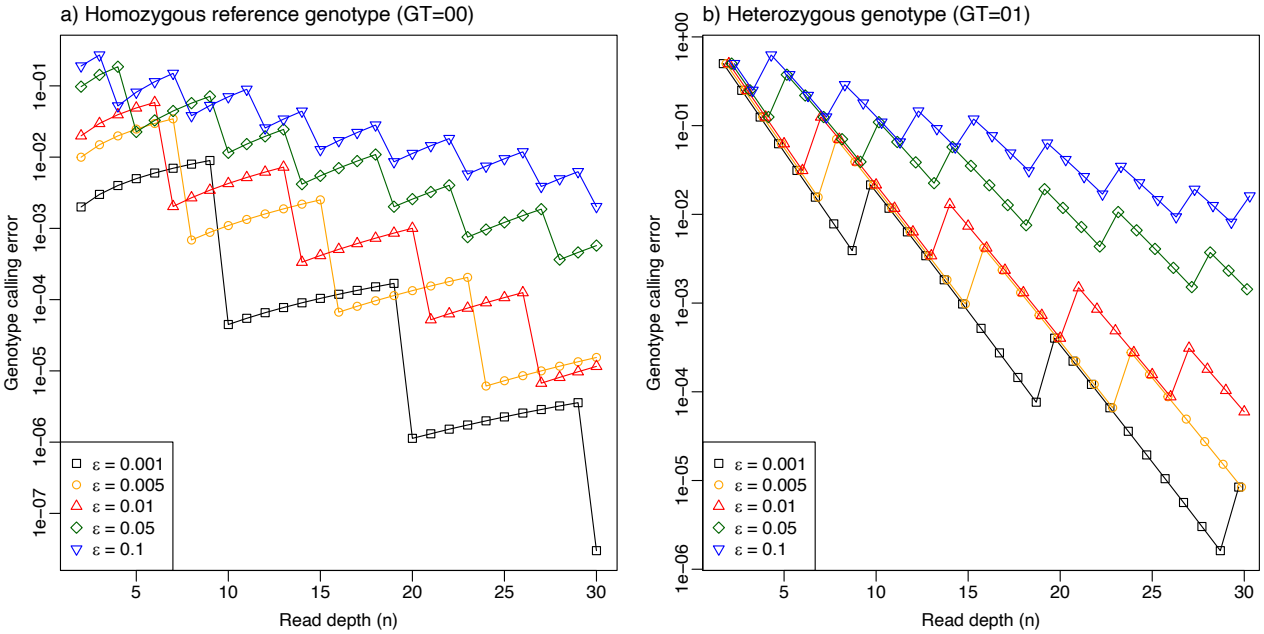


Figure S1. The genotype-calling error rate for a given base-calling error (ε) and read depth (n) when the true genotype is (**a**) a homozygote for the reference allele (GT = 00) or (**b**) a heterozygote (GT = 01). The data are the same shown as in **Figure 1** but a log-10 scale is used for the y-axis.

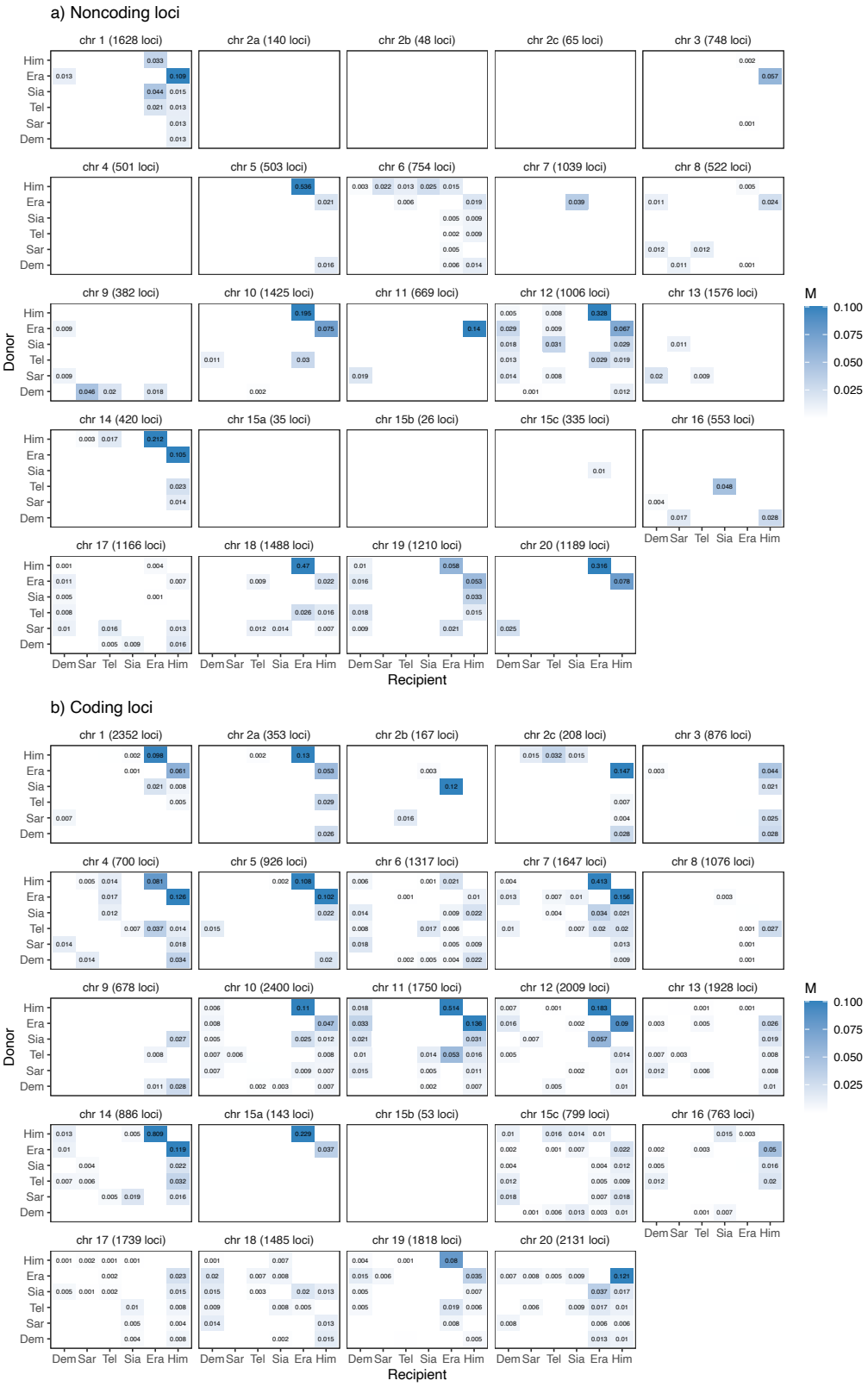


Figure S2. Maximum-likelihood estimates of the pairwise migration rates (*M* = *Nm*) under the IM model from 3S for each chromosomal region in the autosomes using (**a**) noncoding and (**b**) coding loci. The donor and recipient species of gene flow are given in the y- and x-axis, respectively. *H. melpomene* was used as an outgroup. Only significant migration rate estimates (by likelihood ratio test at $p < 0.01$) larger than 0.001 are shown. See **Figure S3** and **Table S8** for estimates of other parameters.

Figure S3. Maximum likelihood estimates of all parameters obtained in the 3S analysis under the IM model for each chromosomal region (the x-axis). The model assumes the species tree ((*S*$_1$, *S*$_2$), *S*$_3$) and allows for gene flow between *S*$_1$ and *S*$_2$ at rates *M*$_{12}$ and *M*$_{21}$. Each locus had three sequences, with one or two sequences from *S*$_1$ or *S*$_2$, and at most one sequence from *S*$_3$. Rows are different species pairs *S*$_1$ and *S*$_2$, with *H. melpomene* used as the outgroup *S*$_3$ in all cases. Columns are parameters in the model. Error bars indicate two standard errors. Standard errors for some parameters for certain datasets were not shown since there were not reliably estimated. A few missing points indicate that the program did not produce reliable estimates due to numerical issues, e.g. chr 2b and 15b noncoding loci from Era/Him. See **Table S8** for complete results.

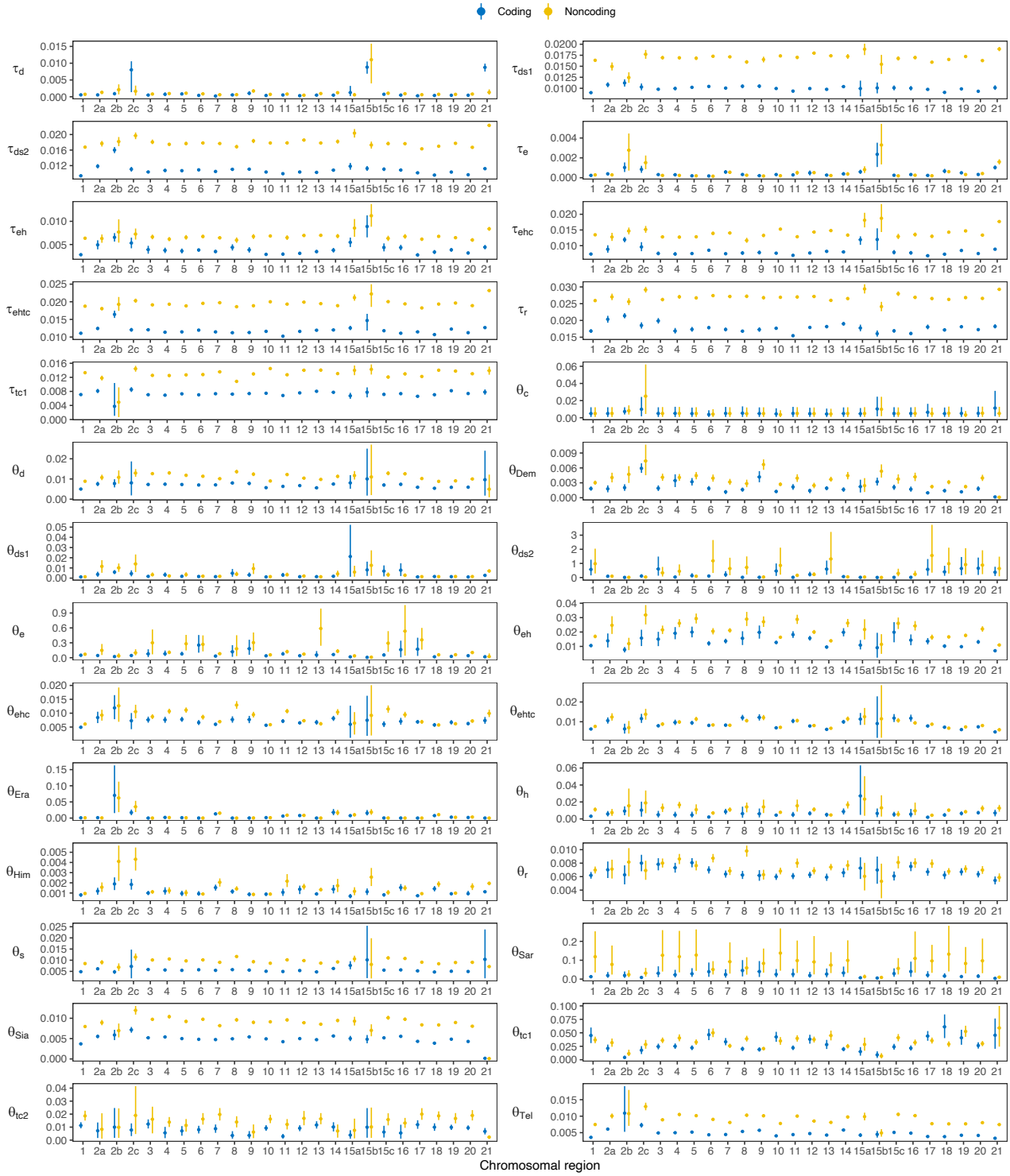


Figure S4. Posterior means and 95% HPD intervals of nineteen population sizes (θ) and nine divergence or introgression times (τ) under the MSci model (**Fig. 4a**) obtained using BPP for the 25 chromosomal regions (see **Table S4** for the number of loci). Only non-redundant parameters are shown; for example, $\tau_d \equiv \tau_s$, so only τ_d is shown. Results for the six introgression probabilities (φs) are in **Figure 4b**.

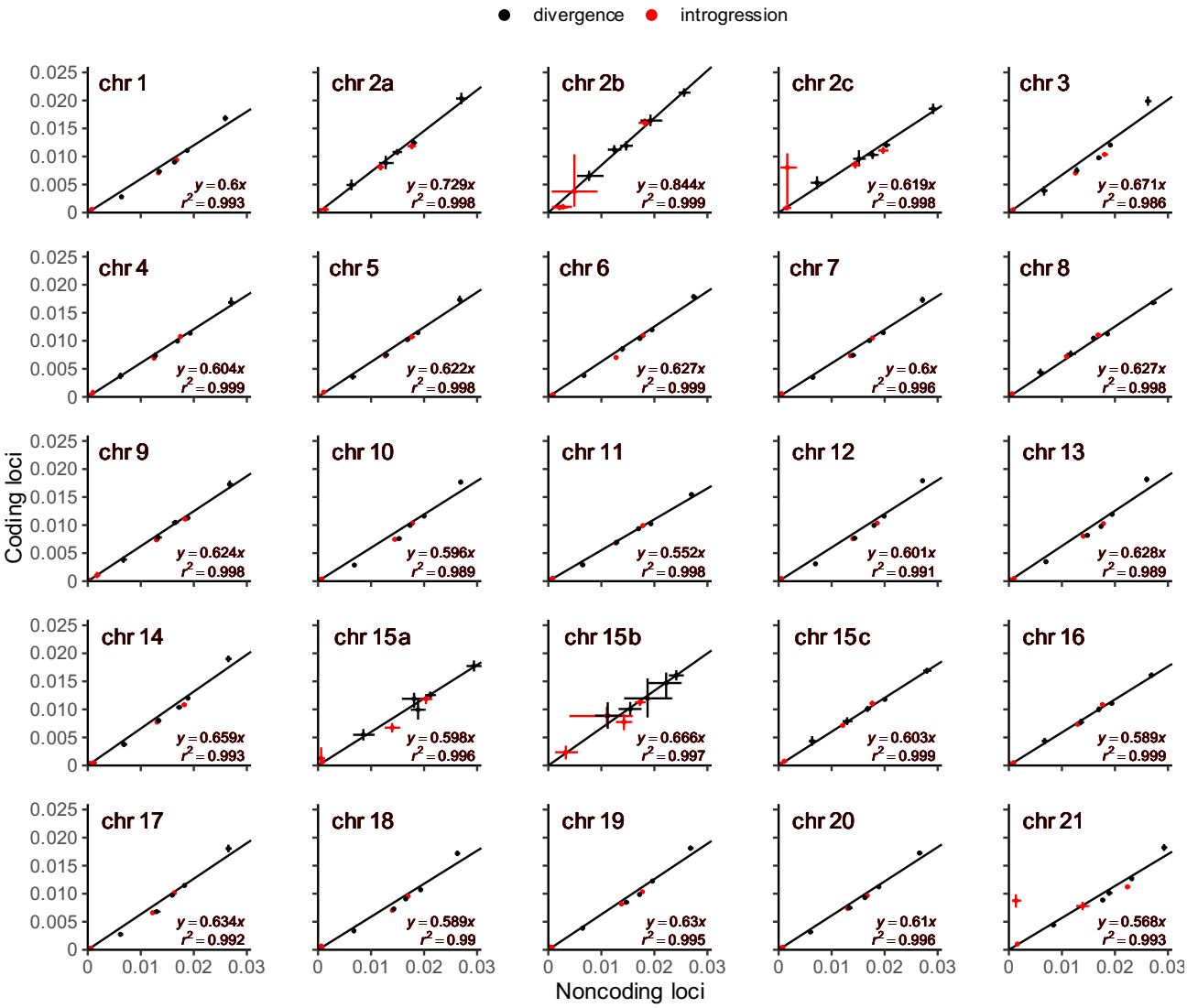


Figure S5. Posterior means and 95% HPD intervals of divergence times (τ) estimated from the noncoding (x-axis) versus coding (y-axis) loci under the MSci model of **Figure 4a**. Linear regression $y = c\,x$ was fitted to the divergence times (black points) only, with the introgression times (red points) excluded. The outlier introgression time for chr 2c and 21 corresponds to *H. sara* → *H. demeter* introgression (τ_d), which was estimated to be close to the present day for noncoding loci like other chromosomal regions, but was poorly estimated and close to the species divergence time for coding loci (**Fig. S4**).

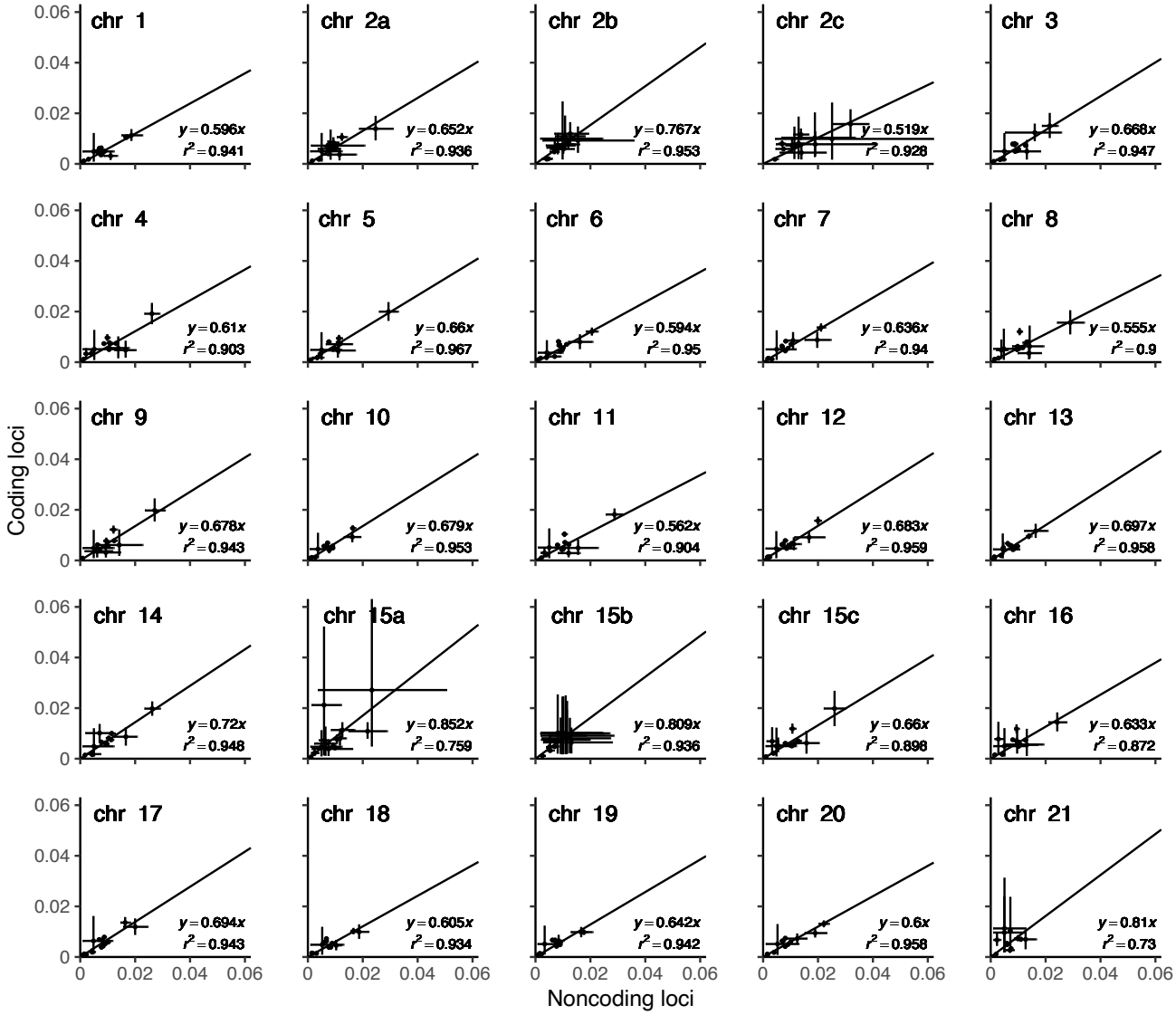
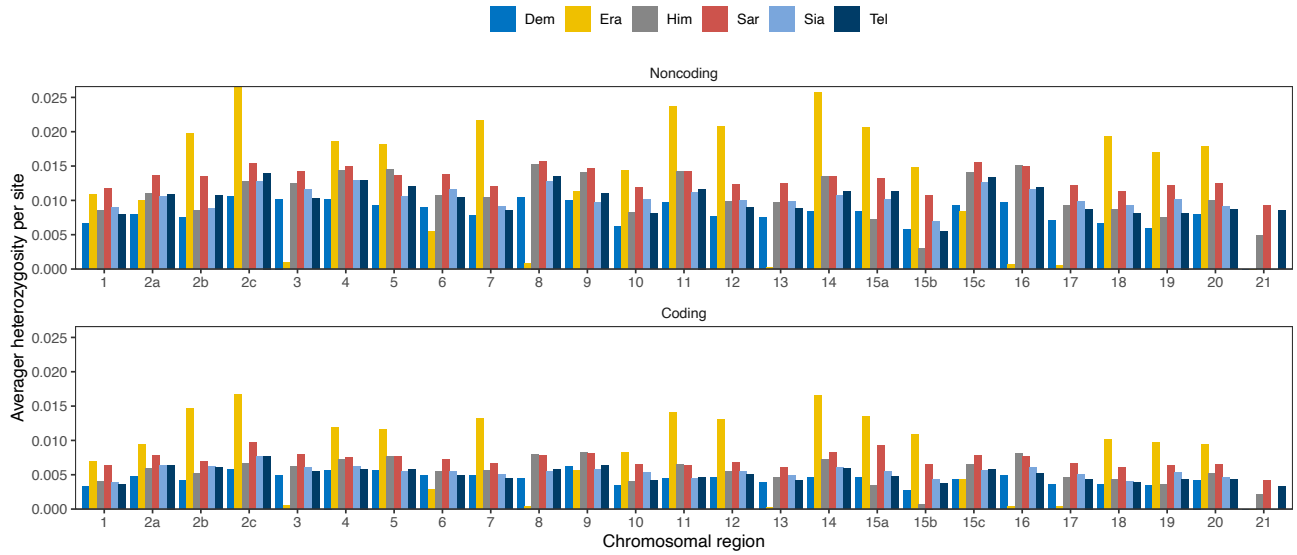


Figure S6. Posterior means and 95% HPD intervals of present-day and ancestral population sizes (θ) estimated from the noncoding (x-axis) versus coding (y-axis) loci under the MSci model (**Fig. 4a**). Linear regression $y = c\,x$ was fitted to well-estimated population sizes, with poorly estimated θ_{ds2}, θ_e, θ_{tc1}, θ_{Sar} and θ_{Era} excluded (see **Fig. S4**).

a)



b)

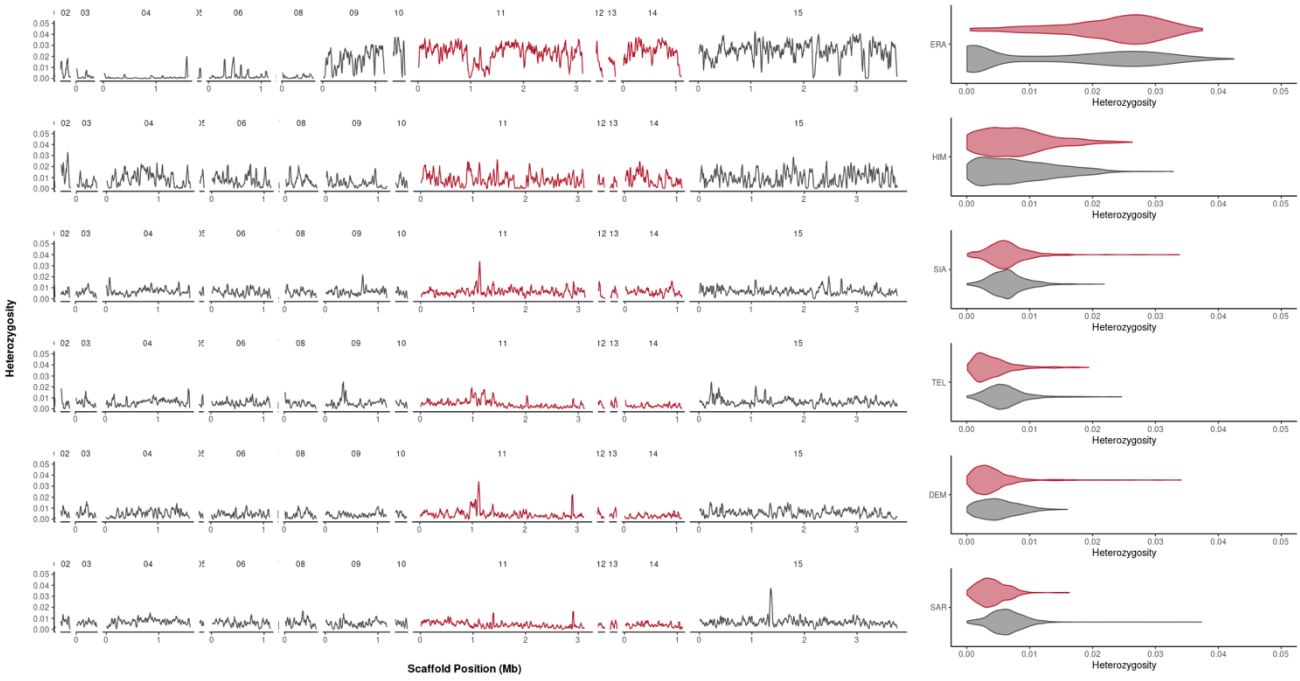


Figure S7. (**a**) Heterozygosity across chromosomal regions for each of the six genomes used in this study. *H. erato* individual (Era) was partially inbred and had large fluctuation of the heterozygosity level across the genome. Note that three individuals Dem, Era and Sia were females which had only one copy of the Z chromosome. (**b**) Heterozygosity in sliding windows (window size of 25 kb, step size of 5 kb) along chromosome 2 shows that the inversion region (red) is not more polymorphic than the flanking regions.

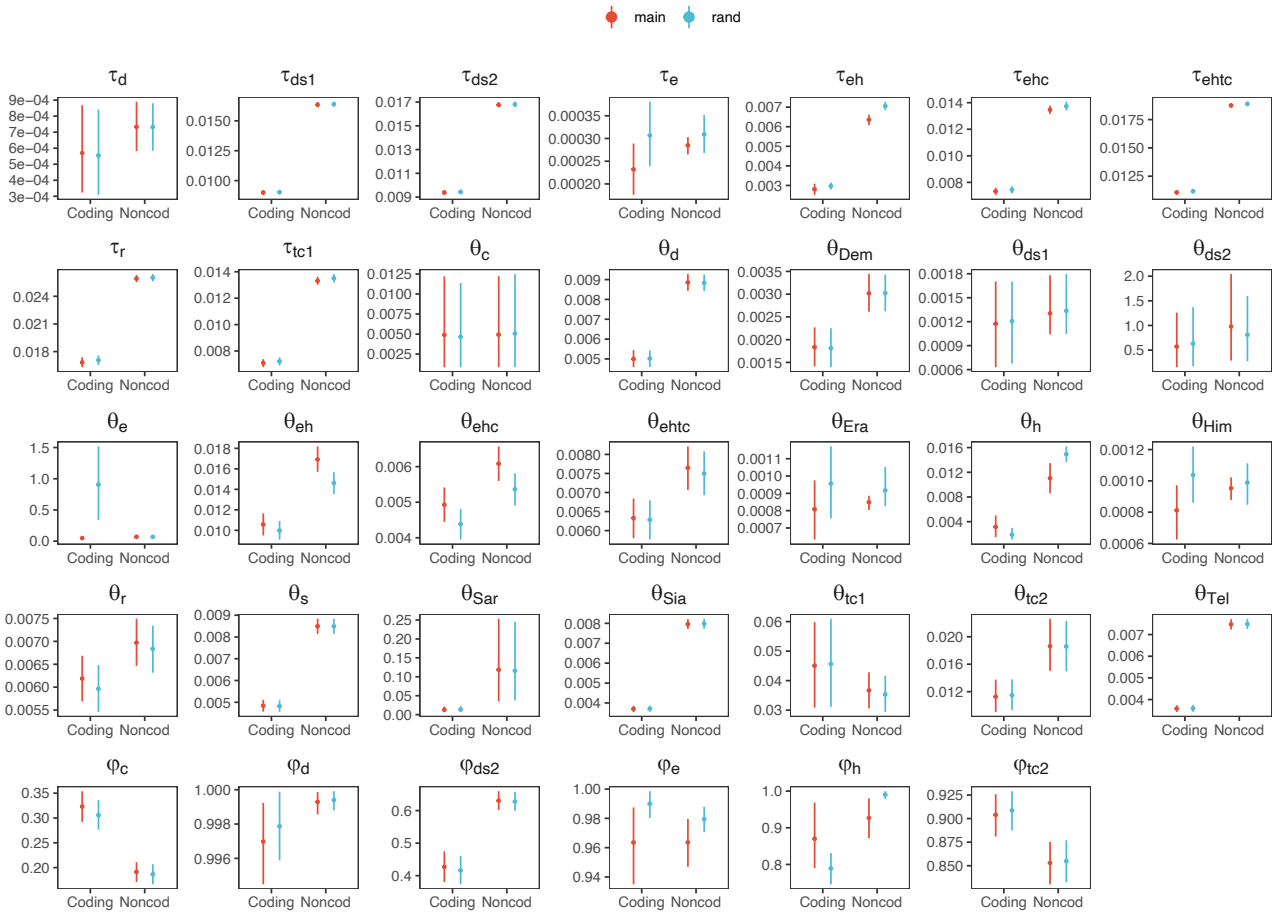


Figure S8. Posterior means and 95% HPD intervals of divergence/introgression times (τ), population sizes (θ) and introgression probabilities (φ) under the MSci model (**Fig. 4a**) obtained from BPP using unphased diploid sequences ('main'; **Table S9**) and randomly phased sequences ('rand') for chromosome 1 (4,902 coding loci, 6,030 noncoding loci). Only non-redundant parameters are shown; for example, $\tau_d \equiv \tau_s$, so only τ_d is shown.

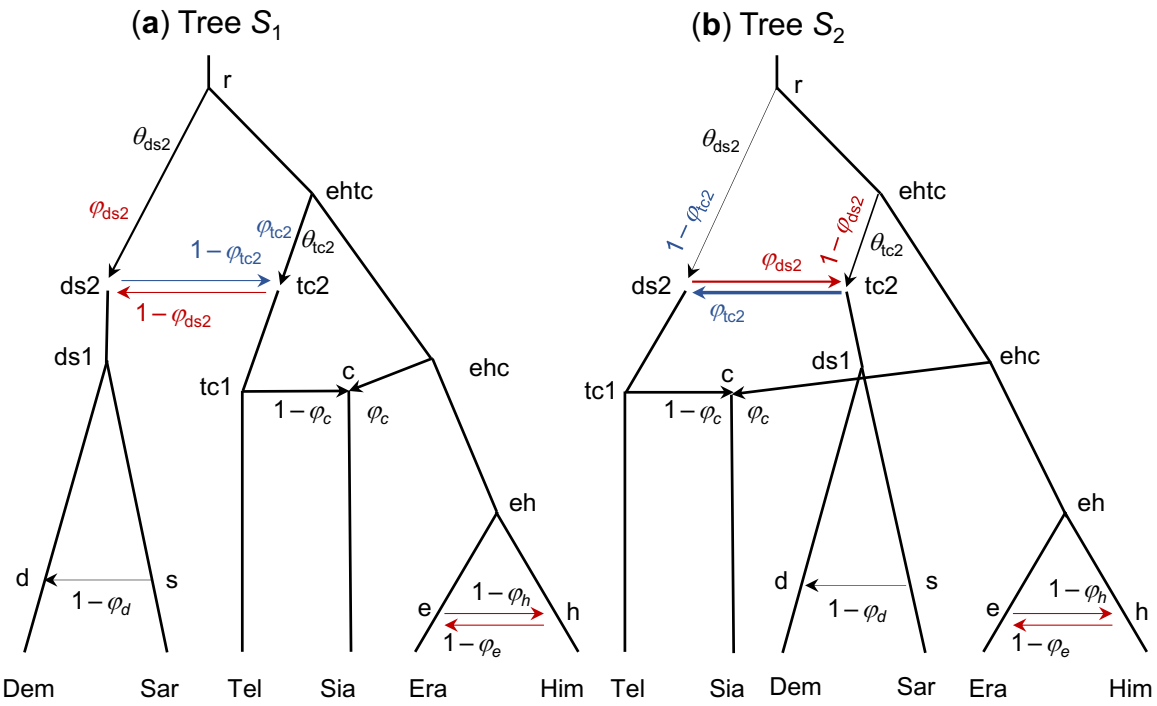


Figure S9. Cross-model unidentifiability of the MSci model (**Fig. 4a**). (**a**) Tree S_1 is the model of **Figure 4a** used in our main analysis. (**b**) Tree S_2 is another unidentifiable model with the same posterior distribution. Each of models S_1 and S_2 has a within-model unidentifiability due to the bidirectional introgression event at nodes e-h; see main text. Thinner lines indicate less likely paths that sequences will pass through, based on posterior estimates of introgression probabilities from BPP under the MSci model (**Fig. 4b**).

Figure S10. Trace plots of MCMC samples from BPP analysis of the MSci model (**Fig. 4a**) using 822 noncoding loci from chromosome 2c (**Table S4**). The x-axis is the MCMC iteration. Colours represent ten independent runs. Two issues were identified in this analysis. First, runs a, c and i had mixing issues (for parameters φ_d, θ_d and θ_s) and those runs were discarded. Second, there were label-switching unidentifiability issues for the Era–Him introgression and thus the samples were pre-processed before summarizing: if $\varphi_e < 0.5$, we changed φ_e to $1 - \varphi_e$, and φ_h to $1 - \varphi_h$, and swapped θ_e and θ_h (Flouri et al., 2020). See **Figure S11** for box plots of the parameters from each run.

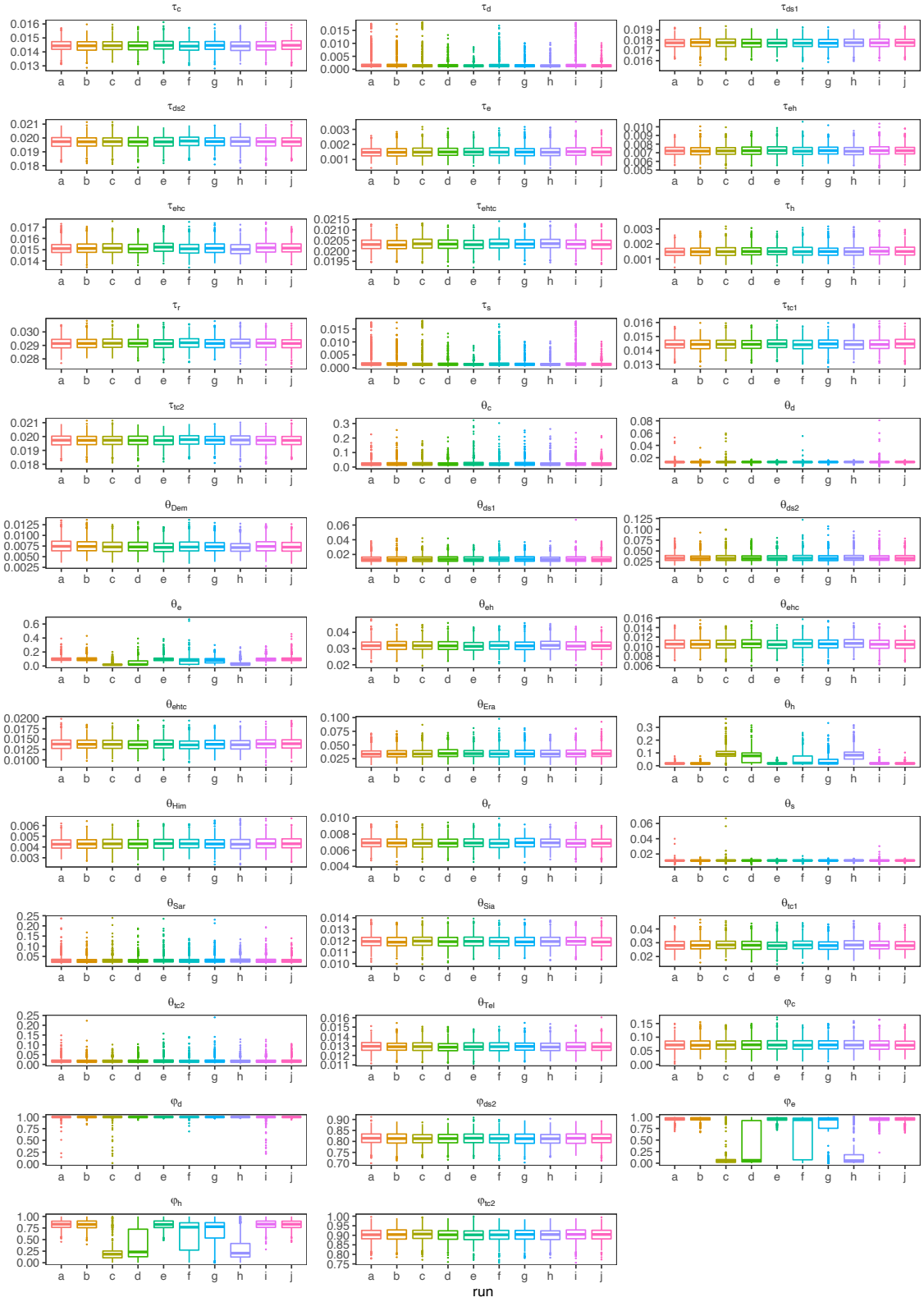


Figure S11. Box plots of the MCMC samples from each of the ten independent runs in **Figure S10**.

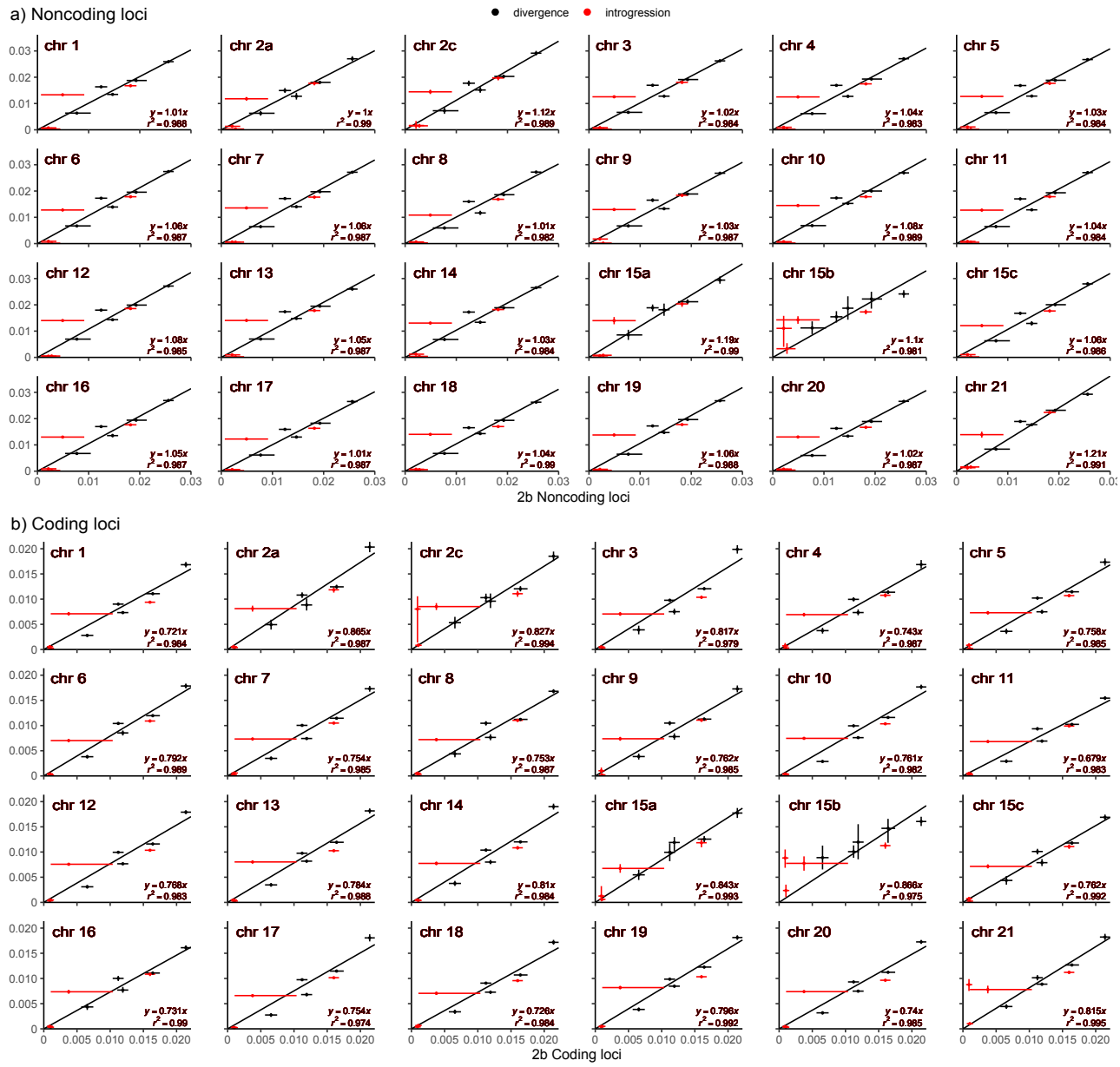


Figure S12. Posterior means and 95% HPD intervals of divergence times (τ) estimated from the chromosome 2b inversion region and other chromosomal regions for (**a**) noncoding and (**b**) coding loci. The slope *c* of the regression $y = c\,x$ measures the relative mutation rate of other region of the genome (y) relative to the 2b region (x). The long horizontal bar in each plot corresponds to an uncertain estimate of the introgression time from *H. telesiphe* into *H. hecalesia* (τ_{tc1}), indicating the absence of such introgression in the 2b region. The divergence time between *H. demeter* and *H. sara* (τ_{ds1}) is always above the regression line, corresponding to the younger estimate of τ_{ds1} in the chromosome 2b region compared to other chromosomes (see **Fig. S4**).

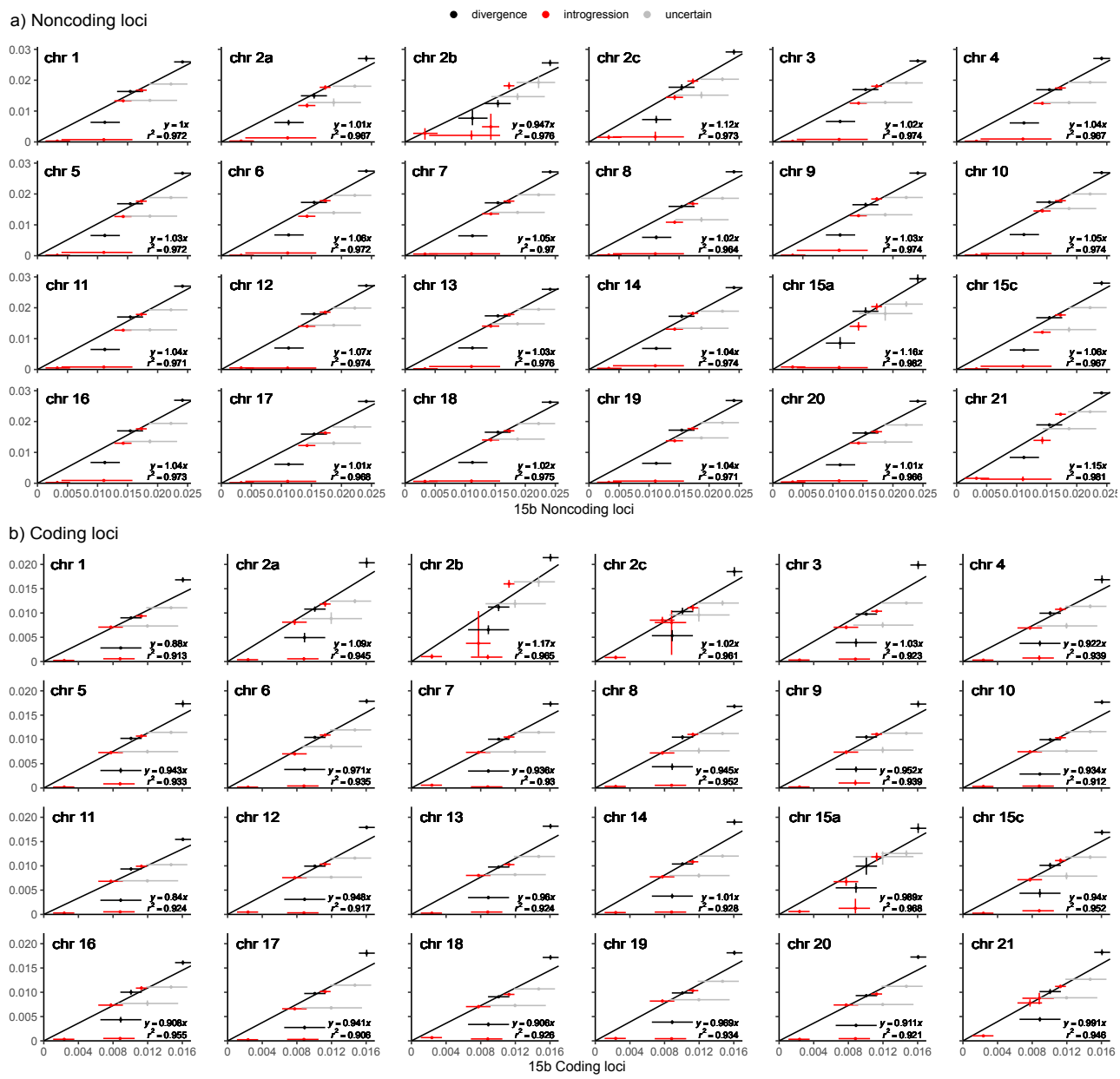


Figure S13. Posterior means and 95% HPD intervals of divergence times (τ) estimated from the chromosome 15b inversion region and other chromosomal regions for (**a**) noncoding and (**b**) coding loci. The slope c of the regression $y = c\,x$ measures the relative mutation rate of other region of the genome (y) relative to the 15b region (x). The grey points correspond to τ_{ehtc} and τ_{ehc} that were poorly estimated (see **Fig. 6b**, Scenario 1) and were excluded from the regression fitting. The time τ_d for *H. sara* → *H. demeter* introgression was estimated to be close to the divergence time in 15b, but with high uncertainty.

Table S1. Sample information.

Clade	Species	Subspecies	Abbreviation	Sex	BROAD ID	Mean depth		Dataset	
						hmelv25 ref	heradem ref	bpp	3s
erato	*H. erato*	*demophoon*	Era	F	SM-6OSJR	37.5	66.6	yes	yes
	H. himera	-	Him	M	SM-6OSJQ	22.0	31.5	yes	yes
	H. hecalesia	*formosus*	Sia	F	SM-ADRBM	53.2	71.8	yes	yes
	H. telesiphe	*telesiphe*	Tel	M	SM-ADRBQ	22.0	28.2	yes	yes
sara	*H. demeter*	-	Dem	F	SM-6OSKP	40.5	50.4	yes	yes
	H. sara	*magdalena*	Sar	M	SM-ADRBN	47.5	57.7	yes	yes
melpomene	*H. melpomene*	*melpomene*	Mel	F	SM-5BSJX	104.2	48.7	no	yes

Table S2. Estimates of the base-calling error rate for homozygous reference allele (GT00) and homozygous alternative allele (GT11).

minDP	Genotype	Dem	Era	Him	Sar	Sia	Tel	Mel
20	GT00	0.06%	0.08%	0.11%	0.06%	0.06%	0.10%	0.08%
	GT11	0.30%	0.33%	0.29%	0.21%	0.22%	0.27%	0.20%
50	GT00	0.07%	0.08%	0.53%	0.06%	0.06%	0.30%	0.08%
	GT11	0.29%	0.32%	1.33%	0.20%	0.21%	0.72%	0.18%

Table S3. Estimates of the base-calling error rate for homozygous reference allele (GT00) and homozygous alternative allele (GT11) by genomic region (coding and noncoding) using different depth (DP) filters: (1) Dpdef: minDP ≥ 12, (2) Dpmin: minDP ≥ 0.5*meanDP, (3) Dpmax: maxDP ≤ 2*meanDP, (4) Dpbot: minDP ≥ 0.5*meanDP and maxDP ≤ 2*meanDP.

Genotype	Region	Filter	Dem	Era	Him	Sar	Sia	Tel	Mel
GT00	noncoding	Dpdef	0.07%	0.08%	0.10%	0.06%	0.06%	0.12%	0.07%
		Dpmin	0.07%	0.08%	0.10%	0.06%	0.06%	0.12%	0.07%
		Dpmax	0.05%	0.05%	0.07%	0.05%	0.05%	0.06%	0.07%
		Dpbot	0.05%	0.05%	0.07%	0.05%	0.05%	0.06%	0.07%
	coding	Dpdef	0.06%	0.07%	0.09%	0.05%	0.06%	0.08%	0.08%
		Dpmin	0.06%	0.07%	0.09%	0.05%	0.06%	0.08%	0.08%
		Dpmax	0.05%	0.06%	0.08%	0.05%	0.05%	0.05%	0.08%
		Dpbot	0.05%	0.06%	0.08%	0.05%	0.05%	0.05%	0.08%
GT11	noncoding	Dpdef	0.28%	0.29%	0.21%	0.19%	0.20%	0.22%	0.12%
		Dpmin	0.26%	0.28%	0.21%	0.18%	0.19%	0.22%	0.10%
		Dpmax	0.26%	0.27%	0.16%	0.18%	0.18%	0.16%	0.11%
		Dpbot	0.24%	0.25%	0.16%	0.18%	0.17%	0.16%	0.10%
	coding	Dpdef	0.17%	0.20%	0.13%	0.10%	0.11%	0.14%	0.16%
		Dpmin	0.17%	0.20%	0.13%	0.10%	0.11%	0.14%	0.15%
		Dpmax	0.16%	0.17%	0.10%	0.09%	0.09%	0.09%	0.13%
		Dpbot	0.15%	0.17%	0.10%	0.09%	0.09%	0.09%	0.12%

Table S4. Number of loci for each of the 25 chromosomal regions in the BPP dataset (in blocks of 100 loci or 200 loci) and in the 3s dataset.

Chr	BPP dataset (6 genomes)														3s dataset (7 genomes)	
	Noncoding loci							Coding loci								
	#loci	100-locus dataset			200-locus dataset			#loci	100-locus dataset			200-locus dataset			#noncoding loci	#coding loci
		#blocks	#loci in last block	last block discarded	#blocks	#loci in last block	last block discarded		#blocks	#loci in last block	last block discarded	#blocks	#loci in last block	last block discarded		
1	6030	61	30	yes	31	30	yes	4902	50	2	yes	25	102	no	1628	2352
2a	1275	13	75	no	7	75	no	1263	13	63	no	7	63	no	140	353
2b	411	5	11	yes	3	11	yes	516	6	16	yes	3	116	no	48	167
2c	822	9	22	yes	5	22	yes	772	8	72	no	4	172	no	65	208
3	3621	37	21	yes	19	21	yes	2410	25	10	yes	13	10	yes	748	876
4	3351	34	51	no	17	151	no	2025	21	25	yes	11	25	yes	501	700
5	3376	34	76	no	17	176	no	2389	24	89	no	12	189	no	503	926
6	4674	47	74	no	24	74	no	3932	40	32	yes	20	132	no	754	1317
7	5060	51	60	no	26	60	no	3935	40	35	yes	20	135	no	1039	1647
8	3147	32	47	no	16	147	no	2829	29	29	yes	15	29	yes	522	1076
9	2870	29	70	no	15	70	no	1828	19	28	yes	10	28	yes	382	678
10	6462	65	62	no	33	62	no	5519	56	19	yes	28	119	no	1425	2400
11	3964	40	64	no	20	164	no	4090	41	90	no	21	90	no	669	1750
12	5602	57	2	yes	29	2	yes	5312	54	12	yes	27	112	no	1006	2009
13	6186	62	86	no	31	186	no	4471	45	71	no	23	71	no	1576	1928
14	3062	31	62	no	16	62	no	2580	26	80	no	13	180	no	420	886
15a	491	5	91	no	3	91	no	493	5	93	no	3	93	no	35	143
15b	149	2	49	no	1	149	no	167	2	67	no	1	167	no	69	138
15c	2832	29	32	yes	15	32	yes	2228	23	28	yes	12	28	yes	292	714
16	3479	35	79	no	18	79	no	2090	21	90	no	11	90	no	553	763
17	5017	51	17	yes	26	17	yes	4531	46	31	yes	23	131	no	1166	1739
18	5914	60	14	yes	30	114	no	3692	37	92	no	19	92	no	1488	1485
19	5734	58	34	yes	29	134	no	4533	46	33	yes	23	133	no	1210	1818
20	5087	51	87	no	26	87	no	4864	49	64	no	25	64	no	1189	2131
21	4350	44	50	no	22	150	no	3628	37	28	yes	19	28	yes	574	1348
Total	92966	933	-	-	472	-	-	74999	749	-	-	382	-	-	18002	29552

Table S5. Proportions of species tree estimates (MAP trees, with minimum, median and maximum posterior probabilities shown in parentheses) from the blockwise BPP MSC analysis using blocks of 100 loci and 200 loci, summarized into four major regions: auto (all autosomes excluding 2b and 15b inversions), 2b inversion, 15b inversion and the Z chromosome (chr 21). n is the number of blocks. Trees are ordered in decreasing proportions of blocks for the 100-locus dataset. Tree indices correspond to those of **Figure 2**.

Region	Group	Tree	Tree index	100-locus dataset		200-locus dataset	
				n	Proportion	n	Proportion
Noncoding	auto	((Dem, Sar), ((Era, Him), (Sia, Tel)));	i	511	0.579 (0.39, 1.00, 1.00)	278	0.622 (0.44, 1.00, 1.00)
		((Dem, Sar), (((Era, Him), Sia), Tel));	ii	356	0.403 (0.48, 1.00, 1.00)	168	0.376 (0.50, 1.00, 1.00)
		((Dem, Sar), (((Era, Him), Tel), Sia));	vi	11	0.013 (0.44, 0.69, 0.95)	1	0.002 (0.50, 0.50, 0.50)
		(((Dem, Sar), (Sia, Tel)), (Era, Him));	iv	5	0.006 (0.87, 0.95, 1.00)	0	0
	2b	(((Dem, Sar), Tel), ((Era, Him), Sia));	iii	4	1.000 (1.00, 1.00, 1.00)	2	1.000 (1.00, 1.00, 1.00)
	15b	(((Dem, Sar), (Sia, Tel)), (Era, Him));	iv	2	1.000 (0.66, 0.83, 1.00)	1	1.000 (1.00, 1.00, 1.00)
	21	((Dem, Sar), (((Era, Him), Sia), Tel));	ii	43	0.977 (0.92, 1.00, 1.00)	22	1.000 (1.00, 1.00, 1.00)
		(((Dem, Sar), Tel), ((Era, Him), Sia));	iii	1	0.023 (0.98, 0.98, 0.98)	0	0
Coding	auto	((Dem, Sar), ((Era, Him), (Sia, Tel)));	i	331	0.469 (0.43, 0.99, 1.00)	179	0.497 (0.55, 1.00, 1.00)
		((Dem, Sar), (((Era, Him), Sia), Tel));	ii	327	0.463 (0.42, 0.98, 1.00)	171	0.475 (0.52, 1.00, 1.00)
		(((Dem, Sar), (Sia, Tel)), (Era, Him));	iv	21	0.030 (0.47, 0.75, 1.00)	6	0.017 (0.53, 0.98, 1.00)
		((Dem, Sar), (((Era, Him), Tel), Sia));	vi	13	0.018 (0.40, 0.61, 0.96)	3	0.008 (0.37, 0.38, 0.86)
		(((Dem, Sar), Tel), ((Era, Him), Sia));	iii	5	0.007 (0.59, 0.68, 0.94)	0	0
		(((Dem, Sar), ((Era, Him), Sia)), Tel);	viii	3	0.004 (0.46, 0.49, 0.68)	0	0
		((Dem, ((Era, Him), (Sia, Tel))), Sar);	x	3	0.004 (0.48, 0.53, 0.85)	0	0
		(((Dem, ((Era, Him), Sia)), Tel), Sar);	v	1	0.001 (0.95, 0.95, 0.95)	1	0.003 (1.00, 1.00, 1.00)
		(((Dem, Sar), Sia), ((Era, Him), Tel));	vii	1	0.001 (0.69, 0.69, 0.69)	0	0
		((Dem, (((Era, Him), Sia), Tel)), Sar);	ix	1	0.001 (0.90, 0.90, 0.90)	0	0
	2b	(((Dem, Sar), Tel), ((Era, Him), Sia));	iii	5	1.000 (1.00, 1.00, 1.00)	3	1.000 (1.00, 1.00, 1.00)
	15b	(((Dem, Sar), (Sia, Tel)), (Era, Him));	iv	2	1.000 (0.84, 0.92, 1.00)	1	1.000 (1.00, 1.00, 1.00)
	21	((Dem, Sar), (((Era, Him), Sia), Tel));	ii	35	0.972 (0.85, 1.00, 1.00)	18	1.000 (0.99, 1.00, 1.00)
		(((Dem, Sar), Tel), ((Era, Him), Sia));	iii	1	0.028 (0.56, 0.56, 0.56)	0	0

Table S6. Proportions of species tree estimates (MAP trees), with minimum (pr_min), median (pr_med) and maximum (pr_max) posterior probabilities, from the BPP MSC analysis using blocks of 100 loci, summarized by chromosomal region.
See excel file.

Table S7. Proportions of species tree estimates (MAP trees), with minimum (pr_min), median (pr_med) and maximum (pr_max) posterior probabilities, from the BPP MSC analysis using blocks of 200 loci, summarized by chromosomal region.
See excel file.

Table S8. Maximum likelihood estimates (MLEs) of parameters under the isolation-with-migration (IM) model obtained from 3s for each of the fifteen pairs of species. LRT (likelihood ratio test) statistic was used to select the best-fit model with or without gene flow (M2 vs M0), using the p-value threshold of 1%. See **Figure S3** for plots of these estimates. 'auto' used all autosomal loci excluding the two inversion regions (see **Table S4** for the number of loci).
See excel file.

Table S9. Posterior means and 95% HPD intervals (in parentheses) of parameters under the MSci model in **Figure 4a** obtained from BPP using noncoding and coding loci from each chromosomal region.
See excel file.